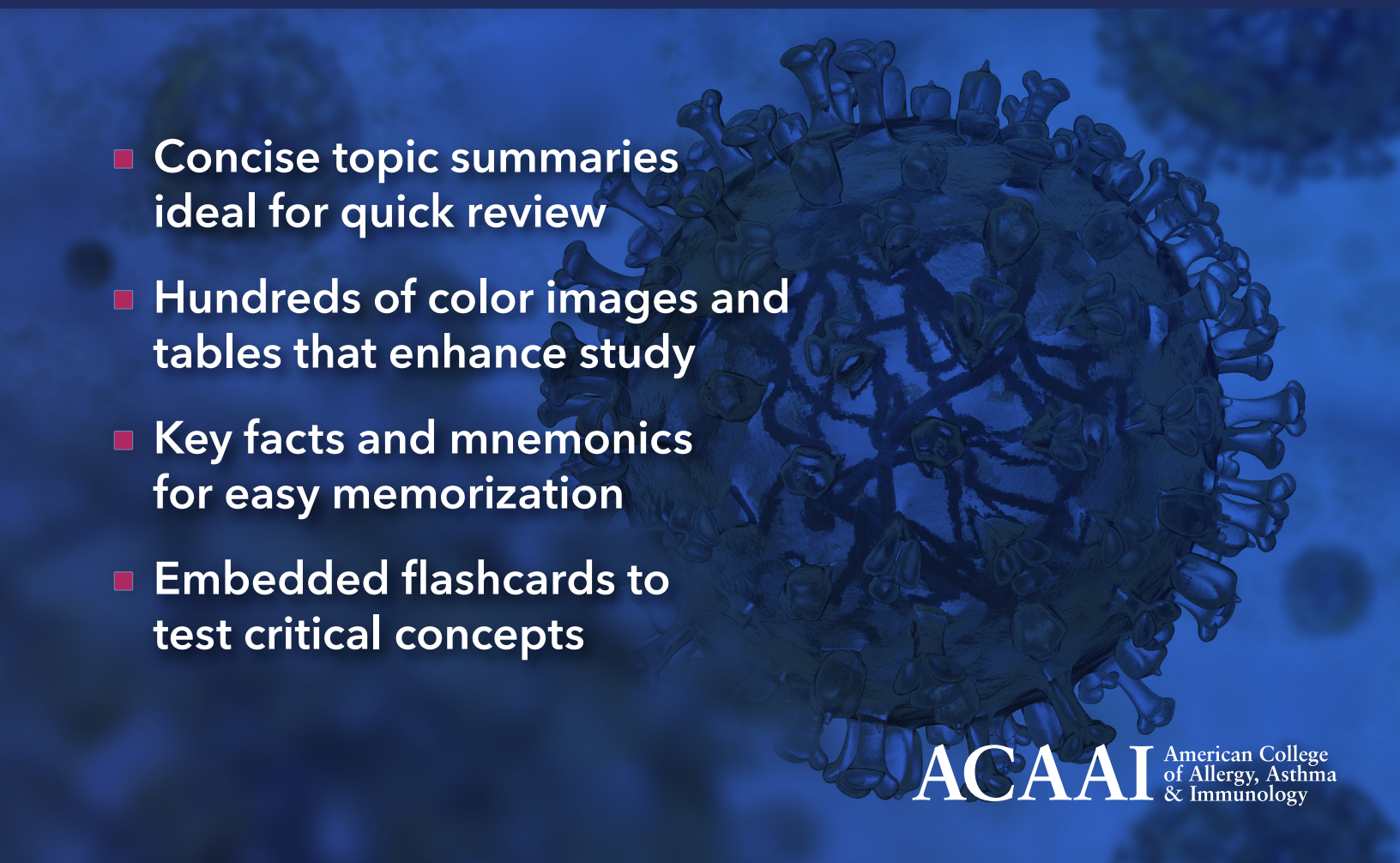
TAO **LE** BRET **HAYMORE**
VIVIAN **HERNANDEZ-TRUJILLO** GERALD **LEE**

ACAAI REVIEW FOR THE ALLERGY & IMMUNOLOGY BOARDS

SECOND EDITION



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ACAAI American College of Allergy, Asthma & Immunology

ACAAI REVIEW FOR THE ALLERGY & IMMUNOLOGY BOARDS

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Tao Le, MD, MHS
Associate Clinical Professor of Medicine and Pediatrics
Chief, Section of Allergy and Immunology
Department of Medicine
University of Louisville School of Medicine, Kentucky

Bret Haymore, MD
Assistant Professor
University of Oklahoma Health Science Center
Medical Director, *Breathe*America
Tulsa, Oklahoma

Vivian Hernandez-Trujillo, MD
Director, Division of Allergy and Immunology
Miami Children's Hospital
Clinical Assistant Professor
Herbert Wertheim College of Medicine
Miami, Florida

Gerald Lee, MD
Assistant Professor
Section of Allergy and Immunology
Department of Pediatrics
University of Louisville School of Medicine, Kentucky

American College of Allergy, Asthma & Immunology

ACAAI Review for the Allergy and Immunology Boards, Second Edition

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DEDICATION

To our families, friends, and loved ones, who encouraged and assisted us in the task of assembling this guide.

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CONTRIBUTING AUTHORS

Cindy Salm Bauer, MD
Physician, Division of Allergy and Immunology, Department of Pediatric Pulmonology, Phoenix Children's Hospital; Clinical Assistant Professor, Department of Child Health, University of Arizona College of Medicine, Phoenix, Arizona

Neeti Bhardwaj MD, MS
Assistant Professor of Pediatrics, Division of Pediatric Allergy and Immunology, Department of Pediatrics, Penn State Milton S. Hershey Medical Center, Penn State College of Medicine, Hershey, Pennsylvania

Ahmed Butt, MD
Allergist/Immunologist, Allergy and Asthma Centers of Fredericksburg and Fairfax, Fredericksburg, Virginia

Timothy J. Campbell, MD
Fellow, Division of Allergy and Immunology, Department of Pulmonology, Allergy and Critical Care Medicine. Respiratory Institute, Cleveland Clinic, Ohio

Howard C. Crisp, MD
Fellow-in-Training, Department of Allergy and Immunology, Wilford Hall Ambulatory Surgical Center, San Antonio, Texas

Justin C. Greiwe, MD
Fellow, Division of Allergy and Immunology, Department of Pulmonology, Allergy and Critical Care Medicine, Respiratory Institute, Cleveland Clinic, Ohio

Michelle A Halbrich, MD
Fellow, Paediatric Clinical Immunology and Allergy, McGill University, Montreal Children’s Hospital, Quebec, Canada

Hillary S. Hernandez-Trujillo, MD
Attending Physician, Connecticut Asthma & Allergy Center; Clinical Assistant Professor, Department of Pediatrics, University of Connecticut School of Medicine, Division of Infectious Diseases and Immunology, Connecticut Children's Medical Center, West Harford, Connecticut

Fatima S. Khan, MD
Physician, Allergy/Immunology, Grand Forks, North Dakota

Yong Luo MD, PhD
Fellow, Division of Allergy and Immunology, Departments of Medicine and Pediatrics, North Shore-LIJ Health System, Great Neck, New York

Lisanne P. Newton, MD
Fellow, Division of Allergy and Immunology, Department of Pulmonology, Allergy and Critical Care Medicine, Respiratory Institute, Cleveland Clinic, Ohio

Joon H. Park, MD
Allergist and Immunologist, Clinical Allergy Center, Fairfax, Virginia

Pavadee Poowuttikul, MD
Assistant Professor, Assistant Program Director, Allergy/Immunology, Children's Hospital of Michigan, Wayne State University, Detroit, Michigan

Mariam Rasheed, MD
Assistant Professor of Pediatrics, Albert Einstein College of Medicine, Division of Allergy and Immunology, Department of Pediatrics; Attending Physician, The Children's Hospital at Montefiore, New York, New York

Jonathan Romeo, DO
Instructor in Internal Medicine and Pediatrics, Section of Pulmonary, Critical Care, Allergy, and Immunologic Diseases, Wake Forest University School of Medicine, Winston Salem, North Carolina

Ahila Subramanian, MD, MPH
Fellow, Division of Allergy and Immunology, Department of Pulmonology, Allergy and Critical Care Medicine, Respiratory Institute, Cleveland Clinic, Ohio

Jonathan S. Tam MD
Assistant Professor of Pediatrics, Division of Clinical Immunology and Allergy, Children's Hospital Los Angeles, Department of Pediatrics University of Southern California, Los Angeles, California

SENIOR REVIEWERS

Elizabeth Secord, MD
Associate Professor of Pediatrics; Chief and Program Director, Division of Allergy/ Immunology, Department of Pediatrics Wayne State University, Detroit, Michigan

Monica Vasudev, MD
Associate Professor of Medicine and Pediatrics, Division of Allergy and Clinical Immunology, Medical College of Wisconsin, Milwaukee

Frank S. Virant MD
Clinical Professor of Pediatrics, University of Washington School of Medicine; Division Chief, Allergy, Seattle Children's Hospital; Associate Director, Allergy/Immunology Training Program, Seattle, Washington

Julie Wang, MD
Assistant Professor of Pediatrics, Division of Pediatric Allergy and Immunology, Mount Sinai School of Medicine, New York

Kevin M. White, MD
Staff Physician, Department of Allergy and Immunology, Wilford Hall Ambulatory Surgical Center, San Antonio, Texas

PREFACE

With this second edition of *ACAAI Review for the Allergy and Immunology Boards,* we continue our commitment to providing fellows-in-training and A/I physicians with the most useful and up to-date preparation guide for the ABAI examination. This text was written like other publications in the *First Aid* board review series and is designed to fill the need for a high-quality, in-depth, concept driven study guide for ABAI exam preparation. The second edition features all new "embedded flashcards" to test your learning as well as revised and expanded high-yield topic summaries and illustrations.

This book would not have been possible without the help of the many fellows-in-training, physicians, and faculty members who contributed their feedback and suggestions. We invite you to share your thoughts and ideas to help us improve *ACAAI Review for the Allergy & Immunology Boards.* (See How to Contribute, p. xvii.)

Tao Le, MD, MHS
Louisville, Kentucky

Bret Haymore, MD
Tulsa, Oklahoma

Vivian Hernandez-Trujillo, MD
Miami, Florida

Gerald Lee, MD
Louisville, Kentucky

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Tao Le, MD, MHS
Louisville, Kentucky

Bret Haymore, MD
Tulsa, Oklahoma

Vivian Hernandez-Trujillo, MD
Miami, Florida

Gerald Lee, MD
Louisville, Kentucky

HOW TO CONTRIBUTE

To continue to produce a current review source for the ABAI exam, you are invited to submit any suggestions or corrections. Please send us your suggestions for:

- Study and test-taking strategies
- New facts, mnemonics, diagrams, and illustrations
- Relevant topics that are likely to be tested in the future

For each entry incorporated into the next edition, you will receive a personal acknowledgment in the next edition. Also let us know about material in this edition that you feel is low yield and should be deleted.

The preferred way to submit entries, suggestions, or corrections is via our email address:

boardreview@acaai.org

NOTE TO CONTRIBUTORS

All submissions become property of the ACAAI and are subject to editing and reviewing. Please verify all data and spellings carefully. Include a reference to a standard textbook to facilitate verification of the fact. Please follow the style, punctuation, and format of this edition if possible.

Section 1. Basic Science

1 Immune Mechanisms

ANTIGENS

The term antigen—a contraction of "**anti**body **gen**erator"—is a molecule that is recognized by the immune system.

Definitions

- **Antigen** is a molecule that is recognized by the immune system.
- **Immunogen** is a molecule that induces immune responses other than immune tolerance.
- **Hapten** is a small-molecule antigen that requires covalent linkage to a larger carrier to stimulate immune response (e.g., penicillin). Once an antibody to hapten is generated, hapten can be recognized by the antibody itself.
- **Carrier** is a macromolecular substance to which a hapten is coupled in order to produce an immune response against the hapten.
- **Adjuvants** are molecules that enhance the immune response. Adjuvants release bound antigens to antigen-presenting cells (APCs) over a prolonged period, interact with Toll-like receptors (TLRs), and stimulate chemokine and cytokine release. Examples:
  - Alum
  - Freund's adjuvant: Emulsified bacterial products (e.g., bacille Calmette-Guérin [BCGs])
  - Incomplete Freund's adjuvant: Water in oil emulsification
  - Ribi adjuvant system: Squalene–Tween80–water and oil emulsification
  - Titermax: Copolymers polyoxypropylene (POP) and polyoxyethylene (POE)
- **Superantigen:** Antigens that activate a large number of polyclonal T lymphocytes. Examples of microbial toxin superantigens are provided in Table 1-1.

Flash Card Q1

What is the hapten-carrier effect?

Flash Card Q2

What are the common superantigens and related diseases?

Table 1-1. Superantigens

Source	Toxin	Disease
Staphylococcus aureus	SEB	Food poisoning
	SEC2	Food poisoning
	TSST	Toxic shock syndrome
Streptococcus pyogenes	SPE-C	Streptococcal toxic shock syndrome

Abbreviations: SEB, staphylococcal enterotoxin B; SEC, staphylococcal enterotoxin C; TSST, toxic shock syndrome toxin; SPE-C, streptococcal pyrogenic exotoxins C.

Key Fact

Superantigens bind the V_β region of TCRs (CDR4) and outside of the peptide-binding groove on the MHC molecule.

Superantigens bind to a particular family of V_β chain of the T-cell receptor (TCR), bypassing the need for the specific major histocompatibility complex (MHC), peptide, or TCR complex required for signal 1 (Figure 1-1). Stimulation of T cells by superantigens can lead to "cytokine storm."

Flash Card A1

Small-molecule antigen requires covalent linkage to a larger carrier to stimulate adaptive immune response. The process is achieved in collaboration between hapten-specific B cells and carrier-specific T cells. This is the basis of developing conjugated vaccines.

Flash Card A2

SEB and SEC cause food poisoning; TSST and SPE-C cause toxic shock syndrome.

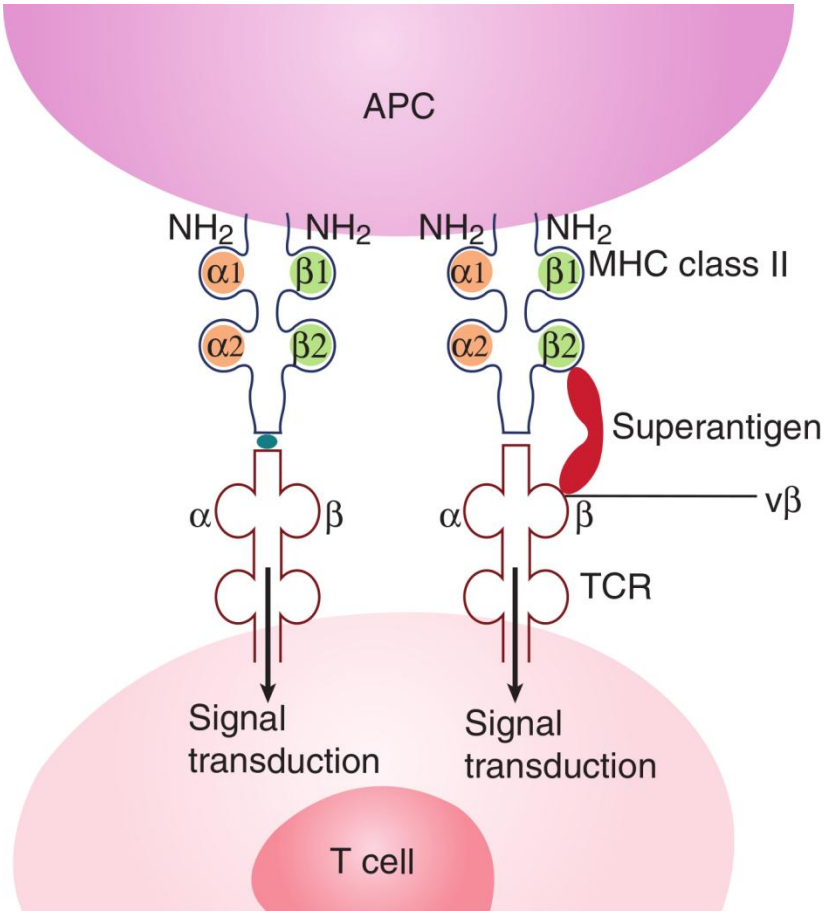


Figure 1-1. Superantigen.

Composition of Antigens

Table 1-2 summarizes the composition of antigens.

Antigen Structure

Epitope (Antigenic Determinant)—Antigenic component identified by a unique antibody.

Recognized by B lymphocytes:
- Linear determinants or tertiary structure
- Carbohydrates, amino acids (four to eight residues), and nucleic acids

Recognized by T lymphocyte:
- Linear determinants
- Amino acid peptides
- Eight to 30 amino acids (MHC class I 8–11 aa and MHC class II 10–30 aa).

An understanding of antigen composition and factors influencing immunogenicity is critical to immunization development and the assessment of response to immunizations.
Table 1-3 reviews factors that influence the immunogenicity of antigens.

Table 1-2. Composition of Antigens

Antigen	Immune Cell Involved	Surface Molecule Involved	B-Cell Response	Vaccines
Protein	T–cell-dependent	MHC class I MHC class II	Isotype switch Affinity maturation Induced memory response	Diphtheria Tetanus
Polysaccharide	T–cell-independent Marginal zone B cells and B1 cells		No isotype switch Predominantly IgM No affinity maturation Limited memory	Meningococcal vaccine (MSPV4—Menomune) Pneumococcal vaccine 23-valent
Nucleic acids	T–cell-dependent/independent CTL and DCs	MHC class I MHC class II TLR9 (CpG recognization on DCs)		DNA vaccines in clinical trials
Lipids	NKT cells γδ T cells	MHC-like CD1 (NKT)		

Abbreviations: CTL, cytotoxic T lymphocyte; DCs, dendritic cells; MHC, major histocompatibility complex; NKT, natural killer T cell.; TLR, Toll-like receptor

Key Fact

Conjugated vaccines are T-independent antigens linked to a carrier protein, which can trigger a T-dependent response and memory. Examples of conjugated vaccines include 13-valent pneumococcal vaccine (Prevnar 13), Hib vaccines, and meningococcal vaccines (MCV4–Menactra and Menveo).

Flash Card Q3

Which type of T cells recognizes lipid antigens and what is the molecule involved?

Table 1-3. Factors Influencing Immunogenicity of Antigens

Factors		Immunogenic	Non or Less Immunogenic
Immunogen	Origin	Foreign	Self
	Size	Larger (usually)	Smaller (usually)
	Chemical composition	Proteins, polysaccharides (less), and lipids (activate NKT via CD1)	Nucleic acids Lipids
	Physical form	Particulate and denatured	Soluble and native
	Degradability	Easily degradable by APCs (T-lymphocyte-dependent)	Not easily degradable
Biological system	Genetics	Specific HLA-antigen interactions (e.g.: HLA-B* 5701 and abacavir)	
	Age		Extremes of age
Method of administration	Dose	High dose Prolonged course Repetitive exposure	Low dose Single exposure
	Route	Parenteral Cutaneous	Oral
	Adjuvant	Use of adjuvant increases immunogenicity	

Abbreviations: APCs, antigen-presenting cells; HAL, human leukocyte antigen; NKT, natural killer T cell.

MAJOR HISTOCOMPATIBILITY COMPLEX (MHC)

MHC molecules are also known as human leukocyte antigens (HLA), which are encoded on the MHC locus.

MHC molecules share certain features:

- Each MHC molecule has one binding site.
- MHC molecules are bound to cell membranes.
- Interaction with T lymphocyte requires direct contact.
- MHC molecules are expressed codominantly (MHC from both parents is expressed on cell surfaces).

Flash Card A3

Natural killer T (NKT) cells and CD1 molecule.

Structure

Table 1-4 summarizes differences between MHC class I and MHC class II molecules.

Anchor residues:

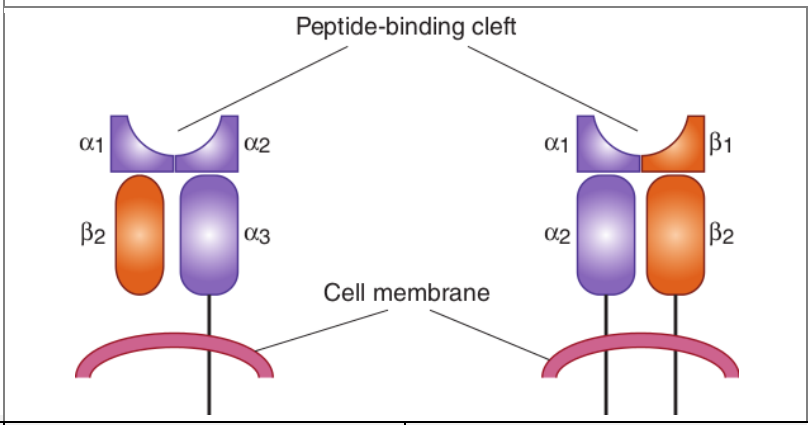
- Side chains of peptide, which strongly bind to pockets in the peptide-binding cleft of MHC molecule. This interaction stabilizes the peptide in the cleft.

MHC Distribution

MHC class I is expressed on most nucleated cells. The expression of MHC class II varies by cell type. Their expression is induced by cytokines produced by innate and adaptive immune responses. APCs express both MHC class I and MHC class II.

Features of MHC expression are reviewed in Table 1-5.

Table 1-4. Structure of MHC Class I and Class II Molecules

	MHC Class I	MHC Class II
		
Genes	HLA-A, -B and -C	HLA-DP, -DQ and -DR
Polypeptide chains (domains)	α chain (α_1, α_2, α_3) β_2-microglobulin	α chain (α_1, α_2) β chain (β_1, β_2)
Restriction	CD8	CD4
Binding site for TCR (nonpolymorphic)	α_3 binds CD8	β_2 binds CD4
Binding site for peptide (polymorphic)	α_1 and α_2	α_1 and β_1
Peptide-binding cleft	Peptides 8–11 amino acids	Peptides 10–30 amino acids
Antigenic sampling	Intracellular	Extracellular

Flash Card Q4

What are the binding sites for TCR on MHC class I and class II molecules, respectively?

Table 1-5. Cells That Express MHC Class I and MHC Class II

	MHC Class I	MHC Class II
Constitutive	Most nucleated cells	APC (dendritic cells, macrophages, and B lymphocytes), thymic epithelia, and activated T lymphocytes
Inducting cytokines	Interferon (IFN)α, IFNβ, and IFNγ	IFNγ

Abbreviation: APC, antigen-presenting cell.

MHC Genome

Genes that encode MHC molecules are encoded on the short arm of chromosome 6, whereas the β_2-microglobulin chain is encoded on chromosome 15. A map of the human MHC is shown in Figure 1-2.

In addition to encoding the MHC polypeptides, the MHC genome encodes proteins involved in the processing of peptides that occupy the peptide-binding clefts.

The class III region encodes:

- Proteins of the complement system: Factor B, C4a, C4b, and C2
- Cytokines: Tumor necrosis factor (TNF)α, and lymphotoxins α and β
- Heat shock proteins

The class I-like proteins are highly conserved. They include:

- HLA-E: NK cell recognition
- HLA-F: Localized to endoplasmic reticulum and Golgi apparatus
- HLA-G: On fetal-derived placental cells
- HLA-H: Involved in iron metabolism

Antigen Processing and MHC Presentation

Intracellular and extracellular proteins can be processed by specific pathways and are presented in association with MHC class I or MHC class II molecules. Summaries of class I and class II antigen-processing pathways can be found in Figures 1-3 and 1-4**,** respectively.

Flash Card A4

α_3 and β_2.

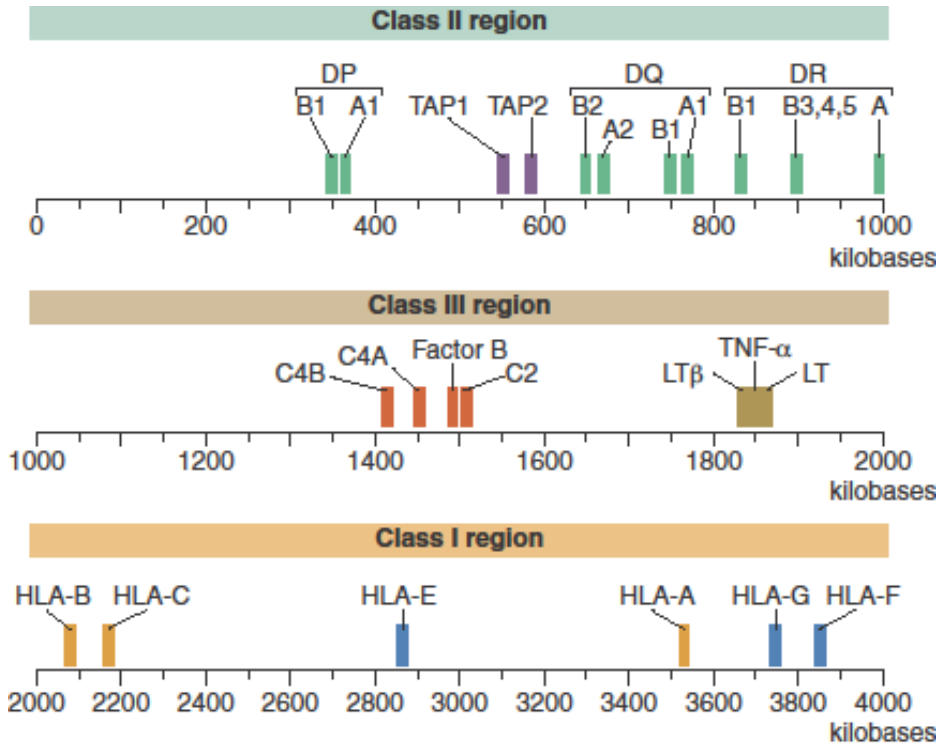


Figure 1-2. Map of the human MHC genome.

MHC I Pathway

- Newly synthesized MHC class I polypeptides remain sequestered in the endoplasmic reticulum by interacting with calnexin, calreticulin, Erp57, and tapasin.
- Cytoplasmic proteins that enter the cytoplasm are degraded to antigenic peptides by the **proteasome**:
  - The proteasome is a multisubunit proteinase. Four seven-membrane rings have catalytic subunits.
  - Examples of subunits are: Low-molecular-mass polypeptide (LMP) 7 and LMP2.
  - LMPs are encoded in MHC class II locus.
- Antigenic peptides are transported into the endoplasmic reticulum by transporter of antigenic-processing (TAP) proteins.
  - Energy-dependent transport of peptides.
  - Composed of two subunits: TAP1 and TAP2, both of which must be present for function.
  - TAP proteins are encoded in MHC class II locus.
- Antigenic peptides are loaded onto newly synthesized MHC class I polypeptides.
- MHC class I and antigenic peptide are transported to cell surface.
- Stable MHC class I expression requires presence of antigenic peptide.

Key Fact

Viruses develop strategies to evade MHC class I presentation. Herpes simplex virus (HSV) can block TAP transportation, and cytomegalovirus (CMV) can remove MHC class I molecule from ER.

Flash Card Q5

MHC class I molecule presents which type of antigens and where does the antigen-MHC class I loading happen?

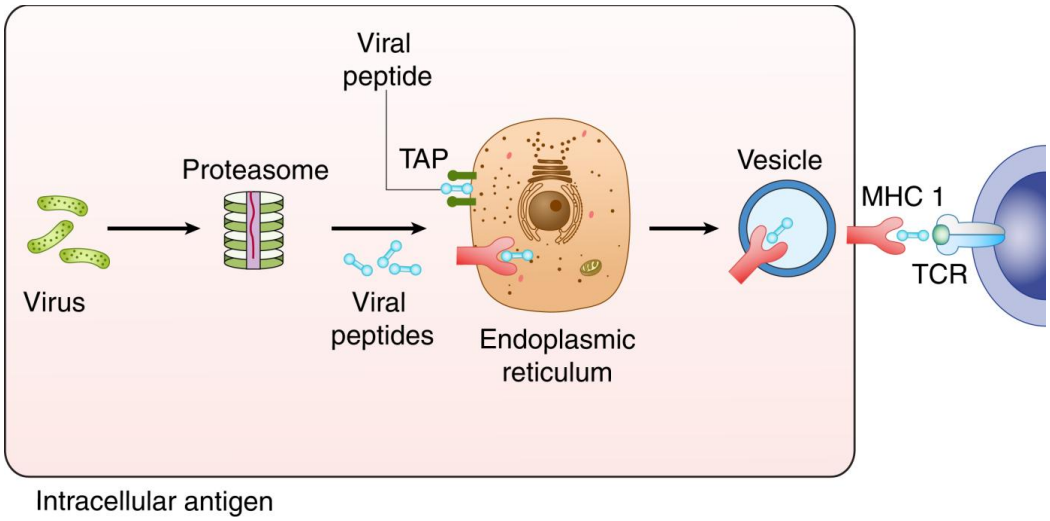


Figure 1-3. MHC class I antigen-processing pathway.

MHC II Pathway

- Extracellular antigen is endocytosed and compartmentalized in cytosolic phagosomes.
- Phagosomes fuse with lysosomes. The resulting phagolysosome degrades the microbe into antigenic peptides by endosomal and lysosomal proteases (cathepsins).
- Newly synthesized MHC class II molecules are synthesized in the ER and transported to the phagolysosome, forming the MHC class II vesicle. The MHC class II-binding cleft is occupied by the invariant chain (Ii) prior to peptide loading.
- In the MHC class II vesicle, the Ii is degraded by proteolytic enzymes, leaving behind a short peptide named class II-associated invariant chain peptide (CLIP).
- HLA-DM removes CLIP and allows antigenic peptides to be loaded in the MHC-binding cleft.
- MHC class II and peptide are transported to cell surface.
- Stable MHC class II expression requires presence of antigenic peptide.

Key Fact

HLA-DM is an intracellular protein involved in MHC class II antigen processing and does not present antigenic peptides nor is it a component of MHC class II.

Defect in MHC Expression and Disease

Bare Lymphocyte Syndromes (MHC Class I and MHC Class II Deficiencies)—The bare lymphocyte syndromes are primary immune deficiencies due to a lack of MHC expression. Features of MHC class I and MHC class II deficiencies are reviewed in Table 1-6.

Flash Card A5

Intracellular antigens (e.g., viral antigen in cytoplasm) and the loading site is endoplasmic reticulum (ER).

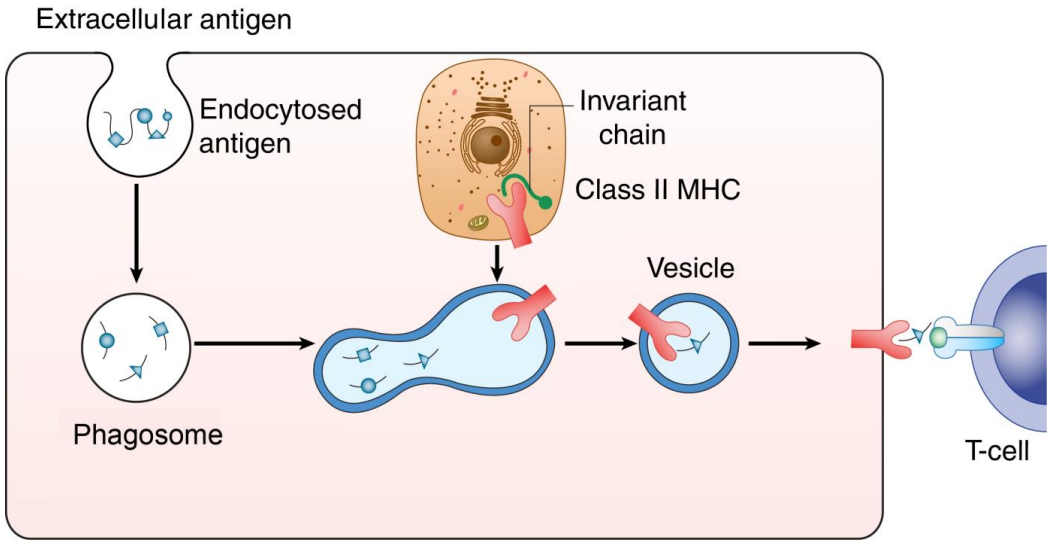


Figure 1-4. MHC class II antigen-processing pathway.

Table 1-6. Bare Lymphocyte (MHC Deficiency) Syndromes

	MHC Class I Deficiency	MHC Class II Deficiency
Mutation	Genes encoding for TAP—essential for MHC class I expression.	Genes encoding for mutations in several transcription factors required for MHC class II expression: MHC2TA, RFX5, FRXAP, and FRXANK
Inheritance	Autosomal recessive (rare)	Autosomal recessive
Clinical features	Sinopulmonary infections, granulomatous skin lesions, and necrobiosis lipoidica	Diarrhea, hepatosplenomegaly, transaminitis, sclerosing cholangitis (*Cryptosporidium parvum*), pulmonary infections (*Pneumocystis jiroveci*, encapsulated bacteria, Herpesviridae, and RSV), and meningitis
Laboratory	CD8 lymphopenia, PBMC on flow cytometry lack MHC class I	CD4 lymphopenia (reversed CD4:CD8) Lack of HLA DR/DP/DQ on lymphocytes, DTH Hypogammaglobulinemia Absent germinal centers from lymph nodes
Treatment	Treat pulmonary infections like CF (aggressive toileting and chest PT)	Hematopoietic stem cell transplantation (HSCT)

Abbreviations: CF, cystic fibrosis; DTH, delayed-type hypersensitivity test; MHC, major histocompatability complex; PMBC, peripheral blood mononuclear cells; RSV, respiratory syncytial virus; TAP, . transporter-associated with antigen presentation.

Flash Card Q6

MHC class II molecule presents which type of antigens and where does the antigen-MHC class II loading happen?

IMMUNOLOGIC TOLERANCE

Definitions

- **Tolerance** is unresponsiveness to an antigen. This can be to self-antigens (i.e., self-tolerance) or to foreign antigens. Self-tolerance is part of the normal function of educating the immune system not to react to itself.
- **Tolerogens** are antigens that induce tolerance. A foreign antigen that becomes a tolerogen is conditional. The antigen may only induce tolerance under certain conditions, like age or the amount of antigen being at a very low or high concentration.
- **Anergy** is a state of unresponsiveness to antigenic stimulation. The antigen is recognized by the immune cell; but weak signaling, due to a lack of costimulation, leads to anergy. Other factors include antigen type and antigen dose.

Central Tolerance

Key Fact

Mutation in the AIRE gene produces disorders such as autoimmune polyglandular syndrome (APS). Lymphocytes are not deleted or tolerized to endocrine-related self-antigens. The endocrine organs are attacked by autoreactive T lymphocytes and autoantibodies.

Central tolerance occurs in the lymph organs.

Central T-Lymphocyte Tolerance

Key Fact

κ light chains are rearranged first. If receptor editing is needed, a λ light chain will be used.

In T-lymphocyte central tolerance, a T-lymphocyte **precursor** is exposed to a **self-antigen** in the **thymus.** The T lymphocyte is exposed to a self-antigen, which yields two fates: apoptosis, which is also known as **negative selection,** or development into a regulatory T (Treg) cell, which will migrate to the periphery. The two main factors determining tolerance or negative selection are antigen **concentration** and **affinity** to the TCR. High concentration and high affinity promote negative selection.

The thymus presents self-antigens through thymic antigen-presenting lymphocytes that process antigen in the context of HLA class I and II. The **autoimmune regulatory gene (AIRE)** is expressed in the thymus. This gene promotes expression of **nonthymic** tissue antigens in the thymus!

Central B-Lymphocyte Tolerance

Flash Card A6

Extracellular antigens (e.g., antigens from phagocytosed bacteria) and loading site is phagolysosome.

In B-lymphocyte central tolerance, the **precursor** B lymphocyte is exposed to a self-antigen in the **bone marrow** during development. The immature B lymphocyte is exposed to self-antigen, which yields three fates: apoptosis (or

negative selection), **receptor editing,** or anergy. **Receptor editing** involves reactivation of RAG1 and RAG2 when a high-affinity self-antigen is recognized by a B-cell receptor (BCR). The RAG enzymes will delete the previously rearranged $V_\kappa J_\kappa$ exon and give the BCR a new light chain. As a result, the self-reactive immature B cell will have a new specificity. If both recombinations recognize a self-antigen (failure of editing), the immature B lymphocyte will be deleted by apoptosis. In low antigen concentration, the B lymphocyte may become anergic to the self-antigen.

Peripheral Tolerance

In both T and B lymphocytes, peripheral tolerance occurs in peripheral tissues when a **mature** lymphocyte encounters a self-antigen. In the case of a T lymphocyte, if recognition of self-antigen occurs, the T lymphocyte may be induced to undergo apoptosis, may become anergic, or a Treg cell that confers suppression. In this situation, a B lymphocyte will either become anergic or be deleted through apoptosis.

Peripheral T-Lymphocyte Tolerance

Peripheral tolerance has the same outcomes as central tolerance: Anergy, deletion, or regulation. Lack of a second signal or lack of innate costimulation (e.g., microenvironment) produces the anergy of the peripheral T lymphocytes.

Anergy in these T lymphocytes is maintained by blockade of TCR signaling, ubiquitin ligases (which target proteins for degradation), and inhibitor costimulatory molecules (e.g., CTLA-4 and PD-1). Dendritic cells may also present self-antigen without expression of costimulatory molecules.

Maintenance of Tolerance

Dendritic cells that are not activated or that are immature still present self-antigen on their surfaces. These cells in an immature state do not express receptors, thus antigen presented to T lymphocytes will not have a second signal, resulting in tolerance. This presentation of antigen by the dendritic cell is ongoing, which reminds cells not to be self-reactive (Table 1-7.)

Treg cells are a component of the immune system involved in suppressing immune response of other cells. They are mainly thymic emigrants that respond to self-antigen. Development of Treg cells in thymus depends on the binding affinity between the cells and self-peptide/MHC II complex. T cells with strong binding will undergo negative selection (apoptosis). T cells with weak binding will undergo positive selection (effector cells). T cells with intermediate binding will

Key Fact

Lack of costimulation, or lack of an innate immune system response to the antigen, blunts the required upregulation to achieve costimulation (i.e., a **second signal**). T lymphocytes recognize the antigen, but receive no support to activate. After repeated recognition without costimulation, the lymphocyte becomes unresponsive to that antigen (i.e., **anergic**). Once a cell is anergic, costimulation will not restore activation.

Flash Card Q7

Which B-cell-exclusive process is involved in developing central tolerance and which two enzymes are important in this process?

Table 1-7. To Be or Not to Be: Tolerance to Self-Protein Antigen

Determinant	Immunogenicity	Tolerance	Comments
Dose	Optimal		Optimal dose varies.
Exposure			Gets tired of seeing the same thing and ignores it, until it becomes "background."
Delivery route	Cutaneous and intradermal	Oral	Think about where the APCs are located. We have to eat.
Adjuvant	T-lymphocyte help	Without an adjuvant	Adjuvant can promote costimulation. It gets the team involved.
APCs	Activated and costimulators	Inactivated and naïve	Without costimulation, there is no activation, which yields immune ignorance or tolerance.

Abbreviation: APCs, antigen-presenting cells.

Key Fact

Treg cells play a critical role in maintaining normal immune function. Classically Treg cells express CD4, CD25 and FoxP3. FoxP3 mutation in human causes immune dysregulation, polyendocrinopathy, enteropathy X-linked (IPEX) syndrome, which is a fatal autoimmune disorder characterized by a triad of watery diarrhea, eczema, and endocrinopathy.

Key Fact

Mutations in Fas or caspase 10 manifest as autoimmune lymphoproliferative syndrome (ALPS). The lymphocytes do not know when to die. They accumulate in the lymph organs. There is a lack of tolerance, producing autoimmune problems.

Flash Card A7

Receptor editing. RAG1, RAG2.

become Treg cells. Despite being self-reactive, they are allowed to escape the thymus and, instead, help to maintain self-tolerance.

Characteristically, Treg express CD4, CD25 (interleukin [IL-2R] α chain) and FoxP3 (Foxhead box P3, a master transcription factor of Treg cells). Their survival depends on IL-2 and transforming growth factor beta (TGFβ). Tolerance or regulation is maintained by secretion of IL-10 and TGFβ. IL-10 targets macrophages and dendritic cells, and TGFβ inhibits lymphocytes and macrophages.

Apoptosis is a key regulator of self-reacting T lymphocytes. Self-antigens repeatedly recognized by a T lymphocyte without costimulation can activate **Bim**, which is a proapoptotic member of the Bcl-2 protein family**.** Bim leads to cell apoptosis through **mitochondrial** pathway.

Cells presenting self-antigen without innate response or costimulation have other receptors on their surfaces, such as **Fas ligand (FasL) (CD95L)** on the T lymphocyte. FasL is upregulated on repeatedly activated T lymphocytes. FasL can interact with **Fas (CD95)** on the same cell or nearby cells, either deleting a self-reactive T lymphocyte or causing the death of an activated cell, thereby downregulating the immune response. The Fas:FasL interaction signals through the **caspase** system.

Peripheral B-Lymphocyte Tolerance

Because antigens cannot cross-link the BCR on their own, B cells cannot be activated if there is no help from T cells. B cells will then become anergic or be induced to apoptosis.

Chronic antigen recognition downregulates CXCR5, inhibiting B-lymphocyte homing and interaction with T lymphocytes, which yields death.

To summarize, tolerance is a process by which the immune system teaches itself not to react to self-antigens (Table 1-8). In central tolerance, the T lymphocyte can be deleted by negative selection of high-affinity self-antigens or apoptosis; or some "self-recognizers" can be released into the periphery to become regulator cells to maintain tolerance. B-lymphocyte central tolerance can yield deletion; but, prior to deletion, receptor editing may save the B lymphocyte from negative selection. In low concentration of antigen, anergy is also a possibility. Peripheral tolerance can result in deletion or anergy. Anergy occurs with antigen exposure, without a second signal and/or inflammation.

Table 1-8. Summary of T- and B-Lymphocyte Tolerance

	T Lymphocytes		B Lymphocytes	
	Central	**Peripheral**	**Central**	**Peripheral**
Location of tolerance	Thymus		Bone marrow	
Educational phenotype	CD4+CD8+ T lymphocyte (double positive)	CD4+ or CD8+ T lymphocyte (single positive)	IgM+, IgD+ B lymphocyte (immature)	
Why become tolerant?	High avidity	No co-stimulators No inflammation (innate) Repeated stimulation	Multivalent antigen	No T lymphocyte help Chronic stimulation No costimulation
Fate of self-recognition	Deletion Become regulatory	Anergy (no signal) Death (apoptosis) Regulatory suppression	Receptor editing Deletion	Anergy (no signal) Follicular exclusion Death (apoptosis)

IMMUNOGENETICS

DNA

- DNA is stored in the nucleus of cells.
- Composed of subunits (or bases) called **nucleotides,** which include **adenine (A), guanine (G), thymine (T),** and **cytosine (C).**
- A and G are purines.
- T and C are pyrimidines.
- Organized into a double helix (the Watson-Crick model), in which A forms a base pair with T and G forms a base pair with C.

RNA

- Protein synthesis occurs via RNA.
- Contains the pyrimidine **uracil (U)**, instead of T.
- mRNA is copied from DNA and travels to ribosome.
- tRNA transports amino acids to ribosome.
- rRNA and protein combine to make ribosomes.

Transcription is the synthesis of mRNA from DNA. **Translation** is the synthesis of proteins from mRNA.

Genetic Mutations

- Mutations result from changes in the nucleotide sequence of genes (Table 1-9).
- Germ-line mutations can be passed down via reproductive cells.
- Somatic mutations involve cells outside the reproductive system and generally do not get passed to subsequent generations.

Table 1-9. Types of Mutations

Mutation	Consequence	Code	Translation
Frameshift	Insertion or deletion causes a shift in the translational reading frame. More dramatic effect on peptide sequence	UUU UAC AAA GAC UU**U ACA AAG ACA**	Phe Tyr Lys Asp Phe **Thr Lys Thr**
Missense	Single-nucleotide substitution causes the translation of a different amino acid	UUU UAC AAA GAC UU**G** UAC AAA GAC	Phe Tyr Lys Asp **Leu** Tyr Lys Asp
Nonsense	Single-nucleotide substitution causes an early stop (or termination) codon	UUU UAC AAA GAC UUU UA**A** AAA GAC	Phe Tyr Lys Asp Phe **STOP**
Silent	Single-nucleotide substitution does not cause a change in amino acid sequence	UUU UAC AAA GAC UUU UAC AA**G** GAC	Phe Tyr Lys Asp Phe Tyr **Lys** Asp
Neutral	Single-nucleotide substitution causes a different but similar amino acid to be translated	UUU UAC AAA GAC U**A**U UAC AAA GAC	Phe Tyr Lys Asp **Tyr** Tyr Lys Asp

Single-Nucleotide Polymorphism

- A single-nucleotide polymorphism (SNP) is a variation in DNA sequence that occurs when a single nucleotide in a gene of an individual is different from that of other individuals.
- SNPs are not mutations. SNPs usually occur more frequently in noncoding DNA sequences. Overall, these occur at a higher frequency than mutations.
- SNPs occur in varying frequency between different geographic and ethnic groups. Therefore, they are useful markers of human genetic variations, which sometimes underlie different susceptibilities to diseases.
- SNPs are used in genome-wide association studies (GWAS) as high-resolution gene-mapping markers related to various diseases.
- Several SNPs have been identified that associate a higher risk of atopy or change the response to medications used to treat atopic conditions (Table 1-10).

Key Fact

SNPs, which manifest human genome variations across different geographic and ethnic groups, are widely used in GWAS as gene-mapping tools to study the association between genes and diseases.

Key Fact

Histone acetylation opens the chromatin to allow transcription. Histone deacetylation represses gene expression and is reduced in chronic obstructive pulmonary disease (COPD).

Table 1-10. Selected SNPs Associated with Development of Atopic Disease

Gene	Protein	Protein Function	Relevance in Atopy
Filaggrin gene	Filaggrin	Essential for epidermal barrier	Increased risk of eczema and asthma
17q12-21	ORMDL3	Unknown	Increased risk of asthma
5q22-32	CD14	Lipopolysaccharide (LPS) receptor	Both increased as well as reduced risk of asthma and atopy

Table 1-10. Selected SNPs Associated with Development of Atopic Disease, cont.

Gene	Protein	Protein Function	Relevance in Atopy
3p21-22	CCR5	Chemokine receptor	Protection against nonatopic asthma
Xp22	TLR 7 and 8	Pattern recognition receptor for viral ssRNA	Increased risk for asthma, rhinitis, atopic dermatitis, and increased specific IgE
5q31	IL-13	Cytokine that induces IgE secretion, mucus production, and collagen synthesis	Increased risk of asthma, bronchial hyper-responsiveness, and skin-test responsiveness. Linked to response to montelukast
ADRB2	β_2-Adrenergic receptor	Adrenaline and noradrenaline receptor	Arg/Arg phenotype with decreased albuterol response compared with Gly/Gly phenotype at residue 16
ADAM33	Type 1 transmembrane protein	Involved in cell-to-cell interactions	Increased risk of asthma and bronchial hyperresponsiveness

Abbreviations: Arg, arginine; Gly, glycine; ssRNA, single-stranded RNA; TLR, Toll-like receptor.

Epigenetics

Epigenetics can be described as changes in gene function that occur without a change in the sequence of DNA. These changes occur as a result of the interaction of the environment with the genome. **DNA methylation** and **histone modification** likely play a crucial role in the epigenetic regulation of immune system genes.

IMMUNOGLOBULINS (Ig)

Igs are glycoprotein molecules produced by B lymphocytes and plasma cells in response to an immunogen. Ig is the key component of humoral immunity. The earliest cell in B-lymphocyte lineage that produces Ig is the pre-B lymphocyte. An adult human produces approximately 2–3 g of Ig every day.

Ig Structure

The Ig molecule is a polypeptide heterodimer composed of two identical light chains and two identical heavy chains connected by disulfide bonds (Figure 1-5). Each chain consists of two or more Ig domains, which are compact, globular structures of approximately 110 amino acids containing intrachain disulfide bonds.

Heavy chains are designated by letters of the Greek alphabet (i.e., γ, α, μ, ε, δ) for Ig classes: G, A, M, E, and D, respectively. Human IgG consists of four isotypes: IgG1, IgG2, IgG3, and IgG4. For example, IgG1 contains $C_\gamma 1$ as its heavy chain. The constant (C) regions of IgG, IgA, and IgD consist of only three C_H domains. In IgM and IgE, the C regions consist of four C_H domains.

Light chains κ and λ are identified by their C regions. κ is encoded on chromosome 2 and λ is encoded on chromosome 22. An Ig molecule has either κκ (60%) or λλ (40%) but never one of each. An individual B lymphocyte will produce only κ or λ chains but never both.

Hinge regions are proline-rich and provide Ig flexibility. Interchain disulfide bonds exist between the heavy-heavy and heavy-light chains.

Ig fragments are produced from enzymatic cleavage of the Ig molecule. **Papain** cleaves Ig above the hinge (as seen in Figure 1-5) and results in two Fab (antigen-binding) fragments and one Fc (crystallizable) fragment. **Pepsin** cleaves Ig below the hinge at multiple sites and produces $F(ab')_2$, which contains interchain disulfide bonds, and exhibits two antigen-binding sites. F(ab) can bind but not cross-link; and $F(ab')_2$ both binds and cross-links. Neither F(ab) nor $F(ab')_2$ will fix complement or bind to the Fc receptor on the cell surface.

Variable regions V_L and V_H form the antigen-binding sites that consist of complementarity-determining regions (CDRs) of about 10 amino acids and account for antibody diversity. There are three CDRs in each V region; CDR3 is the most variable and, typically, has the most extensive contact with the antigen.

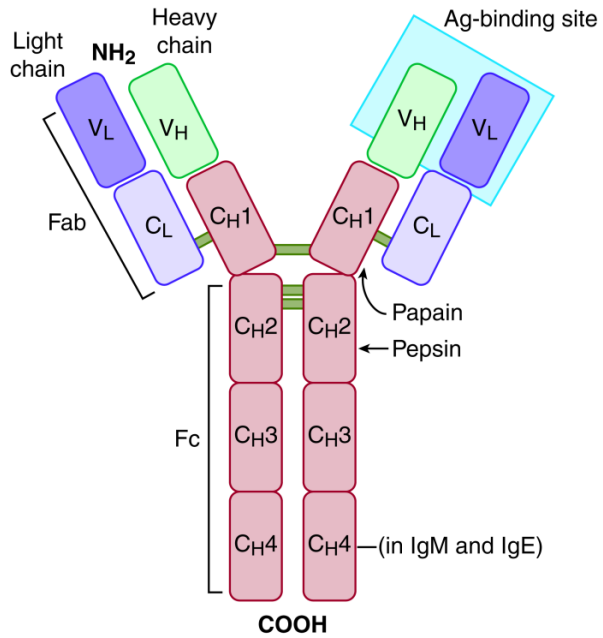


Figure 1-5. Immunoglobulin structure.

Key Fact

Omalizumab binds to $C_\varepsilon 3$.

Key Fact

The ratio of κ-bearing lymphocytes to λ-bearing lymphocytes can be used as an indication of clonality and is, therefore, useful in diagnosing and typing B-lymphocyte lymphomas.

Key Fact

The most variable part of Ig molecule is CDR3.

Flash Card Q8

What molecules belong to Ig superfamily?

Constant regions C_H and **C_L** are located at **C**-terminals of the Ig molecule. Only **C_H** mediates effector functions by binding to Fc receptors or binding complement.

Glycosylation of Igs is important in maintaining their structural stability and effector functions. Human IgG has one conserved glycosylation site in the $C_\gamma 2$ domain (asparagine-297). Deglycosylated IgG cannot bind FcγRs and C1q effectively and therefore is unable to trigger antibody-dependent cell-mediated cytotoxicity (ADCC) and complement activation. Decreased galactosylation has been associated with many inflammatory and infectious diseases, such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), Crohn's disease, and tunerculosis (TB). Sialic acid enrichment in intravenous immunoglobulin (IVIG) preparation significantly increases its anti-inflammatory activity.

Ig Forms

Two forms of Ig exist that differ in the amino acid sequence of the C-terminal end of the C_H.

Membrane-bound Ig (or surface Ig) is attached to the B-lymphocyte surface by its transmembrane region. Once Ig molecules bind to antigens and are cross-linked, they serve as B-lymphocyte antigen receptors that mediate B-lymphocyte activation.

Key Fact

The shortest half-life of all IgG subclasses is IgG3.
IgM fixes complement most efficiently of any Ig isotype.
Rheumatoid factor (RF) is an antibody against the Fc portion of IgG. RF is most commonly IgM, but can also be any other isotype.

Secreted Ig molecules lack transmembrane regions and circulate in the plasma, mucosal sites, and interstitial fluids. Secreted Ig can be in the form of monomers (all Ig), dimers (IgA), or pentamers (IgM). Dimers or pentamers are formed by tail pieces connected by disulfide bonds to joining (J) chain.

Ig Characteristics

Affinity is the strength of the binding between each molecule of Ig and antigen epitopes, and is indicated by K_d. A numerically lower K_d indicates higher affinity.

Avidity is determined by the net effect of affinity and valence. It is an estimate of the overall strength of the binding between Ig and antigen. A low-affinity IgM can produce a high-avidity interaction by simultaneous binding to multiple antigen epitopes, through 10 contact sites on each IgM molecule.

Flash Card A8

TCR, MHC molecules, CD4, CD8, CD19, B7-1, B7-2, Fc receptors, KIR, and VCAM-1.

Ig Superfamily

The Ig superfamily is a group of proteins that share similar structure to Ig by having one or more domains composed of 70–110 amino acids, most typically containing an intrachain disulfide loop. Examples of Ig superfamily members are

TCR, MHC molecules, CD4, CD8, CD19, B7-1, B7-2, Fc receptors, killer cell immunoglobulin-like receptor (KIR), and vascular cell adhesion molecule 1 (VCAM-1).

Antigen Recognition

Ig can recognize highly diverse antigens through linear and conformational determinants found in various macromolecules (i.e., proteins, polysaccharides, and lipids). TCRs only recognize linear determinants of peptides presented by MHC molecules.

Ig Production

- IgM is the first Ig produced after birth, the first to reach adult level, and the first to be synthesized following antigenic stimulation.
- **Only IgG crosses the placenta**. IgG level reaches a nadir around 4–6 months after birth due to decline in passively transferred maternal IgG.
- IgA is produced in the highest quantity daily, and is found in higher concentrations in the respiratory and GI mucosal surfaces. It may take several years for IgA to reach adult levels. In the gastrointestinal tract, IgA is produced by plasma cells in the lamina propria and is transported across the mucosal epithelium by poly-Ig receptor (transcytosis).

Key Fact

The only Ig to cross the placenta is IgG.
The Ig class with highest **plasma** concentration is IgG.
The Ig class with highest **total body** concentration and daily production is IgA.
The poly-Ig receptor is synthesized by mucosal epithelial cells and expressed on their basolateral surfaces. Once inside the epithelial cell, IgA bound to the poly-Ig receptor is actively transported in vesicles to the luminal surface.

Ig Isotypes

There are five Ig heavy chain isotypes. The isotypes differ in their biological properties, functional locations and interactions with different antigens, as depicted in Table 1-11.

Table 1-11. Immunoglobulin Isotypes

Isotype	**IgG**				**IgA**		**IgM**	**IgE**	**IgD**
Subclasses	IgG1	IgG2	IgG3	IgG4	IgA1	IgA2	none	none	none
Heavy chain	γ1	γ2	γ3	γ4	α1	α2	μ	ε	δ
# Heavy chains (C_H)	3				3		4	4	3
Secreted form (MW in kDa)	Monomer (150)				Monomer (170), dimer (390)		Monomer (180), Pentamer (900)	Monomer (190)	Monomer (180)
Serum level in adult (mg/dL)	430–1050	100–300	30–90	15–60	90–325	80–290	50–250	0.0015–0.2	0.3–30
	Total: 700–1500				Total: 60–450				
Half-life (days)	23	23	8	23	6	6	5	2	3
Cytokines inducing class switch	IFNγ, IL-4	IFNγ, TGFβ	IFNγ	IL-4, IL-13	TGFβ, IL-5		N/A	IL-4, IL-13	N/A
Complement fixation	++	+	++	–	–		+++	–	–
Placental transport	++	+	++	++	–		–	–	–
Properties	T_h1 response, opsonization Best for ADCC	Antipolysaccharide Ab Latest to reach adult level of all IgG subclasses	Shortest half-life in IgG subclasses Opsonization	Antipolysaccharide Ab T_h2 response Elevated in immunotherapy	Mucosal immunity IgA1 in serum and respiratory tract IgA2 in lower GI tract		Primary response. (e.g., isohemagglutinin and rheumatoid factor)	Allergic reaction The only Ig bind to mast cells	Do not require isotype switching Exact function unknown B-cell maturation marker

Abbreviations: Ab, antibody; ADCC, antibody-dependent cell-mediated cytotoxicity kDa, kilodaltons; MW, molecular weight..

General Functions of Ig

Antigen recognition by Ig initiates a humoral immune response. Ig selectively captures antigens and microbial pathogens, including bacteria and viruses, through noncovalent, reversible binding through the Ig V regions.

Ig-mediated **effector functions** include neutralization of microbes or toxins, opsonization, ADCC, and immediate hypersensitivity (IgE). These effector mechanisms often require interaction of Ig with complement proteins or other immune cells, such as phagocytes, eosinophils, and mast cells, through Fc receptors. The functional features of Ig are summarized in Table 1-12. Some of these characteristics are also shared by TCR.

Key Fact

Somatic hypermutation leads to changes in the V but not the C regions.

Class switch recombination changes the C but not the V regions.

Alternative splicing changes Ig from transmembrane to secretory form.

Table 1-12. Functional Features of Ig

Feature	Description	Results
Specificity	Ability of Ig and TCR to distinguish subtle differences in molecular structures of antigen	Distinct antigens elicit specific responses
Diversity	Variability in the structures of the antigen-binding site of Ig and TCR	Large structurally distinct antibody and TCR repertoires
Germ-line variation	Variation in inherited (germ-line) V, D and, J elements in Ig and TCR	Inherited structural differences create different basic structural frameworks
Combinatorial diversity (somatic recombination)	Result of different V, D, and J segment rearrangement in developing B and T lymphocytes	Moderate levels of immune receptor diversity
Junctional diversity	Random (nontemplated) addition or removal of nucleotide sequences at junctions between V, D, and, J regions	Extensive somatic variability in immune receptors
Somatic hypermutation	Point mutations in V regions of Ig in rapidly dividing B lymphocytes	Selected increase (or decrease) in antibody affinity
Receptor editing	Changes in Ig specificity that express self-reactive antibody achieved through secondary rearrangements	Elimination of self-reactive Ig
Class switch recombination (Isotype switching)	Change in heavy-chain C regions, with same V region at the gene locus	Switch in Ig isotype from IgM or IgD to IgG, IgA, or IgE
Affinity maturation	Process of somatic hypermutation and selective survival of B lymphocytes that produce high-affinity antibodies	↑Ig affinity
Alternative splicing	Splicing at different locations in the 3′ of C region exons	Production of membrane-bound or secreted Ig forms.
	Splicing at different location of the C-terminal of the IgM gene	Production of IgD from the same RNA transcript with IgM. Not conventional class switch

Flash Card Q9

Which somatic recombination process introduces the greatest diversity in immune receptors and which enzyme is important in this process?

Fc Receptors (FcRs)

FcRs are members of the Ig superfamily. Each Fc receptor functions as a receptor specific for the C_H region of the Ig molecule (Table 1-13). FcRs contain domains for Ig-binding and signaling components.

T-CELL RECEPTORS AND SIGNALING

T-Cell Activation

T-lymphocyte progenitors arise in the bone morrow and travel to the thymus as **double-negative** (CD4– and CD8–) and **CD3+** cells. Once in the thymus, they are educated and screened for reactivity. Then single-positive CD4+ **or** CD8+ T lymphocytes enter the blood and lymph system as naïve T lymphocytes. Naïve T lymphocytes recirculate through the lymph nodes looking for their unique protein antigen as displayed in the context of an HLA molecule, class I for CD8+ T cells and HLA class II for CD4+ T cells.

Table 1-13. Fc Receptors on Leukocytes

	FcR	CD Marker	Affinity for Ig	Cell Distribution	Function
For IgG	FcγRI	CD64	High	Macrophages, neutrophils eosinophils	Phagocytosis
	FcγRIIA	CD32	Low	Macrophages, neutrophils, eosinophils, platelets	Phagocytosis (poor)
	FcγRIIB	CD32	Low	B lymphocytes	Feedback inhibition of B lymphocytes
	FcγRIIIA	CD16	Low	NK cells, macrophages	ADCC
	FcγRIIIB	CD16	Low	Neutrophils, macrophages, eosinophils	Phagocytosis (poor)
For IgE	FcεRI		High	Mast cells, basophils, eosinophils	Degranulation, ADCC
	FcεRII	CD23 (not 32!)	Low	Neutrophils, eosinophils, monocytes	Unknown

Abbreviations: ADCC, antibody-dependent cell-mediated cytotoxicity; NK, natural killer.

Flash Card A9

Junctional diversity and TdT.

T-Cell Differentiation

Once the TCR-antigen HLA complex is formed, then activation can occur as follows:

- **Activation requires a second signal or costimulation**.
- The most important cytokine produced during activation is the T-lymphocyte survival signal, provided by **IL-2** and its receptor **CD 25**.
- **Proliferation is clonal**. It is stimulated by IL-2. Clonal expansion preserves the specificity of the T lymphocyte for its particular antigen.

Once T lymphocytes are activated, they become either effector or memory T lymphocytes (Table 1-14). Effector cells react to antigens. In the CD4+ lineage, they induce differentiation of the T lymphocyte response, T_h1, T_h2, Treg, and T_h17. In the CD8+ population, the cells become cytolytic.

- Effector cells function to eliminate antigen. With the decline in antigen stimulation, there is a decline in T-lymphocyte activity and achievement of homeostasis.
- Memory cells are a subset of the clonally expanded population. These cells are long-lived and functionally quiet. They provide a rapid secondary response.

Costimulation Between Antigen-Presenting Cells and T Cells

The costimulatory molecules are on the APCs. Their ligands are on the T lymphocytes. There is no T-lymphocyte response without costimulation. The T lymphocyte that recognizes the antigen, but is not costimulated, is said to be in a state of **anergy** (nonresponsiveness).

Costimulators may produce activation or induce negative regulation of the immune response (Table 1-15).

The TCR comes in two forms: αβ and γδ.

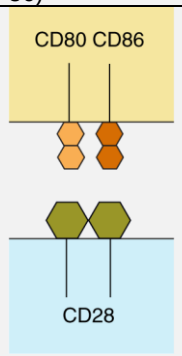
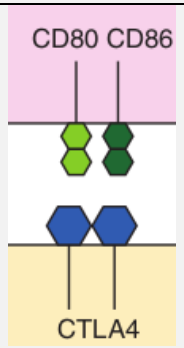
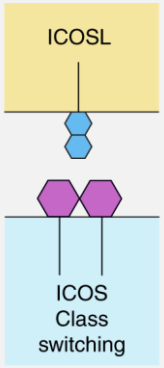
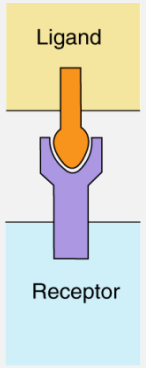
Key Fact

CD3 deficiency produces severe combined immunodeficiency (SCID).

Table 1-14. T-Cell Differentiation

Antigen Recognition	Cell Activation	Clonal Expansion	Functional Differentiation
→	→	→	→
Naïve CD4+ T cells Dendritic cells ⇕ Naïve CD8+ T cells	IL-2 production Upregulation IL-2 receptor	Proliferation of cell with same antigen recognition	Effector CD4+ T cells (helper) Memory CD4+ T cells Effector CD8+ T cells (killer) Memory CD8+ T cells

Table 1-15. Costimulator Expression and Function

APC	DC/Mø/B cells	DC/Mø/B cells	DC/Mø/B cells	DC/Mø/B cells	DC/Mø/B cells
Costimulator on APC	B7-1 (CD 80), B7-2 (CD 86)	B7-1 (CD 80), B7-2 (CD 86)	ICOS-L	CD40	PD-L1/PD-L2
APC					
T cells					
Receptor on T cells	CD 28	CTLA4 (CD152) (Inhibitory through ITIM)	ICOS	CD40L	PD-1 (Inhibitory through ITIM)
Expression	Constitutive	Inducible	Inducible	Inducible	T, B, myeloid cells/inducible
Effect	Activation of naïve cells Induction of CD40L, OX40, CXR5, ICOS, CTLA-4	T-lymphocyte tolerance T_h1 development	Costimulation of effector T lymphocytes, implicated in Ab class switching	APC activation, germinal center development, class switching	Negative regulation, cell death

Abbreviations: APC, antigen-presenting cells; CTLA, cytotoxic T-lymphocyte antigen; DC, dendritic cell; ICOS, inducible costimulator; ITIM, immuno-receptor tyrosine-inhibitory motifs; Mø, macrophage; PD-1, programmed death.

αβ TCR

Structure—It is a heterodimer of an α and β chain, each with two Ig-like domains. One is the variable domain, antigen contact, and HLA contact. The second Ig-like domain is the constant domain. The particular variable β or V_β region of the TCR is the binding site for superantigen.

Both chains have an extracellular region, constant region, transmembrane region, and a short cytoplasmic tail with no signaling molecules. The CDRs of both chains are the sites of recognition of the peptide-HLA complex.

αβ TCR affinity is less than that of antibodies; this, and the lack of signaling motifs on the αβ receptor, sets the stage for the necessity for accessory molecules and adhesion molecules to and from the TCR complex.

TCR Complex—TCR complex consists of the TCR, CD3 and two zeta (ξ) chains (Figure 1-6). CD4 recognizes antigens presented in the context of HLA class II, and CD 8 (not shown in Figure 1-6) recognizes antigens presented in the context of HLA class I antigens.

In order for antigen-signaling transduction to be initiated, the entire TCR complex is required to be expressed. The accessory molecules contain immunoreceptor tyrosine-based activation motifs (ITAMs), which are required for signal transduction. Antigen recognition is connected to activation through the TCR complex.

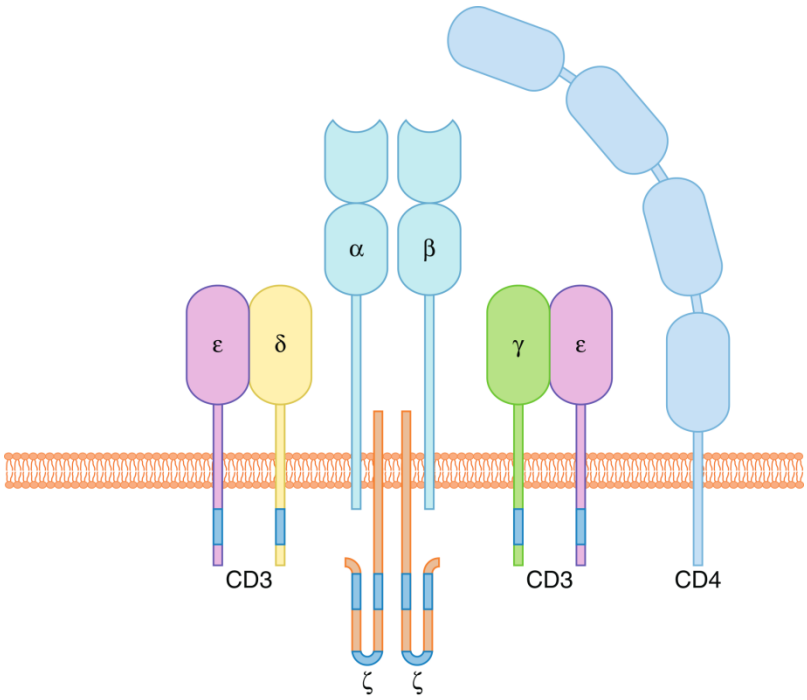


Figure 1-6. TCR complex with CD4.

Key Fact

αβ TCRs recognize a single antigen only in the context of an HLA molecule. The receptor has no signaling ability and requires accessory molecules for signal transduction.

Key Fact

The combination of the chains and their Ig-like domains are analogous to the immunoglobulin Fab fragment. These molecules undergo **recombination** for their diversity. Both chains have complementarity-determining regions (CDRs), and like in an immunoglobulin, the αβ CDR3 region of the TCR imparts the most significant sequence variability.

Flash Card Q10

Mutation in SAP cause which disease?

γδ T Lymphocytes

Key Fact

CD40 ligand: CD40 stimulates activation-induced cytosine deaminase (AID), which is crucial for somatic mutation and isotype switching.

γδ T lymphocytes are similar in structure and association to αβ T lymphocytes. γδ T lymphocytes require CD3 and the ζ chain for signal transduction. Most do not have CD4 or CD8. **They are not HLA-restricted.** Some antigens **do not** require processing. Some antigens are presented in MHC-like class I molecules. They are thought to be a bridge between innate and acquired immunity.

Natural Killer T (NKT) Cells

Key Fact

NKT cells recognize lipids in the context of CD1.

These cells express NK cell and T lymphocytes markers.

TCR-Signaling Pathways

Role of CD4 and CD8 Molecules—These molecules dictate HLA restriction. CD4 binds to HLA II molecules at a nonpolymorphic site. CD8 binds to HLA I molecules at a nonpolymorphic site. The class II-binding site is the β_2 region and the class I-binding site is the α_3 region; both contain Ig-like domains. These molecules stabilize the immune synapse. They are also involved in signaling through a Src family tyrosine kinase called **lck**. Lck is noncovalently associated with CD4 and CD8, and it is **required** for T-lymphocyte activation and maturation (Figure 1-7).

Costimulation—The interaction between CD28 and both CD80 and 86, CD2 and CD58, and a signaling lymphocytic activation molecule (SLAM) provides signals of cosimulation for activation, survival, and stability of the immune synapse.

Key Fact

ZAP-70 deficiency is a SCID with *no* CD8 cells and no T-lymphocyte function, but normal B lymphocytes and NK cells.

SLAM has an immunoreceptor tyrosine switch motif (ITSM). SLAM binds to SLAM-associated protein (SAP), which links SLAM to Fyn. Mutations in SAP cause X-linked lymphoproliferative syndrome (XLPS).

- **P** equals phosphorylation.
- **Lck** is a Src family kinase. It is noncovalently associated with CD4 and CD8.
- **Fyn** is a Src family kinase. It is noncovalently associated with CD3.
- **ZAP-70** is a Syk family kinase. It has two Src homology domains (SH), where ZAP-70 binds to the P on the ξ chain, which results in docking.
- **LAT** phosphorylation recruits adapter proteins that mediate different signaling pathways.
- **Sos** is a GDP/GTP exchanger.
- **ERK is one of the MAP kinases which** activates the transcription factor activation protein 1 (AP-1).
- **IP_3** is1,4,5-trisphosphate.

Flash Card A10

XLPS

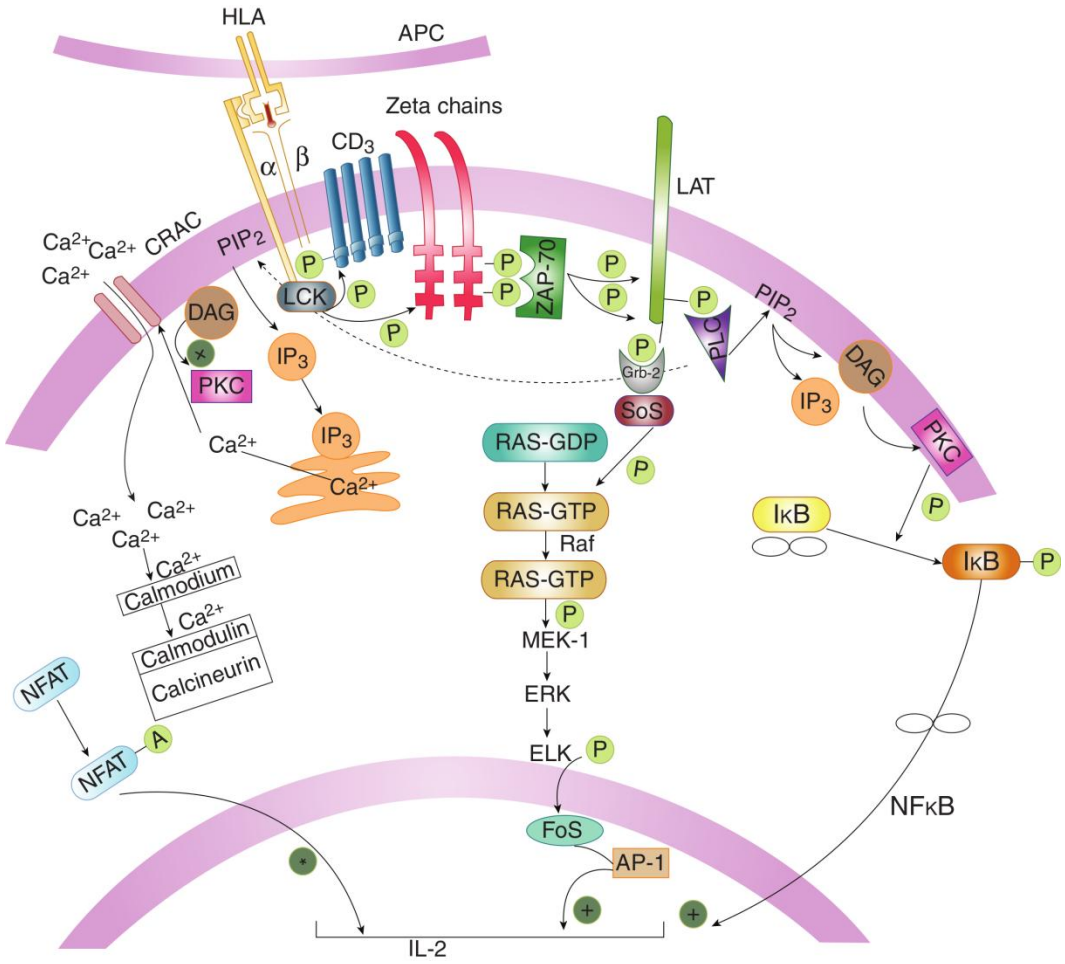


Figure 1-7. T–cell-receptor signaling.

- **CRAC (calcium release-activated calcium channel)** is on the cell membrane.
- **Calmodulin** is an ubiquitous, calcium-dependent regulatory protein that binds calcium and interacts with calcineurin.
- **Calcineurin** is an activator of nuclear factor of activated T lymphocytes (NFAT) by dephosphorylation, allowing it to travel to the nucleus.
- **NFAT** is an antigen-activated transcription factor for cytokines including IL-2, IL4, and TNF.
- **Rac-GTP** is another molecule activated by another GDP/GTP exchanger that activates JNK to phosphorylate Jun, which also turns on AP-1.

Flash Card Q11

Immunosuppressant cyclosporine binds to which molecule in T-cell signaling pathway?

B-CELL RECEPTOR SIGNALING

(See Figure 1-8.)

B–lymphocyte-receptor complex (BCR)—made up of the surface immunoglobulin and the associated Igα and Igβ chains.

Cross-linking—Recognition of the antigen by at least two receptors. Activation **will not** occur without receptor cross-linking.

Igα and Igβ chains—similar to the CD3 molecules and the ξ chain of the TCR; they contain the ITAMs, are noncovalently associated, and required for signal transduction.

Lipid rafts—formed with cross-linking, bringing the BCR and the Src family of kinases in close proximity. The BCR Src kinases are Lyn, Fyn, and BTK. Like in the TCR, they phosphorylate the ITAMs, providing a docking site for Syk. These are, again, Src homology domains.

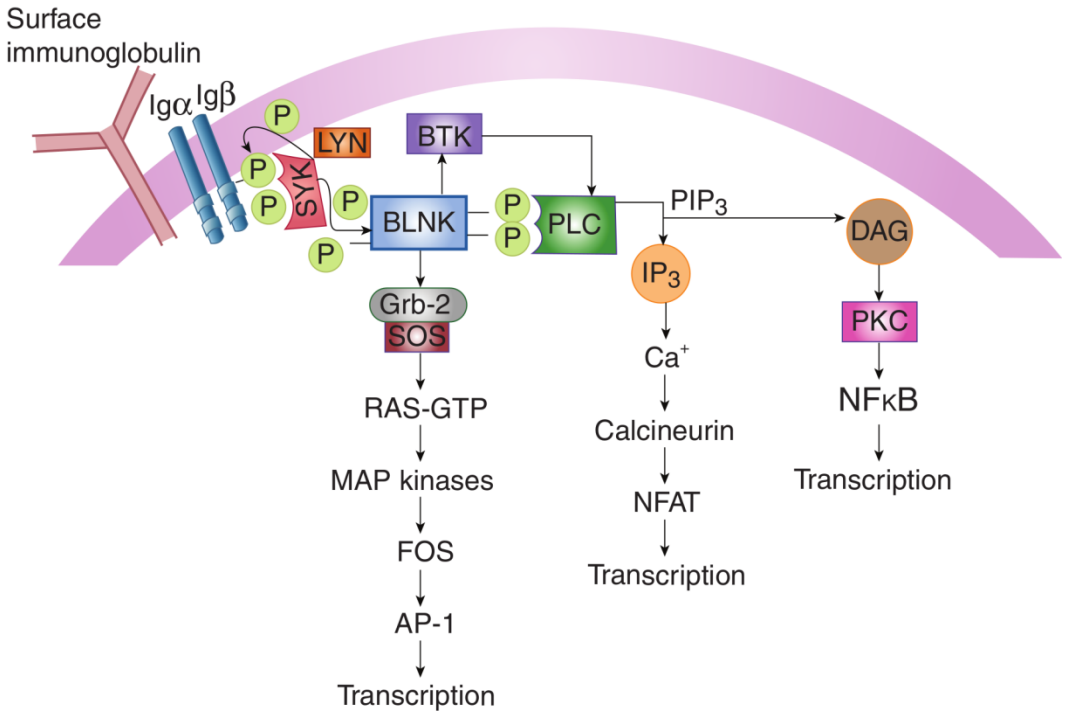


Figure 1-8. B-lymphocyte signaling.

Flash Card A11

Cyclosporin bind to proteins called immunophilins. Then drug protein complex inhibits calcineurin and therefore NFAT translocation to the nucleus.

- **Syk** is the ZAP-70 analog, and its phosphorylation of molecules (e.g., PLC and accessory molecules) is active in the same pathways as for transcription factor activation.
- **B-lymphocyte linker protein (BLNK)** when phosphorylated by Syk, it will further activate Ras and Rac, PLC, and Bruton's tyrosine kinase (BTK).
- **BTK** is unique to B lymphocytes. BTK and Syk activate PLC to break PIP_2 down to IP_3 and make diacylglycerol (DAG) analogous to the TCR pathway. **Note:** BTK is also involved in B-lymphocyte maturation. Mutation in BTK produces Bruton's agammaglobulinemia or X-linked agammaglobulinemia.
- **CD21 (CR2)** provides signals that enhance the BCR if the antigen is opsonized by C3b. On the surface, C3b is covalently bound to the antigen and degraded to C3d, which is the ligand for CD21.
- CD21-CD19-CD81 complex is expressed on the surface of B lymphocytes. When CD21 interacts with C3d, the complex is brought into the BCR. CD19 has an ITAM that is phosphorylated, thus recruiting Lyn to enhance phosphorylation of the Igα and Igβ chains; it also activates IP_3 kinase, which helps in BTK and PLC recruitment.

CYTOKINES, CHEMOKINES, AND THEIR RECEPTORS

CYTOKINES

Cytokines are proteins secreted by the cells of the innate and adaptive immunity that mediate many of the functions of these cells

Cytokines That Mediate and Regulate Innate Immunity

Cytokines are Produced mainly by mononuclear phagocytes in response to infectious agents (Table 1-16).

Flash Card Q12

What are the CD molecules in BCR coreceptor?

Table 1-16. Cytokines of the Innate Immune System

Cytokine	Source	Receptor	Action
TNF	Activated mononuclear phagocytes Antigen-stimulated T cells NK cells Mast cells Most potent stimulus is TLR engagement with LPS and other microbial products Synthesis augmented by IFNγ	Two types: Type I TNF and type II TNF Binding to TNF-RII leads to recruitment of TRAFs to cytoplasmic domains, activating transcription factors (NFκB, and AP-1) Binding of TNF-RI leads to apoptosis via caspase 8	Mediates the acute inflammatory response to infectious microbes (ESP gram negative rods) Stimulates the recruitment of neutrophils and monocytes to sites of infection Induces vascular endothelial cells to express adhesion molecules Stimulates endothelial cells and macrophages to induce leukocyte chemotaxis and recruitment Acts on mononuclear phagocytes to stimulate IL-1 secretion
IL-1 2 forms: IL-1α and IL-1β	Activated mononuclear phagocytes Neutrophils Epithelial cells Endothelial cells Production is induced by bacterial products, such as LPS and other cytokines (TNF)	Binding to type I IL-I R leads to Myd88 recruitment to the TIR domain and protein kinases (IRAK4, IRAK1 and TRAF6), leading to activation of NFκB	Low concentrations: Mediates local inflammation and acts on endothelial cells to increase expression of surface molecules that mediate leukocyte adhesion Larger quantities: Induce fever and the synthesis of acute phase reactants by the liver (via IL-6 production), and neutrophil and platelet production by the bone marrow
IL-12 Made of p35 and p40 subunits	Activated dendritic cells Macrophages Produced in response to TLR signaling induced by many microbial stimuli Stimulated by IFNγ from NK cells or T lymphocytes CD40L/CD40 interaction on macrophages and dendritic cells	Type I receptor family, composed of β1 and β2 subunits p35 binds to β2 receptor, leading to Jak 2 → STAT4 p40 binds to β1 receptor, leading to Tyk 2 → STAT 4	Stimulates production of IFNγ by NK cells and T lymphocytes **Promotes differentiation of CD4 helper T lymphocytes into IFNγ producing TH1 cells** Enhances cytotoxicity of NK cells and CD8 cells

Key Fact

1β is cleaved by caspase-1, which requires activation by a complex of proteins, including NALP. Gain-of-function mutations of NALP lead to uncontrolled IL-1 production and auto-inflammatory syndromes. IL-1ra is a competitive inhibitor of IL-1 made by mononuclear phagocytes. It is available commercially (Anakinra) to treat auto-inflammatory syndromes.

Key Fact

Patients with mutations in the IL-12 Rβ1 are susceptible to infections with intracellular bacteria notably *Salmonella* and atypical mycobacteria.

Key Fact

IRAK-4 deficiency leads to susceptibility to pyogenic infections, especially with *Streptococcus pneumoniae.*

Flash Card A12

CD21, CD19, and CD81.

Table 1-16. Cytokines of the Innate Immune System, cont.

Cytokine	Source	Receptor	Action
Type 1 interferons	IFNα: Plasmacytoid dendritic cells and mononuclear phagocytes IFN β: Produced by many cells, including fibroblasts Most potent stimulus is viral nucleic acids	Type II cytokine receptor family IFNAR1/Tyk2 and IFNAR2/Jak1, leading to STAT1 and STAT2 phosphorylation and recruitment of IRF9	**Inhibit viral replication, thereby eradicating viral infections** Increase expression of class I MHC molecules; Stimulate development of T_h1 cells Promote sequestration of lymphocytes in lymph nodes Inhibit proliferation of many cell types
IL-10	Macrophages Regulatory T cells	Type II cytokine receptor family, Jak1 and Tyk2; Janus family kinases, which induce STAT3 signaling molecule	Inhibits production of IL-12 by activated macrophages and dendritic cells Inhibits expression on costimulators, and class II molecules on macrophages and dendritic cells Inhibits costimulatory receptors
IL-6	Mononuclear phagocytes Vascular endothelial cells Fibroblasts Produced in response to IL-1 and TNF	Type I cytokine receptor family Signaling pathway involves Jak1 and STAT3 activation	Stimulates synthesis of acute phase protein by hepatocytes Stimulates production of neutrophils from bone marrow progenitors Stimulates growth of B lymphocytes that have differentiated into antibody producers Growth factor for neoplastic plasma cells (myelomas)
IL-15	Mononuclear phagocytes in response to viral infection and LPS	Activates Jak3, STAT5 and Akt-dependent signaling pathways	**Survival of memory CD8 T lymphocytes, NK cells, and NK-T cells** Required for NK cell differentiation and activation
IL-18	Macrophages; Dendritic cells. Production is dependent on caspase-1	IL-1 or TLR family signals through TIR domain that recruits IRAK and TRAF, leading to activation of NFκB and AP-1 transcription factors	Enhances IFNγ production by T lymphocytes Promotes differentiation of IFNγ, producing T_h1 CD4 cells Synergistic with IL-12

Table 1-16. Cytokines of the Innate Immune System, cont.

Cytokine	Source	Receptor	Action
IL-23	Macrophages and dendritic cells in response to microbial infection Production depends on caspase-1 (similar to IL-1)	On T lymphocytes and NK cells IL-23R is a heterodimer of a unique IL-23R chain and the IL-12Rβ1 chain	Contributes to inflammation in autoimmunity Important for resistance to *Klebsiella pneumoniae* **Promotes differentiation and maintenance of T lymphocytes that produce IL-17**
IL-27	Macrophages Dendritic cell	IL-27R composed of IL-6 gp30 subunit and a second homologous chain Expressed on resting NK cells and NK-T lymphocytes, effector and memory T lymphocytes, and regulatory T lymphocytes	Promotes T_h1 differentiation Promotes IFNγ production by T lymphocytes Role in controlling ongoing T-lymphocyte responses

Abbreviations: CTL, cytotoxic T lymphocyte; DCs, dendritic cells; IFN, interferon; IL, interleukin; MHC, major histocompatibility complex; NFκB, nuclear factor kappa B; NK, natural killer; TLR, Toll-like receptor; TNF, tumor necrosis factor.

Cytokines That Mediate and Regulate Adaptive Immunity

Produced mainly by T lymphocytes in response to specific recognition of foreign antigens (Table 1-17).

Table 1-17. Cytokines of the Adaptive Immune System

Cytokine	Source	Receptor	Actions
IL-2	Mainly CD4 T lymphocytes (8–12 hr after activation)	IL-2R (composed of 3 proteins:IL-2Rα, IL-2/15Rβ, γ_c) Engages Jak3-STAT5 signal transduction pathways	**Required for survival and function of Treg cells** Stimulates the survival, proliferation, and differentiation of antigen-activated T lymphocytes Promotes the proliferation and differentiation of NK cells Induces expression of Bcl-2 (anti-apoptotic protein)

Key Fact

Receptors for IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21 contain γc, which is affected in X-linked SCID.

Table 1-17. Cytokines of the Adaptive Immune System, cont.

Cytokine	Source	Receptor	Actions
IL-4	T_h2 CD4 T lymphocytes Activated mast cells	Type I cytokine receptor family; IL-4R$\alpha\gamma_c$ signals through the Jak-STAT pathway (STAT6).	**T_h2 differentiation** Inhibition of T_h1 and T_h17 cell development **B lymphocyte switching to IgE** Acts on macrophages with IL-13 to induce arginase induction, leading to collagen production and increased mannose receptor expression, which promotes phagocytosis of microbes
IL-5	T_h2 CD4 T lymphocytes Activated mast cells	IL-5Rα and IL-5Rβc heterodimer induces Jak2 and STAT 3 signaling pathway	**Activates immature eosinophils, and stimulates growth and differentiation of eosinophils** Stimulates the proliferation of B lymphocytes and production of IgA antibodies
IL-13	T_h2 CD4 T lymphocytes CD8 T lymphocytes NK T lymphocytes Basophils Eosinophils	IL-4Rα and IL-13Rα1 heterodimer Binds to IL-4 and IL-13 with high affinity	Promotes fibrosis as part of the tissue repair phase of chronic inflammatory state Stimulates mucus production by lung epithelial cells Induces IgE class switching in B lymphocytes Promotes inflammation by inducing expression of endothelial expression factors and chemokines

Key Fact

The receptors IL-3, IL-5, and granulocyte–monocyte colony-stimulating factor (GM-CSF) share a common β chain.

Table 1-17. Cytokines of the Adaptive Immune System, cont.

Cytokine	Source	Receptor	Actions
IFNγ	NK cells T_h1 CD4 T lymphocytes CD8 T lymphocytes	Type II cytokine receptor family, IFNγR1, and IFNγR2 heterodimer IFNγR1 associates with Jak1 kinase and IFNγR2 associates with Jak2 kinase Leads to STAT1 phosphorylation and dimerization	Promote macrophage-rich inflammatory reactions while inhibiting IgE-dependent eosinophil rich reactions Activates macrophages to kill phagocytosed microbes **Promotes the differentiation of naïve CD4 T lymphocytes to the T_h1 subset (via T-bet)** Inhibits the differentiation of TH2 cells; Acts on B lymphocytes to promote switching to certain IgG subclasses Stimulates expression of class I and class II MHC molecules, and costimulators on APCs
TGFβ(1)	Antigen-stimulated T lymphocytes LPS-activated mononuclear phagocytes	Serine or threonine kinase domain that phosphorylates transcription factors of Smads ALK5 phosphorylates Smad2 and Smad3, which, with Smad4, translocates to the nucleus, binds to promoters of target genes, and regulates their transcription	**Inhibits the proliferation and effector functions of T lymphocytes, and the activation of macrophages** Regulates the differentiation of T-lymphocyte subsets Stimulates the production of IgA antibodies by inducing B lymphocytes to switch to this isotype Regulates tissue repair
Lymphotoxin	T lymphocytes	TNF receptors	Activates endothelial cells and neutrophils

Table 1-17. Cytokines of the Adaptive Immune System, cont.

Cytokine	Source	Receptor	Actions
IL-21	Activated CD4 T lymphocytes	Type I family receptor that activates Jak1, Jak2, STAT1, and STAT3	Stimulation of proliferation and augmentation of effector CD8 T lymphocytes Enhancement of class switching and Ig production by B lymphocytes Induction of differentiation and enhancement of effector function of NK cells
BAFF	Neutrophils Monocytes Macrophages Dendritic cells Follicular dendritic cells Activated T lymphocytes	TACI, BCMA, and BAFF-R	Up-regulation of anti-apoptotic protein Bcl-2 activation of NFκB.
APRIL	Monocytes Macrophages Dendritic cells Activated T lymphocytes	TACI and BCMA	Up-regulation of anti-apoptotic protein Bcl-2 activation of NFκB.

Abbreviations: MHC, major histocompatibility complex; NK, natural killer; NFκB, nuclear factor kappa B; TNF, tumor necrosis factor

Cytokines and Hematopoiesis

Cytokines that stimulate hematopoiesis are also involved in the differentiation and expansion of bone marrow progenitor cells (Table 1-18).

Families of Cytokine Receptors

Table 1-19 lists families of cytokine receptors and their signaling pathways.

Flash Card Q13

Which chemokine receptor is associated with WHIM (warts, hypogammaglobulinemia, infections, myelokathexis) syndrome?

Table 1-18. Cytokines That Stimulate Hematopoiesis

Cytokine	Source	Receptor	Actions
Stem cell factor (c-*kit* ligand)	Stromal cells of the bone marrow	Act on immature stem cells with c-*kit*	Enhances response to other colony-stimulating factors (CSFs) Sustains the viability of T lymphocytes in the thymus **Mast cell growth factor**
IL-7	Fibroblasts Bone marrow stromal cells	IL-7Rα chain associated with γ_c chain Associated with Jak3 kinase	Survival of mature, naïve and memory T lymphocytes (especially CD4)
IL-3	CD4 T lymphocytes	Type I cytokine receptor family Signal transduction involves Jak-STAT	Promotes the growth and development of mast cells from bone marrow **Basophil-differentiating cytokine**
Erythropoietin (Epo)	Produced in the kidney in response to low oxygen tension	Type I cytokine receptor that signals through Jak2-STAT5 and PI-3 kinase-Akt pathways	Promotes production of RBC from committed erythroid progenitors
IL-11	Bone marrow stromal cells	gp130 signaling Jak-STAT	Megakaryocytopoiesis

Mnemonic

Hot T-Bone stEAk

IL-1: fever (**Hot**)
IL-2: stimulates **T** lymphocytes
IL-3: stimulates **Bone** marrow
IL-4: stimulates Ig**E**
IL-5: stimulates Ig**A**

Table 1-19. Cytokine Receptor Families

Receptors	Pathways
Type I cytokine R (hematopoietin receptors)	Engage Jak-STAT signaling pathways
Type II cytokine R	Engage Jak-STAT-signaling pathways
IL-1 family R	Share Toll-like/IL-1 receptor (TIR) domain.
TNFR	Intracellular signaling mechanisms induce apoptosis and/or stimulate gene expression
Seven transmembrane α-helical R	Signaling pathways involve GTP-binding proteins.

Abbreviations: IL, interleukin; TNFR, tumor necrosis factor receptor.

Flash Card A13

CXCR4

CHEMOKINES

Chemokines are a subgroup of cytokines that are divided into four families, based on the number and location of terminal cysteine residue.

Source—Leukocytes, endothelial cells, epithelial cells, and fibroblasts stimulated by microbes via TLR signaling and inflammatory cytokines (TNF and IL-1).

Receptors—Found on leukocytes (greatest number and diversity on T lymphocytes); and G protein-coupled receptors (GPCRs) with seven-transmembrane α-helical domains (GTP) modulate cytoskeletal protein configuration and integrin affinity.

Actions—Recruit cells of host defense to sites of infection; induce migration of leukocytes toward the chemical gradient of the cytokine by stimulating alternating polymerization/depolymerization of actin filaments; regulate the traffic of lymphocytes and other leukocytes through peripheral lymphoid tissues; and promote angiogenesis and wound healing.

Table 1-20 lists the known chemokines or receptors that are associated with certain diseases.

Table 1-20. Select Chemokine or Receptor Defects and Their Associated Diseases

Chemokines or Receptors	Associated Disease
CCR5 or CCL3L1 and CXCR4	HIV and/or AIDS
CXCR4	WHIM syndrome
CXCL4	Heparin-induced thrombocytopenia
CX3CR1, CCL5	Atherosclerosis
CCL2, 5, 7, 11 and CXCL8	Asthma or allergies
CXCL12 or CXCR4	Cancer metastases
CCL25 or CCR9	Crohn’s disease

Abbreviations: WHIM., warts, hypogammaglobulinemia, infections, and myelokathexis.

CELL ADHESION MOLECULES

Think of cell adhesion molecules (CAMs) as traffic cops: they aid in directing the traffic of leukocytes to areas of inflammation.

The following are the major players that are most important to know:

- Chemokines
- Selectins
- Integrins
- Immunoglobulin superfamily

Chemokines

Figure 1-9 outlines chemokine pathways and functions.

The following four families of chemokines are the most important to know (see Table 1-21 for a list of notable chemokine ligands and their receptors):

- C
- CC → Eo's, Baso's, Mono's → ALLERGY
- CXC → PMN's → INFLAMMATION
  - ELR—angiogenic, acts through CXCR2
  - Non-ELR—angiostatic, acts via CXCR3B, induced by interferons
- CX3

Homeostatic: CCL19/CCL21 → CCR7 → Lymphocyte Homing
Inflammatory: CCL17/CCL22 → CCR4 → pro T_h2 response

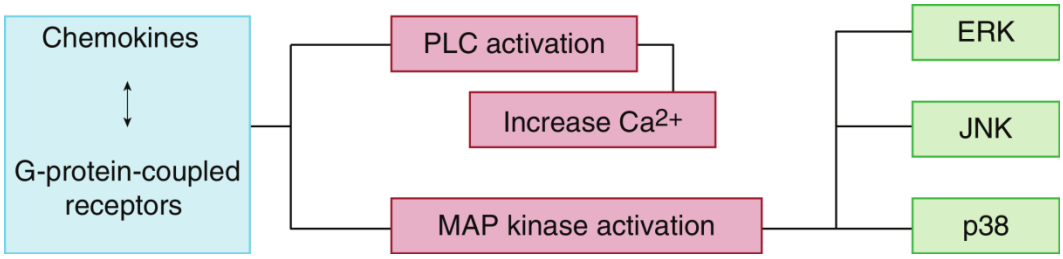


Figure 1-9. Chemokines signal via G protein-coupled receptors and result in the activation of several pathways as shown.

Table 1-21. Important Chemokine Ligands and Receptors

Ligand	Receptor
CCL5 (RANTES)	CCR 1,3,5
CCL11 (Eotaxin)	CCR3
CCL17 (TARC)	CCR4
CXCL8 (IL-8)	CXCR 1,2

Abbreviations: IL, interleuking; RANTES, regulated on activation, normal T expressed and secreted; TARC, thymus and activation-regulated cytokine.

Decoy Chemokine Receptors

- **DARC (Duffy):** Protects against metastasis; mutation in GATA in DARC gene confers malaria protection in African Americans.
- D6
- CCX-CKR: Target for CCL19 or CCL21.

Selectins

All three types of selectins are involved in the rolling of leukocytes, and they bind carbohydrates (Table 1-22).

In LAD-2, polymorphonuclear neutrophils (PMNs) cannot express carbohydrate ligands for E and P selectin.

Integrins

All integrins are involved in adhesive interplay between APCs and lymphocytes as well as lymphocyte homing. There are three families of integrins (Table 1-23).

Table 1-22. Summary of Selectins

Selectin	Location	Ligand	Function
P Selectin (CD62**P**)	**P**latelets, and Weibel-Palade bodies of the endothelium	**P**GSL and Sialyl-Lewis X	Binds **P**MNs, T lymphocytes, and monocytes
E Selectin (**E**LAM[a] and CD62**E**)	**E**ndothelium	**E**SL-1, CD15, PGSL, and Sialyl-Lewis X	Homing of T lymphocytes to peripheral sites of inflammation
L Selectin (**L**AM-1 and CD62**L**)	**L**ymphocytes and **l**eukocytes	G**L**YCAM-1, MADCAM-1, CD34, and Sialyl-Lewis X	Homing to lymph node HEV (G**L**YCAM) and PMN rolling (MAdCAM)

[a]ELAM increases in Kawasaki's disease. Abbreviations: ELAM, endothelial leukocyte adhesion molecule; MAdCAM, mucosal addressin cell adhesion molecule; PMN, polymorphonuclear neutrophil.

Flash Card Q14

Which integrin molecule is important for gut homing by binding to MAdCAM?

Table 1-23. Integrin Families

Name	Synonyms	Ligand	Function
B1			
A4β1	**V**LA4	**V**CAM	Important for neuronal homing; used as treatment for multiple sclerosis
B2			
AIβ2	LFA1, CD11a/CD18	ICAM-1	Rhinovirus binds here
Amβ2	MAC1, CD11b/CD18	ICAM-1, iC3B (CR3)	
Axβ2	CD11c/CD18	ICAM3, C3dg, iC3B (CR4)	
Amβ2	MAC1, CD11b/CD18	ICAM-1, iC3B (CR3)	
B3			
Avβ3			Important for platelets and GIIbIIIa

The fourth β integrins family, **α4β7** is also important. This family is a mucosal addressin that binds to mucosal addressin cell adhesion molecule (MAdCAM) and it is important for gut homing.

Immunoglobulin Superfamily (IgSF)

All IgSF molecules contain at least one immunoglobulin or immunoglobulin-like domain. These molecules are involved in cell-to-cell interaction.

ICAM1 (CD54) → binds LFA1, MAC1, and rhinovirus

ICAM2 (CD102) → binds LFA1

ICAM3 (CD50) → binds LFA1 and CD18

ICAM4 (CD242) → binds LFA1 and CD18

VCAM1 (CD106) → binds **V**LA4 (**α4β1**); vascular

PECAM (CD31) → binds CD31 and CD38; platelets

MAdCAM → binds mucosal addressin (**α4β7**); mucosa

NCAM (CD56) → binds VLA4 (**α4β1);** neuronal

Clinical Implications

Flash Card A14

α4β7

Chemokines are implicated in a number of clinically relevant disorders. Please see Table 1-24 for further details.

Table 1-24. Chemokines and Associated Conditions

Condition	Implicated Chemokines	
Heparin-induced thrombocytopenia	CXCL4	
Asthma and allergies	CCL2, 5, 7, 11; CXCL8	
HIV	CCR5 (mono/macro)	Homozygotes ⟶ No infection Heterozygote ⟶ Slow progressors of infection
	CXCR4	T trophic
	CCL3L1	Low level ⟶ Higher HIV acquisition, high viral load, worse disease
Atherosclerosis (also known as fractalkine)	CXC3CL1	
	CX3CR1	V249I ⟶ Increased risk of acute coronary syndrome T280M ⟶ Prevents plaque entry (confers protection)
Glanzmann's thrombasthenia	GIIbIIIa defect leading to platelet defect	

COMPLEMENTS AND KININS

The complement system is an important effector mechanism of both humoral and innate immunity. Its functions are to promote phagocytosis of microbes, to stimulate inflammation, and to induce lysis of these organisms.

Pathways of Complement Activation

Alternative Pathway (AP) (Figure 1-10)

- C3 is continuously undergoing small-scale cleavage in the plasma (C3 tick-over) to C3b ⟶ activates an internal thioester bond ⟶ covalent binding of C3b to hydroxyl groups on microbial cell surfaces.
- Bound C3b binds factor B to form C3bB.
- Factor D cleaves factor B to form the **AP C3 convertase (C3bBb)**, stabilized by properdin.
- Regulation of this pathway occurs through factor I-mediated cleavage of C3b.
- Factor H, membrane cofactor protein (MCP/CD46) and decay-accelerating factor (DAF/CD55) serve as cofactors (only found on mammalian cells).

Flash Card Q15

Which complement is the most potent mediator of basophil and cutaneous mast cell degranulation?

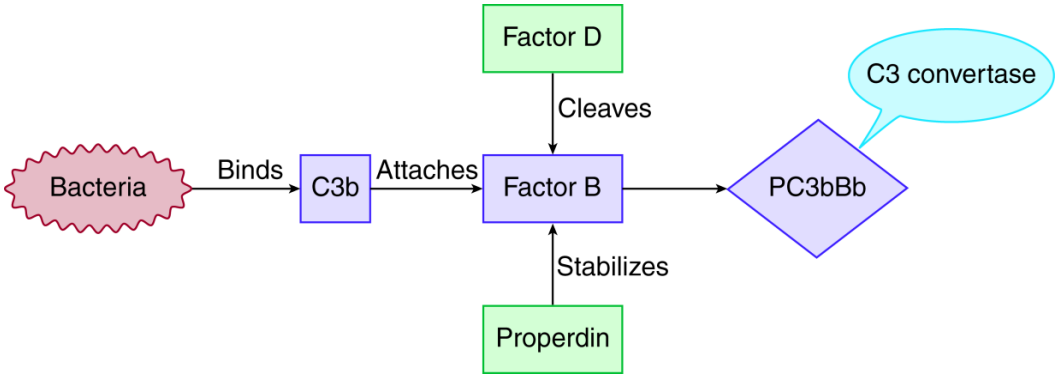


Figure 1-10. Alternative pathway.

Classical Pathway (CP) (Figure 1-11)

- C1q binds to the Fc portion of an immunoglobulin cross-linked by antigen.
- Binding affinity of C1q for **IgM > IgG3 > IgG1 > IgG2.**
- C1q is associated with C1r serine proteases, which cleave and autoactivate C1s proteins.
- Activated C1s cleave C4 and C2 to yield **C4b2a**, which is the **C3 convertase of the CP.**
- C1 inhibitor inhibits C1r and C1s, thus regulating the pathway.
- C4b deposited on cell surfaces is bound by DAF, complement receptor type 1 (CR1) and C4-binding protein (C4BP), thus blocking further progression of the cascade.
- C4b bound to C4BP is inactivated to C4bi by factor I

Key Fact

Binding affinity of C1q for IgM > IgG3 and IgG1 or IgG2. Factor H regulates only the alternative pathway.

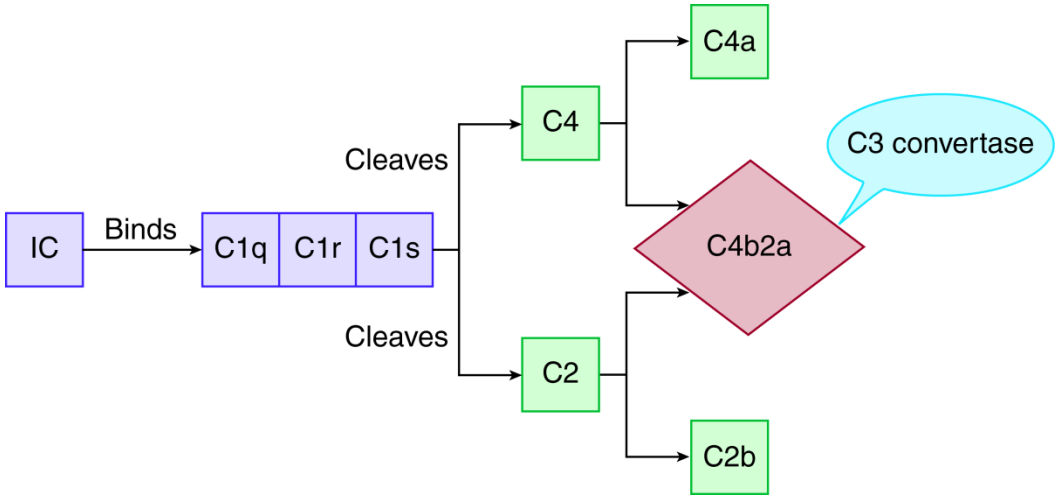


Figure 1-11. Classical pathway.
Abbreviation: IC, immune complex.

Flash Card A15

C5a.

Lectin Pathway (LP)—Activated in the absence of antibody. Mannose-binding lectin (MBL) binds to mannose residues on microbial polysaccharides, and also to MBL-associated protease-1 (MASP-1) and MBL-associated protease-2 (MASP-2). MASP-2 cleaves C4 and C2 to form the **C3 convertase C4b2a** (Figure 1-12).

- C3 convertase cleaves C3 to C3b, which binds to the convertase and form C5 convertase: C3bBb3b in the AP, and C4b2a3b in the CP and LP.
- C5 convertase cleaves C5 and initiates the formation of the membrane attack complex (MAC), and C5a is released.
- MAC is formed by (C5b-8) polyC9 and it creates pores in the membrane and induces cell lysis.
- C9 is structurally homologous to perforin.
- S Protein and CD59 inhibit formation of the MAC.
- C5a is the most potent mediator of basophil and cutaneous mast cell degranulation; C5a > C3a > C4a.
- C5a is chemotactic for neutrophils, eosinophils, monocytes, and basophils.
- C3a is chemotactic for only eosinophils.

Complement Receptors

Biologic activities of the complement system are performed by binding of complement proteins to membrane receptors on various cell types. However, many microorganisms can also bind to these receptors, thus entering human cells (Table 1-25). Leukocyte adhesion deficiency (LAD) type 1 is observed in patients with CR3 or CR4 deficiency and is due to a rare mutation in the β chain (CD18) common to the CD11 or CD18 family of integrin molecules.

Key Fact

Leukocyte adhesion deficiency (LAD) type 1 is observed in patients with CR3 or CR4 deficiency → due to a rare mutation in the **β chain (CD18)** common to the CD11 or CD18 family of integrin molecules.

Key Fact

CR4 is a marker for dendritic cells.
C3d binds CR2 (CD21) and provides a second signal for B-lymphocyte activation by antigen.

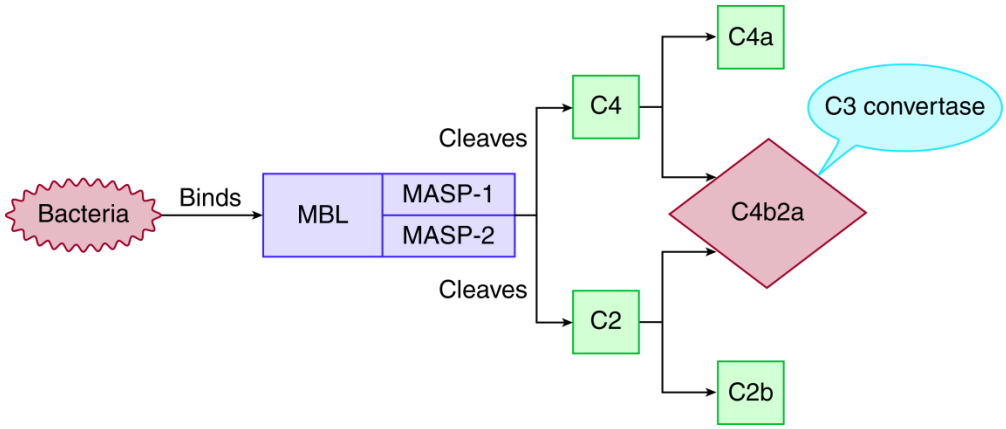


Figure 1-12. Lectin pathway.
Abbreviations: MASP, mannose-associated serine protease; MLB, mannose-binding lectin.

Flash Card Q16

Which complement receptor is implicated in PNH (paroxysmal nocturnal hemoglobinuria)?

Table 1-25. Receptors of the Complement System

Receptor (Alternative Names)	Ligand	Function	Associated Pathology	Complement System Used by Micro-organisms to Enter Human Cells
CR1 (CD35)	C3b, C4b, iC3b	Regulates complement activation Phagocytosis of C3b- and C4b-coated particles Clearance of immune complexes (CR1 on RBCs)		HIV
CR2 (CD21)	C3d, iC3b, and C3dg	Part of B lymphocyte coreceptor with CD19 and CD81 (TAPA) Trapping antigens in the germinal center		EBV and HIV
CR3 (Mac-1, CD11b/CD18)	iC3b and ICAM-1	Phagocytosis Leukocyte adhesion to endothelial cells	LAD type I	*Mycobacterium tuberculosis,* HIV, and West Nile virus.
CR4 (gp 150/95, CD11c/CD18)	iC3b	Phagocytosis	LAD type I	
CD46 (MCP)				Measles virus
CD55 (DAF)	C4b and capsid	Regulates formation of C3 convertase	PNH	Echovirus and coxsackie virus
CD59 (Protectin	C5b-8 and monomeric C9	Disrupts formation of (C5b-8)polyC9	PNH	

Abbreviations: DAF, decay-accelerating factor; EBV, Epstein-Barr virus; HIV, human immunodeficiency virus; LAD, leukocyte adhesion; MCP, membrane cofactor protein; PNH, paroxysmal nocturnal hemoglobinuria.

Flash Card A16

CD55 (DAF); CD59.

Pathologic Effects of Complement Activation

C3 Nephritic Factor

- An autoantibody that binds and stabilizes C3bBb, and protects it from factor H-mediated dissociation and factor I cleavage, resulting in unregulated consumption of C3
- Found in a few patients with systemic lupus erythematosus (SLE)
- Associated with type II membranoproliferative glomerulonephritis (MPGN) and partial lipodystrophy
- MPGN: Mesangial proliferation, capillary wall thickening, and subendothelial deposits of Ig and C3
- Partial lipodystrophy, a disfiguring condition that affects the body from the waist upward but spares the legs
- Adipose cells are the main source of factor D, which completes the formation of C3bBb
    - There is a gradient in the concentration of factor D; more is present in the upper-half than the lower-half of the body, thus causing the distribution of fat loss observed in partial lipodystrophy. C3 nephritic factor stabilizes C3bBb that forms in the vicinity of adipocytes and cleaves C3 to allow assembly of membrane attack complex, which lyses the adipocytes.

Anti-C1q Antibody

- Autoantibody to the collagen-like region of C1q
- Found in patients with hypocomplementemic urticarial vasculitis (HUVS)
- Results in activation of creatine phosphate pathway with tissue deposition of immune complexes
- HUVS is responsive to treatment with hydroxychloroquine

Complement Deficiencies

Testing (Figure 1-13)

- CP: CH50: Assesses ability of serum to lyse sheep RBCs sensitized with rabbit IgM
- AP: AH50: Measures lysis of unsensitized rabbit RBCs
- C3 and C4 levels are measured in nephelometric immunoassays
- C4a and C4d: markers of CP or LP activation; and Bb: AP activation
- C3a, iC3b, C5a, and soluble C5b-9: markers of terminal pathway activation

Flash Card Q17

Which complement deficiency is inherited as X-linked?

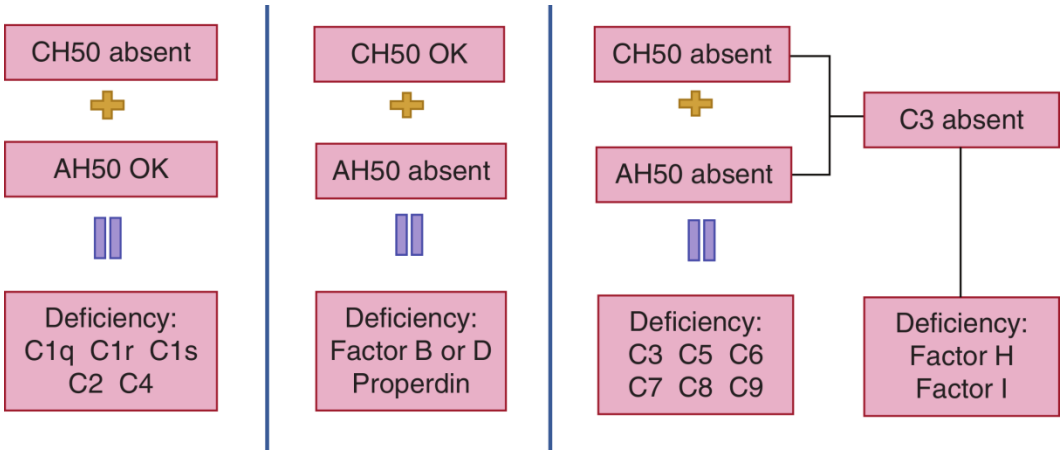


Figure 1-13. Diagram summarizes deficient complement components based on the results of CH50 and AH50.

Complement Component Deficiency—Generally autosomal recessive, but properdin deficiency is the only X-linked deficiency; so, all known cases are male. Patients are predisposed to recurrent infections and autoimmune disorders at an early age (Table 1-26).

Key Fact

SLE is seen much more frequently with deficiency of C1q than any other complement deficiency. Also caused by C1r/C1s, C4 (1% whites C4A), and C2 deficiency.

Table 1-26. Complement Component Deficiency

Complement Component Deficiency	Associated Disease	Increased Frequency of Infection With Microorganism
C1q, C1r, C1s, C4, and C2	SLE and MPGN	Recurrent pyogenic bacterial infections: *Streptococcus pneumonia* and *Haemophilus influenzae* type b.
C3	MPGN	Severe, recurrent pyogenic infections beginning shortly after birth
MBL	SLE	Pyogenic infections
C5-C9		*Neisseria*
Factor D, Factor B, and Properdin		
Factor H	HUS, MPGN, and ARMD	
Factor I	MPGN	
CD46 (MCP)	MPGN	
C1 inhibitor	HAE	
CD59 (MAC) and CD55 (DAF)	PNH	

Abbreviations: ARMD, age-related macular degeneration; HAE, hereditary angioedema; HUS, hemolytic uremic syndrome; MPGN, membranoproliferative glomerulonephritis; PNH, paroxysmal nocturnal hemoglobinuria; SLE, systemic lupus erythematosus.

Flash Card A17

Properdin.

Kallikrein-Kinin System Activation

There are two pathways of bradykinin generation. In the first, simpler one, tissue kallikrein cleaves low-molecular-weight kininogen to yield lys-bradykinin, which is further cleaved by an aminopeptidase to bradykinin. This latter cleaving is the second pathway.

Contact Activation Pathway—Initiated when circulating factor XII (Hageman factor) binds to a negatively charged surface and autoactivates (contact activates) to form factor XIIa. Prekallikrein (PK) circulates as a complex with high-molecular-weight kininogen (HK), and HK binds to the initiating cell surface. Factor XIIa then cleaves PK to kallikrein, 10–20% of which dissociates into the fluid phase, and kallikrein digests HK to liberate the vasoactive peptide bradykinin (Figure 1-14). A positive feedback loop is shown in which kallikrein cleaves and activates factor XII at a rate at least 50-fold faster than the factor XII autoactivation. Factor XIIa also cleaves XI to form factor Xia, which initiates the intrinsic coagulation pathway.

Key Fact

C1 inhibitor is the sole plasma inhibitor of factor XIIa and factor XIIf, a cleavage product derived from factor XIIa by digestion with kallikrein or plasmin. C1 inhibitor is also one of the major inhibitors of kallikrein and factor XIa as well as C1r and C1s. C1 inhibitor is consumed by plasmin.

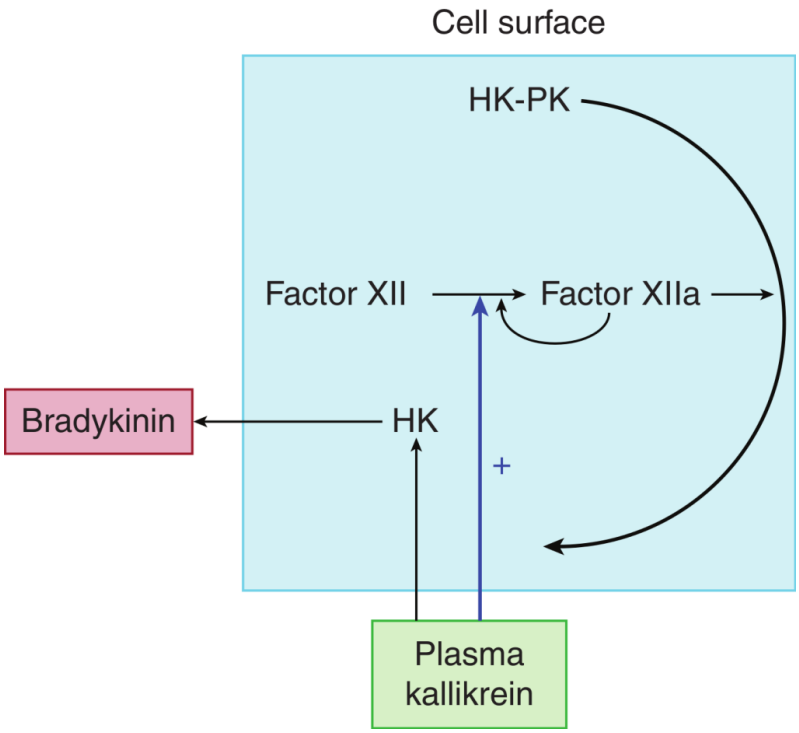


Figure 1-14. Contact activation pathway leading to bradykinin generation.
Abbreviations: HK, high-molecular-weight kininogen; PK, plasma kallikrein; + = activates.

Bradykinin—The mediator of swelling during angioedema episodes, via the B-2 receptor on endothelial cells.

- Hereditary angioedema (HAE) is characterized by bradykinin overproduction caused by lack of C1 inhibitor function; activation is most pronounced at the site of swelling although enzyme activation is detectable throughout the plasma.
- Angiotensin-converting enzyme (ACE) is identical to kininase II, which is the major enzyme responsible for bradykinin degradation. Thus treatment with ACE inhibitors leads to bradykinin accumulation caused by inhibition of degradation.
- The natural surface is likely the endothelial cell membrane (Figure 1-15). Factor XII and HK bind to cell-surface complexes of urokinase plasminogen activator receptor (u-PAR), cytokeratin 1, and receptor for the globular head of C1q (gC1qR), cytokeratin 1, respectively in the presence of zinc.
- Although cell-bound factor XII may initiate the cascade, it is also possible that cell-derived heat shock protein (HSP) 90 or prolylcarboxypeptidase can activate the PK-HK complex stoichiometrically (but not unbound PK) to generate kallikrein. The kallikrein formed can activate factor XII.

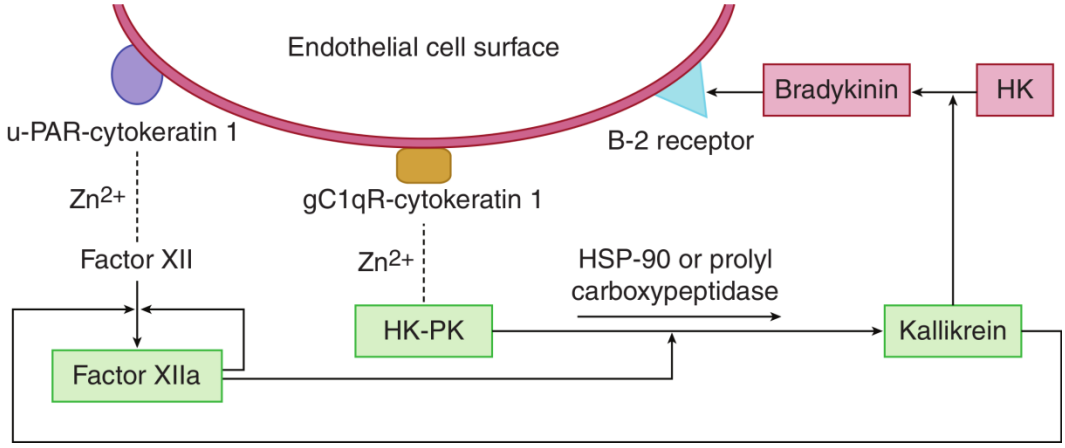


Figure 1-15. Contact activation pathway: Cell-surface receptors.
Abbreviations: + = activates; B-2 receptor, B_2 bradykinin receptor; gC1qR, receptor for the globular head of C1q; HK, high-molecular-weight kininogen; HSP-90, heat shock protein-90; PK, plasma kallikrein; u-PAR, urokinase plasminogen activator.

MUCOSAL IMMUNITY

Role of Mucosal Surfaces

The gastrointestinal (GI) mucosal barrier is a complex physical (i.e., mucus, epithelial cell tight junctions, acid, and enzymes) and immunologic structure. The mucosal immune system is a barrier between the internal and external environment; therefore, it is an important site of entry for microbes.

- Mucosal surfaces of the GI and respiratory tracts are colonized by lymphocytes and APCs that initiate immune responses to ingested and inhaled organisms.
- Removal or alterations to this barrier may promote food allergy and respiratory allergy.
- Secretory immunity (IgA) is the major mechanism of protection by oral vaccines such as oral polio.

Oral vaccines such as the oral polio vaccine use the mucosal immune system to provide protection.

Pathophysiology

When food is ingested, intestinal and pancreatic enzymes break proteins into amino acids and small peptides. Figure 1-16 highlights cells of the gastrointestinal mucosa and their role in immunity.

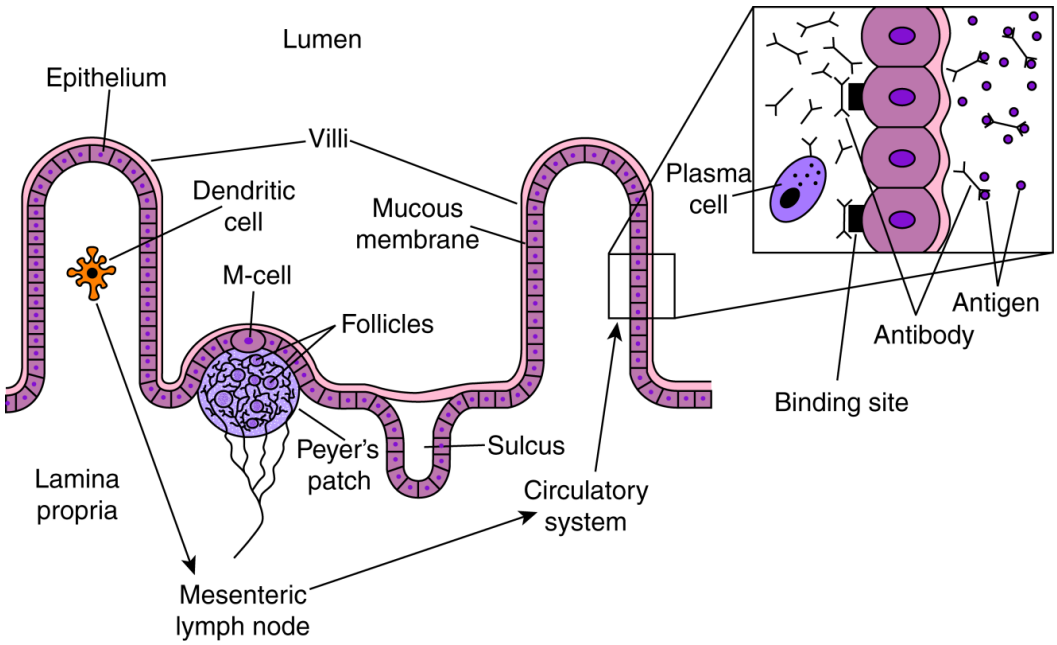


Figure 1-16. Gastrointestinal tract mucosa.
(Reproduced, with permission, from by Daniel Muller).

- It is estimated that 2% of the proteins are absorbed in immunologically active forms.
- Specialized cells in the GI tract selectively absorb peptides.
- Mucosa of the GI has lymphocytes in large numbers in three main regions. Cells in each site have distinct phenotype and functional characteristics (Table 1-27).

Peyer's patches are organized collections of lymphoid tissue and are most prominent in the small intestine. They are also the major site of IgA and B-lymphocyte development that begins from cellular signals from dendritic cells and T lymphocytes.

Cells Involved in Mucosal Immunity

***M Cells*—**Specialized membranous cells that play a role in delivering antigens to Peyer's patches.

- M cells are not APCs. M cells within the Peyer's patch help create an invaginated subdomain or pocket, where memory T lymphocytes, naïve B cells, and memory B lymphocytes interact with antigen.

Table 1-27. Three Main Regions of the Gastrointestinal Tract Mucosa

	Predominant T Lymphocyte Types	Other Cells	Function of Various Cells
Epithelial layer	CD8+	Paneth cells	Participates in innate immunity
		Intestinal epithelial cells	Important in nutrient absorption. Transports secretory IgA
		Intraepithelial lymphs (IELs)	Predominantly effector and effector memory cells
Lamina propria	Mixed population of cells, including activated CD4+ T lymphocytes scattered throughout	Activated B lymphocytes and plasma cells	IgA+
		T lymphocytes	Memory phenotypes. Produce cytokines IFNγ, IL-4, and IL-5
		Macrophages	
		Mast cells	Fight parasites
		Eosinophils	Allergic response Fight parasite
Peyer's patches	Interfollicular regions with CD4+ T lymphocytes	B lymphocytes	Central region is B-lymphocyte rich area that contains germinal centers
		M cells	Specialized membranous cells that play a role in delivering antigens to Peyer's patches

- M cells assist in transport of antigens such as protein, bacteria, virus, and noninfectious particles from the gut lumen to APCs and lymphoid tissue via transcytosis.
- "Sampling" of these antigens is important in the development of the immune response and tolerance.

T Lymphocytes—Localize to small intestine due to $\alpha4\beta7$ + CCR9.

- CD4+ and CD8+ T lymphocytes occur throughout lamina propria. These T lymphocytes have memory phenotype. CD4+ T lymphocytes produce cytokines IFNγ, IL-4, and IL-5.
- CD8+ T lymphocytes are preferential to epithelium.

Epithelial Cells

- Paneth cells: Participate in innate immunity. Located in crypts of Lieberkühn in small intestine.
- Intestinal epithelial cells: Important in nutrient absorption. Transport secretory IgA. Act as nonprofessional APCs by recognizing bacterial and viral motifs such as TLRs. Intraepithelial lymphocytes (IELs): Reside above the basement membrane, between epithelial cells. Migration is influenced by CCR9 or CCL25 and CD103 or E cadherin. All IEL subunits express CD8$\alpha\alpha$, which is a characteristic of activated mucosal T lymphocytes. IELs are mostly effector and effector memory cells with mainly CD8+. See Table 1-28 for phenotype of IELs.

Regulatory T lymphocytes—Five regulatory T lymphocytes have been identified in conjunction with intestinal immunity:

- T_h3 cells, a subset of CD4+ cells that secrete TGFβ
- TR1 cells, CD4 cells that secrete IL-10
- CD4+CD25+ regulatory T lymphocytes
- CD8+ suppressor T lymphocytes
- γ or δ T lymphocytes

Table 1-28. Phenotype of Intraepithelial Lymphocytes

$\gamma\delta$ T cells	CD8+ –10% of total population
$\alpha\beta$ T lymphocytes	CD4+ or CD8+
Double-negative T lymphocytes	$\alpha\beta$ T lymphocytes with no coreceptor

Oral Tolerance

In both children and adults, intact food antigens are ingested and absorbed through the GI tract. These antigens enter circulation and are transported throughout the body in an immunologically intact form. These antigens do not normally cause symptoms because most people develop tolerance.

Mechanisms That Promote Tolerance

- ACPs, especially intestinal epithelial cells, dendritic cells, and Treg cells play central role in oral tolerance.
- APCs in the gut lumen and elsewhere in the body present the potentially allergenic proteins to the T lymphocytes that, in a genetically predisposed individual, results in T_h2 allergic response.
- Intestinal epithelial cells process luminal antigens and present it to T lymphocytes on a MHC class II complex; but these lack a "second signal," thus suggesting their potential to play a role in tolerance induction.
- Dendritic cells residing within lamina propria and a noninflammatory environment of Peyer's patches express IL-10 and IL-4. IL-10 promotes Tregs.
- **Commensal gut flora** can influence mucosal immune response. Gut flora are established within 24 hours after birth and depend on both maternal flora and local environment. Studies feeding lactating moms and their offspring *Lactobacillus* GG suggest that probiotics may be of benefit in preventing atopic dermatitis, possibly by enhancing T_h1 cytokine response (IFNγ). Whether probiotics will be useful to prevent food allergy has yet be determined.

Key Fact

High-dose tolerance involves deletion of effector T lymphocytes.
Low-dose tolerance is mediated by activation of regulatory T lymphocytes with suppressor functions.

Mechanisms That Hinder Tolerance

- Developmental immaturity of components of gut barrier (decreased enzymatic activity in newborn period and secretory IgA) accounts for high prevalence of food allergy in infancy.
- Studies show that neutralizing pH (decreased acidity from antacids) can promote allergy sensitization by changes to the physiologic barrier.

IgA and Mucosal Immunity

Key Fact

Gut homing lymphocyte integrins are $\alpha4\beta7$ and MAdCAM-1.

IgA is the major class of antibodies produced by the mucosal immune system. IgA binds to microbes and toxins present in lumen and neutralizes them by blocking entry into the host. Secretory immunity is mechanism of protection by oral vaccines such as oral polio. More IgA is produced than any other antibody because of the size of the intestinal surface.

Secretion of IgA is shown within the square insert of Figure 1-16 and detailed as follows:

- IgA synthesis occurs primarily in gut mucosal lymphoid tissue (lamina propria), and transport across mucosal lumen is efficient (i.e., not lost to the bloodstream).
- IgA+ B lymphocytes migrate from Peyer's patches to mesenteric lymph nodes. They circulate in lymphatics, ending up in lamina propria of gut.
- Arrival in laminal propria is regulated by site-specific adhesion molecules, $\alpha 4\beta 7$ + and MAdCAM-1.
- Secretory IgA molecules in the gut lumen bind foreign proteins and block absorption.
- Switching to IgA isotype is stimulated by TGFβ and IL-5.
- IgA is secreted in dimerized form that is held together by J chain. Serum IgA is a monomer. IgA is transported across the epithelium by a poly-Ig receptor.
- Once the dimerized IgA + poly-Ig receptor is on the luminal surface, the receptor is proteolytically cleaved, leaving behind its transmembrane and cytoplasmic forms. The IgA molecule plus extracellular domain of receptor is released into the intestinal lumen. Poly-Ig receptor transports IgA into bile, milk, sputum, saliva, and sweat as well as IgM into intestinal secretions.

Breast Feeding—Provides immune protection and decreases incidence of food allergies. Colostrum has very high levels of IgA in the first 4 days postpartum, then drops to serum levels. Other components of breast milk include lysozyme, lactoferrin, and TNFα.

Key Fact

A normal adult secretes 2 g of IgA per day. More IgA is produced than any other antibody. Switching to the IgA isotype is stimulated by TGFβ and IL-5.

TRANSPLANTATION AND TUMOR IMMUNOLOGY

TRANSPLANTATION

Transplantation Antigens

ABO System—ABO incompatibility does not cause stimulation in mixed leukocyte cultures, indicating that ABO incompatibility is of much less importance than HLA compatibility in graft survival. **ABO incompatibility can result in hyperacute rejection of primary vascularized solid-organ grafts such as kidney and heart.** This occurs because of the following:

- ABO blood group antigens are present on all tissues, including kidney and cardiac grafts.
- Preformed, naturally occurring antibodies to blood group substances are present in mismatched recipients.

Key Fact

In **kidney transplants**, the degree of HLA compatibility correlates with long-term survival. If less HLA compatibility, cyclosporine helps improve survival.

In **bone marrow transplant**, an HLA-matched sibling is preferred so as to avoid rejection, and lethal graft-versus-host disease (GVHD). If using a haploidentical match, depletion of post-thymic T lymphocytes helps decrease GVHD.

There is an increase of CD3 HLA-DR cells in patients with idiopathic anaphylaxis.

Flash Card Q18

Which receptor facilitates transport of IgA across the epithelium in the gut?

Major Histocompatibility Complex (MHC)—HLA antigens are inherited in a Mendelian dominant manner, but HLA genes are almost always inherited together because of the closeness of the different loci of the MHC and the resultant low cross-over frequency.

- The fixed combination of genetic determinants is referred to as a **haplotype**.
- Since chromosome 6 is an autosome, all individuals have two expressed HLA haplotypes (maternal and paternal).
- There are only four possible combinations of haplotypes among the offspring of any two parents
- **HLA typing is very useful in organ transplants.**

HLA Antibodies—Individuals exposed to non-self-HLA antigens can develop anti-HLA antibodies. These antibodies, like ABO incompatibility, can cause hyperacute rejection of a solid organ expressing those HLA antigens.

Methods to Detect Antibody Mismatches Between Donor and Recipient

Cross-Matching—Serologic cross-matching is of particular importance to success of highly vascularized grafts **such as kidney and heart**. Serum from the prospective recipient is tested against cells from the potential donor for presence of antibodies to RBC or HLA antigens.

- **Presence of such antibodies correlates with hyperacute renal graft rejection.**
- Positive serologic crossmatch is an **absolute** contraindication to renal transplantation.

Donor-Recipient Matching—Two general methods are used to pair donors and recipients for transplantation:

- Detect HLA antigens on donor and recipient leukocytes by either serologic or DNA typing.
- Measure response of immunocompetent cells from recipient to antigens present on donor cells (and vice versa for bone marrow transplantation).

Key Fact

Heart, lung, and liver transplants are often not HLA-matched (primarily due to time considerations).

Antigen Mismatches = disparities that are serologically detected.
Allele Mismatches = differences that can be identified only by DNA-based typing.

Flash Card A18

Poly-Ig-receptor.

Mechanisms Involved in Rejection

The mechanisms involved in rejection are summarized in Table 1-29 and include following:

- **Hyperacute rejection** is often associated with deposits of neutrophils, endothelial damage, and thrombosis. It is mediated by preformed ABO natural antibodies, HLA antibodies, and complement (Figure 1-17).
- **Accelerated rejection** results in vascular disruption and hemorrhage. It is mediated by noncomplement-fixing antibodies, NK cells, and monocytes.
- **Acute rejection** is associated with tubulitis, interstitial inflammation, parenchymal cell damage, and endovasculitis. It is mediated by T lymphocytes (CD4+ and CD8+) and antibodies.
- **Chronic rejection** results in vascular onion skinning (fibrosis) on biopsy, along with delayed-type hypersensitivity (DTH) in vessel wall, intimal proliferation, and vessel occlusion. It is mediated by antibodies.

Postoperative immunosuppression, using corticosteroids, antithymocyte globulin, tacrolimus, and cyclosporine, can be used to reduce risk of rejection. (See section on immunomodulators for more details.)

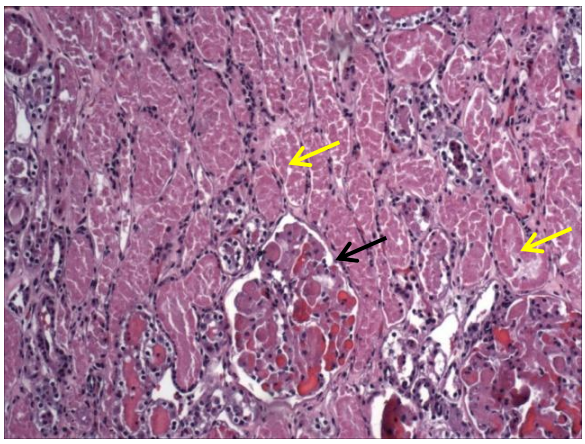


Figure 1-17. Hyperacute rejection in a transplanted kidney demonstrating acute tubular necrosis. All of the tubules are necrotic with sloughed pink epithelial cells and debris, with loss of nuclear detail (examples indicated by yellow arrows). The glomerulus is indicated by the black arrows.
(Reproduced, with permission, from USMLERx.com.

Key Fact

Chance of rejection of transplants in order from highest to lowest:
- Mismatched
- Matched unrelated
- Matched 1st-degree relative
- Syngeneic (identical twin)

Table 1-29. Key Features of Mechanisms Involved in Rejection

Types	Timing	Onset	Immune Component
Hyperacute	<24 hours	Hours	Ab and complement
Accelerated	3–5 days	1 day	Noncomplement-fixing Ab
Acute	6–90 days	Days to weeks	T lymphocytes and Ab
Chronic	>60 days	Months to years	Ab and inflammatory cytokines

Abbreviation: Ab, antibody.

Screening for Preformed Antibodies

Key Fact

High-risk groups for hyperacute and accelerated rejection are multiparous women and multiple transfusion recipients.

Screening involves the following:

- Pretransplant screen for presence of antidonor HLA antibodies to panel of potential donors
- Expressed as percentage of reactive antibodies (PRA)
- Predicts hyperacute and accelerated rejection
- High-risk groups (i.e., previously sensitized) include recipients of multiple transfusions and multiparous women

Mechanisms of Graft Rejection

Antibodies—Antibodies are involved in hyperacute rejection of a highly vascularized organ such as kidney and heart. Antidonor antibodies are seen in recipients undergoing those reactions.

- Antibodies combine with HLA antigens on endothelial cells with subsequent complement fixation and accumulation of polymorphonuclear cells.
- Endothelial damage then occurs secondary to enzymes released form polymorphonuclear (PMN) leukocytes.
- Platelets later accumulate, thrombi develop, and the result is renal cortical necrosis or myocardial infarction.

Innate Immunity—Natural killer cells and monocytes are effector cells of accelerated rejection via antigen-dependent cell-mediated cytotoxicity (ADCC).

Leukocytes and Cytokines—Allograft rejection results from coordinated activation of alloreactive T lymphocytes and APCs. Acute rejection is a T–lymphocyte-dependent process, but destruction of allograft results from a broad array of effector mechanisms.

- Primed helper T lymphocytes release multiple cytokines: IL-2, IL-4, IL-5, IL-7, IL-10, IL-15, TNFα, and IFNγ.

- Cause recruitment of primarily immunocompetent donor-specific CD4+ T lymphocytes; also CD8+ cytotoxic T lymphocytes, antibody-forming B lymphocytes, and nonspecific inflammatory cells.

CD4 T Cells—Stimulation of CD4+ T lymphocytes through antigen receptor is not sufficient to initiate T-lymphocyte activation. **Activation of CD4+ T lymphocytes needs costimulation.**

- Signaling must occur through one of the costimulators:
  - Receptor: Ligand interactions (Table 1-30)
  - IL-1 and IL-6 on APCs
  - CD4 T lymphocyte anergy
- Tolerance induction occurs when T-cell receptor interacts with APCs. As a result, T–lymphocyte-accessory proteins and ligands on APCs are target molecules for antirejection therapy.

CD8+ T Lymphocyte—CD8+ T lymphocytes recognize antigenic peptides on MHC class I molecules and represent a major cytotoxic effector lymphocyte population in graft rejection.

- CD8+ activation requires second signal + IL-2.
- CD8+ activation signaling occurs through γ chain of multiple cytokine receptors.
- Activated CD8 cells proliferate and mature into specific alloreactive clones capable of releasing granzymes, perforin, and toxic cytokines such as TNFα.

T-Lymphocyte Activation—Once T lymphocyte activation occurs:

- Autocrine T-lymphocyte proliferation continues secondary to expression of IL-2R. The IL-2 or IL-2R interactions trigger kinases, leading to Raf-1 activation and expression of DNA binding proteins, Jun, c-Fos, and c-Myc. This results in graft-specific T lymphocytes.
- T-lymphocyte cytokines also activate macrophages, leukocytes, and upregulate HLA on graft cells.
- Activated T lymphocytes stimulate B lymphocytes to make autograft antibody.
- Ultimately, all cellular and humoral factors destroy graft.

Table 1-30. T lymphocyte-Ligand Combinations for Activation of CD4+ T Lymphocytes

T Lymphocyte	Ligand on APC
CD2	CD58
CD11a/CD18	CD54
CD5	CD72
CD40L	CD40
CD28	CD80 or CD86

Abbreviations: APC: antigen-presenting cell.

Key Fact

Cyclosporine and tacrolimus interfere with activating process involving T-cell receptors and costimulatory molecule CD28:CD80 or CD86.

Immunosuppressive agents used in the management of graft rejection target some of these components discussed earlier. See Table 1-31 for specific mechanism of action for immunosuppression.

TUMOR IMMUNOLOGY

A tumor is a mixture of many individual clones that are all from a single common precursor. Each clone contains one or more genetic alterations with different mechanisms to resist the host's antitumor defense systems.

Tumors develop from a single, transformed cell to a mass of malignant cells through a progression of genetic changes, which arise over many years in the descendants of a transformed cell. The genetic changes result in the creation of a tumor that can grow without restraints.

It is likely that tumors that are discovered by the immune system are destroyed and that only the tumor cells that evade the immune system can survive. The growth of a tumor results after a series of interactions between the immune system and the tumor, in which the immune system tries to limit the expansion of the tumor and the tumor responds by modifying itself to become impervious to the immune system.

These interactions are referred to as **immune surveillance** and **immune selection**.

Table 1-31. Immunosuppression in the Management of Graft Rejection

Category	Drug	Mechanism of Action
Inhibitors of T lymphocytes and their activation	Cyclosporin (CsA) FK 506 (tacrolimus)	CsA binds to cyclophilin → **inhibition of calcineurin** → blockade of **NFAT** activation → inhibition of transcription of **IL-2** → inhibition of T-cell differentiation
	Rapamycin	Rapamycin binds to FKBP → binds to mTOR → **inhibition of mTOR** → blockade of T-cell proliferation
Metabolic toxins inhibiting proliferating T cells	Mycophenolate mofetil (MMF)	Inhibition of **guanine nucleotide synthesis** → T-cell proliferation halted
	Azathioprine	Blockade of **lymphocyte precursors** (less-specific than MMF → higher toxicity)
Antibodies to T-cell antigens	Anti-CD3	Monoclonal Ab binding to **CD3** → promote phagocytosis or complement-mediated lysis of T cells
	Anti-CD25 (**α subunit of IL2R**)	Blockade of IL-2 binding to activated T cells that express CD25 → prevention of T-cell activation
Anti-inflammatory agents	Corticosteroids	Block synthesis and secretion of cytokines from macrophage

Tumor Progression

- Early lesions are infiltrated with hematopoietic cells, including lymphocytes, macrophages, and occasionally granulocytes.
- Late stages of tumor development of colon, breast, and oral carcinomas have an abundance of tumor infiltrating lymphocytes (TILs).
  - TILs: Small-to-large lymphocytes that are mostly CD3+ CD95+ TCR-α/β + T lymphocytes.

Key Fact

The presence of the TILs with a normal ζ chain is associated with improved patient survival. These cells are typically CD95+ (Fas).

Human tumors produce molecularly defined immunoinhibitory factors:

- Tumor necrosis factor (TNF) family ligands: Induce apoptosis through the TNF family receptors. Include FasL, TRAIL, and TNF.
- Cytokines: TGFβ, IL-10, GM-CSF, and ζ-inhibiting protein (ZIP).
- Small molecules: Prostaglandin E_2, epinephrine, and reactive oxygen metabolites (ROMs). Inhibit leukocyte functions via increased cyclic adenosine monophosphate (cAMP) or superoxide generation.
- Virally related products: p15E and EBI-3.
- Tumor-associated gangliosides: Inhibit IL-2-dependent lymphocyte proliferation, induce apoptotic signals, suppress NFkB activation, and interfere with dendritic cell generation.

Tumors are not ignored by the immune system:

- Cancer patients have a higher amount of tumor-specific T lymphocytes in circulation that recognize class I or class II MHC-restricted tumor epitopes.
- Tumor-specific T lymphocytes, in the presence of cytokines, can increase and target tumor cells for destruction.
- Tumor-specific T lymphocyte can be used to recognize T–lymphocyte-defined tumor epitopes.
- TILs with normal ζ chain expression (resulting in normal signaling through the TCR) is associated with improved patient survival.
- Antitumor antibodies in patients allow for identification of serologically defined tumor antigens.
- NK cells are important in preventing metastasis of tumors by eliminating tumor cells in the circulation through perforin-mediated lysis and apoptosis of tumor cell targets.
- Inhibitor receptors on NK cells known as **killer cell immunoglobulin-like receptors (KIR)** recognize MHC class I and assist NK cells in sampling healthy cells to look for abnormal cells that have lost the self-determinants on their surface.
- This evidence is leading researchers to consider new ways to protect immune cells from the tumor and the factors they release.

Flash Card Q19

Cyclosporin inhibits calcineurin and blocks NFAT activation by binding to which molecule?

INNATE IMMUNITY AND TOLL-LIKE RECEPTORS

INNATE IMMUNITY

Innate immunity is initiated in response to microbial invasion. Recognition of conserved microbe sequences stimulates immediate host defense mechanisms (Table 1-32). It differs from adaptive immunity in several ways, which are summarized in Table 1-33.

Table 1-32. Innate Immune Mechanisms

Microbe		Immune Mechanisms
Bacteria	Extracellular	Complement activation Phagocytosis: Recognition by PRR
	Intracellular	**N**ucleotide binding **o**ligomerization **d**omain-like receptors (NLRs)
Fungi		Neutrophil phagocytosis Complement activation
Viruses		Phagocytosis: Toll-like receptors (TLRs) and NK cells
Parasites		Phagocytosis; Complement (alternative pathway)

Abbreviations: PRR, pattern recognition receptor.

Table 1-33. Innate Versus Adaptive Immune Mechanisms

	Innate Immunity	Adaptive Immunity
Genetics	Response encoded in host germ line DNA	Somatic gene rearrangement
Recognition	Recognize conserved sequences such as pathogen-associated molecular patterns (PAMPs)	Recognize unique antigenic determinants: TCR and (MHC restricted-) Ig receptors
Components	Complement Pattern recognition receptors (PRRs) on immune cells Phagocytes Mast cell or basophils NK cells	T lymphocytes, B lymphocytes, and complement

Flash Card A19

Cyclophilin

Components

Antimicrobial Peptides (AMP)—AMP are ubiquitous cationic proteins that play a role in innate immunity. They provide defense against bacteria and fungi viruses.

> ***Key Fact***
>
> AMPs are decreased in a T_h2 environment. Atopic dermatitis patients are susceptible to *Staphylococcus aureus* infection due to a decrease in AMP.

Features:

- Produced by keratinocytes
- Two families in humans:
  - Human β defensins: HBD1, 2, 3
  - Human cathelicidins: LL37
  - Lactoferrins: hLF1-11
  - Histatins
- Interact with phospholipids of microbial membranes, enter cell and mediate antiproliferative effects

Complement

Refer to Chapter 4 for a discussion on Complement.

Cells of the Innate Immune System

Cells involved in innate immunity are reviewed in Table 1-34.

Table 1-34. Cells of the Innate Immune System

Cell	Function
Neutrophil	Phagocytosis and oxidative burst or free radical production
Monocyte or macrophage	Phagocytosis and secretion of proinflammatory cytokines
Dendritic cells	APCs (conventional) and antiviral (plasmacytoid)
Eosinophil	Antihelminthic, antibacterial, and secretion of proinflammatory mediators or free radicals
Mast cell	Antibacterial, antiviral, and secretion of proinflammatory cytokines
Basophil	Antibacterial, antiviral, and secretion of proinflammatory cytokines
NK Cell	Eliminates virus-infected cells and tumor cells (missing “self” hypothesis), and secretion of cytokines
Intraepithelial lymphocytes	Secretion of proinflammatory cytokines (phagocyte activation and killing of infected cells)
B-1 B lymphocytes	Produce natural serum antibodies

Abbreviation: APCs, antigen-presenting cells.

Innate Response: Recognition

Pathogen-Associated Molecular Patterns (PAMPs)

- PAMPs are conserved microbial sequences found on microorganisms.
- Their structures are consistent and conserved.
- Examples include lipopolysaccharide (gram-negative bacteria) and teichoic acid (gram-positive bacteria).
- They are recognized by receptors of the innate immune system.

Pattern Recognition Receptors (PRRs)

- PRRs on APCs (conventional dendritic cells, B lymphocytes, and macrophages).
- PRRs identify PAMPs on microorganisms to detect infection.

A summary of PRRs is provided in Table 1-35.

Table 1-35. Pattern Recognition Receptors

Receptors		Location	Class	Structure	Function
fMLP	*N*-formyl Met-Leu Phe receptors	Cell surface	Signaling	G protein-coupled 7-transmembrane domain receptor	Antibacterial
CARD	Caspase activation and recruitment domains (e.g., retinoic acid-inducible gene-I-like receptors)	Cytoplasm	Signaling	RNA helicase, and caspase recruitment domains Component of inflammasome	Cytoplasmic virus detection and to induce type 1 IFN production
CLR	C-type lectin receptors: Mannose-binding lectin and macrophage mannose receptor	Cell surface	Secreted or endocytic	Calcium-dependent carbohydrate-binding domains	Antifungal immunity
NLR or NACT-LRR	Nucleotide-binding **o**ligomerization **d**omains (NODs) NACT-LRR and pyrin domain-containing proteins (NALPS)	Cytoplasm	Signaling	C-terminal leucine-rich repeat and nucleotide-binding domain Central proteins of inflammasome	IL-1β and IL-18 secretion
TLR	Toll-like receptors	Cell-surface cytoplasm (TLR3)	Signaling		Antibacterial, antifungal, and antiviral immunity

Inflammasome

The inflammasome is multiprotein complex located in the cytoplasm that activates caspases 1 and 5, leading to production and secretion of IL-1 and IL-18. NALPs provide a platform to which the adaptor protein ASC and caspases bind.

> ***Key Fact***
>
> Activating mutations in cold-induced auto-inflammatory syndrome 1(CIAS1) is seen in Muckle-Wells syndrome (MWS), familial cold urticaria, and chronic infantile neurologic, cutaneous, and articular autoinflammatory disease (CINCA).

TOLL-LIKE RECEPTORS (TLRs)

TLRs are a family of receptors that recognize PAMPs and initiate signaling pathways, which activate innate immunity. The known TLRs and their ligands are summarized in Table 1-36.

TLR Ligands

Subcellular Localization and Signaling Pathways of TLRs—TLRs are found on the cell surface or intracellularly.

- TLR 3, 7, 8, and 9 are found in the **intracellular compartment** and detect nucleic acids.
- TLRs initiate multiple signaling pathways by use of adapter proteins:
  - The adapter protein MyD88 plays a role in TLR signaling.

Table 1-36. Toll-Like Receptors and Their Ligands

Toll-Like Receptor	Ligand	Source
1	Lipoarabinomannan	Mycobacteria
2	Zymosan	Fungi
3	dsRNA	Virus
4	Lipopolysaccharide, peptidoglycan, RSV fusion protein, and HSP 70 and HSP 90	Gram-negative bacteria, gram-positive bacteria, RSV, and endogenous acute phase proteins
5	Flagellin	Flagellated bacteria
6	Diacyl lipopeptides	Mycoplasma
7	Imidazolquinolones	Synthetic
8	ssRNA	Virus
9	Unmethylated CpG motifs	Bacteria and DNA viruses
10	Unknown	
11	Profilin	*Toxoplasma gondii*

Abbreviations: CpG, cytosine-phosphate-guanosine; dsRNA, double-stranded RNA; HSP, heat shock protein; RSV, respiratory syncytial virus; ssRNA, single-stranded RNA.

- MyD88-dependent pathways are similar to signaling through IL-1 receptors, and are involved in all TLR-signaling pathways **except for TLR 3**.
- TLR3 signaling is mediated by the Toll-interleukin-1-receptor domain containing adapter-inducing interferon β (TRIF) and is MyD88-independent.
- TLR4 can signal through both MyD88-dependent and -independent pathways (Figure 1-18).TLR 4 binds to lipopolysaccharide (LPS) via lipopolysaccharide-binding protein (LBP), MD2 (lymphocyte antigen 96), and CD14.

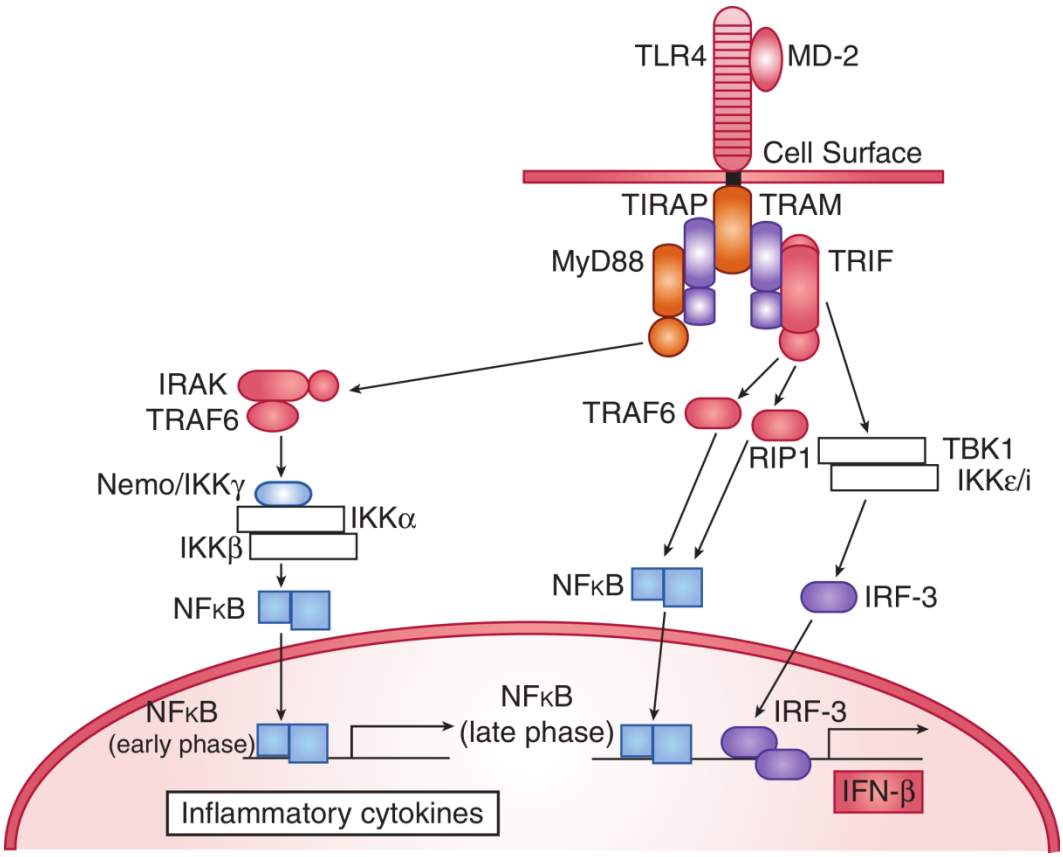


Figure 1-18. TLR 4 signaling occurs through MyD88-dependent and -independent pathways.

TLR and Disease

TLR mutation has been associated with infections and other diseases. Examples of such diseases are reviewed in Table 1-37.

TLR agonists, such as imiquimod (TLR 7) and resiquimod (TLR 7 and 8), are used topically for its antiviral and antitumor effects.

TLR function can be measured by flow cytometry, as seen in Figure 1-19.

Once recognition by the innate immune system occurs, phagocytosis, rapid production of proinflammatory mediators, and engagement of the adaptive immune system leads to eradication of the foreign organism.

Innate immunity confers a constitutive first line of defense to a myriad of pathogens. Mutations in innate immunity have implications beyond immune deficiency.

Table 1-37. Pattern Recognition Receptors and Clinical Disease

Disease	TLR, PRR or Signal Pathway Affected
HSV1 encephalitis	TLR 3, 7, 8, 9
Aspergillosis	TLR4
Adrenal insufficiency	TLR 2, 4
Crohn's disease or Blau's syndrome	NOD2
Leprosy and TB	TLR2
IRAK4 or MyD88 deficiency (recurrent infections with pyogenic bacteria)	IRAK4 or MyD88 (all TLR)
Primary immune deficiency with infectious and mycobacterial susceptibilities	NEMO

Abbreviations: HSV, herpes simplex virus; NEMO, NFκB essential modifier; NOD, nucleotide oligomerization domain; TLR, Toll-like receptor.

Flash Card Q20

Which TLR binds lipopolysaccharide (LPS) on gram-negative bacteria?

Flash Card Q21

Which TLRs are present in the intracellular compartment and implicated in HSV1 encephalitis?

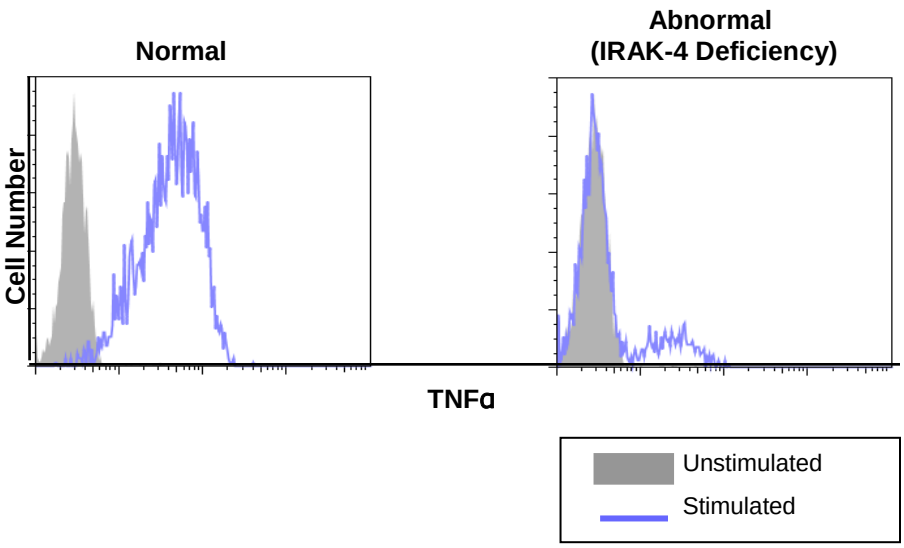


Figure 1-19. Measuring TLR function. Histogram plot shows induction of cytokine TNFα in lipopolysaccharide (LPS)-stimulated monocytes in normal healthy controls. Reduced expression of TNFα is indicative of defective TLR4-signaling pathway.
(Reproduced, with permission, from Dennis W. Schauer, Jr., Trivikram Dasu, PhD, James W. Verbsky, MD, PhD, Clinical Immunodiagnostic & Research Lab, Medical College of Wisconsin.)

Flash Card A20

TLR 4.

Flash Card A21

TLR 3, 7, 8, 9.

2 Cells Involved in Immune Responses

LYMPHOCYTES

Lymphocytes function to ward off infection. They are the primary players in adaptive immunity.

TYPES OF LYMPHOCYTES

The three *major* types of lymphocytes are B cells, T cells, and natural killer (NK) cells.

B lymphocytes

Humoral immunity; produce immunoglobulins; derived from and mature in bone marrow; recognize linear or conformational epitopes; major ligands/markers/associations: Fc receptors, clusters of differentiation (CD19, CD20, CD21), and major histocompatibility complex II (MHCII).

T lymphocytes

Cellular immunity; precursors arise in bone marrow, but then mature in thymus; recognize linear epitopes.

- **Helper T lymphocytes (T_h, CD4+)(Table 2-1):** Stimulate B-cell growth; secrete cytokines to activate macrophages; MHCII-restricted; markers CD3+/CD4+/CD8–.
  - **T_h1 cells, T_h2 cells, T_h17 cells:** See Table 2-1.
  - **T regulatory (Treg) cells:** Do not make IL-2; use cytotoxic T-lymphocyte antigen 4 (CTLA4) as a means to suppress other T cells (Tables 2-1 and 2-2).
  - **T_h9 cells:** Induced by transforming growth factor beta (TGFβ) and interleukin 4 (IL-4); produce IL-9 +/– IL-4; IRF4 and PU.1 required for their differentiation.

Table 2-1. Major CD4+ Families

T Helper Family	Induced By	Produce	Transcription Factors	Major Function	Other
T_h1	**IL-12**; IL-27, 18	**IFNγ;** IL-2, TNF	**Tbet, STAT4,** STAT1	Intracellular defense	Express CXCR3 and CCR5; T_h1 cells are associated with type I DM and MS
T_h2	**IL-4;** IL25, 33; TSLP	**IL-4,5,13;** IL-6, 10, 21,25,31,33	**GATA3, STAT6,** STAT5	Humoral immunity; antiparasitic; allergy	
T_h17	**IL-6;** IL-1, 21, 23; TGFβ	**IL-17;** IL1,6,21, 22; TNFα; GM-CSF	**RORγT, STAT3**	Extracellular defense; neutrophil recruitment; autoimmunity	Express CCR6; involved in RA, IBD, MS, and psoriasis
Treg	**TGFβ** and retinoic acid	**IL-10, TGFβ**	**FOXP3, STAT5**	Immunosuppression; prevents autoimmunity	See Table 2-2

Abbreviations: DM, diabetes mellitus; GM-CSF, granulocyte–monocyte colony-stimulating factor; IBD, inflammatory bowel disease; IFN, interferon; IL, interleukin; MS, multiple sclerosis; RA, rheumatoid arthritis; TGF, transforming growth factor; T_h, helper T cell; TNF, tumor necrosis factor.

Key Fact

Know what induces Th1, Th2, Th17, and Treg cell development. Also, know what these cells produce and the transcription factors used.

  - **T_{fh} (follicular helper T cells)**: In the follicles of active germinal centers and play a major role in helping B cells make antibodies; mediated by transcription factor Bcl-6 (which causes downregulation of CCR7 and upregulation of CXCR5); produce IL-21.
- **Cytolytic T lymphocytes (CTLs** and **CD8):** Kill viral-infected cells and tumor cells via perforin and granzyme; express IFNγ, TNF, and lymphotoxin; harder to activate than CD4s; involved in allograft rejection; use eomesodermin homologs (EOMES) as a transcription factor; MHCI-restricted; markers CD3+/CD8+/CD4–.
- **γδ T cells:** These cells use γ and δ chains (instead of α and β chains); they develop in the thymus and have limited diversity; they can bind lipids and heat shock proteins; they are not associated with CD4 or CD8, but do require CD3 and the ζ chain for signal transduction; they do not recognize MHC-associated peptides and are not MHC-restricted; produce IFNγ and TNF.

Table 2-2. Regulatory T-Cell Subsets

Natural Treg's	Induced Treg's
Thymically derived and develop in response to thymically presented autologous antigens Constitutively express IL-2-Rα(CD25+) FOXP3+ Mediate self-tolerance	Peripherally derived and develop in response to peripherally expressed self-antigens and external antigens Inducible IL-2–Rα(CD25) expression Include: Tr1(produce IL-10; involved in mechanism of immunotherapy), T_h3 (produce TGFβ; in gut; important in IgA production)

Abbreviations: Ig, immunoglobulin; IL, interleukin; TGF, transforming growth factor; T_h, helper T cell.

Natural Killer (NK) cells

These cells function mostly in innate immunity. They kill viral-infected cells and tumor cells (due to these cells losing MHCI and gaining danger receptors). IL-15 is very important for NK cell development. Markers include: CD16+ (FCγRIII), CD56+ (NCAM), natural cytotoxicity receptors (NCRs), and killer inhibitory receptors (KIRs); do not express CD3. NK cell killing is mediated via perforin, granzymes, serglycine, and Fas. See Table 2-3 for summary of NK cell receptors.

Natural Killer T (NKT) Cells

These are not NK cells; limited T-cell receptor (TCR) repertoire (Vα24/Jα18 and Vβ11); CD3+, CD16+, CD56+, and usually CD4+; recognize glycolipid antigens in context of CD1; produce IFNγ and IL-13 rapidly.

Innate Lymphoid Cells

There are several types of innate lymphoid cells.

Innate lymphoid cells (ILC2; nuocytes) arise from a common lymphoid progenitor, but lack rearranged antigen receptors; secrete IL-5, IL-9, and IL-13; proliferate in response to IL-25 and IL-33; play a central role in type-2-mediated immunity.

Table 2-3. Activating and Inhibitory Receptors for NK Cells

Activating (activate to kill)	Inhibitory (inhibit killing)
EBV-infected cell expresses CD48 → binds 2B4 receptor on NK cell	MHCI → binds KIR (long tail containing ITIM)
Stressed cell expresses MICA/B, ULBP → binds NKG2D on NK cell	
Viral hemagglutinin on target cell → binds NKp44/46 on NK cell	
HLA-E → binds NKG2C on NK cell	HLA-E → binds NKG2A/B
IgG-bound target cell → binds FcγRIIIA on NK cell (ADCC)	

Abbreviations: EBV, Epstein-Barr virus; HLA-E, human leukocyte antigen E; Ig, immunoglobulin; IL, interleukin; ITIM, immunoreceptor tyrosine-based inhibitory motif; KIR, killer cell immunoglobulin-like receptor; MHC, major histocompatibility complex; NK, natural killer .

Flash Card Q1

Which cytokines induce development of Th17 cells?

LYMPHOCYTE DEVELOPMENT, MATURATION AND APOPTOSIS

Anatomic segregation of B and T cells:

- T-cell zone (parafollicular zone) → T cell expresses CCR7, which binds CCL19/21 in the T cell/parafollicular zone (as the concentration/gradient of CCL19/21 is highest there).
- B-cell zone (follicles) → B cell expresses CXCR5, which binds CXCL13 in the B-cell zone/follicles (as the concentration/gradient of CXCL13 is highest there).

This segregation ensures that each subset of lymphocytes is talking to the right **antigen-presenting cell (APC)** in the lymph node.

Maturation of B and T lymphocytes is characterized early on by high levels of IL-7 (lack of this cytokine can lead to X-Linked severe combined immunodeficiency disease [SCID]); then, somatic recombination of antigen receptor genes and, finally, positive (active) selection or negative (passive) selection of mature lymphocyte repertoires.

- **Positive selection:** Interaction between lymphocyte and MHC molecules, which ensures maturation; for T cells; this ensures that an individual's T cells can respond to peptides bound to her/her own MHC molecules.
- **Negative selection:** Interaction between lymphocytes and MHC molecules, which serves to eliminate self-reactive lymphocytes (and prevent autoimmunity); important for T and B cells; results in anergy, or elimination [by apoptosis or receptor editing (for B cells)].

VDJ recombinase is the collective term for the set of enzymes needed to recombine V, D, and J gene segments; the combining of these gene segments results in **combinatorial diversity;** it recognizes DNA sequences called **recombination signal sequences (RSS)**; recombination occurs between two gene segments (immunoglobulin or TCR) only if one of the segments is flanked by one 12-nucleotide spacer and by one 23-nucleotide spacer → **12/23 rule**. Important enzymes in lymphocyte (immunoglobulin or TCR) gene rearrangement appear in the following list and are summarized in Figure 2-1.

- RAG 1/RAG 2: Expressed in immature lymphocytes (i.e., when antigen receptors are being assembled); cleaves double-stranded DNA between the coding segment and its recombination signal sequence.
- Ku: Binds DNA ends (hairpin in the case of the coding segment).
- DNA-PK (DNA-dependent protein kinase complex) and Artemis: Open DNA hairpin at a random site.
- Terminal deoxynucleotidyl transferase (TdT): Adds nucleotides for **junctional diversity** (creates the greatest variability for diversity).
- Endonuclease: Removes nucleotides for junctional diversity.
- DNA ligase IV:XRCC4: Ligates DNA.

Flash Card A1

IL-6, TGFβ, IL-1, IL-21, and IL-23

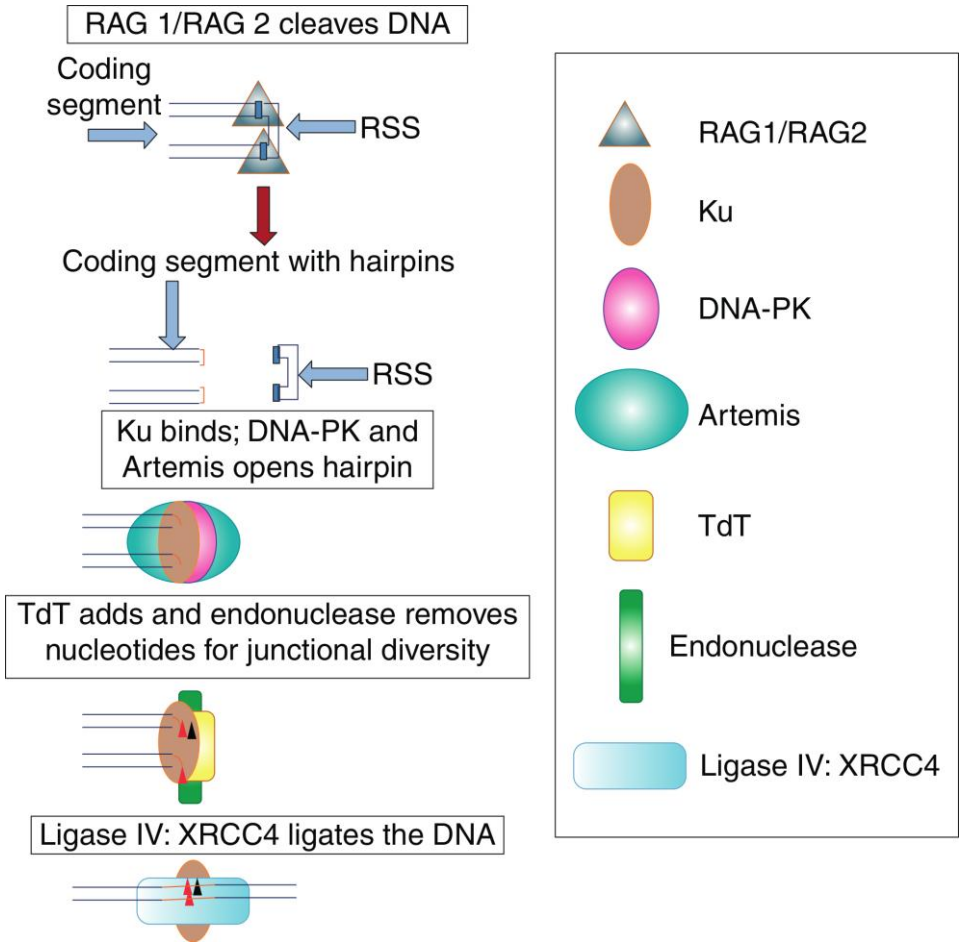


Figure 2-1. V(D)J rearrangement.
(Reproduced, with permission, from Cindy Salm Bauer, MD, Medical College of Wisconsin.)

B-Cell Development

B-cell maturation is presented in Table 2-4 and Figure 2-2.

Table 2-4. B-Cell Maturation

Pro B Cell	Pre B Cell	Immature Naïve B Cell	Mature Naïve B Cell	Memory B Cell or Plasma Cell
CD 19+, CD20+; Heavy-chain D-J then V-DJ rearrangement	Cytoplasmic μ chain produced here; signaling through pre-B–cell receptor and then light-chain V-J rearrangement	Light-chain product (κ or λ) binds μ heavy chain to make IgM; receptor editing occurs	Express δ heavy chain (as well as μ) via alternative splicing so that IgM and IgD are expressed on the cell surface	Memory cell: Isotype switch and somatic hypermutation occurs Plasma cell: Alternative splicing yields membrane and secreted Ig

Flash Card Q2

Which enzyme is responsible for adding nucleotides during junctional diversity?

Flash Card Q3

At which stage does signaling through the pre-B–cell receptor occur?

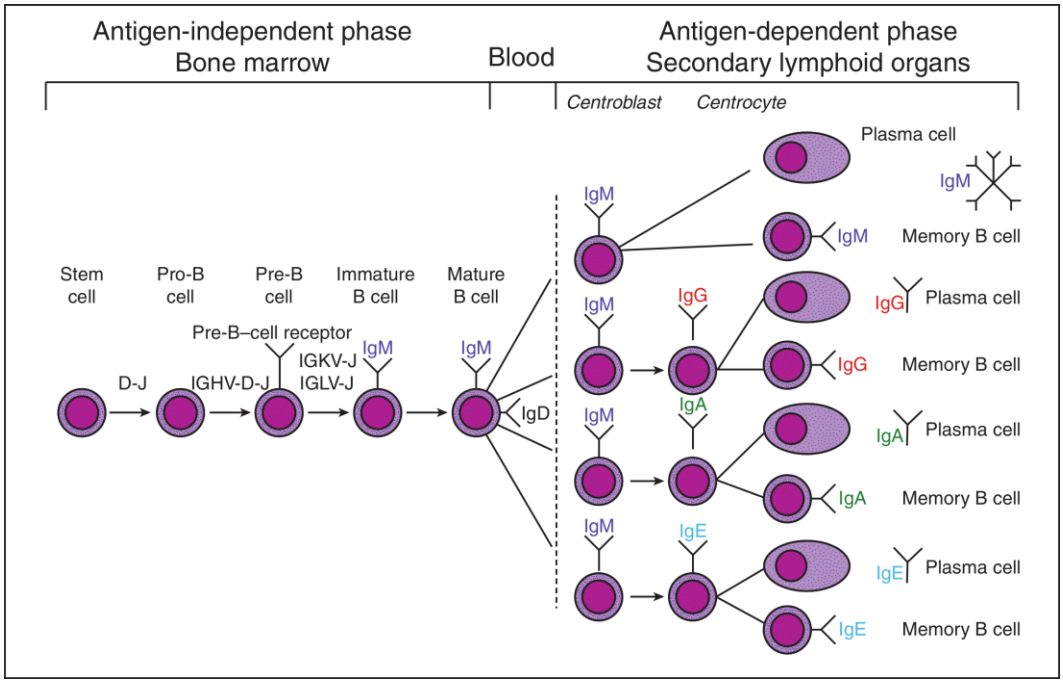


Figure 2-2. B-cell maturation.
(Reproduced, with permission, from Marie-Paule Lefranc, IMGT, the International ImMunoGeneTics Information System/Wikimedia Commons.)

- **Alternative splicing** yields membrane versus secretory Ig.
- **Differential splicing** produces different immunoglobulin isotypes. IgD is the only Ig without a switch region; so, class switching will never apply to it.
    - AID, UNG, CD40, CD40L → if lacking, cannot class switch → Ig gets 'stuck' at IgM → Hyper IgM phenotype ensues.

Transcription Factors associated with commitment to the B cell include: PU.1, IKAROS, E2A, EBF, PAX5, and IRF8.

B-cell survival in the periphery depends on survival signals such as BLYSS, BAFF, and APRIL. The receptors for these on the B cells are BR3 and TACI (early on) and BCMA (later on). No TACI → CVID risk factor.

***Mature B Cells*—**There are three types of mature B cells:

- **B1 cells**: Innate; limited diversity; analogous to γδ cells; make up 5–10% of total B lymphocytes; self-renewing; produce natural antibodies (low-affinity polyreactive immunoglobulins) that respond to microbes and lipids; found in peritoneal cavity and fetus; **T-cell-independent.**
- **Marginal zone B cells:** Developed by 2 years of age (which explains why infants have poor polysaccharide responses); first responders (especially to polysaccharide antigens); **T–cell-independent.**
- **B2 cells (conventional B cells or follicular B cells):** includes memory B cells and plasma cells; adaptive; respond to protein antigens; **T–cell-dependent.**

Flash Card A2
TdT

Flash Card A3
Pre B cell

T-Cell Development

T-cell maturation is presented in Table 2-5.

When T cells are immature (DN; CD4–/CD8–), they are located in the subcapsular cortex. As they undergo **positive selection**, they move to the cortex. As they undergo **negative selection**, they move to the medulla.

T cells leave lymphoid organs by expressing sphingosine-1-phosphate (S1P) receptors. After activation, expansion, and differentiation, memory or effector T cells form:

- **Central memory T cells**: CD45RA–, CD27+, CCR7+, CD62L+ (L-selectin).
- **Effector memory T cells**: CD45RA–, CD27–, CCR7–, CD62L– (L-selectin).

Expression of a molecule called **Notch** commits T cells to develop.

Lymphocyte Apoptosis

There are two types of lymphocyte apoptosis:

- **Passive/Intrinsic Pathway:** Programmed death by neglect; mitochondrial pathways; mediated by **caspase 9**; bcl-2 and bcl-XL (antiapoptotic); bid/bim (proapoptotic).
- **Active/Extrinsic Pathway:** Repeated lymphocyte activation causes increased Fas (CD95)/FasL(CD178) expression, which causes increased Fas-associated protein with Death Domain (FADD); mediated by **caspase 8.**

Both pathways converge on caspase 3.

Key Fact

Mutations in Fas(CD95) are the most common defect seen in autoimmune lymphoproliferative syndrome (ALPS), which results in defective lymphocyte apoptosis.

Table 2-5. T-Cell Maturation

Double Negative (DN)	Double Positive (DP)	Single-Positive Thymocyte
Does not express T-cell receptor; stages: DN1, DN2 (β chain D-J then V-DJ rearranges; analogous to pro B cell), DN3 (signaling through pre-T–cell receptor; analogous to pre B cell), and DN4 (proliferation)	CD4+/CD8+; positive and negative selection occurs; α chain V-J rearranges	Cells undergo selection to become CD4+ or CD8+

Flash Card Q4

Where does negative selection occur for T cells?

MONOCYTES, MACROPHAGES, AND DENDRITIC CELLS

Monocytes are white blood cells that replenish macrophages and dendritic cells in the periphery. In tissue, macrophages and dendritic cells engulf invaders and cellular debris; they also initiate the adaptive immune system as APCs.

Monocyte Morphology

- Monocytes are cells with one, kidney-shaped nucleus and are 10–15 μm in size.
- Express surface receptors for IgG, IgA, and IgE.

Monocyte Growth and Differentiation

- Originate from monoblasts (Figure 2-3).
- Highly express **CD14** and **CD16** after further maturation.
- Migrate to peripheral tissue and become macrophages and dendritic cells.

Monocyte Function

- Can act as phagocytes and present antigen.

Macrophage Morphology

- Mononuclear cells with a diameter of 21 μm (Figure 2-4).
- Move in tissue via amoeboid movement and capture particles with pseudopodia.
- Express CD14, CD11b/CD18 (Mac-1), and CD36.
- Have several subtypes named by location, including Kupffer cells (liver), histiocytes (tissue), osteoclasts (bone), mesangial cells (kidney), and alveolar macrophages (lung).

Macrophage Growth and Differentiation

- Develop in response to granulocyte–monocyte colony-stimulating factor (GM-CSF) and macrophage colony-stimulating factor (M-CSF).
- Can survive weeks in tissue.

Flash Card A4

The medulla, which is also where the AIRE protein functions

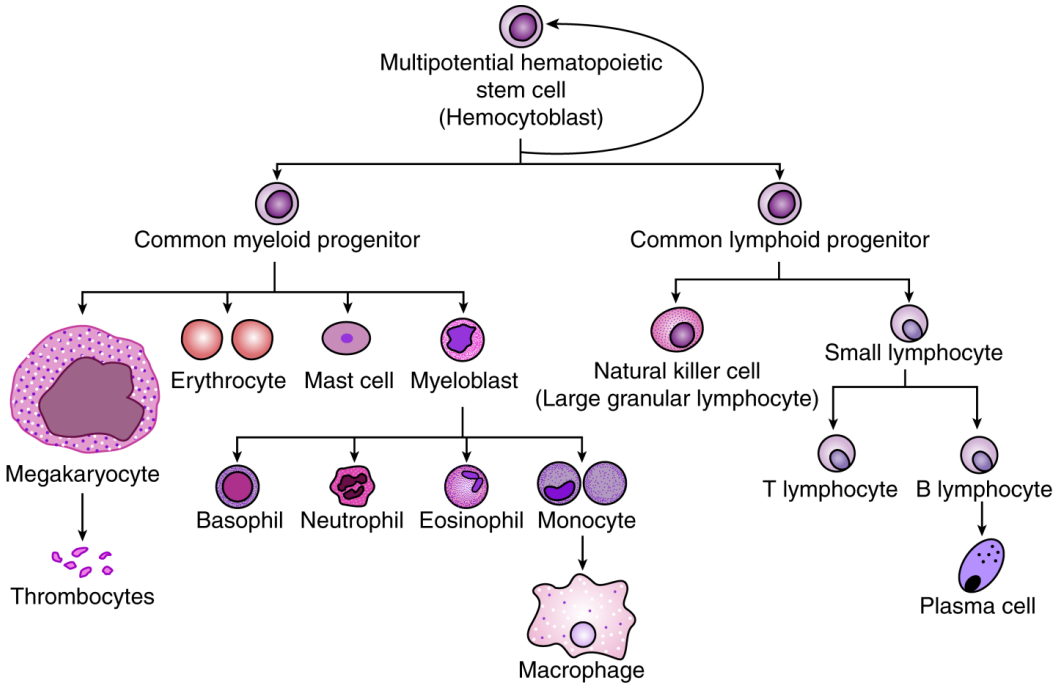


Figure 2-3. Blood cell lineage.
(Adapted, with permission, from Wikimedia Commons.)

Macrophage Function

- Clear cellular debris as well as bacterial invaders.
- Can be activated by signals, such as **IFNγ** and **LPS** (which binds CD14).
- Present pathogens to helper T cells via MHCII molecules.
- Secrete cytokines, such as **TNFα,** IL-12, IL-18, IL-1, IL-6 (CXCL-8).

Key Fact

CD14 is a highly expressed receptor on monocytes and macrophages, which binds lipopolysaccharide and activates an immune response. It is a component of Toll-like receptor 4 (TLR4).

Diseases Associated with Macrophages

Many macrophages (see Figure 2-4) fuse to form **granulomas** in order to wall off a pathogen when they are unable to clear it. Granulomas are characteristic of many infectious and noninfectious inflammatory reactions, including sarcoidosis (noncaseating granulomas) and tuberculosis (caseating granulomas). TNFα, which is secreted by macrophages, promotes granuloma formation.

Uncontrolled activation of macrophages with a marked increase in circulating cytokines is called **hemophagocytic lymphohistiocytosis (HLH)** or **macrophage activation syndrome,** when secondary to chronic rheumatoid disease. This potentially life-threatening syndrome is characterized by high fever, hepatosplenomegaly, high ferritin and triglyceride levels, and low fibrinogen and

Key Fact

HLH is often a fatal consequence of Epstein-Barr virus (EBV) infection in boys with X-linked lymphoproliferative (XLP) syndrome, which is due to mutations in the *SH2D1A* gene (encodes SAP protein).

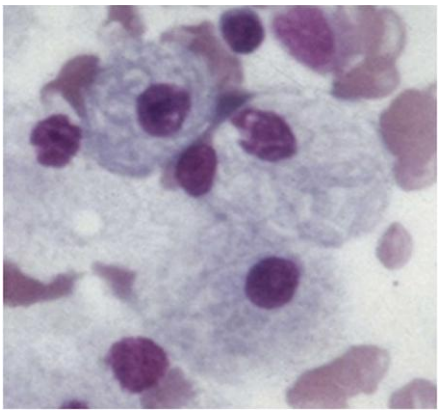


Figure 2-4. Gaucher cell. Macrophage with "crumpled tissue" appearance.
(Reproduced, with permission, from the National Institutes of Health.)

circulating NK levels. Hemophagocytosis can often be seen on bone marrow biopsy. The diagnosis is made when five of the eight following criteria are met: fever, splenomegaly, cytopenia involving two or more cell lines, hypertriglyceridemia or hypofibrinogenemia, hemophagocytosis, hepatitis, low or absent NK cell activity, ferritin level >500 µg/L, and soluble CD25 (sIL-2 receptor) >2400 U/mL.

Dendritic Cell Morphology

- Mononuclear cells with multiple projections, called **dendrites** (Figure 2-5).
- Classified into subsets (Table 2-6).

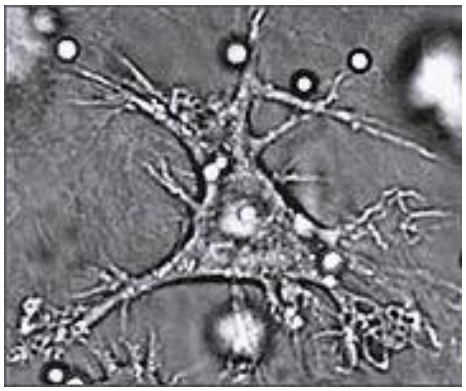


Figure 2-5. Dendritic cell.
(Reproduced, with permission, from Wikimedia Commons.)

Table 2-6. Dendritic Cell (DC) Subsets

Subset	Markers	Precursor	Key Points
Myeloid (DC1)(Conventional DCs)	CD1, CD11b/c, CD13, CD14	Myeloid	Primarily act in phagocytosis and **antigen presentation**
Plasmacytoid (DC2)		Lymphoid	Secrete large amounts of IFNα and associated with **viral infections**
Langerhans	CD11c, CD207 **Birbeck granules**	Myeloid (CLA+)	Prime CD8 T cells
Interstitial	CD2, CD9, CD68	Myeloid (CLA–)	Activate B cells

Dendritic Cell Growth and Differentiation

- Develop in response to GM-CSF and IL-4 from the common myeloid or lymphoid progenitor.

Dendritic Cell Function

- Are the **major antigen presenting cells (APCs)** and are critical in starting germinal center reaction.
  - Are immature in the tissue (where they specialize in antigen uptake) and mature in the lymphoid organs (where they specialize in antigen presentation; increased MHC II expression).
- Express B7.1 and B7.2 (CD80/CD86) which interacts with CD28 on T cells to provide costimulation.

Key Fact

CD1 isoforms (CD1a–CD1e) are class I MHC like molecules that present nonpeptide (lipid and glycolipid) antigens to T cells. CD1 maps outside the MHC locus on chromosome 15.

Flash Card Q5

Which subset of dendritic cells produces type I IFN during viral infections?

MAST CELLS

Mast cells (MC) are ubiquitous granulocytes found in skin and mucosal membranes; known primarily for their role in immediate hypersensitivity, but now better known as a regulator of innate immunity.

Mast Cell Morphology

- Metachromatically staining cell; mast means "feeding" or "fattening."
- Contain large cytoplasmic granules, which are filled with preformed mediators (e.g., histamine) that can be released quickly.
- Have peripheral, unsegmented nuclei and are 9–14 μm in size (Figure 2-6).

Mast Cell Growth and Differentiation

- Derived from pluripotent, kit+ (CD117+), CD34+ stem cells in the bone marrow.
- Develop phenotype after arrival in the tissue (Table 2-7)—mature in the **tissue** (unlike basophils and eosinophils).

Key Fact

Kit is the receptor for the cytokine stem cell factor, the critical growth factor of mast cells and a target of imatinib. The gene that encodes the receptor kit, c-*kit,* is a proto-oncogene.

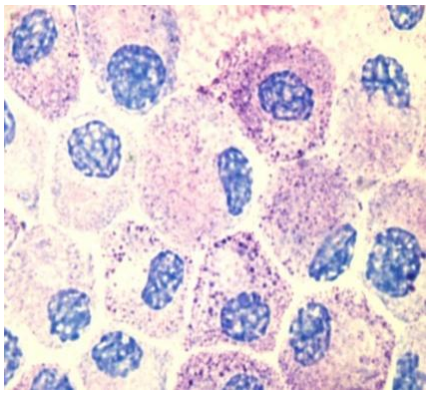


Figure 2-6. Mast cell.
(Reproduced, with permission, from Wikimedia Commons.)

Flash Card A5

Plasmacytoid dendritic cells

Table 2-7. Mast Cells Phenotypes

Phenotype	Mediators	Location	Other
MC_T	Tryptase	Respiratory tract and intestinal mucosa	Do not express C5aR (CD88); scroll-like appearance; require T cells for their development; do not respond to opiates, C5a, C3a, and vancomycin
MC_{TC}	Tryptase, chymase, carboxypeptidase, cathepsin G	Skin, blood vessels, **eyes**, synovium, intestinal, and respiratory submucosa	Express C5aR (CD88); whorled appearance; do not require T cells for their development; respond to opiates, C5a, C3a, and vancomycin
MC_C	Chymase	Lymph nodes, intestinal submucosa, and salivary glands	

Mnemonic

For MC_T, think **T**issue. For MC_{TC}, think **C**onnective **T**issue.

IgE Receptor/FcεR

- High-Affinity IgE Receptor/FcεRI:
  - Tetrameric form: Composed of **one α chain, one β chain, and two γ chains**; it is found on mast cells and basophils (Figure 2-7).
    - The α chain contains two immunoglobulin domains that bind with high affinity to IgE.
    - The β chain contains four transmembrane domains and one immunoreceptor tyrosine-based activation motif (ITAM).
    - The γ chains are covalently linked, and each contains one ITAM. This is the main signaling component.
  - Trimeric form: Composed of one α chain and two γ chains; it is found on dendritic cells, monocytes, and eosinophils.
- Low-Affinity IgE Receptor/ FcεRII/CD23: C-type lectin; it is found on mature B cells, activated macrophages, eosinophils, dendritic cells, and platelets; the allergen responsible in dust mite allergy (**Der-p-1) is known to cleave CD23** from the cell surface, increasing the production of allergen-specific IgE.

Key Fact

Some CIU patients have IgG directed against the α chain of FcεRI, though this is also seen in patients without urticaria. There is no evidence that these antibodies are pathogenic in urticaria.

Key Fact

IgE binds to FcεRI via the α chain. Signaling of mast cells is similar to that in B cells with Lyn, Fyn, and Syk.

Mast Cell Activation

- Allergens activate MC via cross-linking IgE receptor (Figure 2-7).

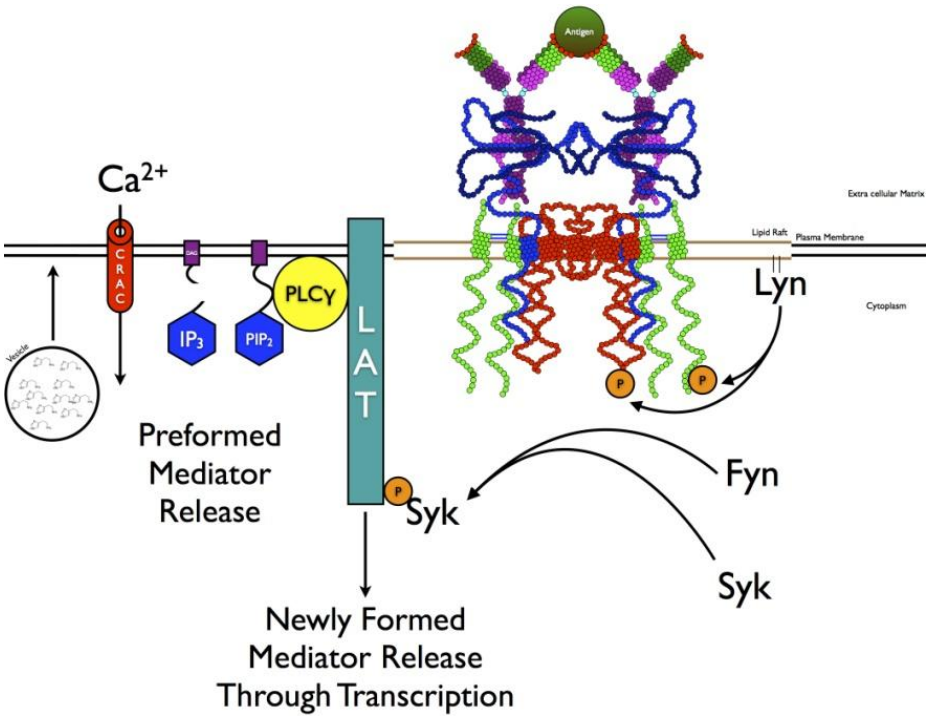


Figure 2-7. Mast cell activation and signaling.
(Reproduced, with permission, from Wikimedia Commons.)

- Mast cells can also be activated by: IgG via FcγRI; bacterial antigens via TLR 2/6 or 4; C3a or C5a (anaphylatoxins)(MC_{TC} only); cytokines; neuropeptides; physical stimuli (e.g., heat, pressure); substance P; and drugs [radiocontrast media; opioids (MC_{TC} only); muscle relaxants].

Mast Cell Mediators

- Release **preformed** mediators (<15 minutes) (e.g., histamine, tryptase, chymase, TNF, and heparin).
- Produce and release **lipid-derived mediators** (~10–30 minutes) after activation via the arachidonic acid pathway; responsible for late allergic reactions (e.g., PGD_2, LTC_4, LTB_4, PAF).
- Produce and release **cytokines** and chemokines (hours to days) after activation (eg, IL-3, IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, GM-CSF, TNFα, MCP-1, MIP-1α, and RANTES [CCL-5]).

Table 2-8 lists selected mast cell mediators.

Table 2-8. Select Mast Cell Mediators

Mediator	Function	Preformed	Lipid-Derived
Histamine	Smooth muscle contraction; mucus production; vasodilation; gastric acid secretion; and wakefulness	X	
Tryptase	Serine (protease) endopeptidase	X	
Carboxypeptidase	Peptidase for C-terminal end of peptide	X	
Chymase	Serine (protease) endopeptidase	X	
Heparin	Anticoagulant	X	
Prostaglandin D_2	Increases vascular permeability; bronchoconstriction; inhibits platelet aggregation; chemoattraction		X
Leukotriene C_4 and B_4	Increase vascular permeability; bronchoconstriction		X
Platelet-activating factor	Bronchoconstriction; vasodilation; and platelet aggregation		X

Key Fact

Both α- (mostly immature) and β- (mostly mature) tryptase are elevated in mastocytosis, but only β-tryptase (i.e., mature tryptase) is elevated in anaphylaxis. Mast cells are the major producers of tryptase.

BASOPHILS

Basophils are the least common circulating granulocytes that appear in many inflammatory reactions (especially allergic diseases) and share many characteristics of mast cells. They are important in the late phase of IgE-mediated disease.

Basophil Morphology

- Contain large cytoplasmic granules, which are filled with preformed mediators (e.g., histamine) that can be released quickly.
- Have bilobed nuclei and are 10–14μm in size (Figure 2-8).

Basophil Growth and Differentiation

- Derived from bone marrow progenitors distinct from those of mast cells.
- Mature in **bone marrow.**
- Found primarily circulating in **blood**; also in bronchoalveolar lavage (BAL) from late-phase reactions.
- Have CD123 (IL-3 receptor) and IL-3 is important for their expansion/differentiation.

Flash Card Q6

Which mast cell mediator is both preformed and produced in the late phase?

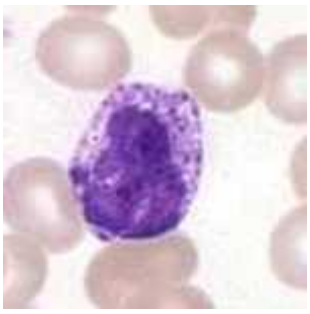


Figure 2-8. Basophil.
(Reproduced, with permission, from Wikipedia Commons.)

Basophil Mediators

- Release **preformed** mediators (<15 minutes) that cause symptoms of immediate allergic reactions (e.g., histamine, chondroitin, MBP, and Charcot-Leyden protein).
- Produce and release **lipid-derived mediators** (~10–30 minutes) after activation via the arachidonic acid pathway responsible for late allergic reactions (e.g. LTC4).
- Produce and release **cytokines** and chemokines (minutes to days) (e.g. IL-3, IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, GM-CSF, TNFα, MCP-1, MIP-1α, RANTES (CCL-5), and PAF).

Key Fact

Unlike mast cells, basophils do not produce (or produce very little) tryptase, chymase, carboxypeptidase, heparin, PGD2, and LTB4.

Table 2-9 compares mast cells and basophils.

Table 2-9. Comparison of Mast Cells and Basophils

Mediator/Characteristic	Mast Cell	Basophil
Location	Tissue	Circulation/blood
Nucleus	Unsegmented	Multilobed
Granules	More, smaller	Fewer, larger
Histamine	+	+
Tryptase	+++	+
Chymase	+	–
Carboxypeptidase	+	–
LTC4	+	+
LTB4	+	–
PGD_2	+	–
FCεRI	+	+
IL-3 receptor (CD123)	–	+
C5a receptor	+	–

Abbreviations: IL, interleukin; LT, leukotriene; PGD, prostaglandin.

Flash Card A6

TNF

EOSINOPHILS

Eosinophils are granulocytic leukocytes that combat infection (namely, helminth) and mediate allergic responses.

Eosinophil Morphology

- Have a bilobed nucleus and are 12–14 μm in size (Figure 2-9).
- Primary granules contain Charcot-Leyden crystals
- Specific/secondary granules contain preformed mediators that include **major basic protein, eosinophil cationic protein, eosinophil peroxidase, and eosinophil-derived neurotoxin,** which are all toxic to surrounding tissue and are released during cytolysis.
- Express Fc receptors for immunoglobulins, β_2-integrins (CD11a-cCD18), β_1-integrin (VLA-4), β_4-integrin ($\alpha_4\beta_7$), and PSGl-1.

Eosinophil Growth and Differentiation

- Derived from myeloid precursor (Figure 2-3).
- Differentiation, maturation, and activation stimulated by GM-CSF, IL-3, and **IL-5**.
  - Mature in the **bone marrow**.
  - GATA1 transcription factor
- Migrate to tissue in response to **RANTES** (CCL-5) and **eotaxin** (eotaxin-1, CCL-11; eotaxin-2, CCL-24).
  - Most eosinophils reside in the **tissue** (lower GI tract, mammary gland, female reproductive tract, lymph tissue).
  - 1–2% in peripheral blood.
- Half-life of approximately 18 hours.
  - Levels vary throughout the day (diurnal variation).

Key Fact

Major basic protein can be used to detect the recent presence of eosinophils in tissue, even when eosinophils cannot be seen on biopsy.

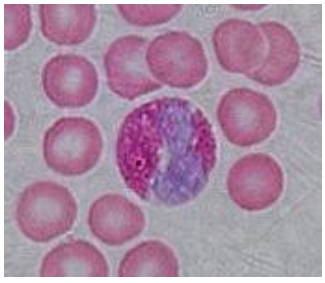


Figure 2-9. Eosinophil.
(Reproduced, with permission, from Wikimedia Commons.)

Flash Card Q7

What is contained in eosinophil primary granules?

Eosinophil Function

- Modulate immune response, fight helminthic infections, and has antitumor effects:
  - Release of toxic granule proteins.
  - Produce reactive oxygen species.
  - Release leukotrienes and prostaglandins.
  - Release cytokines, including IL-1, IL-2, IL-4, IL-5, IL-6, IL-8, IL-13, and TNF.
- Mammary gland development.
- Wound repair and tissue remodeling.

Associated Disorders

Hypereosinophilia (>450 cells/μL) the following causes:

Mnemonic

Causes for eosinophilia: **NAACP**

Neoplasm
Atopy
Addison's disease
Collagen vascular disease
Parasites

- Malignancy (Hodgkin's lymphoma)
- Adrenal insufficiency (Addison's disease)
- Allergic disease and asthma (usually only mild to moderate eosinophila in asthma)
- Collagen vascular disorders
- Drug reactions (antiepileptics, NSAIDs, fluoroquinolones, tetracycline, penicillin, nitrofurantoin, others)
- Hematologic disorders:
  - Hypereosinophilic syndrome
  - Mastocytosis
- Eosinophilic esophagitis
- Skin disease (eczema and pemphigoid)
- Kimura's disease
- Lung disease:
  - Allergic bronchopulmonary aspergillosis (ABPA)
  - Loeffler's syndrome
  - Eosinophilic pneumonia
  - Churg-Strauss vasculitis
- Infectious diseases:
  - HIV (secondary to adrenal insufficiency, HIV medications, or HIV virus itself)
  - Parasitic infections, especially those that invade tissue (**strongyloidiasis)**
  - *Isospora belli* and *Sarcocystis,* but not other protozoa
  - Chronic TB

Key Fact

Drug reactions are the #1 cause of marked eosinophilia in the U.S., whereas parasitic infections are the #1 cause worldwide.

Flash Card A7

Charcot-Leyden crystals

Suppression of eosinophil levels occurs by:

- Most bacterial/viral infections (note the exceptions listed earlier, such as HIV).
- **Fever**
- **Steroids**

NEUTROPHILS

Neutrophils are the most abundant white blood cells in the adult human body and an essential component of the innate immune system.

Key Fact

Neutrophils are attracted to tissue by IL-8 (CXCL8) and LTB4.

Neutrophil Morphology

- Have a multilobulated nucleus and are 12–15 μm in size (Figure 2-10).
- Contain granules of preformed mediators (Table 2-10).
- Express surface receptors for IgG, IgA.

Neutrophil Growth and Differentiation

- Derived from myeloid precursor (Figure 2-3).
- Survival, differentiation, and proliferation stimulated by IL-3, GM-CSF and G-CSF.
- Approximately 1×10^{11} is made per day in a healthy individual.
- Circulate for only 4–10 hours (mostly in the bone marrow).
- Migrate into tissues via **IL-8 (CXCL-8)**, IFNγ, f-met-leu-phe (fMLP), MIP-1, LTB4, and C5a as well as interactions between **Sialyl-Lewis X, E-/P-selectins,** and **LFA-1/ICAM-1.**

Key Fact

Neutrophils, eosinophils, and basophils are each considered polymorpho-nuclear cells because of the multilobulated appearance of their respective nuclei. They are named for their characteristic appearance after H&E stain. Basophils stain a basic blue. Eosinophils stain an acidic, bright red. Neutrophils stain a neutral pink.

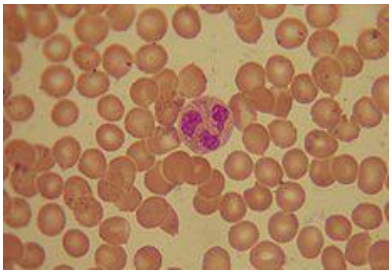


Figure 2-10. Neutrophil.
(Reproduced, with permission, from Wikimedia Commons.)

Table 2-10. Neutrophil Granules

Granule Type	Mediators
Azurophilic (**1° granules**)	Myeloperoxidase, defensins, elastase, lysozyme, and cathepsin
Specific (**2° granules**)	Lactoferrin, cathelicidin, fMLP, and CD11b
Gelatinase	fMLP, CD11b, lysozyme, and gelatinase
Secretory	fMLP, CD11b, and alkaline phosphatase

Key Fact

Primary granules are enlarged in Chediak-Higashi syndrome, but secondary granules are absent in specific granule deficiency. The exact mediators in each are less important.

Neutrophil Function

- Function primarily as phagocytes.
- Phagosomes fuse to lysosomal granules (see Table 2-10) and kill invaders via respiratory burst.
- Also secrete detectable amounts of cytokines.
- Undergo apoptosis in tissue and can "feed" surrounding cells.

Key Fact

Neutrophils are activated by cytokines secreted by T_h17 cells.

Associated Disorders

- Neutropenia causes a predisposition to severe bacterial infections, ulcerations, abscess formation, and gingivitis.
  - Mild: ANCA < 1500 cells/μL
  - Moderate: ANCA < 1000 cells/μL
  - Severe: ANCA < 500 cells/μL
- Several congenital causes of neutropenia as well as defects in migration and effective killing have been identified. See section in Chapter 8, Congenital (Primary) Immunodeficiencies.

PLATELETS

Platelets are small, anuclear cells in the blood circulation whose primary role is in homeostasis.

Morphology, Growth, and Differentiation

- Anucleated cells that are 2–3 μm in diameter (Figure 2-11).
- Derived from megakaryocyte precursor (Figure 2-3).
- Average life span is 8–12 days.

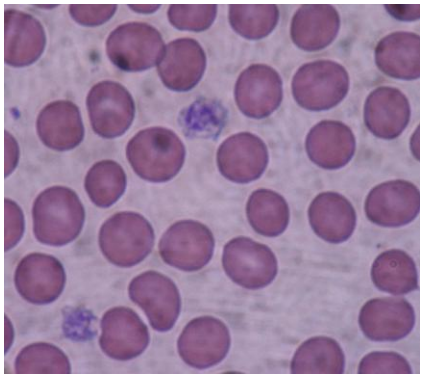


Figure 2-11. Two Platelets (purple).
(Reproduced, with permission, from Wikimedia Commons.)

- Formation regulated by thrombopoietin secreted by the liver and kidney.
- Express surface receptors for IgG and CD23.

Function

- Thrombus formation (Figure 2-12).
  - After endothelial damage, platelets are activated by exposed collagen and von Willebrand factor (vWF).
  - Activated platelets release contents of granules (ADP/ATP, calcium, serotonin, TGFβ, platelet factor 4 (PF4), platelet-derived growth factor (PDGF), vWF, and fibrinogen).
  - Arachidonic acid pathway initiated to produce thromboxane A_2, which activates more platelets.
  - Platelets clump together via fibrinogen and vWF interaction with the GPIIb/IIIa receptor
- Cytokine release

Associated Disorders

- In general, thrombocytopenia increases bleeding risk (however, heparin-induced thrombocytopenia may lead to thrombus formation), and thrombocytosis leads to increased coagulation.

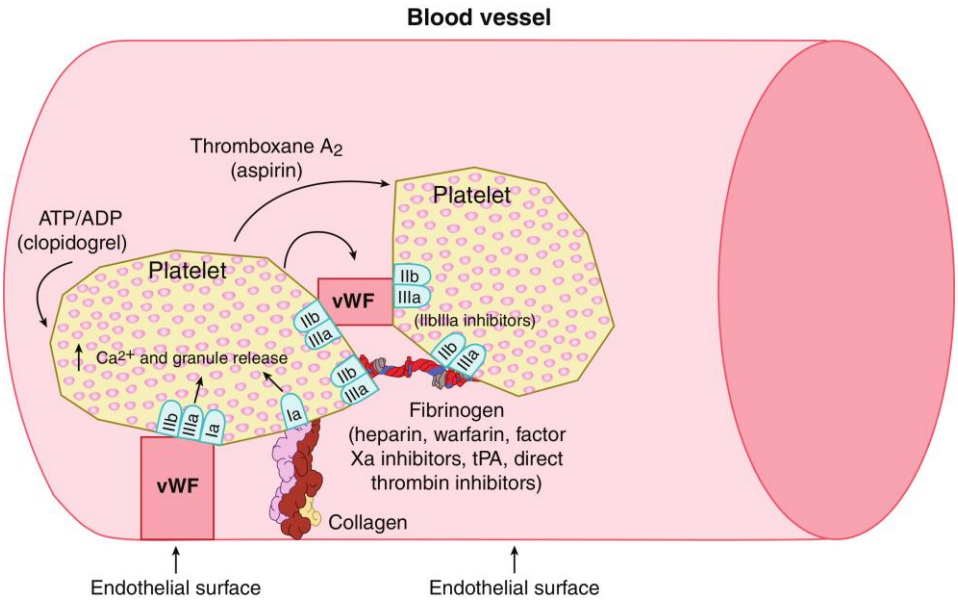


Figure 2-12. Thrombus formation (and inhibitors).

- Diseases with reduced platelet counts (thrombocytopenia) or function:
  - Aplastic anemia
  - Bernard-Soulier syndrome
  - Drug-induced thrombocytopenia
  - Gaucher's disease
  - Glanzmann's thrombasthenia
  - HELLP syndrome
  - Hemophilia
  - Hermansky-Pudlak syndrome
  - Wiskott-Aldrich syndrome
  - Idiopathic thrombocytopenic purpura (ITP) (due to antibodies to platelet's **GP2b3a**)
  - Thrombotic thrombocytopenic purpura (TTP) (due to antibodies to **ADAMTS13**)
  - Von Willebrand disease
  - Heparin-induced thrombocytopenia (due to antibodies to **PF4**)

Key Fact

Wiskott-Aldrich syndrome is an X-linked disorder characterized by eczema, infections, and thrombocytopenia (as well as small platelet volume). The defective gene encodes for the Wiskott-Aldrich syndrome protein (WASp) used in cell signaling.

EPITHELIAL CELLS

Mnemonic

Layers of the epidermis:
Before **S**igning **G**et **L**egal **C**ounsel

Stratum **B**asale
Stratum **S**pinosum
Stratum **G**ranulosum
Stratum **L**ucidum
Stratum **C**orneum

Epithelial cells form a type of tissue that lines the surface of structures throughout the body, serving in innate and acquired host defense as both a barrier and an effector (secretory) tissue.

Epithelial Cell Morphology

- Classified by structure into four distinct types.

- Layers of keratinized epithelial cells make up the skin epidermis and contain an extensively cross-linked protein called **transglutaminase**.
- Defined by the expression of certain adhesion molecules called **e-cadherins**.
- Attached to adjacent epithelial cells by tight junctions, intermediate junctions, and **desmosomes** (which contain e-cadherins).
- Attached to extracellular matrix via **hemidesmosomes** (which use **integrins** for adhesion instead of cadherins).
- Contain filament-associated proteins, which bind keratin fibers called **filaggrins**.

Key Fact

Oligomeric IgA binds to a polymeric immunoglobulin receptor on epithelial cells, is transported through the cell, and secreted, while still attached to part of the receptor called the **secretory component**.

Epithelial Cell Function

- Forms primary barrier against pathogens.
- Secretes numerous mediators used in defense, including cytokines, chemokines, growth factors, mucous, and antimicrobials.
- Removes potential pathogens via beating motion of cilia.
- Also functions in absorption and diffusion of liquids and gases.

Key Fact

Intestinal epithelial cells can process antigens from the gut lumen and present them to T cells via MHCII.

Epithelial Cells in Disease

- Pathogens exploit epithelial receptors to initiate infection:
  - Rhinovirus/ICAM-1
  - Influenza/Glycans
  - Adenovirus/Integrins and CD80/CD86
- Filaggrin mutations are associated with the development of eczema, allergic rhinitis, and asthma.
- Autoantibodies against components of epithelial cells cause several bullous skin disorders (Table 2-11).
- The innate immune function of the human airway epithelium is to orchestrate key inflammatory events following insult (virus, fungus, allergen, pollution) which may be important in allergic disease such as asthma and atopic dermatitis.
  - Airway epithelial cells produce CCL2 and CCL20 in response to house dust mite inhalation, which attracts monocytes and immature DCs to the lung.
  - Produce thymic stromal lymphopoietin (**TSLP**), **GM-CSF**, **IL-25** and **IL-33**, which share the propensity to activate DCs that prime T_h2 and coordinate the subsequent immune response.
  - **IL-33** and other members of the IL-1 family of cytokines, such as IL-1α, IL-1β and IL-36γ (IL-1F9), are released from bronchial epithelial cells in response to allergen exposure.
  - Suppression of β defensins and cathelicidin due to the expression of IL-4 and IL-13 may contribute in the pathogenesis of atopic dermatitis.

Flash Card Q8

In which platelet disorder are platelets abnormally large? *Hint:* Antibodies to platelet's GP2b3a are also seen.

Flash Card Q9

Which chemokines produced by keratinocytes are responsible for homing T cells to the skin? What are the corresponding receptors on the T cells?

Table 2-11. Bullous Skin Disorders

Disease	Direct IF	Target Antigen	Location
Pemphigus vulgaris	Intercellular IgG and IgM	Desmoglein 3	Desmosome
Pemphigus foliaceous	Intercellular IgG	Desmoglein 1	Desmosome
Bullous pemphigoid	Linear IgG at BMZ	BP 230 (BPAg1) or BP180 (BPAg2)	Hemidesmosome
Pemphigoid gestationis (formerly herpes gestationis)	Linear IgG at BMZ	BP 230 (BPAg1) or BP180 (BPAg2)	Hemidesmosome
Dermatitis herpetiformis	Granular IgA in dermis	Transglutaminase	Subepidermal
Linear IgA dermatosis	Linear IgA at BMZ	BP 180 (BPAg2)	Hemidesmosome

Abbreviations: BMZ, basement membrane zone; Ig, immunoglobulin.

Study note: Be able to look at an immunofluorescence and know the most likely bullous skin disorder and target antigen.

ENDOTHELIAL CELLS

Endothelial cells are specialized, mesenchymally derived cells related to epithelium; they are found on the inside line of blood vessels throughout the body.

Endothelial Cell Function

Flash Card A8

ITP

- Maintains homeostasis via vasodilation and vasoconstriction, coagulation and fibrinolysis, wound healing, inflammation, and immunity.
- Mediates adhesion and transmigration of circulating leukocytes via selectins and integrins (Table 2-12).
- Certain chemokines can increase the affinity of integrins for their ligands.
- Endothelial cells in the high endothelial vessels display adhesion molecules and chemokines that are responsible for the homing of naive T cells to the lymph nodes.

Flash Card A9

Keratinocytes produce CCL17 (TARC) and CCL22 (MDC). Skin-homing T cells express cutaneous lymphocyte antigen (CLA) and CCR4 and CCR10.

Table 2-12. Adhesion Molecules on Endothelium Used for Transmigration of Leukocytes

Adhesion Molecule	Adhesion Molecule Location	Ligand	Ligand Location
E-Selectin	Endothelium	Sialyl-Lewis X	Leukocytes
P-Selectin	Endothelium	Sialyl-Lewis X	Leukocytes
L-Selectin	Leukocytes (**not endothelium**)	Sialyl-Lewis X on GlyCAM-1/ MadCAM-1	High endothelial venules
Integrins	Leukocytes	ICAM and VCAM	Endothelium

Abbreviations: GlyCAM, glycosylation-dependent adhesion molecule; ICAM, intercellular adhesion molecule; MadCAM, mucosal addressin cell adhesion molecule, VCAM, vascular cell adhesion molecule.

Mnemonic

"**P**" selectin: think "**P**"reformed (actually named after platelets)

Endothelial Cells in Disease

- Activation and deposition of inflammatory cells can lead to endothelial disruption and endothelial death, both locally and systemically.
- Antineutrophil cytoplasmic antibodies (ANCA) can lead to widespread endothelial inflammation.
- Deposition of inflammatory cells (in particular, eosinophils) in lung endothelium correlates with airway hyperresponsiveness in asthma.

SMOOTH MUSCLE

Smooth muscle is an involuntary, nonstriated muscle found in vessels walls. An IgG autoantibody (anti-SMA) is directed against smooth muscle in type I autoimmune hepatitis.

Flash Card Q10

Which selectin is not found on endothelium?

Flash Card Q11

What surface markers on the high endothelial venules and naive T cells are responsible for homing to the lymph nodes?

FIBROBLASTS

Fibroblasts are a type of cell that makes collagen and other components of the extracellular matrix. Fibroblasts secrete growth factors, including stem cell factor, which is essential for mast cell differentiation and survival. Fibroblasts are particularly important in wound healing.

Flash Card Q12

What integrin ligands are found on the surface of activated endothelium?

Flash Card A10

L-selectin (this is found on leukocytes. E-selectin and P-selectin are found on endothelium)

Flash Card A11

CCL19 and CCL21 by the high endothelial venule, and CCR7 on the naive T cell

Flash Card A12

ICAM-1, ICAM-2, VCAM-1

3 Specific Immune Responses

OVERVIEW OF HYPERSENSITIVITY REACTIONS

Hypersensitivity reactions have been grouped into four types (types I–IV) based primarily on the mechanisms involved. This has been summarized in Table 3-1. A particular disease may however involve more than one type of reaction.

TYPE I HYPERSENSITIVITY REACTIONS

Mast cells and basophils are the primary cells in immediate hypersensitivity or type I hypersensitivity reactions. The reaction is amplified or modified by platelets, neutrophils and eosinophils. **IgE** is the primary immunoglobulin. Examples include: allergic asthma, allergic conjunctivitis, allergic rhinitis ("hay fever"), anaphylaxis, drug allergy, and food allergy.

Table 3-1. Overview of Hypersensitivity Reactions

Type of Hypersensitivity Reactions	Key Players	Antigen	Example(s)
Type I	Mast cells Basophils IgE	Soluble	Allergic asthma Allergic rhinitis Anaphylaxis Food allergy
Type II	IgG IgM Complement Phagocytes	Cell/matrix associated	Hemolytic anemia Goodpasture's syndrome Graves' disease
Type III	Complement IgG	Soluble	SLE Glomerulonephritis Serum sickness
Type IV	T cells		Contact dermatitis Psoriasis Celiac disease

Abbreviations: Ig, immunoglobulin; SLE, systemic lupus erythematosus.

Pathophysiology

IgE Production—Upon initial exposure, allergen/antigen is presented by antigen-presenting cells (APCs) to CD4+ T_h2 cells specific to the antigen. These T_h2 cells then drive B cells to produce IgE specific for the antigen (which is called **sensitization**) through cytokines such as interleukin 4 (IL-4) (which binds IL-4Rα/γc and IL-4Rα/IL-13Rα1) and IL-13 (which binds IL-4Rα/IL-13Rα1). This process occurs primarily in the peripheral lymphoid organs.

The specific IgE (sIgE) binds high-affinity IgE receptors (FcεRI) on mast cells and basophils.

Degranulation—Upon re-exposure, allergen binds the surface-bound sIgE on mast cells or basophils. When the receptors are cross-linked, intracellular signaling occurs and leads to cell degranulation (see Figure 2-6). This releases preformed mediators, enzymes, and cytokines (Table 2-8). These mediators act directly on tissues and recruit and activate additional inflammatory cells (eosinophils among others) which release more mediators and propagate the reaction.

- Immediate reaction—results from the release of preformed mediators
- Late reaction—results from the influx of inflammatory cells and generation of cysteinyl leukotrienes (lipid-derived mediators)

For mediators, mast cell kininogenase and basophil **kallikrein** activate the contact system; **tryptase** has kallikrein activity [which can activate the contact system, complement cascade, and clotting cascade (via cleaving fibrinogen, which is a chemoattractant for neutrophils and eosinophils)]; **platelet-activating factor** (PAF) from tumor necrosis factor (TNF) activation of nuclear factor kappa B (NFκB) induces clotting and disseminated intravascular coagulation; PAF also activates mast cells; **heparin** inhibits clotting; and, **chymase** can convert angiotensin I to angiotensin II, which modulates hypotension.

The process is summarized in Figure 3-1.

Anaphylaxis

IgE-mediated immunologic anaphylaxis (sometimes called allergic anaphylaxis) is a key example of a type I hypersensitivity reaction.

Subtypes of Anaphylaxis

- Immunologic anaphylaxis—IgE-mediated reactions; non-IgE-mediated (i.e., not type I reactions), which include IgG-mediated reactions (which have not been identified in humans) and immune complex/complement-mediated reactions
- Nonimmunologic anaphylaxis—IgE-independent mast cell or basophil degranulation. This is also known as nonallergic anaphylaxis.

Pathophysiology of Immunologic/IgE-mediated Anaphylaxis—As described earlier in detail, these reactions consist of allergens reacting with IgE antibodies bound to Fc receptors on mast cells and basophils, leading to activation of the cells and mediator release.

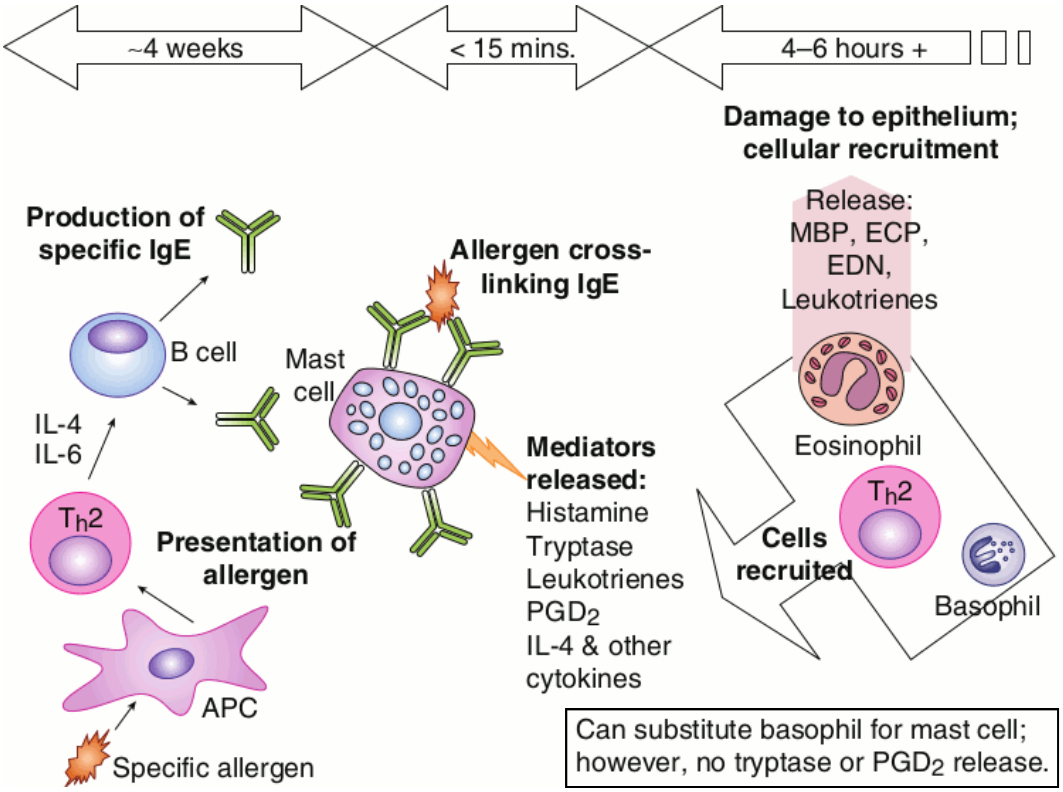


Figure 3-1. Steps in a type I hypersensitivity reaction.
Abbreviations: APC = antigen-presenting cell, ECP, EDN, MBP = eosinophil granule proteins, IgE = immunoglobulin E, IL = interleukin, PGD_2 = prostaglandin 2, T_h2 = type 2 helper T cell.
(Reproduced, with permission, from Mitchell Grayson, MD, Medical College of Wisconsin.)

Flash Card Q1

What are the steps that occur during primary exposure to antigen?

TYPE II HYPERSENSITIVITY REACTIONS

IgG and IgM are the primary immunoglobulins in type II hypersensitivity reactions with **complement or phagocytes** also frequently being involved. Examples include: hemolytic anemia, hemolytic disease of the newborn, Goodpasture's syndrome, myasthenia gravis, and Graves' disease.

Pathophysiology

- The effector function of antibodies is to eliminate microbes and toxins (Table 3-2).
- This antibody-mediated elimination of antigens requires the involvement of other effector mechanisms, such as complement and phagocytes.
- In summary, type II reactions occur when antibodies bind antigens on a target cell (e.g., for example, penicillin bound to red blood cells); target cell damage then occurs through:
    - Cellular neutralization/blocking (i.e., myasthenia gravis),
    - Cytotoxicity (i.e., hemolytic anemia), or
    - Cellular stimulation (i.e., Graves' disease)

Key Fact

Know all of the effector functions of immunoglobulins listed in Table 3-2 as well as the how the processes are carried out.

Neutralization of Microbes and Toxins

- Antibodies block the binding of microbes and toxins to cellular receptors.
    - Example: Influenza virus uses its envelope hemagglutinin to infect respiratory epithelial cells. An antibody will bind the hemagglutinin and "neutralize" the microbe so it does not interact with cellular receptors. This is an example of **steric hindrance.**

Flash Card A1

(1) Allergen/antigen is presented by APCs to CD4+ Th2 cells specific to the antigen; (2) these Th2 cells then drive B cells to produce IgE specific for the antigen (which is **sensitization**) through cytokines such as IL4 and IL-13; (3) the specific IgE (sIgE) binds high-affinity IgE receptors (FcεRI) on mast cells and basophils.

Table 3-2. Effector Functions of Antibody Isotypes

Antibody Isotype	Effector Functions
IgG	**Opsonization** (namely, IgG1 and IgG3) Activation of classical pathway of **complement** (namely, IgG1 and IgG3) Antibody-dependent cell-mediated immunity (ADCC) Neonatal immunity as a result of placental transport
IgM	Activation of classical pathway of **complement**
IgA	Mucosal immunity Neonatal immunity provided by breast milk
IgE	Mucosal immunity

Cytotoxicity by Opsonization and Phagocytosis

- IgG antibodies coat or opsonize microbes and promote phagocytosis (Figure 3-2).
- Mononuclear phagocytes and neutrophils have receptors for the Fc portions of the antibodies that specifically bind the opsonized microbes for intracellular killing.
- The phagocyte **Fc receptors**:
  - Promote phagocytosis of opsonized particles.
  - Deliver signals that promote killing of the microbes by the phagocyte.
- **FcγRI (CD64)** is a high-affinity phagocyte receptor that strongly binds **IgG_1** and **IgG_3** (two of the most efficient opsonins).

Cytotoxicity by Complement Fixation

- Out of the three major pathways of complement activation, the **classical pathway** is the only one activated by an antibody (IgG or IgM).
- The **alternative pathway** is activated by microbial cell surfaces and the **lectin pathway** is activated by plasma lectins, which bind to microbe mannose residues, both in the absence of antibody.
- All three pathways result in the generation of **C3**, the most abundant complement protein.
- With complement activation, **C3** is proteolyzed to produce an active product **C3b** that attaches to microbes.
- As a result, microbes can be opsonized by complement particles, specifically **C3b**, and engulfed by phagocytes expressing receptors (**CR1=CD35**) specific for C3b.

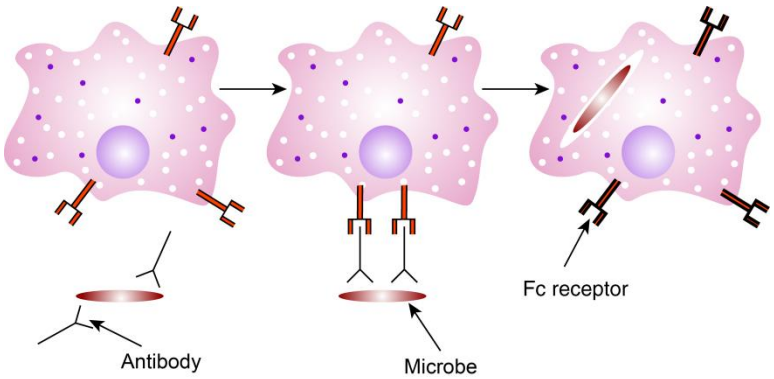


Figure 3-2. Antibody-mediated opsonization and phagocytosis of a microbe.

Flash Card Q2

Which immunoglobulin(s) is/are involved in opsonization?

Cytotoxicity by Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC)

- A process whereby natural killer (NK) cells (and other leukocytes) bind to antibody-coated microbes to destroy them.
- NK cells express the FcγRIII receptor (a low-affinity Fc receptor; CD16) that binds to clustered IgG molecules (not to monomeric-circulating IgG) (Figure 3-3)
- An antibody-coated particle engages the **FcγRIII receptor** on the NK cell, which subsequently activates the NK cell to:
    - Produce and secrete cytokines such as IFNγ.
    - Discharge its granules.
    - Kill the infected cell.

Antibody-Mediated Immune Regulation

IgA and Mucosal Immunity: Gut/Oral—The gastrointestinal and pulmonary tracts are the most common entry point for microbes. The major defense against microbes on those mucosal surfaces is provided by **IgA antibody**.

Key Fact

Although it constitutes less than one quarter of antibody in plasma, the amount of **IgA** produced in the body is **greater** than that of any other antibody isotype (60–70% of total antibody output), with most of the antibody located in or near the mucosal tissues.

- IgA binds microbes and toxins in the gut and respiratory lumen, neutralizing them and preventing infection.
- Isotype switching to IgA occurs most efficiently in the mucosal lymphoid tissue and is stimulated by transforming growth factor **(TGFβ)** and **IL-5**.
- Secreted IgA is a dimer that is transported into lumen via an IgA-specific receptor called the **poly-Ig receptor** (Figure 3-4).
    - Note: Poly-Ig receptor can also transport IgM into intestinal secretions; thus, the use of the prefix "poly" in its name.
- The poly-Ig receptor is responsible for secreting IgA into milk, bile, saliva, and sweat.

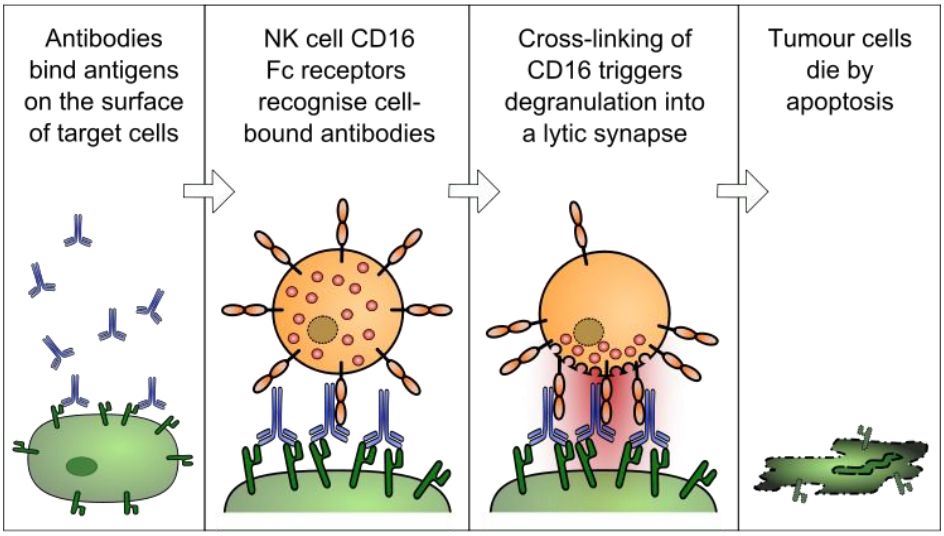


Figure 3-3. Antibody-dependent cell-mediated cytotoxicity.
(Reproduced, with permission, from Wikipedia.)

Flash Card A2

IgG

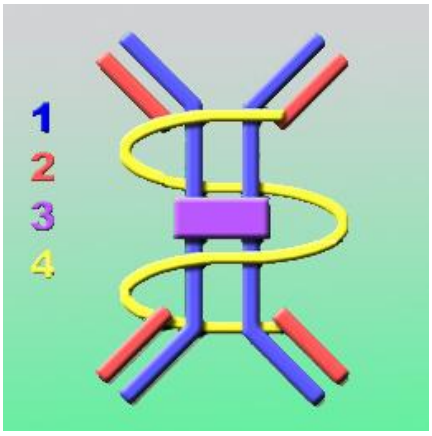


Figure 3-4. IgA dimer. 1 = H chain; 2 = L chain; 3 = J chain; 4 = secretory component.
(Adapted, with permission, from Wikimedia Commons.)

IgG and Neonatal Immunity—Neonates are not capable of producing an effective immune response and, for the first several months after birth, they are protected passively by maternal antibodies.

- IgG is transported across the placenta from mother to infant.
- Maternal IgA more than IgG are ingested from the breast milk by the infant.
- IgG is transported across the placenta and infant gut lumen by an IgG-specific Fc receptor called **neonatal Fc receptor (FcRn)**, which resembles the major histocompatibility complex (MHC) class I molecule.

TYPE III HYPERSENSITIVITY REACTIONS

Immune complexes, hence **antigen, antibody**, and **complement,** are the major players in immune complex-mediated or type III hypersensitivity reactions. Examples include systemic lupus erythematosus (SLE), glomerulonephritis, serum sickness, and vasculitis.

Pathophysiology

Immune complexes are clusters of antigens and antibodies that are typically cleared by the spleen and liver. Under some circumstances, immune complexes are deposited in the blood vessels or tissues where they can cause disease. Damage is caused by formation or deposition of antigen-antibody complexes in vessels or tissue. The deposition of immune complexes causes complement

activation and recruitment of neutrophils by interaction of immune complexes with Fc IgG receptors

Immune Complex Clearance—The relative concentration of antigen to antibody and their charge (amongst other factors) determine whether immune complexes will be cleared or deposited in the tissue.

Immune complexes formed at **equivalence** (antibody number = antigen number) form a lattice and are rapidly removed from the circulation by mononuclear phagocytes, such as Kupffer cells in the liver.

Immune complexes that are formed when either the antibody or the antigen are in excess are smaller and not removed efficiently from the circulation. It is thought that the most pathogenic state (i.e., the case of many pathological syndromes in vivo) occurs when immune complexes are created in a state of moderate **antigen excess.** Immune complexes in this state are soluble (and hence difficult to phagocytose) and can easily fix complement and generate potent cleavage products (contributing to increased vascular permeability and extravascular movement of inflammatory cells)

Precipitin curves illustrate immune complex formation (Figure 3-5).

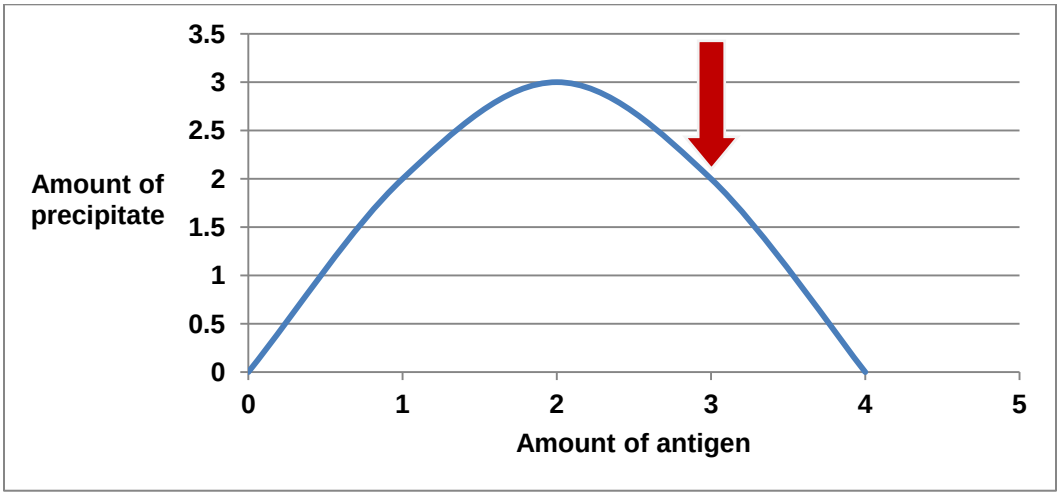


Figure 3-5. Precipitin curve. As antigen is added to serum with a fixed amount of antibody, small immune complexes are first formed in a state of antibody excess (left side of curve). As equivalence is reached, immune complexes begin to precipitate (middle of curve). With antigen excess, immune complexes again become smaller and do not precipitate (right side of curve). The most pathogenic state is thought to be moderate antigen excess (arrow). This is a representative curve of an Ouchterlony plate with the visible precipitate graphed against the increasing antigen concentration using a serum with a fixed antibody concentration *(*x-axis = amount of antigen added; *y*-axis = concentration of precipitate in serum.)

The basement membrane is negatively charged. **Positively** charged immune complexes tend to deposit in the basement membrane of skin and kidneys (Figure 3-6), whereas neutral or negatively charged complexes do not. Hemodynamics also influence immune complex deposition.

Immune Response—In general, early in an immune response, immune complexes that form with a low amount of antibody (antigen excess) tend to elicit further responses to antigen. Late in the immune response, complexes formed in antibody excess tend to suppress the response.

- Exposure to a large amount of antigen tends to be more immunogenic.
- The longer the duration of exposure to an antigen, the more potent the immunologic stimulus.
- Example: SQ immunization offers better antibody production than a brief IV exposure to antigen
- Immune complex injury in tissues is associated with the release of inflammatory cytokines, such as IL-1β, TNF, IL-2, and IFN-γ.

Examples

Arthus Reaction—When antigen is injected into the skin or tissue of an immunized individual, local edema, neutrophil migration, hemorrhage, and necrosis occur. This is called an Arthus reaction. This cutaneous inflammation is caused by local vasculitis due to immune complex deposition in vessel walls, with peak intensity occurring at 4–10 hours. It is a **local** type III hypersensitivity reaction as the target organs is the blood vessels of the dermis.

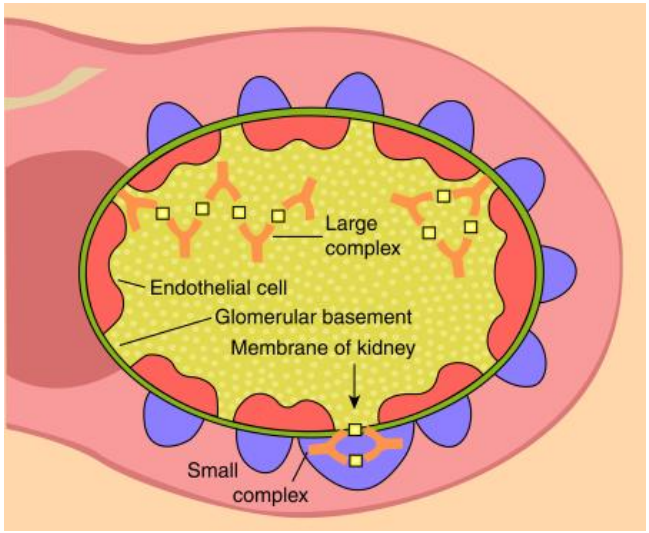


Figure 3-6. Immune complexes deposited in the kidney.
(Adapted, with permission, from Wikimedia Commons.)

Serum Sickness—Exposure to foreign proteins or haptens in unimmunized persons leads to antibody production and formation of soluble immune complexes in the circulation. This typically develops in 4–10 days (due to time needed to class switch). Pathogenesis is related to (1) immune complex deposition in blood vessels of the skin, joints, kidneys, and/or lungs and (2) immune complex fixing and activating of complement. Symptoms include: fever, rash, joint pain, and lymphadenopathy (all which are all self-limited once the foreign antigen is removed). Common causes of serum sickness are hypersensitivity reaction to medications (antibiotics) and foreign proteins. In contrast to the Arthus reaction, serum sickness is a systemic type III hypersensitivity reaction since the target organs are the blood vessels of the skin, joints, kidneys, lungs, etc.

Upon re-exposure (i.e., previously immunized person), the amnestic IgG response is much more rapid. Thus, the symptoms of serum sickness may occur in 12–36 hours and be more severe.

Vasculitis—Inflammation of small or large blood vessels can result in tissue damage. Immune complex deposition in endothelial basement membrane usually occurs in this disease. Inflammation can vary from necrosis and granulomatous change to fibrosis and scarring. In some individuals bacterial, viral and mycobacterial antigens have been detected in vessel walls.

Systemic Lupus Erythematosus (SLE)—SLE is a systemic autoimmune disease characterized by autoantibody production (against DNA), low levels of complement, hypergammaglobulinemia, and the presence of circulating immune complexes. In patients with SLE, high levels of circulating immune complexes correlate with disease activity. Immunoglobulin and complement deposits can be found in blood vessel walls and kidney glomeruli of SLE patients.

Glomerulonephritis—Immune-mediated glomerular disease with many subtypes mediated by antigen-antibody complexes, including post-streptococcal glomerulonephritis, lupus nephritis, and membranoproliferative glomerulonephritis. In these diseases, immunoglobulin and complement deposits are seen in the glomeruli.

TYPE IV HYPERSENSITIVITY REACTIONS

The major player of cell-mediated or delayed hypersensitivity (also called type IV hypersensitivity) reactions is the **T-cell (CD4+ or CD8+)**; other cells involved depends on the subtype. Examples include: contact dermatitis, psoriasis, celiac disease, etc.

Pathophysiology

T Cells—T cells originate from hematopoietic stem cells in the bone marrow and mature in the thymus.

- T_h0 (Naïve T cells)—Naïve T cells migrate from blood to peripheral lymphoid organs in search of antigen they recognize. Naïve T cells express L-selectin (CD-62L). After seeing their antigen, they become activated, proliferate, and differentiate into effector T cells or memory T cells.
- CD4+ T cells (Helper T Cells)—Subsets are listed in Chapter 2. CD4+ T cells respond to antigens that are internalized by APCs in association with MHC class II molecules.
- CD8+ T Cells (Cytotoxic T Cells)—Naïve CD8+ T cells differentiate into effector T cells (CTLs) that recognize and kill cells expressing foreign peptides (specifically intracellular peptides, such as viruses) in association with class I MHC molecules. Refer to Chapter 1.
    - CD8+ T cells contain membrane-bound cytotoxic granules that contain perforin and granzymes. Like T_h1 cells, CTLs are able to secrete cytokines: IFN-γ, TNF, and lymphotoxin. CTL killing is antigen-specific and contact-dependent. Lysis of target cells can occur via two mechanisms: perforin and granzyme released from the CTL that enters target cell and induces apoptosis; and, Fas ligand (FasL) expressed on CTL that binds target cell Fas, leading to apoptosis.

Subtypes—Type IV reactions have been subdivided in IVa, IVb, IVc, and IVd. Certain diseases may fit into more than one subtype (i.e., contact dermatitis). Type IVc and IVd are less well-characterized. These are summarized in Table 3-3.

Table 3-3. Subtypes of Type IV Hypersensitivity Reactions

Reaction Type	T-Cell Involved	Other Cells	Target Organs	Example
IVa	CD4+ Th1 cells (IFNγ, TNFα, IL2)	Macrophages NK cells	Skin, lung, GI	Contact dermatitis TB
IVb	CD4+ Th2 cells (IL4, IL5, IL13)	Eosinophils B cells	Skin, lung, GI	Chronic allergic disease
IVc	CD4+ T17 cells (IL17, IL21, IL22)	Neutrophils	Skin, lung, GI	Psoriasis
IVd	CD8+ cells		Skin, systemic	Contact dermatitis

Abbreviations: IFN, interferon; IL, interleukin; NK, natural killer; T_h, helper T cell; TNF, tumor necrosis factor.

Flash Card Q3

Upon first exposure to a medication, when might a patient develop symptoms of serum sickness?

Delayed-type Hypersensitivity (DTH)—These reactions occur by contact sensitization to chemicals or subQ/ID injection of protein antigen (e.g., PPD). Chemicals can bind and modify self-proteins, creating new antigens that are presented to CD4+ or CD8+ T cells. This is a type IV delayed hypersensitivity reaction involving an induction/sensitization phase (priming of the immune system) and elicitation phase (triggering of the reaction) and involves a cell-mediated response. An example of DTH is allergic contact dermatitis. Agents implicated in allergic contact dermatitis include: nickel, Quaternium-15 in cosmetics, bacitracin, poison ivy (urushiol, which is a hapten) and resin. Evidenced by the name, these reactions are "delayed" (i.e., 48–72 hours).

Flash Card A3

4–10 days

4 Laboratory Tests

IMMUNOGLOBULIN MEASUREMENT

Methods for Determining Immunoglobulin Levels

Immunoassays detect specific antibody-antigen interactions. Due to the bivalent binding properties of immunoglobulins, the resulting antibody-antigen interaction is dependent upon the ratio of antigen to antibody. Complexes that form are classified into three classes, based upon the immunoprecipitin curve (Figure 4-1).

- **Zone of Antibody Excess**: Complexes exist as single antibody to single antigen.
- **Zone of Equivalence**: Single antibody bind to two antigens, forming large insoluble lattices (i.e., can increase turbidity of solution, appear as precipitin lines).
- **Zone of Antigen Excess:** Binding sites on the antibodies are saturated, and antibodies exist as single antibody to two antigens.

Immunoassays can be classified based on the immunoprecipitin curve (Table 4-1).

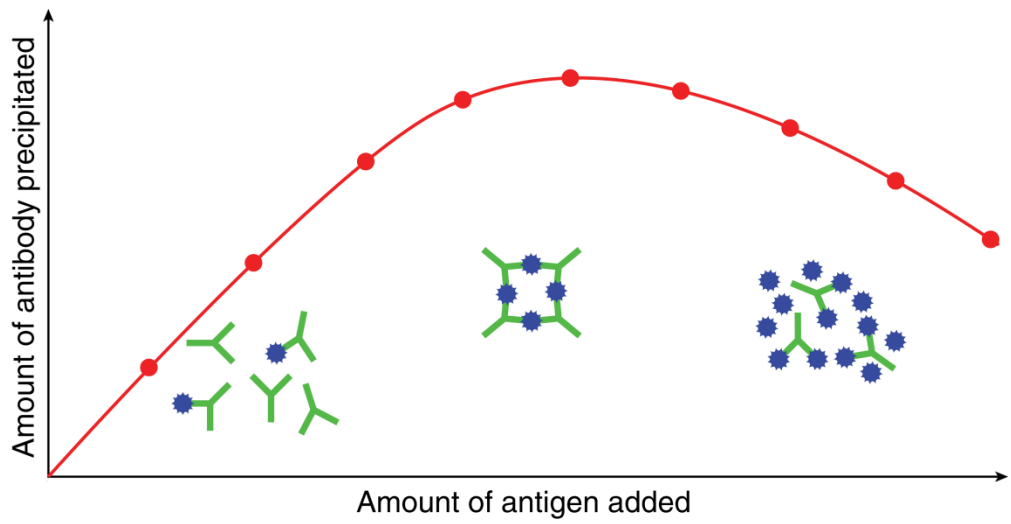


Figure 4-1. Immunoprecipitin curve.

Table 4-1. Immunoassays	
Antibody excess	Immunoblotting Enzyme-Linked Immunosorbent Assay (ELISA)
Antigen-antibody equivalence	Radial Immunodiffusion (RID) Double Immunodiffusion (Ouchterlony) Nephelometry
Antigen excess	Radioimmunoassay (RIA) ELISA

Serum immunoglobulins (Ig) IgG, IgG subclasses, IgM, and IgA are usually measured by automated nephelometry or immunoturbidimetric assay systems.

Nephelometry—Nephelometry is a method by which the turbidity of a solution is determined by a pattern of scattered light (Figure 4-2). In the determination of Ig levels, Ig are injected into a reaction chamber with antigen-specific antibody. As antibody-antigen complexes form, light is applied and the extent of light scatter is measured by a nephelometer. Photons are reflected from the immune complexes at an angle and measured with a photomultiplier tube.

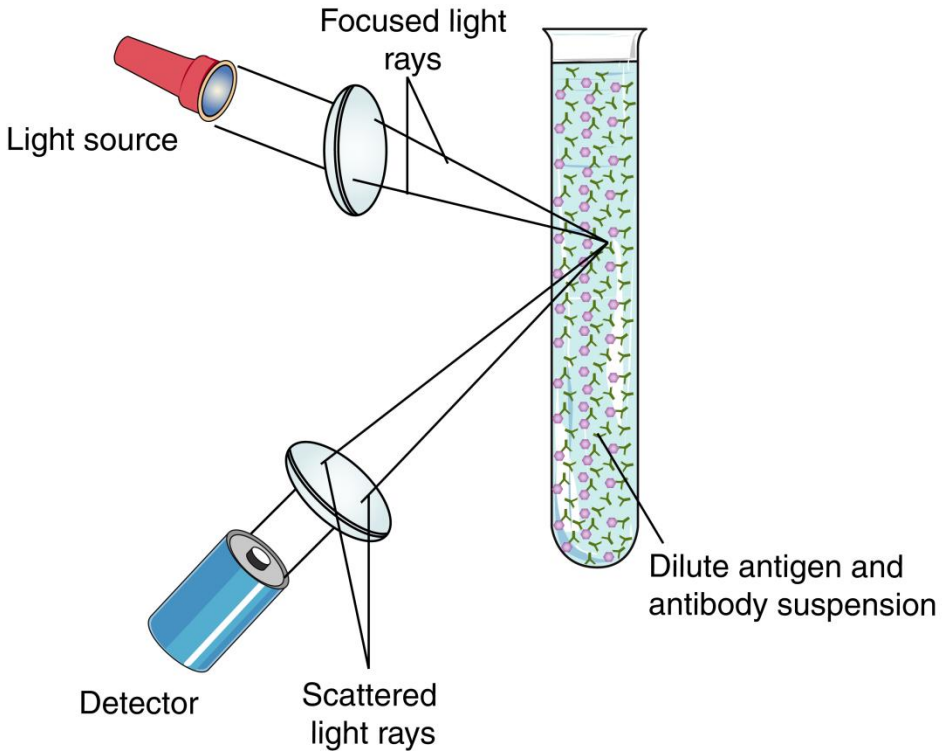


Figure 4-2. Nephelometry determination of serum immunoglobulin levels.

The amount of light detected is quantified as a function of time and the concentrations of antibody and antigen. This automated technique allows laboratories to process a high volume of samples. Nephelometry is considered to be more sensitive than RID and can measure quantities of protein from 1–10 µg/mL.

Radial Immunodiffusion (RID)—Antiserum is mixed into a warm gel matrix while it is still in liquid form, then poured into a flat plate and allowed to cool. Antigen (IgG) is then placed into wells that have been cut into the gel and diffuses into the gel matrix. At the zone of equivalence, a precipitin line is seen (Figure 4-3). The amount of antigen is determined by the diameter of the precipitin ring, which is compared with a standard curve, created by known serially diluted concentrations of the antigen (see Figure 4-3). This is a quantitative method. Limitations include the inability to accurately measure serum protein concentrations less than 10 µg/mL.

Laurell Rocket—The Laurell rocket method is considered to be less time-consuming and more accurate than RID. This method incorporates features of immunoelectrophoresis and the immunodiffusion technique. The addition of immunoelectrophoresis decreases the time involved considerably, from up to 48 hours in RID to several minutes for the Laurell rocket technique. Samples are added to gel plates containing antiserum in holes made throughout the gel layer. A voltage is applied for a predetermined length of time. A precipitin line is seen at the zone of equivalence, which adopts the shape of a "rocket" (Figure 4-4). The height of the precipitin line is used to determine the amount of antigen concentration, compared against a standard curve. This represents both a qualitative and quantitative method of the antigen placed in the gel.

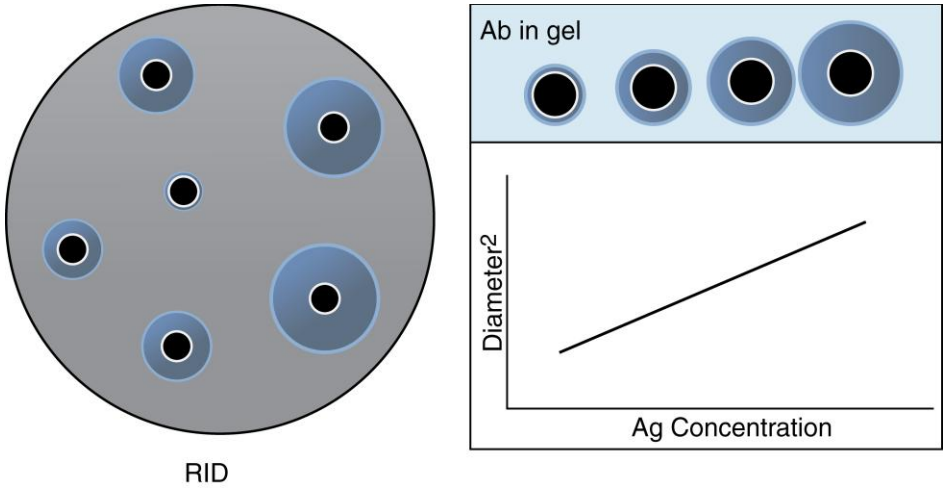


Figure 4-3. Radial Immunodiffusion (RID).

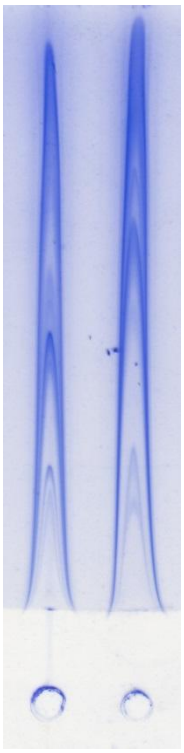


Figure 4-4. Laurell rocket electroimmunoassay.
(Reproduced, with permission, from Nancy Elms and Jordan N Fink, MD, Medical College of Wisconsin.)

A summary of immunoassays used to determine immunoglobulin levels are found in Table 4-2.

Table 4-2. Methods for Determining Immunoglobulin Levels

	Method	Features
Nephelometry	Scatter light to determine turbidity Turbidity created by immune complexes in solution Zone of equivalence	Automated Method of choice
Radial immunodiffusion (RID)	Antiserum mixed in gel Study Ig placed in well, looking for ring of precipitin Zone of equivalence	Time-consuming 48 hr to process a sample Insensitive, semiquantitative
Laurel Rocket	Immunodiffusion + electrophoresis. Precipitin appears rocket-shaped, need to measure height of peak Zone of equivalence	Minutes to perform Insensitive, semiquantitative

Methods for Characterizing Immunoglobulins

Immunodiffusion (Ouchterlony)—The Ouchterlony method utilizes a double-immunodiffusion (DID) technique. Holes are punched into an agarose gel. Antigens are placed in wells, and serum is placed in adjacent wells. Antigen and antibodies diffuse into the gel, and complexes form at the zone of equivalence, which is seen as a white precipitin line (Figure 4-5). The Wadsworth method simplifies the Ouchterlony method and utilizes agar on glass slides. Diffusion occurs within hours and requires fewer reagents.

***Enzyme-Linked Immunosorbent Assay* (ELISA)—**ELISA refers to assays performed on a microtiter plate in either a zone of antigen excess or zone of antibody excess. Antigens and reagents are immobilized by adsorption onto microtiter plates

ELISAs can be performed in many different ways by modifying the basic principle of identifying the bound protein by an antibody, which is conjugated to a label (e.g., enzymes such as horseradish peroxidase, alkaline phosphatase, streptavidin, and fluorescent tags). Figure 4-6 illustrates common ELISA formats. The appropriate substrate is added, and the signal is detected by use of a spectrophotometer, fluorometer, or luminometer.

ELISAs have been used to diagnose a variety of clinical conditions. It is used to determine the presence of allergen-specific IgE (see later discussion), B-natriuretic peptide, complement, toxins, histamine, leukotriene C4, and tumor markers.

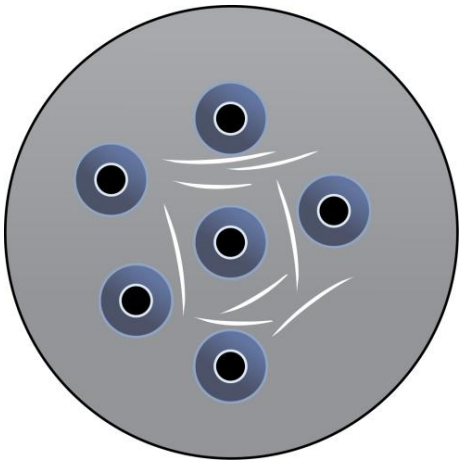


Figure 4-5. Ouchterlony's double-immunodiffusion method.

Flash Card Q1

Which test can be used to detect both excess antibody and excess antigen?

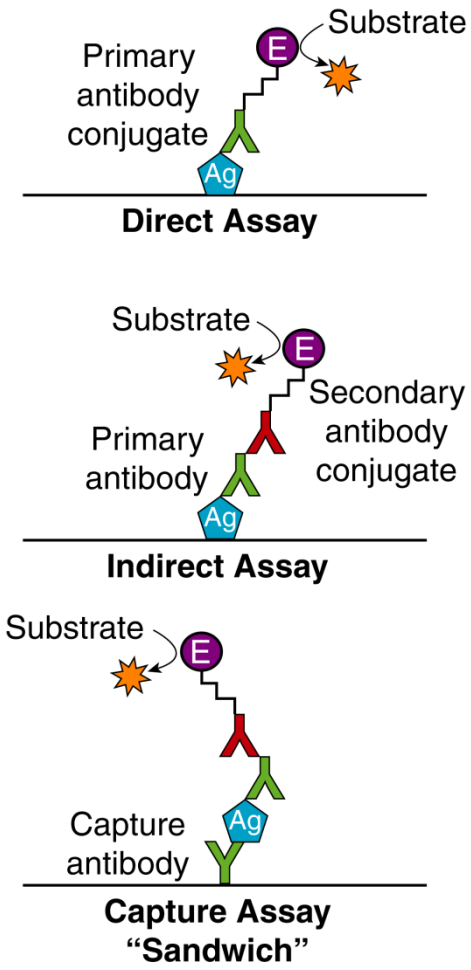


Figure 4-6. ELISA formats.

Western Blot (Protein Immunoblot)—The Western blot represents an antibody excess assay for recognizing specific proteins within soluble tissue or cell samples. The method is summarized in Figure 4-7 and a Western blot is shown in Figure 4-8.

- Proteins are separated by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). SDS is a detergent that unfolds a protein's tertiary structure and provides a negative charge. Immunoglobulins separate by migrating to a positive pole when placed under an electric field. Shorter, denatured immunoglobulins will migrate further in the polyacrylamide gel.
- Separated immunoglobulins are then transferred to a nitrocellulose sheet or polyvinylidene difluoride (PVDF) membrane while maintaining the same separation pattern created in the polyacrylamide gel.
- Labeled specific immunoglobulin antibody is added, binding to the separated immunoglobulins. A secondary antibody is added and then conjugated to an enzyme, such as horseradish peroxidase or alkaline phosphatase.
- Enzyme substrate is added to provide a colorimetric reaction (see Figure 4-8).

Flash Card A1

ELISA

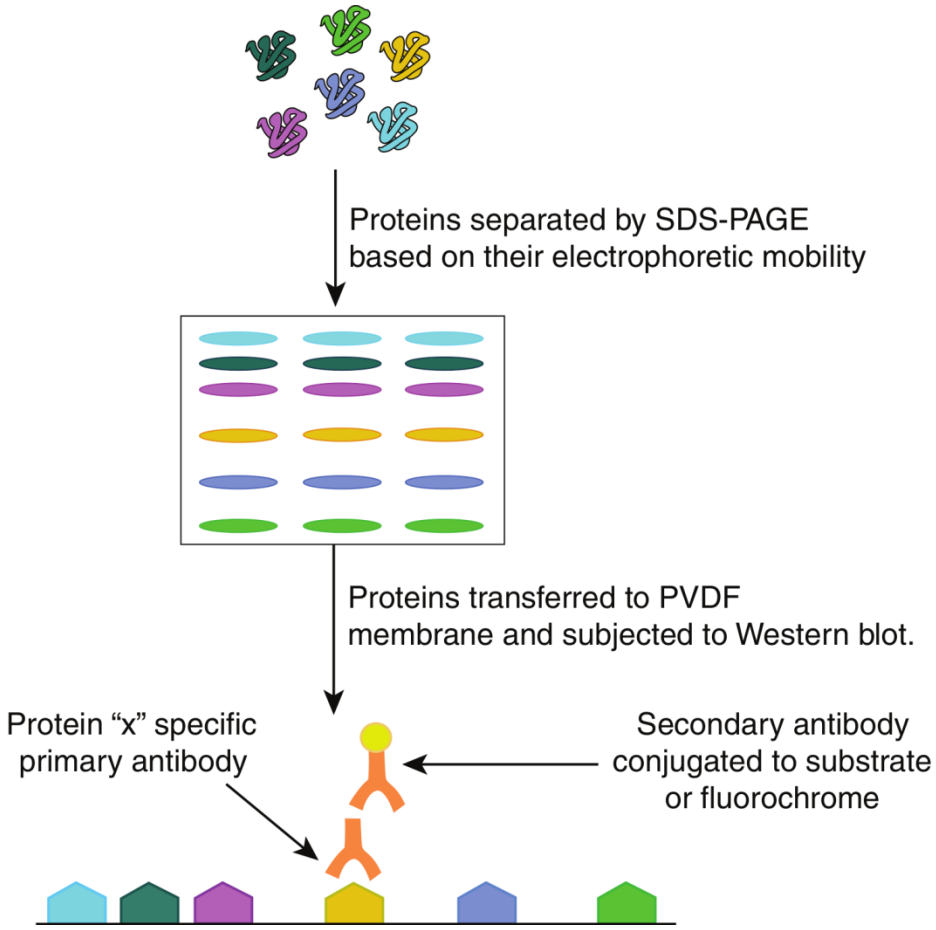


Figure 4-7. Western blot method of protein detection.
(Reproduced, with permission, from Stephen B. Gauld, PhD, Medical College of Wisconsin.)

Clinically, the Western blot has been used to determine infection by:

- *Borrelia burgdorferi*
- HHV6 and HHV7
- HIV (confirmatory test after positive ELISA)
- Human T-lymphocyte leukemia virus
- Prion diseases
- Respiratory syncytial virus
- Severe acute respiratory syndrome (SARS) coronavirus
- Varicella-zoster virus
- Confirmatory test for hepatitis B
- Lyme disease

It can also be used to determine the presence of antiglomerular basement antibodies, antinuclear antibodies, and antiretinal antibodies. Western blotting can essentially be used to detect expression of virtually any protein as long as a detection antibody exists.

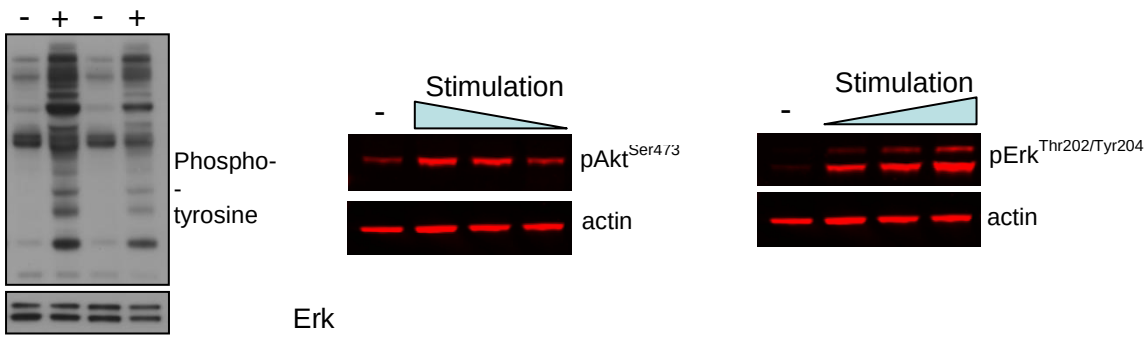


Figure: 4-8. Western blot. Phosphorylated proteins separated by one-dimensional SDS-PAGE and analyzed by Western blot.
(Reproduced, with permission, from Stephen B. Gauld, PhD, Medical College of Wisconsin**.)**

Immunofixation Electrophoresis (IFE)—IFE determines levels of serum immunoglobulins by electrophoresis by measuring immunodiffusion against an antiserum. The test replaces the less sensitive and slower immunoelectrophoresis method and is used to confirm monoclonal gammopathy and determine heavy- or light-chain class type.

Serum proteins are separated by high-resolution agarose gel electrophoresis. Proteins are separated by size and charge. Once separated, immunoglobulins are exposed to specific antisera and the immunoprecipitate is visualized with a protein stain.

Clinical Applications of Immunoglobulin Measurement

Immunoglobulin levels vary with age. Maternal IgG levels contribute to the high IgG seen at birth. A nadir is seen at 6 months of age, when maternal IgG levels wane, and before the child synthesizes its own IgG (Figure 4-9). Serum IgG and IgA also increase with age (Figure 4-10).

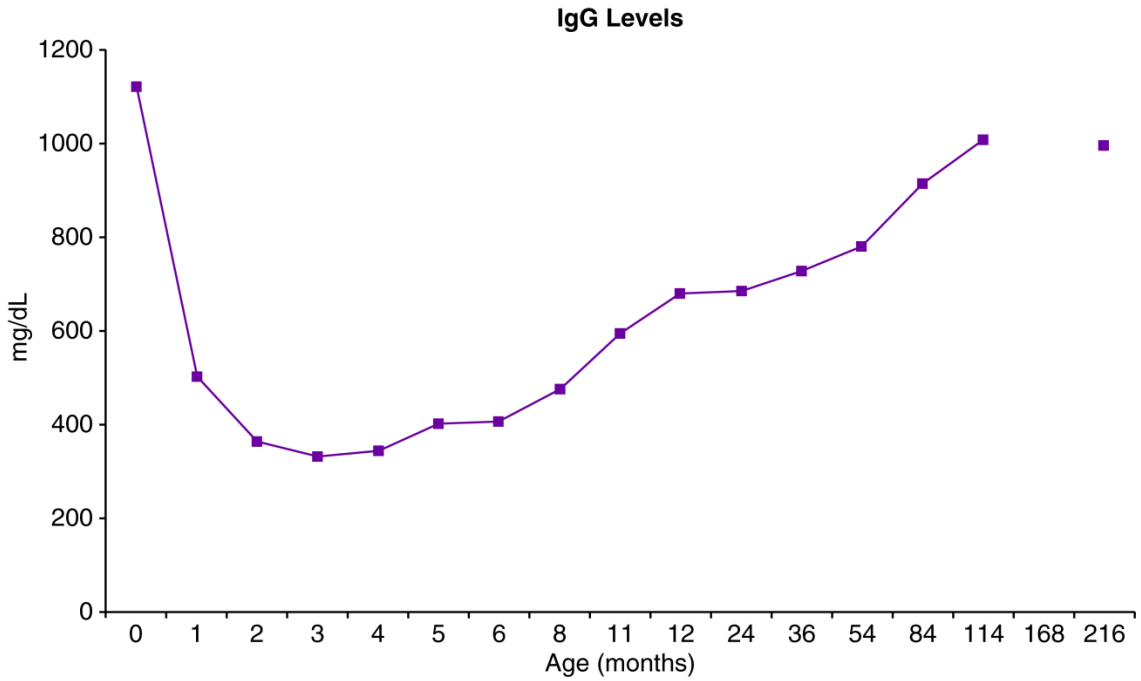


Figure 4-9. IgG versus age in months.

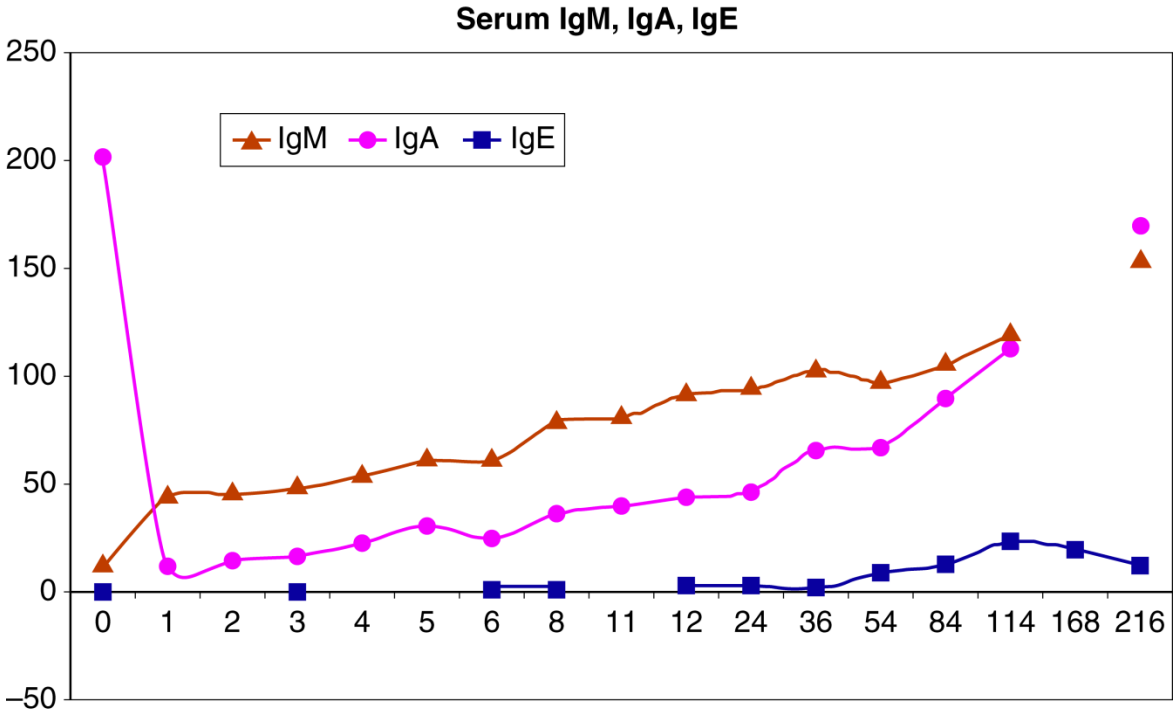


Figure 4-10. Serum IgM and IgA (mg/mL) and IgE (IU/mL) with age (months)

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Specific Ig Levels Determine Prior Immunization, Infection, Exposure, or Autoimmune Disease

Specific immunoglobulins are routinely measured in the evaluation of clinical disease. Examples of the use of specific immunoglobulins are summarized in Table 4-3.

Specific IgE Testing

HyCor Turbo-RAST: Radioallergosorbent Test—The first-generation method for determining allergen-specific IgE was the radioallergosorbent test. Paper discs (allergosorbent material) coated with allergens were incubated with a patient's serum. Antigen would bind specific immunoglobulins of all classes (IgG, IgM, IgA, IgE), and unbound immunoglobulins were washed away. Radiolabeled antihuman IgE was used to detect antiallergen IgE. Results were then compared with a reference curve.

Currently, Phadia ImmunoCAP (cellulose sponge as allergosorbent) and DPC Immulite 2000 (allergen conjugated to biotin) are used due to their excellent degree of quantification and automation.

ImmunoCAP or FEIA (Fully Automated Fluroroenzyme Immunoassay)— ImmunoCAP was developed by Phadia and is the technique employed by both Phadia and Quest. It utilizes a basic "sandwich" ELISA technique (Figure 4-11). The anti-IgE conjugate has a fluorescent enzymatic tag. Fluorescence quantifies the amount of sIgE captured in this process.

Table 4-3. Examples of Clinical Disease and Methods to Determine Specific Ig Levels

Clinical Disease	Method
Bacterial polysaccharide (*Pneumococcus, Meningococcus,* Hib)	ELISA
Tetanus	ELISA
Escherichia coli O157 LPS/HUS infection	ELISA (IgG, IgM, IgA)
Helicobacter pylori infection	ELISA (IgG, IgM, IgA)
Sm autoantibody	DID (Ouchterlony)
Alport's syndrome (anti GBM antibody)	Western blot

Abbreviations: DID, double immunodiffusion; ELISHA, enzyme-linked immunosorbent assay; Hib. *Haemophilus influenzae* type B.

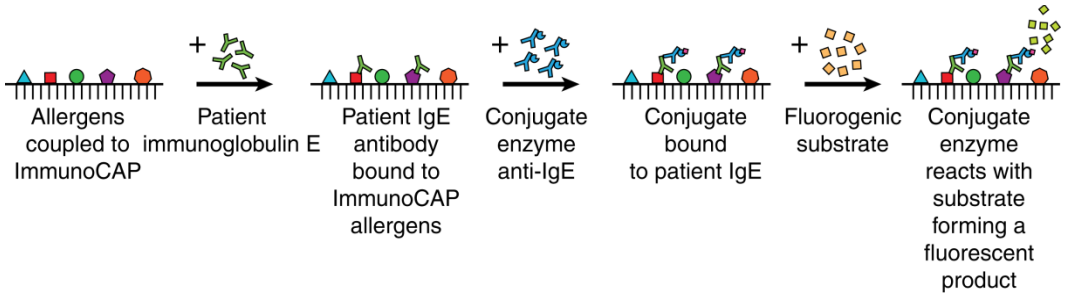


Figure 4-11. ImmunoCAP.

DPC Immulite 2000—This is a chemiluminescent enzyme-labeled immunoassay (Figure 4-12).

Specific egg, milk, peanut, cat, birch, and *Dermatophagoides farinae* IgE levels were obtained by ImmunoCAP and compared with specific IgE levels obtained by Turbo-MP (Aglient Technologies Co, Santa Clara, CA); and Immulite 2000 (Siemens Medical Solutions Diagnostics, Tarrytown, NY) assay systems by Dr. Julie Wang (Mount Sinai School of Medicine, New York, NY) and her colleagues. **She found that allergen-specific IgE levels were not comparable across the different testing modalities.** She highlighted that predictive values of specific IgE levels, which exist for the management of food allergies, is based on studies using the ImmunoCAP assay; and that these predictive values should not be applied to specific IgE levels from other assay systems.

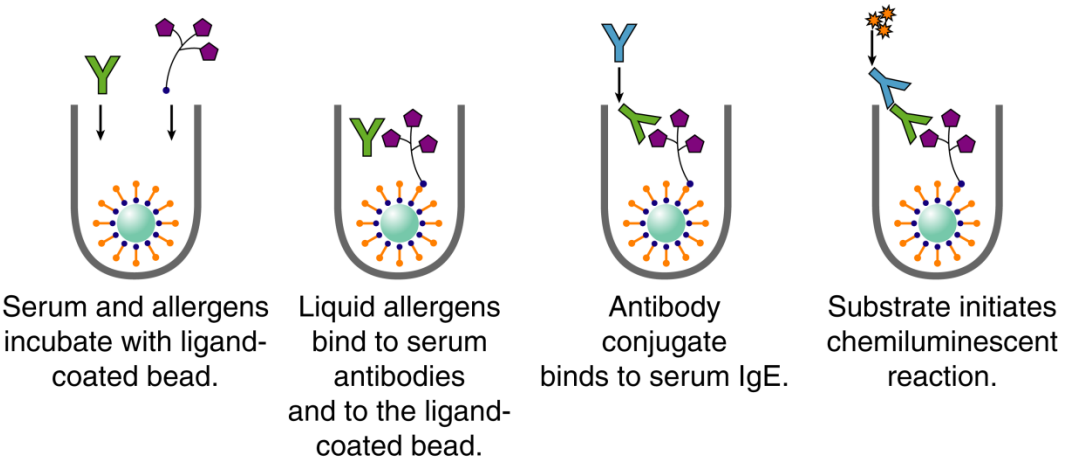


Figure 4-12. DPC Immulite 2000.

Flash Card Q2

What test(s) would you use to diagnose Lyme disease?

MEDIATOR DETECTION

Immediate hypersensitivity reactions are associated with the release of mediators, including proteases, biologic amines, proteoglycans, prostaglandins, leukotrienes, and cytokines.

Leukotrienes

- Basophils are the main source of LTC4, LTD4, and LTE4 during IgE-mediated reactions, as well as during some non-IgE-mediated reactions (e.g., nonsteroidal anti-inflammatory drug [NSAID] hypersensitivity).
- Basophil activation test uses IL-3-primed basophils that are stimulated by allergens in vitro. The release of LTC4, LTD4, and LTE4 is measured by ELISA.

Cellular Allergen Stimulation Test (CAST)

- This test became available commercially in 1993 and provides a quantitative measurement of leukotriene release.
- May be used in conjunction with the flow cytometric basophil activation test (FAST), which analyzes surface expression of CD63 on basophils following allergen stimulation.
- CAST demonstrates high sensitivity but low specificity for diagnosis of IgE-mediated allergy to inhalant allergens.
- Studies have shown a correlation between the results from food-specific basophil activation studies and food-specific IgE levels. Prediction of clinical sensitivity has not been shown to be increased. Further studies are needed.
- Up to 33% of patients with stinging insect allergy proven by provocation have negative skin tests. The combined CAST and FAST test appears to have high diagnostic efficiency in this scenario.
- Technical issues: The mixed results found in various studies may be due to the lack of standardized allergens used and the varying dose of allergen used to perform the CAST assay. The future use of recombinant allergens may overcome these problems.
- Although the histamine release test (HRT) has been used as a research tool for 40 years, it has not been applied routinely in laboratory diagnosis, mainly because it is relatively cumbersome and not very sensitive. In principle, it is very similar to CAST and shows good correlation with it; however, it has been found to be less sensitive than CAST. An additional drawback is that histamine release may be nonspecifically high (often observed in food allergy)

Flash Card A2

ELISA as a screen and Western blot to confirm

and lead to false positives in cases of allergen cytotoxicity and/or following recent in vivo allergen exposure.

Interleukins and TNF

Measured using commercially available sandwich ELISA.

Prostaglandins

Measured using radioimmunoassay (RIA).

Toll-Like Receptor (TLR)

- Real-time reverse transcription polymerase chain reaction (RT-PCR) can be performed on ribonucleic acid (RNA) samples using commercially available TaqMan assays for TLR1 through TLR10.
- Flow cytometry can also be performed to analyze cell surface and intracellular expression of TLR1 through TLR10.
- Functional Study: The responsiveness of cells to TLR stimulation can be assessed using incubation with a panel of ligands for TLR1 through TLR9, followed by measurement of inflammatory molecule production by real time RT-PCR, ELISA, and flow cytometry.

Fas (CD95 or Apo 1)

- Fas and FasL expression on the cell surface can be measured using flow cytometry.
- sFas and sFasL can be measured via sandwich ELISA using monoclonal human antibodies.
- Several assays quantify apoptosis. Labeling of DNA strand breaks by the terminal deoxynucleotidyl transferase dUTP nick end-labeling (TUNEL) reaction is the most specific of all the methods tested.

Bradykinin

RIA may be used for the measurement of plasma bradykinin levels.

Study note: ELISA or RIA assays are available for many cytokines and mediators, but they are used primarily in research settings.

CELL SURFACE MARKERS AND RECEPTORS

Function

Cell surface makers play a role in the following:

- Recognition
- Adhesion
- Signal transduction
- Cell recognition in research

Cell Surface Markers

Cluster of differentiation (CD) markers signal the presence of cell surface proteins, which leads to the identification and characterization of leukocytes.

CD markers have multiple roles:

- Cell identification
- Antigen or cytokine receptors

Table 4-4 offers a summary of CD markers.

Table 4-4. Cell Surface CD Markers

CD Marker	Other Names	Expression	Structure	Function
CD1	Five subsets CD1a– e CD1a (Leu6)	APCs	Member of Ig superfamily, binds to β_2-microglobulin	Presents autologous and bacterial lipid antigen to T lymphocytes
CD2	LFA 2 E rosette receptor	Early T and NK cells	Ig superfamily	Binds LFA3 (CD58) on APC Activates T lymphocytes Induces cytokine production Mediates adhesion between T lymphocytes and APCs Inhibits apoptosis of activated T lymphocytes

Table 4-4. Cell Surface CD Markers, cont.

CD Marker	Other Names	Expression	Structure	Function
CD3		T lymphocytes (plasma cells, macrophage) **Not found on NK cells**	Ig superfamily δ, ε, γ, and ζ chains	Required for TCR expression and signal transduction δ, ε, γ, and ζ defects causes T-B+NK+ SCID OKT3—monoclonal antibody clone, which recognizes human CD3 in the treatment of solid-organ transplant rejection and acute T-lymphocyte ALL. Leads to activation then apoptosis of T-cells causing immuno-suppression
CD14	LPS receptor	Macrophage and monocytes	Pattern recognition receptor	Detects lipoteichoic acid on GPB, and LPS on GNB, mycobacteria, and fungi Mediates IL-12 and IFNγ production
CD16	FcγRIIIA Low-affinity IgGR	NK cells, granulocytes, and macrophages		ADCC
	FcγRIIIB Low-affinity IgGR	Neutrophils	Most common IgG FcR	Phagocytosis
CD18	β_2 chain	Neutrophils, macrophages monocytes, and NK cells	Combines with α_L: LFA1, (CD11aCD18), α_M: MAC-1 and CR3 (CD11bCD18), α_X: p150,95 and CR4 (CD11cCD18)	Adhesion and signaling Defect in common β chain responsible for LAD1
CD19		Pre B lymphocytes, B lymphocytes, and follicular dendritic cells	Coreceptor with CD21	B-lymphocyte ontogeny and activation
CD20	L26 MS4A1	On B lymphocytes after CD19 expression Follicular dendritic cells	Transmembrane phosphoprotein—forms structure like ion channel—Ca influx	B lymphocyte activation and signaling
CD21	CR2 C3d receptor EBV receptor	Mature B lymphocytes and follicular dendritic cells		Binds EBV, HHV8, C3d, and CD23 High levels of CD21lo B cells are associated with CVID class Ia

Flash Card Q3

Which cell marker of the following is not found on NK cells: CD3, CD16, CD56?

Table 4-4. Cell Surface CD Markers, cont.

CD Marker	Other Names	Expression	Structure	Function
CD22	B-lymphocyte cell adhesion molecule (BL-CAM)	B lymphocytes		Inhibits B signaling
CD23	Low-affinity IgE receptor FCεRII	Activated, mature B lymphocytes and follicular dendritic cells	Type C lectin	B-lymphocyte ontogeny and activation
CD25	IL-2 Rα chain	Activated B and T lymphocytes		Suppress self-reactive T lymphocytes. prevent CTL cytolysis, and suppress NK cells. Elevated in HLH
CD27	TNFRSF7	Memory B lymphocytes	TNF receptor superfamily	B-lymphocyte activation and Ig production Memory B lymphocytes: CD27+ Memory T lymphocytes: CD27-
CD31	PECAM-1	Endothelial cells, platelets, monocytes, and macrophage	Ig superfamily	Cell adhesion and binds CD38
CD32	FcγRII (types a, b, and c)	WBCs		Binds Fc of IgG immune complexes to remove foreign antigens Binding of FcγRIIb proposed mechanism of IVIG
CD34		Adult hematopoietic stem cells		Adhesion molecule and binds CD62L (L selectin)
CD35	CR1 C3b and C4b receptor	WBCs		Binds immune complexes coated with C3b and C4b Cofactor for factor I-mediated cleavage
CD40	TNFRSF5	APCs		T–lymphocyte-dependent Ig switching Expressed on B cells Defective in HIGM3
CD44		Activated B and T lymphocytes	Surface glycoprotein	Cell adhesion
CD45	Leukocyte common antigen (LCA)	CD45RA naïve T lymphocytes and CD45RO memory or activated T lymphocytes	Protein tyrosine phosphatase	Defective in T-B+NK- SCID

Flash Card A3

CD3

Table 4-4. Cell Surface CD Markers, cont.

CD Marker	Other Names	Expression	Structure	Function
CD46	Membrane cofactor protein (MCP)	All cells (no RBC)		Cofactor for factor I-mediated cleavage Adenovirus receptor
CD49	Very late antigen (VLA a–f)	WBCs		Receptors for fibronectin, VCAM, and others in cell adhesion
CD52	CAMPATH-1 antigen	Mature lymphocytes		Target for alemtuzumab (Campath)—monoclonal antibody used in the treatment of CLL
CD54	ICAM-1	WBCs		Binds LFA-1 (CD50) Receptor for rhinovirus
CD55	Decay-accelerating factor (DAF)	Hematopoietic cells, epithelial cells, and cell matrix		Binds C3bBb and C4b2a to accelerate decay of C3 convertase Deficient in paroxysmal nocturnal hemoglobinuria
CD58	LFA-3	WBCs		Binds CD2 and adhesion
CD59	Protectin Complement regulatory molecule	All cells		Inhibits MAC formation by binding to C8 or C9
CD62	CD62E E selectin ELAM1 SELE	Endothelium		Ligand for CD15s, CD44, and CD162 Leukocyte rolling Defective in LAD2
	CD62L LECAM-1 SELL L Selectin	B and T lymphocytes, and NK cells		Lymphocyte homing to HEV of LN Binds CD34, CD15s,and MAdCAM-1
	CD62P PADGEM SELP P Selectin	Platelets Activated endothelial cells (membranes of Weibel-Palade bodies)		Binds CD162, rolling on activated endothelial cells Defective in LAD2
CD64	FcγRI High affinity IgG receptor	APC (macrophages, neutrophils, eos)		ADCC

Flash Card Q4

What is the CD marker for FcγRII?

Flash Card Q5

What is the CD marker for CD40L expressed on T-cells?

Table 4-4. Cell Surface CD Markers, cont.

CD Marker	Other Names	Expression	Structure	Function
CD95	Fas Apo-1 TNFRSF6	Activated B and T lymphocytes		Apoptosis when ligated by FasL Defective in ALPS
CD106	VCAM-1	Endothelium, fibroblasts, and respiratory epithelium		VLA-4 (α4:β1) α4β7 (act-1, LPAM-1)
CD154	CD40L TRAP	T lymphocytes		Regulates B lymphocyte function Expressed on T-cells Defective in XHIGM
CD158	KIR (KIR2DL, NKG2A, and others)	NK and T		Binds HLA class I and inhibits NK or T cell cytotoxicity
CD159	NKG2A	NK		Modulates NK killing
CD162	P-selectin glycoprotein ligand-1 (PSGL-1)	Myeloid cells Activated T lymphocytes		Adhesion with endothelial cells
CD178	CD95 ligand FasL	Activated, cytotoxic T lymphocytes		Apoptosis Fas-expressing cells

Abbreviations: ADCC, antibody-dependent cell-mediated cytotoxicity; ALL, acute lymphoblastic leukemia; ALPS, autoimmune lymphoproliferative syndrome; APC, antigen-presenting cell; CIVD, common variable immunodeficiency; CLL, chronic lymphocytic leukemia; EBV, Epstein-Barr virus; GNB, gram-negative bacillus; GPB, gram-positive bacillus; HEV, high endothelial venule; HIGM, hyper-IgM; HHV, human herpesvirus; HLA, human leukocyte antigen; HLH, hemophagocytic lymphohistiocytosis; IVIG, intravenous immunoglobulin; LAD, leukocyte-adhesion deficiency; LFA, leukocyte function-associated antigen; LN, lymph node; LPS, lipopolysaccharide; MAC, membrane attack complex; MAdCam, mucosal addressin cell adhesion molecule; NK, natural killer; SCID, severe combined immunodeficiency disease; TCR, T-cell receptor; TNF, tumor necrosis factor; XHIGM, X-linked hyper-IgM.

CD4 and CD8 are T–cell-receptor coreceptors. A comparison between CD4 and CD8 is summarized in Table 4-5.

Flash Card A4

CD32

Flash Card A5

CD154

Table 4-5. CD4 versus CD8

	CD4	CD8
Other names	OKT4 CD223	OKT8
MHC restriction	MHC class II	MHC class I
Cell expression	T Helper cells Monocytes Phagocytes Dendritic cells Langerhans cells Thymocytes	Cytotoxic T lymphocytes Cortical thymocytes NK cells Dendritic cells
% T-lymphocyte expression	65%	35%
Structure	Ig Superfamily Monomer	Ig Superfamily Heterodimer CD8α CDβ
Domain-binding MHC	β2	α3
Function or biological properties	MHC-restricted antigen-induced T-lymphocyte activation HIV receptor on T lymphocytes Downregulated by HIV nef protein during infection	MHC-restricted antigen-induced cytolysis

Abbreviations: MHC, major histocompatibility complex; NK, natural killer,

CD28 Family

Costimulation provides signal 2, which leads to proliferation of T lymphocytes. The CD28 family of receptors plays an important role in signal 2 and is summarized in Table 4-6.

Table 4-6. CD28 Family of Receptors, Their Ligands, and Functions

CD28 Receptors		B-7 Family Ligand		Function
CD28	T lymphocytes	CD80 (B71) CD86 (B72)	(APCs) DC Macrophage B lymphocytes	Costimulation or activation
CTLA4 (CD152)	Activated T lymphocytes	CD80 (B71) CD86 (B72)		Costimulation or inhibition
ICOS	T lymphocytes	ICOS-L		Costimulation
B7-H1 (PD-L1)	DCs Macrophages B lymphocytes	PD 1	B lymphocytes T lymphocytes	Inhibition
B7-DC				

Abbreviations: APCs, antigen-presenting cells; DC, dendritic cells.

Flash Card Q6

What is the MHC class restriction for CD4 and CD8?

Cell Surface Receptors

Cell surface receptors have been classified by function and structure. A summary of commonly encountered receptors are found in Tables 4-7, 4-8, and 4-9.

Table 4-7. FcγR Family

	Other Names	Cell	Function	Ligand
CD16	FcγRIIIA	NK cells	ADCC	IgG—low affinity
	FcγRIIIB	Neutrophils	Phagocytosis	IgG—low affinity
CD32	FcγRIIB	B lymphocytes, dendritic cells, macrophages	Feedback inhibition of B cells, dendritic cells, macrophages	IgG—low affinity
	FcγRIIA	Macrophage Neutrophils Eosinophils Platelets	Phagocytosis	IgG—low affinity
CD64	FcγRI	Macrophage Neutrophils Eosinophils	Phagocytosis	High-affinity IgG (IgG1, IgG3 and monomeric IgG)

Abbreviation: ADCC, antibody-dependent cell-mediated cytotoxicity.

Table 4-8. FcεR Family

Receptor	Cell	Function	Ligand
FcεRI	Mast cells Basophils Eosinophils Dendritic cells	Degranulation Antigen uptake	Monomeric IgE high affinity
FcεRII (CD23)	B lymphocytes Eosinophils	Unknown	IgE Low affinity

Flash Card A6

CD4 = MHC II and CD8 = MHC I

Tumor Necrosis Family Receptor Family (TNFR Family)

The TNFR family includes the following cell surface receptors, which have a role in cellular apoptosis. They are reviewed in Table 4-9.

Complement Receptors

Complement receptors have an important role in bridging innate immunity with cell-mediated immunity. Innate responses generate complement components, which bind to receptors on WBCs. Ligand-receptor interaction leads to signaling pathways, cytokine release, and engagement of cell-mediated immunity. Complement receptors have also been utilized by microbes as the mechanism of entry into host cells. A summary of complement receptors is provided in Table 4-10.

Key Fact

Activating mutation of CXCR4 causes the phagocytic defect, WHIM (or wart, hypogammaglobulinemia, infection, and myelokathexis) syndrome.

CR3 is the most potent complement receptor and binds iC3b, which is an opsonin like Ig.

A hereditary deficiency of C3 will lead to defective phagocytosis of encapsulated bacteria and will present with autoimmune disease and repeated infections.

Key Fact

CCR5 and CXCR4 are HIV coreceptors

Chemokine receptors are G-coupled, seven-transmembrane proteins that play a role in lymphocyte trafficking. A summary of chemokine receptors is found on Table 4-11.

Table 4-9. TNFR Family

Receptor	Cell	Function	Ligand
TNF RI/p55	Most cells	Apoptosis	TNF (membrane bound and trimeric soluble forms)
TNFR2/p75	Immune cells		TNF (membrane-bound homotrimer form)
LT-βR	Epithelial cell Myeloid cells (Not B and T lymphocytes)	Apoptosis IL-8 release	TRAF Lymphotoxin
Fas	Activated B and T lymphocytes	Apoptosis	FasL
CD40/CD134	Activated T lymphocytes	Second signal	CD40L TRAF
OX40 Ligand	Activated T lymphocytes	Implicated in cytokine storm of H1N1	TRAF
RANK	Osteoclasts Dendritic cells Some macrophages	Activation of osteoclasts	RANK ligand TRAF

Abbreviations: RANK, receptor activator of nuclear factor κ-B; TNF, tumor necrosis factor; TRAF, TNF receptor-associated factor.

Flash Card Q7

Which PID is associated with defective CD40 and which is associated with defective CD40L?

Table 4-10. Complement Receptors

Complement Receptor	Other Names	Cell	Function	Ligand
CR1	CD35	Monocytes Neutrophils T and B lymphocytes Eosinophils RBCs	Phagocytosis Cofactor for cleavage of C3b, c4b Clears immune complexes	C3b C4b iC3b
CR2	CD21	B lymphocytes FDC	B-lymphocytes activation EBV receptor	C3d iC3b c3dg
CR3	Mac-1 CD11b/CD18	Monocytes Neutrophils NK cells	Phagocytosis Adhesion	iC3b ICAM-1 bacteria
CR4	p150.95 CD11c/CD18	Monocytes Neutrophils NK cells	Phagocytosis	iC3b, ICAM-3
CRIg	Complement receptor of the immunoglobulin family	Macrophage in liver (Kupffer cells)	Phagocytosis	C3b iC3b Inhibits alternative pathway convertases

Abbreviations: EBV, Epstein-Barr virus; FDC, follicular dendritic cell.

Table 4-11. Chemokine Receptors

Chemokine Receptor	Cell	Function	Ligand	Chemokine Receptor
CC	CCR3	Macrophage Eosinophils Basophils T_h1 T_h2 Airway epithelial cells	Implicated in allergic disease	CCL11 (eotaxin-1) CCL5 (RANTES) CCL7 (MCP-3) CCL8 (MCP2) CCL13 (MCP4) CCL26 (eotaxin-3)
	CCR4	CD4 T lymphocytes DCs Basophil Macrophage Platelets	T-lymphocyte trafficking	CCL17 (TARC) CCL22 (MDC)
	CCR5	T lymphocytes Monocytes	Cell trafficking. HIV coreceptor	CCL3 (MIP 1α) CCL4 (MIP 1β) CCL5 (RANTES) CCL11 (eotaxin-1) CCL14 (HHC-1) CCL16 (HHC-4)
	CCR7	T lymphocytes DCs (EBV-infected B lymphocytes)	Naïve T lymphocyte and DC trafficking to LN	CCL19 (MIP-3β/ELC) CCL21 (SLC)

Flash Card A7

Defective CD40 = HIGM3 and defective CD40L = XHIGM

Table 4-11. Chemokine Receptors, cont.

Chemokine Receptor	Cell	Function	Ligand	Chemokine Receptor
CXC	CXCR4	Mature blood cells, blood progenitor cells, epithelial cells	B lymphocyte development HIV co-receptor	CXCL12 (SDF-1)
	CXCR5	B lymphocytes	Home to B-T junction in LN	CXCL13 (BCA-1)
XCR	XCR1		T and NK cells recruitment	XCL1 (lymphotactin)
CX3CRI	CX3CRI		Recruits T, NK, and Macrophages Activates CTL and NK	CX3CL1(factalkine)

Abbreviations: DCs, dendritic cells; EBV, Epstein-Barr virus; LN, lymph node; RANTES, regulated on activation, normal T expressed and secreted.

Cell Surface Markers on Specific Cell Types

Cell surface markers on lymphocytes are expressed differentially, based on the maturity of the cell. For example, some of these markers allow lymphocytes to follow a restricted pattern of circulation. Naïve lymphocytes home in preferentially on secondary lymphoid organs such as lymph nodes, spleen, and Peyer's patches. This ensures a maximal probability of a diverse TCR repertoire coming in contact with unique antigens. Once activated, lymphocytes enter the circulation, whereby they encounter antigen residing in different tissues to elicit a strong memory response.

A summary of cell surface makers and activating and inhibitory receptors is shown in Table 4-12.

- All nucleated cells express MHC I.
- B lymphocytes express MHC I and MHC II.
- CD4 T-cells express MHC II.
- Switched memory B lymphocytes lose expression of surface IgD.
- CLA-1 on T lymphocytes mediates homing to skin.
- A4β7 on T lymphocytes mediate homing in on colonic tissue by binding to MAdCAM-1.

In addition, key cell surface markers of other cell types are reviewed in Table 4-13.

Key Fact

NK cells lack expression of CD3 and TCR.

Flash Card Q8

Which complement receptor is also known as CD11c/CD18?

Flash Card Q9

What lymphocyte cell surface marker is present on memory B cells and absent on memory T cells?

Table 4-12. Lymphocyte Cell Surface Markers

	B lymphocyte	T lymphocyte	NK Cells
Naïve	IgM/IgD CXCR5	L Selectin CCR7 CD45RA	CD2
Activated	IgA IgE IgG CD27	CD40L (CD154) CD28 CTLA4 CD 25 CD44 CD45RO CXCR3 CCR5 HLADR	
Memory	IgG IgA IgE CD27+	CCR7 L-Selectin (CD62L) IL-7R IL-15 (CD8 only) CD44 CD45RO MHCI CD27-	
Receptors			
Inhibitory	CD22		NKG2A (CD94) ILT-2 KIR
Activating		IL-12 Rβ IL-18 R	CD16 NCR KIR2DS CD92/NKG2C NKG2D

Abbreviation: NK, natural killer.

Table 4-13. Cell Surface Markers

Cell	Surface Markers
Monocyte or macrophage	FcγR
Endothelial cell	Selectins (CD62E)
NKT cell	Vα24-Jα18 Vβ11
Mast cell	CD117 (c-KIT)

Abbreviations: KKT, natural killer T cell.

Flash Card A8

CR4

Flash Card A9

CD27

Dendritic cells are divided into:

- Conventional dendritic cells (myeloid, lymphoid)
    - Act as antigen-presenting cells
- Plasmacytoid dendritic cells
    - Produce IFNα

Surface markers differentiate between the subsets of dendritic cells, as shown in Table 4-14.

Cell surface markers play an important role in cell function and survival. Clinical disease results as a defect in expression or in function, or by opportunistic use by a pathogenic organism. Further evaluation of cell surface markers will provide greater insight into the normal physiology and disease manifestation using cells of the immune system.

Table 4-14. Human Dendritic Cell Surface Markers

	Conventional Dendritic Cells	Plasmacytoid Dendritic Cells
CD1a-3	High	Negative
CD11c	High	Negative
CD11b	Variable	Negative
TLR	TLR 4 TLR5 TLR8	TLR 7
CD80/CD86	Inducible	
ILT7 (CD85g)	Negative	High
CD33 (Siglec - 3)	High	
CD209 (DC SIGN)	High	
CMKLR1	High	In vitro expression
TCL1	Negative	High

Abbreviations: DC SIGN: dendritic dell-sp

LYMPHOCYTE FUNCTION: CELL PROLIFERATION, CYTOKINE PRODUCTION, AND CYTOTOXICITY

Adaptive immunity reflects the function of T lymphocytes, B lymphocytes, and NK cells. The first step performed in an immunologic evaluation is a complete blood count (CBC) that provides an absolute lymphocyte count (ALC). The ALC reflects the sum of all lymphocyte populations in the given peripheral blood specimen. The ALC is normal or decreased in most of the cellular primary

immunodeficiency disorders; however, it is profoundly decreased in most forms of severe combined immune deficiency (SCID), and increased ALC does not rule out these disorders. Flow cytometry enables the quantitative enumeration of lymphocyte subsets (T and B lymphocytes, and NK cells) in peripheral blood.

Measuring T-Cell Number and Function

Enumeration of T Lymphocyte Subsets—Flow cytometric analysis of lymphocytes enumerates the T-lymphocyte compartment. T lymphocyte subsets are identified by their cell surface molecules (Table 4-15).

Measuring in Vitro T-Cell Function

In Vitro T-Cell Proliferation Assay by ^{3}H Thymidine Incorporation—The lymphocyte proliferation assay (LPA) is an in vitro method of assessing lymphocyte-mediated immunity. T lymphocytes undergo clonal proliferation when stimulated in vitro by a mitogen (polyclonal) or recall antigen. (Table 4-16).

Table 4-15. Cell Surface Markers on T Lymphocyte Subsets

Cell Surface Marker	T-Cell Subset
CD3	T cells
CD3 CD4	CD4 T lymphocytes (helper)
CD3 CD8	CD8 T lymphocytes (cytotoxic)
CD4 HLA-DR	Activated CD4 cells
CD8 HLA-DR	Activated CD8 cells
CD45RA	Naïve T lymphocytes
CD45RO	Activated T lymphocytes
CD4-CD8-	Double-negative T lymphocytes
CD4-CD8-γδ	γδ T lymphocytes
CD3 CD25	T regulatory cells (highly expressed); activated T cells

Table 4-16. T Cell Proliferation in Response to Recall Antigens or Mitogens

	Antigen-Specific T-Lymphocyte Proliferation (Recall Antigen)	Polyclonal T Lymphocyte Proliferation (Mitogen)	
T cells	Activated and memory	Naïve, activated, and memory	
Prior exposure required	Yes	No	
Antigens	CMV Tetanus VZV antigens HIV antigens gp 120, p24 Candida	Cell surface signaling Phytohemagglutinin (PHA) Pokeweed mitogen (PWM) Concanavalin A (ConA) anti-CD3 antibody	Bypass proximal signaling Phorbol ester (PMA) Calcium ionophore (Ionomycin)
Costimulation needed	No (but requires antigen presenting cells)	No	No

Abbreviation: VZV, varicella-zoster virus.

Lymphocyte Proliferation Assay (LPA) Stimulation Index

- Cells are incubated in culture for a week. On day 3 after mitogen stimulation, and on day 6 for antigens specific T lymphocyte-specific stimulation, radioactive [^{3}H] (tritiated) thymidine is added and is incorporated into the newly synthesized DNA of the dividing cells.
- The amount of radioactivity incorporated into the DNA of each culture well is measured in a scintillation counter and is proportional to the number of proliferating cells. In the absence of proliferation of defective lymphocytes, less radioactivity is detected.
- The readout is measured in counts per minute (cpm).
- Note that pokeweed mitogen also has B-cell proliferation activity along the T-cell proliferation capacity.

Stimulation Index (SI) = (cpm Mitogen / cpm background Unstimulated)
Net counts or cpm = (cpm Mitogen – cpm background Unstimulated)

In Vitro T-Lymphocyte Proliferation Assay by Flow Cytometry

Peripheral blood mononuclear cells (PBMCs) are labeled with a proliferation tracking fluorescent dye (carboxyfluorescein diacetate succinimidyl ester, CFSE) and stimulated with appropriate mitogens for 4 days. Flow cytometric evaluation of T-cell proliferation is shown in Figure 4-13.

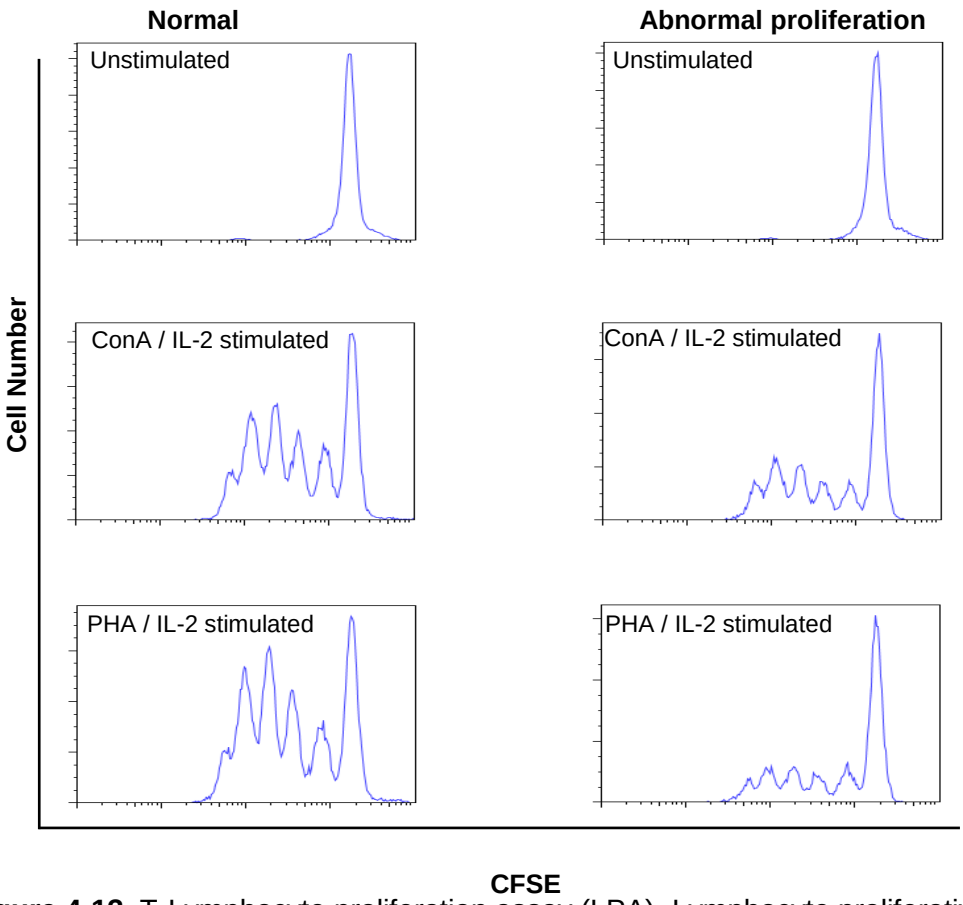


Figure 4-13. T-Lymphocyte proliferation assay (LPA). Lymphocyte proliferative response is demonstrated by a progressive twofold reduction in the fluorescence intensity of CFSE (read from right to left, in the graphs above) as measured by flow cytometry.
(Reproduced, with permission, from Dennis W. Schauer, Jr., Trivikram Dasu, PhD, and James W. Verbsky, MD, PhD, Clinical Immunodiagnostic & Research Lab, Medical College of Wisconsin.)

Upon stimulation and following proliferation of dividing T cells, there is a dilution of CFSC. This will lead to its decreased concentration. In the absence of proliferation of defective lymphocytes, there will be no decrease of fluorescence detected by the machine given the absence of dilutional effect.

Cytokine Production

Measuring cytokine production by activated T lymphocytes is more often done in experimental laboratories and is often not used routinely in clinical evaluation.

In Vivo T-Lymphocyte Function

Delayed-type hypersensitivity (DTH) is a cost-effective and widely available method to screen for cell-mediated immunity. Clinically, it is used most commonly to determine prior tuberculosis exposure (purified protein derivative, PPD), although the test can be done with a number of other recall antigens (*Candida*, tetanus, *Tricophyton*, etc.).

DTH assesses the integrity of cell-mediated immunity. Patients who have a lack of response to ubiquitous antigens are susceptible to intracellular pathogens, such as viruses, fungi, protozoa, and parasites. In acquired immunodeficiency due to HIV, DTH could used to clinically monitor the progression of disease. Anergy (i.e., lack of DTH response) indicates the likelihood of disease progression.

Anergy can be seen in < 5% of the general healthy population who have been exposed to a antigen. In this case, an in vitro T-lymphocyte function assay should be employed. However, a lack of exposure, as well as a number of other conditions, could explain a negative DTH response.

Caution should also be advised if a patient has had repeat DTH testing. A booster effect can be seen by testing that occurs within 3 months between tests.

The DTH test can be used to determine the presence or history of exposure to an infectious agent. Tuberculosis, histoplasmosis, blastomycosis, and leishmaniasis can be diagnosed with DTH testing. DTH testing employs the Mantoux skin testing method as described in Figure 4-14.

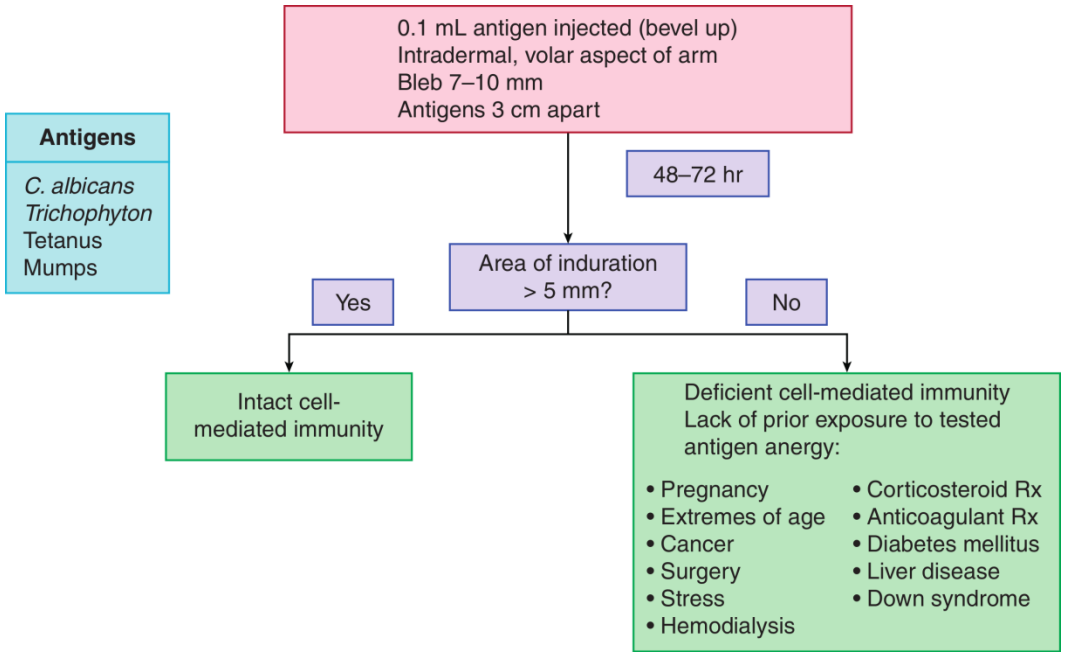


Figure 4-14. Mantoux test and interpretation.

Measuring B-Lymphocyte Function

- ***Enumeration of B-Lymphocyte Subsets—***Enumeration of B lymphocytes in the peripheral blood is accomplished with flow cytometry. B-lymphocyte numbers are characterized by their hallmark cell surface markers, CD19 and CD20.
- CD19 is part of the CD19, CD21, CD81 coreceptor complex and functions to amplify B-lymphocyte receptor-generated signals. CD19 is expressed on early B-lymphocyte progenitors up until the B-lymphocyte plasmablast stage of differentiation.
- CD20 is expressed on pre-B lymphocytes and B-lymphocyte blasts, but are not seen in early B lymphocytes or plasma cells.

In patients with X-linked agammaglobulinemia (XLA), Bruton agamma-globulinemia tyrosine kinase (BTK) is absent in B lymphocytes and monocytes. CD19+ B lymphocytes are absent.

For examples of flow cytometric patterns for XLA, see Figures 4-15; for patterns in common variable immunodeficiency (CVID), see Figure 4-16.

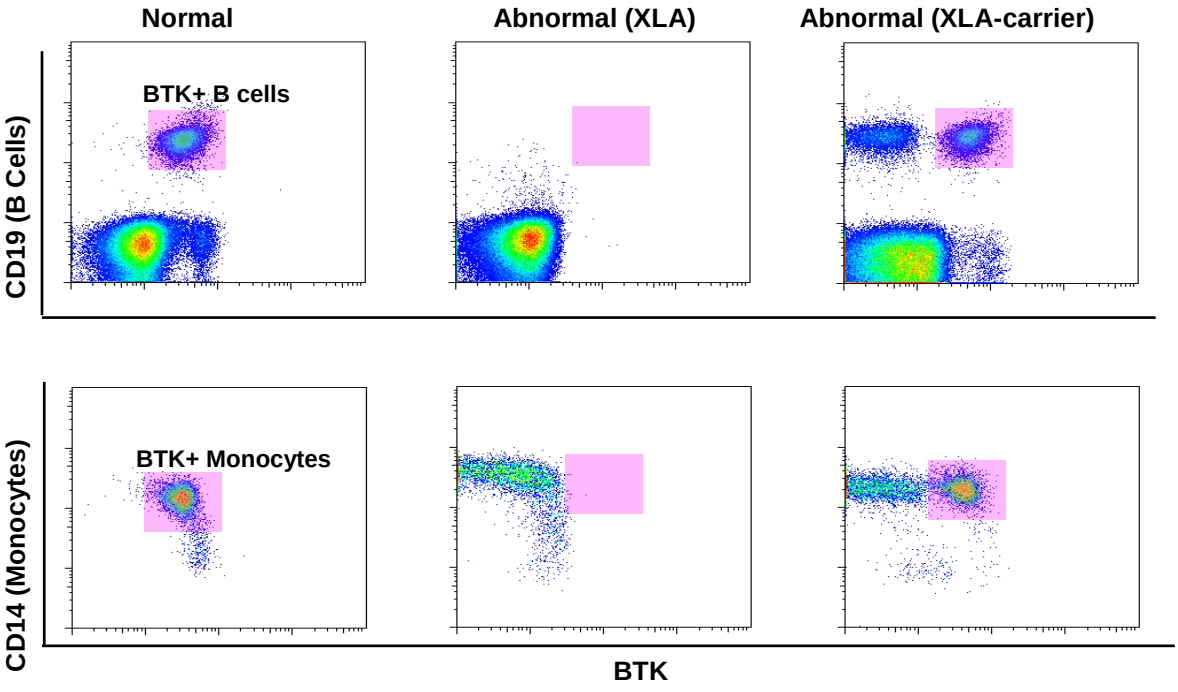
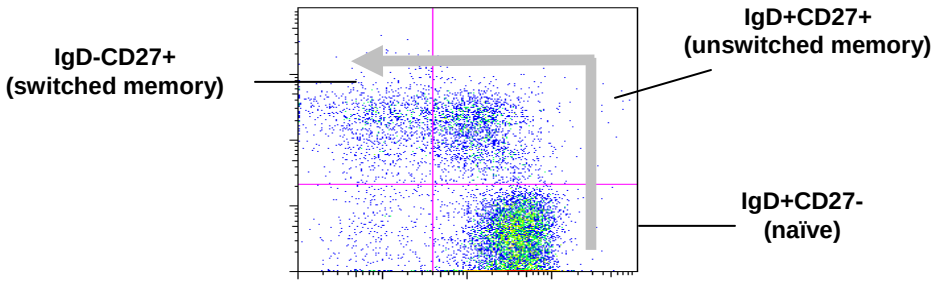


Figure 4-15. Flow cytometric assessment of B lymphocytes: XLA. Bruton's tyrosine kinase (BTK) is expressed in normal B lymphocytes and monocytes. In patients with X-linked agammaglobulinemia (XLA), CD19 B lymphocytes and BTK expression in monocytes are absent. Carrier status is determined by reduced CD19 B lymphocytes and BTK expression.
(Reproduced, with permission, from Dennis W. Schauer, Jr., Trivikram Dasu, PhD, and James W. Verbsky, MD, PhD, Clinical Immunodiagnostic & Research Lab, Medical College of Wisconsin.)



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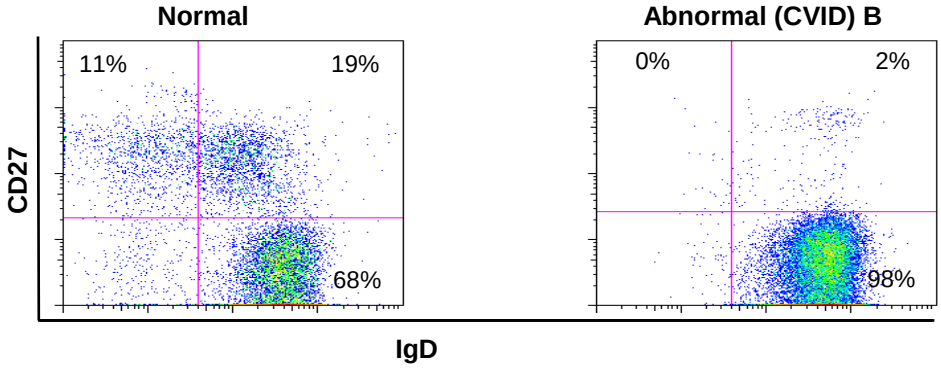


B

Figure 4-16. Flow cytometric assessment of memory B lymphocytes: CVID. Abnormal populations of naïve and memory B lymphocytes in patients with common variable immune deficiency (CVID): (A) Arrow demonstrates normal B-lymphocyte maturation. (B) Case scenarios of normal and abnormal B-lymphocyte maturation.
(Reproduced, with permission, from Dennis W. Schauer, Jr., Trivikram Dasu, PhD, and James W. Verbsky, MD, PhD, Clinical Immunodiagnostic & Research Lab, Medical College of Wisconsin.)

B-Lymphocyte Proliferation

B-lymphocyte proliferation can also be measured by the lymphocyte proliferation assay. Whole blood or peripheral blood mononuclear cells (PBMCs) are cultured with mitogens [i.e., *Staphylococcus aureus* Cowan I (SAC), anti-IgM, pokeweed mitogen (PWM), and tetanus toxoid] for up to a week.

A stimulation index is generated by measuring the amount of $[H]^3$ thymidine incorporated into DNA and expressed as:

- [counts per minute of stimulated wells] / [counts per minute of control wells]
- [counts per minute of stimulated wells] – [counts per minute of control wells]

Alternatively, flow cytometry can be used.

Flash Card Q10

Which mitogens stimulate T cells?

Flash Card Q11

Which mitogens stimulate B cells?

B-lymphocyte secretion of immunoglobulins in cell culture supernatants after stimulation can be determined by ELISA or ELISPOT.

Immunoglobulin Measurement

- Total serum immunoglobulin measurement (IgM, IgG, IgA, and IgE) is the best screening test for B-lymphocyte function.
- Response to immunizations can be evaluated by serology.

Controversy still exists around what constitutes an adequate response to immunization. Table 4-17 provides a summary of immunizations and their role in assessing B-lymphocyte function.

Measuring NK Cell Function

In Vitro NK or CD8 T Lymphocyte Cytotoxicity—Perforin and granzyme B expression in NK and CD8 T lymphocyte can be used to determine cytotoxic cell function (Figure 4-17).

Key Fact

Protein vaccines and conjugated vaccines enlist T-cell help.

The granules of NK cells coat in the interior with CD107a. Expression of CD107a on the surface of an NK cell is indicative of degranulation. Absence of CD107a on the surface suggests failure of degranulation.

The assessment of lymphocyte function is a useful clinical tool in the assessment of some primary immune deficiencies.

Table 4-17. Immunizations Assess B-Lymphocyte Function with or Without T-Lymphocyte Help

	Protein	Conjugated Polysaccharide	Unconjugated Polysaccharide
Immunizations	Diphtheria Tetanus	*Haemophiuls influenzae* Prevnar	*Neisseria meningitides* Pneumovax
T-lymphocyte help?	Yes	Yes	No
Age	All ages	All ages	Only >2 years of age can induce response

Flash Card A10

Pokeweed, phytohemagglutinin, and concanavalin A

Flash Card A11

Pokeweed, LPS, and SAC

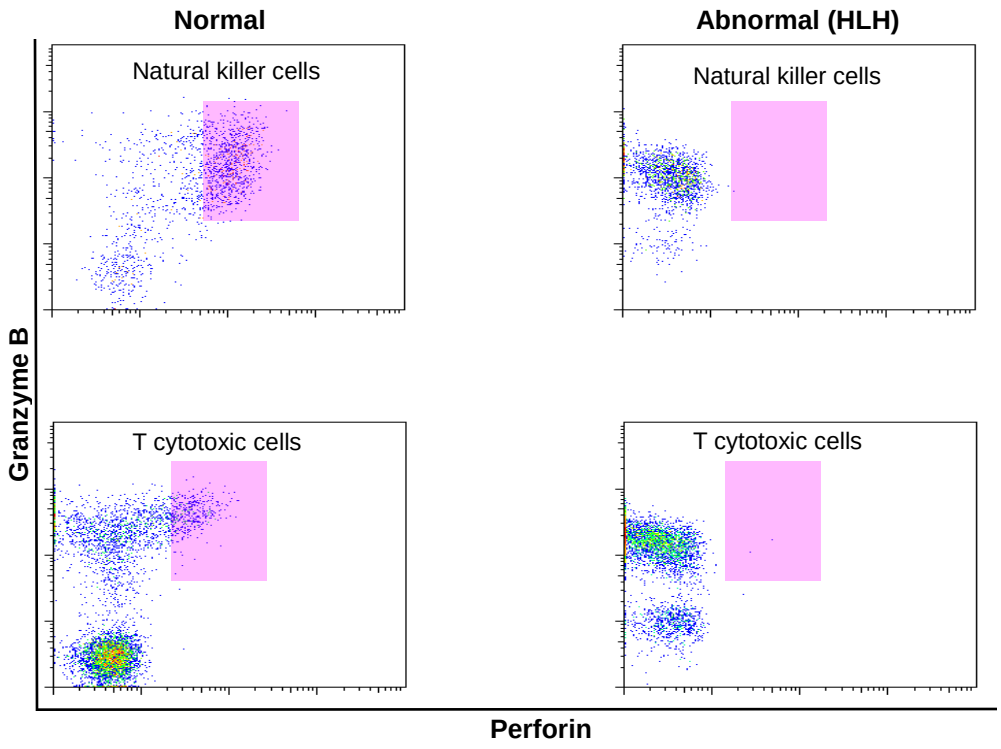


Figure 4-17. Perforin and granzyme B expression in cytotoxic lymphocytes and NK cells. Deficient expression can be seen in patients with hemophocytic lymphohistiocytosis (HLH).
(Reproduced, with permission, from Dennis W. Schauer, Jr., Trivikram Dasu, PhD, and James W. Verbsky, MD, PhD, Clinical Immunodiagnostic & Research Lab, Medical College of Wisconsin.)

CHEMOTAXIS

Chemotaxis is the ability of a protein to direct the traffic and migration patterns of a specific cell. Basically, an assay creates a gradient of the chemotactic agent and allows cells to migrate through a membrane toward said agent. If the agent is not chemotactic for the cell, then the cells will remain on the membrane. If the agent is chemotactic, then the cells will move through the membrane and settle on the chemotaxis plate.

Flash Card Q12

Which surface markers are not present in leukocyte adhesion deficiency 1?

This type of test is used generally for testing neutrophils, and it can reveal specific defects in this molecule. It is important for diagnosing neutrophilic disorders, such as the following:

- **Chédiak-Higashi syndrome:** Neutropenia, recurrent skin, sinus, pulmonary infections, oculocutaneous albinism, mitral regurgitation (MR), neuropathy. Diagnosis made with detection of giant granules seen on a manual smear, and mutations in LYST 1q42
- **Leukocyte adhesion deficiency 1 (LAD1):** Missing CD18 protein; chemotaxis and adhesion of leukocyte to wall of endothelium and diapedesis impaired; recurrent necrotizing infections of lung, GI tract and skin; umbilical stump separation delayed beyond 1 month of age; poor wound healing; cigarette paper scarring. Diagnosis made through impaired chemotaxis, but can also be made with flow cytometry that shows abnormal CD18
- **Ras-related C3 botulinum toxin substrate 2 (RAC2) deficiency:** RAC facilitates actin cytoskeleton regulation; therefore deficiency causes adhesion problems and impaired chemotaxis. Clinically resembles LAD 1. Transmission autosomal dominant.

Another area of chemotactic importance is in the complement system. Active metabolites C4a, C3a, and C5a are potent anaphylatoxins.

PHAGOCYTOSIS AND CELL KILLING

Phagocytosis is an active process by which solid material (i.e., >500 nm in diameter), such as microbes and apoptotic cells, are engulfed within a cell and killed. Classic examples of phagocytic cells include neutrophils and macrophages.

PHAGOCYTOSIS

- Phagocytic cells express various receptors, such as **mannose receptors**, **scavenger receptors**, and **Toll-like receptors,** that can recognize pathogens.
- They also express **opsonin receptors**, which increase phagocytosis of bacteria coated with IgG antibodies or with complement.
- Bound pathogen is surrounded by the phagocyte cell membrane and then internalized in a membrane-bound vesicle known as a **phagosome** (Figure 4-18).

Flash Card A12

CD18, which is part of CD11a/CD18 (LFA-1), CD11b/CD18 (Mac-1), and CD11c/CD18 (p150/95)

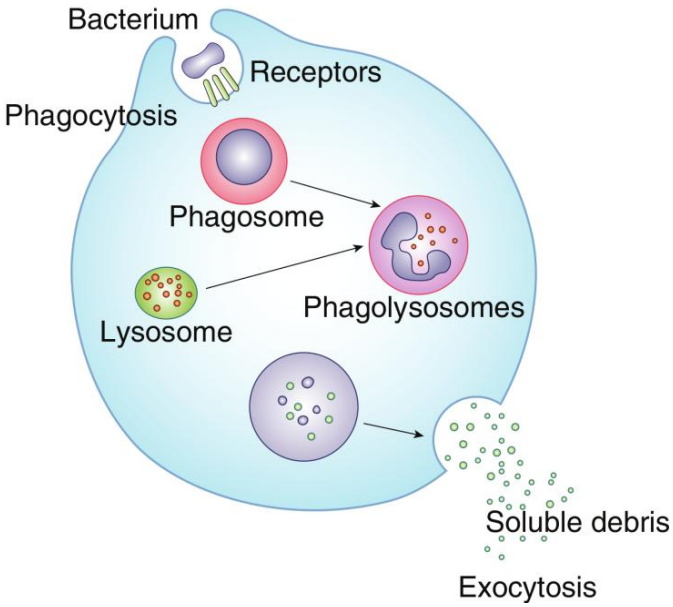


Figure 4-18. Phagosome.

KILLING

- The phagosome becomes **acidified**, killing most pathogens.
- Macrophages and neutrophils have lysosomal granules that contain microbicidal enzymes, proteins, and peptides.
- The phagosome fuses with the lysosome, producing the **phagolysosome,** in which lysosomal contents are released to destroy the ingested pathogen.
- Ingestion of microorganisms activates the phagocyte to assemble the multisubunit enzyme **NADPH oxidase** from its components. The enzyme consists of several subunits and converts molecular oxygen to oxygen free radicals, which are toxic to bacteria (Figure 4-19).
- Myeloperoxidase (MPO) uses hydrogen peroxide to convert normally unreactive halide ions (Cl^- and Br^-) into reactive hypohalous acids [i.e., hypochlorite (HOCL) and hybromite] that are toxic to bacteria.
    - MPO deficiency is a phagocytic disorder that is asymptomatic in most individuals. Recurrent candida infections (skin, bones, blood) have been described rarely in patients with diabetes mellitus and partial/complete MPO deficiency.

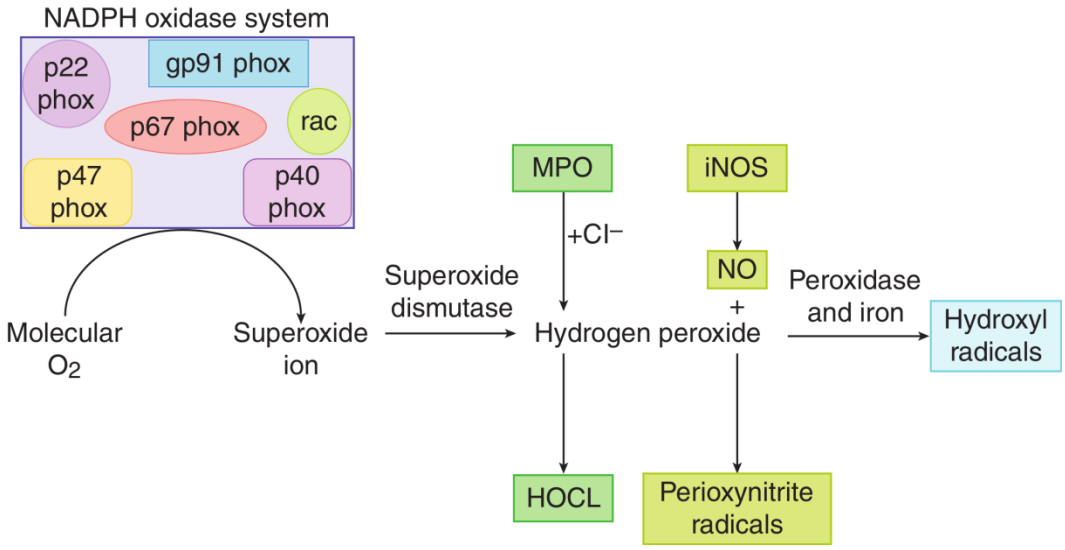


Figure 4-19. Respiratory burst and intracellular killing.

- **Macrophages produce** reactive nitrogen intermediates, mainly **nitric oxide**, by the action of inducible **nitric oxide synthase (iNOS)**, which can combine with hydrogen peroxide to form peroxynitrite radicals, which are toxic to bacteria.

Key Fact

The importance of the respiratory burst is highlighted in patients with **chronic granulomatous disease (CGD)**, which is caused by **mutations in the genes encoding the NADPH oxidase system**. Individuals with CGD have defective intracellular killing of bacteria and fungi that predispose them to infections with **catalase-positive organisms** (e.g., *Staphylococcus aureus*, *Serratia marcescens*, *Pseudomonas,* and *Salmonella* spp.).

Bactericidal Killing Assay

This assay measures the respiratory burst, which is responsible for the generation of reactive oxygen species and intracellular microbial killing. Respiratory burst activity can be measured by several methods, including nitro blue tetrazolium (NBT) reduction test, dihydrorhodamine 123 (DHR) oxidation test, and chemiluminescence assay.

NBT Reduction Test—The oldest and best-known laboratory test for CGD is the NBT test. This provides a simple and rapid (but largely **qualitative**) determination of phagocyte NADPH oxidase activity. Neutrophils are mixed with NBT and stimulated with phorbol myristate acetate. Normal neutrophils reduce the NBT to formazan, which changes color from yellow to blue. (PAM) Patients with CGD lack a component of the oxidase system that produces superoxide anion and, thus, cannot reduce NBT to formazan. Figure 4-20 depicts a visual representation of normal and abnormal NBT test results.

This test is no longer the gold standard for the diagnosis of CGD.

Normal (positive test)	Abnormal (negative test)
Large cells with an orange-red nucleus and blue cytoplasm 	Small cells with an orange-red nucleus and colorless cytoplasm 

Figure 4-20. Interpreting NBT reduction test results.

- Most patients with X-linked CGD will have nearly absent reduction of the NBT, so **mostly** negative cells (yellow).
- Patients with **autosomal recessive CGD** may have some positive cells, leading to a missed diagnosis.
- **Carriers of the autosomal recessive form of CGD** usually have normal NBT results.
- In the X-linked carrier state, usually between 30–80% of the cells will be negative (yellow). The results are variable and can range between 10–90% negative cells.

Dihydrorhodamine 123 (DHR) Oxidation Test—In this test, the nonfluorescent rhodamine derivative, **DHR, is taken up by phagocytes and oxidized to a green fluorescent compound by products of the NADPH oxidase**. The fluorescence emitted is detected by flow cytometry. The DHR test is preferred because it is a **quantitative** assay, offers relative ease of use, enables **distinction between X-linked and autosomal forms of CGD**, as well as providing the ability to **detect gp91phox carriers**. Other tests can provide reliable diagnosis of CGD, but cannot distinguish carrier status or they require significant operator experience. Figure 4-21 shows the normal and abnormal results with a DHR assay.

Chemiluminescence Assay—In this assay, light is generated from the interaction of reactive oxygen species (generated during respiratory burst) with ingested microorganisms. The light can be detected using a scintillation counter, with the amount released reflecting the respiratory burst activity of the cells. It is more sensitive than the NBT test.

Interpretation:

- Patients with CGD lack a chemiluminescence response.
- Maternal carriers of X-linked CGD have intermediate values.

Flash Card Q13

Which test can distinguish among X-linked, autosomal, AND X-linked carrier forms of CGD?

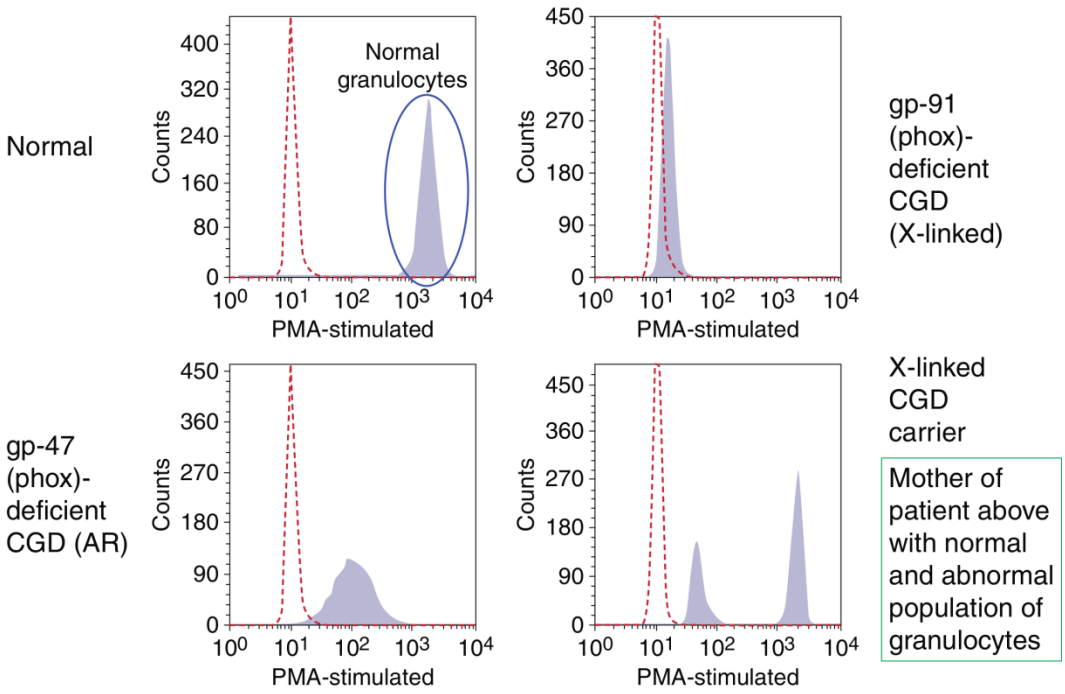


Figure 4-21: DHR assay results.
(Reproduced, with permission, from Thomas A. Fleisher, MD, National Institutes of Health, DHHS Bethesda, MD.)

- Patients with CD11 or CD18 surface glycoprotein deficiency may have normal chemiluminescence after stimulation with soluble stimulators, such as PMA; but, they may have delayed and diminished chemiluminescence after stimulation with opsonized particles.

HYBRIDOMA AND MONOCLONAL ANTIBODIES

HYBRIDOMA

A hybridoma is product of cell fusion between a normal antibody producing B lymphocyte and a myeloma cell, followed by selection of fused cells that secrete antibody of the desired specificity.

Flash Card A13

Dihydrorhodamine 123 assay (DHR)

Synthesis

- Spleen cells from an immunized mouse are fused with myeloma cells by using polyethylene glycol (PEG) to produce a hybrid cell line called a **hybridoma**.
- Myeloma cells lacking both antibody production and the enzyme **hypoxanthine-guanine phosphoribosyl transferase (HGPRT)** are selected out. Absence of this enzyme prevents growth in hypoxanthine-aminopterin-thymidine (HAT) medium.
- After fusion, cells are transferred to HAT medium, where unfused spleen and myeloma cells die. Only hybrid cells with HGPRT enzyme (i.e., gene contributed by spleen cells) can grow in this medium and are, thus, selected.
- Hybridomas are screened for antibody production, and cells that make antibody of desired specificity are cloned by growing them from a single cell (Figure 4-22).

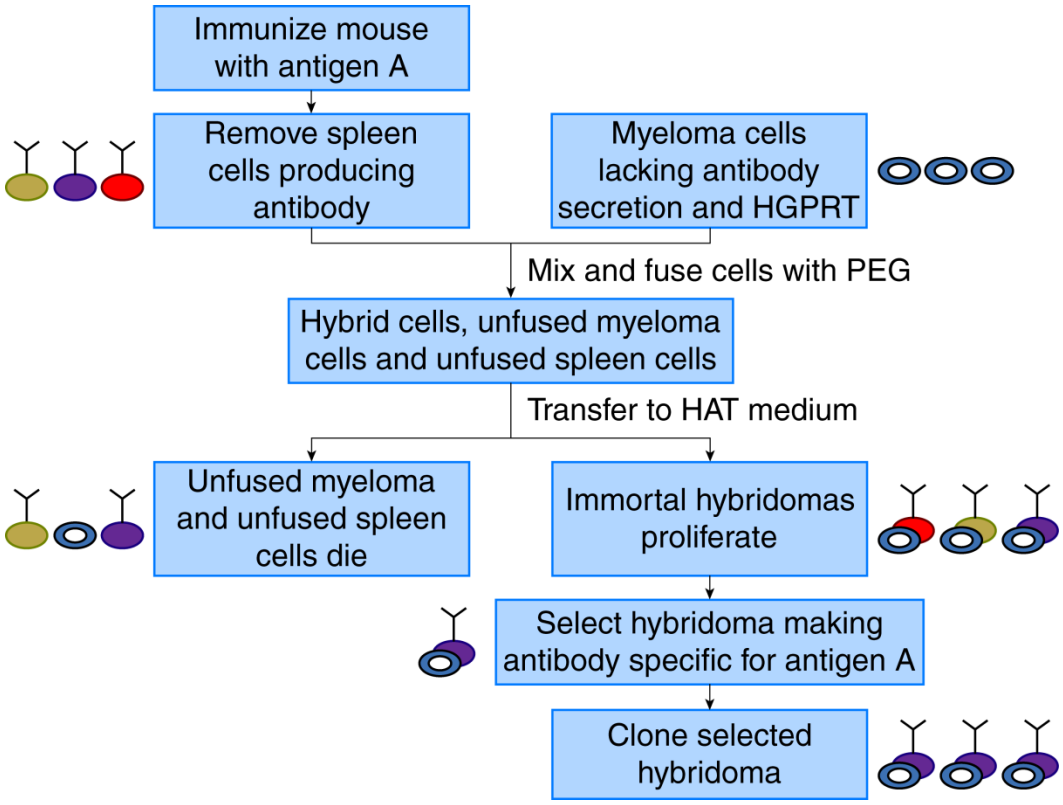


Figure 4-22. Production of monoclonal antibodies.

MONOCLONAL ANTIBODY

As each hybridoma is descended from a single cell, all the antibodies it produces are identical in structure, including their antigen-binding site and isotype. These antibodies are called **monoclonal antibodies (mAbs)**.

The initial mAbs produced were all composed of mouse protein; but, when used in humans, they elicited production of human antimouse antibodies with resultant side effects. A number of techniques have been developed to make the murine mAbs less immunogenic. The different types of mAbs and their proposed nomenclature are detailed in Table 4-18. Nomenclature for product source identifiers is listed in Table 4-19. Sketches of the various types of monoclonal antibodies are depicted in Figure 4-23.

Uses—Monoclonal antibodies are used in serologic assays, as diagnostic probes, and as therapeutic agents (Table 4-20).

Mnemonic

XImab: mi**Xi**ng human and mouse makes **Chi**meric

Umab: h**U**man

Zumab: humans at the **Z**oo are humani**Z**ed

Omab: m**O**use

Table 4-18. Monoclonal Antibodies and Fusion Protein

Nomenclature[a]	Type	Means of Synthesis	Murine Component (%)
-omab	Mouse	Murine-variable and constant antibody regions	100
-ximab	Chimera	Combines murine mAb variable regions with the constant region of human antibody	30–35
-zumab	Humanized	Combines murine mAb hypervariable or CD regions to remaining portion of human IgG molecule	<10
-umab	Human	Human heavy- and light-chain variable (V) region genes are cloned into a bacteriophage—V region is expressed as a fusion protein on phage cell surface → **phage-display library**—multiplied in bacteria-select phage with desired antigen specificity. May replace hybridoma technology for production of mAbs	None
-cept		**Fusion proteins** created by joining two or more genes that were originally coded for separate proteins	

[a]All mAbs end in the suffix -mab. These identifiers are used as infixes preceding the -mab suffix stem.

Table 4-19. Nomenclature of Product Source Identifiers

	Protein
u	Human
o	Mouse
a	Rat
e	Hamster
i	Primate
xi	Chimeric
axo	Rat/mouse
xizu	Combination of humanized and chimeric chains

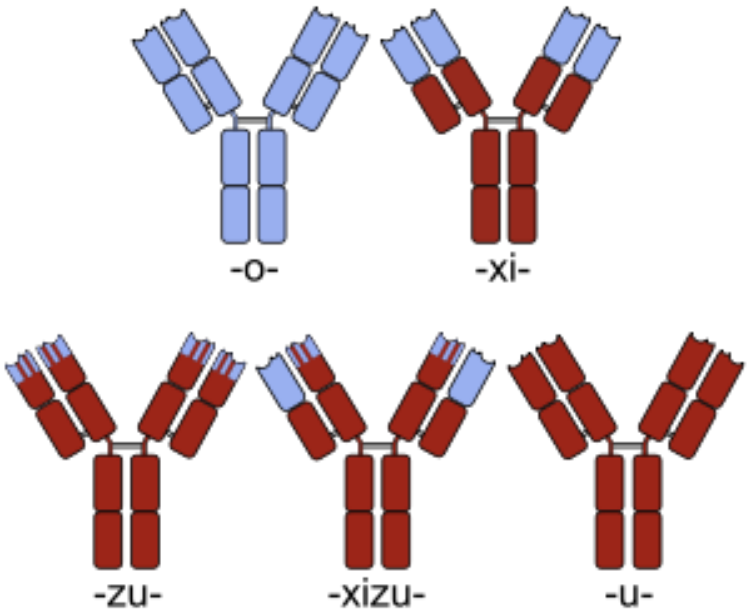


Figure 4-23: Monoclonal antibody figures. Mouse (top left), chimeric (top right), humanized (bottom left). Human parts are shown in brown, nonhuman parts in blue.
(Reproduced, with permission, from Wikimedia Commons.)

Table 4-20. Examples of Monoclonal Antibodies Used as Medications

Antibody	Target Antigen Molecule	Application
Abciximab	Integrin αIIβ3	Cardiac ischemic complications
Adalimumab	TNFα	Rheumatoid arthritis
Alemtuzumab	CD52	Chronic lymphocytic leukemia
Basiliximab	IL-2 receptor α chain (CD25)	Transplant rejection
Belimumab	B-cell activating factor	Systemic lupus erythematosus (SLE)
Bevacizumab	Vascular endothelial growth factor (VEGF)	Colorectal cancer
Brentuximab vedotin	CD30	Anaplastic large-cell lymphoma (ALCL), Hodgkin's lymphoma
Canakinumab	IL-1β	Cyropyrin-associated periodic syndrome (CAPS)
Cetuximab	Epidermal growth factor receptor	Colorectal cancer, head and neck cancer
Certolizumab pegol	TNFα	Crohn's disease
Daclizumab	IL-2 receptor α chain (CD25)	Transplant rejection
Denosumab	RANK ligand	Postmenopausal osteoporosis, solid tumor's bony metasteses
Eculizumab	Complement system protein C5	Paroxysmal nocturnal hemoglobinuria
Efalizumab	CD11a	Psoriasis
Gemtuzumab	CD33	Acute myelogenous leukemia
Golimumab	TNF-α	Rheumatoid arthritis, psoriatic arthritis, Ankylosing spondylitis
Ibritumomab tiuxetan	CD20	Non-Hodgkin's lymphoma
Infliximab	TNFα	Several autoimmune disorders
Ipilimumab (MDX-101)	CTLA-4	Melanoma
Keliximab	CD4	Multiple sclerosis
Mepolizumab	IL-5	Hypereosinophilic syndrome
Muromonab-CD3 (OKT3)	T cell CD3 receptor	Transplant rejection
Natalizumab	α 4 chain of integrin molecule	Multiple sclerosis
Ofatumumab	CD20	Chronic lymphocytic leukemia
Omalizumab	FcεRI (cε3)	Asthma
Palivizumab	RSV F-protein	Infants with bronchopulmonary dysplasia
Panitumumab	Epidermal growth factor receptor	Colorectal cancer
Ranibizumab	Vascular endothelial growth factor A (VEGF-A)	Macular degeneration
Rituximab	CD20	Autoimmune diseases, B-lymphocyte lymphomas
Tocilizumab	Anti-IL6R	Rheumatoid arthritis
Tositumomab	CD20	Non-Hodgkin's lymphoma
Trastuzumab	ErbB2	Breast cancer

Limitations—There is a risk of hypersensitivity reactions, ranging from mild allergic reaction to anaphylaxis. However, with the development of humanized and human mAbs, the risk of hypersensitivity has decreased significantly by reducing exposure to murine antibody immunogenicity.

Fusion Proteins

- Fusion proteins are created by joining two or more genes that originally coded for separate proteins.
- Translation of the fusion gene results in a single polypeptide with functional properties derived from each original protein.
- Recombinant fusion proteins are created artificially using recombinant DNA technology for use in research and therapeutics.
- One example is **etanercept**, a TNFα antagonist created through the combination of a tumor necrosis factor receptor (TNFR) with the immunoglobulin G1 Fc segment. TNFR provides specificity for the drug target, and the antibody Fc segment adds stability and deliverability of the drug. It is used for treatment of autoimmune conditions, including rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, plaque psoriasis, and juvenile idiopathic arthritis.

IMMUNE COMPLEXES

Synthesis

- IgM or IgG antibodies specific for a soluble antigen bind to it, forming circulating **immune complexes (ICs)**.
- If IgM or IgG binds to an antigen on a normal host cell, regulatory complement proteins, such as **factor H** and **I**, **inactivate C3b** to **iC3b**, thereby inhibiting IC formation. Thus, **IC formation normally occurs only on foreign cells.**
- C3b binds CR1 on RBCs, thus allowing transport of ICs to the liver and spleen, where they are **eliminated by macrophages**.
- ICs only cause disease if produced in excessive amounts, are not cleared efficiently, and are deposited in tissues.
- Large circulating ICs are formed at the **zone of equivalence** (Figure 4-24).
- Small circulating ICs are formed at the **zone of antigen** or **antibody excess**. (see Figure 4-24).
- IgM and IgG (subclass IgG1, IgG2, and IgG3) can activate the classical complement pathway.

Key Fact

Degree of complement activation by immuno-globulin in descending order:
IgM → IgG3 → IgG1 → IgG2.

Flash Card Q14

What is the target antigen for omalizumab?

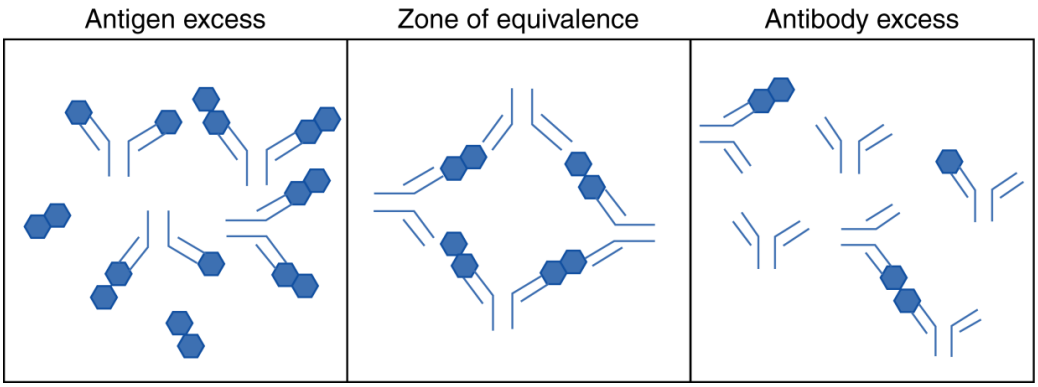


Figure 4-24. Immune complex formation.

IC-Mediated Diseases

Key Fact

Serum sickness is a type III hypersensitivity reaction in which excess immune complex formation leads to clinical symptoms of fever, rash (urticarial), joint pain, and lymphadenopathy.

Circulating ICs are deposited in vascular beds and complement, which leads to complement consumption and reduced levels of C3 and C4. **This produces a neutrophilic inflammation, usually affecting small arteries, glomeruli, and synovial of joints, which results in clinical symptoms of vasculitis, nephritis, and arthritis.** (See Table 4-21 for specific IC-mediated diseases).

Serum sickness is a type III hypersensitivity reaction in which excess immune complex formation leads to clinical symptoms of fever, rash (urticarial), joint pain, and lymphadenopathy.

Laboratory Testing for Detection of Circulating IC

Key Fact

ICs that are not properly eliminated can cause inflammation, resulting in tissue injury. Excessive IC formation also results in complement consumption. Measuring, IC formation via complement consumption, allows for estimation of disease burden. Thus, **C3 and C4 levels can be used to monitor disease activity** (e.g., in SLE).

- IC formation leads to the activation of the classical complement pathway.
- **C1q**, **C4b**, and **C3b** coat the IC, maintain its solubility, and act as ligands for receptors on WBCs and RBCs.
- **Conglutinin**: Binds iC3b, which can then be detected in an assay. Does not detect C3dg. iC3b is converted to C3dg within 1–8 hours of its generation.

Flash Card A14

FcεRI

Table 4-21. Examples of Immune Complex-Mediated Diseases

Disease	Antigen Involved
Systemic lupus erythematosus	DNA, nucleoproteins, and others
Serum sickness	Various antigens
Poststreptococcal glomerulonephritis	Streptococcal cell wall antigen
Polyarteritis nodosa	Hepatitis B virus surface antigen
Cryoglobulinemia	Hepatitis C virus or rheumatoid factor

- **Raji cell**: Burkitt's cell line with receptors for C1q, C3b, C3bi, and C3d. ICs bind to Raji cells, which are detected by antihuman IgG (IgA or M). False positive with warm reactive antilymphocyte Abs (e.g., SLE).
- **C1q-binding assay**: Radiolabeled C1q binds to circulating ICs and is then detected. False positives may occur with autoantibody to C1q, aggregated immunoglobulins, or heparin in test samples. C1q will also bind to polyanionic substances in serum, such as bacterial endotoxin or DNA, giving false positive results.

COMPLEMENT

Complement deficiency may manifest with increased susceptibility to infection, autoimmune or IC-mediated diseases, or with angioedema. The specific illness depends upon which complement component is involved.

DIAGNOSTIC TESTS FOR COMPLEMENT DEFICIENCY

Functional Screening Tests

- **AH50:** Measures lysis of unsensitized **rabbit** RBCs. Screens the **alternative pathway**.
- **CH50:** Assesses ability of serum to lyse **sheep** RBCs sensitized with rabbit IgM. Screens the **classical pathway**. Best single screen for complement abnormalities. Low CH50 implies that at least one of the components is missing or low.
- **C1 inhibitor levels and function:** Several assays available. Evaluates for presence of type II hereditary angioedema.
- **Function of MBL pathway:** Measured by an ELISA assay. Wells coated with mannan are incubated with patient's serum-activation of MBL pathway, which results in production of C4b and C4d that are measured by using enzyme conjugated monoclonal antibodies.

See Table 4-22 for a summary of screening test results for complement deficiency.

Key Fact

The most common cause of low complement level is a poorly handled specimen. Complement assays are very sensitive to breakdown at room temperature. Consequently, when abnormal results are obtained, the test should be repeated to confirm true low/absent complement.

Mnemonic

rAbbit — **A**H50 — **A**lternative pathway

Classic image to sleep → **sheep** jumping over **C**louds — **C**H50 — **C**lassical

Flash Card Q15

Type III hypersensitivity occurs with antigen or antibody excess?

Table 4-22. Interpretation of Laboratory Results for Complement Deficiency

CH50	AH50	Missing Factors
Absent	OK	C1q, C1r, C1s, C2, or C4
OK	Absent	B or D (very rare), or Properdin
Absent	Absent	C3, C5, C6, C7, C8, or C9
Absent	Absent, and C3 absent	Factor H or I

Distinguishing Hereditary from Acquired Complement Deficiency

In an acquired complement deficiency, CH50 and/or AH50 will be low, as opposed to a hereditary defect, in which case CH50 and/or AH50 will be absent. Also, an acquired defect will manifest with multiple low complement components; whereas, a hereditary deficiency will manifest with only one component low or absent (Table 4-23).

Quantitative Tests for Component Concentrations

Individual complement components are measured using immunoprecipitation assays, including nephelometry, RIA, RID, and ELISA techniques. The most definitive method for evaluating complement activation is quantitation of the fragments formed during the enzymatic cleavage steps. Because many of the complement components are acute-phase reactants, decreases due to activation might be masked by increases in the synthesis rates during an inflammatory episode. The split products can be used to determine whether activation has occurred, because their increase occurs only when the complement enzymes are formed and active.

- **C4a and C4d**: Markers of classical pathway or lectin pathway activation
- **Bb**: Marker of alternative pathway activation
- **C3a, iC3b, C5a, and soluble C5b-9**: Markers of terminal pathway activation
- **SLE: C3 and C4:**
  - Reduced levels in the setting of lupus nephritis is an important predictor of more severe disease and poor outcome.
  - Total deficiency of C3 is associated with development of membranoproliferative glomerulonephritis.
- Hereditary versus acquired angioedema (see Table 4-23)

Flash Card A15

Antigen excess results in the formation of **small immune complexes** that do not fix complement and are not cleared from circulation, leading to deposition in glomeruli, vessels, and joints.

Table 4-23. Hereditary Versus Acquired Angioedema

	C4[a]	C1 Inhibitor Level	C1 Inhibitor Function	C1q
HAE type I	Low	Low	Low	Normal
HAE type II	Low	Normal or elevated	Low	Normal
AAE	Low	Low	Low	Low
HAE type III	Normal	Normal	Normal	Normal

[a]Note: C4 is a sufficient screening test to evaluate patients with angioedema. Further testing is not necessary if C4 is normal.
Abbreviations: AAE, acquired angioedema HAE, hereditary angioedema.

Complement Autoantibody Tests

- **C1q autoantibody:** Found in patients with hypocomplementemic vasculitis
- **C1 inhibitor autoantibody:** Found in some patients with acquired angioedema
- **C3 nephritic factor:** Found in a few patients with SLE, associated with dense deposit disease (membranoproliferative glomerulonephritis type II) and partial lipodystrophy

Very few complement assays have been standardized and validated for FDA clearance. However, it is fairly easy to measure individual complement component, especially C3 and C4 levels, which can be used as screening tests for various disorders.

MOLECULAR BIOLOGY TECHNOLOGY

Flash Card Q16

What complement defect would be expected in an individual with frequent *Neisseria* infections?

The following techniques are used to characterize, isolate, and manipulate the molecular components of cells and organisms.

T-Cell Receptor Excision Circles (TRECs)

Methods—T lymphocytes develop in the bone marrow and undergo maturation in the thymus. During T-cell receptor rearrangement, extrachromosomal DNA byproducts, called T-cell receptor excision circles (TRECs) are generated. **Measurement of TRECs via PCR allows monitoring of the development of**

Flash Card Q17

Various mutations in which gene have been associated with type III HAE?

naïve T lymphocytes that have recently emigrated from the thymus. In healthy individuals, the number of TRECs declines with increasing age.

Disadvantages—TREC concentration can be affected by T-lymphocyte division and T-lymphocyte death.

Uses

- Diagnosis of **SCID** and **22q11 deletion syndromes**.
- **HIV-1** infection causes decreased levels of TRECs in peripheral blood T lymphocytes. Successful treatment with highly active antiretroviral therapy (HAART) increases TREC levels in peripheral blood T lymphocytes, possibly by decreasing T-lymphocyte turnover.
- **Downs syndrome can present with low TREC levels in a newborn.**
- **Monitoring of immune reconstitution after bone marrow transplant.**

See Figure 4-25 for an illustration of TRECs.

Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

Methods—RT-PCR is a sensitive method for detecting mRNA expression levels. RNA is first reverse transcribed into cDNA using a reverse transcriptase. The resulting cDNA is used as templates for subsequent PCR amplification with primers specific for one or more genes.

Advantages—Most sensitive technique for mRNA detection and quantitation. It can be used to quantify mRNA levels from much smaller samples and is sensitive enough to enable quantitation of RNA from a single cell.

Flash Card A16

Terminal complement complex (C5, C6, C7, C8, C9)

Flash Card A17

Factor 12 mutation (Hageman Factor). The most common mutation involves a gain of function resulting in abnormal generation of bradykinin.

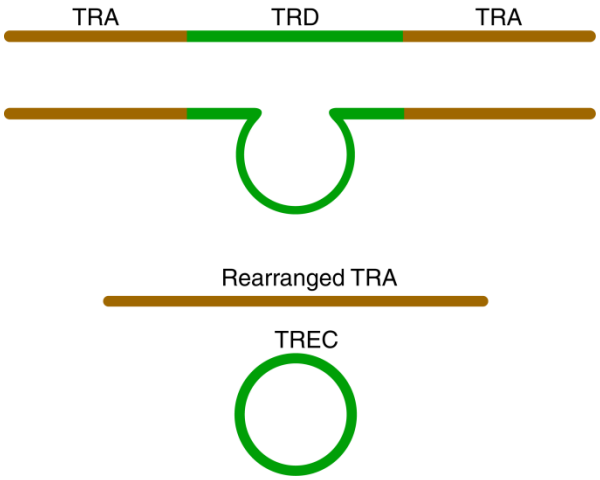


Figure 4-25. T–cell-receptor excision circles.

Disadvantages—Accuracy is dependent on variations during amplification.

Uses—Widely used in the diagnosis of genetic diseases and commonly used in studying the genomes of viruses whose genomes are composed of RNA, such as **influenza A** and **retroviruses** like **HIV**.

Figure 4-26 illustrates the RT-PCR.

In Situ Hybridization (ISH)

Methods—A method of localizing and detecting specific mRNA sequences in morphologically preserved tissue sections or cell preparations by hybridizing the complementary strand of a nucleotide probe to the sequence of interest.

Fluorescent in situ hybridization (FISH) uses fluorescence microscopy to find where the fluorescent probe binds to the chromosomes (Figure 4-27).

Advantages—Does not require living cells and can be quantified automatically. Can be used to determine the structure of chromosomes. ISH can also use two or more probes, labeled with a radioactive label or the other nonradioactive labels, to simultaneously detect two or more transcripts.

Uses

- FISH is often used for **finding specific features in DNA for use in genetic counseling, medicine, and species identification**.
- FISH can also be used to detect and localize specific mRNAs within tissue samples.
- Diseases that can be diagnosed using FISH include **acute lymphoblastic leukemia (ALL); DiGeorge syndrome [Deletion 22q11 or velocardiofacial syndrome (VCSF)]; and Down syndrome.**

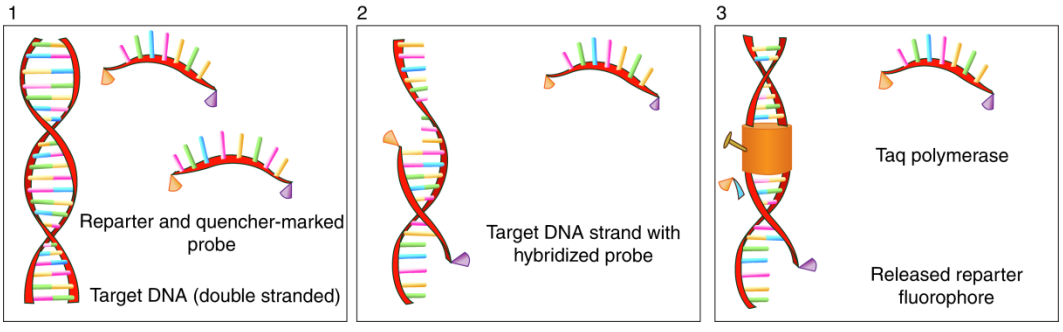


Figure 4-26. Reverse transcriptase PCR.

Flash Card Q18

TREC assay has been introduced in the newborn screens of several states for diagnosis of which disease?

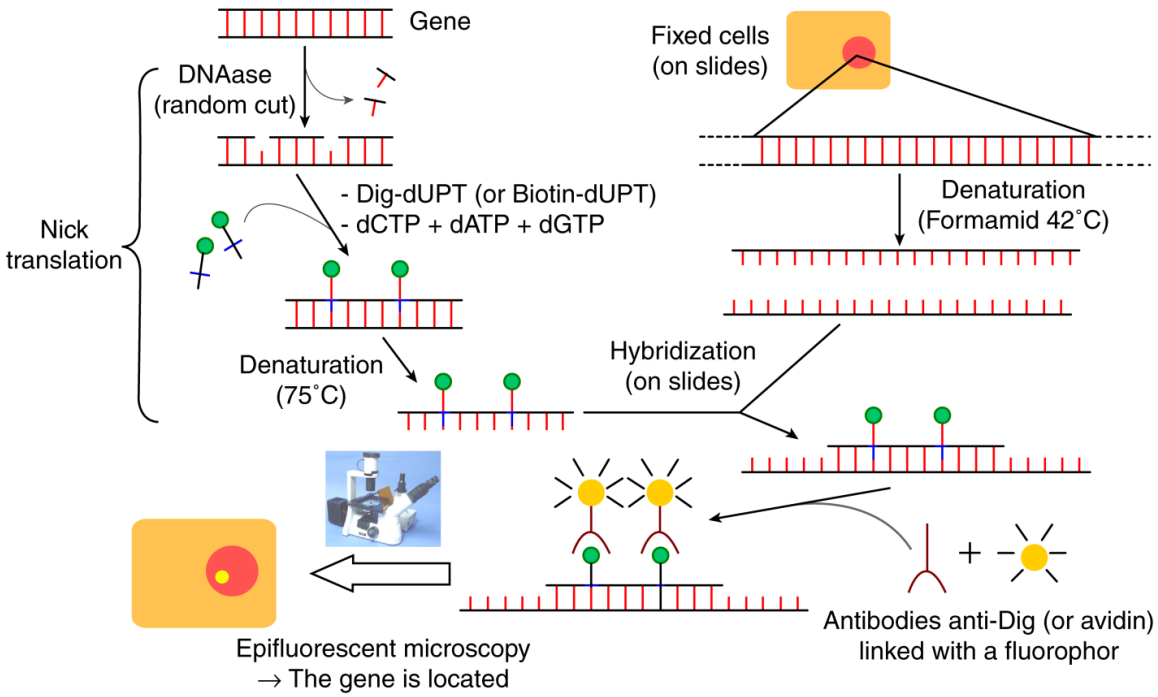


Figure 4-27. Fluorescent in situ hybridization.

Gene Chips

Methods—Gene chips are glass substrate wafers containing many tiny cells, each of which holds DNA from a different human gene; the genetic material and a fluorescent probe react. Hybridization of the added nucleic acid and a piece of the tethered DNA will occur if the sequences complement one another. The development of fluorescence on the chip's surface identifies regions of binding, and the known pattern of the tethered DNA can be used to deduce the identity of the added sample (Figure 4-28).

Advantages—Possible to carry out a very large number of genetic tests on one sample simultaneously (e.g., up to 260,000 genes can be probed on a single chip). This is **useful when one wants to survey a large number of genes quickly or when the sample to be studied is small**.

Uses

- To genotype or resequence mutant genomes
- To identify **single-nucleotide polymorphism (SNP)** among **alleles**

Flash Card A18

SCID. The absence of TRECs in a newborn reflects T-cell lymphocytopenia. The assay can also detect 22q11 deletion syndromes and trisomy 21.

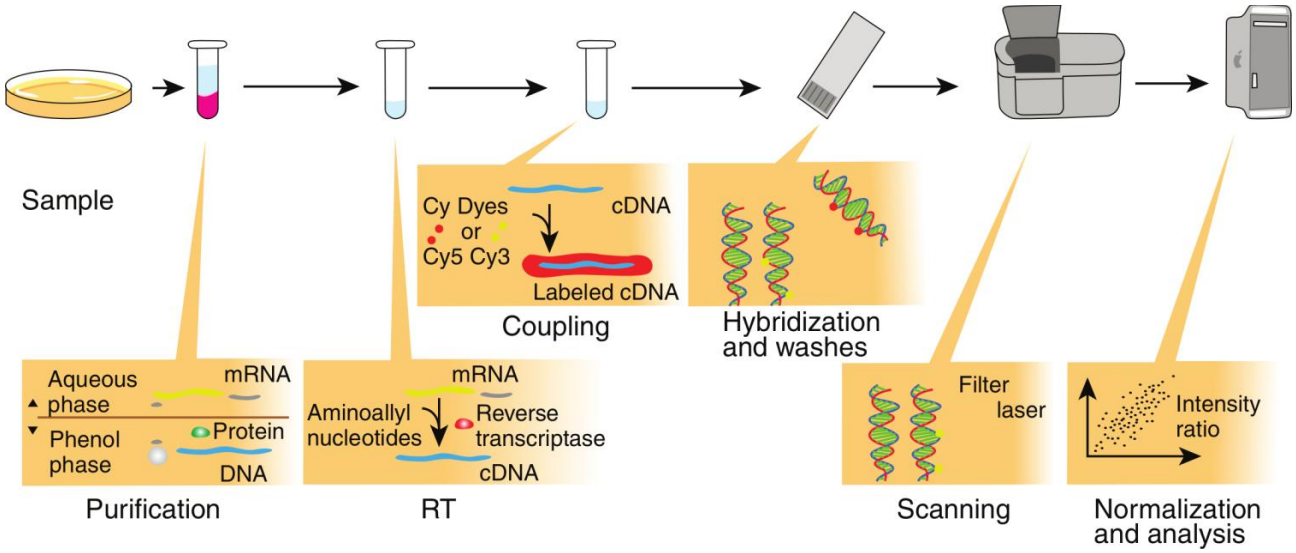


Figure 4-28. Gene chips.

DNA Sequencing

***Methods*—**Technique for determining the precise order of nucleotides within a DNA molecule. Several different techniques have been developed to sequence DNA.

- Developed in 1977, the **Sanger method** is based on the concept of the "chain-terminating" technique. It became the method of choice for its relative ease and reliability. This was the most commonly used method until the advent of next generation of techniques in early 2000.
- Second-generation techniques (**Next-gen**) utilize a variety of methods, including fluorescent labeling, capillary electrophoresis, and general automation. Examples include pyrosequencing, sequencing by synthesis, ion semiconductor, and sequencing by ligation. Goals of next-gen sequencing include lowering the cost of DNA sequencing while increasing productivity by replicating thousands or millions of sequences concurrently.

Advantages

- Sanger method: Simple and sensitive. Long individual reads useful for many applications.
- Next-generation methods: Variable. Overall lower cost per base in DNA sequence with potential for higher sequence yield.

***Uses*—**Identification of specific mutations in genes associated with disease can aid in diagnosis, prognosis, and possible use of therapeutic gene therapy in the future.

- For example, the location (exon) and type of mutation (loss of /gain of function) in the WAS protein determines clinical phenotype of disease.

5 Anatomy, Physiology, and Pathology

LYMPHOID SYSTEM AND ORGANS

Lymphoid organs are organized aggregations of lymphocytic cells in a framework of nonlymphoid cells.

PRIMARY (CENTRAL) LYMPHOID ORGANS

Bone marrow and thymus are the central lymphoid organs. They are the site for generation and maturation of lymphocytes.

Bone Marrow

Bone marrow generates both B- and T-lymphocyte precursors.

B-lymphocyte precursors complete most of their development in bone marrow. However, B lymphocytes also originate in the **fetal liver** and **neonatal spleen**.

Interleukin 7 (IL-7) is a hematopoietic growth factor produced by stromal cells in the bone marrow and thymus that is required for development of B and T lymphocytes.

B-lymphocyte precursors in contact with bone marrow stromal cells undergo successive cell divisions:

- Autoreactive or nonfunctional B-cell–receptor-bearing B lymphocytes undergo apoptosis and are removed by marrow macrophages by **negative selection**.
- Functional IgM-bearing B lymphocytes migrate from the periphery of bone to the central sinusoid along reticulum processes. They then enter central sinus and blood vessels to migrate to secondary lymphoid tissues.

Mnemonic

BONE

B-lymphocyte education and development occur in **BO**ne marrow. **NEg**ative selection occurs during B-lymphocyte development.

Thymus

Mnemonic

THYMUS

T-lymphocyte maturation occurs in the thymus.
Hassall's corpuscles are in the thymus (cortex).
Young individuals have a thymus that is relatively large in childhood, peaks during puberty, and decreases in size thereafter with age.
Medulla, cortex, and subcapsular zone.
U should remember both positive (cortex) and negative (medulla) selection occur in thymus.
Subcapsular epithelium provides blood-thymus barrier.

Once generated in bone marrow, T-lymphocyte precursors migrate to the thymus for maturation. The thymus is formed of the endoderm and mesoderm of **third and fourth pharyngeal pouches**. It grows in size from birth, peaks at puberty, and then undergoes gradual atrophy with age by thymic involution caused by circulating hormone levels.

The thymus is composed of two identical lobes and located in the anterior superior mediastinum. Thymic histology and architecture is detailed in Figure 5-1.

The thymic epithelium is divided into the subcapsular zone, the cortex, and the medulla:

- The **subcapsular zone** contains the most primitive lymphocyte progenitors derived from bone marrow.
- The **cortex** contains small lymphocytes engaged in the division, expression, and selection process of T-cell receptors.
- The **medulla** contains lymphocytes that are undergoing their final stages of selection and maturation. It also contains **Hassall's corpuscles**, which are small bodies of granular cells surrounded by concentric layers of modified epithelial cells.

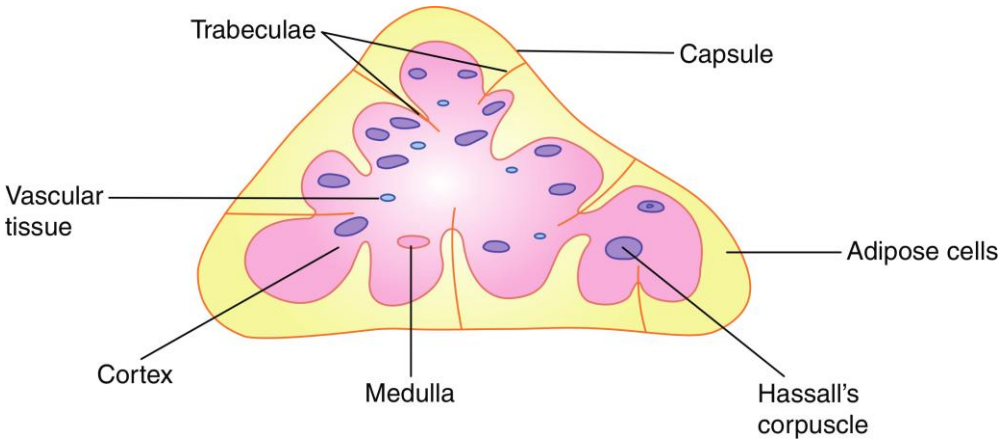
Within the cortex:

- **Positive selection:** T-cell receptor gene rearrangement in immature thymocytes creates T-cell receptors compatible with self-MHC molecules displayed on the thymic epithelium or stroma. The functional T-cell receptors transmit a survival signal for positive selection.
- Limited **negative selection** begins in the cortex where autoreactive or nonfunctional T-cell receptor–bearing T lymphocytes undergo apoptosis.

By way of the medulla:

- **Negative selection:** Thymocytes undergo further rounds of negative selection to remove autoreactive T cells and contribute to **central tolerance**.
- Mature T lymphocytes leave the thymus to circulate at the periphery or go to secondary lymphoid tissues.

It should also be noted that, other than migrating to the thymus for maturation, some T-lymphocyte precursors from bone marrow migrate to **cryptopatches (CPs)**, tiny mucosal lymphoid aggregates found just under the intestinal epithelium, to form specialized T-lymphocyte populations.



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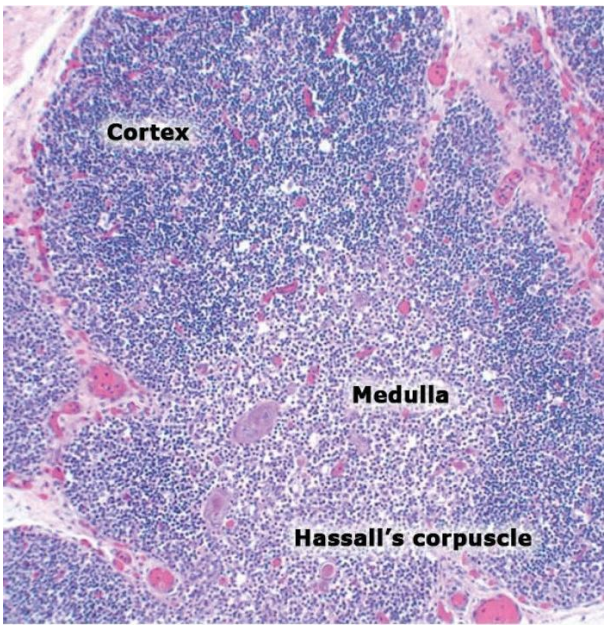

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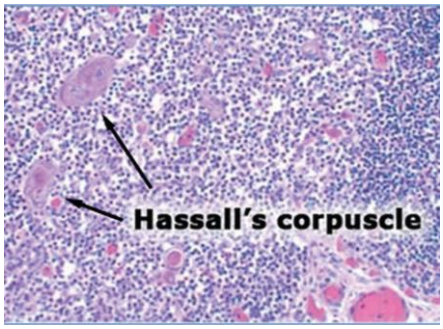


C

Figure 5-1. (A) Thymus architecture showing Hassall's corpuscle. (B, C) Histologic images of human thymic tissue.
(Figure A adapted, with permission, from Ankita Nair and Nikita Nair; Figures B and C reproduced, with permission, from USMLERx.com.)

Flash Card Q1

What diseases are associated with congenital thymic aplasia in humans?

SECONDARY (PERIPHERAL) LYMPHOID ORGANS

The secondary or peripheral lymphoid glands are the sites for mature lymphocyte activation and the initiation of adaptive immune responses. They also provide signals that sustain recirculating lymphocytes.

Flash Card Q2

In which condition is immunodeficiency (hypogammaglobulinemia) associated with a thymoma in adults?

Lymph Node

Lymph nodes (LN) are located along the course of lymphatic vessels. They are small, oval- or kidney-shaped, with hilum that contains the blood vessels and efferent lymphatic vessel. Figure 5-2 shows the structure of the lymph node:

- The outermost layer is the **capsule** with several afferent lymphatics that drain in the subcapsular or marginal sinus (Figure 5-2A).
- The **cortex** contains B-lymphocyte-rich follicles and T-lymphocyte-rich parafollicular areas. Dendritic cells and macrophages are interspersed (Figures 5-2B and 5-2C, respectively).

The **medulla** has less densely packed lymphocytes, forming medullary cords, macrophages, plasma cells, and few granulocytes.

- Postcapillary venules are lined by high cuboidal endothelium and are called **high endothelial venules (HEV)**, which are designed for lymphocyte cell adhesion and exit.
- Structures:
  - Lymph: Extracellular fluid collected from tissues, which is filtered by the LNs, then returned to blood
  - Follicle: Site of B-lymphocyte localization within the LN
  - Primary follicle: Site of resting B lymphocytes
  - Secondary follicle: Has germinal-center area of B-cell proliferation in response to antigen stimulation or T-lymphocyte help.
  - Ratio of primary and secondary follicles defines LN activity
  - Parafollicular cortex: T-lymphocyte zone surrounding B-lymphocyte follicle

Lymph node size is generally larger in adolescence than later in life. Normal lymph nodes are typically less than 1 cm in diameter. Enlarged peripheral lymph nodes, or **peripheral lymphadenopathy**, can result from a variety of diseases and drugs. See Table 5-1 for common causes of peripheral lymphadopathy.

Table 5-1. Differential Diagnosis of Peripheral Lymphadenopathy

Etiology	Examples
Infections	Bacterial, viral , fungal, parasitic
Malignancy	Lymphoma, SSC head/neck, metastatic, leukemia
Lymphoproliferative	ALPS, hemophagocytic lymphohistiocytosis
Immunologic	Serum sickness, drug reactions
Endocrine	Hypothyroidism, Addison's disease
Miscellaneous	Sarcoidosis, amyloidosis, Churg-Strauss syndrome

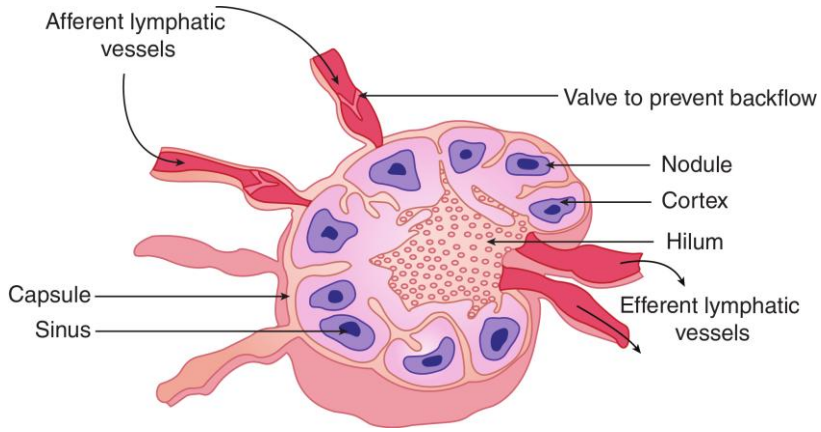
Abbreviations: ALPS, autoimmune lymphoproliferative syndrome; SSC, squamous cell carcinoma.

Flash Card A1

DiGeorge syndrome, SCID

Flash Card A2

Good's syndrome



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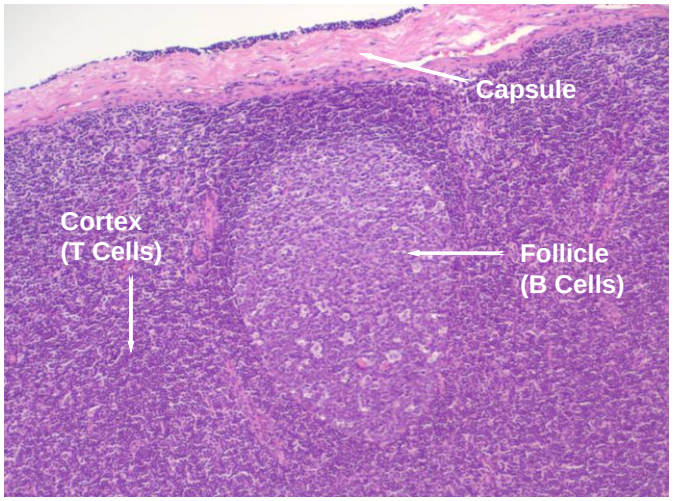

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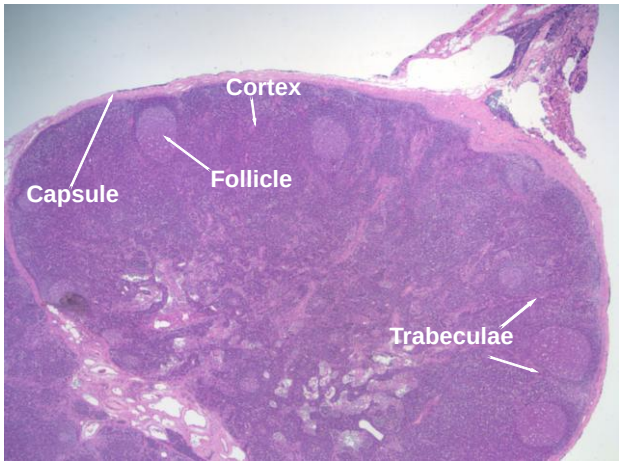


C

Figure 5-2. Lymph node. (A) Lymph node architecture; (B, C) histologic images of lymph node.
(Figures B and C reproduced, with permission, from Dr. Harpreet Chopra.)

Spleen

The spleen is the largest specialized lymph organ. It contains **25% of total blood lymphocytes** and consists primarily of red pulp with only a relatively small amount of white pulp (20%).

- **Red pulp** is the site of senescent RBC collection and disposal (Figure 5-3).
- **White pulp** includes the areas rich in lymphocytes that surround arterioles entering the spleen.

The spleen **does not have afferent lymphatic supply**, but it receives lymphocytes and antigen from the vasculature via the splenic artery. It also has efferent lymphatic vessels that carry lymphocytes out:

- **Periarteriolar lymphoid sheath (PALS)** has an inner region of white pulp with T lymphocytes.
- **B-lymphocyte corona** surround PALS and B-lymphocyte follicles.
- **Splenic artery** branches along invaginations of capsule (trabeculae).

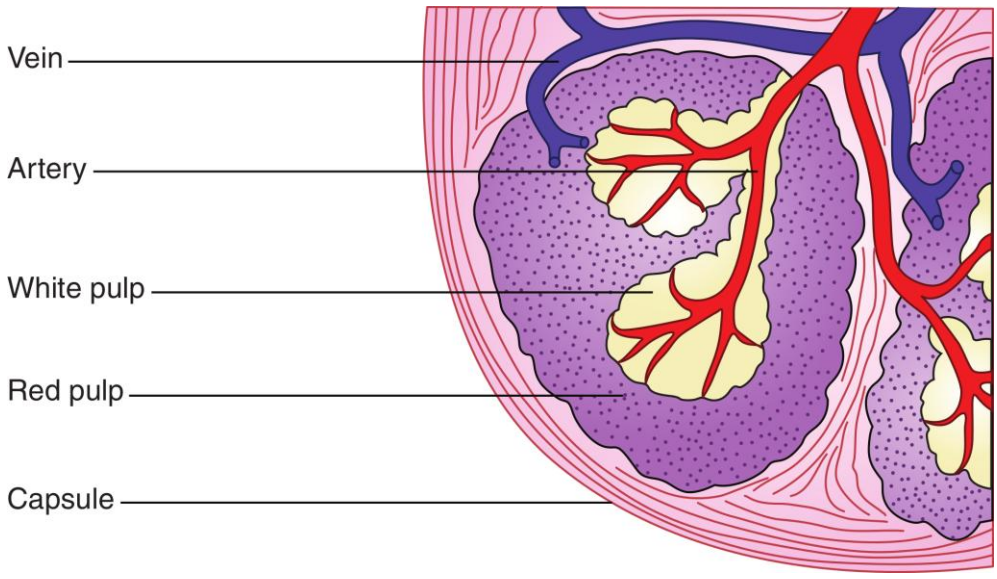


Figure 5-3. Spleen architecture.

Mucosa-Associated Lymphoid Tissue

Mucosa-associated lymphoid tissue (MALT) is unencapsulated lymphoid tissue found in the walls of the gut-associated lymphoid tissue (GALT), respiratory epithelium (bronchus-associated lymphoid tissue, BALT), reproductive tract, urinary tract, and skin.

Key Fact

Remember: **B lymphocytes** undergo only **negative** selection, whereas **T lymphocytes** undergo **both positive and negative** selection.

Most MALT presents as microscopic populations of lymphocytes, located in the **lamina propria** and **submucosa**, where they are discrete follicles as well as dispersed in the base of the epithelium.

Some MALT presents as macroscopic masses, such as **peripharyngeal lymphoid ring** (i.e., **Waldeyer's ring** containing tonsils and adenoids) and **Peyer's patches**.

Similar to lymph nodes, MALT serves as a center for activation of B and T lymphocytes after antigen presentation.

UPPER AIRWAY (NOSE, SINUSES, AND MIDDLE EAR)

NOSE

Anatomy

The lateral walls of nose are rigid bony structures with bony **conchae** that protrude into the **nasal cavity** (Figures 5-4A and 5-4B, respectively).

The **concha bullosa** is the pneumatization of the concha and is one of the most common variations of the sinonasal anatomy (Figure 5-5).

Flash Card Q3

The absence of a spleen leads to increased susceptibility to which organisms?

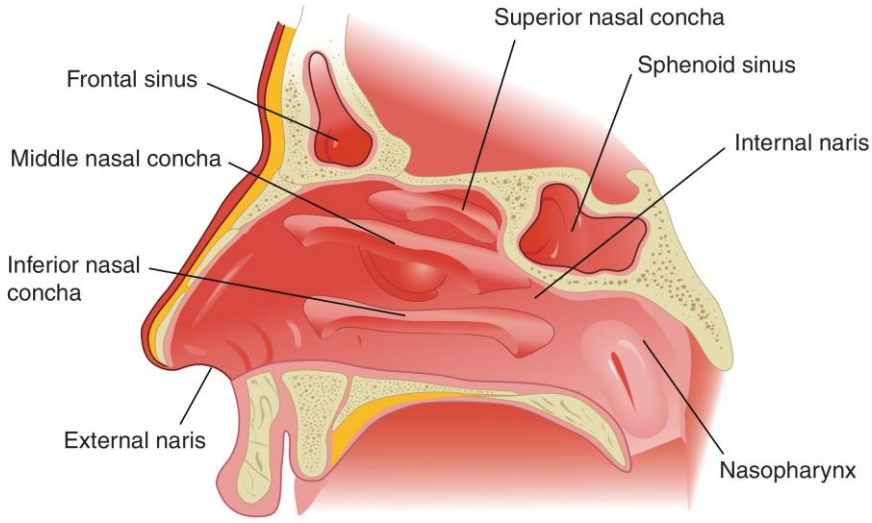
The **nasal septum** has bony and cartilaginous components. Trauma or other factors can cause septal deviation, leading to blockage and sometimes formation of nasal spurs that can cause facial pain syndromes.

Conchae are covered by an erectile mucosa with a rich blood supply, forming **turbinates**.

Flash Card Q4

What pathogen is frequently associated with gastric MALT lymphoma?

The **nasal valve,** or internal ostium, is the narrowest point of the whole airway. It is located at the junction between the nasal vestibule and the main nasal cavity, just anterior to the tip of the inferior turbinate.



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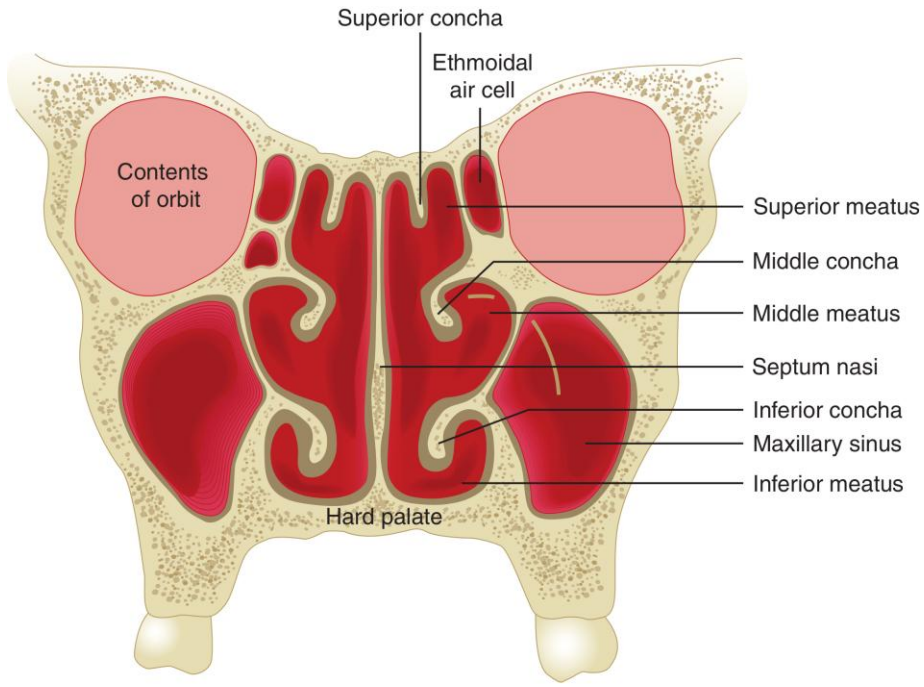


B

Figure 5-4. Nasal structures. (A) Sagittal section and (B) coronal section.

Flash Card A3

Encapsulated bacteria (*Neisseria meningitidis*, *Haemophilus influenzae*, and *Streptococcus pneumoniae*)

Flash Card A4

Helicobacter pylori. Gastric MALT lymphoma is frequently associated (72–98%) with chronic inflammation from *H. pylori*.

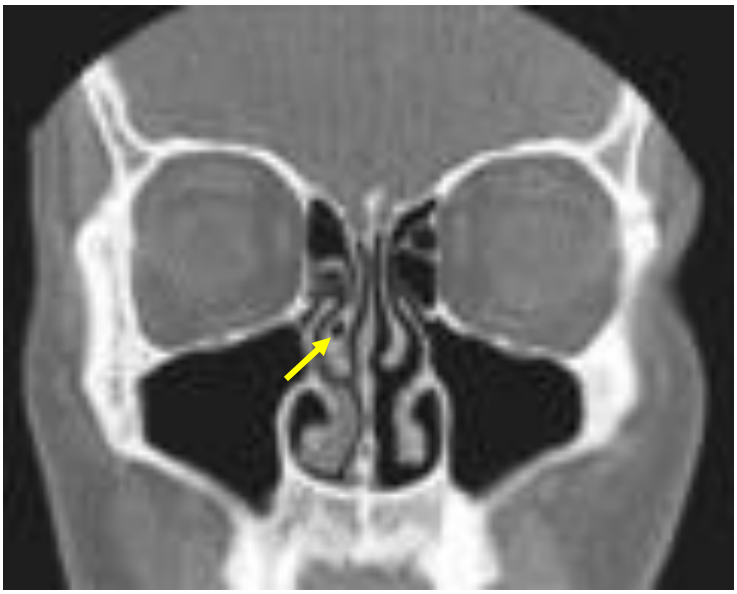


Figure 5-5. CT scan shows concha bullosa (arrow).
(Reproduced, with permission, from Wikimedia Commons.)

Function

The primary function of the nose is to provide filtration of foreign particles, humidification, and thermal regulation of inspired air as well as olfaction.

The turbinates increase the surface area of the nasal cavity, thereby providing turbulence to the **airflow**. Airflow is essential for the humidification and thermal regulation of all inspired air, not just the portion in contact with nasal mucosa.

Nasal airway resistance is regulated at the level of the nasal valve, which is controlled by the swelling of the inferior turbinate. Dilator naris muscles voluntarily increase patency of the nasal vestibule.

Nasal Epithelium

The nasal epithelium is the stratified squamous membranous tissue in the nasal vestibule and nasopharynx.

- Pseudostratified ciliated columnar epithelia are found in the lines of the trachea and the respiratory area of the nasal cavity.
- Specialized olfactory epithelium is found in the olfactory area.

Submucosa contains serous and mucous glands, fibroblasts, inflammatory cells, nerves, and blood vessels. Smooth muscle is not present in the submucosa.

Nasal polyps are nontender, mobile masses of mucosal overgrowth occurring in the nose and paranasal sinuses (Figure 5-6).

Differential diagnosis for nasal polyps includes chronic rhinosinusitis, cystic fibrosis, NSAID sensitivity (with coexisting asthma), and tumors.

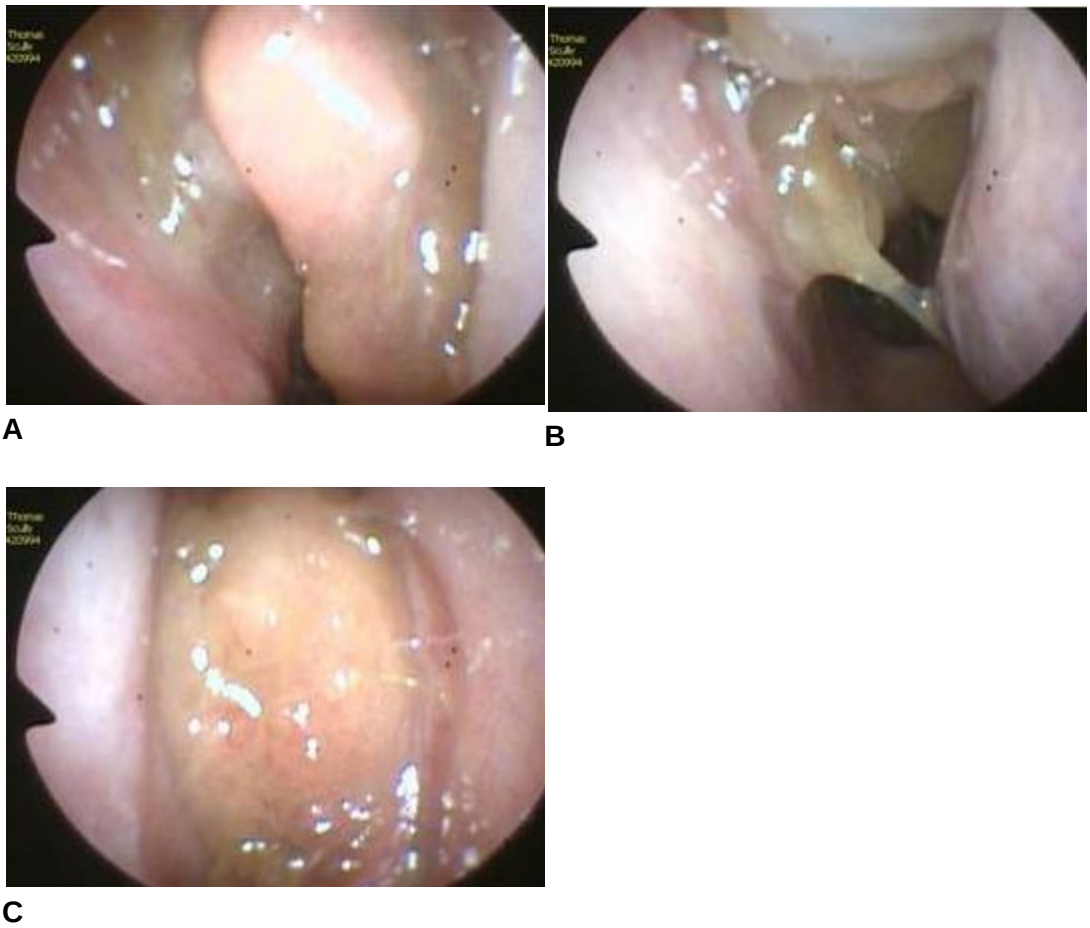


Figure 5-6. Example of diffuse nasal polyposis. (A) Right nasal cavity illustrating nasal polyps in the middle meatus and olfactory cleft (on both sides of the middle turbinate). (B) Lower left nasal cavity, showing polyps extending beneath the left middle turbinate and touching the inferior turbinate. (C) Upper left nasal cavity showing the middle turbinate enveloped by polyps in the middle meatus and olfactory cleft.
(Reproduced, with permission, from Dr. Daniel Hamilos, Massachusetts General Hospital, Allergy and Immunology, Department of Medicine)

Nasal Fluid and Mucociliary Clearance

The adult nose makes approximately a quart of thin mucus each day.

Nasal fluid contains immunoglobulins (IgA) and other serum proteins, enzymes, antioxidants (glutathione, uric acid and vitamin C), and cellular mediators.

Mucus is normally swallowed via mucociliary clearance, subconsciously, along with trapped particles, potentially infectious agents, and other debris.

Conditions that increase mucus production or thicken it, such as inflammation (viral, bacterial, or allergic), irritation, altered ciliary clearance, and autonomic dysregulation, etc., will produce postnasal drip.

Seromucous glands in nasal epithelium have parasympathetic cholinergic innervations, causing watery nasal secretion; hence, an anticholinergic is used to decrease rhinorrhea.

Mucociliary clearance can be tested by placing saccharin on the anterior end of an inferior turbinate and timing the onset of sweet taste in the mouth. (The normal range is 7–11 minutes.)

Primary ciliary dyskinesia (PCD) is a disease that results in decreased mucociliary clearance. PCD is a rare autosomal recessive genetic disorder that causes a defect in the function of cilia lining in the respiratory tract, fallopian tubes, and flagella of sperm. This causes reduced/absent mucous clearing from the lungs, leading to chronic recurrent respiratory infections (sinusitis, bronchitis, pneumonia, otits media), bronchiectasis, infertility, and in some cases hearing loss.

Blood Vessels

- **Resistance vessels** (i.e., small arteries and arterioles) control nasal blood flow via local mediators and sympathetic stimulation, which causes vasoconstriction.
- **Capacitance vessels**, or venous erectile tissue located primarily over inferior and middle turbinates and nasal septum, have sympathetic adrenergic innervation, which controls nasal airway resistance.
- **Subepithelial capillaries** and veins are fenestrated, leading to the high permeability-increased absorption of intranasal drugs.

Flash Card Q5

What syndrome is characterized by ciliary dyskinesia, situs inversus, bronchiectasis, and chronic sinusitis?

Nerve Supply

The olfactory nerve is a chemoreceptor that governs the sense of smell.

Ophthalmic and maxillary branches of the trigeminal nerve are responsible for sensory innervation.

The nasal epithelium has both a parasympathetic and sympathetic supply.

- Parasympathetic innervation to nasal glands controls glandular secretion and is mediated primarily by acetylcholine.
- Sympathetic innervation of arterial vessels controls nasal blood flow (i.e., nasal patency), and venous erectile tissue controls airflow resistance.

Nasal Airflow

Key Fact

The nasal airflow is usually asymmetrical due to the "nasal cycle" of alternating congestion and decongestion of nasal venous sinuses. About 80% of the population exhibits a **nasal cycle**, with reciprocal changes in airflow over time as controlled by the sympathetic nervous system.

With nasal airflow there is normal asymmetry of nasal mucosal swelling, in which one side of the nose is **swollen** as a result of dilatation of venous sinuses in the inferior turbinate and the other side is **open**.

Table 5-2 shows dynamic factors that affect nasal airflow. Static factors like septal deviation and adenoids also affect nasal flow.

Table 5-2. Factors Affecting Nasal Airflow

Factors Affecting Nasal Flow	Mechanism	Effect on Airflow
Sympathetic activity	Vasoconstriction	Increased flow
Posture, sitting to supine	Increased venous filling	Decreased flow
Lateral recumbent position	Reflex vasomotor activity	Increased flow in upper nostril Decreased flow in lower nostril
Mild to moderate exercise	Increased sympathetic activity	Increased flow
Increase in blood carbon dioxide levels	Sympathetic supply to blood vessels	Increased flow
Epinephrine	Vasoconstriction	Increased flow
Puberty, menstruation, and pregnancy	Vasodilatation and glandular hypersecretion	Decreased flow

Flash Card A5

Kartagener's syndrome—50% of individuals with PCD have Kartagener's syndrome

SINUSES

Sinus is a Latin word meaning "fold" or "pocket."

Paranasal sinuses develop in the embryo as an excavation of bone by air-filled sacs (pneumatic diverticula) from the nasal cavity. They are rudimentary at birth and slowly grow to be well developed by about 8 years of age. They are lined with ciliated pseudostratified columnar epithelia with goblet cells, which secrete mucus.

Ostia are 2–6 mm wide sinus openings into the nose. Ciliary action sweeps mucus produced in the sinus through the ostium into the nose. An open ostium allows ventilation of the sinus. **A blocked ostium contributes to development of sinusitis.**

Mnemonic

Sinuses listen to the following radio channels: **FM AM PS SS**

Frontal sinus, **M**axillary sinus, and **A**nterior ethmoids all drain into **M**iddle meatus.

Posterior ethmoids and **S**phenoid sinus drain into **S**phenoethmoidal recess above **S**uperior turbinate.

Function of Sinuses

The function of the sinuses is debatable. The following have been proposed:

- Decreasing the relative weight of the front of the skull
- Increasing resonance of the voice
- Insulating intracranial structures
- Humidifying and heating of inhaled air
- Source of nitric oxide

Opening of Sinuses

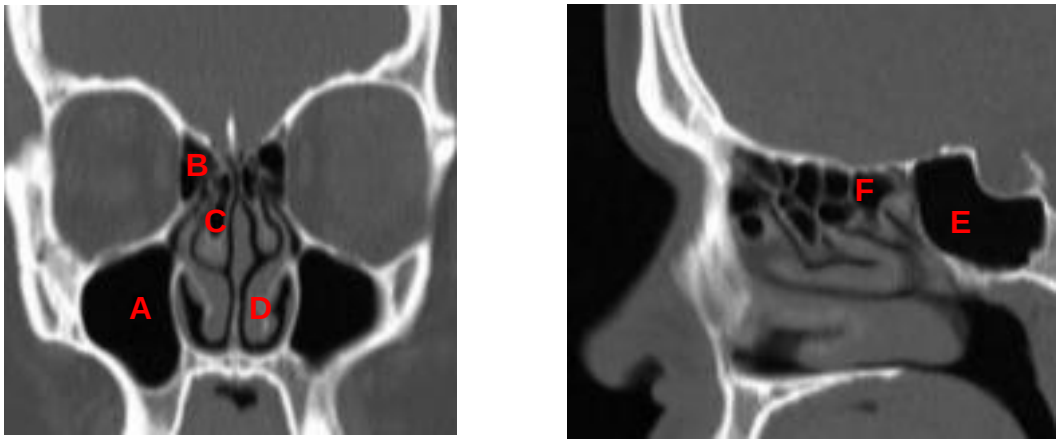
- **Inferior meatus:** Opening of nasolacrimal duct
- **Middle meatus:** Frontal, maxillary and anterior ethmoids open into hiatus semilunaris
- **Superior turbinate:** Has sphenoethmoidal recess above it where posteth-moids and sphenoid sinuses drain

Flash Card Q6

Identify the structures labeled (A–F) in the sinus CT images (Figure 5-7).

Flash Card Q7

Which paranasal sinus cavity is absent at birth?



A B

Figure 5-7. CT scan images with coronal (A) and sagittal (B) views of the sinuses.
(Reproduced, with permission, from Dr. Ahila Subramanian)

MIDDLE EAR

The tympanic cavity, or middle ear, is an irregular space in the petrous part of the temporal bone. It is lined by mucous membrane and filled with air that reaches it from the nasopharynx via the **eustachian tube (ET)**, which is also known as either the auditory or pharyngotympanic tube.

The **middle ear contains the three auditory ossicles** that transmit vibrations of the tympanic membrane to the cochlea.

Infection to the middle ear usually spreads from the nasopharynx via the pharyngeal ostium of the ET (Figure 5-8).

Flash Card A6

(A) maxillary sinus; (B) ethmoid sinus; (C) concha bullosa; (D) inferior turbinate; (E) sphenoid sinus; (F) posterior ethmoid sinus

Flash Card A7

Frontal sinus. It is generally well developed by 7–8 years of age and reaches full size after puberty.

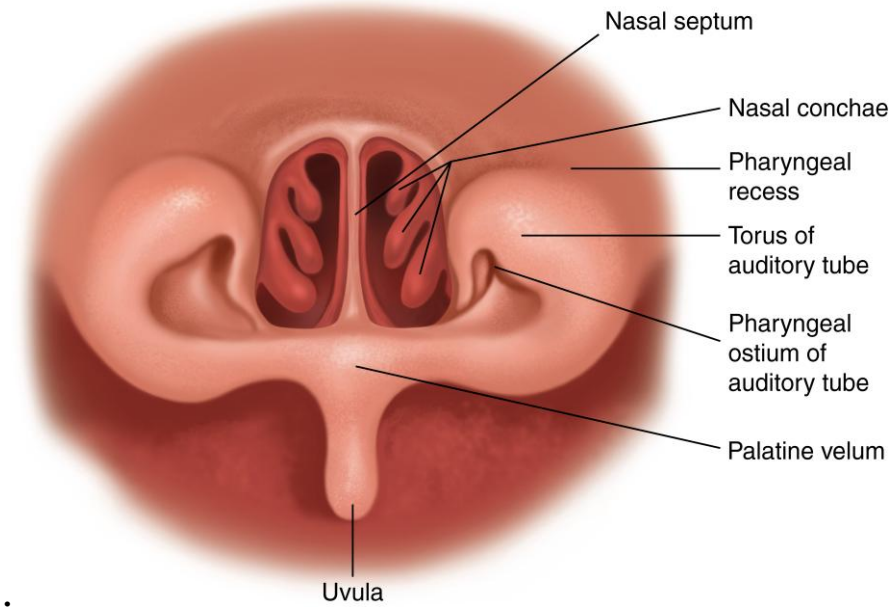


Figure 5-8. Endoscopic view of nasopharynx.

Posterior to the middle ear are the **mastoid antrum** and **mastoid air cells**.

The tympanic membrane is innervated by the **auriculotemporal nerve**, which can perceive only pain.

Key Fact

ET blockage in children can occur due to mucosal swelling caused by infection, allergic inflammation, or enlarged adenoids. Fluid unable to drain from middle ear causes middle ear effusion, which is prone to infection.

REMODELING OF THE LOWER AIRWAY

Lower airway changes are seen in both asthma and chronic obstructive pulmonary disease (COPD).

REMODELING

Remodeling is defined as structural change evident in asthmatic airways compared with normal airways. With remodeling, the airway wall is thickened by cellular infiltration, extracellular matrix deposition, and expansion of airways smooth muscle. There is also pronounced neovascularization.

Sputum Characteristics in Asthma

- **Curschmann's spirals:** Corkscrew-shaped twists of condensed mucus (Figure 5-9)
- **Creola bodies:** Clusters of surface epithelial cells (Figure 5-9)
- **Charcot-Leyden crystals:** Eosinophil cell and granule membrane lysophospholipase (Figure 5-9)
- **Eosinophils**
- Metachromatic cells

Sputum Characteristics in COPD

- Though eosinophils may be present, **eosinophilia is NOT predominant** in sputum of patients with COPD.
- Sputum macrophages and neutrophils during exacerbations are caused by infections.

LOWER AIRWAY CHANGES SEEN IN ASTHMA AND COPD

Table 5-3 illustrates the changes seen in lower airways in cases of asthma and COPD.

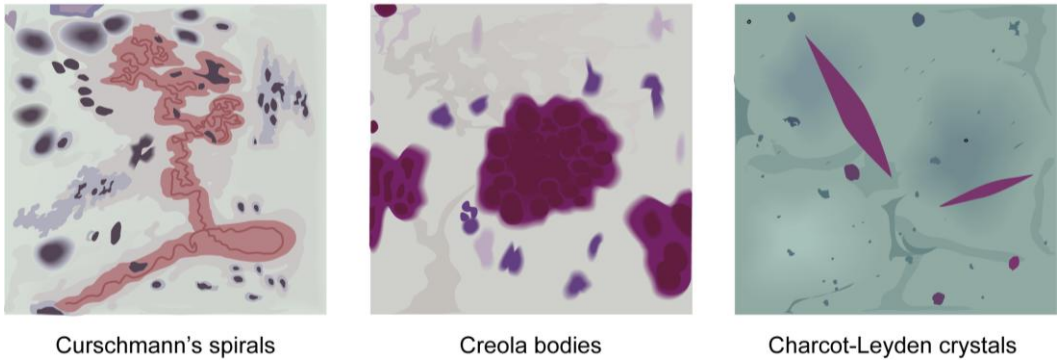


Figure 5-9. Sputum findings seen in asthma.

Table 5-3. Lower Airway Changes in Asthma and COPD

Characteristic	Asthma	COPD
Surface epithelium	**Fragile** or denuded	Usually **not fragile**
Reticular basement membrane	**Thickened** and hyaline	Variable or normal
Bronchiolar mucous cells	Possible mucous metaplasia	Metaplasia or hyperplasia
Bronchial smooth muscle	Enlarged in **large airways**	Enlarged in **small airways**
Mucus histochemistry	No change	**Increased acidic glycoprotein**
Cellular infiltrate	Predominantly CD3, **CD4**, CD25 (IL-2R) positive, **marked eosinophilia**, and increase in mast cells	Predominantly CD3, **CD8**, CD68, CD25, VLA-1 positive, HLA-DR positive, mild eosinophilia, and mast cell increase
Cytokines	**Increase in IL-4 and IL-5** gene expression	GM-CSF protein, ±IL-4; **No increase in IL-5**
Airflow obstruction	Variable, **reversible**; Nonreversible after remodeling	Usually progressive deterioration of lung function
Postmortem findings	Hyperinflation, airway plugs; **Usually no emphysema**	Excessive mucus, more small airway involvement; **Emphysema prominent**

SKIN

The skin is the largest organ in the body. The structure of the skin is depicted in Figure 5-10.

Functions of the skin include thermoregulation, sensation, metabolism (vitamin-D synthesis), physical barrier to external environment, and active participation in host defense with ability to generate and support local immune and inflammatory reactions

Epidermis

Principal cell populations in the epidermis include the following:

- **Keratinocytes** and **melanocytes** do not appear to be important mediators of adaptive immunity. However, keratinocytes do express major histocompatability complex (MHC)-type II proteins and produce several molecules (IL-1, antimicrobial peptides, thymic stromal lymphopoietin [TSLP], and receptor activator of nuclear factor κ-B ligand [RANKL]) that contribute to both the adaptive and innate immune response, respectively. These cytokines also mediate cutaneous inflammation.

Flash Card Q8

What are the pathologic features of airway remodeling?

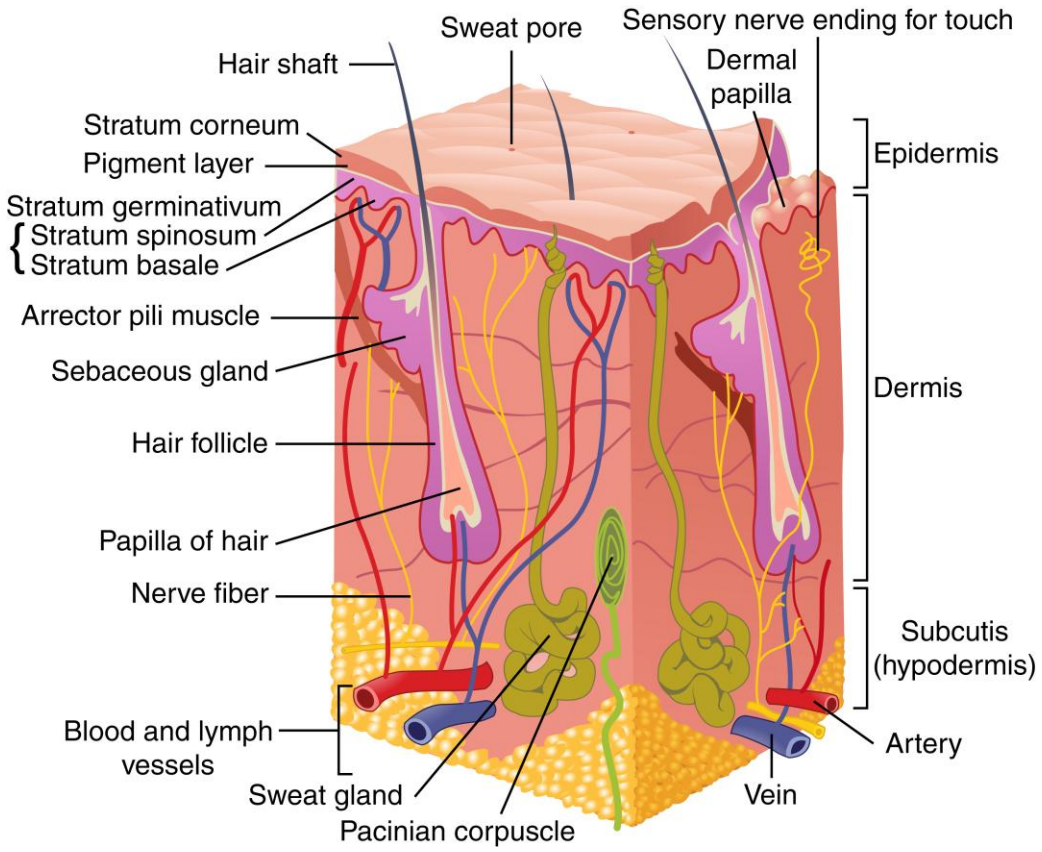


Figure 5-10. Skin structure.

- **Langerhans' cells** are immature dendritic cells of the cutaneous immune system located in the suprabasal portion of the epidermis.
    - They have dendritic processes that enable them to capture antigens entering through the skin.
    - On stimulation by proinflammatory cytokines, Langerhans' cells retract their processes, lose their adhesiveness for epidermal cells, and migrate to the dermis.
    - They subsequently migrate to lymph nodes through afferent lymphatic vessels.
- **Epidermal lymphocytes** constitute only about 2% of skin-associated lymphocytes (the rest reside in dermis), and the majority are CD8+ T lymphocytes.
    - Express a more restricted set of antigen receptors than T lymphocytes in most extracutaneous tissues.
    - The majority express an effector or memory phenotype.
- Langerhans' cell histiocytosis (LCH) is a rare disease caused by clonal proliferation of Langerhans' cells. Clinical presentation involves a nonspecific inflammatory response (fever, lethargy, weight loss) and multifocal organ involvement, including bone, skin, bone marrow, lymph nodes, endocrine glands, and lungs.

Flash Card A8

Airway wall thickening with increased collagen deposition and airway smooth muscle, goblet cell hyperplasia, subepithelial fibrosis, and angiogenesis

Dermoepidermal Junction

The dermoepidermal junction joins the epidermis to the dermis. Its function is to protect against mechanical shear and to serve as a semipermeable barrier.

The basement membrane has three main layers:

- **Lamina lucida**: Includes the extracellular portion of the hemidesmosomes and anchoring filaments
- **Lamina densa**: Includes collagen IV and laminin network
- **Sublamina densa**: Includes anchoring fibrils (collagen VII)

Dermis

Principal cell populations in the dermis include the following and their related functions:

- **Fibroblasts** synthesize and degrade extracellular matrix proteins, collagen, and elastic fibers. They also secrete soluble mediators (e.g., eotaxin when stimulated by IL-4, as well as IL-1 and IL-6).
- **Mast cells** have granules containing both tryptase and chymase. Their development depends on c-*kit* receptor and its ligand stem cell factor (SCF).
- **Macrophages** in dermis have several functions, including phagocytosis, antigen presentation, wound healing, and secretory functions. They express **CD68**.
- **Dermal dendritic cells** can be labeled by factor XIIIa, and can express DC-SIGN/CD 209+, CD1b, CD1c, and CD11c. They play a role in antigen presentation.
- **Dermal T lymphocytes** (both CD4+ and CD8+ cells) are located predominantly in perivascular locations. They express phenotypic markers typical of activated or memory T lymphocytes. Many dermal T lymphocytes also express a carbohydrate epitope called cutaneous lymphocyte antigen (CLA)-1, which may play a role in homing to the skin.

Key Fact

Connective tissue mast cells with tryptase and chymase (MC_{TC}) are present in skin, conjunctiva, heart, and intestinal submucosa (and have the CD88 receptor for C5a anaphylatoxin).

Mucosal mast cells with tryptase (MC_T) present in the surface of the alveolar wall, the respiratory epithelium, and the small intestinal mucosa.

Specialized Structures

Specialized structures of the skin include: hair follicles, sebaceous glands, Meissner's and Pacinian corpuscles, and apocrine and eccrine sweat glands.

Role in Immunity

Skin homing of memory, **effector**, **and regulatory T-cell subtypes (CLA+)** are programmed by skin-derived dendritic cells via **cytokines,** such as **CCR4/CCL17** and **CCR10/CCL27**.

Key Fact

Filaggrin (FLG) serves as the matrix protein promoting aggregation and disulfide bonding of keratin filaments. **Mutations in the FLG gene** have been linked to **ichthyosis vulgaris** and **atopic eczema**. Some of these mutations have also been associated with the propensity to develop asthma in eczema patients and asthma severity that is independent of eczema.

Immunobullous Skin Diseases

Table 5-4 lists immunobullous skin diseases.

Key Fact

In all suspected immunobullous diseases, it is best to obtain two biopsies for diagnosis. One shave biopsy of an intact vesicle or bulla for routine hematoxylin and eosin (H&E) stain; and a second biopsy of perilesional tissue for direct immunofluorescence. (**Note:** Immunoreactants may not be present in lesional tissue.)

Key Fact

Nikolsky's sign is the formation of erosion from shearing pressure applied on normal-appearing skin.

Table 5-4. Immunobullous Skin Diseases

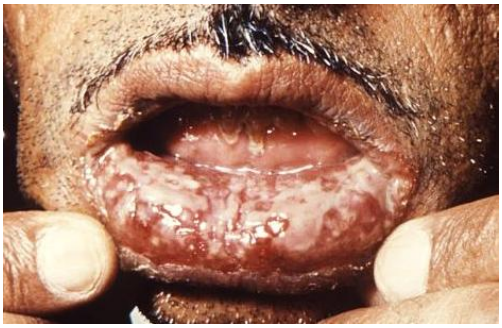
Immunobullous Disease	Clinical Presentation	Serum Autoantibodies	Tissue Immuno-fluorescence
Pemphigus vulgaris (Figure 5-11A)	Flaccid>tense bullae Crusting Nikolsky's sign present Affects scalp, chest, intertriginous areas, and oral mucosa	IgG autoantibodies to desmoglein 1 and 3	Epidermal IgG and C3 cell surface staining of the suprabasal layers
Pemphigus foliaceous	Superficial bullae Erosions Scale with crusting Nikolsky's sign present	IgG autoantibodies to desmoglein 1	Epidermal IgG and C3 cell surface staining of the granular layer
Paraneoplastic pemphigus	Flaccid bullae Lichenoid or erythema multiforme-like lesions Affects mucosa Nikolsky's sign present	IgG autoantibodies to plakin proteins and desmoglein 1 and 3	Epidermal IgG and C3 cell surface and basement membrane zone staining
IgA pemphigus	Pustules, erythema, and flaccid lakes of pus	IgA autoantibodies to desmoglein 3, desmocollin 1	Epidermal IgA cell surface staining
Bullous pemphigoid (Figure 5-11B)	Tense > flaccid bullae Prominent pruritus Nikolsky's sign in some (~10%)	IgG autoantibodies to **BP180** and **BP 230**	Linear basement membrane zone IgG and C3
Cicatricial pemphigoid	Erosions, rare vesicles or bullae Scarring sequelae	IgG autoantibodies to integrins, BP180, and laminins	Linear basement membrane zone IgG and C3
Herpes gestationis	Tense bullae Onset in second trimester pregnancy Pruritus	Complement fixing, basement membrane zone, and epidermal; Autoantibody to BP180	Linear basement membrane zone C3
Epidermolysis bullosa acquisita	Tense bullae, erosions, and scarring Sites of trauma and oral mucosa	IgG autoantibody to collagen VII	Linear basement membrane zone IgG and C3
Linear IgA bullous dermatosis	Tense bullae Oral involvement common	IgA autoantibody LABD97, LAD-1, and others	Linear basement membrane zone IgA

Table 5-4. Immunobullous Skin Diseases, cont.

Immunobullous Disease	Clinical Presentation	Serum Autoantibodies	Tissue Immuno-fluorescence
Dermatitis herpetiformis (Figure 5-11C)	Small bullae on elbows and knees Markedly pruritic Associated with intestinal gluten sensitivity (celiac disease)	IgA autoantibody to epidermal transglutaminase	Granular basement membrane zone IgA with stippling in dermal papillae
Bullous lupus erythematosus	Tense bullae Photo distributed	IgG autoantibody to collagen VII	Linear basement membrane zone IgG; May show granular IgM and C3 basement membrane zone

Key Fact

Pemphigus vulgaris (PV), bullous pemphigoid (BP), and dermatitis herpetiformis (DH) must be known for the boards! (Figure 5-11.)



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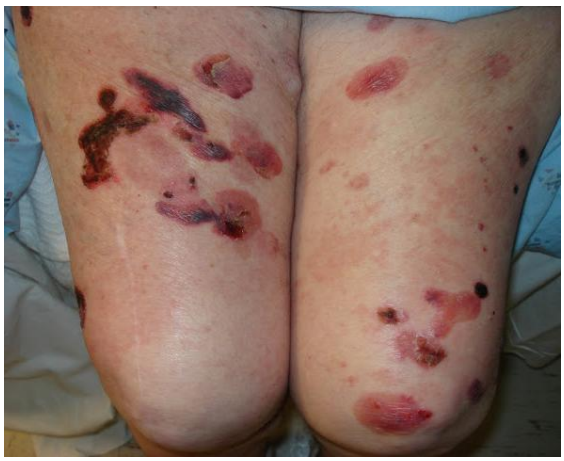
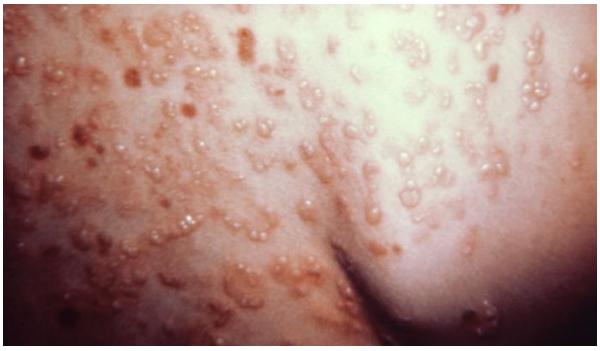


B



C

Figure 5-11. (A) Pemphigus vulgaris lesions on the mucosal surface of the lower lip. (B) Bullous pemphigoid. (C) Dermatitis herpetiformis on a four-year-old child with a vesiculo-papular skin eruption due to dermatitis herpetiformis.
(Figure A reproduced, with permission, from the Public Health Image Library, Centers for Disease Control and Prevention, Atlanta, Georgia, courtesy of Dr. J. Lieberman; Dr. Freideen Farzin, Univ. of Tehran;Figure B reproduced, with permission, from USMLERx.com; Figure C reproduced, with permission, from the Public Health Image Library, Centers for Disease Control and Prevention, Atlanta, Georgia.)

Flash Card Q9

Which condition, PV, BP, or DH, is most likely to show a positive Nikolsky's sign?

Flash Card Q10

Which condition(s), PV, BP, and DH, is(are) extremely pruritic?

Flash Card Q11

In PV and BP, what two molecules react with antigens in the basement membrane zone?

Autoimmune Skin Diseases

Table 5-5 lists autoimmune skin diseases.

Table 5-5. Autoimmune Skin Diseases

Autoimmune Disease	Indirect Immuno-fluorescence Pattern (Serum)	Nuclear Antigens to Which Autoantibodies Are Directed	Direct Immuno-fluorescence of Tissue
Systemic lupus erythematosus (SLE)	Peripheral Homogeneous Nucleolar Speckled	nDNA or dsDNA, ssDNA, histones, nucleolar RNA, various ribonucleo-proteins, cardiolipin, Sm (Smith), U1-snRNP, and HMG-17	Two or more granular immunoglobulin and complement deposits at BMZ, IgG, IgM, and/or IgA with C3 in involved and uninvolved skin (lupus band)
Discoid lupus erythematosus (DLE)	No circulating antibodies	Usually none detected	Two or more granular immunoglobulin and complement deposits at BMZ, IgG, IgM, and/or IgA with C3 in involved skin
Subacute cutaneous lupus erythematosus (SCLE)	Speckled	SS-A/Ro and SS-B/La	Particulate intercellular staining with or without granular immune deposits at BMZ or lichenoid changes
Neonatal lupus erythematosus	Speckled	SS-A/Ro and SS-B/La	Granular IgG (transplacental) at BMZ
Drug-induced lupus erythematosus	Peripheral Homogeneous	Histones	Granular immune deposits at the BMZ
Cutaneous scleroderma	Peripheral	Scl-70, SS-A/Ro, and SS-B/La	No characteristic changes; vascular staining may be observed
Limited scleroderma (CREST)	Centromere	Centromere, Scl-70, U1-snRNP, and HMG-17	No characteristic changes; vascular staining may be observed
Progressive systemic sclerosis	Nucleolar Speckled	Scl-70, U1- and U3-snRNP, fibrillarin, and RNA pol I, II, and III	No characteristic changes; vascular staining may be observed
Dermatomyositis, polymyositis	Speckled Nucleolar	Jo-1, PM-Scl, Mi-2, U1-snRNP and SS-A/Ro s	No characteristic changes; lichenoid features and vascular staining may be observed
Sjögren's syndrome	Fine speckled Nucleolar	SS-A/Ro, SS-B/La, histones, and U1-snRNP	No characteristic changes; vascular staining may be observed
Mixed connective tissue disease (MCTD)	Speckled	U1-snRNP and PM-scl	No characteristic changes; granular immune deposits at the BMZ lichenoid features and vascular staining may be observed

Abbreviations: BMZ, basement membrane zone; ds, doubled-stranded; ss, single-stranded.

Flash Card A9

Pemphigus vulgaris (PV). Only approximately 10% of individuals with BP will have a positive Nikolsky's sign.

Flash Card A10

Dermatitis herpetiformis (DH) and bullous pemphigoid (BP)

Flash Card A11

Immunoglobulin G and C3 complement

Antimicrobial Peptides (AMPs) in the Skin

Defensins are cysteine-rich peptides made up of 29–34 amino acids that are present in skin. They are abundant in neutrophil granules and constitute about 5% of all cellular proteins of human neutrophils.

Defensins are broad in spectrum, and kill a wide variety of bacteria and fungi. **Synthesis of defensins is increased in response to inflammatory cytokines such as IL-1 and tumor necrosis factor (TNF)**, which are produced by macrophages and other cells in response to microbes.

GASTROINTESTINAL

Gut-Associated Lymphoid Tissue (GALT)

- Samples antigens from the gut.
- **Peyer's patches** are located in small and large intestine (Figure 5-12).
- Microfold (M) cells are overlying specialized epithelial cells with microvilli that help in antigen sampling.
- **Follicles** located within lamina propria of intestinal mucosa have **B lymphocytes**.
- **Parafollicular** area have **T lymphocytes**.
- No afferent lymphatics, **lymphocytes reach here via high endothelial venules (HEV)** and leave via efferent lymphatics.

Key Fact

Peyer's patches follicles differ from LN in that there is never a resting appearance due to a constant response to gut flora.

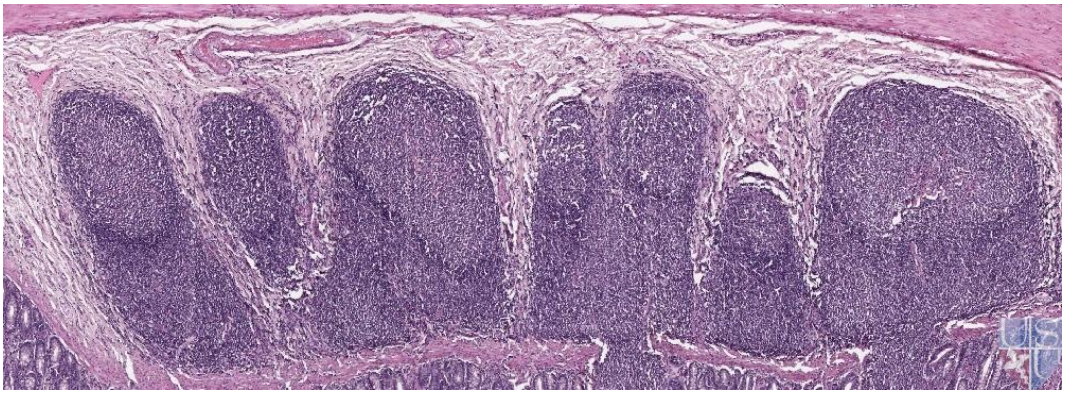


Figure 5-12. Peyer's patches lymphoid follicle.
(Reproduced, with permission, from Uniformed Services University of the Health Sciences, Huck Finne.)

Flash Card Q12

What is a diagnostic biopsy characteristic for eosinophilic esophagitis (EoE)?

Allergic Eosinophilic Esophagitis and Gastroenteritis

Key Fact

Eosinophils are present in all segments of gastrointestinal tract mucosa at baseline EXCEPT for the esophagus.

- Eosinophil-associated gastrointestinal disorders (EGID) comprise a range of disorders, including eosinophilic esophagitis (EoE), eosinophilic gastritis, eosinophilic enteritis, eosinophilic colitis, and eosinophilic gastroenteritis (involves more than one gastrointestinal segment).
- EGID are believed to be primarily a T_h type 2 lymphocyte-mediated process driven by food allergens.
- Characterized by eosinophilic infiltrates in the mucosal, vascular, and/or serosal layers of the gut (Figure 5-13).
- **IL-5** and **Eotaxin-3** are implicated in the pathogenesis of EoE.
- Therapy for EGID involves a combination of interventions including allergen avoidance, elemental diets, and anti-inflammatory agents.
- Allergic proctocolitis, or eosinophilic proctocolitis, typically presents with bloody stools in an otherwise well-appearing infant. The condition occurs in infants, is strongly associated with breast-fed infants, and has a benign and limited course. Dietary proteins, most commonly cow's milk protein, excreted in the mother's milk are responsible for the majority of cases. There is no association with atopy or specific IgE, and greater than 90% will tolerate an unrestricted diet by 1 year of age.

On endoscopy, linear furrows, white papules or plaques, or concentric rings may be found (Figure 5-14).

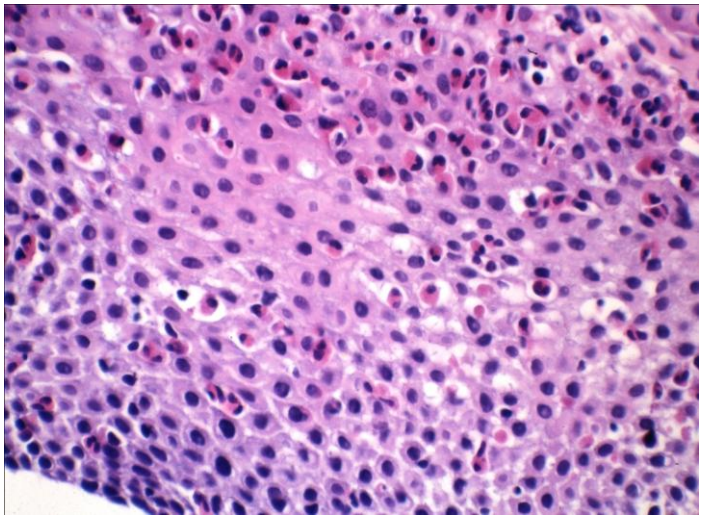


Figure 5-13. Esophageal mucosa infiltrated by eosinophils.
(Reproduced, with permission, from Dr. Douglas Field).

Flash Card A12

Greater than 15 eosinophils per high powered field while on a proton pump inhibitor (PPI) for at least 4–8 weeks.

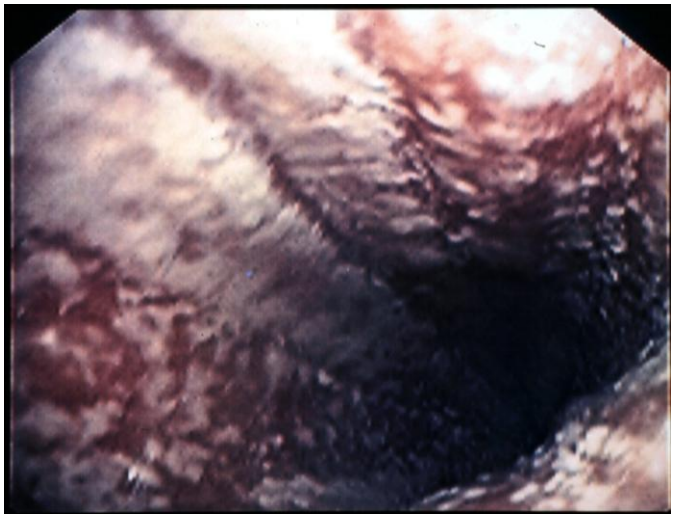


Figure 5-14. Linear furrows and white plaques in esophagus.
(Reproduced, with permission, from Dr. Douglas Field).

Celiac Disease

Celiac disease is an autoimmune disorder of the small intestine that presents in genetically predisposed people of all ages, from infancy onward. It occurs due to sensitivity to **gliadin**, the alcohol-soluble portion of gluten.

With celiac disease, there is **loss of intestinal villi** and **hyperplasia of the crypts** along with **lymphocytic infiltrate** (Figure 5-15).

Clinical presentation involves malabsorption, chronic diarrhea, steatorrhea, abdominal distension, flatulence, weight loss, or failure to thrive. **Oral ulcers** or **dermatitis herpetiformis** may occur in the absence of GI symptoms.

Celiac disease is screened with **IgA antibodies to tissue transglutaminase** (tTG) and **confirmed with intestinal biopsy** when patient is consuming gluten.

Key Fact

Diagnostic screening for celiac disease with anti-tTG IgA should always be accompanied by a serum IgA level. IgA deficiency is often undiagnosed as it can commonly occur without clinical symptoms. Therefore low Anti-tTG IgA levels can be a false-negative result in the setting of IgA deficiency and warrants screening for total IgA in conjunction with anti-tTG IgA.

Flash Card Q13

What mediator is important in the pathogenesis of EoE?

Flash Card Q14

What skin manifestation of celiac disease can present in absence of GI symptoms?

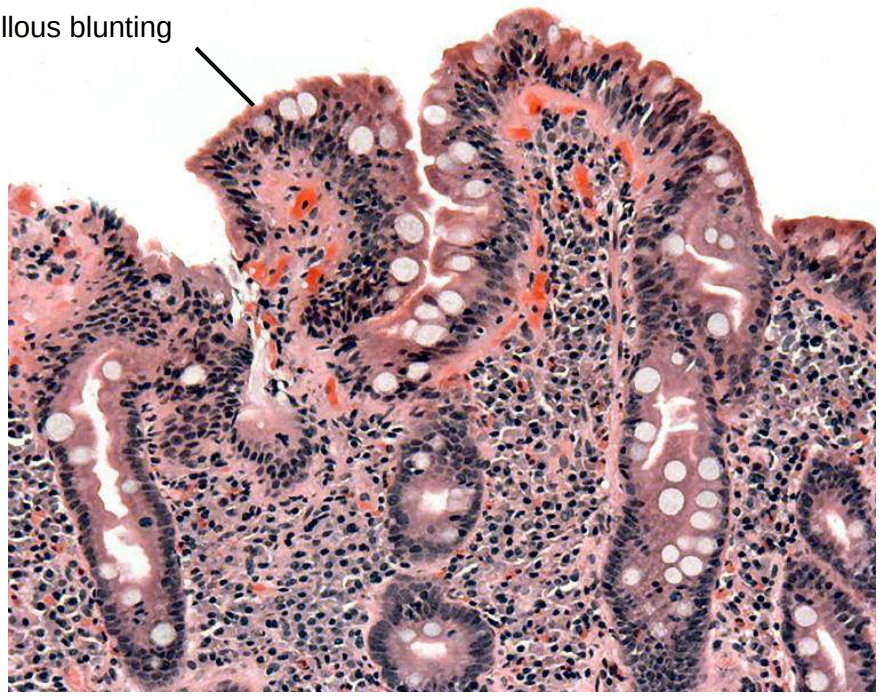


Figure 5-15. Small-bowel biopsy specimen in celiac disease.
(Reproduced, with permission, from Wikimedia Commons.)

Flash Card A13

Eotaxin-3.

Flash Card A14

Dermatitis herpetiformis

6 Research Principles

EXPERIMENTAL DESIGN

TYPES OF STUDIES

There are six main types of experimental design studies: cross-sectional, case series, case control, cohort studies, and randomized control studies.

Cross-Sectional

- **Defined:** Subjects sampled from a population and data regarding presence or absence of exposure and disease are collected at the same time
- **Example:** In a specific group, is there a relationship between smoking and lung cancer?
- **Strengths:** Inexpensive and quick to perform; population-based; and provides a timely "snapshot"
- **Weaknesses:** Recall bias; lacks time sequence (i.e., it is not always possible to discern whether the exposure preceded or followed the disease)

Case Series

- **Defined:** Tracks patients with a known exposure who have been given similar treatment, or examines their records for exposure and outcome
- **Example:** A clinical report on a series of patients
- **Strengths:** Provides a method of investigating uncommon diseases; inexpensive; and can be hypothesis-generating
- **Weaknesses:** No control group with which to compare outcomes; no statistical validity; and may be confounded by selection bias

Flash Card Q1

If cost and time were unlimited, which two studies would yield the most robust data?

Case Control

- **Defined:** Compare subjects who have a condition (the "cases") with patients who do not have the condition but are otherwise similar (the "controls"), examining how frequently the risk factor is present in each group
- **Example:** Do women who use hormone replacement therapy (HRT) have reductions in the incidence of heart disease?
- **Strengths:** Inexpensive and quick study of (several) risk factors; useful in studying infrequent events or when populations would have to be tracked for long periods of time (e.g., development of cancer); and useful for generating odds ratio (OR)
- **Weaknesses:** Do not indicate the absolute risk of factor in question; suffer from confounders since it can be difficult to separate the "chooser" from the "choice" (e.g., those who wear bike helmets vs. those who choose not to wear bike helmets); and do not show cause and effect; recall bias

Cohort Study

- **Defined:** Form of longitudinal study comparing a group of people who share a common characteristic or experience within a defined period with another group. Importantly, cohort identified before appearance of the disease or condition under investigation
- **Examples:** Framingham Heart Study and Nurses' Health Study
- **Strengths:** Longitudinal observations over time; collection of data at regular intervals; reduced recall error; considered gold standard in observational epidemiology; and useful for generating relative risk (RR)
- **Weaknesses:** Expensive to conduct, sensitive to attrition, and requires lengthy follow-up time to generate useful data

Randomized Controlled Trial (RCT)

- **Defined:** Random allocation of different interventions to subjects
- **Example:** Comparison of a standard drug therapy with a new experimental medication regimen; comparison of a new drug with placebo group
- **Strengths:** Consistent selection of subjects and randomization removes most forms of bias
- **Weaknesses:** Expensive; attrition to or loss of follow-up occurs; and treated individuals may not be fully compliant with treatment

Flash Card A1

Cohort and RCT

DATA ANALYSIS AND BIOSTATISTICS

Types of Data

***Variable Defined*—**When the variable is defined, three main types of categorical data are collected: nominal, ordinal, and interval. Table 6-1 provides a summary of these types of categorical data.

Parametric statistical tests are used if the dependent variable is reasonably symmetric or of normal distribution (i.e., not skewed) or if variances of the group seem close. Table 6-2 provides a summary of parametric statistical tests and analogous nonparametric tests.

Table 6-1. Three Main Types of Categorical Data

Nominal	Ordinal (Ranked)	Interval
Data in the form of frequencies fitting discrete, distinct categories	Measures of physical quantities that can be ranked	Differences between the values correspond to real differences between the physical quantities that the scale measures
Example: Race; gender; counting a class, where each individual is either a male or a female, and they cannot be ranked numerically by this data	**Example**: Small, medium, large; responses on a Likert scale	**Example**: Differences in height correspond to actual physical differences

Table 6-2. Parametric Statistical Tests and Analogous Nonparametric

Parametric Tests	Analogous Nonparametric Tests
Student's t-test	Wilcoxon rank-sum test
Analysis of variance (ANOVA)	Kruskal-Wallis test
Paired *t*-test	Wilcoxon signed-rank test (or sign test)
Least square correlation	Spearman rank-order correlation

Error and Power

Type I Error—Occurs when the null hypothesis is falsely rejected. A *p* value indicates the chance that an error is made by accepting the difference between treatments when, in reality, there was no true difference.

- **Example:** The null hypothesis states no difference exists between the response to drug A and the response to drug B.
  - Drug A increased (forced expiratory volume in 1 second) (FEV_1) by 0.41 L
  - Drug B increased FEV_1 by 0.05 L
  - $p < 0.05$
- **Conclusion:** Drug A is "significantly" better than drug B, $p < 0.05$. This means that there is less than a 5% chance that no difference exists between drug A and drug B. The risk of type I error is less than 5%.

Type II Error—Occurs when the null hypothesis when the null hypothesis is not rejected when it is false. In other words, the study fails to find a true difference when one is actually present. A common reason for a type II error is that the sample size is too small.

- **Example:** The null hypothesis states that there is no difference between the response to drug A and drug B.
  - Drug A increased FEV_1 by 0.26 L
  - Drug B increased FEV_1 by 0.09 L
  - $p = 0.25$
- **Conclusion:** Drug A is "not significantly" better than drug B.

Statistical Power—The percentage chance that a difference will be detected if a difference does exist.

- **Calculation:** 1 minus the probability of a type II error. For example, if the probability of a type II error in a study is 5%, the statistical power of the study is 95%.

Probability

Normal Distribution—Produces a bell-shaped curve, where 68% of observations are within one standard deviation (1 SD), and 95% of observations are within 2 SD. Thus, 2.5% of observations are greater than 2 SD above the 95% level, and 2.5% are less than the 95% level. These two populations of 2.5% of observations represent the "two tails" of the bell-shaped curve.

p Values—Express the probability of rejecting the null hypothesis due to chance, when the null hypothesis is true (type I error).

- **Example:** If $p = 0.05$, there is a 5% chance that the observed results are due to chance alone.

Odds Ratio—The probability of occurrence of an event over the probability of nonoccurrence. For example, OR = odds that a case was exposed / odds that a control was exposed. Here, however, the term **odds** can be defined differently, according to the situation.

Relative Risk—The ratio of the risk of disease (or death) among people who are exposed to the risk factor compared with the risk among people who are not exposed. Alternatively, relative risk can be defined as the ratio of the cumulative incidence rate among those exposed compared with the rate among those not exposed. In either case, the term *relative risk* is synonymous with risk ratio.

Prevalence—The percentage of the population with existing disease (at one time point or during one time period) and, as such, is a measure of present disease (**p**revalence = **p**resent).

Incidence—The number of new disease cases in the population over an interval of observation (i**n**cidence = **n**ew).

Sensitivity—The fraction of all true cases the test detects (i.e., among those who have the disease, it refers to how many test positive).

- Defined as true-positive tests or number with disease
- **S**ensitivity is associated with the false-**n**egative rate of a test; and, therefore, can be used to rule **out** disease. For tests with a low false-negative rate, a negative result rules out disease. (Recall tip: **SNout**.)
- **Calculation:** Sensitivity = true positive / (true positive + false negative) or $a/(a+c)$

Specificity—The fraction of all negative cases the test detects (i.e., among those who do not have the disease, it refers to how many test negative).

- Defined as true-negative tests / number without the disease
- Tests with high **s**pecificity are associated with a low number of false **p**ositives and can be used to rule **in** disease. (Recall tip: **SPin**.)
- **Calculation:** Specificity = true negative / (false positive + true negative) or $d/(b+d)$

Positive Predictive Value (PPV)—Describes the probability that a positive test indicates disease.

Flash Card Q2

What type of error occurs when the null hypothesis is falsely rejected?

- **Calculation:** PPV = True positive / (true positive + false positive) or $a/(a + b)$
- PPV determines how many actually have the disease from among those who test positive. This information is found on the first row of a 2 × 2 table (Table 6-3).
-

Negative Predictive Value (NPV) —Describes the probability that a negative test indicates no disease

- **Calculation:** NPV = True negative / (true negative + false negative) or $c/(c + d)$.
- Values for NPV calculation are found on the second row of a 2 × 2 table (Table 6-3).
- **Example:** A new allergy test finds that, in patients with the disease, 80 are positive (true positives), whereas 5 without the disease tested positive (false positives). Of those testing negative, 95 do not have the disease (true negatives); but 20 that had the disease tested negative (false negatives). Table 6-4 shows details of the constructed 2 × 2 table in this scenario.

Table 6-3. Mechanics of a 2 × 2 Table

		Disease Status	
		Positive (+)	**Negative (–)**
Test Result	**Positive (+)**	True positive (a)	False positive (b)
	Negative (–)	False negative (c)	True negative (d)

Flash Card A2

Type I error

Table 6-4. Example With Detailed Calculations for Mechanics of a 2 × 2

		Disease Status		
		Positive (+)	**Negative (–)**	
Test result	**Positive (+)**	80	5	85
	Negative (–)	20	95	115
		100	100	

Sensitivity = $a/(a + c)$ = 80/100 = 80%
Specificity = $d/(b + d)$ = 95/100 = 95%
PPV = $a/(a + b)$ = 80/85 = 94%
NPV = $d/(c + d)$ = 95/115 = 82.6%

Additional formulas:
Odds ratio = $a \times d / b \times c$

This measure is used for case control studies because persons are selected based on disease status so one cannot calculate the risk of getting disease.

Relative risk = $\frac{\text{Incidence rate among exposed}}{\text{Incidence rate among unexposed}} = \frac{a/(a + b)}{c/(c + d)}$

Where:
1 = No association between exposure and disease
>1 = Positive association, and
<1 = Negative association or protective effect
Absolute risk (AR) = $\frac{\text{number of events in treated or control groups}}{\text{number of people in that group}}$
ARC = the AR of events in the control group
ART = the AR of events in the treatment group
Absolute risk reduction (ARR) = ARC – ART
Relative risk (RR) = ART/ARC
Relative risk reduction (RRR) = (ARC – ART)/ARC
RRR = 1 – RR
Number needed to treat (NNT) = 1/ARR

EPIDEMIOLOGY

Validity

The validity of a study is dependent on the degree of the systematic error. Validity is usually separated into two components:

- **Internal validity** is dependent on the amount of error in measurements, including exposure, disease, and the associations between these variables. High internal validity implies a lack of error in measurement and suggests that inferences may be drawn at least as they pertain to the subjects under study.
- **External validity** pertains to the process of generalizing the findings of the study to the population from which the sample was drawn (or, even beyond

that population, to a more universal statement). Internal validity is a prerequisite for external validity.

Bias is any deviation of results or inferences that results in an erroneous estimate of effect of an exposure on the risk of disease. This can be due to flaws in study design, conduct, or analysis of the study. Examples of bias include selection bias or information bias (e.g., recall bias, surveillance bias, misclassification bias, wish bias).

Precision in epidemiologic variables is a measure of random error. Precision is also inversely related to random error, such that a reduction in random error increases precision. Confidence intervals are computed to demonstrate the precision of relative risk estimates. The narrower the confidence interval, the more precise the relative risk estimate.

Clinical Evidence

Evidence classes are used to stratify evidence by quality, such as this one by the US Preventive Services Task Force (USPSTF) for ranking evidence about the effectiveness of treatments or screening:

- **Level I:** Evidence obtained from at least one properly designed randomized controlled trial
- **Level II-1:** Evidence obtained from well-designed controlled trials without randomization
- **Level II-2:** Evidence obtained from well-designed cohort or case-control analytic studies, preferably from more than one center or research group
- **Level II-3:** Evidence obtained from multiple time series with or without the intervention. Dramatic results in uncontrolled trials might also be regarded as this type of evidence
- **Level III:** Opinions of respected authorities, based on clinical experience, descriptive studies, or reports of expert committees

Recommendations for a clinical service are classified by the balance of risk versus the benefit of the service **and** the level of evidence on which this information is based. The USPSTF uses the following recommendations:

- **Level A:** Good scientific evidence suggests that the benefits of the clinical service substantially outweigh the potential risks. Clinicians should discuss the service with eligible patients.

- **Level B:** At least fair scientific evidence suggests that the benefits of the clinical intervention outweigh the potential risks. Clinicians should discuss the treatment with eligible patients.
- **Level C:** At least fair scientific evidence suggests that the clinical treatment does provide benefits, but the balance between benefits and risks are too close for making general recommendations. Clinicians need not offer it unless there are individual considerations.
- **Level D:** At least fair scientific evidence suggests that the risks of the clinical intervention outweigh the potential benefits. Clinicians should not routinely offer the treatment to asymptomatic patients.
- **Level I**: Scientific evidence is lacking; it is of poor quality, or conflicting, such that the risk versus the benefit balance cannot be assessed. Clinicians should help patients understand the uncertainty surrounding the clinical treatment.

INFORMED CONSENT

The *Belmont Report* was issued in 1979. It explains the unifying ethical principles that form the basis for the National Commission's topic-specific reports and the regulations that incorporate its recommendations.

The three fundamental ethical principles for using any human subjects for research are:

- **Respect for persons:** Protecting the autonomy of all people and treating them with courtesy and respect, and allowing for informed consent
- **Beneficence:** Maximizing the benefits for the research project while minimizing the risks to the research subjects
- **Justice:** Ensuring that reasonable, nonexploitative, and well-considered procedures are administered fairly (e.g., the fair distribution of costs and benefits to **potential** research participants)

These principles remain the basis for the Health and Human Service's (HHS) human subject protection regulations.

Elements of informed consent include: (1) competence; (2) disclosure; (3) understanding; (4) voluntariness; and (5) consent. In addition, the patient should have an opportunity to ask questions to elicit a better understanding of the treatment or procedure. This communication process (or any variation thereof) is both an ethical obligation and a legal requirement as spelled out in statutes and case law in all 50 states.

Flash Card Q3

Name the three fundamental ethical principles of human research?

If a patient is competent to act, receives thorough disclosure, has an understanding, and is voluntary in his or her consent, then informed consent is viable. For informed consent to be legally recognized in medical practice, the following steps need to be clearly articulated:

- **Preconditions:** These include competence (to understand and decide) and voluntariness (in deciding).
- **Information elements:** These include disclosure (of risks/benefits), recommendation (plan), and understanding (of information and plan).
- **Consent elements:** These include authorization (based on patient autonomy).

ADVERSE EVENT REPORTING

The **Adverse Event Reporting System (AERS)** is a computerized information database designed to support the Food and Drug Administration's (FDA) postmarketing safety surveillance program for all approved drug and therapeutic biologic products. The FDA uses AERS to monitor for new adverse events and medication errors that might occur with these marketed products.

Reporting of adverse events from the point of care is voluntary in the United States. The FDA receives some adverse event and medication error reports directly from health care professionals and consumers. Health care professionals and consumers may also report these events to the products' manufacturers. If a manufacturer receives an adverse event report, it is required to send the report to the FDA as specified by regulations. However, AERS data do have limitations:

- There is no certainty that the reported event was actually due to the product. (The FDA does not require that a causal relationship between a product and event be proven.)
- The FDA does not receive all adverse event reports that occur with a product. Therefore, AERS cannot be used to calculate the incidence of an adverse event in the US population.

Mandatory reporting is required for the following areas:

- Over-the-counter products and dietary supplements
- Drug, biologic, or human cell tissues, and cellular and tissue-based product manufacturers, distributors, and packers
- Adverse reactions (ARs) relating to human cell and tissue products (HCT/P)

Flash Card A3

Respect for persons, beneficence, and justice

Section 2. Clinical Science

7 Hypersensitivity Disorders

RHINITIS

The nose knows that not all that sneezes are allergic. The nose also knows that this is too routine a topic and far too "bread and butter" to be high yield for the boards.

ALLERGIC RHINITIS (AR)

Epidemiology

- Prevalence 15–25%
- Uncommon in patients younger than 2 years old (especially rare for seasonal AR younger than 2 years old)
- Most common chronic disease of childhood. Male predominance in childhood with no gender disparity by adulthood
- Mean age of onset is 10 years
- 80% of cases develop before 20 years of age
- 20% of AR is seasonal, 40% is perennial, and 40% is mixed (i.e., perennial with seasonal exacerbation)
- About half of chronic rhinitis allergic in nature

Risk Factors

Although the prevalence is generally increasing, this trend varies markedly from country to country by up to 10-fold. Generally, industrialized countries are showing a more pronounced increase; however, even this trend varies among countries.

The prevalence rate is increasing at a faster pace in younger age groups than in adults.

Decreased exposure to infections in childhood (i.e., the hygiene hypothesis) is one posited mechanism of the increase. This is broadly supported by data showing a decrease in the risk of allergen sensitization and clinical allergy with:

- The protective effect of the number of siblings
- A rural upbringing, with exposure to farmyard animals early in life (with higher endotoxin),
- Day care attendance
- Large family size
- Exposure to household pets

Morbidity

As shown in the 2010 National Allergy Survey Assessing Limitations study, significant differences between those with AR and the general population include:

- Those with AR rate their health significantly lower
- Significant effect on sleep: Difficulty getting to sleep and/or waking up during the night
- Significant effect on daily life for more than 50% of patients with AR
- More of a limitation with work, with an estimated 20% decrease in productivity

Pathophysiology

AR is usually due to aeroallergen sensitivity to dust mites, pets, pollens, mold, and cockroaches. Allergens cross-link the allergen-specific IgE receptor (FcεRI) on previously sensitized mast cells, which leads to degranulation. More recent evidence suggests that IgE may be made in nasal tissues and upper airway lymphatics even in the absence of systemic production.

Immediate Allergic Response—Occurs within 15 minutes. Mast cells release **preformed mediators:** histamine, proteases such as tryptase, and kinins such as kallidin and bradykinin. Mast cells also release **newly formed mediators**: prostaglandin-2 (PGD_2) and leukotrienes (LT) LTC4, LTD4, and LTE4.

Acute symptoms are influenced by specific mediators:

- Histamine: Itch, sneeze, rhinorrhea
- PGD2: Nasal congestion
- Leukotrienes: Nasal congestion
- Kinins: Nasal congestion and/or blockage

Late Allergic Response—Begins within 4–8 hours and can last for more than 24 hours. Cellular infiltrates occur with **basophils,** eosinophils, and infiltrating lymphocytes, especially T_h2 CD4 T lymphocytes. Since the mast cell is less important, there is no increase in tryptase. T_h2 cells release cytokines, such as IL-3 and IL-5 (eosinophil factors), as well as IL-4 and IL-13 (IgE class switch). Nasal mucosal epithelial cells have been shown to generate granulocyte–monocyte colony-stimulating factor (GM-CSF, an eosinophil growth factor) and stem cell factor (i.e., mast cell growth factor), as well as eotaxin and regulated on activation, normal T expressed and secreted (RANTES, also known as chemokine (C-C motif) ligand 5, or CCL5). Predominant symptoms include nasal congestion and mucus production.

Nasal hyperresponsiveness to both allergic and nonallergic triggers may be observed. AR is a risk factor for asthma, and most asthmatics (80%) have AR. AR may be associated with lower airway abnormalities, such as basement membrane thickening, even in the absence of asthma.

Key Fact

30–50% of patients with chronic rhinitis have nonallergic triggers

Exhaled nitric oxide (eNO) is a sensitive marker of inflammation that is increased in AR and decreased in sinusitis.

Priming is the effect by which progressively lower doses of allergen are needed to trigger subsequent symptoms, perhaps due to the recruitment of inflammatory cells such as eosinophils and mast cells that cause progressive inflammation and increased mucosal sensitivity. Clinically, residence in a geographic location is required for at least 1 year, and usually longer for sensitization to take place.

Signs and Symptoms

Classically, symptoms of AR include rhinorrhea, nasal pruritus, nasal congestion, and sneezing.

Signs of AR include:

- Allergic shiners
- The “allergic crease”
- Dennie-Morgan lines (linear creases or furrows underneath the lower eyelids)
- Pale nasal mucosa
- Turbinate hypertrophy
- Mouth breathing
- Cobblestoning of the oropharynx

Common comorbid conditions are allergic conjunctivitis, rhinosinusitis, asthma (up to 40% of AR patients have asthma) and pharyngeal lymphoid hyperplasia with resultant obstructive sleep apnea (OSA) and disordered sleep.

Flash Card Q1

What symptom is particularly prominent in the late-phase response of allergic rhinitis?

Diagnosis

Diagnosis of AR requires skin testing (prick or intradermal) or serum-specific IgE in the appropriate clinical context. Although both methods generally have good agreement, skin tests are more sensitive but less specific than in vitro allergen-specific IgE tests. History has a reasonable positive predictive value (PPV) of about 70% in patients with symptoms.

Less useful clinically is the seasonal-versus-perennial distinction. AR may be classified based on symptom severity (i.e., mild, moderate, or severe) and frequency (i.e., intermittent vs. persistent) as defined by allergic rhinitis and its impact on asthma (ARIA) guidelines.

Treatment

Treatment of choice is allergen avoidance, but this is not always practical (i.e., pollen allergy). **Intranasal steroids are the most effective medication for AR and, therefore, the medication of choice.** They work by causing vasoconstriction, reducing mucosal edema, and inhibiting the expression of cytokines and mediators. First-generation antihistamines are also effective but limited by side effects due to their relative lack of selectivity for the histamine (H_1) receptor and their ability to cross the blood-brain barrier. Nonsedating (second-generation) oral antihistamines are also effective but have fewer side effects as they are more selective for peripheral H_1 receptors. They are often used in combination with intranasal steroids despite the fact that the addition of an oral antihistamine to daily intranasal steroid therapy has not clearly demonstrated any added efficacy in controlled clinical trials. Additional medications may include:

- Nasal antihistamines
- Antileukotriene therapy
- Oral and intranasal decongestants (short-term use only)
- Cromoglycolates
- Allergen immunotherapy

The best treatment for patients with inadequately controlled AR, who have failed avoidance and standard medications, is allergen immunotherapy. Allergen immunotherapy improves AR symptoms, decreases the risk of developing new sensitizations, and helps decrease the risk of developing asthma in children.

Flash Card A1

Nasal congestion

NONALLERGIC RHINITIS

Table 7-1 provides a summary of differential diagnosis of nonallergic rhinitis.

Table 7-1. Summary of Differential Diagnosis of Nonallergic Rhinitis

Type of Rhinitis	Associations
NARES[a]	Eosinophilia on nasal smear
Vasomotor	Irritant, gustatory, cold- or temperature-induced
Medications	ASA, NSAIDs, topical decongestants, α antagonists, β blockers, other antihypertensives
Hormonal	OCPs, pregnancy, menstrual associations, hypothyroidism
Atrophic	Elderly
Infectious	Common cold viruses
Occupational	Flour (bakers), latex (health care workers), pet dander (animal handlers)
Miscellaneous	Anatomic, tumor, systemic disease, CSF rhinorrhea

[a]NARES: Nonallergic rhinitis with eosinophilic syndrome.
ASA, aspirin; CSF, cerebrospinal fluid; NSAIDs, nonsteroidal anti-inflammatory drugs; OCPs, oral contraceptive pills.

Vasomotor or Irritant Rhinitis

Vasomotor or irritant rhinitis indicates noninflammatory nasal hyperresponsiveness with sympathetic and parasympathetic nerve imbalance. Common triggers are:

- Cold air
- Dry air
- Barometric pressure or temperature shifts
- Strong scents (i.e., tobacco, perfume, or cleaning materials)
- Other respiratory irritants

Rhinorrhea may be the only presenting symptom. Treatment usually consists of intranasal antihistamines such as azelastine. **Gustatory rhinitis**, a common subtype of vasomotor or irritant rhinitis, is triggered by eating, possibly by stimulation of vagal (parasympathetic) innervation. A common treatment option is an anticholinergic medication—intranasal ipratropium.

Nonallergic Rhinitis with Eosinophilia

Nonallergic rhinitis with eosinophilia syndrome (NARES) is a poorly understood condition with typical symptoms of AR and documented eosinophilia on nasal smear cytology in the absence of increased total or specific IgE. It typically

Flash Card Q2

Intranasal corticosteroids block which phase(s) of the allergic response?

occurs in middle-aged adults. These patients are prone to nasal polyps, and this condition may represent a prodrome to aspirin-exacerbated respiratory disease (AERD). NARES responds well to intranasal corticosteroids.

Atrophic Rhinitis

Atrophic rhinitis is a noninflammatory condition associated with loss of the normal secretory/humidifying function of the nose. It occurs more frequently in young to middle-aged patients, especially females at onset of puberty. The primary (idiopathic) form is more predominant in developing countries with a warm climate and is generally associated with microbial colonization (e.g., *Klebsiella ozaenae*). Secondary causes are frequent in the developed world and occur in those with a history extensive nasal and sinus surgeries, chronic granulomatous nasal infection, or irradiation). Symptoms of atrophic rhinitis include:

- Nasal congestion
- Nasal pain upon inspiration from excess mucosal dryness
- Nasal crusting
- A foul smell in the nasal vault (fetor)

Treatment may include nasal saline irrigation and topical antibiotics.

Medication-Induced Rhinitis

Some common medications and drugs that can cause medication-induced rhinitis include:

- ASA and NSAIDs (as a feature of AERD)
- β blockers and various other antihypertensive medications
- Rhinitis medicamentosa: Prolonged use of intranasal decongestants (rebound congestion)
- Angiotensin-converting enzyme (ACE) inhibitors
- OCPs
- **Sildenafil**
- Intranasal cocaine or methamphetamine.

Key Fact

Intranasal corticosteroids are ineffective in rhinitis of pregnancy.

Hormone-Induced Rhinitis

Flash Card A2

They block both the early- and late-phase responses

Rhinitis may occur due to fluctuations in estrogen or progesterone and may thus occur perimenstrually or during OCP use, puberty, or pregnancy. Hormone-induced rhinitis may be associated with hypothyroidism. Any of the causes of rhinitis may occur during pregnancy. True pregnancy-induced rhinitis occurs in 1

in 5 pregnant patients, is not present prior to pregnancy, does not present before the second trimester, and usually resolves within 2 weeks postpartum.

Infectious Rhinitis

This is the most common cause of pediatric nonallergic rhinitis. It is distinguished from AR by a lack of pruritus and limited duration. More than 200 different viruses may cause infectious rhinitis, but common culprits are:

- Rhinovirus
- Parainfluenza
- Adenovirus
- Coronavirus
- Influenza virus

Miscellaneous Rhinitis

Common miscellaneous causes include:

- Rhinitis triggered by emotion
- Occupational rhinitis
- Anatomic or mechanical factors (e.g., foreign body or septal deviation)
- Systemic disease (e.g., Wegener's granulomatosis)
- Tumor (e.g., nasopharyngeal carcinoma)
- Diseases of ciliary dysfunction

Persistent unexplained rhinorrhea, particularly with a history of trauma and/or if unilateral, should trigger a search for CSF rhinorrhea (e.g., check β_2-transferrin).

SINUSITIS

ACUTE RHINOSINUSITIS

Acute rhinosinusitis (ARS) is defined as inflammation of the mucosal lining of the nasal and sinus cavities lasting up to 4 weeks. (Subacute rhinosinusitis: >4 weeks, chronic rhinosinusitis: >8–12 weeks).

Flash Card Q3

What is the most common cause of rhinitis during pregnancy?

Flash Card Q4

Why can nasal congestion or rhinorrhea develop in hypothyroidism?

Epidemiology

Rhinitis and sinusitis usually coexist in most patients as the nasal mucosa is connected to that of the paranasal sinuses, hence the term rhinosinusitis. Annually, nearly 1 in 7 adults older than 18 years are diagnosed with rhinosinusitis; adults between 45–75 years old are most commonly affected. Incidence is higher in females.

Pathogenesis

The vast majority of ARS cases are viral (>90%), including:

- Rhinovirus
- Coronavirus
- Influenza virus
- Parainfluenza
- Respiratory syncytial virus (RSV)
- Adenovirus
- Enterovirus and others

Anatomic obstruction is the usual inciting event (e.g., **blocked ostiomeatal complex**). For bacterial sinusitis, the most common pathogens are the same that cause otitis media: ***Streptococcus pneumoniae***, ***Moraxella catarrhalis***, and ***Haemophilus influenzae.*** Less frequent but also common etiologies include group A strep, *Staphylococcus aureus*, and anaerobes. Resistant strains of *S. pneumoniae* occur due to altered penicillin-binding proteins which can be compensated for by increasing the dose of amoxicillin. Up to 50% of *H. influenzae* is β-lactamase-positive, as is nearly 100% of *M. catarrhalis*; therefore the addition of a β-lactamase inhibitor is useful.

Flash Card A3

Pre-existing allergic rhinitis: About 1/3 of females with AR experience symptom exacerbation during gestation.

Predisposing factors of ARS include:

- Allergy
- Nasal polyps
- Anatomic variations (e.g., nasal septal deviation)
- Preceding upper respiratory infection (URI)
- Conditions that impair mucociliary clearance (e.g., cystic fibrosis [CF] or smoke exposure)
- Immunodeficiency

Flash Card A4

Release of thyroid-stimulating hormone (TSH) results in increased edema in the turbinates

Signs and Symptoms

There are both major and minor symptoms for rhinosinusitis.

Major symptoms include:
- Purulent anterior or posterior (postnasal drip) rhinorrhea
- Nasal congestion and/or obstruction
- Facial congestion and/or "fullness"
- Facial pain/ pressure
- Fever
- Hyposmia and/or anosmia

Minor symptoms include:
- Headache
- Ear pain and/or pressure and/or fullness
- Bad breath
- Dental pain
- Fatigue
- Cough

Diagnosis for ARS requires at least 2 major symptoms or 1 major and at least 2 minor symptoms.

Differential Diagnosis

It can be difficult to distinguish acute bacterial rhinosinusitis (ABRS) from viral rhinosinusitis. The following clinical scenarios best identify patients with ABRS:
- Persistent signs/symptoms compatible with ARS for more than 10 days, no sign of clinical improvement
- **Severe** signs/symptoms (fever $\geq 102^{o}$F) and either purulent rhinorrhea or facial pain lasting for at least 3–4 consecutive days (at start of illness)
- URI symptoms for 5–6 days followed by worsening signs/symptoms (new fever onset, headache or increase in rhinorrhea)—this is known as "double sickening."

Work-up

During uncomplicated URI, patients may have significant radiographic abnormalities; the presence of these positive findings can't confirm the presence of rhinosinusitis. CT scans should be performed in ABRS with suspected suppurative complications. Recurrent ARS or sinusitis with complications warrants a search for predisposing conditions (e.g., allergy, anatomic abnormality, or immunodeficiency). Cases refractory to standard treatment may warrant ENT

Flash Card Q5

Gravity cannot drain which sinus?

consultation for culture of the organism via direct sinus aspiration (recommended) or endoscopically guided cultures of middle meatus (adults only). Note: A nasal swab culture is unreliable for diagnosis of ABRS.

Treatment

Most cases of ARS (viral) may be treated supportively and with reassurance. Empiric treatment should be started as soon as ABRS has been diagnosed (refer to the three earlier scenarios). Children should be treated for 10–14 days, whereas adults can be treated for 5–7 days.

The initial antibiotic treatment of choice for children and adults with ABRS is **amoxicillin-clavulanate**, rather than amoxicillin alone. High-dose amoxicillin-clavulanate should be given to patients living in areas with high endemic rates of invasive resistant *S. pneumoniae*, fever >102^{o}F, threat of suppurative complications, participating in day care, younger than 2 years or older than 65 years of age, recently hospitalized, treated with antibiotics in preceding month, or those who are immunocompromised.

Penicillin (PCN)-allergic adults may be treated with a fluoroquinolone or doxycycline. Children with a history of type I reaction to PCN may be treated with levofloxacin, and children with other types of allergic reactions to PCN can be treated with clindamycin in combination with a third-generation cephalosporin. Levofloxacin is not licensed by the FDA for routine use in children.

Empiric therapy with macrolides or trimethoprim-sulfamethoxyzole (TMP-SMX) is not recommended due to high resistance rates. Coverage should be broadened in those who do not improve or worsen after 3–5 days.

RECURRENT SINUSITIS

Recurrent sinusitis is defined as repeated (typically ≥4 episodes in 1 year) occurrences of acute sinusitis. Patients with chronic or recurrent sinusitis should be evaluated for underlying inflammation, allergy, **immunodeficiency**, and anatomic abnormalities. The majority of immunodeficient patients with recurrent sinusitis have defects in humoral immunity. However, other types of immunodeficiencies might present with recurrent sinusitis as one of their clinical features, including AIDS.

Flash Card A5

Because of the position of the maxillary ostia, gravity cannot drain the contents of the maxillary sinus when the head is upright.

CHRONIC RHINOSINUSITIS

Chronic rhinosinusitis (CRS) is an inflammatory (rather than infectious) condition that is defined by radiographic evidence of sinusitis with symptoms persisting >8–12 weeks. The presence of ≥2 symptoms (i.e., nasal obstruction/congestion, facial pain/pressure/fullness, purulent anterior/posterior rhinorrhea, hyponosmia /anosmia) for >12 weeks in conjunction with documented inflammation (i.e., purulence or edema in middle meatus or ethmoid region, polyps in middle meatus or nasal passages, and/or imaging documenting paranasal sinus inflammation) is highly sensitive for diagnosing CRS. There is less evidence to support the use of antibiotics in CRS than in ABRS because microorganisms (typically anaerobes) are thought to be secondary to the inflammatory mechanisms causing CRS or suggestive of colonization only. However, acute exacerbations of CRS are commonly treated with antibiotics.

Differential Diagnosis

The differential diagnosis of CRS is similar to that of AR and includes:

- Infectious rhinitis
- AR
- Nonallergic rhinitis (e.g., vasomotor, eosinophilic, etc.)
- AERD
- Rhinitis medicamentosa
- Anatomic abnormalities (e.g., nasal septum deformity)
- Tumors (e.g., inverted papillomas)
- Granulomatosis with polyangiitis (Wegener's)
- Eosinophilic granulomatosis with polyangiitis (Churg-Strauss)
- Asthma
- Fungal sinus disease
- Nonrhinogenic causes of facial pain (e.g., migraine, trigeminal neuralgia, etc.)

Flash Card Q6

Which inflammatory mediators are elevated in ARS?

Work-up

Work-up for CRS is likely to include sinus imaging (i.e., CT scan), allergy testing, and rhinoscopy in refractory or persistent cases. Depending on the clinical context, work-up may also include:

- Quantitative immunoglobulins, pneumococcal titers
- Ciliary function tests
- Sweat test (to rule out cystic fibrosis, CF)
- HIV testing
- Sinus puncture with cultures.

Flash Card Q7

Which of the following is a risk factor for the development of sinusitis: concha bullosa or Haller cell?

Treatment

Treatment for CRS may include:

- Antibiotics for acute exacerbations
- Treatment of underlying or exacerbating conditions (e.g., AR, gastroesophageal reflux disease [GERD], AERD, CF)
- Topical nasal steroids or short courses of systemic steroids
- Surgical consultation (e.g., for functional endoscopic sinus surgery [FESS]).

Surgery is indicated in sinusitis with complications or in cases of medical treatment failure. Note: Antifungals are indicated in invasive fungal disease (usually only seen in diabetics or immunodeficient patients).

Complications of Sinusitis

Complications of sinusitis are rare in general. However, these are seen in greater frequency with sphenoid and frontal sinusitis, which include:

- Orbital cellulitis
- Subperiosteal abscess
- Cavernous sinus thrombosis
- Meningitis
- Subdural or epidural brain abscess
- Osteomyelitis
- Mucocele

CRS with Nasal Polyps

Flash Card A6

IL-1β, IL-6, and especially IL-8

CRS with nasal polyps (NPs), also known as hyperplastic eosinophilic sinusitis, represents about one third of CRS cases. Polyps most commonly arise from the **ethmoid sinuses.** Compared with CRS without NPs, CRS with NPs has an increased incidence of:

- Anosmia
- Dust mite sensitization
- Eosinophils on biopsy
- Asthma
- AERD
- **Allergic fungal rhinosinusitis (AFRS)**

Flash Card A7

Haller cell—a pneumatized ethmoid cell that blocks the ostiomeatal complex. Concha bullosa, an aerated middle turbinate, is not a risk factor.

CRS with NPs is not usually associated with bacterial causes, except in cases of local production of staphylococcal enterotoxins in NPs, which may increase local IgE production and eosinophilic inflammation via T_h2 skewing.

Both CRS with and without NPs have significant nasal obstruction/congestion and rhinorrhea. In CRS without NPs there is a higher rate of facial pain. Patient with CRS with NPs tend to have more problems with hyponosmia/anosmia, whereas these is rare in CRS without NPs.

Nasal steroids or short courses of systemic steroids are of clear benefit, and surgery may be indicated for treatment failures.

CRS Without Nasal Polyps

A summary of inflammatory mediators in CRS with and without NPs is given in Table 7-2.

Table 7-2. Summary of Inflammatory Mediators in CRS with and Without Nasal Polyps

	CRS Without NP	CRS with NP
IL-3	↑↑	–
GM-CSF	↑	–
Eosinophil cationic protein	↑	↑↑
IL-5	–	↑↑
IgE	–	↑↑
Eotaxin	–	↑↑
LTC4/D4/E4	↑	↑↑
LTB4	↑	↑
PGE_2	↑↑	↓
Lipoxin A4	↑	↑↑

Ig, immunoglobulin; IL, interleukin; LT, leukotriene; PG, prostaglandin.

OTITIS MEDIA

ACUTE OTITIS MEDIA

Epidemiology and Risk Factors

Acute otitis media (AOM) typically follows URI. Peak incidence occurs in children 6–18 months old. Risk factors include:

- Male gender
- Day care attendance or older sibling in home
- Low socioeconomic status
- Bottle propping (i.e., feeding while supine)
- Passive smoke exposure
- Craniofacial abnormalities such as cleft lip or palate
- Factors affecting immunity (e.g., immunodeficiency, prematurity, altered host defenses such as allergic rhinitis or age younger than 3 years)

Key Fact

Children younger than 3 years of age are at increased risk for AOM because of a lack of pneumococcal antibodies and the horizontal position of the eustachian tube interferes with drainage.

Recurrent AOM is defined as ≥3 episodes within 6 months or ≥4 episodes within 1 year with at least 1 episode in the past 6 months. A blocked eustachian tube, which drains from the middle ear into the nasopharynx, causes an effusion; and, when the effusion becomes infected, AOM results. Sixty percent to 80% of children have an episode by the age of 1 year, and 80–90% have at least one episode by age 2–3 years. Breast feeding is protective.

Microbiology

AOM is 50–90% bacterial in different series. The microorganisms most commonly involved are the same as those for acute sinusitis: ***Streptococcus pneumoniae***, **nontypeable *Haemophilus influenzae***, and ***Moraxella catarrhalis***. Traditionally, *S. pneumoniae* was more common. Since the introduction of pneumococcal vaccination, *S. pneumoniae* = *H. influenzae*, and *M. catarrhalis* is third. In neonates, otitis may be caused by group B Strep. Viral causes can typically occur secondarily to RSV or rhinovirus.

Key Fact

Conjunctivitis with otitis (otitis-conjunctivitis) is more likely caused by nontypeable *H. influenzae* and suggests that a broader-spectrum antibiotic like amoxicillin-clavulanate may be indicated.

Signs and Symptoms

Signs of AOM include:

- Fever
- Fussiness
- Tugging at ear
- Otalgia

The physician should assess for bulging of the tympanic membrane (TM), quintessential sign distinguishing AOM from OM with effusion, decreased TM mobility on pneumatic otoscopy, or intense TM erythema. AOM can be diagnosed in children who present with:

- Moderate to severe bulging of the TM or new onset of otorrhea not due to acute otitis externa
- Mild bulging of TM and recent (<48 hours) onset of ear pain (holding, tugging, rubbing of ear in the nonverbal child) or intense erythema of the TM

Treatment

Table 7-3 shows treatment guidelines for AOM.

Table 7-3. Criteria for Therapy

Age	Presentation	Decision
<6 months of age	Unilateral or bilateral involvement with severe signs/symptoms: • Moderate-to-severe otalgia • Otalgia for at least 48 hr • Fever >102.2°F)	Treat × 10 days
6–23 months of age	Bilateral involvement without severe signs/symptoms: • Mild otalgia for < 48 hr • Temperature <102.2°F	Treat × 10 days
6–23 months of age	Unilateral involvement without severe signs/symptoms	Joint decision making with parent(s)/caregiver regarding: • Treatment × 10 days or • Observation with close follow-up
>24 months of age	Unilateral or bilateral involvement without severe signs/symptoms	Joint decision making with parent(s)/caregiver regarding: • Treat (2–5 years: 7 days; >6 years 5–7 days) or • Observation with close follow-up

If a patient has not received amoxicillin in the past 30 days or does not have concurrent purulent conjunctivitis, then high-dose amoxicillin can be prescribed. Otherwise another antibiotic with additional β-lactamase coverage (e.g., amoxicillin/clavulanate) should be prescribed. A patient history of recurrent AOM unresponsive to amoxicillin is also an indication to prescribe additional β-lactamase coverage. During the close follow-up period, if a patient's symptoms have worsened or failed to respond within 48–72 hours, reevaluation and consideration for a different coverage is necessary. Patients with type I PCN allergy and negative testing/challenge to cephalosporins may be treated with cefdinir, cefuroxime, cefpodoxime, or ceftriaxone. Patients with AOM that fails treatment with amoxicillin should be given amoxicillin-clavulanate. Amoxicillin-clavulanate treatment failures should be treated with 3 days of ceftriaxone. Bactrim (TMP-SMX) is not useful due to substantial resistance rates. Consider ENT referral for tympanocentesis and culture, and possible tympanostomy tubes in patients with refractory or recurrent AOM. Don't forget to assess for pain and give treatment to reduce it.

OTITIS MEDIA WITH EFFUSION

Unlike AOM, otitis media with effusion (OME) is a condition that does not warrant antibiotic treatment, as the effusion in this case is sterile. Most commonly, it occurs in children younger than 2 years of age, may occur in some children from 2–6 years of age, and very rarely occurs after the age of 6. The only symptoms may be subtly decreased hearing and the sensation of fullness in the ear. Pneumatic otoscopy is sensitive (nearly 90%), but not as specific (50–88%), whereas a tympanogram may be helpful for confirming uncertain cases or documenting hearing loss (Figure 7-1). This is a common, potentially incidental finding in children; but, a persistent effusion in adults could indicate a structural abnormality such as a tumor. The most common cause is recent AOM with lingering eustachian tube dysfunction. There is insufficient evidence to suggest a causal link between atopy and OME, although there is a strong increased relative risk of OME in atopic patients.

After AOM, the majority will have an effusion at 2 weeks, nearly 50% at 1 month, 10–25% at 3 months, and 5–10% will last 1 year or longer. **OME is the leading cause of hearing loss in children (usually conductive)**, and it may be associated with language delay in children younger than 10 years of age. The average hearing loss from OME is 25 dB (mild = 21–39 dB loss; moderate ≥ 40 dB).

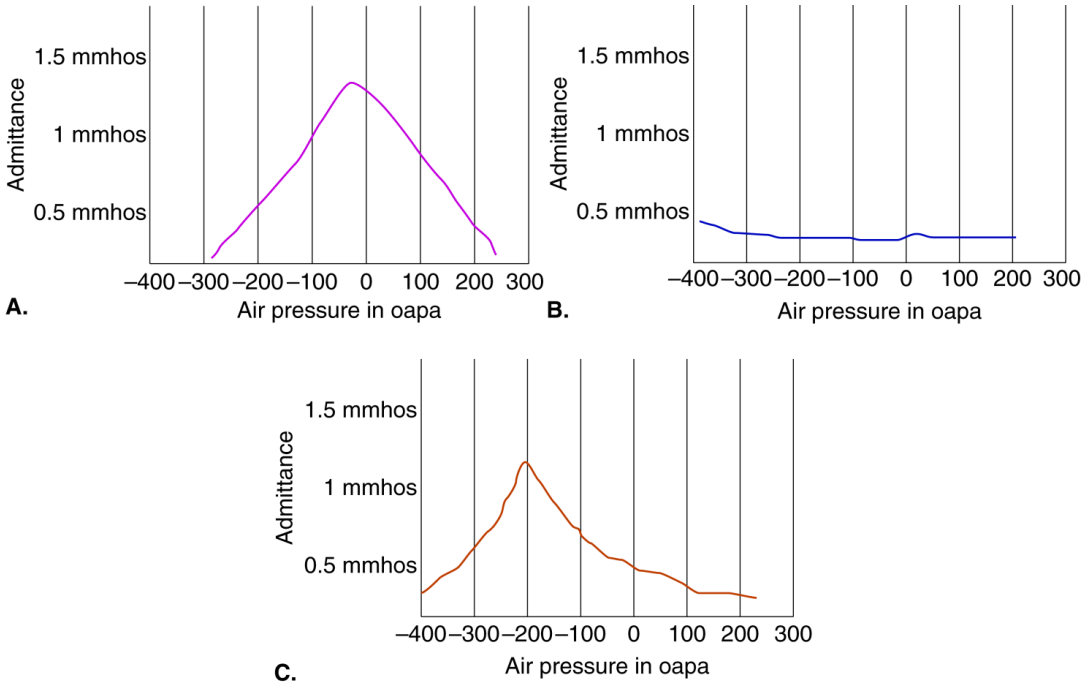


Figure 7-1. Tympanograms. (A) Type A is normal and resembles a symmetrical upside down "V". (B) Type B is seen in effusion or perforation and appears relatively flat. (C) Type C represents eustachian tube dysfunction and is shifted to the left.
(Reproduced, with permission, from Wikimedia Commons.)

Treatment

Medical—Examination 3 months after onset of OME with baseline hearing test; then serial evaluation every 3–6 months. These evaluations should continue until the effusion resolves, hearing loss is documented, or structural changes of the TM or middle ear are noted. Antihistamines, decongestants, antibiotics, or intranasal steroids have no proven benefit. Identify at-risk children (i.e., those at greater risk for developmental or language delay), who may require more aggressive management.

Surgical—May be indicated in cases of OME with structural damage, recurrent OME, hearing loss of 40 dB or higher, or hearing loss 21–39 dB in at-risk children. (Note: Both thresholds apply to the better ear.) Surgery may include myringotomy (incision of TM) with or without tympanostomy tubes, adenoidectomy, or both. Any repeat surgery should include an adenoidectomy, which results in improved eustachian tube drainage with less of a local nidus for infection.

Complications

AOM complications may be extracranial or intracranial.

Extracranial complications include:

- TM perforation
- Chronic AOM
- Labyrinthitis or vestibular disturbance (secondary to fistula formation)
- **Mastoiditis**
- Facial paralysis
- Subperiosteal abscess

Intracranial complications of AOM include:

- Meningitis
- Brain abscess
- Sinus thrombosis
- Epidural abscess
- Subdural empyema

OME complications may include hearing loss, TM retraction, or **cholesteatoma** (Figure 7-2). A cholesteatoma is a destructive, expanding accumulation of keratinized squamous epithelium in the middle ear or mastoid (see arrow in Figure 7-2), which usually occurs as a result of chronic or recurrent infection.

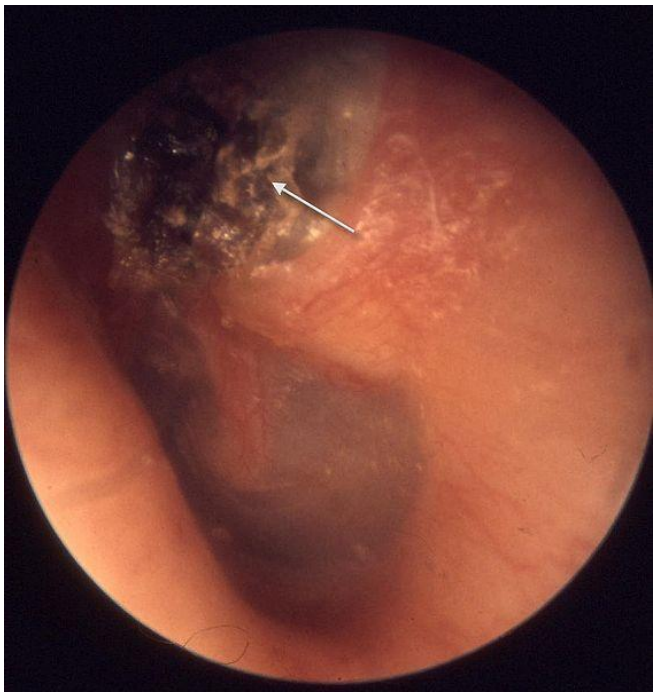


Figure 7-2. Cholesteatoma.
(Reproduced, with permission, from Wikimedia Commons.)

CONJUNCTIVITIS

ALLERGIC CONJUNCTIVITIS (SEASONAL AND PERENNIAL)

Allergic conjunctivitis (AC) is such a basic topic that it is **not** high yield for the boards. A few key points, however, are worth remembering. AC is a self-limited, bilateral inflammation of the conjunctiva with **limbal sparing** (lack of or less injection around the limbus, where the cornea fuses with the conjunctiva) (Figure 7-3). This is considered under-reported, with an estimated 20% of the general population being affected, 60% of whom present with allergic rhinitis. Vision, pupil shape, and ocular movement remain normal in AC. **Allergic shiners** are a transient increase in periorbital pigmentation from decreased venous return to skin and tissue. The best treatment for AC includes dual-acting topical medications with combination of H_1-receptor antagonist and mast cell stabilizers (e.g., olopatadine, ketotifen, and azelastine).

VERNAL KERATOCONJUNCTIVITIS

Epidemiology and Risk Factors

Vernal keratoconjunctivitis (VKC) is a **sight-threatening**, bilateral chronic inflammation of the conjunctiva. It occurs more in young atopic males (ages 3–20 years) residing in warm and dry climates.

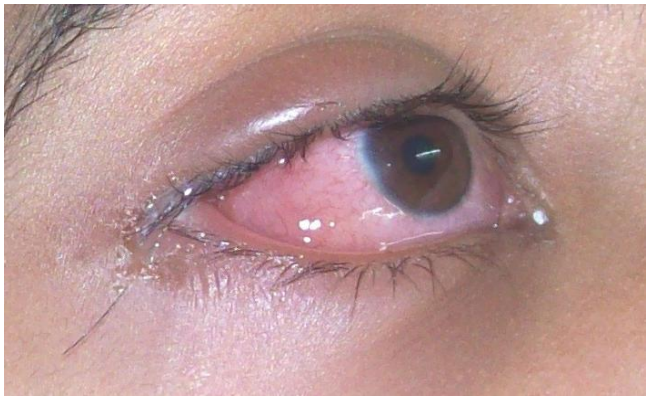


Figure 7-3. Edema of the conjunctiva due to hay fever allergy.
(Reproduced, with permission, from Wikimedia Commons.)

Flash Card Q8

What is ciliary flushing, and in which conditions is it found?

Pathology

The exact mechanism is incompletely understood, but mast cells and eosinophils are increased in conjunctival epithelium and substantia propria.

Clinical Features

VKC presents with a severe photophobia and intense ocular itching**.** Key features include **papillary hypertrophy** (>1 mm), resulting in possible ptosis of the upper eyelid, **thick, ropey discharge, and Horner-Trantas dots.**

Differential Diagnosis

Differential diagnosis of VKC

- Atopic keratoconjunctivitis (AKC)
- Giant papillary conjunctivitis (GPC)
- AC
- Infective conjunctivitis
- Blepharitis

Treatment

Treatment of VKC includes allergen avoidance (i.e., alternate occlusive eye therapy) and high-dose pulse topical corticosteroids. Mast cell stabilizers (i.e., cromolyn) have shown to be effective. Other treatments include dual-acting medications (i.e., H_1-receptor antagonist and mast cell stabilizers) oral antihistamines, and antibiotic and steroid ointments (for shield ulcers).

ATOPIC KERATOCONJUNCTIVITIS

Epidemiology and Risk Factors

Flash Card A8

Ciliary flushing is an injection of the deep episcleral vessels, causing redness around the cornea. It is seen in corneal inflammation, iridocyclitis, and acute glaucoma.

Atopic keratoconjunctivitis (AKC) is a **sight-threatening**, bilateral chronic inflammation of the conjunctiva and eyelids. It occurs mostly in teenagers or young adults in their twenties with a personal or family history of atopic dermatitis. The conjunctival activity parallels the skin involvement and can occur perennially (no seasonal predisposition).

Pathology

Similarly to AC, AKC involves IgE, mast cells, and eosinophils.

Clinical Features

The key feature of AKC is **chronic ocular pruritus/burning with findings of atopic dermatitis**. Loss of vision can occur from corneal pathology, which includes:

- Superficial punctuate keratitis
- Corneal infiltrates
- Scarring
- Keratoconus
- Anterior subcapsular cataracts (Figure 7-4)

Key Fact

Steroid administration results in formation of posterior subcapsular cataracts.
(Prednisone = posterior)

Differential Diagnosis

Differential diagnosis of AKC includes:

- Contact dermatitis
- Infective conjunctivitis
- Blepharitis
- Pemphigoid
- VKC
- AC
- GPC

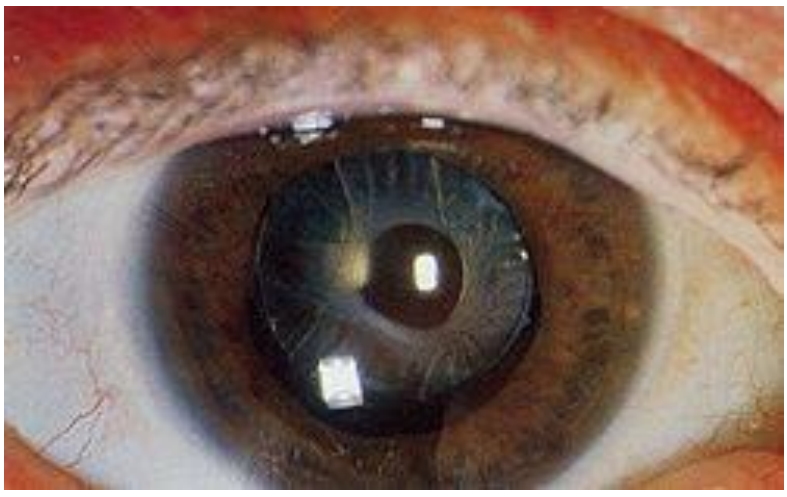


Figure 7-4. Anterior subcapsular cataract.
(Reproduced, with permission, from Wikimedia Commons.)

Treatment

Treatment of AKC usually involves environmental allergen controls and a transient topical corticosteroid. Mast cells stabilizers (i.e., cromolyn) or dual-acting medications (e.g., H_1-receptor antagonist and mast-cell stabilizers) are effective. Other treatments include systemic antihistamines, cyclosporine A (oral or topical), and topical tacrolimus.

GIANT PAPILLARY CONJUNCTIVITIS

Epidemiology and Risk Factors

GPC is accompanied by a chronic, bilateral inflammation associated with foreign body intolerance (e.g., ocular prostheses, sutures), which may affect 20% of contact-lens wearers (Figure 7-5). Extended-wear soft contact lens > hard contact lens > soft contact lens (daily disposables). This condition can be aggravated by concomitant allergy.

Pathology

Mechanical trauma with irritation of the upper lid and protein buildup on the lens causes an allergic reaction. Tear deficiency may also be a contributing factor of GPC.

Figure 7-5. Contact lenses are a risk for GPC.
(Reproduced, with permission, from Wikimedia Commons.)

Clinical Features

Key features of GPC are:

- Ocular itching after lens removal
- Morning mucus discharge
- Photophobia or blurred vision
- Contact lens intolerance

Tarsal papillary hypertrophy is smaller than in VKC (i.e., >0.3mm).

Differential Diagnosis

Differential diagnosis of GPC includes:

- Infective conjunctivitis
- Irritant or toxin conjunctivitis
- AC
- AKC
- VKC

Treatment

The most effective treatment of GPC involves the reduction in contact lens wearing and/or a change in lens style, plus "artificial tears."

Several eye diseases may threaten sight and need to be considered when examining patients with ophthalmic concerns (Table 7-4).

Table 7-4. Sight-Threatening Conditions and Signs

Sight-Threatening Conditions	Red Eye Danger Signs
Acute glaucoma	Photophobia
Scleritis	Blurry vision
Iritis	Severe pain
Uveitis	Seeing colored halos
Herpes simplex keratitis	Abnormal pupil size (sluggish or fixed)
	Ciliary flush

ATOPIC DERMATITIS (ECZEMA)

Atopic dermatitis (AD) is a chronic, relapsing skin disorder that starts in childhood and, generally, improves with age. It can be associated with allergic rhinitis and asthma. AD manifests in adults as relapsing. Note that environmental and bacterial stimuli cause T_h2 bias.

Epidemiology

Key Fact

In an adult patient presenting with new-onset dermatitis and no history of childhood eczema, asthma, or allergic rhinitis, the physician should consider other causes, such as cutaneous T-cell lymphoma (CTCL), which may require a skin biopsy for diagnosis.

AD is the most common chronic skin disease of young children, but can affect patients of any age. Its prevalence (8–18%) has continued to increase, paralleling respiratory allergies and asthma.

Atopic March—More than 50% of patients with AD will develop asthma, and a higher percentage will develop allergies. Increased numbers of IgE+ Langerhans cells present in both active AD and asthma (as opposed to inactive AD or asthma) suggests the active allergic disease may be controlled systemically.

Natural History—AD presents in early childhood, often by 2–6 months of age. Onset is usually before 5 years of age in approximately 90% of patients. Other disease causes need to be considered in adults with new-onset dermatitis, especially without a history of childhood eczema, asthma, or allergic rhinitis.

Basic Science

Key Advances

- High levels of FcεRI-expressing IgE+ Langerhans cells in active AD, asthma, and allergic rhinitis.
- IgE to *Staphylococcus aureus* toxins is produced in both extrinsic and intrinsic forms of AD.
- Two distinct Ag-presenting dendritic cells in atopic skin, Langerhans cells and inflammatory dendritic epidermal cells (IDECs), upregulate surface FcεRI to catch allergens that have infiltrated the injured skin.
- Cutaneous T–lymphocyte-attracting chemokine (CTACK and CCL27) and thymus and activatation-regulated chemokine (TARC) levels have been found to be specific for AD, increasing with acute symptoms and decreasing with improvement of symptoms.
- Superantigen stimulation causes CD4+CD25+ Treg cells to lose their immune suppressive activity.

- A decrease or absence of antimicrobial peptides, human β defensins (hBD-2 and hBD-3) and human cathelicidin (LL-37) in keratinocytes place patients at risk for infections with bacteria, fungi, and viruses. Deficiency in hBD-3 expression results in impaired killing of *S. aureus.* Downregulation of antimicrobial genes, due to local upregulation of T_h2 cytokines, could explain the susceptibility of atopic dermatitis skin to bacterial, viral, and fungal infections.
- A *TLR2*-gene polymorphism that results in an impaired *TLR2* expression has been linked to severe AD with frequent bacterial infections.
- Loss of function variants of the epidermal barrier protein filaggrin can predispose patients to have earlier onset, more severe, and persistent AD. Loss-of-function mutations in filaggrin (*FLG*) are associated with increased risk for asthma when it occurs with atopic dermatitis.
- TSLP (thymic stromal lymphopoietin) is expressed by keratinocytes in both acute and chronic lesions of atopic dermatitis.

Mnemonic

Cytokines involved in acute and chronic atopic dermatitis:

Acute: IL-4, IL-13:"**4** Suits with **13** cards each in a deck, including the **ac**es."
Chronic: IL-5, IL-12, IFNγ: "Education is **chronic** starting at **5** through **G**rade **12.**"

Cells Involved in Atopic Dermatitis

- **T lymphocytes**: Predominantly CD3, CD4, CD45RO memory T lymphocytes that also express CD25 and human leukocyte antigen (HLA) DR on their surface. Most memory T lymphocytes express high levels of the homing receptor cutaneous lymphocyte-associated antigen (CLA), a ligand for vascular adhesion molecule, E selectin.
- **Langerhans cells**: Express FcεRI that bind IgE. FcεRI on Langerhans cells lack the classic β chain. Langerhans cells contain Birbeck granules.
- **Inflammatory Dendritic Epidermal Cells (IDECs)**: FcεRI-activated IDECs stimulate naïve T lymphocytes to become IFNγ-producing T lymphocytes. In combination with IL-12 and IL-18, this leads to switch from T_h1 to T_h2. IDECs do not contain Birbeck granules.
- **Eosinophils**: Activated eosinophils are present in significantly greater numbers in chronic than in acute lesions. Deposition of eosinophil, a major basic protein, can be detected throughout the dermis of involved areas. Serum levels of eosinophil cationic protein are elevated and correlate with disease severity.

Mnemonic

You beta remember that the FcεRI of Langerhans cells lack the classic beta chain

Flash Card Q9

Where is filaggrin expressed?
A. Anterior vestibulum of nose
B. Transitional nasal epithelium
C. Respiratory nasal epithelium
D. Human bronchial epithelium

Table 7-5 highlights the differences between extrinsic and intrinsic AD as well as the cells and cytokines involved.

Flash Card Q10

Which cell type expresses higher levels of FcεRI: Langerhans cells or IDECs?

Table 7-5. Extrinsic Versus Intrinsic Atopic Dermatitis

	Extrinsic = Atopic Eczema 70–80%	Intrinsic = Nonatopic Eczema 20–30%
IgE	IgE-mediated sensitization	No IgE-mediated sensitization
Eosinophils	Eosinophilia	Eosinophilia
IL-4 and IL-13	Increased production Cause increased IL-5 production	Less production
IL-10	Plays immune-modulating role	Plays immune-modulating role

Clinical Features

Presentation—Initially, patients with AD have intense itching accompanied by red macules and papules. Scratching leads to excoriation with crusting and formation of exudates.

Distribution

- **Involvement of face and extensor surfaces of extremities** occurs in children younger than 2 years old (Figure 7-6).
- **Flexural involvement** occurs in children older than 2 years of age and in adults.

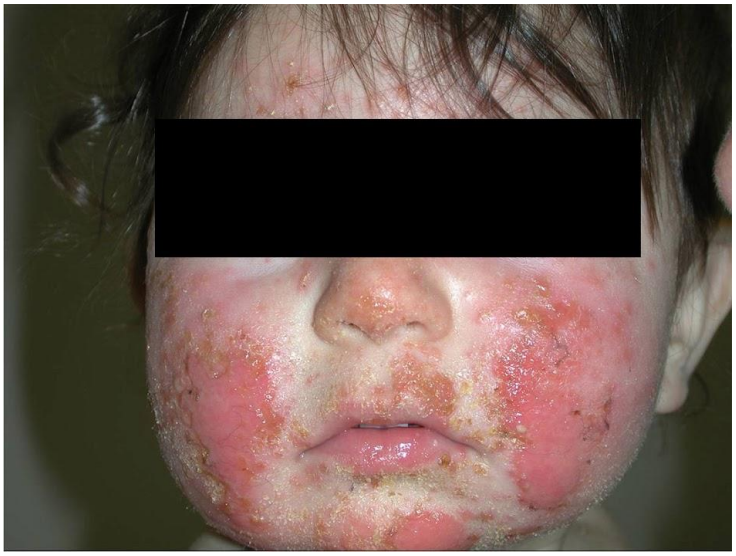


Figure 7-6. Severe atopic dermatitis.
(Reproduced, with permission, from USMLERx.com).

Flash Card A9

A. Anterior vestibulum of nose

Flash Card A10

IDECs

Major Clinical Features

- Pruritus
- Chronic or relapsing course
- Personal or family history of atopy

Other Characteristic Features

- Early age of onset (i.e., 2–6 months of age)
- Dry skin
- Hand and/or food dermatitis
- Cheilitis
- Nipple eczema
- Immediate hypersensitivity skin test responses
- Anergy

Diagnosis

Diagnosis of AD is based on itching and characteristic rash, with specific distribution and history.

Major Diagnostic Criteria—Criteria include pruritus, chronic or chronically relapsing dermatitis, personal or family history of atopy (asthma, allergic rhinoconjunctivitis, or atopic dermatitis).

Specific distribution and appearance based on age:

- **Older children and adults** have lichenification of flexor surfaces, including hands, feet, face, antecubital and popliteal fossae.
- **Infants** have involvement of extensor surfaces, neck, trunk, and face.
- Spares nasolabial skin.

Laboratory Tests—Diagnostic tests for AD are primarily supportive. Elevated serum total IgE supports diagnosis of AD. Prick skin tests or in vitro testing of suspected allergens can help identify specific triggers of AD. Negative tests can help exclude triggers.

- **Skin biopsy** can be useful in patients who do not respond well to corticosteroids.
- **Patch testing** is useful in patients with suspected contact allergen such as nickel or cosmetics. It has also been used to identify aeroallergen triggers such as:

Flash Card Q11

Which cytokine is primarily associated with pruritus in atopic dermatitis?

- Dust mites
- Animal dander
- Weeds
- Molds

Complications

AD is accompanied by an increased susceptibility to infections or colonization with a variety of organisms: *S. aureus*, herpes simplex, molluscum contagiosum, *Pityrosporum orbiculare* (formerly known as *Malassezia furfur)*, and *Pityrosporum ovale.*

Mnemonic

Anterior cataracts are associated with **a**topic keratoconjunctivitis. **P**osterior cataracts are associated with **P**rednisone.

- AKC presents with bilateral intense ocular pruritus, burning, tearing, and copious mucoid discharge. Horner-Trantas dots may be seen. Activity parallels that of the skin and may result in visual loss from corneal scarring. Can be complicated by herpes infection, keratoconus (noninflammatory thinning of cornea), and anterior subcapsular cataracts.
- Nonspecific hand dermatitis is aggravated by repeated wetting, especially in the work environment, leading to occupational disability.
- **Eczema vaccinatum** is a rare, severe reaction to smallpox vaccination in patients with history of AD.

Triggers of AD

Food—Double-blind, placebo-controlled food challenges have demonstrated that food allergens can trigger flares in some AD patients (e.g., 25–33% of those with moderate to severe disease).

- Seven foods (milk, egg, soy, wheat, fish, peanuts, and tree nuts) account for nearly 90% of positive challenges.
- Elimination of the causative food allergens results in the improvement of skin disease and a decrease in spontaneous basophil histamine release.

Aeroallergens—Evidence supporting a role for aeroallergens includes finding of both allergen-specific IgE antibodies and antigen-specific T lymphocytes. Environmental control measures, such as dust mite avoidance, resulted in clinical improvement in AD.

Autoantigens—Sera from some patients with severe AD have been found to have IgE antibodies directed against human protein. The release of allergens against self from damaged tissues triggers IgE- or T–lymphocyte-mediated responses and maintains chronic allergic inflammation in severe AD.

Microbial Agents and Toxins—Patients with AD are colonized with high numbers of *S. aureus.* Exotoxins secreted by *S. aureus* can act as superantigens, contributing to persistent inflammation or inflammation of AD.

Flash Card A11

IL-31

- Increased colonization may be associated with decreased antimicrobial peptides, H-β defensin (HβD-2 and 3), and human cathelicidin (LL-37) by keratinocytes.
- AD patients can make specific IgE antibodies against the toxins on their skin, and their disease severity appears to correlate with the presence of these antibodies.

Differential Diagnosis

Table 7-6 lists the differential diagnosis of AD.

Table 7-6. Differential Diagnosis of Atopic Dermatitis

Disorder	Specific Diseases	Distinguishing Features
Chronic dermatoses	Netherton syndrome	Congenitally acquired scaly dermatitis with short, spiky, brittle hair, called "bamboo hair"
	Seborrheic dermatitis	Greasy, scaly rash with well-defined edges in areas with sebaceous glands (i.e., scalp, face, and periauricular)
	Contact dermatitis (allergic or irritant)	History of exposure Erythema, papules, and vesicles
	Nummular eczema	Clearly demarcated edge Lesions on limbs more so than on trunk Variable intermittent course
	Lichen simplex chronicus	Cutaneous response to repeated rubbing or scratching Common in neck or fold behind ear (areas commonly reached) Accentuation of surface marking (e.g., resembles tree bark)
Infections and infestations	Scabies	Eczematous vesicles on hands and feet Pityriasis that is rosea-like
	HIV-associated dermatitis	
Malignancy	Cutaneous T-cell lymphoma (CTCL) (mycosis fungoides)	Adult without history of eczema Diagnosis by biopsy
Immune deficiencies	Wiskott-Aldrich syndrome (WAS)	Child with dermatitis, low platelets, and recurrent infection
	Severe combined immunodeficiency (SCID)	Dermatitis, failure to thrive, diarrhea, and life-threatening infections
	Hyper-IgE syndrome	Chronic eczema with impetigo

Flash Card Q12

Deficiency of which antimicrobial peptide in atopic skin may contribute to eczema vaccinatum?

Table 7-6. Differential Diagnosis of Atopic Dermatitis, cont.

Disorder	Specific Diseases	Distinguishing Features
Immune deficiencies	IPEX[a] syndrome	Infant with dermatitis, intractable diarrhea, diabetes, and hypothyroid *FOXP3* mutation
	Dock8 deficiency	Respiratory tract infections, difficult to treat viral skin infections (HPV-associated warts, orolabial/anogenital/corneal HSV, molluscum, severe varicella or herpes zoster), bacterial skin infections and/or mucocutaneous candidiasis. High incidence of malignancy
Metabolic disorders	Zinc deficiency; acrodermatitis enteropathica	Infant with eczema that does not respond to steroids Periorificial rash Necrotic areas around nose
	Pyridoxine (vitamin B_6 and niacin deficiency)	Seizures, irritability, and cheilitis
	Multiple carboxylase deficiency	Infant with skin rash, alopecia, and lethargy
	Phenylketonuria	Pale pigmentation, blue eyes, and scleroderma If untreated, leads to mental retardation
Proliferative disorder	Letterer-Siwe disease	Infants with anemia, thrombocytopenia, lymphadenopathy, and histiocytic infiltration of liver, spleen, and lymph nodes

[a] Immunodysregulation, polyendocrinopathy, enteropathy, X-linked.

Treatment

- **Cutaneous hydration and emollients:** Lukewarm soaking baths, followed by emollient application, can control itching and hydrate dry skin. Effective hydration and emollient therapy may reduce need for topical corticosteroids.
- **Topical corticosteroids:** Mainstay of treatment. Careful patient instruction essential to minimize side effects.
- **Topical calcineurin inhibitors:** Early treatment with topical calcineurin inhibitors might prevent relapses and decrease need for steroid rescue. Black box warning has raised concerns about long-term use.
- **Avoidance of triggers of AD:**
  - **Antibiotics, antivirals, and antifungals:** Helpful in reducing secondary infections that can complicate AD such as *S. aureus*, herpes simplex, *P. ovale*, *Candida*, or scabies
  - **Antihistamines** to control itching
  - **Vitamin D,** which has been shown to enhance innate immune responses in patients with AD. Especially helpful in patients with house dust mite sensitization
  - **Bleach baths**: Decreases bacterial infection and reduces symptoms

Flash Card A12

LL-37

ASTHMA

Asthma can be defined as a complex disorder with variable and recurring respiratory symptoms, airflow obstruction, bronchial hyperresponsiveness, and underlying inflammation.

Pathogenesis

Infiltration of the airways with inflammatory cells is a hallmark of asthma. These are mainly eosinophils; however, neutrophils, lymphocytes, and other cells are also typical. In contrast to neutrophils, eosinophils, and T lymphocytes that are recruited from the circulation, airway mast cells are primarily longstanding tissue-dwelling cells. In asthma, the number of mast cells is not increased, but they are activated and show frequent degranulation. **Neutrophilic accumulation is a hallmark of fatal asthma**.

Airway cellular recruitment involves several proinflammatory processes, including:

- Upregulation of adhesion molecule expression
- Arachidonic acid metabolite production (including LTB4)
- Chemokine synthesis (including IL-8, monocyte chemotactic protein 1 (MCP-1), and RANTES)
- Cytokine secretion (IL-1β, 4, 5, 9, 10, 13, 16, TNFα IL-6, GM-CSF, and transforming growth factor beta [TGFβ]).

In particular, **T_h2 cytokines (IL-4, 5, 13) induce isotype switching of B lymphocytes to IgE-producing plasma cells** and support eosinophil survival, **whereas others promote mast cell (stem cell factor, SCF) and basophil (IL-3) development.**

Airway smooth muscle is hypertrophied in the smaller airways and hyperplastic in the larger airways. Asthmatics have increased airway wall thickness (50–300% in fatal asthma and 10–100% in nonfatal asthma compared with nonasthmatics). Thickening occurs in smooth muscle, epithelium, submucosa, adventitia, and mucosal glands. **Angiogenesis** also occurs. **Thickening of the lamina reticularis** occurs below the basement membrane, composed largely of collagen types III and V, likely produced by myofibroblasts beneath the epithelium. **Mucus plugs** worsen airway obstruction. These plugs are composed of mucus, serum proteins, inflammatory cells, and cellular debris. Excess mucus is due to hypertrophy and hyperplasia of submucosal glands, promoted by IL-9. **Damaged epithelia** are a characteristic feature of chronic asthma. Damage to the epithelial cells may be due to eosinophil-derived products, active radicals of oxygen, or proteins from neutrophils or mast cells (e.g., tryptase, chymase). When these cells are repaired

Flash Card Q13

A 3-year-old boy presents to the pediatrician with bloody stools, draining ears, and eczema. What primary immunodeficiency should be considered?

Flash Card Q14

Oral vitamin D induces production of which antimicrobial peptide in atopic individuals?

or regenerated, instead of pseudostratified, ciliated columnar cells, the regenerated cells are simple, stratified, nonciliated epithelium or goblet cells. Characteristic features in asthmatic sputum are:

- **Curschmann's spirals:** Associated with excess mucus production (see Figure 5-9).
- **Creola bodies:** Clusters of surface airway epithelial cells (Figure 5-9).
- **Charcot-Leyden crystals (CLC)**: A classic finding. The CLC protein is produced in eosinophils and released in eosinophilic disorders, including asthma. These appear as colorless, needle-shaped structures (Figure 5-9).)

Development of Asthma

Both genetic predisposition and environmental interactions are thought to determine the asthma phenotype. **Atopy** is the strongest identifiable predisposing factor. Allergen exposure plays a complex role, both in the onset and triggering of asthma. Early life exposure to **pets and farm animals** may exert a protective effect on the development of allergy but not asthma. Epidemiologic studies have found that a child's sensitization to ***Alternaria*** by 6 years of age is associated with persistent asthma by 11-years-old. In older children, exposure to higher levels of **dust mites** correlates with wheezing and airway hyperresponsiveness. **Gender** differences are seen (e.g., boys have a higher prevalence than girls until the ages of 15–17; the opposite is seen after that age). The **hygiene hypothesis** suggests that some types of microbial exposure may *decrease* the development of asthma. **Viruses and other infections** are associated with asthma in several ways:

- Viruses such as RSV may produce symptoms of asthma in infants (see Bronchiolitis section).
- In asthmatics, viral infections are a common trigger.

Genetic factors may contribute to the heritability of asthma. On **chromosome 5q,** the genes 5q31-33, known as the interleukin-4 (IL-4) gene cluster, may play an important role in inflammation in atopy and asthma. IL-4 plays a key role in inducing synthesis of IgE and T_h2 differentiation. A polymorphism in the IL-4 promoter was identified that increased transcription of IL-4 with resultant high serum levels of IgE. The **β_2-adrenoreceptor gene**, also in this region, is highly polymorphic. Polymorphisms including Arg-16 → Gly and Gln 27 → Glu are associated with decreased responses to β_2 agonists. **CD14**, is a recognition coreceptor with TLR4 for endotoxin (bacterial lipopolysaccharide) and is important for innate responses to bacterial infection, leading to a shift towards T_h1 cell responses. A polymorphism in a soluble form of CD14 (sCD14) was associated with high levels of sCD14 and low levels of IgE. Lastly, on a genome-wide scan, chromosome 20p13 was found to contain a locus that was linked to asthma. On the locus, the disintegrin and metalloproteinase (***ADAM 33***) gene was associated with asthma. It may play a role in the function of airway smooth muscle leading to airway hyperreactivity or remodeling. **Chitinase-like proteins**

Flash Card A13

Wiskott-Aldrich syndrome

Flash Card A14

Cathelicidin

(including chitotriosidase [CHIT 1] and YKL-40) are also an area of research as susceptibility genes for asthma.

Diagnosis

Features of asthma include recurrent episodes of **airflow obstruction** (i.e., obstruction is at least partially reversible; an increase in forced expiratory volume in 1 second (FEV_1) >200 mL and > 12% from baseline after inhaling short-acting β_2 agonist [SABA] is specified in the American Thoracic Society definition of reversibility) or **airway hyperresponsiveness** (the airways react too readily and too much; demonstrated by methacholine challenge). Declining and irreversible loss of lung function over time is generally attributed to **airway remodeling,** which is thought to be a tissue response to recurrent injury or inflammation. Features of remodeling include:

- Subepithelial fibrosis
- Increase in thickness of the small airways
- Angiogenesis
- Mucosal gland hypertrophy

Differential Diagnosis

All that wheezes is not asthma. A differential diagnosis of adult asthma includes:

- Chronic obstructive pulmonary disease (COPD)
- Congestive heart failure
- Pulmonary embolism
- Mechanical obstruction of airways (e.g., tumor)
- GERD
- Bronchiectasis
- Lower respiratory tract infection
- Vocal cord dysfunction (VCD).

Table 7-7 shows the differential diagnosis for asthma in infants and children.

Table 7-7. Differential Diagnoses of Asthma in Infants and Children

Upper Airway Disease	Obstruction of Large Airways	Obstruction of Small Airways	Other
Allergic rhinitis Sinusitis	Foreign body in trachea or bronchus Vocal cord dysfunction Vascular ring or laryngeal web Laryngotracheomalacia, tracheal stenosis, and bronchostenosis	Viral or obliterative bronchiolitis Cystic fibrosis Bronchopulmonary dysplasia Heart disease	Aspiration Gastroesophageal reflux Tracheoesophageal fistula

Flash Card Q15

What is the most frequent infectious cause of asthma exacerbations?

Flash Card Q16

What percentage of children will have episodes of wheezing in the first 3 years of life attributable to viral respiratory tract infections?

Infants and Children

Epidemiology—About 50–80% of children who have asthma developed symptoms before their fifth birthday. **Lung function declines very early in childhood, but this may be irreversible.** Approximately 50% of children under the age of 3 years have wheezing episodes with viral respiratory infections, with many responding to asthma therapy even though the diagnosis of asthma is not clearly established. Longitudinal studies looking at the natural history of asthma in young children have defined three different phenotypes among children who wheeze:

- **Transient early wheezers**, children who wheezed when they were younger than 3 years old and resolved by the time they were 6 years old, the most prevalent type
- **Persistent wheezers,** children whose wheezing began when they were younger than 3 years old and continued through 6 years old
- **Late-onset wheezers,** children whose onset of wheezing was at 6 years old

The **asthma predictive index** (Table 7-8) was developed to estimate which children with wheezing would have persistent asthma. Roughly, two thirds of children with frequent wheezing and a positive asthma predictive index will have asthma during school years; by contrast, > 95% of wheezing toddlers with a negative index did not have asthma during school years. In addition, the 2007 National Heart Lung and Blood Institute (NHLBI) guidelines suggest the use of the asthma predictive index as a tool to help determine when to initiate long-term control therapy. In children younger than 4 years of age, long-term control therapy can be initiated for any of the following scenarios:

- At least four episodes of wheezing in the past year that lasted more than 1 day and affected sleep, and that had a positive asthma predictive index (see Table 7-8)
- Consider for patients who require symptomatic treatment more than 2 days per week for more than 4 weeks
- Consider in patients requiring oral steroids twice in 6 months
- Consider during periods or seasons of previously documented risk (e.g., during seasons of viral respiratory infections)

Table 7-8. Asthma Predictive Index

Any One of the Following Major Criteria	Or	Any Two of the Following Minor Criteria
Parental asthma		Sensitization to foods
Physician diagnosis of atopic dermatitis		>4% eosinophils
Sensitization to aeroallergens		Wheezing apart from colds

Flash Card A15

Rhinovirus

Flash Card A16

Fifty percent

National Heart, Lung, and Blood Institute (NHLBI) Guidelines

It is very important to memorize the summary tables of the NHLBI guideline for the boards (Tables 7-9 through 7-15). The long-term management of asthma is divided into four components as follows:

- **Assessing and monitoring asthma severity and asthma control. Impairment** is the frequency and intensity of symptoms and the functional limitation of the patient. **Risk** is the likelihood of exacerbation, decline in lung function, or adverse effects of medication. **Severity** is the intrinsic intensity of disease and should be assessed prior to a patient being on long-term control medications. **Control** is the degree to which asthma is controlled by treatment and the goals of treatment are met. For patients on long term-control medications, access control and step-up therapy if asthma is not well controlled. Be sure to check adherence, inhaler technique, and environmental control measures prior to stepping up.
- **Education for a partnership in care,** including the importance of giving all patients a written asthma action plan.
- **Control of environmental factors and comorbid conditions that affect asthma.**
    - Allergens: Common trigger. Major contributor to both acute symptoms and chronic inflammation. **Immunotherapy can reduce incidence of additional aeroallergen sensitization and the subsequent development of asthma in children.**
    - Allergic bronchopulmonary aspergillosis (ABPA) (discussed later). GERD treatment may be beneficial for patients with frequent nighttime asthma symptoms, even in absence of GERD symptoms. Up to 45–65% of asthmatics have GERD. Possible mechanisms include bronchospasm due to esophageal irritation and vagal reflex, or microaspiration.
- Nonsteroidal antiinflammatory drugs (NSAIDs): 5–10% of adults may have asthma episodes triggered by NSAIDs. Some have Samter's triad, consisting of asthma, nasal polyps, and aspirin sensitivity. Symptoms to aspirin (and NSAIDs that inhibit COX-1) occur within 1–2 hours or ingestion and include rhinorrhea, nasal congestion, tearing of the eyes, and bronchospasm. These reactions are mediated by eicosanoid metabolism with leukotriene production, not IgE. The onset of NSAID sensitivity can occur years after asthma and nasal polyps.

For asthma medications, see Tables 7-9 through 7-15 for steps of care.

Flash Card Q17

Which of the following is the biggest risk factor for persistent asthma at age 6 in a 3-year-old with recurrent wheezing?

A. Asthma in a parent
B. Asthma in a sibling
C. Wheezing apart from colds
D. Allergic rhinitis in a sibling

Exercise

Exercise is a common trigger in uncontrolled asthma; however, some patients experience bronchospasm, which is only triggered by exercise. Classically, symptoms have their onset after 10 minutes of aerobic activity and usually resolve 15–30 minutes after exercise. **Exercise-induced bronchospasm is diagnosed by an FEV_1 decrease > 15% after exercise challenge test or history and an appropriate bronchodilator response**. SABA used shortly before exercise may last for 2–3 hours and be helpful in 80% of patients. A daily leukotriene receptor antagonist can help in 50% of patients.

Pregnancy

The classic rule of thumb is that, during pregnancy, approximately one third of asthmatic women improve, one third worsen, and one third remain the same. Uncontrolled asthma increases perinatal mortality, preeclampsia, preterm birth, and the likelihood of a low-birth-weight infant. It is generally safer for pregnant women to be treated with asthma medications than to risk asthma exacerbations. Albuterol is the preferred SABA. Budesonide is the preferred inhaled corticosteroid.

Flash Card A17

A. Asthma in a parent.

Table 7-9. Classifying Asthma Severity in Children 0–4 Years of Age

		Intermittent	Mild Persistent	Moderate Persistent	Severe Persistent
Impairment	**Symptoms**	≤2 days/week	>2 days/week	Daily	Throughout the day
	Nighttime awakenings	0	1-2×/month	3-4×/month	>1×/week
	Short-acting β agonist (SABA) use	≤2 days/week	>2 days/week	Daily	Several times per day
	Interference with normal activity	None	Minor limitation	Some limitation	Extremely limited
Risk	**Exacerbations requiring oral steroids**	0–1/year	≥2 exacerbations in 6 months, or >4 wheezing episodes/1 year lasting >1 day AND risk factors for persistent asthma		
Recommended step for initiating therapy		Step 1	Step 2	Step 3 and consider short course of oral steroids.	Step 4 and consider short course of oral steroids.

Table 7-10. Assessing Asthma Control in Children 0–4 Years of Age

		Well Controlled	Not Well Controlled	Very Poorly Controlled
Impairment	**Symptoms**	≤2 days/week	>2 days/week or multiple times on ≤2 days/week	Throughout the day
	Nighttime awakenings	≤1/month	>1/month	>1×/week
	Interference with normal activity	None	Some	Extremely limited
	SABA use	≤2 days/week	>2 days/week	Several times per day
Risk	**Exacerbations requiring oral steroids**	0–1/year	2–3×/year	>3×/year
	Treatment-related adverse effects	Side effects can vary in intensity. Level of intensity should be considered in overall assessment of risk.		
Recommended action for treatment		Maintain current step. Follow up every 1–6 months. Consider step-down if well controlled ≥3 months.	Step up one step.	Step up one to two steps. Consider short course of oral steroids.

Table 7-11 Classifying Asthma Severity in Children 5–11 Years of Age

		Intermittent	Mild Persistent	Moderate Persistent	Severe Persistent
Impairment	**Symptoms**	≤2 days/week	>2 days/week	Daily	Throughout the day
	Nighttime awakenings	≤2/month	3-4 × /month	>1×/week	Often nightly
	SABA use	≤2 days/week	>2 days/week	Daily	Several times per day
	Interference with normal activity	None	Minor limitation	Some limitation	Extremely limited
	Lung function	Normal FEV_1 between exacerbations FEV_1 > 80% predicted; FEV_1/FVC > 85%	FEV_1 >80% predicted FEV_1/FVC > 80%	FEV_1 60–80% predicted; FEV_1/FVC 75–80%	FEV_1 < 60% predicted; FEV_1/FVC < 75%
Risk	**Exacerbations requiring oral steroids**	0–1/year		≥2/year	
Recommended step for initiating therapy		Step 1	Step 2	Step 3 and consider short course of oral steroids	Step 3 or 4 and consider short course of oral steroids

Abbreviations: FEV_1, forced expiratory volume in 1 second; FEV_1/FVC, forced expiratory volume in 1 second/ forced vital capacity; SABA, short-acting β_2 agonist.

Table 7-12. Assessing Asthma Control in Children 5–11 Years of Age

		Well Controlled	Not Well Controlled	Very Poorly Controlled
Impairment	**Symptoms**	≤2 days/week	>2 days/week or multiple times on ≤2 days/week	Throughout the day
	Nighttime awakenings	≤ 1/month	>1/month	>1×/week
	Interference with normal activity	None	Some	Extremely limited
	SABA use	≤2 days/week	>2 days/week	Several times per day
	Lung function	FEV_1>80% predicted FEV_1/FVC > 80%	FEV1 60–80% predicted; FEV1/FVC 75–80%	FEV_1 <60% predicted; FEV_1/FVC <75%
Risk	**Exacerbations requiring oral steroids**	0–1/year	2-3×/year	>3×/year
	Treatment-related adverse effects	Side effects can vary in intensity. Level of intensity should be considered in overall assessment of risk.		
	Progressive loss of lung function	Evaluation requires long-term follow-up care.		
Recommended action for treatment		Maintain current step. Follow up every 1–6 months. Consider step-down if well controlled ≥3 months.	Step up one step.	Step up one to two steps. Consider short course of oral steroids.

Abbreviations: FEV_1, forced expiratory volume in 1 second; FEV_1/FVC, forced expiratory volume in 1 second/ forced vital capacity; SABA, short-acting β_2 agonist.

Table 7-13. Classifying Asthma Severity in Adults and Children ≥ 12 Years of Age

		Intermittent	Mild Persistent	Moderate Persistent	Severe Persistent
Impairment	**Symptoms**	≤2 days/week	>2 days/week	Daily	Throughout the day
	Nighttime awakenings	<2×/month	3–4×/month	>1×/week	Often nightly
	SABA use	≤2 days/week	>2 days/week	Daily	Several times per day
	Interference with normal activity	None	Minor limitation	Some limitation	Extremely limited
Normal FEV_1/FVC: **8–19 yr 85%** **20–39 yr 80%** **40–59 yr 75%** **60–80 yr 70%**	**Lung function**	Normal FEV_1 between exacerbations FEV_1 >80% predicted FEV_1/FVC normal	FEV_1 >80% predicted; FEV_1/FVC normal	FEV_1 60–80% predicted FEV_1/FVC reduced 5%	FEV_1 <60% predicted FEV_1/FVC reduced >5%
Risk	**Exacerbations requiring oral steroids**	0–1/year		>2/year	
Recommended step for initiating therapy		Step 1	Step 2	Step 3 and consider short course of oral steroids.	Step 4 or 5 and consider short course of oral steroids.

Abbreviations: FEV_1, forced expiratory volume in 1 second; FEV_1/FVC, forced expiratory volume in 1 second/ forced vital capacity; SABA, short-acting β_2 agonist.

Table 7-14. Assessing Asthma Control in Adults and Children ≥ 12 Years of Age

		Well Controlled	Not Well Controlled	Very Poorly Controlled
Impairment	**Symptoms**	≤2 days/week	>2 days/week or multiple times on ≤ 2 days/week	Throughout the day
	Nighttime awakenings	≥2/month	1–3×/week	≥4×/week
	Interference with normal activity	None	Some limitation	Extremely limited
	SABA use	≤2 days/week	>2 days/week	Several times per day
	FEV_1 or peak flow	≥80% predicted/personal best	60–80% predicted/personal best	<60% predicted/personal best
	Validated questionnaires **ATAQ** **ACQ** **ACT**	 0 ≤0.75 ≥20	 1–2 ≥1.5 16–19	 3-4 n/a ≤15
Risk	**Exacerbations requiring oral steroids**	0–1/year	≥2/year	
	Treatment-related adverse effects	Side effects can vary in intensity. Level of intensity should be considered in overall assessment of risk.		
	Progressive loss of lung function	Evaluation requires long-term follow-up care		
Recommended action for treatment		Maintain current step. Follow up every 1–6 months. Consider step-down if well controlled ≥3 months.	Step up one step.	Step up one to two steps. Consider short course of oral steroids.

Abbreviations: ACT, Asthma Control Test; ACQ, Asthma Control Questionnaire; ATAQ, Asthma Therapy Assessment Questionnaire; FEV_1, forced expiratory volume in 1 second; FEV_1/FVC, forced expiratory volume in 1 second/ forced vital capacity; SABA, short-acting β_2 agonist.

Table 7-15. Stepwise Approach for Managing Asthma

		Step1	Step 2	Step 3	Step 4	Step 5	Step 6
Children 0–4	**Preferred**	SABA prn	Low-dose ICS	Medium-dose ICS	Medium-dose ICS + LABA or montelukast	High-dose ICS + LABA or montelukast	High-dose ICS + LABA or montelukast + oral corticosteroids
	Alternative		Cromolyn or montelukast				
Children 5–11	**Preferred**	SABA prn	Low-dose ICS	Low-dose ICS + LABA, LTRA, or theophylline or medium-dose ICS	Medium-dose ICS + LABA	High-dose ICS + LABA	High-dose ICS + LABA + oral corticosteroids
	Alternative		Cromolyn, LTRA,nedo-cromil, or theophylline		Medium-dose ICS + LTRA or theophylline	High-dose ICS + LTRA or theophylline	High-dose ICS + LTRA or theophylline + oral corticosteroids
			Consider subcutaneous immunotherapy for persistent allergic asthma.				
Children ≥12 and Adults	**Preferred**	SABA prn	Low-dose ICS	Low-dose ICS + LABA or Medium-dose ICS	Medium-dose ICS + LABA	High-dose ICS + LABA and consider omalizumab	High-dose ICS + LABA + oral corticosteroids and consider omalizumab
	Alternative		Cromolyn, LTRA, nedocromil, or theophylline	Low-dose ICS + LTRA, theophylline, or zileuton	Medium-dose ICS + LTRA or theophylline, or zileuton		
			Consider subcutaneous immunotherapy for persistent allergic asthma				

Abbreviations: ICS, inhaled corticosteroid; LABA, long-acting β agonist, LTRA, leukotriene receptor antagonist; SABA, short-acting β agonist.

OCCUPATIONAL DISEASE

Occupational Asthma (OA)

- Form of work-related asthma characterized by bronchial hyperresponsiveness, airway inflammation and obstruction related to specific exposure only in the workplace
- 10–25% of adult asthma due to OA
- Variable latency period that is dependent on duration of exposure, dose, agent, host susceptibility factors. Refer to Table 7-16.
- Methacholine challenge performed when patient symptomatic and at work if negative excludes OA

Table 7-16. Mechanisms of OA

	Immunologic		Nonimmunologic
Mechanism	IgE Can be polyimmunogenic (IgE/IgG)	Haptens with protein (albumin)	Irritant—RADS/IrIA*
Agents	High molecular weight (HMW)	Low molecular weight (LMW)	Gases/fumes
	Protein/polysaccharide	Chemical	
Timing of symptoms	Latency period, variable onset		Immediate no latency

Abbreviations: IrIA; irritant-induced asthma; RADS, reactive airways dysfunction syndrome..

Key Fact

Animal laboratory workers take 2 years to sensitize; in contrast, flour workers take significantly longer.

Allergic rhinitis usually precedes OA for HMW agents, not LMW agents.

Epidemiology—It is reported in up to 30% of animal workers, 10% of snow crab workers, 10% of bakers, and 10% of isocyanate handlers (Table 7-17).

Different means of sensitization can lead to the same clinical outcome. Symptoms generally get worse as the work week goes on.

Flash Card Q18

What agents are the most common causes of OA?

Natural Progression—The natural progression of OA leads to variable airflow limitation and/or airway hyper-responsiveness. The diagnosis is confirmed by objective testing as well as the relation between asthma symptoms and work. OA should be considered in any adult with new-onset asthma. The treatment is similar to any other asthmatic. Early diagnosis and removal of the patient from exposure prevents permanent loss of lung function.

Flash Card Q19

Smoking is a risk factor for sensitization to which agent?

Table 7-17. Common Associations for Occupational Asthma

Industry	Agent
	Low molecular weight:
Western red cedar mill, carpenters, woodworkers	Plicatic acid
Body shop, spray paint, and insulation, roofers, auto industry	Isocyanates
Nail salon workers	Acrylates
Welder, metal/chemical workers	Platinum salts, potassium dichromate
Hairdressers	Ammonium persulfate
Adhesives and epoxy resin, paint/plastics	Phthalic anhydride
Plastics, paint	Trimellitic anhydride
Shellac and lacquer, plastics/cleaners	Amines
Hospital workers, laboratories	Formaldehyde
Drug industry workers	β-Lactam agents
	High molecular weight:
Lab workers, vets	Animal proteins
Seafood handlers	Crab/lobster
Baker	Flour (wheat, soya dust)
Baker/textiles	Wheat, coffee, tobacco dust, psyllium, latex
Baker/pharmaceuticals	Enzymes (amylase, lipase, pectinase)

Key Fact

Plicatic acid activates complement.

Diisocyanates can induce reactions via immunologic and nonimmunologic mechanisms.

Risk Factors

- History of atopy (especially cat/dog in laboratory workers/vets)
- Smoking
- HLA DQB1*0503/0201/0301 increase risk of TDI OA
- HLA DR3, DR7 increases risk for OA in general
- Glutamine S transferase (GSTP1) enzyme defects (increased diisocyanate OA)

Diagnosis

- Obtain thorough history, especially of exposures/timing/pattern of symptoms, atopy history, current occupation and past work exposures
- Skin prick test (SPT)/sIgE testing to occupational allergens, correlate with symptoms and airflow obstruction via spirometry on exposure
- Pulmonary function test at baseline and serially, may need to check after exposure to agent if normal initially
- Methacholine challenge
- Radiograph of the chest
- Gold standard is inhalational challenge but false-positive and -negative results can occur and has limited availability; only at specific centers

Flash Card A18

Plicatic acid, isocyanates, wheat flour, latex

Flash Card A19

Platinum

Treatment

- Change work environmental exposures if can't change jobs
  - Protective equipment
- Inhaled corticosteroids and other standard asthma therapies
- Smoking cessation

Reactive Airway Dysfunction Syndrome (RADS)

Also called irritant induced asthma (IrIA); nonimmunologic process secondary to high-dose single exposure to irritant agent. Symptoms are immediate, there is no latency period, and can last a long time.

Lung Diseases

Vocal Cord Dysfunction (VCD)

VCD is known as "the asthma imitator." It is the paradoxical adduction of vocal cords during inspiration; however, it can happen in expiration as well.

Clinical Features

- Wheezing
- Stridor
- Hoarseness
- Dysphonia
- Chest tightness
- Cough

There is a high preponderance of VCD found in treatment-resistant asthma. Patients are usually either:

- Young female overachievers and athletes
- Middle-aged women with psychiatric history or health care association

Diagnosis—Physical examination reveals inspiratory wheezing over larynx. Bronchodilators can worsen the condition. Spirometry shows reduced FEV_1 and FVC with a preserved ratio as well as a blunted inspiratory loop. Refer to Chapter 10, Figure 10-2 for pulmonary function test (PFT). Diagnosis is confirmed with fiberoptic laryngoscopy while patient is symptomatic.

Treatment—(Refer to Table 7-18).

Table 7-18. VCD Treatment

Long Term	Short Term
Panting	Heliox inhalation
Speech therapy	Topical lidocaine spray
Botox injections into the vocal cords	Intermittent positive pressure ventilation

ALLERGIC BRONCHOPULMONARY ASPERGILLOSIS (ABPA) AND ALLERGIC FUNGAL SINUSITIS

Allergic Bronchopulmonary Aspergillosis

Allergic bronchopulmonary aspergillosis (ABPA) is an inflammatory disease of lungs and airways, occurring as an immune response to *Aspergillus fumigatus* colonization. Other fungi are rarely implicated.

Key Fact

ABPA is due to fungal colonization not local invasion.

Pathophysiology—*Aspergillus* colonization in the bronchi leads to an inflammatory and immune response to *Aspergillus* antigens, resulting in poor mucus clearance and recurrent bronchial obstruction. The chronic mucoid impaction of bronchi leads to the development of bronchiectasis, pulmonary fibrosis, and pulmonary function compromise. The dectin-1 receptor on alveolar macrophages plays a critical role in defense against *Aspergillus.* β-1,3-glucan in fungal cell walls binds dectin-1, which leads to production of various cytokines, including, TNFα, IL-1β, IL-1α, IL-6, IL-10, and IFNγ leading to T_h1 response critical in host defense against this pathogen.

Clinical Features

- **Asthmatics:**
  - Exacerbation of asthma symptoms (shortness of breath, cough, and wheezing), brown/tan sputum production, and systemic symptoms (fever and malaise)
  - Pulmonary infiltrates on radiograph of the chest mid/upper lung fields (tram line, parallel lines, hilar adenopathy)
  - Central- or upper-lobe bronchiectasis, pulmonary nodules and/or air trapping on CT of the chest.
- **Cystic Fibrosis:** Similar to symptoms and radiographic findings seen in primary disease.

Diagnosis—Table 7-19 provides a diagnostic overview of APBA in patients with asthma versus those with cystic fibrosis.

See Figures 11-3, 11-5 through 11-24 for micrographs of various pollens. See Figure 11-27A for a micrograph of *Aspergillus.*

Treatment—Treat with long-term corticosteroids. Daily prednisone (0.5–1 mg/kg) for at least 14 days; taper and continue for 3–6 months. Steroid therapy can be combined with **itraconazole for at least 16 weeks. Voriconazole can be used as it has better absorption and requires less frequent dosing.** Antifungal therapy decreases antigenic stimulus for bronchial inflammation and decreases *Aspergillus* IgG. It may also affect steroid levels, thus allowing decreased doses of steroid with concomitant use. Monitor response to steroid therapy with monthly total serum IgE.
Omalizumab and immunotherapy have been discussed for treatment of ABPA in children with CF but there is insufficient evidence at this time to support their use in this patient population.

Key Fact

In cases with ABPA, the treatment goal is to prevent the development or progression of bronchiectasis and any worsening of pulmonary function.

Key Fact

Rising IgE levels can help predict an ABPA recurrence, with a doubling of the baseline IgE often indicating a flare of ABPA.

Allergic Fungal Sinusitis (AFS)

Fungal sinusitis is divided into acute/fulminant (invasive form), chronic/indolent (also invasive), fungus ball, and AFS categories. AFS is the most common form of fungal sinusitis. It is more prevalent in southern US than in northern states.

AFS is a **noninvasive hypersensitivity** reaction to fungi affecting the nasal cavity and paranasal sinuses, resulting in the formation of thick fungal debris, mucin, and polyposis. Causative fungi are from the dematiaceous family (i.e., genera *Bipolaris, Curvularia, Alternaria*, *Rhizopus, Drechslera*, *Helminthosporium, Fusarium,* and *Aspergillus*). Approximately 5–10% of patients with chronic rhinosinusitis requiring surgery have AFS. Refer to Figures 11-25 through 11-32 for fungal images.

Flash Card Q20

A 15-year-old soccer player complains of chest tightness that is worsening despite high-dose ICS/LABA combination therapy and frequent albuterol use. What alternative diagnosis should be considered?

Table 7-19. Commonly Used Diagnostic Criteria

Patients with Asthma	Patients with Cystic Fibrosis
Positive immediate skin test to *Aspergillus fumigatus*	Clinical deterioration not due to other causes
Total serum IgE >1000 ng/mL (417 IU/mL)	Total serum IgE >1200 ng/mL (500 IU/mL)
Elevated *A. fumigatus* specific IgG and IgE	Positive immediate skin test to *A. fumigatus*
Central bronchiectasis[a]	Serum IgE or IgG antibodies to *A. fumigatus*
Peripheral eosinophilia (>1000/mm^3)	Fixed chest film abnormalities (e.g., infiltrates or mucus plugging)

[a]Absence of bronchiectasis with presence of other criteria is seropositive ABPA (ABPA-S).

Flash Card Q21

In the setting of which two lung diseases does ABPA typically occur?

Key Fact

Charcot-Leyden crystals are composed of eosinophil-degradation products.

Pathophysiology—AFS is due to an exaggerated immunologic reaction following exposure to a fungus to which the patient is sensitized. This results in the development of chronic edema and obstruction, leading to the obstruction of the sinuses, mucosal stasis, and fungal proliferation. The fungal proliferation further contributes to the hypersensitivity response and worsening of nasal symptoms.

Clinical Features—Refer to Table 7-20.

Imaging—CT shows hyperattenuation, heterogeneous opacification, and calcification in affected sinus(es).

Key Fact

Positive fungal culture does not confirm the diagnosis of AFS, nor does a negative culture exclude it.

Laboratory Findings—Allergic mucin (**gross**): thick, tenacious, highly viscous, light tan to brown colored; (**histologically)** branching noninvasive fungal hyphae, sheets of eosinophils, and Charcot-Leyden crystals. Positive fungal skin testing, elevated IgE and fungal IgG may be seen.

Diagnosis—Table 7-21 provides a summary of diagnostic criteria for AFS.

Treatment—Courses of treatment include:

- Endoscopic sinus surgery: Surgical debridement of fungal debris and polyps
- Nasal saline irrigations: Provides mild symptomatic relief
- Oral corticosteroids: Usually initiated before surgery and most often continued 3–4 weeks postoperatively delayed the need for repeat surgery
- Topical nasal steroids: Most effective when begun postoperatively and chronically continued for control of inflammation
- Immunotherapy: Effectiveness is controversial, but this is frequently used as an adjuvant treatment
- Antifungals: Have not been shown to significantly modify clinical course

Prognosis—Chronic condition that requires life-long therapy, with recurrence of disease varying from 10–100%.

Table 7-20. Symptoms and Signs AFS

Acute	Chronic
Fever	Vision changes/loss due to ophthalmic nerve compression
Nasal congestion	Proptosis
Acute facial pain	Sinus tenderness to palpation/pressure
Headache	Malaise
Diplopia	Fatigue
Acute loss of sense of smell	

Flash Card A20

VCD

Flash Card A21

Asthma and cystic fibrosis

Table 7-21. Most Commonly Accepted Diagnostic Criteria for AFS

Type I hypersensitivity to fungi confirmed on skin testing or by specific IgE
Nasal polyposis
Positive fungal stain of sinus contents
Characteristic histology: Eosinophil-rich allergic mucin and fungal elements with no invasion of respiratory mucosa

HYPERSENSITIVITY PNEUMONITIS (HP)

This is an important topic, key elements are discussed in Tables 7-22, 7-23, and 7-24.

Table 7-22. Immunologic Lung Disease

TH1	TH2
CD8: HP and COPD	Asthma, ABPA, pulmonary eosinophilia, HIV, and Graves' disease
CD4: granulomatous → TB, sarcoid, and berylliosis	

Abbreviations: ABPA, allergic bronchopulmonary aspergellosis; COPD, chronic obstructive pulmonary disease; HIV, human immunodeficiency virus; HP, hypersensitivity pneumonitis; TB, tuberculosis.

Table 7-23. Three Types of HP

Acute	Subacute	Chronic
Explosive respiratory symptoms Takes 4–6 hours Nonproductive cough Fever present Recovery spontaneous On HRCT: Normal or fleeting ground glass opacities seen	Progressive Slow Productive cough No systemic symptoms HRCT: Diffuse micronodules, air trapping, mild fibrosis	No explosive symptoms No fever Fibrotic lung Anorexia Weight loss Permanent damage likely HRCT: Ground-glass opacities, emphysema, honeycombing, and parenchymal micronodules

Abbreviations: HRCT, high-resolution computed tomography.

Flash Card Q22

What treatment(s) are recommended for AFS?

Table 7-24. Important HP Associations

Antigen	Source
Faenia rectivirgula or *Micropolyspora faeni*	Farmer's lung (moldy hay and compost)
Thermoactinomyces vulgaris	Bagassosis (moldy sugar cane)
Thermoactinomyces sacchari, Naegleria gruberi, or *Acanthamoeba*	Ventilation pneumonitis (humidifier and A/C)
Bacillus cereus or *Klebsiella oxytoca*	Humidifier's lung (cool mist humidifier)
Mycobacterium avium intracellulare (MAI)	Hot tub lung
Aspergillus species	Malt worker's lung (malt, tobacco, and soy)
Penicillium casei or *P. roqueforti*	Cheese worker's lung (moldy cheese)
Pseudomonas, Acinetobacter, or *Mycobacter*	Machine operator's lung
Avian proteins or *Aspergillus* species	Bird fancier's lung (kids have good prognosis)
Bacillus subtilis	Detergent worker's lung
Alternaria species	Woodworker's lung
Stachybotrys	Winegrower's lung
Aureobasidium	Air-conditioner lung
Trichosporum cutaneum	Summer-type HP (Japan)
Pezizia	El Niño lung
Candida albicans	Saxophonist's lung
Epicoccum nigricans	Basement shower lung
Sitophilus	Wheat weevil lung
Toluene or toluene diisocyanate (TDI)	Paint finisher's lung
Diphenylmethane diisocyanate (MDI)	Chemical worker's lung
Phthalic acid	Epoxy resin worker's lung
Trimellitic anhydride	Plastic worker's lung

Diagnosis—The major criteria for diagnosis are:

- HP symptoms
- Evidence of exposure (history and +IgG)
- + Radiograph of chest or high-resolution computed tomography (HRCT, reticular, nodular, or ground-glass opacities)
- Bronchoalveolar lavage (BAL) lymphocytosis > 20% (low CD4:CD8 ratio <1)
- Symptoms on re-exposure

Flash Card A22

Systemic and topical steroids with surgical intervention. IgE level increase can predict recurrence when following these patients.

Additional criteria include bibasilar dry rales, decrease in diffusion lung capacity for carbon monoxide (DLCO), and decrease in partial arterial pressure of oxygen (PaO_2) with exercise.

Laboratory Findings—(See Table 7-25).

Table 7-25. Laboratory Findings in HP

Laboratory Test	Result
BAL	↑CD8 (CD4:CD8 <1) Lymphocytosis >20% ↑ neutrophils ↑ eosinophils >1% of WBCs in BAL are mast cells ↑ IgG, IgA, IgM ↑ hyaluronic acid
Lung biopsy	Noncaseating granulomas Mononuclear cell infiltrates of alveolar walls (lymphocytes, plasma cells) Foamy histiocytes in alveoli Giant cells, peribronchiolar fibrosis
PFT findings	↓FEV_1 after 4–6 hr of exposure ↓FVC after 4–6 hr of exposure ↓DLCO after 4–6 hr of exposure

Abbreviations: BAL, bronchoalveolar lavage DLCO, diffusion lung capacity for carbon monoxide; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; PFT, pulmonary function test; WBCs, white blood cell count.

For a summary of laboratory studies for asymptomatic versus symptomatic HP, see Table 7-26.

Table 7-26. Laboratory Studies for Asymptomatic Versus Symptomatic HP

Asymptomatic	Symptomatic
CD4 > CD8	CD8 > CD4
Normal CD8 numbers	Low CD8
Normal IgM	High IgM

Differential Diagnosis

- **Organic dust toxic syndrome (ODTS, pulmonary mycotoxicosis):** Noninfectious, febrile illness that occurs in workers after exposure to dust contaminated by bacterial and/or fungal spores (from inhalation of toxin-producing fungi-contaminated aerosols [i.e., grain, hay])
  30 to 50 times more common than HP; young patients; usually contracted in the summer months; complete recovery.

- **Fire-eater's lung:** Choking, coughing, respiratory symptoms from aspiration of the flammable petrochemicals used during the fire-eating demonstration. Fever and pleuritic chest pain also seen
- **Humidifier fever:** Toxic alveolitis, recirculated water with endotoxin, chest tightness occurs 4–8 hours after exposure; resolves in hours
- **Silo unloaders disease**: Acute exposure to NO_2, leading to asphyxia (i.e., a farmer entering a silo).
- **Byssinosis:** Dust inhalation of cotton, flax, and hemp; bronchoconstriction first day of exposure; tends to improve as the week goes on
- **Histiocytosis**: Pneumothorax in 10% of cases
- **Usual interstitial pneumonia (UIP)**: Clubbing present and neutrophil predominant
- **Occasional interstitial lung disease (ILD)**: Secondary to flock or nylon; lifeguarding at the pool, with endotoxin as the culprit

***Clinical History and Findings*—Farmer's lung is the most common form of HP**. But, far more common, in farmers is ODTS (nonimmunologic) up to 50 times more common; thus it is important to distinguish between the two. Farmer's lung occurs after prolonged exposure, whereas ODTS occurs after a single high-dose exposure.

Mnemonic

Drugs that can cause HP

A Boy **C**ame **F**rom a **G**ood **H**ome to **M**ake **N**ice **P**urple **S**weets

Amiodarone
Beta Blockers
Chlorambucil, clozapine, cyclosporine
Fluoxetine
Gold
Heroin, HMG Co-A reductase inhibitors
Methotrexate, minocycline
Nitrofurantoin
Procarbazine
Sulfasalazine

- HP seen with contamination of water and ventilation systems. MAI (*Mycobacterium avium intracellulare*) most common pathogen
- Smoking is protective in HP, thought to be due to decreased antibody response to antigen in the lung
- The particle size of the inciting agent must be <5 μm to enter to smaller airways
- Timing of cells involved: Eosinophils and PMNs after a few hours, lymphocytes infiltrate after a few days and macrophages over months

Key Fact

ODTS is 50 times more common in farmers than farmer's lung.

***Treatment*—**Avoidance of offending agent. Glucocorticoids in severe or persistent symptoms accelerate recovery. Use daily for 1–2 weeks and taper over next 2–4 weeks.

INTERSTITIAL LUNG DISEASE (ILD)

ILD refers to a group of lung diseases affecting the interstitium.

Interstitial Pneumonitis

The acute injury pattern of interstitial pneumonitis is characterized by fibroblastic foci. IL-1, -6, -8, and TNF are important cytokines involved via macrophages. "Ground-glass" appearance on early radiography versus "honeycombing" in the late phases. (Note: It is synonymous with usual interstitial pneumonitis.).

Usual Interstitial Pneumonitis (UIP)

UIP is the most common of the interstitial pneumonias and is also known as **idiopathic pulmonary fibrosis (IPF)**. Males have poorer prognosis. It is a chronic, progressive fibrotic disease. Symptoms include:

- Dyspnea and/or nonproductive cough
- Diffuse interstitial infiltrates on chest radiographs
- Honeycombing on HRCT
- Restrictive pattern with low DLCO on spirometry

The etiology of UIP is unknown and it has a poor prognosis. Although steroids are used in treatment, lung transplant is definitive.

Desquamative Interstitial Pneumonitis (DIP)

DIP is an uncommon ILD. Ninety percent of these patients have a smoking history and present with "smoker's bronchiolitis." DIP has a better prognosis than UIP.

Acute Interstitial Pneumonitis (AIP)

AIP is another uncommon ILD. It is also called **Hamman-Rich syndrome** and has a pathologic pattern of diffuse alveolar damage. It is characterized by:

- High fever
- Severe dyspnea over 1 day
- Flu-like symptoms
- Rapid respiratory decompensation

Flash Card Q23

What are typical laboratory findings in HP?

Nonspecific Interstitial Pneumonia (NSIP) or Fibrosis

NSIP is yet another uncommon ILD, which is based on a diagnosis of exclusion. One must rule out collagen vascular disorders, organic dust inhalation, drug reactions, etc. Overall, NSIP has a good prognosis.

Flash Card Q24

Usual interstitial pneumonia is the typical pathologic pattern in patients with which clinical condition?

CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD)

Characteristics—COPD has the following characteristics:

- Progressive airflow limitation characterized by $FEV_1/FVC < 70\%$
- Spirometric severity is based on postbronchodilator FEV_1 percent predicted

Spirometry is essential for test of COPD staging. GOLD criteria are used for staging purposes as follows:

GOLD 1: $FEV_1 \geq 80\%$ predicted = Mild
GOLD 2: 50% to < 80% predicted FEV_1 = Moderate
GOLD 3: 30 % to < 50% predicted FEV_1 = Severe
GOLD 4: $FEV_1 < 30\%$ predicted, or <50% normal with chronic respiratory failure present

COPD is caused by chronic inflammation of the airways and lung parenchyma. The primary risk factor is tobacco smoke exposure (current or former). In severe COPD, an inverse relationship between body mass and respiratory mortality has been described.

***Airway Inflammation—*Neutrophils and macrophages play a major role** in COPD. Activated macrophages and neutrophils release matrix-degrading enzymes (including matrix metallo-, serine, and cysteine proteases) that cause the parenchymal lung destruction that underlies emphysema. Nicotine in cigarette smoke may also enhance tissue destruction by inhibiting tissue inhibitors of matrix metalloproteinases (TIMPs). **Sputum eosinophils are a marker of viral exacerbation**, whereas sputum neutrophils are seen in both bacterial and viral exacerbations.

***Clinical Phenotypes—*Chronic bronchitis** involves inflammation of small- and medium-sized airways, with symptoms of dyspnea, chronic cough, and sputum production. **Emphysema** involves destruction of elastic tissue in terminal airspaces, loss of lung elastic recoil, and parenchymal loss. Signs and symptoms include dyspnea and hypoxemia.

- Centrilobular emphysema: Involves upper lobes; associated with cigarette smoke
- Panlobular emphysema: Involves lower lobes; seen with α_1-antitrypsin deficiency

Flash Card A23

Increased CD8 in BAL fluid with decreased CD4:CD8 <1

Flash Card A24

Idiopathic pulmonary fibrosis

Lung Function—There is an **accelerated loss of lung function** in smokers with a decline in FEV_1 of almost 60 mL/year (compared with a normal loss of 30 mL/year typical of nonsmokers). **Hyperinflation** is seen in moderate-to-severe COPD. Destruction of the alveolar-capillary interface (particularly with emphysema) and alveolar hypoventilation leads to a **reduced diffusing capacity** for carbon monoxide along with hypoxemia.

Treatment Considerations in COPD Compared with Asthma—Medications useful for COPD include short- and long-acting anticholinergics, which improve symptoms and airflow while decreasing hyperinflation in COPD. Long-acting β agonists, which are used only with inhaled corticosteroids for asthma, can be used as isolated agents for COPD.

Although inhaled corticosteroids (ICS) are a mainstay of treatment for persistent asthma, ICSs are considered only for moderate-to-severe COPD with symptoms despite bronchodilators. A proposed mechanism explaining why COPD may be less steroid-responsive than asthma includes the following:

- Histone acetyltransferase acetylates (unwinds) the DNA-histone complex, leading to the generation of nuclear factor kappa B (NFκB) and activator protein 1 (AP-1), which are proinflammatory transcription factors.
- Histone deacetylases (HDACs) deacetylate (compacts) DNA, which suppresses gene transcription of NFκB and AP-1 (thus less inflammation).
- **Corticosteroids increase HDACs in asthma, but not in COPD**.

Smoking cessation is critical and returns rate of loss of lung function almost to normal. Supplemental oxygen decreases mortality. There is a role for pulmonary rehabilitation, lung volume reduction surgery, and/or lung transplant for some patients. Use of anticholinergic therapy as adjunct to ICS has been shown to preserve lung function.

Flash Card Q25

What is the only treatment that prolongs life in COPD?

FOOD ALLERGY

Adverse Food Reactions

Nonimmune-mediated adverse food reactions include the following categories:

- Metabolic: Lactose intolerance, galactosemia, alcohol intolerance
- Pharmacologic: Caffeine (makes you jittery), tyramine in aged cheeses (headaches and migraines), Scombroid fish poisoning (releases histamine-like chemicals)
- Toxins (not host-specific): Food poisoning
- Psychologic: Food aversion, anorexia nervosa

Flash Card Q26

On a vacation in Florida, a 39-year-old man orders mahi mahi in a restaurant and, within 20 minutes of eating it, develops abdominal cramps, vomiting, swelling of the tongue, and trouble breathing. He has eaten fish all of his life. Skin testing to all white fish is negative. What is the cause of this patient's illness?

- Other: Auriculotemporal syndrome (vasodilatation), gustatory rhinitis (runny nose from spicy or hot foods), Frey's syndrome (transient, unilateral and bilateral facial flushing or sweating after ingestion of spicy or flavored foods; due to damage to auriculotemporal nerve)

IgE-Mediated and Non-IgE-Mediated Food Allergy

Reactions to foods can be mediated by the immune system. These can be IgE- or non-IgE-mediated.

IgE-mediated food allergy is acute in onset (within 2 hours of exposure) and includes skin symptoms (e.g., hives), gastrointestinal symptoms (e.g., vomiting) , respiratory (e.g., wheezing) or cardiovascular symptoms (e.g., hypotension). The most severe clinical manifestation of an IgE-mediated reaction is defined as anaphylaxis.

Non-IgE-mediated reactions involve mainly T-cell mediated mechanisms. Typically, these reactions are not immediate and involve primarily the gastrointestinal system.

Finally, some reactions to food are considered to be mixed IgE- and cell-mediated (Figure 7-7).

Skin	Urticaria Angioedema	Atopic dermatitis	Dermatitis herpetiformis
Respiratory	Asthma Rhinitis		Heiner's syndrome
Gastrointestinal	GI anaphylaxis Oral allergy	Eosinophilic disorders	Enterocolitis Enteropathy Proctocolitis Celiac

Figure 7-7. Types of food allergy showing spectrum of IgE-mediated, non-IgE-mediated, and mixed reactions.

Flash Card A25

Use of supplemental oxygen

Flash Card A26

Scombroid fish poisoning

Prevalence of Food Allergy

Public perception is that 20–25% of the population has food allergies. Actually, about 6% of young children and 3–4% of adults have food allergies (Table 7-27).

- Reactions to fruits and vegetables are approximately 5%, but usually are not severe.
- Soy or wheat each cause reactions in up to 0.4% of young children.
- Food is the most common cause of outpatient anaphylaxis; and peanut accounts for the most common trigger of fatal reactions in North America.
- Sensitization to one food may increase risk of sensitization to other foods.

Pathophysiology

***Class 1 Allergens*—**These allergens result primarily from food that is ingested (i.e., **GI sensitization)**. Such a breach in oral tolerance typically occurs when food proteins stable to digestion are encountered by sensitive individuals. Class I allergens such as egg or peanut may invade through the skin. Type 1 allergens are usually characterized by the following:

- 10–70 kD glycoproteins; heat resistant, acid stable, and water soluble
- Examples include cow's milk protein (casein and whey), egg (ovalbumin and ovomucoid), peanut (vicilin, conglutin, and glycinin), fish (parvalbumin), and shellfish (tropomyosin)
- This class of allergens also includes nonspecific lipid-transfer proteins in apple and corn. (See the food section within Chapter 11 on allergens for more details and nomenclature.)

Table 7-27. Prevalence of Food Allergy in the U.S.

Food	Young Children (%)	Adults (%)
Milk	2.5	0.3
Egg	1.3	0.2
Peanut	0.8	0.6
Tree nuts	0.2	0.5
Fish	0.1	0.4
Shellfish	0.1	2.0
Overall	6	3.7

Class 2 Allergens—These allergens are formed primarily from **respiratory sensitization**. Sensitization to labile proteins encountered via the respiratory route, such as pollens, results in IgE antibodies that recognize homologous epitopes on food proteins from plants. Some patients exhibit symptoms of pruritus limited to the oral mucosa when eating fresh fruits and vegetables, termed **pollen-food allergy syndrome.** Type 2 allergens are characterized by the following:

- Plant-derived and labile
- Predominantly in superfamilies of cupin and prolamin, and the protein families of the plant defense system

> ***Key Fact***
>
> Linear epitope = more prolonged allergy, allergen is "stable" and persistent
>
> Conformational epitope = mild, transient allergy.

Linear vs. Conformational Epitopes—A single food is composed of many proteins, and these proteins have multiple areas, termed epitopes, to which the immune system can respond. Epitopes that are dependent upon the folding of the proteins are termed **conformational epitopes;** those that are not dependent upon folding are termed **linear epitopes**.

Clinical Syndromes

Specific syndromes that are associated with food allergy are listed in Table 7-28. A few are highlighted here.

Anaphylaxis—Rapid-onset organ response involving at least two body systems or hypotension in response to a potential allergen that is potentially fatal. Reaction may be biphasic (i.e., symptoms may recur after initial resolution) . Tryptase levels (a mast cell mediator) may be increased. Any food can cause a reaction, but the main culprits are peanuts, tree nuts, and seafood.

- Factors associated with increased risk for fatal reaction include delayed epinephrine administration, young adult or teen, underlying asthma, and absence of skin symptoms.

Food-Associated, Exercise-Induced—Often associated with particular foods such as celery and wheat (omega-5 gliadin) in the context of physical activity.

Pollen-Food Allergy Syndrome—Sensitization to allergens such as Bet v 1 (birch) through the respiratory route may result in cross-reactivity to type 2 allergens such as Mal d 1 (apple, peach), and present with oral pruritus when eating fresh fruit. This is IgE-mediated, rarely progresses to more severe symptoms, and certain forms of fruit/vegetables may be tolerated. Symptoms may vary by season.

Plant food allergens can be divided into a number of categories:

- **PR-10:** Bet v 1 homologues in the pulp of the fruit; sensitive to heat, therefore, no reaction to cooked/processed fruit; symptoms usually restricted to the mouth

- **Nonspecific lipid transfer proteins:** Located primarily in the peel; stable to heat and digestion, therefore reacts to cooked/processed fruit as well; reaction may be more severe
- **Profilin:** Bet v 2 homologues, sensitive to heat, therefore, no reaction to cooked/processed fruit; symptoms usually restricted to the mouth
- **Storage proteins:** Located in the fruit stone (seed/nut/kernel); stable to heat and proteases, therefore, react to cooked/processed foods
- **Cross-reacting carbohydrate determinants:** Found in plants and invertebrates (not mammals); may cause no reaction, or anaphylaxis; examples include latex, bee/wasp, cockroach, mite, and shellfish

Table 7-28. Important Disorders Associated with Food Hypersensitivities

Disorder	Mechanism	Symptoms	Diagnosis
Gastrointestinal			
Pollen-food allergy syndrome	IgE-mediated	Pruritus, tingling, angioedema of lips, palate, tongue, or oropharynx	Hx and +SPT to pollens and/or fresh fruit
Anaphylaxis	IgE-mediated	Hives, cough, dyspnea, wheezing, abdominal pain, vomiting, and/or diarrhea	Hx, SPT, specific IgE levels, and +/– oral challenge
Eosinophilic esophagitis	IgE-mediated and/or cell-mediated	Reflux, dysphagia, abdominal pain, and failure to respond to reflux meds; sensation of food impaction	Hx, SPTs, and endoscopy or biopsy, and elimination diet
Eosinophilic gastroenteritis	IgE-mediated and/or cell-mediated	Recurrent abdominal pain, vomiting, early satiety, FTT, and peripheral eosinophilia	Hx, SPTs endoscopy or biopsy and elimination diet
Food protein-induced proctocolitis	Cell-mediated	Blood in stool, first few months of life, and no FTT	SPTs, elimination diet, challenge results in sx in 72 hr
Food protein-induced enterocolitis	Cell-mediated	Vomiting and diarrhea, +/- blood, FTT, vomiting one to three hours after eating, and hypotension	SPTs, elimination diet, challenge results in sx in 1–2 hr
Food protein-induced enteropathy; celiac disease	Cell-mediated	Diarrhea, steatorrhea, abdominal distension, flatulence, oral ulcers, and weight loss	Endoscopy and biopsy, IgA, celiac abs, and elimination diet
Cutaneous			
Urticaria and angioedema	IgE-mediated	Itching, hives, and/or swelling	Hx, SPT, specific IgE levels, and food challenge
Atopic dermatitis	IgE- and cell-mediated	Itching and eczematous rash in “classic distribution”	Hx, SPT, specific IgE levels, elimination diet, and food challenges
Dermatitis herpetiformis	Cell-mediated	Itching, papulovesicular rash over extensor surfaces and buttocks; associated with celiac disease	Skin biopsy (IgA deposition), IgA antigliadin and antitransglutaminase antibodies, and endoscopy
Respiratory			
Heiner’s syndrome	Uncertain	Recurrent pneumonia, pulmonary infiltrates, hemosiderosis, iron deficiency anemia, and FTT	Hx, peripheral eosinophilia, milk precipitins, lung biopsy, and elimination diet

Abbreviations: FTT, failure to thrive; Hx, history; SPT, skin prick test; Sx, symptoms.

Specific food and fruit associations can be found in the Foods section of Chapter 11.

Dermatitis Herpetiformis—An example of non-IgE-associated syndromes that affect the skin. Associated with celiac disease. Patients are gluten-sensitive.

- Symptoms include vesicular, pruritic eruption (usually in acral distribution) on sun-exposed areas of arms and/or legs
- Celiac disease antibodies: IgA-antigliadin antibodies and antitransglutaminase antibodies

Key Fact

Antibodies often positive in patients with celiac disease include IgA-antigliadin antibodies and tissue transglutaminase antibodies. If IgA is absent, serology for celiac disease can be falsely negative.

Galactose-α-1,3-galactose—urticaria, angioedema, or anaphylaxis 3–6 hours after ingesting beef, lamb, or pork. May have a history of a tick bite. Unlike common allergens, which are usually proteins, this allergen is a carbohydrate found as part of the glycoproteins, including the chemotherapeutic monoclonal antibody cetuximab.

Diagnosis

Key Fact

Most children who are unable to consume nonbaked forms of egg and milk are able to tolerate baked egg and milk.

- **History:** Consider symptoms, timing, reproducibility, quantity ingested, and ancillary factors, such as exercise, aspirin, and alcohol. Must consider if cause acute vs. chronic disease.
- **Diet details and/or symptom diary:** Allows identification of specific causal food(s) and "hidden" ingredient(s).
- **Physical examination:** Allows evaluation of disease severity. Look for failure to thrive.
- **General approach:** Consider whether reaction is allergy vs. intolerance and IgE- vs. non-IgE-associated.

Laboratory Evaluation

Suspect IgE-Associated—SPTs (consider fresh food if oral allergy); also consider serum food-specific IgE antibodies. Selection of foods for testing should be guided by the history, given the high level of false-positive tests in the absence of suggestive history.

Suspect Non-IgE-Associated—Consider biopsy of gut and skin in appropriate clinical setting (e.g., suspected celiac disease). The role of atopy patch test in detecting delayed-type hypersensitivity reactions is not established yet.

Suspect Nonallergic—Consider the following:

- Breath hydrogen for lactose intolerance
- Sweat test, especially if malabsorption
- Endoscopy

Interpretation of Tests

- Positive SPT and elevated food-specific serum IgE indicate presence of specific IgE antibody (sensitization), but not necessarily clinical reactivity (PPV < 50%). With increasing size of the skin test and beyond certain cut off levels of specific IgE, especially in the context of a suggestive history of allergic reaction, the likelihood of clinical allergy or reaction is increased.
- Commercially prepared extracts may be inadequate for fruits and vegetables, especially for the diagnosis of the pollen-fruit syndrome, so fresh food for testing is often required.
- Intradermal skin tests with food have a higher risk of systemic reactions and are not used.
- Increasing serum-specific IgE correlates with increasing likelihood of reaction. Not useful in predicting the type or severity of the reaction. Ten percent to 25% of those with negative serum-specific IgE may have clinical reactions.
- Component-resolved diagnostics can identify IgE specific for conformational epitopes, or specific epitopes that may help predict whether a reaction may be systemic (i.e., Ara h 2) or oral (i.e., Ara h 8) in individuals with peanut allergy.
- Unproven and/or experimental tests and likely useless tests include provocation and/or neutralization, cytotoxic tests, applied kinesiology, hair analysis, and IgG4.

Treatment

Dietary Elimination—Teach patients about hidden ingredients, reading labels, cross-contamination (e.g., shared equipment), emergency ID bracelet, and seeking assistance from registered dietitian as well as Food Allergy Research & Education (FARE).

Emergency Medications

- Epinephrine is the drug of choice for reactions. Self-administered epinephrine should be readily available. Epinephrine should be administered through the IM route. Train patients on indications and technique.
- Antihistamines are secondary therapy; they help to manage hives and pruritus.
- Emergency **plan in writing** for schools, spouses, caregivers, and mature siblings or friends.

Oral Food Immunotherapy—: Exposing patients to gradually increased amounts of food allergen under medical supervision is currently done on a research basis only.

Follow-Up—Reevaluate periodically for tolerance. Interval and decision to rechallenge is based on type of food allergy, severity of previous symptoms, confirmatory tests (i.e., SPT and /or specific IgE levels), and the specific allergens.

- Ancillary testing: Repeat SPT and serum IgE. Reduced concentration of specific IgE and smaller SPTs suggest outgrowing food allergy.
- See Contact Hypersensitivity section for specific IgE and SPT size for when to challenge.

Natural History

Natural history is dependent on food and pathogenesis of food allergy.

Key Fact

Approximately 85% of cow's milk, egg, wheat, and soy allergies remit by 5 years of age. Twenty percent of young children "outgrow" their peanut allergy (approximately 9% for tree nut).

- IgE-mediated reaction to cow's milk, egg, wheat, and soy: Approximately 85% remit by 5 years, although recent studies suggest lower rates of resolution. Declining and/or low levels of specific IgE are predictive of tolerance development as well as the extent of IgE binding to conformational epitopes.
- IgE-mediated reaction to peanut, nuts, and seafood allergies typically persist into adulthood. Over a 2-year period 20% of young children "outgrow" peanut allergy (approximately 9% for tree nut). Some (i.e., 7–9%) may redevelop the allergy (increased risk if avoided).
- Non-IgE-associated GI allergy: Infant form resolves by 1–3 years of age. Toddler and adult forms are more persistent.
- Some evidence suggests that consumption of baked milk/egg may expedite resolution of IgE-mediated allergy to nonbaked milk/egg

Food Allergy Prevention

There are limited data for the prevention of food allergy. This is an area of intense study and significant controversy, and it is currently being assessed through randomized controlled trials. Several pediatric and allergy organizations have suggested guidelines that require further study.

- Insufficient evidence to suggest maternal avoidance of allergens for atopic dermatitis (AD).
- Exclusive breast feeding for the first 4 months of life may decrease the risk of AD and cow's milk protein allergy in the first 2 years of life.
- Avoidance of solid food in the first 4 months of life may decrease risk of AD.

- Vitamin D, antioxidants, fats; antacids may play a role in exposure to intact proteins.
- Recently it was suggested that delayed introduction of allergens via the oral route (promoting tolerance) may increase risk of sensitization via other routes such as the skin, which may be more likely to produce clinical allergy. However, randomized controlled trials exploring this hypothesis are currently underway.

ANAPHYLAXIS

Anaphylaxis is an acute-onset, potentially fatal, systemic allergic reaction.

- Lifetime prevalence 0.05–2%
- Largest number of cases in children and/or adolescents
- **Food and drug reactions the most common cause of anaphylaxis**
- Risk has been established for several characteristics (Table 7-29)

Table 7-29. Risk Factors of Anaphylaxis

Risk Factors
• **Atopy:** Increased risk for idiopathic anaphylaxis, exercise-induced anaphylaxis, radiocontrast material and latex-induced reactions Note: Atopy is not a risk factor for anaphylactic reactions to medications (PCN, insulin, or muscle relaxants)
• **Gender:** More common in males until age 15 years; more common in females for those older than age 15 years
• **Age:** More cases in children and/or adolescents
• **Route of administration:** More severe reactions with IV or IM versus oral
• **Intermittent admin:** If start and stop, then more likely to sensitize (e.g., DM of pregnancy where patients take insulin intermittently)
• **Length of admin:** Prolonged antibiotic courses more likely to sensitize than single doses
• **Time since reaction:** The longer the time between administrations, the lower likelihood of an event
• **Geography:** Epinephrine autoinjector prescriptions in the US are found to have a north-south gradient, with more in the northern portion of the country
• **Socioeconomic status: Higher-income groups prescribed epinephrine more frequently**

Pathogenesis

Terminology has changed to favor immunologic vs. nonimmunologic (previously anaphylactoid) anaphylaxis.

Immunologic Anaphylaxis

- IgE-mediated: Most common; initiated by antigen interacting with allergen-specific IgE bound to high-affinity IgE receptors (FcεRI) on mast cells and/or basophils. Aggregation leads to cell activation, mediator release, and immediate hypersensitivity response. Examples include foods, venoms, latex, and drugs
- IgG-mediated: Animal models only
- Immune complex/complement-mediated reactions

Nonimmunologic Anaphylaxis—Initiated by certain drugs or events that induce a sudden release of mast cell or basophil mediators in the absence of IgE or other immunoglobulins. Examples include vancomycin, opiates, radiocontrast media, or cold urticaria.

Idiopathic Anaphylaxis— Diagnosis of exclusion where no specific trigger can be identified and all other diseases have been ruled out including mast cell disorders. Women more commonly affected than men; long-term management with oral prednisone and a nonsedating H_1 antihistamines for patients with frequent episodes.

Table 7-30 lists the mediators involved in anaphylaxis.

Table 7-30. Mediators Involved in Anaphylaxis

Mediators	Pathophysiologic Activity	Clinical Signs
Histamine, leukotrienes, thromboxane, prostaglandins, and platelet activating factor	Smooth muscle spasm, mucus secretion, increased vascular permeability, eosinophil chemotaxis, and activation	Wheezing, urticaria, angioedema, flush, itch, diarrhea, abdominal pain, hypotension, rhinorrhea
Neutral proteases: Tryptase, chymase, carboxypeptides, and cathepsin-G	Cleaves complement components, chemoattractants for eosinophils and neutrophils, and converts angiotensin I to II	Recruits complement, increases blood pressure via conversion to angiotensin II
Proteoglycans: Heparin and chondroitin sulfate	Anticoagulation, inhibits complement, binds phospholipase A_2, chemoattractant for eosinophils	Can prevent intravascular coagulation, complement activation, recruits kinins
Chemoattractants: Chemokines, eosinophils chemotactic factors	Attracts cells to the site	Late-phase reaction symptoms, or protraction of symptoms
Tumor necrosis factor (TNF)	Promotes platelet-activating factor production	Vascular permeability and vasodilation, and late-phase reactions

Clinical Features

Anaphylaxis is likely when any one of the criteria in Table 7-31 is fulfilled.

Target Organs (and Percentage of Time Involved)

- Cutaneous (90%)
- Respiratory (70%)
- Gastrointestinal (30–45%)
- Cardiovascular (10–45%)
- Central nervous system (10–15%)

Patients who do not experience skin manifestations may experience profound shock immediately. Most common organs involved in shock include the heart, vasculature, and lungs with fatalities caused by circulatory collapse or respiratory failure.

Note: The severity of a reaction is not predictable, but a history of anaphylaxis to an allergen is more likely to be associated with severe systemic reactions to the same allergen in the future.

Table 7-31. Diagnostic Criteria for Anaphylaxis

Criterion 1	Criterion 2	Criterion 3
Acute onset of symptoms (minutes to hours) with skin and/or mucosal involvement Generalized hives pruritus Angioedema flushing	Acute onset of symptoms (minutes to hours) after exposure to a **likely allergen** for that patient	Acute decrease in BP (minutes to hours) after exposure to a **known allergen** for that patient
plus at least 1 of the following	**plus 2 or more of the following**	**defining reduced BP**
Respiratory compromise (dyspnea, wheeze, hypoxemia) Decrease in BP or end-organ dysfunction (hypotonia, syncope)	Skin/mucosal involvement Respiratory compromise Decreased BP Gastrointestinal symptoms (abdominal pain, vomiting)	Adults: Systolic < 90 mmHg or >30% decrease from baseline Infants/children: Age-specific low systolic BP or >30% decrease from baseline
Skin findings most common presenting symptom of anaphylaxis (~90%). Therefore criterion 1 is the most useful for diagnosis.	Skin findings absent or unrecognized in ~20% of anaphylactic episodes. Therefore criterion 2 includes symptoms from other organ systems in patients with exposure to a likely allergen.	Criterion 3 is used to detect anaphylactic reactions in patients exposed to a known allergen when only one organ system is involved.

Biphasic Anaphylaxis—Biphasic or late-phase reactions are a recurrence of anaphylactic signs and symptoms several hours after the apparent resolution of the initial anaphylactic episode. These reactions occur in roughly 20% of anaphylactic episodes, with symptoms occurring as late as 72 hours (most within 10 hr) after resolution of the primary event. Observation for as long as 24 hours has been advocated, although 8 hours is sufficient for most reactions. Foods appear to be the most likely to induce this reaction.

Protracted Anaphylaxis—Anaphylactic reaction that lasts for hours, days, or even weeks in extreme cases

Compensatory Mechanisms

Physiological attempts to correct the hypotension that can occur during anaphylactic events include the synthesis and release of epinephrine (adrenal gland) and endothelin (endothelium), activation of the renin-angiotensin axis, and the release of norepinephrine (ganglia). Drugs that interfere or block these compensatory mechanisms may predispose to severe or protracted episodes:

- β-Adrenergic blockers: Interfere with normal compensatory tachycardia and blunt the effect of epinephrine. Relative contraindication to immunotherapy
- Angiotensin-converting enzyme inhibitors (ACEIs): Theoretical increased risk in venom immunotherapy, because it acts at two sites (no data supports its cessation in pollen immunotherapy):
  - Blocks conversion of angiotensin I to angiotensin II
  - Inhibits the same enzyme that destroys kinins, which are known to be active in anaphylactic episodes
- Angiotensin II receptor blockers (ARBs): No data
- Tricyclics: Exaggerate the response to epinephrine by preventing the reuptake of catecholamines at ganglionic sites
- Monoamine oxidase inhibitors (MAOIs): Prevent degradation of epinephrine systemically

Key Fact

Recall that the ratio of total tryptase (pro-β+mature) to mature tryptase is helpful in distinguishing anaphylaxis in mastocytosis from other forms: Total/mature >20 = mastocytosis; <10 = other cause.

Tryptase may not be elevated in food-induced anaphylaxis.

Diagnosis

Clinical History—More than one target organ is involved, and history often involves provocation by known food, drug, or insect allergen exposure.

Laboratory Findings

- Check serum histamine 15–60 minutes: Levels begin to rise by 5 minutes but remain elevated only 30–60 minutes.
- Check urinary histamine: Metabolites may remain elevated as long as 24 hours.

- Check serum tryptase 15–180 minutes: Peaks 60–90 minutes after the onset of symptoms and can remain elevated as long as 5 hours.
- Platelet-activating factor (PAF): Recent studies suggest that PAF levels more accurately correlate with anaphylaxis severity scores than either histamine or tryptase levels.

Evaluation

Investigate suspected allergens or triggers for specific IgE by SPT or in vitro methods. SPT is often performed at least 4–6 weeks after the episode due to refractory period of mast cells that can create false negatives. Selection of foods for testing should be guided by the history.

Management

Short term:

- Epinephrine at dose of 0.01 mg/kg intramuscularly (often as 0.3–0.5 mL of 1:1000)
- ABCs, supine positioning, and establishment of an airway
- Skin inspection
- Supplemental oxygen, insertion of one or more large-bore IVs for fluids
- Even if on β blockers, administer epinephrine first but consider glucagon. (Also, recall IV fluids are particularly important for patients unresponsive to epinephrine)

Long term:

- Epinephrine autoinjector
- Emergency action plan and medical alert bracelet
- Relevant and specific preventive treatment (e.g., avoidance of confirmed triggers and/or immunomodulation)

Pretreatment is effective for radiocontrast, cold, and fluorescein-related anaphylaxis. A common protocol consists of 50 mg prednisone 13, 7, and, 1 hour before the procedure, with 50 mg of diphenhydramineone 1 hour before the procedure, along with the use of low osmolar contrast material. This intervention has been shown to lower the risk for reaction to less than 1%. Histamine receptor roles are shown in Table 7-32.

Table 7-32. Histamine Receptors in Anaphylaxis[a]

H_1[b]	H_2[b]	H_3
Coronary artery constriction, bronchoconstriction, systemic vasodilation, increased capillary permeability, tachycardia, pruritus, rhinorrhea	Coronary artery vasodilation, increase force and rate of atrial and ventricular contractions, increased capillary permeability, increase secretion of mucus	Autonomic receptor downregulator (prevents release of additional epinephrine from autonomic ganglion, leads to heightened hypotension)

[a]The histamine H4 receptor is the most recently identified in the histamine receptor family and in murine models may be involved in pruritus, chemotaxis, and mast cell cytokine release.
[b]Need activation of both receptors for maximal changes in hypotension, headache, and flushing.

PERIOPERATIVE ANAPHYLAXIS

Etiology

- Neuromuscular blocking agents (NMBAs)
- Hypotonic induction agents (barbiturates)
- Antibiotics
- Opioids
- Latex
- Colloids

Risk factors: Asthma, female sex, atopy, multiple past surgeries, mast cell disorders

Perioperative anaphylaxis has a higher mortality rate than other forms of anaphylaxis, likely secondary to impaired early recognition (surgical drapes obscuring view, inability of patient to report symptoms), additional stress of surgery/illness, and IV administration of medications.

Diagnosis

Diagnosis is mainly clinical. Develop timeline of reaction with all medications, including NMBAs, latex, and skin prep used during procedure (local anesthetics like lidocaine or disinfectants like povidone-idoine, chlorhexidine). Elevated serum total tryptase, plasma histamine, or histamine metabolites in the urine obtained appropriately suggest anaphylaxis. Wait 4–6 weeks after reaction to skin test to suspected agents; if negative; consider a nonimmunologic cause.

LATEX ALLERGY

Features

- Natural rubber latex (NRL) is a cytoplasmic exudate of the *Hevea brasiliensis* tree.
- Although there are over 250 NRL proteins, 13 protein allergens from nonammoniated latex have been characterized and designated as Hev b allergens (Table 7-33).
- At-risk populations include health care workers and patients who use latex-containing medical devices. Risk is directly related to duration of exposure and cumulative use of latex-containing products.
- Some latex proteins cross-react with proteins found in certain fruits and vegetables (bananas, kiwi, avocado, chestnut).
- Cornstarch powder in latex gloves absorbs allergens and acts as an airborne vehicle to sensitize nearby people by inhalation.
- Dipped rubber products (gloves, condoms, balloons) cause most reactions because these are heat-vulcanized at lower temperatures and have more proteins intact.

Key Fact

Hev b 1 and 3 are less water-soluble and are more commonly identified as allergens in sensitized patients with spina bifida, but are minor in vivo allergens in health care workers.

Key Fact

Health care workers react most frequently to Hev b 5, 6, and 7.

Table 7-33. Protein Allergens in Natural Rubber Latex-Isolated and Associated Foods

Antigen	Trivial Name	Predicted Physiologic Roles	Associated Food
Hev b 1	Rubber elongation factor	Rubber biosynthesis	
Hev b 2	β-1 and 3-glucosidase	Defense-related protein	Bell pepper, olive
Hev b 3	Prenyltransferase	Rubber biosynthesis	
Hev b 4	Microhelix component	Defense-related protein	
Hev b 5	Acidic protein		Kiwi, potato, and sugar beet
Hev b 6	Hevein and prohevein proteins	Defense-related protein (latex coagulation)	Avocado, banana, chestnut, sweet pepper
Hev b 7	Patatin-like proteins	Defense-related protein or inhibitor of biosynthesis	Potato and tomato
Hev b 8	Profilin	Structural protein	
Hev b 9	Enolase		
Hev b 10	Manganese superoxide dismutase		
Hev b 11	Class I endochitinase		
Hev b 12	Lipid transfer protein	Defense-related protein	
Hev b 13	Latex esterase		Potato

Diagnosis

Identification of NRL-specific IgE associated with symptoms that are consistent with IgE-mediated reactions to latex protein allergens. There are no standardized skin test extracts. The in vitro assays have wide ranges of sensitivity (73–92%) and specificity (73–97%).

FOOD-DEPENDENT EXERCISE-INDUCED ANAPHYLAXIS

Features

Exercise-induced anaphylaxis (EIA): Symptoms occur after physical activity.

Food-dependent exercise-induced anaphylaxis: Variant of EIA that requires ingestion of certain foods within minutes to a few hours prior to vigorous physical activity . The culprit food can be ingested without symptoms if there is no associated exercise, and these patients can exercise as long as they have not eaten the offending food.

Associated foods include:
- Crustaceans
- Cephalopods
- **Celery**
- Grapes
- Chicken
- **Wheat**
- Buckwheat
- Tomato
- Dairy product
- Mushrooms

Key Fact

Celery and wheat are the most common foods associated with food-dependent EIA.

Clinically, there are two subsets of patients with food-dependent EIA:
- Rarely patients develop anaphylaxis when exercising within a short time after ingestion of any type of food (usually solids rather than liquids).
- More commonly patients have anaphylaxis with exercise only after ingestion of a specific food.

Management of food-dependent EIA includes avoiding exercising in proximity (4–6 hr) to food consumption, carrying self-injectable epinephrine, exercising with a partner, and wearing a medical alert bracelet.

STINGING INSECT ALLERGY

STINGING INSECTS (ORDER HYMENOPTERA)

Family Apidae

Apis Mellifera (Honeybee)—Used commercially for honey production and pollination; beeswax nest with numerous vertical combs (Figure 7-8A); not aggressive, and only females will sting when provoked; barbed stinger that remains in victim's skin, killing the insect.

African–European Hybrid Bees (Killer Bee—Escaped from a lab in Brazil in 1957 and have gradually migrated into the southern US. Similar to domestic honeybees and deliver identical allergen protein when it stings; however, they have a tendency to swarm with little provocation and sting in large numbers, causing a toxic reaction that can be fatal. Their aggressive nature has earned them the title "killer bees."

Bombus Spp (Bumblebee)—Subterranean or concealed nests made up of loose fibrous material; not aggressive but will sting when nests are disturbed; attack is loud, and slow so avoidance is fairly easy.

Family Vespidae

Subfamily Vespinae—Multilayered paper nests made of masticated wood. Can sting repeatedly without losing sting apparatus.

- ***Vespula* spp. (yellow jacket):** Picnic and trash can scavengers; highly aggressive, especially in summer and autumn when larger populations compete for food supplies; often sting for no apparent reason; nests found in concealed locations, either underground, in wall cavities, or decaying logs (Figure 7-8B); responsible for most human stings.
- ***Dolichovespula arenaria* and *D. maculata* (yellow hornet and white-faced hornets):** Aerial-nesting yellow hornets found in North America but not Europe; nests found around human dwellings; sensitivity to vibrations sets off their defensive sting behavior.

***Subfamily Polistinae—Polistes* spp. (paper wasp):** Nest constructed of a single layer of open cells (or comb) that are found on eaves or window sills of homes (Figure 7-8C). Narrow "wasp waist" and dangling legs when in flight; less aggressive, but can sting repeatedly without losing sting apparatus.

Key Fact

Think **yellow jacket**! Flying hymenoptera are found around garbage cans or food.

A

B

C

Figure 7-8. (A) Honeybee nest; (B) yellow jacket nest; (C) paper wasp nest. (Images courtesy of Wikipedia.)

Family Formicidae

Solenopsis invicta (Imported Fire Ant). Large subterranean nests. Widespread in the southeastern US. Are aggressive and have a true sting apparatus; they anchor by their mandibles and pivot to administer multiple stings that develop into characteristic sterile pustule at site within 24 hours after sting. Arrived in Mobile, Alabama, in the 1940s and have slowly spread, adapting to colder climates, with nests found as far north as Maryland.

Epidemiology

Systemic reactions reported in 3% of adults and 1% of children; approximately 30–50 fatal stings occur per year in the US, with half of those occurring in people with no prior history of allergic reaction to sting.

Presentation

Table 7-34 is a summary of clinical presentation of stings from insects.

Table 7-34. Clinical Presentation of Insect Stings	
Normal	Immediate, local, and transient erythema, also edema and tenderness at site
Large local	Pronounced erythema, edema, and tenderness extending over a large area, peaking at 48 hr after sting, and lasting up to 1 week
Systemic	Immediate, generalized IgE-mediated anaphylactic reaction
Other	Toxic, serum sickness, neuropathy, and rhabdomyolysis

Diagnosis

Clinical History—The most important diagnostic tool is the clinical history; it should include the nature and timing of stings, the temporal relation between sting and development of reaction as well as all associated symptoms and treatments.

Laboratory Tests

- **Skin tests:** Unlike testing for other allergies, the venom skin prick testing is not always done prior to intradermal testing, although it is reasonable in patients with a history of severe anaphylaxis who may be extremely sensitive to venom (1.0–100 μg/mL). Intradermal technique begins with concentrations between 0.001–0.01 μg/mL and proceeds by 10-fold increments until a postive result or a maximum concentration of 1 μg/mL is reached. At concentrations > 1 μg/mL venom can cause false positives due to irritant effects. Negative skin testing in the days or weeks after a sting reaction may be attributed to a refractory period of "anergy." For these patients, the skin test should be repeated after 4–6 weeks.
- **In vitro tests:** For patients whose skin tests are negative, but who have a history of a severe systemic reaction to an insect sting, serologic testing should be performed before concluding that venom immunotherapy (VIT) is unnecessary. Serologic testing is negative in up to 20% of skin test-positive patients, and skin tests are negative in up to 10% of persons found to have venom-specific IgE. If in vitro testing is negative and suspicion is still high, then repeat skin testing may be considered. A baseline serum tryptase level can be drawn to evaluate for a possible mast cell disorder.
- **Sting challenge tests:** Thirty percent to 60% risk of systemic reaction to subsequent stings in patients with a prior history of systemic reaction and with evidence of serum-specific IgE; therefore, a lack of reaction on sting challenge has limited clinical significance. It is also the **gold standard** for assessing efficacy of VIT in research studies.

Key Fact

Whole-body extracts (WBE) for flying hymenoptera contain little or no venom and are ineffective for diagnosis or treatment of venom allergy, unlike imported fire ant venom, in which WBE is routinely used for diagnosis and treatment of imported fire ant allergy

Key Fact

Degree of skin test sensitivity does not correlate with the degree of sting reaction.

Key Fact

Diagnosis of insect sting allergy must include a clear clinical history and positive-specific IgE to hymenoptera venom.

Flash Card Q27

If severe hypotension occurs after a hymenoptera sting, what key blood test should be done?

Flash Card Q28

False-positive (irritant) results to intradermal testing are more likely above what venom concentration?

Treatment and Prevention

Acute Reactions

- **Large local:** Symptomatic treatment with ice, NSAIDs, and H_1 antihistamines. Topical steroids or oral steroid bursts can be used for more bothersome local reactions**.**
- **Systemic:** Immediate administration of IM epinephrine with additional observation, systemic steroids, and antihistamines. With severe reactions, consider obtaining baseline tryptase to rile out mast cell disease.

Venom Immunotherapy—Treatment of choice for prevention of further systemic reactions to insect stings. It is indicated for patients with clinical history of anaphylaxis to insect sting and evidence of venom-specific IgE with skin test or serologic testing (Table 7-35).

VIT Safety—Fifty percent of patients have large local reactions and 5–15% have systemic symptoms during the build-up phase. The majority of reactions are mild, and < 5% require treatment with epinephrine.

VIT Efficacy—Seventy-five percent to 95% efficacy rate with maintenance dose of 100 μg of venom (for single antigen) or 300 μg of venom (for mixed vespids).

Table 7-35. Recommendations Based on Sting History and Results of Testing

Reaction to Previous Sting	Skin Test or in Vitro Test	Risk of Systemic Reaction (%)	Clinical Advice
No reaction		1–3	
Large local	Not indicated	5–10	No VIT
Urticaria/angioedema	Positive—Child < 16 yr Positive—Adult ≥ 16 yr	10 20	No VIT VIT
Anaphylaxis	Positive—Child Positive—Adult Negative	40 60	VIT VIT Repeat ST/in vitro test

Abbreviations: ST, sting test; VIT, venom immunotherapy.

Flash Card A27

Tryptase

Flash Card A28

Concentrations > 1 μg/mL, therefore not recommended for diagnostic purposes

Doses and Schedules—Standard maintenance dose is 100 μg of venom (for single antigen), but can be increased to a 200 μg maintenance dose if treatment fails. For mixed vespids, a standard maintenance dose of 300 μg is used. Schedules are highly variable, and risk of systemic reactions with rush regimens are not much higher than traditional regimens. Imported fire ant extract is composed of WBE and has a maintenance dose of 0.5 mL of a 1:10 to 1:200 wt/vol extract given monthly.

Discontinuation—General recommendation is to continue VIT for at least 3–5 years. Those patients with a very severe initial reaction, patients with systemic reactions to injection or sting while on therapy, or those with honeybee allergy might need to continue VIT indefinitely.

Key Fact

Patients who have received VIT for at least 5 years have ~10% chance of systemic reaction with each sting after stopping treatment. Patients should therefore continue to have epinephrine available even after discontinuing VIT (general population risk ~3%).

BITING INSECT ALLERGY

Kissing Bug (Triatoma)—Most common cause of systemic reactions to biting insects. Most often a nocturnal painless bite that causes an erythematous urticarial nodule or plaque. Relevant allergens are salivary gland proteins; small studies have shown benefit with immunotherapy with salivary gland extracts.

Key Fact

The allergens in biting insects are found in the saliva.

Mosquito (Culicidae)—Anaphylaxis to mosquito bites is rarely reported. Large local reactions are more common in children, may be accompanied by fever (skeeter syndrome), and are due to sensitization to mosquito salivary secretions; mosquito extracts are not approved for therapeutic use. Antihistamines can be used to relieve large local reactions from mosquito bites.

Horsefly and Deerfly (Tabanidae)—Large, blood-sucking flies that inflict painful bites; anaphylaxis following bites has rarely been reported.

Asian Lady Beetle (Harmonia Axyridis)—Bites in sensitized individuals have been associated with rhinitis, urticaria, and asthma symptoms; considered an indoor allergen and has cross-reactivity with cockroach on skin testing.

DRUG REACTIONS

The term *adverse drug reaction* is broadly used to cover a variety of reactions, including expected side effects (e.g., sedation with antihistamines), intolerances (AERD), allergic reactions (PCN-induced anaphylaxis), or pseudoallergic reactions (radiocontrast media). Only about 10% of adverse drug reactions are allergic.

Flash Card Q29

A 6-year-old boy camping in Texas awakens with diffuse hives, shortness of breath, and wheezing after a painless bug bite. What biting insect would you be most concerned about?

Physicians must know the Gell-Coombs classification **of drug reactions**:

- Type I: Immediate IgE-mediated hypersensitivity (e.g., PCN-induced anaphylaxis).
- Type II: Antibody-dependent cytotoxic reactions.
- Type III: Immune complex reactions (i.e., secondary to an antibiotic)
- Type IV: Cell-mediated or delayed hypersensitivity reactions (e.g., to purified protein derivative [PPD]).

Many drug reactions do not fit neatly into this pattern, and some actually involve multiple components of the Gell-Coombs classification. It is also valuable to know the subclassification of type IV reactions (Table 7-36).

Key Fact

The hapten hypothesis states that small drugs, which are not by themselves immunogenic (haptens), become immunogenic or allergenic after binding to a self-carrier protein. So, PCN is not allergenic until it is haptenized; however, one must remember that the hapten refers to PCN, not to the carrier protein.

Pathophysiology

The **prohapten hypothesis** recognizes that most drugs by themselves are not immunogenic until they are metabolized to a reactive metabolite. More recently described, the **pharmacologic interaction of drugs with immune receptors (p-i concept of drug allergy)** states that once a drug binds to a T-cell receptor (TCR) with sufficient affinity, especially in context of the TCR interacting with major histocompatibility complex (MHC), then it may become immunogenic. This latter concept may explain hypersensitivity reactions that can occur even when receiving a medication for the first time.

Table 7-36. Subclassification of Type IV Reactions

Reaction	Cytokines	Cells Involved	Clinical Manifestations
Type IVa	T_h1 (IFNγ)	Monocyte	Eczema
Type IVb	T_h2 (IL-4 and IL-5)	Eosinophil	Maculopapular or bullous
Type IVc	CTL (perforin and granzyme)	CD4 and CD8	Maculopapular or bullous to pustular and increased CD8 T lymphocytes in skin
Type IVd	T lymphocytes and IL-8	PMNs	Pustular

Abbreviations: IFNγ, interferon gamma; PMNs, polymorphonuclear neutrophils.

Flash Card A29

Triatoma

Risk Factors

Risk factors for drug allergy include:

- Higher dose
- IV route
- Large-molecular-weight agents
- Frequent or repetitive courses
- Duration of previous courses
- Female gender

Reactions are less frequently noted in infants and the elderly. HLA-DR3 is an MHC marker associated with increased reactions to insulin, gold, and penicillamine. HLA-B*5701 is strongly associated with reactions to abacavir and should be checked for prior to starting this drug. **Atopy is not a risk factor for most drug allergy, but is for reactions to latex or radiocontrast reactions**.

Common Drug Reactions

Penicillin

- Major determinants = Benzylpenicilloyl polylysine (Pre-Pen)
- Minor determinants = Penicillin G; penicilloate and penilloate if available
- Ampicillin and/or amoxicillin (3–25 mg/mL) if relevant to the patient's care

Most PCN allergy (80%) is related to the major determinant. Approximately 10% of patients report a history of PCN allergy; however, ~90% of these patients will tolerate PCN.

The predictive value of negative skin tests to PCN's major determinants (i.e., PrePen) and minor determinants is ~97% for ruling out anaphylactic potential. The predictive value of positive skin test to PCN is about 60%. Ten percent to 20% of PCN allergic patients only react to minor determinants, therefore they should be included in any PCN skin test to have adequate predictive value. After losing PCN allergy, resensitization is rare with oral medications, but more common with subsequent exposure to high-dose IV PCN (e.g., ~16%).

Key Fact

PCN-specific IgE antibodies decrease over time (~10% per year). Therefore, ~50% of patients who had immediate reactions to PCN will have a negative skin test after 5 years, and ~80% will be negative at 10 years.

Mnemonic

A**Z**treonam cross-reacts with ceftA**Z**idime.

Ampicillin/Amoxicillin (AMP/AMOX)

Patients may have IgE antibodies against side chains (R-group) rather than the core PCN determinants. Such patients are negative for the PCN skin test and able to tolerate other PCN compounds. Approximately **10% of patients have a delayed maculopapular rash with AMP/AMOX, which is not IgE-mediated.**

If patients have Epstein-Barr virus and get AMP/AMOX, ~80% will develop such a rash.

PCN has moderate cross-reactivity with carbapenems (e.g., imipenem or meropenem) based on skin testing, but clinically important cross-reactivity is much rarer (0–11%), and there are no reactions in patients with negative PCN skin tests to imipenem or meropenem. A monobactam, aztreonam, does not cross-react with other β-lactams, except for ceftazidime.

Cephalosporins

Cross-reactivity with PCN is rare (~2%), but some reactions can be fatal. The low cross-reactivity may be because some cephalosporin allergy can occur due to the R-group side chain and not the β-lactam ring. In general, first- and second-generation cephalosporins cause more allergic reactions than do third- and fourth-generations cephalosporins.

To determine if a patient with a history of PCN allergy may safely receive a cephalosporin, you should perform PCN skin testing. **Patient who are negative on the PCN skin test may safely receive cephalosporins**. Cephalosporin skin testing does not have sufficient negative predictive value to rule out anaphylactic risk in a patient with a convincing history. If PCN skin testing is unavailable, risk stratify the reaction and then either directly administer the drug, perform a graded challenge, or desensitize the patient

Sulfonamides

Prototype here is TMP/SMX. Contains SO_2NH_2 moiety. Most clinically important sulfonamide allergy is due to sulfa antibiotics. The typical reaction is a delayed maculopapular rash, likely T-lymphocyte-mediated. This is a **very common reaction to TMP/SMX in patients with HIV (40–70%).** Type I reactions, which are much rarer, are **due to the N4 sulfonamidoyl hapten acting as the major determinant**. There is little clinically relevant cross-reactivity between sulfonamide antibiotics and sulfonamide nonantibiotics (e.g., furosemide), because nonantibiotic sulfonamides lack the aromatic amine at the N4 position.

Pseudoallergic Reactions

These reactions include “anaphylactoid” reactions (a term that is falling out of favor), which indicates that they are non-IgE-mediated. This includes reactions to radiocontrast dye, ASA and/or NSAIDs, and opiate-induced urticaria. These reactions are typically mediated by basophil and mast cell activation. **Radiocontrast adverse reactions are increased in women, asthma and/or**

atopy, cardiovascular disease, and prior history of reaction. Reactions to radiocontrast media may be attenuated or prevented by the use of lower ionic contrast media (or nonionic), and premedication with steroids and antihistamines. (Note that true anaphylactic reactions are not preventable in this manner.)

Blistering Drug Reactions (SJS and TEN)

Stevens-Johnson syndrome (SJS) is defined by less than 10% epidermal detachment. In SJS, patients present with confluent, purpuric macules on face and/or trunk, mucosal involvement, and often systemic symptoms and fever. Other organ involvement may include ocular, liver, kidney, and lung. By contrast, toxic epidermal necrolysis (TEN) is more severe (>30% involvement).

Although steroids may be helpful in early SJS, they are contraindicated in TEN. High-risk agents include PCN and **sulfonamides**, **anticonvulsants**, NSAIDs, and allopurinol. Mechanisms of action include reactive metabolites causing Fas/FasL-mediated apoptosis of epidermal cells as well as cytotoxic T-lymphocyte activation and perforin release.

Treatment is supportive, although **intravenous immunoglobulin (IVIG) can be helpful** due to the presence of Fas-blocking antibodies.

Vancomycin

"Red man syndrome" is a rate-related infusion reaction characterized by flushing, erythema, and pruritus caused by direct activation of mast cells. May respond to decreased rate, premedication with antihistamine, and stopping concurrent narcotic medications. Vancomycin may also cause **linear IgA bullous dermatitis** with tense blisters. Less commonly, this reaction may occur due to captopril, furosemide, lithium, or TMP-SMX (Bactrim).

Mnemonic

Remember V**A**ncomycin may cause Ig**A** bullous dermatitis.

ACE I Reactions

Cough may occur in up to 20% of patients. ACE I-induced angioedema is usually observed in the first week after starting the medication; however, up to 30% of patients present months or even years afterward. Interferes with degradation of bradykinin and therefore rarely responds to antihistamines or steroids. May occur in up to 0.7% of the population and is increased in African Americans. Intermittent angioedema can develop for up to a month after the patient is off the medication, and around 2% of patients continue to have episodes when switched to another antihypertensive. The risk of recurrent angioedema with use of angiotensin receptor blockers (ARBs) is quite low.

Drug Rash with Eosinophilia and Systemic Symptoms (DRESS)

Common offending agents are anticonvulsants, sulfonamides, allopurinol, and minocycline. May be accompanied by fever, lymphadenopathy, and hepatitis. Facial edema is characteristic. This reaction occurs weeks after therapy, develops over days, and, unlike most drug reactions, symptoms may worsen even after drug discontinuation and last for weeks afterwards. Initial treatment is with systemic corticosteroids. IVIG has been reported to be helpful in patients who did not respond to systemic steroids.

Fixed Drug Eruption

Typically, fixed drug eruption appears as a purple-blue macule that occurs at same location upon each subsequent exposure to the drug but may take any dermatologic manifestation. Due classically to Bactrim or phenolphthalein.

Local Anesthetic Agents

Although lidocaine and other "-caine" medications are commonly blamed for "allergic reactions," these rarely cause any actual hypersensitivity; rather, these are associated most commonly with vagal reactions (e.g., a clinical clue is **bradycardia** as opposed to the usual tachycardia that would accompany anaphylaxis). Positive skin tests do not necessarily indicate actual allergy; therefore, graded challenges are more predictive of allergic reactions.

Perioperative Agents

Perioperative drug reactions are most commonly due to **quaternary ammonium muscle relaxants** such as succinylcholine. These agents do not require haptenation since they act as bivalent antigens able to directly bind adjacent IgE antibodies on cell surfaces. Skin testing has been shown to be helpful in such cases if positive; if negative, the predictive value is uncertain. Other IgE-mediated perioperative reactions can be due to latex, antibiotics, barbiturates, or propofol (contains sulfites).

Insulin

Up to 50% of patients receiving insulin may develop anti-insulin antibodies, but these patients often tolerate insulin without clinical reactions. HLA-DR3 confers increased risk. Allergenicity of insulin preparations = bovine > porcine > human.

Most patients can tolerate continuing insulin treatment despite local reactions; however, if needed, antihistamines or splitting doses may help. Patients receiving NPH insulin may have allergy to the protamine component rather than the insulin, and switching to nonprotamine-containing insulin can be helpful. Anaphylactic reactions are rare but may occur; insulin desensitization has been successfully performed.

Biologics

This wide class of drugs may be associated with a variety of adverse reactions. Many are prone to **cytokine release syndrome** with resultant fever, rash, bronchospasm, capillary leak syndrome, GI symptoms, meningoencephalopathy with abnormal liver functions tests (LFTs), uric acid, lactate dehydrogenase (LDH), IL-6, and TNFα. Inciting agents may include rituximab (anti-CD20) or muromonab (anti-CD3).

Sirolimus (rapamycin), which antagonizes IL-2 signaling in particular, is known to cause capillary leak syndrome with resultant hypotension. Interestingly, high-dose monoclonal antibody therapy with IL-2, used in the treatment of metastatic melanoma, is also known to cause this syndrome (cytokine storm).

Interferon therapy may cause:

- Flu-like symptoms
- Urticaria
- Dermatitis
- Vasculitis
- Idiopathic thrombocytopenic purpura (ITP)
- Autoimmunity
- Depression

TNF inhibitors may cause serum sickness reactions. Etanercept causes injection-site and local reactions. Epoetin may paradoxically provoke pure red cell aplasia. Alteplase and tissue plasminogen activator (tPA) may cause anaphylactoid reactions. Any of the anti-TNF drugs (e.g., infliximab, etanercept) may cause disseminated mycobacterial infection and/or TB, so checking a PPD prior to therapy and annually thereafter is recommended.

More recently, a novel mechanism of anaphylaxis to cetuximab has been described. Cetuximab is a monoclonal antibody against the epidermal growth factor receptor and is used in certain colorectal and head and neck cancers. The rate of hypersensitivity reactions to cetuximab varies by geographic region. Molecular analyses have found that this is predicted by the presence of IgE antibodies against naturally occurring galactose-α-1,3-galactose (i.e., oligosaccharides related to the ABO blood group) even prior to exposure to

cetuximab. These pre-existing IgE antibodies appear to put such patients at risk for anaphylaxis to cetuximab, which also contains galactose-α-1,3-galactose.

Drug-Induced Cytopenias

Immune-induced hemolytic anemia may classically be due to quinidine, methyldopa, or PCN. Immune-induced thrombocytopenia may classically be due to:

- Quinidine
- Propylthiouracil
- Gold
- Sulfonamides
- **Vancomycin**
- **Heparin (i.e., specific IgG to heparin-platelet factor 4 forms immune complexes)**

Pulmonary Drug Hypersensitivity

Pulmonary drug hypersensitivity presents with:

- Cough
- Migratory infiltrates
- Peripheral eosinophilia
- Pulmonary fibrosis

May be seen with bleomycin, methotrexate, and **nitrofurantoin**. Nitrofurantoin may also be associated with pleural effusion or interstitial pneumonitis and/or fibrosis. NSAIDs may be associated with eosinophilic pneumonia.

Serum Sickness

Serum sickness occurs most readily or efficiently in the setting of **slight antigen excess.** It may occur with PCN, sulfonamides, and phenytoin. It is secondary to formation of immune complexes. Classic findings include:

- Fever
- Erythema multiforme or urticaria
- Arthralgias
- Lymphadenopathy (typically appearing 1–3 weeks after starting treatment)

Symptoms may last weeks. Best treatment is to stop the offending medication, steroids, and antihistamines, which **cannot be desensitized**.

Drug-Induced Lupus

Drug-induced cutaneous lupus is associated with anti-Ro (SSA) antibodies. Typically, physicians will see photodistributed erythema or scaly, annular plaques weeks after starting a drug. Common agents are

- Hydrochlorothiazide (HCTZ)
- Calcium channel blockers
- ACE inhibitors
- Antifungals

This is not to be confused with **drug-induced lupus (systemic)** in which you see **antihistone antibodies** and which is commonly secondary to **procainamide**, hydralazine, phenytoin, and isoniazid.

Chemotherapeutics

Taxanes (e.g., paclitaxel, docetaxel) cause anaphylactoid reactions, which may be treated or prevented with steroids and antihistamines. Platinum compounds (e.g., cisplatin, carboplatin, and oxiplaten) can cause IgE-mediated, classic allergic reactions; thus, patients may be desensitized if necessary. Asparaginase may cause either anaphylactoid or anaphylactic reactions; however, some patients only react to asparaginase produced from *Escherichia coli*, so substituting formulations from other sources may avoid future reactions. Skin testing to asparaginase may help identify some at-risk patients.

DIAGNOSIS

Choosing an alternative, non-cross-reactive drug is not always feasible for certain clinical scenarios. Unfortunately, the **relevant epitopes are not known for most drugs**, making predictions based on testing challenging. Since benzylpenicilloyl polylysine (PrePen) is now available commercially again, skin testing can be performed for PCN. Using major and minor determinants, PCN skin testing has a negative predictive value for serious reactions approaching 100%. For medications other than PCN, unstandardized skin testing to liquid or solution forms of the drug may be pursued, and the known irritant concentrations are published for several antibiotics. Positive skin tests to a nonirritating concentration of the drug, especially in the context of a convincing history, suggests that drug-specific IgE may be present.

TREATMENT

If drug allergy is suspected based on history and/or testing, an equally efficacious non-cross-reacting alternative is indicated. If the suspected allergen is absolutely required have the patient undergoes desensitization. Negative skin tests are not sufficient proof that drug allergy (i.e., drug-specific IgE) is absent since the drug metabolite that is the relevant allergen may not be present in the testing reagent. Negative skin tests may be helpful in choosing the initial dose for desensitization or prior to a graded oral challenge, but only if the history suggests a mild reaction.

GRADED CHALLENGES

Drug provocation tests may be pursued in patients with a low pretest probability of being allergic to a given drug. These may consist of two or more doses given in incrementally increasing doses every 30–60 minutes. Generally, these are completed in five or fewer doses and, therefore, this procedure in itself is not designed to induce desensitization or tolerance. The starting doses used are generally higher than those used during drug desensitization.

DRUG DESENSITIZATION

Higher-risk situations may warrant drug desensitization rather than graded challenge. Risk factors would include a concerning clinical history (i.e., initial reaction was anaphylactic or urticarial), recent rather than remote reactions, and certain comorbid conditions (e.g., heart disease). Patients receive progressively increasing doses of the drug every 15–20 minutes for IV medications or every 20–30 minutes for oral dosing until a full therapeutic dose is tolerated. **Using this technique, tolerance may be induced in almost all drug-allergic patients.** Skin testing may help identify acceptable starting doses. Mild-to-moderate reactions do not necessarily preclude continuing desensitization but do warrant repeating the dose at which the reaction occurred or decreasing the dose after the reaction subsides. Using modern protocols, the success rate for tolerance induction is extremely high, and serious systemic reactions are rare. Acute desensitization is drug-specific. After desensitization, the drug needs to be continued at regular intervals, or resensitization will occur. Therefore, repeat desensitization is usually required for future courses of the drug should therapy be interrupted. **Contraindications to desensitization include exfoliative or blistering skin reactions and immune complex-mediated reactions**, which include:

- TEN/SJS
- DRESS
- Serum sickness

- Hepatitis
- Hemolytic anemia
- Nephritis

Urticaria

Urticaria lesions are **raised**, **pruritic**, **erythematous**, and **transient** (i.e., lasting < 24 hr at the same location), and are also known as "hives" (Figure 7-9). Pathologically, urticaria results from the activation of vasoactive mediators, including histamine, leukotriene, and others that lead to dilation and increased permeability of blood vessels and edema in the **superficial dermis.** Angioedema occurs when these mediators are released in the deep dermis and subcutaneous tissue.

Acute Urticaria

Acute urticaria is defined as the presence of urticaria for less than 6 weeks. It occurs in up to 20% of the population and is often associated with drug, food, or other allergy, or with infection.

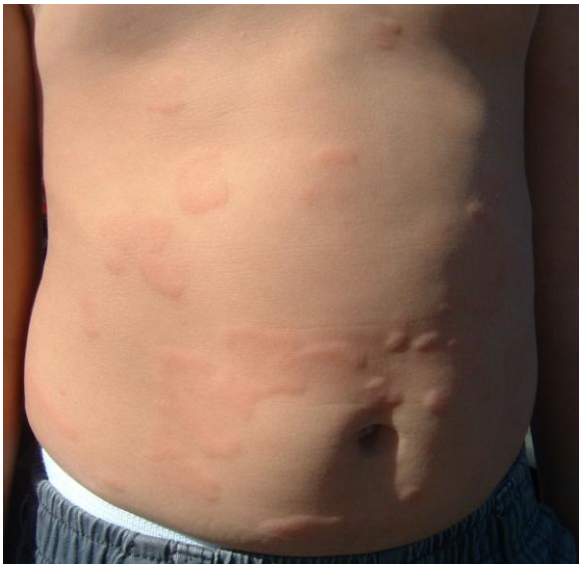


Figure 7-9. Urticaria.
(Reproduced, with permission, from Wikimedia Commons.)

Chronic Urticaria

Chronic urticaria is defined as the presence of urticaria for more than 6 weeks. It occurs in about 1% of the population and is self-limited in most patients; typically presenting in the third to fifth decades of life, with an average duration of 2–5 years. Around 40% have associated angioedema. Chronic urticaria is usually subdivided into two general categories:

- Chronic autoimmune urticaria (40–45%)
- Chronic idiopathic urticaria (55–60%)

Mnemonic

Imagine that urticaria is like raised maps on different parts of skin, **Itching Maps**:

Infections: Bacterial (including *Helicobacter pylori*), fungal, viral, and helminthic
Transfusion reactions
Chronic idiopathic urticaria
Hereditary diseases: Hereditary angioedema, familial cold urticaria, Muckle-Wells syndrome (amyloidosis with deafness and urticaria)
Inhalation or contact with allergens
NSAIDs and/or drug reactions
Gut: Foods or food additives

Mastocytosis (systemic): Urticaria pigmentosa and **M**alignancy
Autoimmune urticaria
Physical urticarias: Different types
Systemic lupus erythematosus and other collagen vascular diseases

Chronic Idiopathic Urticaria (CIU)

- Diagnosis of exclusion after ruling out acute urticaria and physical urticarias; identifiable etiologies may be found in less than 2% of cases.
- Pathogenesis of CIU still unclear. More recent studies point to histamine-releasing factors and defects in basophile signaling and/or function.
- Thyroid autoantibodies (antithyroglobulin or antimicrosomal) are present in more than 20% of patients with CIU. Their presence does not necessarily correlate with abnormal thyroid function. The role of these autoantibodies is unknown but may signify a predilection for autoimmunity.

Mediators

- Mast cell: Histamine, prostaglandin D, leukotrienes LTC4 and LTD4, and PAF
- Complement system: Anaphylatoxins C3a, C4a, and C5a; histamine
- Hageman factor-dependent pathway: Bradykinin
- Mononuclear cells: Histamine-releasing factors and chemokines

Etiologies

Refer to the mnemonic “Itching Maps” for a listing of etiologies.

Physical Urticaria

Table 7-37 summarizes the various types of physical urticarias.

Table 7-37. Types of Physical Urticarias

Physical Urticarias	Triggers	Tests
Dermatographism: very common, 2–5% of population	Stroking of skin results in linear wheals	Stroke skin with a blunt, smooth object (e.g., tongue depressor)
Cold urticaria[a]	Occurs on cold-exposed areas of body, systemic reaction can occur with immersion in lakes or pools	Ice cube test: Place ice cube on skin for 5 min, treat with cyproheptadine (C for cold)
Local heat urticaria	Occurs on warm-exposed areas of body	Apply water heated to 45°C in a test tube on skin for 5 min, treat with hydroxyzine (H for heat)
Cholinergic urticaria or Generalized Heat Urticaria: ~30% of all cases of physical urticaria, characterized by numerous small punctate wheals Autologous sweat sensitivity: Sweat may cause basophil degranulation in sensitive subjects who have positive skin test to own sweat	Elevation of body temperature (e.g., heat, exercise, emotional stress, spicy foods)	Various Testing Methods: Intradermal injection of methacholine Have patient ride stationary bike or run on treadmill until point of sweating, then continue for 15 min Nonexertional elevation of core body temperature by submerging arm of patient in 42ºC hot water bath until core temp increases ≥1°C
Solar urticaria	Exposure of skin to sunlight (triggering wavelengths vary)	Simple exposure of patient's skin to natural sunlight Phototesting: Skin is exposed to UVA and UVB of varying wavelengths using a monochromatic light source
Aquagenic urticaria	Small wheals result from contact with water, independent of temperature; salinity of water important in some cases	Apply 35°C water compress to upper body for 30 min
Delayed-pressure Urticaria/angioedema	Symptoms can develop 30 min to 12 hr after pressure has been applied to skin	Sling attached to a 10–15 lb weight is placed over arm or shoulder for 15 min. Patient reports response over next 4–24 hr

[a] Other cold urticaria syndromes include cold-dependent immunoglobulin diseases (cryoglobulinemia and cold agglutinin disease), delayed cold urticaria, localized cold urticaria, and localized cold reflex urticaria.

Key Fact

Cold urticaria syndromes that have a negative ice cube test: Cold-induced cholinergic urticaria, systemic cold urticaria, and cold-dependent dermographism.

Key Fact

Cholinergic urticaria can also present with hypotension and look clinically similar to exercise-induced anaphylaxis (EIA). The key difference is that patients with EIA will not react with passive heating.

Laboratory Tests

Blood Tests—Blood tests are usually not helpful for determining the cause of acute urticaria. For chronic urticaria without identified etiology, limited or targeted testing may be done for patients who have an otherwise unremarkable history and physical exam. Skin biopsy only if history or physical exam is suggestive of vasculitic process.

Treatment

Goal of therapy is to achieve a level of symptom control that is acceptable to patient while minimizing side effects:

Acute Urticaria

- Elimination of trigger factors when identified
- Second-generation H_1-antihistamines and first-generation H_1-antihistamine at bedtime

Chronic Urticaria: Antihistamine monotherapy first line

- Second-generation H_1-antihistamines
- H_2 receptor antagonist
- Doxepin (tricyclic antidepressant: Both H_1 and H_2 receptor antagonists)
- Leukotriene antagonists: Not great evidence but inexpensive, safe
- Refractory cases may need corticosteroid therapy

Examples of third-line agents used when high-dose antihistamines are ineffective:

- Cyclosporine
- Tacrolimus
- Methotrexate
- Sulfasalazine
- Dapsone
- Hydroxychloroquine
- Omalizumab
- Colchicine
- IVIG

Urticarial Vasculitis and Hypocomplementemic Urticarial Vasculitis Syndrome

Urticarial Vasculitis (UV)—Lesions are less pruritic and described as painful or burning, individual hives last >24 hours, leave residual purpura or hyperpigmentation. Elevated erythrocyte sedimentation rate (ESR), arthralgias,

myalgias, fever, leukocytosis are common associated abnormalities. Histopathology shows leukocytoclasis and vessel wall damage.

Hypocomplementemic Urticarial Vasculitis Syndrome (HUVS)—Urticaria with hypocomplementemia, includes systemic findings such as arthralgias/arthritis, obstructive lung disease, glomerulonephritis, uveitis, angioedema, and recurrent abdominal pain. Laboratory findings include low C3, C4, and C1q, anti-C1q antibodies, and elevated ESR.

CONTACT HYPERSENSITIVITY

Pathogenesis—Contact hypersensitivity (CHS) is a type IV hypersensitivity that is mediated by CD4 and CD8 lymphocytes stimulated by epidermal antigen-presenting cells (Langerhans cells). Once sensitized, dermatitis is **delayed** 12–48 hours upon re-exposure to the triggering antigen.

Diagnosis—Made by patch testing: Place patches on day 1, read and remove on day 3, read again on day 5 (Figure 7-10). **Defining the clinical relevance of positive patch test reactions is of central importance** (Table 7-38 and Figure 7-11).CHS can be divided into **four categories** based on etiology. Differentiating allergic versus irritant reactions can be difficult as both ACD and ICD are indistinguishable clinically and histologically. **Lymphocytic infiltration** and **spongiosis** are the predominant histologic features of contact dermatitis.

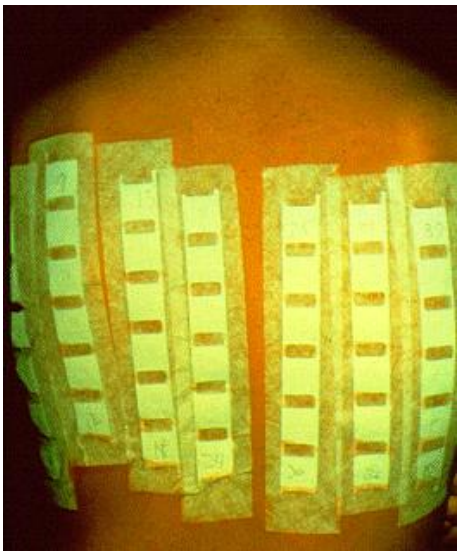


Figure 7-10. Patch test applied in Finn Chambers to a patient's back.
(Reproduced, with permission, from The National Institute for Occupational Safety and Health [NIOSH] of the CDC.)

Table 7-38. Scoring System for Patch Test Reactions

Score	Interpretation	Final Read
-	No evidence of skin changes	Negative reaction
? or +/-	Limited to faint macular erythema; use caution when interpreting, especially with less common allergens	Doubtful reaction
+	Erythema and edema that is palpable with slight infiltration that occupies >50% of patch test site	Weak reaction
++	Microvesicles and erythema that occupy at least 50% of patch test site	Strong reaction
+++	Confluent vesicles or bullae, ulcerative	Extreme reaction
IR	Mild: glazed appearance Moderate: follicular (pustular in atopics) Extreme: can be ulcerative	Irritant reactions

Allergic Contact Dermatitis (ACD)—Delayed type of induced sensitivity (allergy), characterized by an antigen-specific T–lymphocyte-mediated hypersensitivity reaction.

- Location: Hands 27%, generalized 18%, face 16%, eyelids 5%, trunk 5%, and feet 3%
- "Crescendo" phenomenon, where positive reactions to patch test become more marked between first and second readings
- Characterized by severe pruritus, reactions usually take 12–48 hours to develop

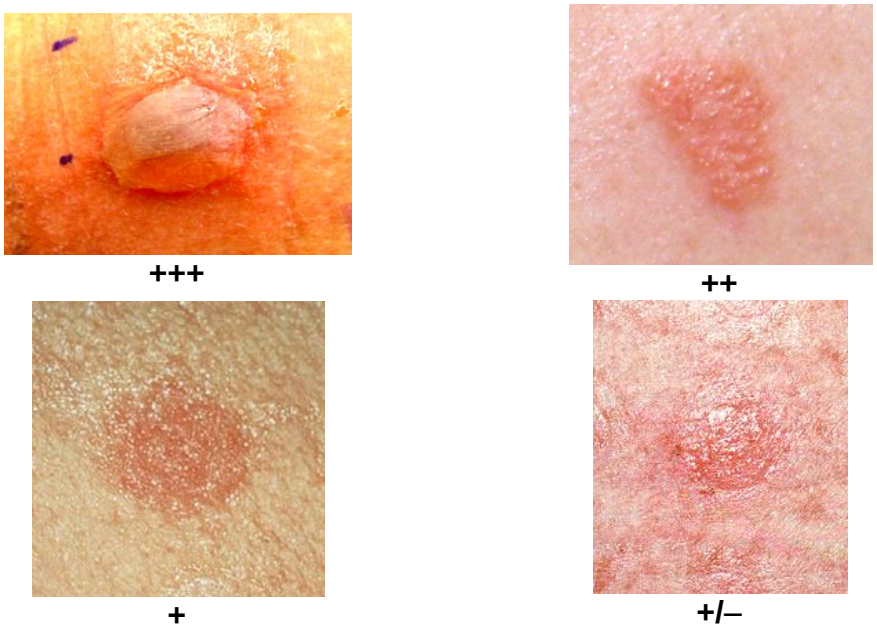


Figure 7-11. Positive patch test reactions. (+++, extreme positive reaction; ++, strong positive reaction; +, weak positive reaction; +/–, equivocal/uncertain reaction.)
(Reproduced, with permission, from Luz Fonacier, MD, State University of New York at Stony Brook)

Irritant Contact Dermatitis (ICD)—Most prevalent form (~80%), irritants cause inflammation of the skin induced by chemicals, oxidants, or alkali, surfactants, and solvents that directly damage the skin. No previous sensitization is required; it reflects nonspecific toxicity of the antigen.

- Characterized by stinging and less pruritic than ACD, reaction almost immediate. "Decrescendo" is typical as reactions tend to decrease in severity between readings.

Photocontact Dermatitis—A photoallergic or phototoxic chemical requires light-induced excitation in the ultraviolet spectrum to cause dermatitis.

- Photoallergic (requires prior sensitization): *para*-Aminobenzoic acid (PABA), chlorhexidine, thiourea, NSAIDs, thiazide diuretics, dapsone, and sulfonylureas
- Phototoxic (no prior sensitization): Psoralens, furocoumarins, tar, lime, celery, parsnip, tetracyclines, amiodarone, diuretics, quinine, and NSAIDs

Contact Urticaria—Can be either immunologic or nonimmunologic.

- IgE-dependent: Dairy products, seafood, various fruits, grains, topical antibiotics, metals, preservatives, and plants
- IgE-independent: Fragrances, arthropods, jellyfish, and coral

Common Allergens for Contact Dermatitis

Ten most common contact allergens in the US:

- Nickel sulfate
- Neomycin
- Myroxylon pereirae (balsam of Peru)
- Fragrance mix
- Thimerosal
- Sodium gold thiosulfate
- Quaternium-15
- Formaldehyde
- Bacitracin
- Cobalt chloride

Metals

- Potassium dichromate: Stainless steel, chrome plating other metals, and tanned leather
- **C**hromates: Textile, leather tanners, and construction workers using wet **c**ement
- Cobalt dichloride: (Uncommon) dental implants, artificial joints, and engines or rockets

- Nickel: Nonoccupational exposure, jewelry; **dimethylglyoxime test of nickel-containing material (pink = positive).**

Plants—*Toxicodendron* dermatitis (aka poison ivy, poison oak, and poison sumac) is the most common form of ACD (Figure 7-12). It is caused by urushiol, an oleoresin that is found in the sap and oozes readily from any crushed part of the plant.

Sensitivity to *Toxicodendron* usually develops after several encounters with the plants, which, in some cases, may occur after many years of exposure. Studies suggest that ~85% of the population will develop a clinical reaction when exposed; however, 10–15% of the population is believed to be highly susceptible to poison ivy and poison oak. These people develop systemic symptoms, which include rashes with swelling of the face, arms, and genitalia. There is also cross-reactivity with **mango peels**.

Although the poison ivy group of plants (Anacardiaceae) causes most cases of plant dermatitis, other plants that are common sensitizers are listed in Table 7-39.

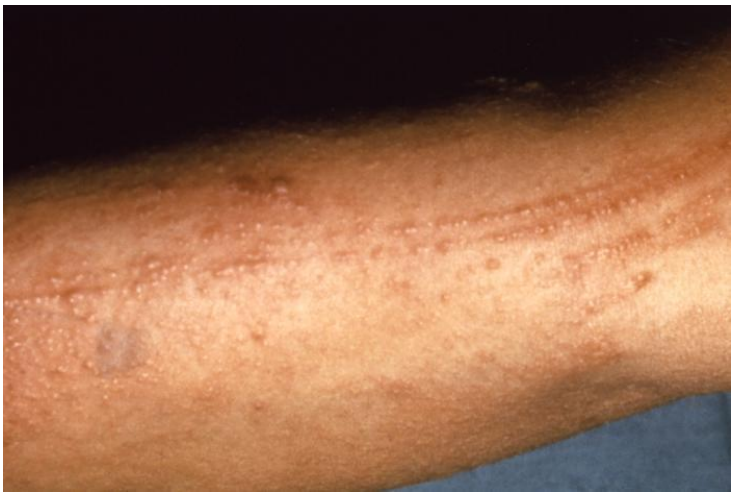


Figure 7-12. Linear papulovesicular presentation of poison oak contact dermatitis.
(Reproduced, with permission, from the CDC's Public Health Image Library)

Table 7-39. Common Non-*Rhus* Plant Contactants

Family	Common Names	Antigen
Ambrosia	Giant and dwarf ragweed	[a]Sesquiterpene lactones
Compositae	Chrysanthemums and daisies	Sesquiterpene
Liliaceae	Tulips, hyacinth, asparagus, and garlic	[a]Tuliposide
Amaryllidaceae	Daffodil and narcissus	Unknown
Primrose	Primula	[b]Primin
Umbelliferae	Carrots, celery, and parsnips	Unknown
Urticaceae	Nettles	Unknown
Rutaceae	Oranges, lemons, and grapefruits	Unknown

[a] Sesquiterpene lactones and tuliposides are large, diverse groups of chemicals found in several plant families that cause ACD in florists and bulb growers. Test the actual chrysanthemum (petal, leaf, and stem) as no single sesquiterpene is sufficient to screen for sensitivity to chrysanthemums.
[b] Primin is the most common ACD in Europe.

The sensitizing substances in most plants are present mainly in the **oleoresin** fraction; in some plants, the allergens are water-soluble glucosides. Most plants must be crushed to release the antigenic chemicals.

In the US, *Alstroemeria*, also called Peruvian lily, is the most frequent cause of hand eczema in flower workers. This classic dermatitis is an intensely pruritic eruption that affects the first three fingers and exposed areas of dorsal hands, forearms, the V-region of the neck, and the face.

Cosmetics—Cosmetics and personal hygiene products contain a variety of potential allergens. Typical contact allergens include:

- Fragrances
- Preservatives
- Formulation excipients
- Glues
- Sunblocks

Fragrances—Fragrances are among the most common causes of CD in the US. Balsam of Peru has a spicy scent and is used in the manufacture of perfumes, but it is also used as a flavoring agent. Balsam of Peru has wide cross-reactivity, but most prominent are cinnamon and vanillin.

Preservatives—Preservatives tend to be grouped into two broad categories: formaldehyde donors (i.e., products that emit formaldehyde) and nonformaldehyde donors (Table 7-40). There is a high prevalence of positive patch tests (approximately 8%) to formaldehyde, and most fabrics, especially cotton and rayon, contain formaldehyde.

Table 7-40. Preservative Agents Classified According to Formaldehyde Releasing

Formaldehyde Releasers	Nonformaldehyde Donors
Diazolinidinyl urea	[a]Parabens
Imidazolindinyl urea	Methylisothiazolinone
[b]**Quaternium-15**	Phenoxyethanol
DMDM hydantoin	PCMX and/or PCMC
Bromonitropropane	Benzalkonium chloride
	Thimerosal

[a]Parabens are the most commonly used preservative in cosmetics; however they are an uncommon cause of ACD.
[b]Quaternium-15 is the preservative that most frequently causes ACD in the US.
Abbreviations: DMDM, dimethylol dimethyl hydantoin; PCMC, parachlorometacresol; PCMX, parachlorometaxylenol.

Formulation excipients—Defined as inert substances that serve to solubilize, emulsify, sequester, thicken foam, lubricate, or color the active component in a product (Table 7-41).

Hair Products—Second only to skin care products as the most common cause of cosmetic allergy. Important causes of CD include:

- Cocamidopropyl betaine: Shampoos, eye and/or facial cleaners, and bath products
- **Paraphenylenediamine**: Most common cause of contact hypersensitivity (CHS) in hair dressers
- Glycerol thioglycolate: Permanent wave solution

Acrylics—In nails can present locally at the distal digit or ectopically on the eyelids and face. Patch testing to a variety of acrylates and nail polish resins may be necessary to delineate the causative agent. **Ethylacrylate** has been demonstrated to detect a higher number of acrylate-allergic patients. Formaldehyde-based nail resins should also be suspected and tested when ectopic facial dermatitis is present.

Table 7-41. Excipient Chemicals that Cause Allergic Contact Dermatitis

Antioxidants (sulfites)	Propylene glycol	Benzylalkonium chloride
EDTA	Ethylenediamine	Cetrimide
Butylene glycol	Vegetable gums	Lanolin
Polyethylene glycol	Chlorocresol	Chloramine-T
Triethanolamine	Thimerosal	Butyl alcohol

Abbreviations: EDTA, ethylenediamine tetraacetic acid.

Sunblocks or Sunscreens—Common causes of photoallergic ACD. These are frequently present in cosmetics such as moisturizers, "facial" creams, lip and hair preparations, and foundations. "Chemical-free" sunblocks use physical-blocking agents (micronized titanium dioxide and zinc oxide) and are rare sensitizers. Photoallergic CD is diagnosed with photopatch testing. This combines patch testing with ultraviolet type A (UVA) exposure (320–400 nm of ultraviolet light). Pure photoallergens only cause a skin hypersensitivity reaction with both UVA light and chemical exposure.

Topical Corticosteroids—Can cause ACD in up to 5% of patients with suspected CD. Risk factors include treatment of refractory eczema, leg ulcers, and stasis dermatitis. The patient usually notes a failure to improve or experiences a flare-up of the underlying dermatitis being treated with the steroid.

- Patch test readings should also be done 7 days after application because of the immunosuppressant nature of the test reagent itself (false negatives are common).
- The most commonly used screening agents in patch testing for topical corticosteroid allergy are budesonide and tixocortol pivalate 1%.
- There are four major chemical classes of sensitizing corticosteroids (designated A–D):
    - Group A: Hydrocortisone type
    - Group B: Triamcinolone type
    - Group C: Betamethasone type
    - Group D: Hydrocortisone-17-butyrate type
- Patients do not usually react to all four classes, so there are safe corticosteroids. It is important to distinguish among the four classes.

Resins

- **Epoxy**, when cured, is nonsensitizing; ACD occurs with uncured resin (90%) or to hardener.
- **Colophony** is made from pine trees and appears in cosmetics, topical medications, and industrial products. Different pine trees mean different forms of colophony (testing is difficult), and Balsam of Peru may cross-react.
- **Ethylenediamine dihydrochloride** appears in topical creams, **aminophylline**, and generic nystatin. EDTA does not appear to cross-react with ethylenediamine. If sensitive, avoid nystatin, aminophylline, and piperazine-based antihistamines (e.g., meclizine and cyclizine).
- **Paraphenylenediamine** is a derivative of benzene and common, epidemic with "henna" tattoos. Patients are not allergic to the henna, but to the contaminating paraphenylenediamine.
- **Topical antibiotics** such as bacitracin (or Neomycin) are common and iatrogenic with risk of anaphylaxis that can be delayed. Neomycin is the most commonly used antibiotic and is an aminoglycoside that cross-reacts with gentamicin, kanamycin, streptomycin, and tobramycin. There is cross-reactivity with bacitracin. If patient is sensitive, the physician should avoid prescribing all of these antibiotics.

Allergic Contact Cheilitis (ACC)—A common form of ACD, because the epithelium of the lips is similar to that of the skin. Common ACC contactants include:

- Dental devices
- Lipsticks
- Lip balms
- Nail polish
- Cigarette paper
- Various essential oils

Surgical Implant Dermatitis—The four criteria for diagnosis of a cutaneous implant-induced reaction are:

- Dermatitis (localized or generalized), appearing after implant surgery
- Persistent dermatitis that is resistant to appropriate therapies
- A positive patch test result to a metallic component of the implant or to acrylic glues
- Resolution of the dermatitis after removal of the implant

Systemic contact dermatitis (SCD) is a generalized ACD rash from systemic administration of a drug, chemical, or food to which the patient previously experienced ACD. Patients allergic to topical antihistamines (e.g., Benadryl cream) may develop systemic CD after systemic administration of diphenhydramine. This has been termed the "baboon syndrome" because of the indurated erythema that may be observed in the groin area of afflicted patients. Reactions have also occurred after systemic and intra-articular use of corticosteroids to which a patient had been topically sensitized.

Mnemonic

The seven **T**s of passive immunity:

Transferred protection from human or animal
Temporary

Examples include:
Transfusion (blood)
Transplacental passage of IgG
Tetanus hyperimmune globulin (IG) (also rabies, hepatitis B, and varicella Ig)
Anti**T**oxins (botulism and diphtheria); cause serum sickness
Monoclonal an**T**ibodies (Synagis) for RSV prevention.

VACCINE PRINCIPLES AND REACTIONS

PRINCIPLES OF VACCINATION

Passive immunity is the transfer of preformed antibodies produced by one human or animal to another that provides temporary protection.

Active (or adaptive) immunity is the stimulation of the immune system by an antigen to produce humoral and cellular immunity. It occurs from natural infection or vaccination and is more permanent.

Classification of Vaccines

Live Attenuated—This is a weakened form of "wild" virus or bacterium that replicates. It is similar to natural infection and usually protective after one dose; IVIG and Ig interferes.

Inactivated—These vaccines are produced by growing the virus or bacterium in culture media and then inactivating it with hear and chemicals (formalin). Inactivated vaccines cannot replicate, require multiple (booster) doses, and are less affected by IVIG or intravenous gamma globulin (IgG). The immune response is mostly humoral, and antibodies decline with time. The following are examples of inactivated vaccines:

- Whole cell: Polio (IPV), hepatitis A, and rabies
- Fractional: Subunit (hepatitis B, trivalent inactivated influenza virus vaccine (TIV), pertussis, and human papillomavirus [HPV]) or toxoid (tetanus and diphtheria)
- Polysaccharide:
  - Typically T–lymphocyte-independent (i.e., stimulates B lymphocytes without help from T lymphocytes)
  - IgM is greater than IgG response
  - Does not work well in patients younger than 2 years of age
  - No booster response
  - Pneumococcal (23-valent and Pneumovax)
  - Meningococcal (Menomune)
- Conjugated vaccine: Immunogenicity improved with conjugation of polysaccharide to protein and is T–lymphocyte-dependent; Hib, pneumococcal (13 serotype, Prevnar), and meningococcal (Menactra)

Mnemonic

The **LMNOP** and **RSV** of live vaccines.

Live vaccines include:
MMR
Nasal flu
Oral **P**olio

Rotavirus
Smallpox (Vaccinia),
Shingles (Zoster)
Varicella

Key Fact

The live, attenuated influenza vaccine is contraindicated in immunocompromised patients, patient with a history of Guillain-Barré syndrome, and in patients with a history of recurrent wheezing or severe asthma.

General Recommendations

Timing and Spacing—All vaccines can be administered at the same visit as for all other vaccines; however, live vaccines must be separated by 28 days if not given same day.

Minimum Intervals—Never give earlier than minimum age or interval; if late, never restart series for missed dose.

Vaccine-Antibody Interactions

- Passively acquired antibody (IVIG and RBC transfusion) can interfere with the response to live vaccines for more than 3 months. Wait before giving live vaccine (i.e., for tetanus IG, wait 3 months; for IVIG replacement therapy (400 mg/kg), wait 8 months; and for packed RBCs, wait 6 months); if live vaccine is given too soon, then repeat the dose.
- If live vaccine given first, wait more than 2 weeks to give antibody-containing product.

- Not a problem with monoclonal antibodies (i.e., palivizumab) and live vaccine.

ADVERSE REACTIONS

- **Local reactions** are common adverse reactions, which occur with 80% of doses. They are more common with inactivated vaccines.
- **Arthus reactions** are severe, local reactions due to high antibody titers. They are most common after the fourth or fifth dose of diphtheria, tetanus, and pertussis (DTaP) vaccine or with frequent boosters. They are type III hypersensitivity reactions with immune complex deposition.
- **Systemic reactions** are common adverse reactions that include fever, malaise, and headache. They can later onset with live vaccines (e.g., 7–21 days).
- **Allergic (IgE)** are rare (i.e., < 1 one in 500,000) due to vaccine component used more commonly than vaccine antigen itself.
    - **Gelatin**: Used as stabilizer; measles, mumps and rubella (MMR), varicella-zoster, rabies, and yellow fever all contain gelatin; MMR is most commonly reported.
    - **Egg**: Influenza, yellow fever prepared with embryonated chicken eggs; MMR is not contraindicated in egg allergy (grown in chick fibroblasts).
    - **Latex**: In patients with anaphylaxis to latex, do not administer vaccine supplied in vials containing natural rubber unless benefits greater than risks; very small risk in reality.
    - **Yeast**: Hepatitis B vaccine contains yeast, which causes rare problems.
    - **Specific vaccines**: Japanese encephalitis virus (JEV) vaccine causes delayed urticaria and angioedema.

Mnemonic

Vaccines containing egg protein: **E**gg **i**n **Y**our **F**ace

Egg
Influenza
Yellow **F**ever

Influenza Vaccine and Egg-Allergic Patients

- No need to divide and administer vaccine by a two-step approach
- No need to skin test with vaccine, unless patient had reaction to vaccine itself
- Allergy with hives only: give influenza vaccine at primary care provider's office and observe for 30 minutes
- Allergy reaction more severe than only hives: Give influenza vaccine at allergist's office and observe for 30 minutes

Non-IgE-Mediated Reactions

Thimerosal: Preservative; there are no data to support the contention that thimerosal causes risk in vaccines, precautionary removal from many vaccines; delayed local reactions to thimerosal are not a contraindication to vaccination.

- **Neomycin:** Delayed-type reactions to neomycin are not a contraindication to vaccination; only neomycin causes anaphylaxis.
- **Unique side effects of specific vaccines:**
    - MMR: Transient rash (5%), thrombocytopenia, and late fever (i.e., within 5–12 days)
    - Tetanus: Brachial neuritis and Arthus reaction
    - Pertussis: Febrile seizures, inconsolable crying, and hypotonic hyporesponsive event
    - Varicella: Varicella (chickenpox)-like rash
    - Yellow fever: Encephalitis.
    - Smallpox (*Vaccinia*): Myopericarditis, eczema vaccinatum

Invalid Contraindications

- Mild illness
- Antibiotics
- Lactation
- Preterm birth
- Immunosuppressed contact (exception: Smallpox)
- Family history of adverse events
- Multiple vaccines
- Disease exposure
- TB skin test (exception: MMR should be given same day or space 4 weeks apart)

Contraindications

- Severe allergic reaction (anaphylaxis) to prior dose
- Encephalopathy less than 7 days after pertussis-containing vaccine
- History of Guillain-Barré syndrome: Flu, meningococcal infection
- **Live vaccine contraindications:** Pregnancy and immunosuppression

Precaution—Might increase chance of adverse reaction; includes prolonged crying or high fever (>105°F) or seizure within 3 days after pertussis vaccination; and moderate or severe illness.

Vaccine Adverse Event Reporting System (VAERS)—This is a passive, national reporting system, to which anyone can send a report. It was created to help detect rare or new adverse events and patient risk factors for events.

Flash Card Q30

A 16-month-old boy is scheduled to receive both the MMR and varicella vaccines during a health maintenance visit but parents only allow him to receive MMR. What is the minimum period of time that must elapse before the varicella vaccine may be administered to this patient?

BRONCHIOLITIS

General Considerations

In infants, bronchiolitis is a lower respiratory tract infection characterized by wheezing and airway obstruction in children <2 years of age that primarily affects the small airways (bronchioles). Respiratory syncytial virus (RSV) is the most common cause of bronchiolitis.

Etiology

Pediatric bronchiolitis:

- RSV
- Rhinovirus
- Parainfluenza virus
- Human metapneumovirus
- Influenza

Adult bronchiolitis:

- Inhalation injury
- Infection (mycoplasma pneumonia)
- Drug reaction
- Hypersensitivity pneumonitis
- Connective tissue disease

Epidemiology

Infants 3–6 months of age are the most prone to symptoms during peak RSV season, from October through May. Approximately 50–65% infants have been infected with RSV during their first year, and nearly 100% infants have been infected by 2 years of age.

Pathophysiology

Necrosis of respiratory epithelium occurs along with ciliary disruption and peribronchiolar lymphocytic infiltration. Obstruction of the small airways is caused by excessive mucus and edema.

Signs and Symptoms

With RSV bronchiolitis, there is a wide spectrum of illness including:

- Cough
- Wheezing
- Fever
- Rhinorrhea

Flash Card A30

Twenty-eight days

- Nasal congestion
- Dehydration

Symptoms typically peak at 3–5 days and resolve in 2 weeks, although wheezing can persist. Some children with severe illness experience hypoxia, tachypnea, and apnea.

Infants at higher risk for severe disease include those with:

- Congenital heart disease
- Immunodeficiency
- Bronchopulmonary dysplasia
- Prematurity

Diagnosis

Bronchiolitis is a clinical diagnosis. Virologic tests and radiographs can be used to support the diagnosis, but they rarely alter management or outcomes and are not routinely required.

Treatment and Prevention

Bronchiolitis is usually a self-limited, mild disease, and treatment is primarily supportive. Patients in moderate-to-severe respiratory distress may require hospitalization. In the inpatient setting a trial of albuterol is appropriate with assessment for bronchodilator response. Ribavirin can be considered for severely ill infants or those at risk for complications. Palivizumab is a monoclonal antibody directed against an epitope of the RSV virus and can be given monthly to high-risk infants younger than 2 years of age as a preventive measure (bronchopulmonary dysplasia, cyanotic congenital heart disease, and prematurity).

Association with Asthma

Recurrent wheezing is a common problem in pediatrics, with around 40% of children developing a wheeze in their first year of life. RSV bronchiolitis is thought to be an independent risk factor for the development of frequent wheezing, occurring in patients until the age of 10 years. It is not known why this occurs in some children but not others; however, there may be a possible role for genetic disposition, gender, lung size, tobacco exposure, or immune response. Differentiating bronchiolitis from other causes of wheezing, including asthma, is often difficult. The Asthma Predictive Index is a helpful tool that allows

physicians to discern infants who wheeze and eventually develop asthma from those with recurrent wheezing whose symptoms are transient.

CROUP

Laryngotracheitis (croup) is a respiratory illness that results in inflammation of the larynx and subglottic airway. It is the most common etiology for stridor in febrile children and is characterized by a barking cough.

Definitions

- **Laryngitis**: Inflammation limited to the larynx; manifests itself as hoarseness; usually occurs in older children and adults; and is frequently caused by viral infections.
- **Laryngotracheobronchitis**: Inflammation extending into the lower airways; symptoms include wheezing, rales, air trapping, and tachypnea.
- **Bacterial tracheitis**: Bacterial infection of the subglottic trachea; thick and purulent exudates; symptoms of upper-airway obstruction; and may occur as a complication of viral respiratory infections or as a primary bacterial infection.
- **Spasmodic croup**: Sudden onset of inspiratory stridor at night often associated with mild URI. Symptoms last several hours with sudden cessation and condition is recurrent. There is evidence that spasmodic croup may be more common in atopic children and is often referred to as "allergic croup."

Etiology

Key Fact

Influenza A is associated with more severe disease and seen in those with respiratory compromise.

Croup is most often caused by viruses with bacterial infections occurring secondarily. Approximately 80% of croup is secondary to parainfluenza virus infection (type one most common).

- Parainfluenza virus
- RSV
- Adenovirus
- Rhinovirus
- Influenza virus
- Measles

The most common secondary bacterial pathogens include *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Streptococcus pneumoniae*

Epidemiology

Croup is most common between 6–36 months of age. Most common in late fall and early winter.

Pathogenesis

Viral infection leads to inflammation and edema of the subglottic larynx and trachea, especially near the cricoid cartilage (i.e., narrowest part of the pediatric airway). Narrowing results in the turbulent airflow, stridor, and chest retractions. Decreased mobility of the vocal cords due to edema leads to the associated hoarseness.

Key Fact

In cases of severe respiratory distress, a tracheal tube that is 0.5–1 mm smaller than would typically be used may be required secondary to laryngeal edema.

Clinical Presentation

The onset of symptoms of croup is usually gradual, beginning with nasal irritation, congestion, and coryza. Symptoms generally progress over 12–48 hours to include fever, hoarseness, barking cough, and stridor. Symptoms can persist for 3–7 days. Identifying patients with severe respiratory distress and/or impending respiratory failure is paramount.

Key Fact

Infants and young children will frequently present with a barking cough, whereas older children and adults will present with hoarseness.

Symptoms of significant upper-airway obstruction include:

- Stridor at rest
- Retractions
- Diminished breath sounds
- Hypoxia and cyanosis

Imaging—Posteroanterior chest radiograph reveals subglottic narrowing; commonly called the "steeple sign."

Laboratory Findings—CBC is usually nonspecific. WBC and differential may reveal a viral pattern with leukocytosis and lymphocytosis.

When evaluating a patient with signs of croup it is important to assess severity and exclude any other causes of upper-airway obstruction, which can include:

- Epiglottitis
- Foreign body aspiration
- Peritonsillar/retropharyngeal abscesses
- Upper-airway injury
- Congenital anomalies of upper airway

Diagnosis

Clinical diagnosis of croup is based on symptoms, specifically barking cough and stridor, and is more common during an epidemic. **Radiographs and laboratory tests are not necessary to make the diagnosis.** Viral culture of secretions from the nasopharynx or throat can be obtained if etiologic diagnosis is desired, antiviral therapy is indicated, or in patients being admitted, whether isolation is required.

Treatment

Providing a sense of comfort and reassurance to both the patient and parents is paramount. A single dose of PO or IM dexamethasone 0.6 mg/kg (maximum dose 10 mg) has been shown to be the most efficacious treatment of croup. Unlike steroids, nebulized epinephrine provides rapid clinical improvement. Exposure to cold air and humidified air mist therapy are often utilized both at home and in the emergency department setting with mixed results. No studies to date have supported its efficacy in reducing symptoms.

8 Immunologic Disorders

HEREDITARY AND ACQUIRED ANGIOEDEMA

Hereditary angioedema (HAE) is a disease characterized by recurrent episodes of angioedema, without urticaria or pruritus, which most often affect the skin or mucosal tissues of the upper respiratory and gastrointestinal tracts.

Acquired angioedema (AAE) is clinically quite similar to HAE but most commonly develops in patients over the age of 40 years, some of whom have associated lymphoproliferative disorders. HAE usually presents in younger patients who are otherwise healthy.

Hereditary Angioedema (HAE)

- Autosomal dominant inheritance
- C1 inhibitor (C1-INH) gene on chromosome 11
- C1esterase inhibitor mutations cause decreased protein (type I) or dysfunctional protein (type II)
- Bradykinin is the major mediator of swelling, and the lack of C1-INH leads to bradykinin overproduction (See Chapter 1,).

Presentation

- Swelling episodes may be spontaneous or precipitated by trauma or stress.
- Swelling usually lasts 2–4 days.
- Gastrointestinal swelling leads to severe abdominal pain and third spacing.
- Laryngeal angioedema may lead to respiratory arrest, occurring in 50% of patients with HAE type I.
- Family history may exist, but 15% mutations are new.
- Nonpruritic rash, erythema marginatum, may precede swelling episodes.

Classification

- **HAE type I:** A mutation of one of the C1-INH gene alleles, leading to **low or absent protein**; 85% of patients present with HAE
- **HAE type II:** A mutation of one of the two gene alleles, leading to normal or **high levels of a nonfunctioning C1 inhibitor protein**; 15% of patients with HAE
- **HAE type III:** Normal C1-INH. E**strogen-dependent**, seen primarily in women, and often triggered by pregnancy or exogenous estrogen administration. Inheritance is dominant, which may be due to a mutation in factor XII that augments its activity as an initiator of bradykinin formation

Diagnosis

Laboratory Tests:

- Decreased C4, which is often undetectable during an attack, is the best screening test for diagnosing HAE (Table 8-1). A decreased or absent C1-INH confirms the diagnosis (type I). 15–20% patients have a normal, even increased C1-INH but a decreased functional C1 assay (type II). C2 is usually normal when asymptomatic, but it is decreased in all types during attacks. To date, no complement abnormality has been discovered in type 3 HAE.

Treatment

Acute attacks:

- Airway management
- Hydration
- Pain control
- HAE-specific therapies: C1-INH concentrate, kallikrein inhibitor (ecallantide), bradykinin receptor antagonist (icatibant)
- Fresh frozen plasma has been historically used, but may lead to paradoxical worsening

Attacks usually abate in 3–4 days, even if no medication is given; however, they can be lethal (e.g., laryngeal edema).

Long-term therapy:

- Attenuated androgens: Androgen derivatives, such as danazol and stanozolol, help prevent attacks by inducing hepatic synthesis of C1-INH. Common adverse effects of this type of long-term therapy include hepatotoxicity, dyslipidemia, masculinization, and headaches.
- Plasma-derived C1-INH: See Table 8-2.

Table 8-1. Complement Levels in Diagnosis of HAE

Type	C1-INH level	C1 INH Function	C1q	C4	C3
HAE type I	↓	↓	N	↓	N
HAE type II	N or ↑	↓	N	↓	N
HAE type III	N	N	N	N	N
AAE type I	↓	↓	↓	↓	N or ↓
AAE type II	↓ or N	↓	↓ or N	↓	N or ↓

Abbreviations: HAE, hereditary angioedema; C1-INH, C1 inhibitor.

Table 8-2. Medications Available for HAE in United States

Therapy	Indication	Dose	Adverse Effect
Plasma-derived C1-INH **Cinryze**	Long-term prophylaxis	1000 U IV q 3–4 days	Thrombotic events (rare)
Plasma-derived C1-INH **Berinert**	Acute attacks	20 Units/kg IV	Thrombotic events (rare)
Kallikrein inhibitor ecallantide (**Kalbitor**)	Acute attacks	30 mg SQ	Anaphylaxis (~3–4% risk; black box warning)
Bradykinin receptor antagonist icatibant (**Firazyr**)	Acute attacks	30 mg SQ May repeat q6h (max 3 doses/24 h)	Injection site reactions

Abbreviations: HAE, hereditary angioedema; C1-INH, C1 inhibitor; SQ, subcutaneous.

Short-term prophylaxis:
Indicated prior to oral or general surgical procedures. Angioedema episodes typically happen within 48 hours of trauma or surgery.

- Androgens: High dose, 3–5 days prior to planned procedure
- C1 esterase inhibitor, infused 1–2 hours prior to procedure

OB/Gyn Considerations in HAE patients:

- Contraception: Estrogens should be avoided. Progestins can be used
- Pregnancy:
  - Attenuated androgens are contraindicated
  - Plasma-derived C1-INH is preferred for acute treatment, short-term and long-term prophylaxis
- Parturition:
  - Complications during vaginal delivery are rare
  - Plasma-derived C1-INH prophylaxis is advised before caesarian section, and forceps or vacuum extraction

Acquired Angioedema

Classification

- **Type I (paraneoplastic)**
  - Associated with B-cell lymphoproliferative diseases
  - Monoclonal gammopathy of uncertain significance (MGUS)
  - Consumption of C1-INH by neoplastic lymphatic tissue
- **Type II (autoimmune)**
  - Autoantibody to C1-INH always present. Impairs enzyme function
  - C1-INH levels are normal

Flash Card Q1

Which complement component is decreased in acquired, but not hereditary, angioedema?

Flash Card Q2

Which medication approved for treatment of acute attacks of HAE carries a black box warning for anaphylaxis?

Other clinical features include:

- **Late onset**, after fourth decade
- Overproduction of bradykinin
- The **low C1q level** distinguishes this condition from the hereditary disorder
- C4, C2, and C3 may also be depleted
- **Low C1-INH level** caused by C1 activation.

Idiopathic Angioedema

Recurrent angioedema may be due to medications, allergen-induced, or physically induced. In about 50% of cases, urticaria and pruritus are associated. **Angiotensin-converting enzyme inhibitor (ACEI) use** is the most common cause of acute angioedema in the emergency room. The mechanism is thought to involve impaired bradykinin degradation. Icatibant, which is a bradykinin receptor antagonist, is being investigated for potential use in ACEI-induced angioedema. When no underlying etiology is identified for the angioedema (truly idiopathic), the therapy is similar to that for chronic urticaria, with antihistamines being the mainstay of therapy.

Key Fact

Warning Signs of PIDs.

1. ≥4 new ear infections within 1 year
2. ≥2 serious sinus infections within 1 year
3. ≥2 months on antibiotics with little effect
4. ≥2 pneumonias within 1 year
5. Failure of an infant to gain weight or grow normally
6. Recurrent, deep skin or organ abscesses
7. Persistent thrush in mouth or fungal infection of skin
8. Need for IV antibiotics to clear infections
9. ≥2 deep-seated infections, including septicemia
10. A family history of PIDs

CONGENITAL (PRIMARY) IMMUNODEFICIENCIES

Overview of Primary Immune Disorders

Primary immune disorders or deficiencies (PIDs) manifest as increased susceptibility to infections and can occur/present with autoimmune diseases and/or malignancy. An underlying genetic basis has been determined for many PIDs. PID can be diagnosed at any age, but most are diagnosed in childhood. Commonly related microbial organisms and PIDs are shown in Table 8-3.

Flash Card A1

C1q

Flash Card A2

Ecallantide (Kalbitor)

Table 8-3. Commonly Related Microbial Organisms and PIDs

Organism	Antibody Deficiency	Combined Immune Deficiencies	Phagocyte Defects	Complement Deficiency
Viruses	*Enterovirus* (XLA)	All, esp CMV, RSV, EBV	No	No
Bacteria	*Streptococcus pneumoniae,* *Haemophilus influenzae,* *Moraxella catarrhalis,* *Pseudomonas aeruginosa*	Also: *Salmonella typhi, Listeria monocytogenes,* enteric flora	*Staphylococcus aureus,* *Burkholderia cepacia,* *Nocardia, Serratia*	Also: *Neisseria meningitidis* (late component)
Mycobacteria	No	Nontuberculous including BCG	Nontuberculous including BCG	No
Fungi	No	*Candida,* *Aspergillus,* *Cryptococcus,* *Histoplasma capsulatum*	*Candida,* *Aspergillus*	No
Protozoa	*Giardia*	*Pneumocystis jiroveci,* *Toxoplasma, Cryptosporidium parvum*	No	No

Abbreviations: BCG, bacille Calmette-Guérin; CMV, cytomegalovirus; EBV, Epstein-Barr virus; RSV, respiratory syncytial virus; XLA, X-linked agammaglobulinemia.

Initial Screening Tests in Suspected Immune Deficiencies

Initial screening tests for immune deficiencies assess distinct compartments of the immune system in a quantitative and qualitative manner. Table 8-4 is a summary of screening tests.

Second-Tier Evaluation of Primary Immune Deficiencies

Abnormalities in screening tests should be followed by specific studies to further characterize the immune deficiency. A review of second-tier evaluation are highlighted under the specific immune deficiencies.

Table 8-4. Initial Work-Up for Suspected Immune Deficiencies

	Humoral Immunity (B Cell)	Cellular Immunity (T and NK Cell)	Phagocytosis (Macrophage and Monocyte)	Complement Systems
Quantitative assessment	Flow cytometry: CD19 and CD20 IgG, IgA, and IgM	Absolute lymphocyte count (CBC with differential counts) Exclude HIV Flow cytometry: CD3CD4, CD3CD8, and CD16CD56	Absolute neutrophil count (CBC with differential counts) Flow cytometry: CD11 and CD18 (LAD type1) and CD15a (LAD type2)	C3 and C4
Qualitative or functional assessment	Isohemagglutinin titer Specific antibody titers to: Protein antigens (diphtheria and tetanus) and Polysaccharide antigens (*Pneumococcus* and *Meningococcus*)	Delayed-type hypersensitivity Enzyme assays (ADA and PNP) NK cytolysis assay Mitogen or antigen stimulation Cytokine production Cytotoxic assays	Oxidative function (DHR, NBT, or chemiluminescence) Enzyme assays (MPO and G6PD) Phagocyte function Antineutrophil antibodies	CH50 (classical pathway) AH50 (alternative pathway)

Abbreviations: ADA, adenosine deaminase; CBC, complete blood clot; DHR, dihydrorhodamine; G6PD, glucose-6-phosphate dehydrogenase; Ig, immunoglobulin; LAD, leukocyte adhesion deficiency; MPO, myeloperoxidase; NBT, nitroblue tetrazolium; PNP, purine nucleoside phosphorylase.
(Adapted, with permission, from Dr. John M Routes, Medical College of Wisconsin.)

COMBINED ANTIBODY AND CELLULAR IMMUNITY DEFICIENCIES

Severe Combined Immune Deficiencies: Defect in T Lymphocytes

Severe combined immune deficiencies (SCIDs) represent a heterogeneous group of disorders characterized by T-cell lymphopenia. The most common form of SCID is X-linked SCID (γ_c chain defect) and, therefore, most cases will be diagnosed in male infants. The etiology of SCID is summarized in Table 8-5.

Table 8-5. Etiology of SCID

Type	SCID	Infections, Clinical Findings	Defect	Gene	Lymphocyte Phenotype		
X-Linked SCID	X-linked SCID (γ_c chain)	FTT, rash, chronic diarrhea, GVHD (maternal T lymphocytes), and absent lymphoid tissue; Opportunistic and live vaccine infections	Cytokine signaling (γ_c chain involves in IL-2, 4, 7, 9, 15, 21 receptor signaling) (IL-7Rα is specific for only T-cell development, therefore B+ NK+) (JAK-3 is a signaling protein of γ_c chain, therefore both have the same phenotype)	IL-2RG	T–	B+	NK–
AR SCID	JAK-3 deficiency			JAK-3	T–	B+	NK–
	IL-7Rα (CD127)			IL-7RA	T–	B+	NK+
	IL-2Rα (CD25)			IL-2RA	T–	B+	NK+
	CD45		CD45 tyrosine phosphatase and CD3 subunits involve in only TCR signaling, therefore B+ NK+	CD45	T–	B+	NK+
	CD3δεζ			CD3δεζ	T–	B+	NK+
	RAG1/2		RAG1/2 involves in VDR rearrangement of T- and B-cell Ag receptor formation, therefore NK+. (Proteins are unique to immune cells, no radiation sensitivity)	RAG1/2	T–	B–	NK+
AR SCID	Omenn's syndrome	Erythroderma, increased lymphoid tissues, alopecia, recurrent infections, eosinophilia, high IgE	Hypomorphic defects in RAG1/2. Also IL-7RA, ADA, Artemis, RNA component of RNAse mitochondrial RNA, and leaky phenotypes	RAG1/2	T[a]	B–	NK+
	ADA deficiency	Skeletal abnormalities: "rachitic rosary" ribcage and abnormal iliac bone, deafness	Premature lymphoid progenitor cell death from accumulating toxic metabolites, therefore all negative phenotypes (Defective nucleotide salvage pathway) (Defective purine nucleotide metabolism) (Defective hematopoietic energy metabolism)	ADA	T–	B–	NK–
	PNP deficiency	Lymphoreticular disease and autoimmune disease		PNP	T–	B+	NK+/–
	Reticular dysgenesis	Severe neutropenia and sensorineural deafness		Adenylate kinase 2 (*AK2*)	T–	B–	NK–

Table 8-5. Etiology of SCID, cont.

Type	SCID	Infections, Clinical Findings	Defect	Gene	Lymphocyte Phenotype		
Radiosensitive SCID	Artemis	Diarrhea and candidiasis Athebascan-speaking Navajo/Apache	Recombinase DNA repair protein defect Defective receptor formation	Artemis	T–	B–	NK+
	Cernunnos	Developmental delay, growth failure, and bird-like facies Microcephaly	Recombinase DNA repair protein defect Hypogammaglobulinemia	Cernunnos	T–	B–	NK+
	Ligase IV	Developmental delay, FTT, Bird-like facies Microcephaly, photosensitivity Pancytopenia and malignancy	Recombinase DNA repair protein defect Pancytopenia ↓CD4/CD8	Ligase IV	T–	B–	NK+
	Nijmegen breakage syndrome	Microcephaly, recurrent infections, bird-like facies, developmental delay, lymphoma, short stature, clinodactyly,syndactyly, and radiatiosensitive	Class switching recombination defect ↓CD4/CD8 ↓proliferation Can have hypogammaglobulinemia and IgA deficiency. Elevated IgM Defect in specific antibody response Absence of Hassel's corpuscles on thymic biopsy	NBS1 (substrate for ATM)	T–	B–	NK+

[a]T lymphocytes in Omenn's are oligoclonal, CD45RO+, and TREC–.
Abbreviations: ATM, ataxia-telangiectasia mutated (kinase); FTT, failure to thrive; GVHD, graft-versus-host disease; Ig, immunoglobulin; NBS, Nijmegen breakage syndrome; NK, natural killer; PNP, purine nucleoside phosphorylase..

While reviewing this table, focus on the B and NK phenotype. T cells are absent with the following exceptions:

- CD 8 lymphopenia can be caused by MHC class I deficiency (TAP1/2 deficiencies, tapasin deficiency) and ZAP70 deficiency (lack of blood CD8 lymphocyte).
- CD4 lymphopenia can be caused by bare lymphocyte syndrome (MHC class II deficiency), LCK deficiency, and HIV infection.

Lymphocyte proliferation to mitogens is markedly reduced as well.

Treatment is geared to:

- Preventing infections with intravenous immunoglobulin (IVIG), prophylactic antibiotics (sulfamethoxazole/trimethoprim), antifungals, withholding live immunizations, and isolation in the sterile environment
- Improving nutritional status
- Hematopoietic stem cell transplantation
- Gene therapy
- Enzyme replacement; pegdemase bovine ADA (ADA-PEG)
- Avoid breast feeding
- CMV negative, irradiated blood products

Deficiency in Cellular Immunity (or Complex Immunodeficiencies)

Cellular immune deficiencies present with opportunistic infections, chronic diarrhea, and failure to thrive (FTT). An approach to the evaluation of cellular immune deficiencies is provided in Table 8-4 and a review of clinical diseases is found in Table 8-6.

Flash Card Q3

What is the lymphocyte phenotype in X-linked SCID caused by the mutation in the γc chain?

Flash Card Q4

Which type of SCID can most easily be missed by newborn TRECs (T-cell receptor excision circles) screening?

Table 8-6. Cellular or Complex Immune Deficiencies

Cellular Deficiency	Inheritance or Mutation	Infections or Clinical Findings	Laboratory Values	Notes	Therapy
Ataxia-telangiectasia	ATM; Ataxia-telangiectasia, mutated	Progressive neuronal loss—normal at birth, ataxia begins ~2 years old, later in some patients, wheelchair-bound ~10 years old. Oculomotor apraxia, dysarthria, and choreoathetosis Telangiectasia (appears years after ataxia) Sterility Risk of leukemia or lymphoma Immune deficiency—sinopulmonary infections	Naïve T-lymphocyte (CD45RA) lymphopenia; poor mitogen responses Can have hypogammaglobulinemia and poor response to immunizations; IgA deficiency (80%) **Elevated AFP** and decrease CSA to assess radiosensitivity	Class-switching recombination defect ATM is PI3 kinase, responsible for repair in DNA ds breaks; as a result, patients radiosensitive	Treatment and prophylactic antibiotics, IVIG, and chemo-therapy Avoid radiation from imaging.
Chronic mucocutaneous candidiasis (CMC); Autoimmune polyglandular syndrome (APS-1); Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED)	AR and **AIRE gene**	Recurrent noninvasive thrush, candidal dermatitis, dystrophic nails, enamel hypoplasia Endocrinopathies: Hypoparathyroid (most common) Hypoadrenalism, pernicious anemia, DM, vitiligo, alopecia, and hepatitis	↓ T proliferation to mitogens and recall antigens Some IgA deficiency		Antifungal; Treat autoimmune disease
CD40L deficiency (HIGM1, hyper-IgM type1)	**X linked** CD40L/CD154 mutation (TNFS5)	Opportunistic infection, fungal, bacterial, and viral; Autoimmune hemolytic anemia and neutropenia	↓ IgG, IgA, and IgE, levels. Variable high/normal IgM; Neutropenia **Absent germinal centers**	Ig class switch recombination deficiencies from defect in CD40-CD40L interaction	IVIG, PCP prophylaxis GCSF, and HSCT
CD40 deficiency (HIGM3, hyper-IgM type3)	AR CD40 absent				IVIG, PCP prophylaxis, and GCSF

Flash Card A3

T–B+NK–

Flash Card A4

ADA deficiency

Table 8-6. Cellular or Complex Immune Deficiencies, cont.

Cellular Deficiency	Inheritance or Mutation	Infections or Clinical Findings	Laboratory Values	Notes	Therapy
DiGeorge's syndrome	22q11.2 deletion (~90%) 10p13-14 deletion (Renal/GU defect)	1. Cellular immune deficiency → infections. +/- absence of part or all of the thymus 2. Hypocalcemia parathyroid deficiency → tetany/seizures 3. Congenital heart disease (tetralogy of Fallot—most common Type B interrupted aortic arch—2nd common) Other features: low-set or posteriorly rotated ears, short philtrum, micrognathia, developmental delay, B-cell lymphoma, and autoimmune disease	Complete: naive T cell < 50/mm^3 + no mitogen proliferation Partial: +/– decrease T cell, usually normal mitogen proliferation Can have low Ig if severe T-cell defect US: kidney agenesis; Autoimmune antibodies, TUPLE1 (FISH), low-copy repeat, and TBX1.	CATCH 22 (**c**ardiac defects, **a**bnormal facies, **t**hymic hypoplasia, **c**left palate, **h**ypocalcemia, chromosome **22**)	Complete DGS: treat like SCID with thymus transplantation and IVIG Antibiotic prophylaxis—depend on T-cell counts and recall Live vaccines for patients who have normal T recall Neuropsychiatric therapy, and surgery of congenital defects
Defects in NFκB regulation (NEMO)	X-linked inconginentia pigmenti (Null mutation of NEMO)	Lethal in utero (male) Scarring, alopecia, and hypodontia (female)	Hypogammaglobulinemia and poor antibody response to polysaccharide TLR defect (NFκB is central in TLR activation) Can have decreased NK cytotoxicity Normal T cell-count and function	Ectodysplasin A receptor cannot induce activation of NFκB + Defect in CD40 activation pathway	IVIG and IFNγ HSCT: under investigation—prior attempts resulted in engraftment difficulties and post transplant complications
	X-linked anhydrotic ectodermal dysplasia with immunodeficiency (Hypomorhic mutation in NEMO)	Male—reduced sweat, hyperthermia, hypotrichosis, and hypodontia, conical incisors, nail abnormality Severe bacterial infections but poor inflammatory responses, opportunistic infections including mycobacteria Female—usually normal			

Table 8-6. Cellular or Complex Immune Deficiencies, cont.

Cellular Deficiency	Inheritance or Mutation	Infections or Clinical Findings	Laboratory Values	Notes	Therapy
Defects in NFκB regulation (NEMO), cont.	LZ-NEMO mutation (X-linked MSMD	Normal ectoderm; *Mycobacterium avium intracellulare* is cause of infection. Disseminated BCG		Impaired production of IL-12 and IFNγ in response to CD40L	Mycobacteria should be typified and treated Antimycobacterial prophylaxis in some patients
	Immune deficiency	Normal ectoderm; Infections: bacteria, viral, and opportunistic	HIGM1/3 phenotype (combined immune defect)	Defect in class switch recombination due to altered CD40 signaling	
Hyper-IgE syndrome (HIES) Job's syndrome	AD-STAT-3	Recurrent Infections: abscesses, mucocutaneous candidiasis Pneumonia with pneumatoceles Severe eczema and eosinophilic pustular folliculitis Characteristic facies: coarse features, prominent mandible, hypertelorism, and broad nose Skeletal abnormalities: retained primary dentition and scoliosis Connective tissue disease: hyperextensibility and aortic aneurysms	IgE > 2000 IU/mL; Eosinophilia Normal IgM T_h17 levels decreased	Triad of: recurrent skin and lung infections, severe eczema, and elevated IgE Has characteristic infection profile: *Staphylococcus aureus*, *Streptococcus pneumonia*, *Haemophilus influenzae*, and *Candida*; Secondary infection by: *Pseudomonas aeruginosa* and *Aspergillus* sp	Prophylactic antibiotics (covering staph) and antifungal IVIG in case of poor antibody response
	AR-DOCK8	Viral skin infections severe difficult to treat: HPV, HSV, VZV, molluscum Mucocutaneous candidiasis Eczema and allergies Risks of malignancies Pneumonias but no pneumatoceles	Low IgM More prominent eosinophilia Lymphopenia		
	AR-Tyk2	As DOCK8 + disseminated BCG Vasculitis		Tyk-2 involves in Il-12 signaling pathway to produce IFN-γ	

Table 8-6. Cellular or Complex Immune Deficiencies, cont.

Cellular Deficiency	Inheritance or Mutation	Infections or Clinical Findings	Laboratory Values	Notes	Therapy
Immune dysregulation, polyendocrinopathy, enteropathy, and X-linked inheritance (IPEX)	X-linked recessive **FOXP3 mutation** (not found in every cases)	Severe diarrhea and FTT (enteropathy) Early-onset type 1 DM thyroid disease, autoimmune cytopenia Variable skin lesions: erythroderma,exfoliative dermatitis, eczema, psoriasis-like Severe infections Coronary artery disease	Villous atrophy with lymphocytic infiltrates in small bowel Autoimmune antibodies Cytopenia Eosinophilia High IgE, normal IgG (↓enteropathy), IgM Normal B/T number and function exp. no Treg	FOXP3 codes protein involved in Treg (CD4+CD25+) cell development Impair Treg → autoimmune	IVIG Rx autoimmune and endocrine disease HSCT Parenteral nutrition
X-linked lymphoproliferative disease (XLP)	XLP-1: mutation in SH2D1A gene encodes SAP	1. Fulminant EBV mononucleosis 2. Dysgammaglobulinemia—combined immunodeficiency with severe infections 3. Lymphoma: B cell/Burkitt's, splenomegaly	HLH on BM biopsy Low IgG, high IgM ↓ CD4 but high CD8. Impair T-cell function. ↓ NK cell number and function Absent NKT cell Anemia	XLP-1: Failure to eliminate EBV-infected B cells → prolonged Ag presentation + hyperactivation of CTL and macrophages → HLH	IVIG, HSCT, and chemo-therapy
	XLP-2: XIAP	XLP-2: Colitis, predisposition to hemophagocytic lymphohistiocytosis		XLP-2: increased susceptibility to apoptotic stimuli	
Wiskott-Aldrich syndrome	WASp X-linked	Triad: 1. Thrombocytopenia + bleeding diathesis, 2. eczema, 3. recurrent infections. Risk for: autoimmunity and malignancy (EBV-related lymphoma)	↑ IgE, IgA; ↓ IgM; and Normal IgG but impaired Ab responses. Impair T-cell proliferation ↓ Platelet size ↓ Platelet function	PET WASP: **p**yogenic infection, **e**czema, **t**hrombocytopenia, and WASp mutation HOT ITCH THROM; Wiskott-hot Aldrich-itch Syndrome Thrombo-cytopenia	HSCT treatment of choice Treatment of infections, IVIG, and splenectomy

Abbreviations: Ab, antibody; AFP, α-fetoprotein; BCG, bacille Calmette-Guérin; CSA, colony survival assay; CTL, cytotoxic T lymphocyte; DM, diabetes mellitus; EBV, Epstein-Barr virus; ds, double-stranded; G-CSF, granulocyte colony-stimulating factor; HLH, hemophagocytic lymphohistiocytosis; HPV, human papillomavirus; HSV, herpes simplex virus; HSCT, hematopoietic stem cell transplantation; MSMD, Mendelian susceptibility to mycobacterial diseases; NEMO, NFκB essential modifier; PCP, *Pneumocystis jiroveci* pneumonia; PI3, phosphatidylinositol 3; SAP, SLAM-associated protein; TLR, Toll-like receptor; VZV, varicella-zoster virus; WASp, Wiskott-Aldrich syndrome protein; XIAP, X-linked inhibitor of apoptosis.

Antibody Deficiencies

Antibody deficiencies should be considered when a patient has:

- Recurrent sinopulmonary infections
- Enteroviral infections (XLA)
- Giardiasis
- Autoimmune phenomenon

Immunoglobulin levels, pre- and postimmunization with protein-based (diphtheria, and tetanus), carbohydrate-based (Pneumovax, *Haemophilus* and meningococcus), or conjugate (pneumococcal 7-valent conjugate vaccine, Prevnar) vaccines are easily obtained from laboratories performing serology. Tables 8-7 and 8-8 summarize laboratory findings in humoral deficiencies.

Table 8-7. Evaluating Antibody Deficiencies

Condition	CD19/ CD20	IgG	IgA	IgM	Postimmunization Titers
sIgAD	Normal	Normal	<10 mg/dL	Normal	Normal
XLA	Very low/absent	<200	Low	Low	Undetectable
CVID[a]	Normal or low	<450	Low	Low	Low or undetectable
HIGM	Normal	<400	Low	Normal or High	Small IgM response

(Reproduced, with permission, from Dr. John M. Routes, Medical College of Wisconsin.)
[a]For CVID, in order to meet the criteria for diagnosis, you need IgG AND IgA OR IgM to be less than 2 SD below the lower limit for age AND impaired antibody responses.
Abbreviations: CVID, common-variable immunodeficiency; HIGM, hyper-IgM syndrome sIgAD, selective IgA deficiency; XLA, X-linked agammaglobulinemia.

Table 8-8. Antibody Deficiencies

Antibody Deficiency	Inheritance/ Mutation	Infections/ Clinical Findings	Laboratory Findings	Notes	Therapy
Transient hypogammaglobulinemia of infancy (THI)	Unknown	Sinopulmonary GI, thrush, meningitis (usually not severe) May be asymptomatic	IgG < 2 SD (95th percentile), which persists older than 6 months of age. Usually normal Ab responses	Prolonged physiologic hypogammaglobulinemic phase after disappearance of maternal IgG	Mostly no Rx, may need prophylactic antibiotics, but rarely IVIG Usually resolved by 2–4 years old
Selective IgA deficiency (SIgAD)	Unknown Some sIgAD + CVID has *TACI* mutation Most common primary immunodeficiency disorder	Majority asymptomatic; Sinopulmonary, GI infections; Autoimmune and atopic disease; Rare: anti-IgA Ab → transfusion reactions	IgA < 7 mg/dL (Age ≥ 4 years old) Normal IgG, IgM, and vaccine titers FP serum β-HCG tests due to presence of heterophile Ab.	2° IgA deficiency: Antiepileptics Sulphasalazin ecaptopril, thyroxin Can be part of AT, IgG2 subclass def, CMC	Treatment and prophylactic antibiotics; and IVIG if concomitant specific antibody defect Monitor progression to CVID
Specific antibody deficiency (SAD)	Unknown	Sinopulmonary infections Allergic rhinitis	Normal IgG, A, M Poor polysaccharide response despite pneumovax	Age ≥ 2 years old	Prophylactic ABx and IVIG Monitor progression to CVID
X Linked aggamaglobulinemia (XLA)	**BTK**	Sinopulmonary infections, atypical bacteria, GI infections, **enteroviral encephalitis**, septic arthritis, lymphoreticular and colorectal malignancies, bronchiectasis **Small/absent lymphoid tissue, no germinal center**	Low IgG, A, M (<2 SD); CD19+ B lymphocytes <2%; Most have absent BTK (monocytes, platelets) BTK mutation on gene sequencing	Maturational **arrest at the pre-B lymphocyte** stage Do not use serologic assays to diagnose infectious diseases—ex. ELISA for HIV Use PCR assays or cultures instead	IVIG treatment and ABx Rx Live immunizations contraindicated

Flash Card Q5

What mutation causes APECED and what is the most common endocrinopathy seen in the disease?

Flash Card Q6

What mutation causes IPEX and what cell is affected by the disease?

Table 8-8. Antibody Deficiencies, cont.

Antibody Deficiency	Inheritance/ Mutation	Infections/ Clinical Findings	Laboratory Findings	Notes	Therapy
Autosomal recessive agamma-globulinemia	Surrogate light chain (V pre-B; λ5), μ IgM heavy chain (Cμ), Igα, Igβ, BLNK	Same as XLA Can be more severe and earlier onset than XLA	IgG, A, M (<2 SD). CD19+ B lymphocytes <2%	μ IgM heavy chain—most common of AR agamma-globulinemia	Same as XLA
Common-variable immuno-deficiency (CVID)	Mostly unknown; ICOS, TACI; BAFF-R, CD19 complex, CD20	Age >2 years old, sinopulmonary, GI infections, meningitis Bronchiectasis, BOOP, autoimmune disease, GI/liver disease, granulomatous disease, non-Hodgkin's lymphoma, and gastric carcinoma	IgG (<2 SD), and ↓ IgA or IgM, or both (<2 SD) and impaired response immunizations Variable T-cell counts and function	CVID + thymoma = Good's syndrome Reduced # of switched memory B cells shown to be associated with certain noninfectious complications (hematologic autoimmunity)	IVIG treatment and ABx Rx No live vaccine Pulmonary hygiene for bronchiectasis Excise thymoma if present
IgG subclass deficiency (IGGSD)	Unknown	Most asymptomatic	Normal IgG and low level of ≥1 subclasses IgG2 deficiency can occur with SIgAD with impaired Ab response to polysaccharide	Controversial if true PIDD—20% of population have subnormal of ≥1 subclasses (esp. IgG4)	Rx as SAD in case of poor polysaccharide response
Hyper-IgM type2 (HIGM 2)	AR AIDdeficiency	Sinopulmonary and GI infections; **Lymphoid hyperplasia and adenopathy** CVID-like, but have increased autoimmune disease	↓IgG, IgA, IgE, and normal or ↑ IgM; Normal T-cell function -↑LN and giant germinal centers.	AID and UNG are required for class switch recombination and somatic hypermutation of B cell No T-cell defect → less severe than HIGM1, 3 and NEMO defect	IVIG ABx Rx No live vaccines
Hyper-IgM type4 (HIGM4)	AR UNG deficiency				

Abbreviations: ABx Rx, antibiotic prescription; AFP, α-fetoprotein; AID, activation-induced cytidine deaminase; AR, autosomal recessive; β-HCG, beta human chorionic gonadotropin; BLNK, B-cell linker; BOOP, bronchiolitis obliterans organizing pneumonia; BTK, Bruton's tyrosine kinase; CMC, chronic mucocutaneous candidiasis ELISHA, enzyme-linked immunosorbent assay; IVIG, intravenous immunoglobulin; LN, lymph nodes; PCR, polymerase chain reaction; PIDD, primary immune disorder or deficiency; UNG, uracil-DNA glycosylane.

Flash Card A5

AIRE mutation, hypoparathyroidism

Flash Card A6

FOXP3 mutation, Treg cell

An example of flow cytometry used to detect Bruton's tyrosine kinase (BTK) expression in monocytes in the diagnosis of X-linked agammaglobulinemia is seen in Figure 8-1.

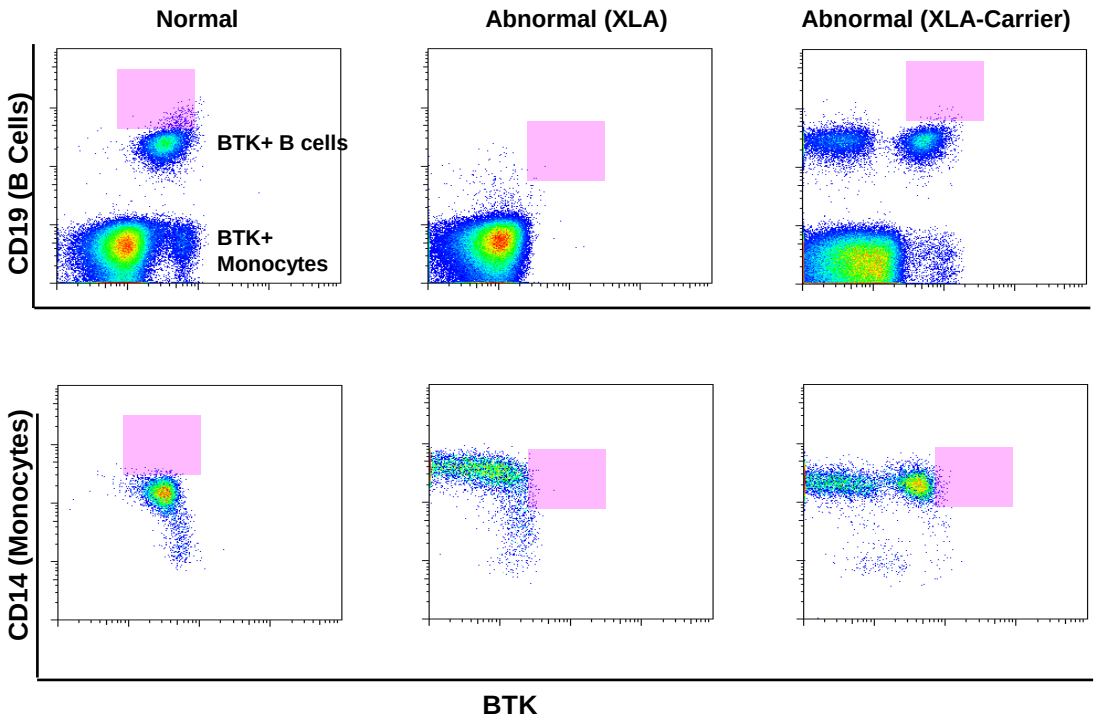


Figure 8-1. Flow cytometry-detected BTK expression in B lymphocytes and monocytes in XLA. Abbreviations: BTK, Bruton tyrosine kinase; XLA, X-linked agammaglobulinemia. (Reproduced, with permission, from Dennis W. Schauer, Jr., Trivikram Dasu, PhD, and James W. Verbsky, MD, PhD, Clinical Immunodiagnostic & Research Laboratory, Medical College of Wisconsin.)

Phagocytic Cell Disorders

- Phagocytes defend against bacteria and fungi. Patients with defects of phagocytic cell number, function, or both experience recurrent and severe infections of fungal (especially *Candida* and *Aspergillus* species) and bacterial origin.
- Respiratory tract and cutaneous infections predominate, but deep-seated abscesses are also common. Recurrent oral stomatitis is present in most cases.
- Suspicion should be raised if infections are associated with neutropenia or lack of neutrophilia.

Suspected primary phagocyte deficiencies can be evaluated by functional assays, which are included in Table 8-9.

Defects intrinsic to the phagocyte are due to:

- Abnormal bone marrow production or release
- Defect in adhesion or chemotaxis
- Leukocyte granule formation
- Oxidative killing

A summary of clinical disorders associated with primary phagocyte cell disorders are found in Table 8-10.

Flash Card Q7

What stage of B-cell development is affected by BTK mutation?

Flash Card Q8

What are the differences between HIGM1/3 and HIGM2/4?

Table 8-9. Evaluating Phagocyte Deficiencies

	Disease
CBC	LAD 1, 2 and 3 Cyclic neutropenia SCN AIN (classical) SDS
NBT	CGD
DHR	CGD
MPO	Primary MPO deficiency and secondary MPO deficiency ex. *Candida albicans* or *C. tropicalis* infection in patient with diabetes mellitus
Chemotaxis	SDS Chediak-Higashi syndrome LAD Rac2 mutation
Bacteriocidal	Neutrophil-specific granule deficiency CGD
CD18	LAD1
CD15a	LAD2

Abbreviations: AIN, autoimmune neutropenia; CGD, chronic granulomatous disease; DHR, dihydrorhodamine flow cytometric assay; LAD,, leukocyte-adhesion deficiency; MPO, myeloperoxidase; NBT, nitroblue tetrazolium chloride; SCN, severe congenital neutropenia; SDS, Shwachman-Diamond syndrome.

Flash Card A7

Arrest in pre-B–cell stage

Flash Card A8

HIGM1/3 are combined immune deficiencies with more severe/wide spectrum of infections with absent LN and germinal centers due to defects in CD40L-CD40 interactions.
HIGM2/4 are antibody deficiencies with less severe infections. Lymphoid hyperplasia and adenopathy are noted. Defects are in B-cell class switching and somatic hypermutation

Table 8-10. Clinical Features, Supporting Laboratory Findings, and Molecular Defects of Phagocytic Cell Disorders

	Disorder	Clinical Presentation & Rx	Laboratory Findings	Molecular Defect
	WHIM syndrome	Sinopulmonary infections Papillomavirus infection Warts with risk of malignant transformation No other viral/opportunistic infections Rx: G-CSF, IVIG, Rx warts, CXCR4 inhibitor (in trial)	Neutropenia (ANC 100–500/mm^3) due to retention of mature granulocytes in bone marrow (myelokathexis) Normal phagocyte functions ALC <1500/mm^3, +/–↓ mitogen responses ↓ IgG, ↓ CD19+ B lymphocytes ↓ CD27+ switched memory B cells	Activating mutation in CXCR4 (important in bone marrow homing and trafficking of progenitor cells)
Defects in Bone Marrow Production or Release	SCN	Early onset, severe bacterial infections: Omphalitis, URTI or LRTI, oral ulcer, skin and liver abscess, cellulitis, meningitis. Rx: G-CSF (not working for G-CSF-R mutation), HSCT, monitor for myelodysplasia, AML	Persistent neutropenia ANC < 200/mm^3 BM → maturation arrest of neutrophil precursors at promyelocyte-myelocyte stage	AR- HAX 1[a] (Kostmann syndrome) AD-Elastase (ELA-2), GFI1, GCSF-R XL- WASP
	Cyclic Neutropenia	Oral ulcers, fever, skin infection/abscess during periods of neutropenia Rx: prophylactic ABx or G-CSF during the cycling nadir	↓ Neutrophils, platelets, monocytes, reticulocytes in 21-day cycle; last for 3–6 days CBC with differential 2–3 times weekly for 6 weeks	ELA-2- AD
Defects in Adhesion and Chemotaxis	LAD 1 (most common form of LAD)	Recurrent pyogenic infections, delayed wound healing, necrotic skin, infections, **impaired pus formation**, gingivoperiodontitis, omphalitis, delayed umbilical cord separation Rx: ABx Rx and prophylaxis, G-CSF, HSCT	Leukocytosis; ↓ CD18 on neutrophils by flow cytometry: Severe: <1-2% Mild-moderate: 2–30% Carrier:40–60% Note: CD18 binds to variable α-chains: LFA-1 (CD11a); Mac-1 (CD11b); P150,95 (CD11c)	Common chain of β2-intergrin family (**CD18**) from ITGB2 gene mutation Defect in WBC adhesion (arrest)

Table 8-10. Clinical Features, Supporting Laboratory Findings, and Molecular Defects of Phagocytic Cell Disorders, cont.

	Disorder	Clinical Presentation & Rx	Laboratory Findings	Molecular Defect
Defects in Adhesion and Chemotaxis, cont.	LAD 2	**Less** severe skin/lung infections, no delayed umbilical cord separation, pus formation is impaired but not absent **Developmental delay, microcephaly, and short stature** Rx: Abx prophylaxis, fucose suplementation	Leukocytosis Absence of CD15a **Bombay blood phenotype (hh)** Sequence analysis of GDPfucose transporter	Mutation in FUCT1 → absence of fucosylation → no Sialyl-Lewis$_X$ (**CD15a**) Defect in WBC rolling
	LAD 3	**LAD 1 + bleeding diathesis**. Rx: as LAD 1	Leukocytosis Normal expression of CD18 Abnormality of Rap1 GTPase function	CalDAG-GEF1 mutation → failure of cytokine activation of integrins
Defect in Leukocyte Granule formation	Chediak-Higashi syndrome	Oculocutaneous albinism, hypopigmented skin, iris, hair, recurrent infections, bleeding tendency, neurologic defects, lymphoma-like syndrome, risk of HLH	Enlarged primary granules in neutrophils, eosinophils, neutropenia, decreased neutrophil chemotaxis, and absent NK cytotoxicity. Evenly distributed larger melanin granules on hair shaft examination	CHS1 or LYST
Defect in Oxidative Killing	CGD	Infections with **catalase-positive organisms** Bacteria: *S. aureus, Burkholderia cepacia, Serratia marcescens, Nocardia* sp. *Aspergillus fumigatus* *Aspergillus nidulans* Fungi: Not associated with *Streptococcus pneumoniae* or *Pneumocystis jiroveci* infections Granuloma formation (GI and GU tract outflow obstruction), poor wound healing, and autoimmune disease	Abnormal NBT and pattern of DHR DHR is a fluorescent dye that is reduced by superoxide radicals (produced by phagocytes stimulated with PMA). This leads to a change in flow cytometry-detected fluorescence(see Fig.8-2) Rx: TMP-SMZ and itraconazole prophylaxis IFNγ Systemic corticosteroids for granuloma formation HSCT	PHOX (phagocytic NADPH oxidase system); >50% g91phox (X-linked form); and AR-p22, p47, and p67.

[a]A mitochondrial protein that protects against apoptosis of myeloid cells.
Abbreviations: ABx, antibiotics; AD, autosomal dominant; ALC, absolute lymphocyte count; AML, acute myelogenous leukemia; ANC, absolute neutrophil count; AR, autosomal recessive; CGD, chronic granulomatus disease; DHR, dihydrorhodamine; G-CSF, granulocyte colony-stimulating factor; HLH, hemophagocytic lymphohistiocytosis; HSCT, hematopoietic stem cell transplantation; IFNγ, interferon gamma; LAD, leukocyte-adhesion deficiency; LRTI, lower respiratory tract infection; NADPH, reduced form of nicotinamide adenine dinucleotide phosphate; NBT, nitrozolium blue test; PAM, phorbol myristic acetate; SCN, severe congenital neutropenia; TMP-SMZ, trimethoprim-sulfamethoxazole; URTI, upper respiratory tract infection; WHIM syndrome, **w**arts, **h**ypogammaglobulinemia, recurrent bacterial **i**nfections, and **m**yelokathexis.

The dihydrorhodamine (DHR) test detects the generation of superoxide free radicals. DHR is a fluorescent dye that is reduced by superoxide radicals, which are produced by phagocytes stimulated with phorbol myristic acetate (PMA). This leads to a change in flow cytometry-detected fluorescence (see Figure 8-2).

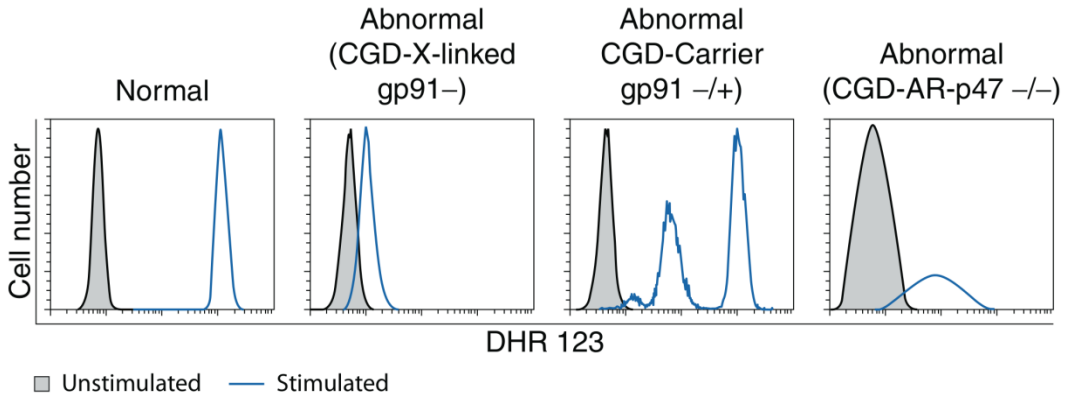


Figure 8-2. Neutrophil oxidative burst patterns on flow cytometry. The inheritance pattern can be determined by the DHR fluorescence pattern.
Abbreviations: CGD, chronic granulomatous disease; DHR, dihydrorhodamine.
(Reproduced, with permission, from Dennis W. Schauer, Jr., Trivikram Dasu, PhD, and James W. Verbsky, MD, PhD, Clinical Immunodiagnostic & Research Laboratory, Medical College of Wisconsin.)

Deficiency in Innate Immunity

See Table 8-11.

Table 8-11. Clinical Features, Supporting Laboratory Findings, and Gene Defect of IRAK4/MyD88 Deficiency

Clinical Presentation	Laboratory Findings	Gene Defect	Therapy
Severe/early-onset recurrent pyogenic bacterial infections (e.g., *Pneumococcus, Staphylococcus*). Meningitis Septicemia Liver abscess Low/absent fever/inflammatory responses	Normal Ig levels, impaired IgG response to polysaccharide antigens, normal B- and T-lymphocyte numbers (may have completely normal screen) Diagnose with decreased PBMC cytokine production when stimulated by TLR agonists (**except TLR 3 agonists**)	IRAK4 MyD88 Involve in signaling of TLRs to the activation of NFκB and AP-1	IVIG therapeutic and prophylactic antibiotics (TMP-SMZ + PenV)

Abbreviations: IRAK4, interleukin 1 receptor-associated kinase 4; NFκB, nuclear factor kappa B; MyD88, myeloid differentiation primary factor 88- adaptor protein; PBMC; peripheral blood mononuclear cells TLR, Toll-like receptor; TMP-SMZ, trimethoprim-sulfamethoxazole.

Flash Card Q9

What molecular defects occur in LAD type 1 and 2?

Defect in IFN γ/IL-12/IL-23 Axis

Interferon gamma (IFNγ) is produced by T_h1 cells, dendritic cells, and NK cells; it is essential for both innate and adaptive immune responses to viral and intracellular infections. T_h1-produced IFNγ induces IL-12 production by dendritic cells and macrophages; and, in turn, IL-12 induces further IFNγ production by NK and T lymphocytes. See Figure 8-3 for IFN γ/IL-12/IL-23 axis.

IL-12 and IL-23, and their receptors are heterodimeric proteins. The IFNγ/1L12/IL23 axis depends on IFNγ and subunits listed in Table 8-12.

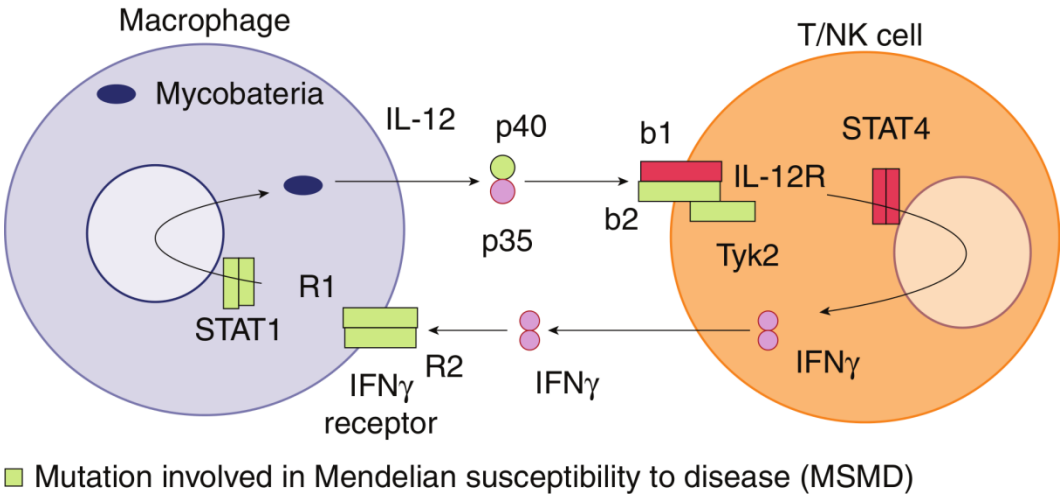


Figure 8-3. IFNγ/IL-12/IL-23 axis between macrophage and T/NK cell.
Abbreviations::IFN, interferon; IL, interleukin; STAT, signal transduction and activation of transcription; T/NK,, natural killer T cell.

Table 8-12 Heterodimeric Components of IL-12 and IL-23 and Their Receptors

Cytokine	**IL-12**	**IL-23**
Subunits	p35	p19
	p40	p40
Cytokine Receptors	**IL-12R**	**IL-23R**
Subunits	IL-12β1	IL-12β1
	IL-12β2	IL-23R

Abbreviation: IL, interleukin.
(Reproduced, with permission, from Dr. John M Routes, Medical College of Wisconsin.)

Flash Card A9

CD18 and CD 15a

NK Cell Deficiency

Table 8-13 summarizes the clinical features, laboratory findings, and genetic defect of NK cell deficiency.

NK deficiency is also seen in:

- All causes of hemophagocytic lymphohistiocytosis (HLH):
  - Primary HLH: XLP, familial, Chediak-Higashi syndrome, and Wiskott-Aldrich syndrome (WAS)
  - Secondary HLH (reactive hemophagocytic syndrome)
- NEMO
- LAD-1
- Defect in IL-12/IFNγ axis
- HIV infection
- Malignancies

Complement Deficiencies

Complement deficiencies are inherited in an autosomal dominant manner except for properdin deficiency, which is inherited in an **X-linked** manner. Complement deficiency versus consumption can be determined by levels of CH50, AH50, individual complement levels, and complement-split products as outlined in Table 8-14.

Primary complement deficiencies are characterized by recurrent infections and autoimmune diseases. A summary of clinical features appears in Table 8-15.

Hereditary angioedema is an autosomal dominant condition caused by a deficiency of functional C1 esterase inhibitor. (Details discussed in Chapter 8).

Table 8-13. Clinical Features, Supporting Laboratory Findings, and Gene Defect of NK Cell Deficiency

Clinical Presentation	Laboratory Findings	Gene Defect
Recurrent infections with herpes viruses, papillomavirus and mycobacteria	↓ CD16 by flow cytometry and ↓ NK function (cytotoxicity)	Classical NK cell deficiency: *GATA2, MCM4* mutation
Recurrent infections with herpes viruses	Normal CD16 by flow cytometry but ↓ NK function (cytotoxicity)	Functional NK cell deficiency: FCRG3A (CD16) mutation CD16 = Low-affinity IgG receptor

Abbreviations: IgG, immunoglobulin G; NK, natural killer.

Flash Card Q10

Patients with a mutation in the IFNγR (IFNγR1 and IFNγR2), p40 subunit, IL-12β1, NFκB essential modifier (NEMO), or signal transducers and activator of transcription (STAT) 1 are susceptible to infections with which organism(s)?

Table 8-14. Evaluating Complement Deficiencies Versus Consumption

Test	Complement Deficiency	Complement Consumption
CH50 or AH50 or both	Low	Low
Individual complement level (e.g., C3)	Low	Low
Two or more individual complement levels of CH50 or AH50	Low of individual complement only	Low
Complement split products	Normal	High

(Reproduced, with permission, from Dr. John M. Routes, Medical College of Wisconsin.)
Abbreviations: AH50, alternate pathway test; CH50, classical pathway test.

Table 8-15. Clinical Features and Characteristic Infections of Complement Deficiencies

	Clinical Features	Infectious Organisms	Laboratory Findings	Gene Defect
Early component (C1q, C1s, C2, and C4)	Sinopulmonary infections Autoimmune disease (SLE-like)	*S. Pneumoniae* *Haemophilus Influenzae* (encapsulated organisms)	↓CH50	
C3	Severe infections (same as antibody deficiency) Glomerulonephritis	Encapsulated organisms (e.g., *Neisseria* sp.)	↓CH50 ↓AH50 ↓C3 C3 NeF: IgG anti-C3 autoantibodies	C3
MBL	Many asymptomatic; Autoimmune disease and respiratory infections	*Neisseria*	↓ MBL	MASP2
Late component	Infections (meningitis, arthritis, sepsis) and C5-7-associated with autoimmunity	*Neisseria meningitidis* (W135 and Y serotypes) and *Neisseria gonorrhoeae*	↓CH50 ↓specific complement ↓AH50	
Alternative pathway (factor B, D and properdin)	Infections	*Neisseria*	↓AH50	

Abbreviations: AH50, alternate pathway test; CH50, classical pathway test; MASP2, mannose-binding lectin-associated protease 2

Flash Card A10

Atypical mycobacteria and *Salmonella*

Acquired (Secondary) Immunodeficiencies

Acquired (secondary) immunodeficiencies are far more common than PID. The conditions can be divided into HIV-related or due to other causes (Table 8-16). Treatment focuses on the underlying disorder.

HIV AND/OR AIDS

HIV Group and Subgroup—2 types of HIV that infect humans:

- HIV-1: More **virulent**, more **infective**, the majority of HIV infections globally
- HIV-2: Largely confined to **West Africa,** lower infectivity

Virus Structure—HIV structure consists of:

- Two identical ssRNAs
- Several enzymes packaged in a core; composed of nucleocapsid **p24** and outer membrane **p17**
- All are surrounded by a host-derived lipid bilayer membrane with two glycoprotein projections: **gp120** and **gp41** (Figure 8-4)

HIV Life Cycle—HIV crosses mucosal surfaces to infect susceptible cells (CD4–+T cells, monocytes/macrophages, dendritic cells, and neurons). The sequential steps of the HIV life cycle are shown in Figure 8-4 and Table 8-17.

Table 8-16. Non-HIV Causes of Secondary Immunodeficiency

Etiology	Conditions
Host factor	Prematurity, malnutrition, metabolic, chromosomal or genetic disorders, and malignancy
Disruptive barrier	Severe eczema and burns.
Defective microorganism clearance	Cystic fibrosis, ciliary dyskinesia, and removal of spleen or autosplectomy
Protein loss	Protein-losing enteropathy, intestinal lymphangiectasia, nephrotic syndrome, and severe burn
Bone marrow disease	Leukemia, myelodysplastic syndromes, and histiocytic disorder
Medication-induced	Immunosuppressive therapy, radiation, and systemic corticosteroid therapy
Stress-related	Trauma, surgery, and exercise

Flash Card Q11

Factor H deficiency is associated with which diseases?

Flash Card Q12

DAF (decay-accelerating factor) and CD59 deficiency are associated with which disease?

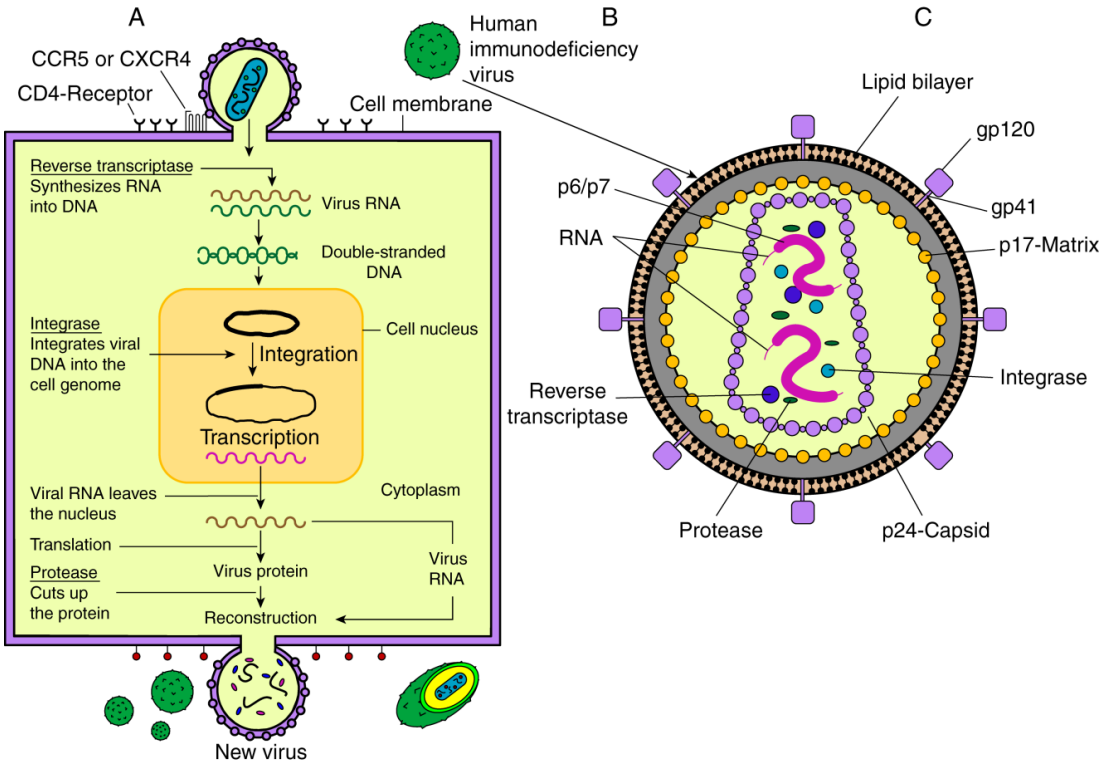


Figure 8-4. Structure and life cycle of HIV.
(Modified, with permission, from Wikimedia Commons.)

Key Fact

During latency period, the integrated proviral DNA may remain transcriptionally inactive for months or years.

Flash Card A11

Atypical hemolytic-uremic syndrome (not associated with diarrhea) or glomerulonephritis and recurrent pyogenic infections due to secondary C3 deficiency. Polymorphism is associated with age-related macular degeneration and HELLP syndrome (hemolytic anemia, elevated liver enzymes and low platelets, occurring during pregnancy.

Flash Card A12

Paroxysmal nocturnal hemoglobinuria

Table 8-17. HIV Life Cycle

Steps of Infection	Key Events
Viral entry	HIV gp120 binds to **CD4** and **chemokine coreceptor CCR5 or CXCR4** on the host cell surface **Coreceptor binding → conformational change in gp41** → fusion of HIV membrane with host cell membrane → entry of viral genome into cytoplasm
Reverse transcription	Activation of enzymes within viral nucleoprotein complex Viral reproductive cycle begins **HIV ssRNA → dsDNA by reverse transcriptase**
Integration	Proviral DNA enters nucleus and is integrated into the host DNA by enzyme **integrase**
Transcription	Proviral DNA transcribes into genomic RNA or mRNA; then translated into viral proteins in the cytoplasm
Maturation	HIV protease cleaves the viral polyprotein into functional peptides and becomes infectious
Viral packaging and budding	Packaging RNA transcripts within a nucleoprotein complex. Enclosing within the host's membrane envelope and released from the cell

Abbreviations: dsDNA, double-stranded DNA; mRNA, memory RNA; ssRNA, single-stranded RNA.

Viral Tropism

HIV coreceptor tropism refers to whichever chemokine coreceptor that a strain of HIV uses to enter cells. Some viruses can enter cells using either of the coreceptors (Table 8-18).

Immune Responses to HIV

Acute Viremia

- HIV infects **CD4+ T lymphocytes**, **macrophages**, and **dendritic cells.**
- Infected cells migrate to the regional lymphoid tissues in 3–5 days.
- Direct cell-to-cell contact between virus-harboring cells and susceptible cells within germinal centers leads to a brisk increase in viral replication within 14 days after exposure.

HIV-Specific Immunity

- The most effective, adaptive immune response to HIV infection during the acute phase is the expansion of HIV-specific **cytotoxic T lymphocytes (CTLs)**.
- Antibody responses to HIV antigens are detectable within a few weeks after infection
- Neutralizing antibodies against gp120 develop 2–3 months after infection, but are not effective.

Key Fact

The most immunogenic HIV molecules are gp120 and gp40. The virus can attach to dendritic cells through the binding of gp120 to the adhesion molecule **DC-SIGN (dendritic cell-specific intercellular adhesion molecule-grabbing nonintegrin).**

Key Fact

Although the hypergammaglobulinemia is partly due to antibody against the HIV itself, it is also in part attributable to polyclonal activation of B cells.

CD4+ T-Cell Lymphopenia Caused by HIV

There are three main mechanisms:

- **Direct viral killing** of infected cells (cytopathic effect)
- Increased **apoptosis** of infected cells
- Killing of infected cells by HIV-specific **CTLs**.

Clinical Features

See Figure 8-5 and Tables 8-19 and 8-20.

Table 8-18. HIV Coreceptor Tropism

Coreceptor	Host Cells and HIV Strain	Stage of Infection
CCR5	Monocytes/macrophages M-tropic (monocytotropic) or R5 strain of virus	Acute infection
CXCR4	T cells T-tropic (T-cell lymphotropic) or X4 strain of virus	Advanced HIV disease

Abbreviations: CCR5, CXCR4, chemokine receptors.

Flash Card Q13

What is the significance of CCR5Δ32 mutation?

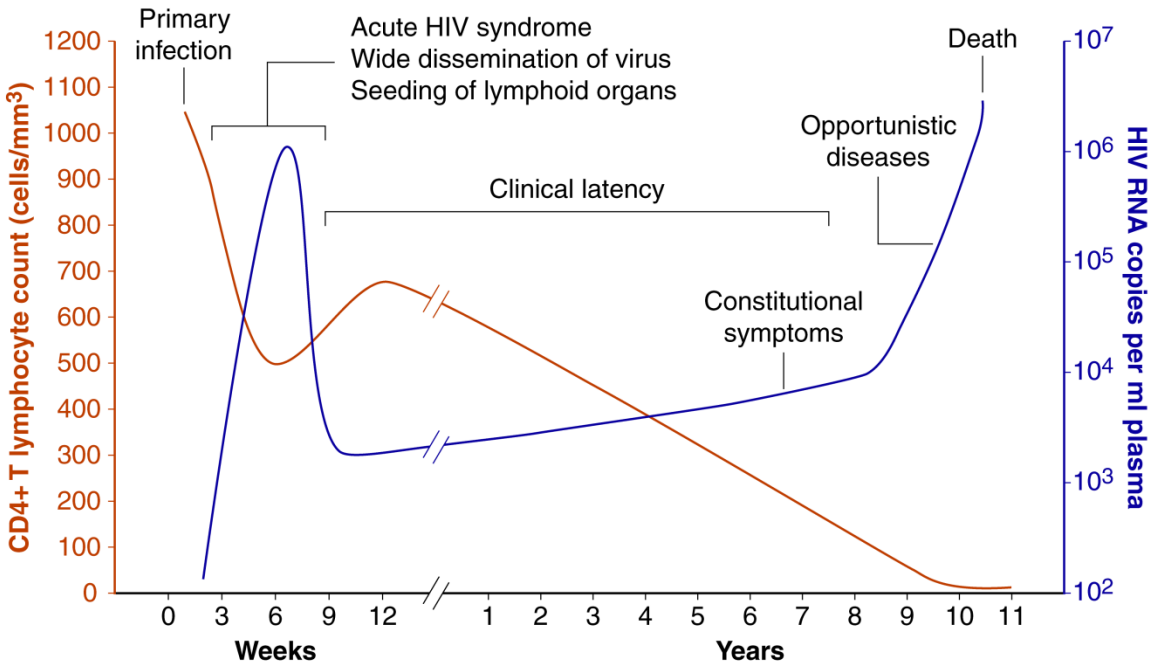


Figure 8-5. Clinical course of HIV disease.
(Modified, with permission, from Wikimedia Commons.)

Table 8-19. Clinical Feature of HIV Disease

Phase of HIV Disease	Clinical Feature
Acute HIV syndrome: Period of viremia (spike of viral load) and most reduction in CD4+ T-cell counts	Flu-like symptoms for several weeks (fever, headaches, pharyngitis, maculopapular rash, generalized lymphadenopathy, arthralgia, nausea, vomiting, and diarrhea)
Clinical latency period	CD4+ T-cell counts returns to normal shortly after the acute phase. Patient enters the chronic latent period. Declining CD4+ T-lymphocyte counts occurs through several years.
AIDS	HIV disease progresses to the final phase, or AIDS, when CD4+ T-cell counts drops **< 200 cells/mm^3 or patients develop AIDS-defining conditions** (category C) see Table 8-20

Flash Card A13

Double-allelic mutations confer resistance to the CCR5 strain of HIV (resistant to infection despite being repeatedly exposed to HIV through sexual contact).
Single allelic mutations are long-term nonprogressors (slow disease).

Table 8-20. Classification System for HIV Infection

Category B Symptomatic Conditions	Category C AIDS-Indicator Conditions
Bacillary angiomatosis	Bacterial pneumonia, recurrent (≥2/12 months)
Oropharyngeal candidiasis (thrush)	Candidiasis of the bronchi, trachea, or lungs
Vulvovaginal candidiasis, persistent or resistant	Candidiasis, esophageal
Pelvic inflammatory disease (PID)	Cervical carcinoma, invasive, confirmed by biopsy
Cervical dysplasia (moderate or severe)/cervical Carcinoma in situ	Coccidioidomycosis, disseminated/extrapulmonary
Hairy leukoplakia, oral	Cryptococcosis, extrapulmonary
Herpes zoster (shingles):	Cryptosporidiosis, chronic intestinal (>1 month)
involving ≥ 2 episodes or ≥ 1dermatome	Cytomegalovirus disease(other than liver/spleen/LN)
Idiopathic thrombocytopenic purpura	Encephalopathy, HIV-related
Constitutional symptoms:	Herpes simplex: Chronic ulcers (>1 month) or
fever > 38.5ºC or diarrhea > 1 month	bronchitis, pneumonitis, or esophagitis
Peripheral neuropathy	Histoplasmosis, disseminated or extrapulmonary
	Isosporiasis, chronic intestinal (>1 month)
	Kaposi's sarcoma
	Lymphoma, Burkitt's, immunoblastic, or primary
	central nervous system
	Mycobacterium avium complex (MAC) or
	Mycobacterium kansasii, disseminated or extrapulmonary
	Mycobacterium tuberculosis,
	pulmonary/extrapulmonary
	Mycobacterium, other species or unidentified
	species, disseminated or extrapulmonary
	Pneumocystis jiroveci pneumonia
	Progressive multifocal leukoencephalopathy (PML)
	Salmonella septicemia, recurrent (nontyphoid)
	Toxoplasmosis of brain
	Wasting syndrome caused by HIV (involuntary weight loss >10% of baseline body weight) with either chronic diarrhea (≥2 loose stools /day for ≥1 month) or chronic weakness and documented fever for ≥1 month

Diagnosis

Table 8-21 summarizes the diagnostic tests for HIV infection.

Table 8-21. Diagnostic Tests for HIV Infection

Antibody Tests	Comments
ELISA or EIA	High sensitivity, moderate specificity; therefore require a confirmatory test. Used as a screening tool for both HIV-1 and 2 **False-positive**: Autoimmune diseases, multiple pregnancies, multiple blood transfusions, following immunizations **False-negative**: Window period (can be up to 6 months after infection)
Rapid HIV test	High sensitivity, moderate specificity; therefore require a confirmatory test Detect anti-HIV antibody in blood/oral fluid. Turnaround time < 20 minutes False-positive results possible
Western blot (WB)	Confirmatory test (high sensitivity, high specificity) Expensive and labor intensive. Used to confirm a reactive EIA/ELISA test or rapid HIV test A positive WB test requires **two of three major bands**: anti-p24, anti-gp41, and anti-gp160/gp120 Follow-up indeterminate or negative test by repeating WB in 4 weeks or considering WB for HIV-2. If results continue to be indeterminate, then virologic testing recommended
Indirect immunofluorescent antibody assays (IFAs)	Confirmatory test Specimens evaluated by fluorescence microscopy
Antigen-based Tests	**Comments**
HIV DNA by PCR	Moderate sensitivity, high specificity Detects HIV-1 DNA within the PBMC Used mainly in **acute viral syndrome** when antibody test is negative and in **exposed infants**
HIV RNA by PCR	Moderate sensitivity, high specificity Quantifies viral load; reported as RNA copies/mL Provides indication for treatment and gauges therapeutic response
HIV p24 antigen	May be used in neonates or as a marker of disease progression and response to treatment Now, replaced by PCR assays **Fourth-generation HIV test** = test for p24 antigen + ELISA antibodies; (Another screening test, can detect infection sooner than ELISA because p24 antigen appear sooner in serum)
HIV culture	Can be diagnostic but not routinely used in clinical practice because the test more expensive, labor-intensive, and takes longer than other diagnostic assays

Abbreviations: EIA, enzyme-linked immunoassay; ELISHA, enzyme-linked immunosorbent assay PCR; polymerase chain reaction; PBMC, peripheral blood mononuclear cells.

HIV-Exposed Infants

HIV-1-exposed infants should be tested by HIV-1 DNA PCR

- At birth, within 14–21 days of age; 1–2 months of age and 4–6 months of age to identify or exclude HIV-1 infection.
- An antibody test by ELISA between 12–18 months should be performed to definitively exclude HIV-1 infection.
- Antibody tests in infants younger than 18 months old are not routinely recommended due to the presence of passively acquired maternal antibody.
- Umbilical cord blood sample is not recommended due to high false positive rate.
- *Pneumocystis jiroveci* pneumonia (PJP) prophylaxis beginning at 4–6 weeks of age is recommended for infants determined to be infected with HIV-1 until at least 1 year old.

HIV Prevention

Table 8-22 represents HIV prevention strategies via mode of transmission.

Treatment

Current U.S. Department of Health and Human Services (DHHS**)** guidelines (revised Feb 2013 from http://www.cdc.gov/hiv/resources/guidelines) recommend the initiation of highly active antiretroviral therapy (HAART) in:

- All symptomatic persons
- Asymptomatic persons
  - To initiate HAART at any CD4+ T-cell count
    - <350 cells/µL (AI*)
    - 350–500 cells/µL (AII*)
    - >500 cells/µL (BIII*)
  - HIV RNA may influence decision to start antiretroviral therapy (ART) and help determine frequency of CD4 monitoring.
- Initial therapy preferably contains a combination of non-nucleoside reverse transcriptase inhibitor (NNRTI), protease inhibitors (PI), or integrase inhibitor (II) + 2 nucleoside reverse transcriptase inhibitors (NRTIs). Currently available antiretroviral drugs are listed in Table 8-23.

(*Strength of recommendation: A. Strong, B: Moderate, C: Optional. Quality of evidence: I: ≥1 randomized controlled trials, II: ≥1 well-designed nonrandomized trials or observational cohort studies with long-term clinical outcomes, III: Expert opinion.)

Table 8-23 summarized the antiretroviral medications, the mechanism of action and phase of inhibition.

Table 8-22. HIV Prevention

Mode of Transmission	Strategies
Mother to child	Antepartum and intrapartum zidovudine-based prophylaxis Elective cesarean section or vaginal delivery without invasive procedures Breastfeeding avoidance
Sexual contact	Condoms, male circumcision, and safe sex practice
Sexual/occupational exposure	Two to three drugs postexposure, with prophylactic regimens Initiate as soon as possible after exposure and continue for 4 weeks Pre-exposure prophylaxis recently FDA-approved

Table 8-23. Antiretroviral Medications

Class	Phase of Inhibition	Mechanism of Action	Examples
Entry inhibitors (fusion inhibitors)	Binding, fusion, and entry of virion	Block one of several targets	Maraviroc (binds to CCR5) and enfuvirtide (binds to gp41).
Integrase inhibitors (II)	Integration of viral DNA into the DNA of the infected cell	Inhibit enzyme integrase	Raltegravir
Reverse transcriptase inhibitor			
Nucleo**s**ide or nucleo**t**ide reverse transcriptase inhibitors (NRTI or N**t**RTI)	Reverse transcription	Incorporated into the newly synthesized viral DNA to terminate the DNA strand	Zidovudine, didanosine, abacavir, and tenofovir
Nonnucleoside reverse transcriptase inhibitors (NNRTI)	Reverse transcription	Bind directly to enzyme reverse transcriptase and inhibit its function	Efavirenz, nevirapine delavirdine and etravirine
Non-nucleoside reverse transcriptase inhibitors (NNRTI)	Reverse transcription	Bind directly to enzyme reverse transcriptase and inhibit its function	Efavirenz, nevirapine delavirdine and etravirine
Protease inhibitors (PIs)	Viral assembly	Inhibit activity of protease	Ritonavir, indinavir, nelfinavir, lopinavir, and atazanavir

Key Fact

It is recommended to screen for HLA-B*5701 before starting a patient with abacavir. Abacavir challenge is absolutely contraindicated if there is a history of hypersensitivity reaction or positive HLA-B*5701 testing, since it can result in a severe and potentially life-threatening reaction.

Immune Reconstitution Inflammatory Syndrome (IRIS)

- A paradoxical deterioration in clinical status, usually 4–8 weeks after HAART initiation.
- Attributed to the reactivation of the immune response (cytokine storm) to an existing opportunistic infection (OI) when the **CD4 count rapidly increases.**
- Most common OIs associated with IRIS include TB and *Pneumocystis jiroveci* pneumonia (PJP), although CMV, herpes zoster, and *Mycobacterium avium* complex (MAC) can be associated with IRIS.
- Patients resent with new or worsening systemic manifestations, such as fever and malaise or local reactions in organs (e.g., lungs/CNS) depending on the location of the OI.

Subjects at high risk for IRIS include:

- Those starting HAART for the first time
- CD4+ T lymphocytes > 50 /mm^3 before starting HAART
- Have recently been treated for OIs

Treatment is mainly limited to supportive measures and anti-inflammatory medications such as NSAIDS or corticosteroids.

Key Fact

If patients have a low initial CD4+ T-lymphocyte count and OI at the time of HIV diagnosis, they should receive treatment to control the OI before HAART is initiated.

Table 8-24 reviews the prophylaxis for HIV-related opportunistic infections.

Table 8-24. Prophylaxis for HIV-Related Opportunistic Infections

Indication	Pathogen	Prophylactic Medications
CD4 < 200 cells/mm^3 **or** CD4 % < 14, thrush, history of AIDS-defining illness or FUO	*Pneumocystis jiroveci*	**TMP-SMX** Dapsone ± pyrimethamine Atovaquone
CD4 < 100 cells/mm^3 **and** positive *Toxoplasma* IgG	*Toxoplasma gondii*	**TMP-SMX** Dapsone + pyrimethamine + leucovorin Atovaquone ± pyrimethamine
CD4 < 150 cells/mm^3 **and** living in endemic area[a] for histoplasmosis	*Histoplasma capsulatum*	**Itraconazole**
CD4 counts are ≤ 250 cells/mm^3 **and** living in endemic area[b] for *Coccidioides* sp. **and** positive IgM or IgG serologies	*Coccidioides* species	**Fluconazole** Itraconazole
CD4 < 50 cells/mm^3	*Mycobacterium avium* complex (MAC)	**Azithromycin** **Clarithromycin** Rifabutin

[a] Ohio and Mississippi River Valleys, central and south America, Asia, and Africa
[b] Sonoran Desert in Arizona and the San Joaquin "Central" Valley in California, New Mexico, western Texas, Nevada, and Utah
Abbreviations: FUO, fever of unknown origin; TMP-SMZ, trimethoprim-sulfamethoxazole.

Flash Card Q14

Which HLA allele is associated with an increase risk in abacavir hypersensitivity?

HIV Vaccine

Candidate HIV vaccines aim at inducing neutralizing antibodies, CTLs, and strong mucosal responses.

SYSTEMIC AUTOIMMUNE DISEASE

Overview of Systemic Autoimmunity

Autoimmunity can result from one of the following:

- Loss of tolerance to host constituents, leading to self-Ag driving immune dysregulation and possible organ damage
- Exposure of genetically susceptible host to inciting antigen(s) in appropriate setting (i.e., innate immunity, inflammation, hormones, or environment, etc.), leading to the development of autoantibodies

In some systemic autoimmune diseases, certain autoantibodies are common and some may even be pathogenic, whereas others serve as markers of disease activity or clinical subsets of disease.

A summary of high-yield autoimmune diseases is presented in Table 8-25.

Factors that contribute to the failure to prevent autoimmunity include the following:

- Infection: Viral and bacterial
- Tissue injury
- Environmental stress
- Advancing age
- General immune dysregulation in predisposed individuals (i.e., HIV infection)

A variety of infection factors are relevant for organ-specific autoimmunity, including the following:

- Self-modification or loss of sequestration (i.e., eye infection)
- Superantigen immune dysregulation (i.e., *Staphylococcal enterotoxin*)
- Molecular mimicry:
  - Glutamic decarboxylase: Coxsackie B
  - Major basic protein: Hepatitis B virus (HBV)
  - Acetylcholine receptor: Herpes simplex virus (HSV)
- Adjuvant effects (i.e., Toll receptor stimulation by endotoxins)

Key Fact

T_h17 cells may be pathogenic in rheumatoid arthritis (RA) and other autoimmune disorders:

- Distinct subset from T_h1 and T_h2
- Induces inflammation by producing IL-17A, IL-17F and IL-22 and synergizes with TNFα and IL-1β
- Induced by IL-6 and TGF-ß
- Survival enhanced by IL-23

Flash Card A14

HLA-B*5701

Specific Systemic Autoimmune Diseases

Table 8-25 summarizes the systemic autoimmune diseases.

Table 8-25. Summary of Systemic Autoimmune Diseases

Condition	Clinical Findings	Autoantibody	Treatment
Sjögren's syndrome	Xerophthalmia, xerostomia (sicca symptoms), arthritis, interstitial nephritis, renal tubular acidosis, and pulmonary involvement	ANA, SS-A, SS-B, and RF	Symptomatic (muscarinic agonists, cyclosporine ophthalmic drops, and artificial tears); and immunosuppressives for severe extraglandular features
Progressive systemic sclerosis or diffuse systemic sclerosis	Fibrosis of skin, vasculopathy, hypertensive renal disease, interstitial lung disease, and CREST symptoms	ANA and Scl-70	Symptomatic: ACE inhibitor for renal crisis; various immune suppressive therapies with varying response
Progressive systemic sclerosis; and limited (CREST syndrome)	Calcinosis, Raynaud's, esophageal motility, sclerodactyly, telangiectasia, and PAH	Anticentromere Ab	Symptomatic (Ca channel blockers, PPI, metoclopramide); and PAH = oral anticoagulation, Ca channel blockers, endothelin receptor antagonist, prostanoids, and epoprostenol in refractory cases
Polymyositis	Idiopathic myositis, weakness of proximal skeletal muscle, increased CPK, and aldolase	ANA, Anti-Jo-1, anti SRP, and anti-PL-7 (antithreonyl-tRNA synthetase)	Corticosteroids and immunosuppressives (azathioprine and MTX)
Dermatomyositis	Similar to polymyositis but also with dermatologic features; Gottron's papules, heliotrope rash, "mechanic hands", and association with malignancy	ANA, Anti-Jo-1, and Anti-PL-7, (antithreonyl tRNA synthetase); andAnti-Mi2 Ab (helicase)	Corticosteroids and immunosuppressives (azathioprine, and MTX)

Abbreviations: ACE, angiotensin-converting enzyme; ANA, antinuclear antibody; CPK, creatine phosphokinase; MTX, methotrexate; PAH, pulmonary arterial hypertension; PPI, proton pump inhibitor; RF, rheumatoid factor; SRP, single recognition particle.

Flash Card Q15

What autoantibody is associated with CREST syndrome?

Flash Card Q16

What is Felty's syndrome?

Rheumatoid Arthritis (RA)

Epidemiology—Female-to-male ratio is 3:1, onset during fourth or fifth decade, but the disease can occur at any age.

Clinical Features— Includes the following:

- Common prodrome of weakness and fatigue
- Initial presentation with multiple symmetrical joints usually involved, most often hands and feet (i.e., metacarpophalangeal [MCP], metatarsophalangeal [MTP], and proximal interphalangeal [PIP] joints). Joint effusions and restricted motion usually present early
- Eventual joint deformities due to chronic inflammation
- Other findings include rheumatoid nodules over bony prominences, splenomegaly, pericarditis, vasculitis, eye disease, and renal amyloidosis

Diagnosis—See Table 8-26 for diagnostic criteria of RA.

Immunologic Features—Features include the following:

- Increase in rheumatoid factor (RF) in 80% of patients. RF is the Ig that binds IgG Fc. Usually IgM isotype. Higher = worse prognosis.
- Elevated acute-phase reactants (ESR and CRP).
- Anti-cyclic citrullinated peptide (CCP) or ACPA: As sensitive as RF, but more specific:
  - May precede onset of symptoms and is associated with worsening prognosis.

Treatment—Options include:

- NSAIDS
- Steroids
- Intrasynovial joint injections
- Disease-modifying antirheumatic drugs (DMARDs) and
- TNFα blocker, α-IL-6R mAb (tocilizumab), CTLA-4-Ig (abatacept), anti-CD20 mAb (rituximab)

Prognosis—RA is a progressive disease. Treatment helps relieve symptoms and slow progression.

Flash Card A15

Anticentomere antibody

Flash Card A16

Triad of RA, neutropenia, and splenomegaly

Table 8-26. American College of Rheumatology Criteria for Diagnosis of RA

Joint Distribution	Score
RA can be classified or diagnosed with a score ≥6.	
1 large joint	0
2–10 large joints	1
1–3 small joints	2
4–10 small joints	3
>10 joints with at least 1 small joint	5
Serology	
Negative RF and anti-CCP	0
Low positive RF or anti-CCP	2
High positive RF or anti-CCP	3
Symptoms Duration	
<6 weeks	0
≥6 weeks	1
Acute-Phase Reactants	
Normal CRP and ESR	0
Abnormal CRP and ESR	1

Abbreviations: CCP, cyclic citrullinated peptide; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; RA, rheumatoid arthritis; RF, rheumatoid factor.

Juvenile Idiopathic Arthritis (JIA)

Definition:

- Age of onset < 16 years of age
- Arthritis
- Duration of symptoms > 6 weeks

It can be divided into four subtypes, each distinguishable by both clinical and demographic factors. An important clinical distinction is the location and number of joints involved. These distinctions are outlined and compared further in Table 8-27.

Treatment—Treatment for JIA is similar to that for RA.

Table 8-27. Comparison of the Four Presentations of JIA

	Pauciarticular Onset	Polyarticular Onset	Juvenile Spondyloarthropathy	Systemic Onset (Still's Disease)
Frequency	50–60%. Most common type.	30–35%	10–5%	10–15%
Common age of onset	18–36 months	18–36 months	Late childhood/adolescent (range 2.8–17.6 years)	No definite peak age of onset
Male : Female	1:5	1:4	4:1	1:1
Arthritis pattern	Mono- or oligoarticular and usually involves larger joints	Symmetrical polyarticular. ≥5 cumulative joints	Axial arthritis, sacroiliitis, can be oligoarthritis or polyarthritis	Polyarticular
Extra-articular pattern	Systemic features are often minimal; Highest uveitis risk (20% patients)	Intermediate risk for uveitis	Enthesopathy, uveitis (20% in adult, less common in children), aortic insufficiency	Fever, rash, serositis, lymphadenopathy, myopericarditis, and Köbner's phenomenon[a]
Relevant laboratory tests	RF almost always negative 65–85% ANA+	15% RF+ 80% ANA+ in RF+ patients, 57% ANA+ in RF-negative patients	90% HLA B27+ (children) RF— absent	Polyclonal elevation of immunoglobulin levels RF, ANA are uncommon and not useful for diagnosis. Increased LFTs, ESR/CRP and ferritin
Prognosis	Variable	Variable, RF + → more disability	Usually chronic	Chronic arthritis in 50%, remission ~1/3 and severe in 20%

[a]Köbner's phenomenon: Still's rash (i.e., faint, evanescent, salmon-colored eruption that occurs with fever) can be triggered by stroking the skin.
Abbreviations: ANA, antinuclear antibody; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; HLA, human leukocyte antigen; LFT, liver function test; RA, rheumatoid arthritis; RF, rheumatoid factor.

Systemic Lupus Erythematosus (SLE)

SLE is a disease seen most often in young women of childbearing age and in cases of drug-induced lupus.

Diagnosis—The clinical features of SLE are listed in Table 8-28.

Table 8-28. Diagnostic Criteria for Systemic Lupus Erythematosus (SLE)

Four or more criteria are needed to make the diagnosis of SLE.

- Malar rash = fixed malar erythema, flat or raised (Figure 8-6)
- Discoid rash = erythematous raised patches, with keratotic scaling and follicular plugging
- Photosensitivity = skin rash as an unusual reaction to sunlight
- Oral or nasopharyngeal ulcers
- Arthritis = nonerosive
- Serositis = pleuritis or pericarditis
- Renal disorder (nephritis): Proteinuria ≥0.5 g or 3+; and cellular casts
- Neurologic disorder: Seizures, and psychosis
- Hematologic disorder: Hemolytic anemia, leukopenia < 4000, lymphopenia < 1500, and thrombocytopenia < 100,000
- Immunologic disorder: Anti-DNA, anti-Sm, positive test for IgM or IgG to anticardiolipin Ab or lupus anticoagulant or false + RPR
- Antinuclear antibody (in absence of drugs known to cause drug-induced lupus syndrome)

Abbreviation: RPR, rapid plasma regain.

Immunologic Features—The mechanism is not completely clear. It is characterized by immune complex deposition. The best example is seen in glomerulonephritis. Genetic factors include HLA-DR2, 3, early complement component deficiencies (C1, 2, 4) and FcγRII, III polymorphisms (Table 8-29).

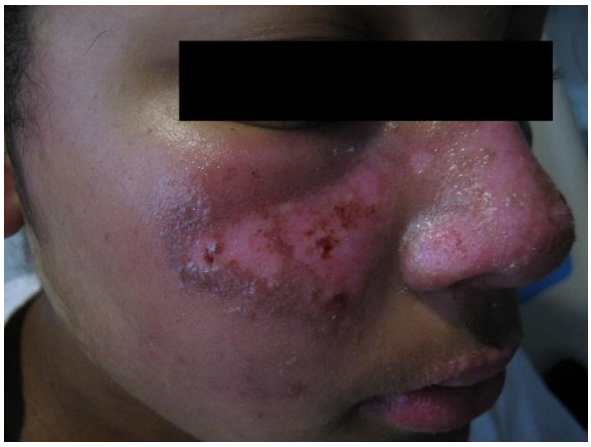


Figure 8-6. Malar rash of systemic lupus erythematosus.
(Reproduced, with permission, from USMLERx.com)

Flash Card Q17

Which type of JIA has the highest risk for uveitis?

Table 8-29. Immunologic Markers in SLE

Antibody	Frequency in SLE	Comments
ANA	99% Very sensitive but specificity ~67%	Negative test essentially rules out SLE Seen in many other connective tissue diseases
Anti ds-DNA	50–60% Very specific but less sensitive	Correlates with active disease and lupus nephritis Very uncommon in other diseases
Anti-Smith	30–40% Very specific but less sensitive	Correlates with interstitial lung disease Very uncommon in other diseases
Anti-Ro (SS-A)	25–30% But see in 60–75% of Sjögren's syndrome	Seen in subacute cutaneous lupus, neonatal lupus syndrome (congenital heart block, thrombocytopenia, and annular rash; "Anti-Ro makes baby's heart GO")
Antihistone	50–70%	Drug-induced lupus

Abbreviation: SLE, systemic lupus erythematosus.

C3 and C4: Decreased in chronic disease because of complement consumption by immune complexes, especially in the setting of glomerulonephritis. Levels return to normal with clinical improvement.

Treatment—Corticosteroids are generally used to treat SLE, but milder forms of the disease can be treated with less potent medications such as NSAIDS. Rash can be treated with topical steroids. Hydroxychloroquine can be used for control of skin disease and arthritis. For kidney disease that threatens kidney function, treatment should involve:

- High-dose corticosteroids
- IV cyclophosphamide
- Mycophenolate mofetil
- Azathioprine
- Methotrexate
- Type I interferons

Antiphospholipid syndrome is commonly seen in patients with SLE, especially in setting of thromboembolic disease, spontaneous abortion, thrombocytopenia, and neuropsychiatric events. Treat with anticoagulants.

Flash Card A17

Pauciarticular JIA

Sjögren's Syndrome (SS)

SS is a chronic inflammatory disorder and lymphoproliferative disease with autoimmune features characterized by a progressive mononuclear cell infiltration of exocrine glands, particularly the lacrimal and salivary glands (autoimmune exocrinopathy).

There are two types of SS:

- **Primary:** Isolated autoimmune disorder. Dry mouth (xerostomia) and dry eyes (xerophthalmia) develop as isolated entities.
- **Secondary:** Complication of connective tissue disorder include RA, SLE, polymyositis (PM), or scleroderma.

Presentation—The most common symptoms of SS are related to xerostomia and xerophthalmia (keratitis sicca). Swelling of parotid gland can be unilateral or symmetrical, and recurrent. Physical findings include:

- Dry skin
- Palpable purpura
- Urticaria
- Arthralgias without arthritis
- Frequent sinus infections
- Obstructive airway disease
- Interstitial pneumonitis (may progress to pulmonary fibrosis)
- Dysphagia
- CNS involvement, including immune-mediated hearing loss, vasculitis, neuropsychiatric manifestations, and mononeuritis multiplex, especially in association with cutaneous vasculitis

SS is also associated with primary biliary cirrhosis, HIV I, and hepatitis C.

Immunologic Markers

- **Ro/SS-A and La/SS-B:** Most clinically significant in primary SS
- Anti-Ro: 60–75%; Anti-La: 40% in primary SS
- ANA + / RF + = 60–80% patients with primary SS

Treatment—In cases of SS, treatment with artificial tears and pilocarpine can improve dryness. Cholinergic agents should be administered for dry mouth. Encourage patients to practice good dental hygiene to prevent dental caries.

Polymyositis, Dermatomyositis, and Inclusion Body Myositis

Inflammatory myopathies are idiopathic diseases of muscle, characterized clinically by muscle weakness and pathologically by inflammation and muscle fiber breakdown. A comparison of the clinical, immunologic, and diagnostic differences can be seen in Table 8-30.

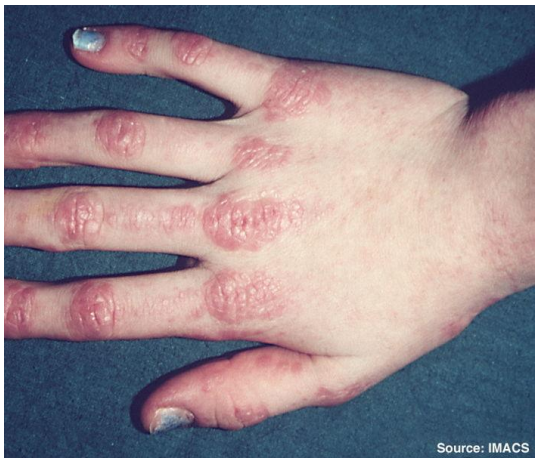
Key Fact

Classic clinical manifestations for polymyositis/dermatomyositis is a difficulty getting up from a chair, climbing stairs, reaching above the head, or the combing hair. Distal muscle and ocular involvement are uncommon (distinguished from myasthenia gravis). Sensation and reflexes are preserved.

Presentation—Cutaneous signs:

- Heliotrope rash on the upper eyelids (Figure 8-7A)
- Erythematous rash on the face
- May also involve the back and shoulders (shawl sign), neck and chest (V-shape), and knees and elbows
- Gottron's papules (i.e., violaceous papules overlying dorsal interphalangeal or metacarpophalangeal areas, elbow, or knee joints) (Figure 8-7B)
- Photosensitivity
- Nail cracking, thickening, and irregularity with periungual telangiectasia
- Mechanic's hand: Fissured, hyperpigmented, scaly, and hyperkeratotic; also associated with increased risk of interstitial lung disease

A



B

Figure 8-7. (A) Heliotrope rash. (B) Gottron’s papules.

(Figure A reproduced, with permission from Dugan, Elizabeth M; Huber, Adam M; Miller, Frederick W; Rider, Lisa G; & the International Myositis Assessment and Clinical Studies (IMACS) Group; Figure B reproduced, with permission, from Wikimedia Commons.)

Table 8-30. Comparison of Autoimmune Myositis

	Polymyositis (PM)	Dermatomyositis (DM)	Inclusion Body Myositis
Epidemiology	Females > Males 18 years of age and older	Females > Males Children & adults	Males > Females 50 years of age and older
Immune markers	Anti-Jo-1 associated with interstitial lung disease Anti-SRP associated with acute onset, refractory to treatment	Anti-Jo-1 Anti-Mi-2 associated with rash, good outcome. Antibodies rare in children	
Pathophysiology	Cell-mediated endomysial inflammatory infiltrate with primarily CD8 T cells. Not Ab-mediated	Perifascicular atrophy due to microvascular damage. Ab-mediated complement attack	CD8 T cells
Clinical presentation	Subacute onset of proximal muscle weakness; systemic autoimmune disease occurs more commonly; and respiratory problems	Subacute onset of proximal muscle weakness; esophageal dysmotility often seen; ECG changes; skin findings (see below); and respiratory problems	Proximal muscle weakness and atrophy that may involve distal spread; progresses over months to years; and dysphagia
Diagnosis	Biopsy, CPK, and EMG and nerve conduction studies		Biopsy may reveal basophilic-rimmed vacuoles as well as characteristic filamentous inclusions and vacuoles (i.e., ragged red fibers on EM); muscle enzymes normal-to-mildly elevated; and no assoc. with ANA
Treatment	Steroids; consider IVIG and cyclophosphamide Hydroxychloroquine for skin lesions of DM		Resistant to therapy; but, consider steroids, IVIG, and immune suppression
Other associations	May be seen in patients with scleroderma, SLE, and MCTD	Associated with cancer in patients older than 50 years of age	Possible association with viral etiology
Prognosis	50% of patients will go into remission and stop therapy within 5 years. Poor prognostic indicators include delay in diagnosis, older age, recalcitrant disease, malignancy, interstitial pulmonary fibrosis, dysphagia, leukocytosis, fever, and anorexia		The older the age at onset of the disease, the more rapid is the loss of strength and function

Abbreviations: ANA, antinuclear antibody; CPK, creatinine kinase, DM, diabetes mellitus; ECG, electrocardiography; EM, electromicroscopy; EMG, electromyelography; IVIG, intravenous immunoglobulin; MCTD, mixed connective tissue disease; SLE, systemic lupus erythematosus.

Scleroderma or Progressive Systemic Sclerosis

This systemic disorder is characterized by excess collagen deposition in skin and viscera. Vascular abnormalities are also present, including vasospasm and microvascular occlusion.

Clinical Features

- Thickening of skin
- Calcinosis and telangiectasias occur later in disease
- **Raynaud's phenomenon:** Universal among patients with systemic sclerosis
- Esophageal dysmotility: Most common GI manifestation and may be accompanied by dysphagia, reflux, and strictures. Decreased motility results in bacterial overgrowth, diarrhea, and malabsorption
- Pulmonary parenchymal fibrosis common but often asymptomatic
- Cardiac involvement
- Renal crisis, especially in diffuse variant, which can lead to hypertension, microangiopathy, and renal insufficiency. Treatment of choice is ACE inhibitors
- **CREST syndrome:**
  - **C**alcinosis
  - **R**aynaud's syndrome
  - **E**sophageal dysmotility
  - **S**clerodactyly
  - **T**elangiectasias (in CREST scleroderma, is limited to distal extremities)

Immunologic Features

- Pathogenesis unknown
- Polyclonal hypergammaglobulinemia common, plus presence of ANA and antiendothelial Abs
- ANA: 80% of patients have ANA in centromere or nucleolar pattern
- Antiendothelial Ab
- Anticentromere antibody (good prognosis if positive)
- Antiscleroderma-70 antibody more common with diffuse disease

Diagnosis—Anticardiolipin antibody or antiscleroderma-70 antibody Ab testing may be used to confirm the clinical suspicion. Skin biopsy is rarely needed.

Treatment—Symptomatic and problem-oriented. Raynaud's phenomenon may respond to a calcium channel blocker. Corticosteroids are reserved for resistant symptoms. Esophageal symptoms are useful for protein pump inhibitors, H_2 blockers, and promotility agents. Inflammatory interstitial lung disease (ILD) is treated with cyclophosphamide.

IMMUNE REJECTION AND ORGAN TRANSPLANTATION

Immune rejection occurs depending on the level of matching and disease.
Solid organ transplantation:

- Rejection revolves around the donor's immune reaction to the vasculature of the transplanted organ.
- GVHD can occur due to harbored, competent immune cells in the donor graft attaching the immunosuppressed host.

Bone marrow transplantation:

- Require human leukocyte antigen (HLA) compatibility, or matching
- Major complication tends to be graft-versus-host disease (GVHD)
- Rejection occurs when the host immune system remains competent. It will recognize the donor cells as foreign and kill them mainly through T-lymphocyte- and natural killer (NK) cell-mediated reactions

IMMUNE REJECTION

Getting to Know HLA (Figure 8-8)

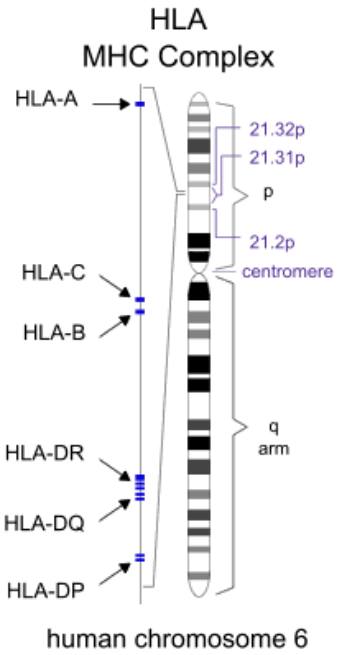


Figure 8-8 MHC Complex. Peptide presentation on cell surface to discriminate self- versus non–self-antigens. Three regions on chromosome 6 with large number of highly polymorphic genes: class I, class II, and class III.
Abbreviations: HLA, human leukocyte antigen; MHC, major histocompatibility complex.
(Reproduced, with permission, from Wikimedia Commons.)

Key Fact

The class I region has both classical (A, B, C) and nonclassical HLA genes. The nonclassical genes (E, G, F) have unique properties. HLA-G and F are expressed on the extra villous trophoblast. Their key role is to protect the fetus from maternal immune rejection. They have a more limited polymorphic profile.

HLA Nomenclature

The HLA is designated by a letter and a single- or a two-digit number.

- Class I antigens = A, B, or C + digit
  - Example: HLA*A0201
- Class II antigens = D + M, O, P, Q, or R + A or B (for α or β chain) + digit
  - Example: HLA-DRB1*0401

The **antigenic designation** is what the antibody sees. One antigen may be coded by numerous alleles.

Alleles are designated first by two-digit numbers, indicating a group of alleles that encode a particular antigen at the serologic level. The allele is further subtyped by a two-digit number, signifying the specific allele at the sequence level.

The difference between the serologic level and the sequence level is that an antigen that the antibody recognizes may be encoded by different polymorphisms. The antibody might not be able to tell the difference between these polymorphic sequences; however, T-lymphocyte epitopes that are linear and presented in the context of the HLA molecules might induce rejection to a polymorphism that was serologically indistinguishable. Thus sequencing gives the so called **high resolution typing**.

HLA is expressed in a codominant fashion. Each locus has two alleles; and, as individuals, we each receive one from our mother and one from our father.

Seeing the Alloantigen

Figure 8-9 represents the direct and indirect allorecognition.

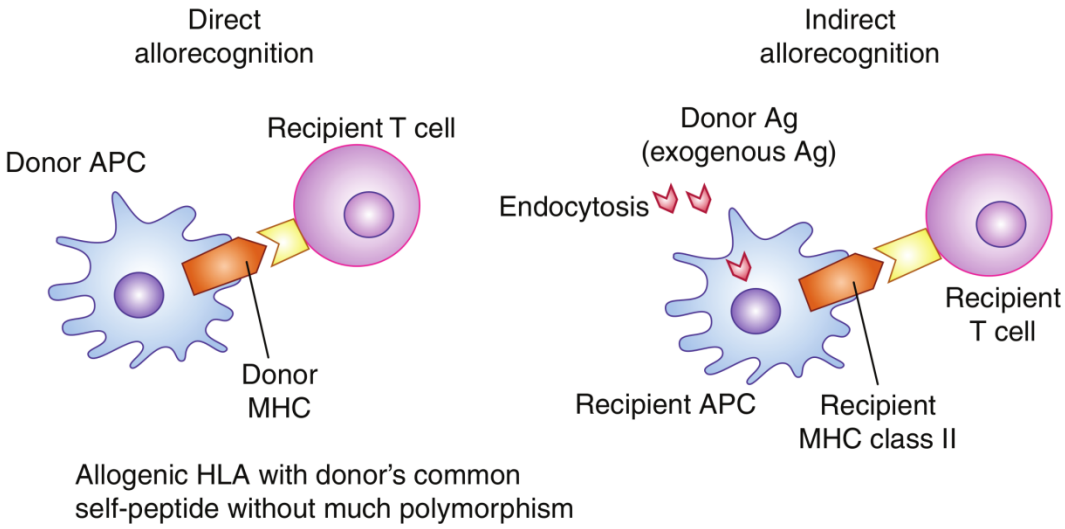


Figure 8-9. Direct and indirect allorecognition. The figure on the left represents the direct allorecognition, the figure in the right represents the indirect allorecognition. Notice that the recipient APC is used in the indirect allorecognition instead of the donor antigen-presenting cell (APC) as in the direct allorecognition.

ORGAN TRANSPLANTATION

Types of Transplants

- **Syngeneic:** Genetically identical, twins
- **Allogeneic:** Nongenetically identical. The reactive antigens are different within the same species (e.g., HLA or other polymorphic gene products): These antigens are **alloantigens**.
- **Xenogeneic:** Graft across species

Allogeneic Transplants

***Solid-Organ Transplantation*—**Kidney, liver, lung, pancreas, cornea, and bone. **Cornea, bone, and joint tissues do not require immunosuppression.** The solid organs require, and survival depends on, immune suppression. Reasons for solid-organ rejection are mediator-specific, which also affects the timing of the rejection from very early to late (Table 8-31).

Table 8-31. Solid-Organ Rejection

Type of Reaction	Mediator	Timing	Pathophysiology	Comments
Hyperacute	Preexisting Ab	On the table after anastomosis Minutes to hours	Ab to antigen compliment activation and endothelial damage thrombosis, PMN	Black organ, ABO-incompatible (natural IgM). Big issue in xenotransplantation: Remove!
Accelerated	Preexisting Ab but minor antigens not in typical screening	Within 2–5 days	IgG non-compliment–fixing Ab, endothelial damage, thrombosis, and vasculitis	Try immunosuppression
Acute	Alloreactive T-lymphocyte +/– Ab	After 7 days to 3 months	Direct killing by T lymphocyte, cell infiltrations, and endovasculitis	CD8+ cell and steroids
Chronic	DTH-like reaction T cells, cytokines	Months to years	Vessel smooth muscle proliferation, fibrosis, and occlusion	Alloreactive CD4+ graft vasculopathy, or accelerated graft arteriosclerosis

Abbreviations: Ab, antibody; PMN, polymorphonuclear neutrophil.

Testing for Transplantation

A test that is rarely administered today, but is still testable, is the **mixed leukocyte reaction (MLR)**.

Donor A's mononuclear cells are cultured with donor B's mononuclear cells. There will be proliferation; however, this will result in a mess since both donors are reacting to each other. Their HLA types are different.

If donor A's cells are irradiated or drug-treated to make them incapable of proliferation. Donor A's cells serve as a stimulatory source. Donor B's cells are **mixed** with the non-HLA identical stimulator cells. Since they are foreign to donor B, the donor B cells will clonally expand in both CD4+ and CD8+ effector cells.

The proliferation can be measured by incorporation of radioactive material in the media. If there was no proliferation, the donor A cells would be seen as self and, thus, ignored.

The other concerns and risks besides HLA associated with transplantation are reviewed in Table 8-32.

Table 8-32. Testing for Transplantation

Test	Target	Method	Outcome
ABO	Natural IgM antibodies	Patients' RBCs with α-A or α-B sera	If A or B is present, the RBCs agglutinate; agglutination to both means AB type
HLA typing	Polymorphic HLA alleles	Past = serologic (standard sera banks) Present = PCR	HLA type Class I or II, loci; Numbers
Antibody screening *Risks: Transfusion Pregnancy Transplantation	Antibodies of allogenic HLA	Complement-mediated lyses **or** flow cytometry of recipient to donor pool	Percent reactive antibodies; percentage of donor pool with which patients react
Cross matching	Ab of recipient to the actual donor	Same as above	Risk of rejection

Abbreviations: HLA, human leukocyte antigen; PCR, polymerase chain reaction.

Bone Marrow Transplantation

Bone marrow transplantation can be used to treat:

- Malignancy
- Inborn errors of metabolism
- Hematopoietic disorders
- Primary immune deficiency

A full match is considered six out of six, at **A, B, and DRB1 alleles**. A match does not have to go out to the four digits and an allele group; the first two numbers will suffice. Trends are changing, and sequence-based four-digit allele typing is being used in some types of BMT transplants. Ten-allele matches are also becoming more common, adding **C** and **DQ** to the search.

STEM CELL TRANSPLANTATION

In the technique of stem cell transplantation (SCT) a donor's stem cells, designated by being CD 34+, are harvested and given to a recipient or host. These hematopoietic stem cells are pluripotent and can **replace** the host's stem cells either completely, creating a chimera, or with a partial engraftment of the stem

Flash Card Q18

Which tissues can be transplanted across ABO?

cell, creating a mixed chimera to provide a **cure** for many diseases. The type of donor and level of engraftment depends on many factors, including:

- Disease
- Host
- Age of donor
- Amount of chimerism required for cure
- Immunologic status of the host.

SCT is by far most widely used to treat malignancy; however, with recent advances, more diseases, particularly immunodeficiency, are being treated by this method. SCT is the only definitive cure for many immunodeficiencies. SCT is also being used to treat aplastic anemia and certain genetic disorders.

Syngeneic, Allogeneic, and Haploidentical SCT

There are two main types of SCT: **syngeneic** and **allogeneic**. The "**s" in syngeneic** stands for the **same**. The source can be autologous, or from an identical twin. Allogeneic transplants are from a nonidentical donor, and are much more common. Allogeneic SCT relies on HLA matching for success and can come from different sources (Table 8-33). Haploidentical (i.e., exactly matching half of another person's HLAs) transplants may also be an option.

Table 8-33. Sources of Stem Cell Transplantation

	Advantages	Disadvantages	Pearls
Bone Marrow	Large doses and low drop in blood counts	Anesthesia, possible injury, and pain	Larger number of CD34+ cells
Peripheral Blood	No anesthesia and leukophoresis	Lower number of CD34+ cells and larger volume must be extracted	Mobilization for enrichment of stem cells and 15 mL/kg of donor for harvest
Cord Blood	Immunologically immature and enriched naturally with CD34+ cells; ease of collection and less stringent HLA matching	Small dose of CD34+ cells, and slower engraftment	Multiple cords have been used

Abbreviation: HLA, human leukocyte antigen.

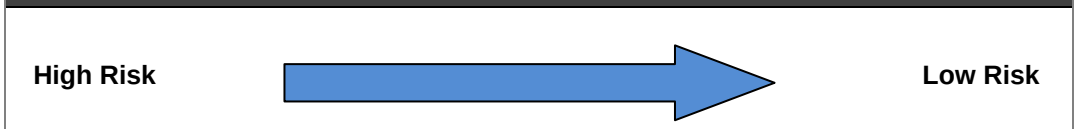
Flash Card A18

Cornea, bone, joint tissues, and hematopoietic stem cells

Risk of Nonengraftment

The order of risk of nonengraftment is shown in Table 8-34.

Table 8-34. Risk for Allogeneic Transplant Rejection



Mismatched Unrelated	Mismatched Cord	Matched Unrelated	Matched Cord	Matched First-Degree Relative	Syngeneic
Class II Mismatch > Class I Mismatch					

Infection and recipient age are related. The older the patients, the more likely they are to be infected chronically with a virus such as Epstein-Barr virus (EBV), cytomegalovirus (CMV), or adenovirus, which can be difficult to control during conditioning. Risk of infection is cause for significant morbidity and mortality.

Mnemonic

Other risks for nonengraftment include: LORD NIC

- **L**ow stem cell dose
- **O**lder age of donor
- **R**ecipient T-lymphocyte function
- **D**egree of HLA mismatch
- **N**atural killer cell function
- **I**nfection
- **C**onditioning regimen

Indications

Malignancies such as leukemia are the most common reasons for SCT. Solid tumors such as neuroblastoma are treated medically, and the patient is rescued with a previously harvested autologous SCT.

Hematopoietic disorders such as aplastic anemia, myelodysplasia, and hemoglobinopathies have been treated with SCT.

Inborn errors of metabolism, such as Hurler's syndrome and osteopetrosis, have been treated using SCT.

In cases of **primary immunodeficiency**, SCT has been used to **cure** the immunologic defects in the following diseases:

- Severe combined immunodeficiency (SCID)
- Chronic granulomatous disease
- X-linked hyper IgM syndrome
- Wiskott-Aldrich syndrome
- Chediak-Higashi syndrome
- Immune dysregulation, polyendocrinopathy, enteropathy, and X-linked inheritance (IPEX)
- Familial hemophagocytic lymphohistiocytosis
- Griscelli's syndrome

Flash Card Q19

To which HLA loci does a 6/6 HLA match refer?

Severe Combined Immunodeficiency (SCID)

> ***Key Fact***
>
> SCID is an immunologic emergency. Survival outcomes are linked to age. Prior to 2 months of age, stem cell transplant in SCID has a survival rate of 95%. After 6 months of age, the survival rate declines rapidly.

SCID deserves some special attention because not all SCIDs are alike. Genotype or phenotype predicts the outcome. The presence or absence of T and B lymphocytes and NK cells help define the particular type of SCID and are also very important in SCT treatment. Patients with T–, B+, and NK– SCID tend to do best. These patients, without matched siblings, have traditionally undergone haploidentical transplant with graft T-lymphocyte depletion and no conditioning. Patients with ADA (T–, B–, and NK–) tend to have the worst prognosis. The presence of NK cells with activity might make conditioning necessary. X-linked SCID (with B-lymphocytes present, but not functional) transplants have been attempted without conditioning; however, the B-lymphocyte engraftment is low and may require lifelong Ig replacement. Conditioning improves B-lymphocyte engraftment, but the agents used for host conditioning have other adverse effects to consider. Graft-versus-host disease (GVHD) is the most common adverse effect.

SCID is unique in the use of haploidentical transplants due to the almost total lack of host immunity. Most other types of immune deficiency disease that require SCT necessitate some kind of conditioning protocol that is disease specific. SCID conditioning may be nonmyeloablative.

> ***Key Fact***
>
> In the graft-versus-leukemia effect the graft T lymphocytes contribute to the eradication of the tumor. These same T lymphocytes can cause GVHD.
>
> The inhibitory killer immunoglobulin-like receptors, also called killer inhibitory receptors (KIRs), on donor NK cells are inhibited by cells that display HLA I markers that they recognize. Recipient leukemia cells express HLA I that is different from donor HLA I and results in donor NK-mediated cellular killing of leukemic cells.

> ***Flash Card A19***
>
> The donor and recipient have been matched at HLA-A, HLA-B, and HLA-DRB1

Conditioning

In primary immunodeficiency, conditioning regimens vary from ablative, to reduced intensity, to no conditioning. If T-lymphocyte function is intact, an ablative conditioning regimen is usually required. This can include medication and/or total body irradiation. In reduced intensity, a combination of monoclonal antibodies and chemotherapeutic drugs is used sometimes at a lower dosage. Conditioning affects outcomes such as GVHD and engraftment. The following drugs are used:

Myaloablative regimen: Destroys the host hematopoietic cells. Bone marrow needs to be replaced. Includes total body irradiation, busulfan, etoposide, cytarabine, cyclophosphamide, cytosine arabinoside, anti-CD45, anti-CD66, anti-CD20 antibodies.

Nonmyeloablative Regimen: Causes minimal cytopenia with significant lymphopenia. Includes fludarabine, cyclophosphamide, antimyocyte globulin (ATG), low-dose total body irradiation, anti-CD52 Ab. Good if graft-versus-tumor effect is desired, minimizes GVHD, minimizes graft rejection, minimizes opportunistic infections. Donor T cells will eliminate host hematopoietic cells with time.

Reduced-Intensity Regimen: Doesn't fit either of the previous categories, causes cytopenia. Includes low-dose busulfan, low-dose melphalan.

Patients with severe organ toxicity, DNA repair defects, or telomere repair defects require minimal-intensity conditioning (cannot tolerate myeloablative or nonmyeloablative regiments).

Toxicities include mucositis, nausea, emesis, alopecia, diarrhea, rash, peripheral neuropathy, infertility, interstitial lung disease, sinusoidal obstruction syndrome. Total body irradiation complications include abnormal pulmonary function, cataracts, sicca syndrome, and thyroid dysfunction.

GRAFT-VERSUS-HOST REACTION

Graft-versus-host (GVH) reaction is the recognition of the host tissues by **mature** donor **T lymphocytes** as being foreign through the recognition of host alloantigens.

Pathophysiology

Traditional HLA matching is based on A, B, and DR alleles. Mismatch at any allele increases the risk of GVH reaction. Even in a six-out-of-six matched transplant, GVH can happen due to the other mismatched HLA alleles or to minor HLA antigens and non-HLA-encoded antigens.

Other important non-HLA factors are polymorphisms in innate immunity components, such as nucleotide oligomerization domain (NOD) and Toll-like receptors (TLRs). The important **cytokines** in GVH reaction are IL-10, TNFα, and IFNγ.

TYPES OF GVH REACTION

Acute and Chronic GVH Reaction

GVH can manifest in multiple organ systems. Acute and chronic GVH differ in timing, immune mechanism, prophylaxis, and treatment (Table 8-35). Acute and chronic reactions may affect the same organ in different ways (Table 8-36).

Table 8-35. Comparison of Acute and Chronic Graft-Versus-Host Reaction

	Acute GVH Reaction	Chronic GVH Reaction
Timing	First 100 days	>100 days
Cells	CD45RO+ T-lymphocyte and neutrophil engraftment (**important**)	CD4+ helper T lymphocytes
Prophylaxis	Methotrexate, cyclosporine, tacrolimus, corticosteroids, antithymocyte globulin, and T-lymphocyte depletion	T-lymphocyte depletion
Treatment	Corticosteroids; alternatives include cyclosporine, tacrolimus, antithymocyte globulin, and mycophenolate	Corticosteroids, corticosteroids plus cyclosporine, thalidomide, and ursodeoxycholic acid

Table 8-36. Clinical Manifestation of Graft-Versus-Host (GVH) Reaction

	Clinical Manifestations	
	Acute GVH Reaction	**Chronic GVH Reaction**
Skin	Maculopapular rash	Dyspigmentation, alopecia, sclerotic features, and nail dystrophy loss
Mouth		Ulcers, xerostomia
Eyes		Dry and sicca syndrome
Musculoskeletal		Fasciitis, myositis, and contractures
Gastrointestinal	Watery diarrhea, severe abdominal pain, bloody diarrhea, and ileus	Weight loss, webs, and strictures
Liver	Cholestatic hyperbilirubinemia	Jaundice transaminitis
Lung		Restrictive or obstructive defects and bronchiolitis obliterans
Heart		Pericarditis
Marrow		Cytopenias
Other	Fever	

Key Fact

Chronic GVH reaction is the major factor in long-term survival in SCT.

Sinusiodal Obstruction Syndrome

Sinusoidal Obstruction Syndrome (SOS) (formerly known as hepatic sinusoidal veno-occlusive disease [VOD]) is associated with conditioning therapy and drug toxicity. Single-dose radiation, alkaloids (e.g., busulfan, cyclosporine, and methotrexate) for prophylaxis, and sirolimus.

Diagnostic criteria for SOS After SCT:

- **Seattle Criteria.** Two of the three symptoms within 20 days of SCT: bilirubin > 2 mg/dL, hepatomegaly or right upper quadrant pain, >2% weight gain due to fluid retention
- **Baltimore Criteria.** Bilirubin >2 mg/dL plus two of the three symptoms: tender hepatomegaly, >5% weight gain, ascites

Graft Manipulation

T-lymphocyte depletion is the major way of preventing or reducing the risk of both acute and chronic GVH reactions. Mature T lymphocytes from the graft are implicated in the disease. Eliminating them might represent an advantage from this perspective; however, if all mature T lymphocytes are purged, engraftment would be severely affected, as would immune reconstitution, graft-versus-tumor effect, and infection complications.

Key Fact

Complete depletion of graft T lymphocytes leads to nonengraftment.

These methods involve ex vivo manipulation of the donor cells with methods such as soybean lectin and E-rosetting, as well as E-rosetting and CD34+ selection, and monoclonal antibodies like anti-CD3, anti-CD2, anti-CD6, anti-CD25, or anti-CD52.

Special Conditions

- **SCID:** During birth a maternal-fetal transfusion of blood and immune cells can occur. When mature, maternal T lymphocytes engraft the baby, a maternal GVH reaction may occur with the infant's tissue as a target. In this case, the cells are likely CD8+ and clonal.
- **Blood transfusions**: In an immunocompromised host, blood transfusion with a **nonirradiated** or **nonleukoreduced** product can result in competent donor T lymphocytes, which are transfusion-derived, causing GVH reaction. Very uncommonly, this can even happen in an immunocompetent host when whole blood is given.

Engraftment

Neutrophil engraftment is defined as 3 consecutive days with an ANC $\geq 0.5 \times 10^9$/L, or one day with a count of $\geq 1.0 \times 10^9$/L.

Platelet engraftment is defined as the first day when the platelet count is $>20 \times 10^9$/L on 3 consecutive measurements within 7 days, with no transfusions in the preceding 7 days

Umbilical cord cells take the longest of the stem cells to engraft.

Flash Card Q20

What are the clinical features of SOS?

IMMUNE ENDOCRINOPATHIES (THYROID, DIABETES, AND ADRENAL)

Immune endocrinopathies are autoimmune pathological changes of endocrine organs in predisposed individuals, induced by infection, tissue injury, or other environmental factors.

Graves' Disease

***Immune Mechanisms*—Antibodies to thyryoid-stimulating hormone (TSH) receptor that stimulate the thyroid gland**, are specific for the disease. Thyroid peroxidase and thyroglobulin antibodies are frequently found, but they are not disease-specific. B lymphocytes and CD4 and CD8 T lymphocytes are involved (i.e., intrathyroid T lymphocytes with B lymphocytes organized in germinal centers), and **T_h2 cells are primarily responsible** for the disease.

***Clinical Features*—Hyperthyroidism** (palpitations, tremor, heat intolerance, sweating, anxiety, emotional lability, weight loss with increased appetite) with diffusely enlarged goiter and **exophthalmopathy** as well as pretibial **myxedema** may occur. The disease occurs more often in women than men, with a ratio of 7:1.

Genetic Association

- Monozygotic twins 20–40%
- 10% occurrence in siblings
- **HLA-DR3** (whites)
- **CTLA-4** alleles
- HLA-DR expression by thyroid epithelium

***Prognosis*—**Fifty percent of patients with hyperthyroid Graves' disease will relapse after 1 year of treatment with antithyroid drugs. The level of autoantibody to TSH receptor is a useful predictor for relapse and remission.

Hashimoto's Thyroiditis

***Immune Mechanisms*—**Antibodies exist for thyroid peroxidase and thyroglobulin, as well as TSH receptor, **correlating with lymphocyte infiltration and decrease with treatment**. Immune mechanisms involved include IgG1/IgG3 complement fixation. There is FAS expression on the thyroid with cytotoxic destruction. Thyroid germinal centers with antibody production are noticed. Epithelial cells are enlarged with distinctive eosinophilic cytoplasm due to increased mitochondria (**Hürthle cells**).

Flash Card A20

Tender hepatomegaly, weight gain due to fluid retention, and hyperbilirubinemia

***Clinical Features*—**Patient usually presents with an **atrophic thyroid** but may have goiter. The disease is defined by chronic progressive **autoimmune thyroiditis**. Clinical features of hypothyroidism (fatigue, weakness, cold intolerance, weight gain, constipation, dry skin, depression, growth failure, delayed puberty). **Prevalence increases with age**, with more women afflicted than men (7:1).

***Genetic Association*—**Monozygotic twins 30–60%, HLA-DR3 and CTLA-4 are often involved.

***Diagnosis*—**Most hypothyroid patients are thought to have Hashimoto's disease, although thyroid antibodies are sent for confirmation. High-titer thyroid Abs are present, with clinical presentation of pyogenic thyroiditis that is characterized by thyroid area pain and fever. Other causes of thyroiditis (postpartum, acute, subacute, and silent) need to be ruled out.

Autoimmune Polyendocrine Syndromes

***Autoimmune Polyendocrine Syndromes-1 (APS-1)*—**Also known as **autoimmune polyendocrinopathy, candidiasis-ectodermal dysplasia (APECED)**. This rare disorder is characterized by chronic **mucocutaneous candidiasis** of the mouth and nails (this is postulated to be mediated by anti- IL-17 or IL-22 antibodies), **hypoparathyroidism**, and **autoimmune adrenal insufficiency**. Usually, the disease first manifests **in childhood,** and the patient then develops these three diseases in the first 20 years of life. The other autoimmune endocrinopathies include:

- GI disease
- Chronic hepatitis
- Autoimmune skin disorders
- **Ectodermal dystrophy**
- Keratoconjunctivitis
- Immunologic defects
- Asplenia
- Cholelithiasis
- Mucosal cancer

APS-1 is associated with **autoimmune regulator (AIRE)** gene mutation on chromosome 21q22.3. In this pathology, autoreactive T cells in the thymus escape negative selection by not being exposed to self-antigen in the thymus. Antibodies are formed to 21-hydroxylase (adrenal), IL-17 and IL-22 (candidiasis), islet cells (DM), IFNω. Autoimmunity affects parathyroid (onset in 20s) > adrenal > gonadal > gut. APS-1 does not usually involve DM or the pituitary gland. APS-1 affects more women than men. Autoantibody to calcium-sensing receptor, CYP1A1, CYP17, CYP21 A2, and tryptophan hydroxylase could also be present.

Flash Card Q21

What is the function of AIRE?

Flash Card Q22

Which of the following glands is not involved in APS-2: adrenal, thyroid, parathyroid, or gonads?

Diagnosis is made by having any two autoimmune conditions, or one autoimmune condition plus a mutation in the AIRE gene.

Autoimmune Polyendocrine Syndrome-2 (APS-2)—This is also known as **Schmidt's syndrome**. It is more common than APS-1. **Adrenal** > type I diabetes = thyroid > gonadal. It is the **most common of the autoimmune endocrinopathies** and occurs more often in women than in men (3:1). There is no evidence of **hypothyroidism**. APS-2 is associated with:

- Celiac disease
- Vitiligo
- Pernicious anemia
- Myasthenia gravis
- Stiff-person syndrome
- Alopecia
- Sjögren's syndrome
- Antiphospholipid antibodies
- Rheumatoid arthritis

Autoantibodies are to cytochrome enzymes, which include CYP21A2, CYP17, and CYP11A1. Antibodies to 21-hydroxylase, tissue transglutaminase (IgA) may be present.

Genetics: Fifty-percent familial (autosomal dominant and polygenic recessive) and HLA DRB0404. CTLA-4 gene polymorphism is associated with APS-2 component disorders.
APS-2 is commonly diagnosed between 20 and 40 years of age.

Immune Dysregulation, Polyendocrinopathy, Enteropathy, X-Linked Syndrome

Flash Card A21

AIRE is a transcription factor expressed in medullary thymic epithelial cells that is responsible for the expression of antigens found elsewhere in the body, and thus guides T-cell–negative selection.

Immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome is a form of **autoimmune enteropathy**. This life-threatening disorder typically presents during infancy and is characterized by dermatitis, intractable diarrhea, and recurrent infections, and is associated with autoimmune endocrinopathy.

Clinical Features—Dermatitis is a key feature of this condition. Diarrhea is severe and a biopsy of the small bowel shows partial or complete villous atrophy, a mononuclear cell infiltration in the lamina propria, and increased expression of MHC class II antigens. This autoimmune disease is diverse and includes:

- Insulin-dependent diabetes often at infancy
- Membranous glomerulonephritis
- Autoimmune cytopenias: Hemolytic anemia, idiopathic thrombocytopenic purpura (ITP), autoimmune neutropenia

Flash Card A22

Parathyroid

- Autoimmune hepatitis
- Hypothyroidism
- 50% of patients have antienterocyte antibodies.

Genetics—Maps to the X-chromosome in the region of the *WAS* gene (Wiskott-Aldrich), but is distinct from that gene—Xp11.23-Xq21.1. **FOXP3 is a transcription factor responsible for the production of T regulatory cells inside the thymus. Mutation of FOXP3 results in depletion of Tregs.** CD25 (IPEX-like syndrome) and STAT5B (autosomal recessive) mutations can also cause an IPEX-like presentation.

Treatment—Without early recognition and treatment**,** children with IPEX usually die in the first 2 years of life from sepsis or failure to thrive. Treatment of IPEX involves bone marrow transplant. There has also been clinical response to cyclosporine, tacrolimus, and sirolimus, which support the role of T lymphocytes.

Autoimmune Lymphoproliferative Syndrome (ALPS)

Autoimmune lymphoproliferative syndrome (ALPS) is characterized by **autoimmunity** (typically autoimmune cytopenias) and **lymphoproliferation** (lymphadenopathy and splenomegaly). There is also an increased risk of **lymphoma.** This disorder is caused by a **defect in lymphocyte apoptosis.**

Clinical Features

- Lymphoadenopathy and/or splenomegaly
- Elevated α/β double-negative T cells
- Defective lymphocyte apoptosis
- Elevated IL-10, vitamin B_{12}, or IL-18 levels
- Autoimmune cytopenias with hypergammaglobulinemia
- Positive family history

Genetics

- Type I—FAS mutation (can be somatic or germline)
- Type Ib—FAS ligand mutation
- Type IIa—Caspase-10 mutation

Treatment—Cytopenias and lymphoproliferation are treated with immunosuppressives, such as corticosteroids, mycophenolate mofetil, cyclosporine, tacrolimus, or sirolimus. Bone marrow transplantation is curative.

Addison's Disease

Addison's disease is a chronic disorder of adrenal cortex characterized by deficient production of glucocorticoids, mineralocorticoids, and androgens, with an increased secretion of adrenocorticotropic hormone (ACTH). In developed countries, the most common cause is autoimmunity, accounting for 75–80% of adrenal insufficiency. In developing countries endemic with TB, TB is the main cause (10–20%) of adrenal insufficiency.

Classification—APS-1, APS-2, and primary adrenal failure. The implicated autoantibody is **to the adrenal cortex enzyme 21-hydroxylase**, and it is found in 90% of recent-onset patients.

Genetics—HLA B8 and DR3.

Clinical Features—Weakness, fatigue, anorexia, orthostasis, nausea, myalgias, salt cravings, hyperpigmentation, shock, fever, abdominal tenderness, neuropsychiatric symptoms.

Diagnosis—Failure of serum cortisol to rise after ACTH stimulation in presence of elevated basal ACTH levels.

Treatment—Addison's disease patients require a lifetime treatment with oral mineralocorticoids and glucocorticoid replacement. Stress doses of steroids are needed for illnesses and surgeries. Monitor for the development of other autoimmune disorders.

Primary Adrenal Failure

Clinical Features—Occurs more often in males than females in the first two decades of life; thereafter, it is seen more often in women than men. Decreased aldosterone with increased renin. Associated with other endocrine diseases such as APS-1 and APS-2.

Immune Mechanisms—Autoantibody to CYP21A2 (enzyme is necessary to make cortisol), involving both cellular and humoral injury.

Genetic Association—HLA DRB*0404, DQ8, and MICA.5.1.

Diabetes Mellitus

***Type IA*—Immune Mechanisms or Immunopathology:** Immune-mediated destruction of islet beta cells in the pancreas. Associated with autoantibodies to glutamic acid dehydrogenase **(anti-GAD65)**, insulin, tyrosine phosphatase, and

zinc T8 transporter. Destruction of beta cells probably requires T lymphocytes. May be related to infections from coxsackievirus B4 and enterovirus. Ninety percent of white children with DM have this disorder. Thirty percent of black and Hispanic children with diabetes do not have anti-islet autoantibody or high-risk HLA alleles. Type 1A is characterized by **low insulin level and normal weight**.

Genetic: Family history of autoimmune diseases, monozygotic twins 30%. Ninety percent of patients will have the following haplotypes: **DR3,DQ2** (DQ2 = DQA1*0501 and DQB1*0201) and **DR4, DQ8** (DQA1*0301 and DQB1*0302).

Type IB—Anti-insulin receptor antibodies block the ability of insulin to bind to its receptor. Thus, insulin levels are high. It is associated with SLE and scleroderma. Patients can present with hyperglycemia, euglycemia, and hypoglycemia.

Type II—Noninsulin-dependent, with anti-islet cell autoantibody. The majority of **adults** with DM have this type. Incidence increases with age and 5–20% express anti-islet cell autoantibody with accelerated loss of insulin secretion.

Insulin Autoimmune Syndrome—Autoantibody that reacts with insulin, which can be monoclonal or polyclonal. The monoclonal antibody is associated with B-lymphocyte tumors. Polyclonal antibody follows therapy with sulfhydryl-containing medications such as methimazole (i.e., treatment for Graves' disease). Patients present with recurrent hypoglycemia.

Other Immune Endocrinopathies

Idiopathic Hypoparathyroidism

- Part of APS-1 in children
- Parathyroid deficiency causes hypocalcemia

Premature Ovarian Failure

- Defined as amenorrhea, increased gonadotropin levels, hypoestrogenism before 40 years of age
- Can be idiopathic and isolated; or also associated with adrenal insufficiency (has antibodies to 21-hydroxylase, 17-hydroxylase that cross-react with theca interna/granulosa layers of ovarian follicles) or only idiopathic

Lymphocytic Hypophysis

- Inflammation of the pituitary gland
- Occurs with pregnancy, postpartum, anti-CTLA-4 Ab (ipilimumab)
- Antipituitary antibodies have been found in some patients
- Fatigue, headache, visual field deficits
- Diagnose with pituitary biopsy
- Treat by replacing anterior pituitary hormones

POEMS Syndrome

- Polyneuropathy, organomegaly, endocrinopathies, M-protein, skin (hyperpigmentation, hypertrichosis)

IMMUNOLOGIC RENAL DISEASES

Multiple renal diseases are mediated by renal or nonrenal antigens, either in the form of self- (autoimmune) or foreign antigens, resulting in renal pathologic changes.
There are two main distinctions: nephrotic and nephritic syndromes (Table 8-37).

Table 8-37. Renal Syndromes

	Nephrotic Syndrome	Nephritic Syndrome
Symptoms	>3.5 g/day proteinuria	Hematuria
	Edema	Edema
	Hyperlipidemia	Hypertension
	Hypoalbuminemia	
	Increased infections due to low IgG secondary protein losses	
Diseases	Minimal change	Poststreptococcal GN
	Membranous nephropathy	IgA nephropathy
	Focal segmental glomerulosclerosis	Henoch-Schönlein purpura
		Membranoproliferative GN
		Rapidly progressive GN

Abbreviation: GN, glomerulonephritis.

NEPHROTIC SYNDROME

Minimal-Change Nephrotic Syndrome

This is the most common nephrotic syndrome **in children**, and 10–15% of nephroses present in adults.

No abnormalities on light microscopy of renal biopsy specimens, usually with **preserved renal function. Immunoglobulin and complement are absent** on immunofluorescence studies.

Biopsy—Effacement of visceral foot processes identified by electron microscopy (EM).

Treatment—Treatment of this disorder involves prednisone. Typically, remission is achieved in 4–6 weeks. For relapses or steroid-resistant cases, administer cyclophosphamide or chlorambucil.

Membranous Nephropathy

This is the most common nephrotic syndrome **in adults**. It can occur secondarily to:

- Hepatitis B
- Malaria
- Syphilis
- Neoplasms
- Drugs
- SLE
- Environmental toxins

Both humoral and cellular immunity are implicated as causes as well as the activated complement.

Clinical Features—Variable. Classic nephrotic syndrome with unremarkable urinalysis. Preserved renal function.

Biopsy—Diffuse thickening of glomerular basement membrane (GBM) with immunofluorescence revealing **prominent granular staining of IgG and C3 along GBM**. EM studies show epithelial foot process effacement with electron-dense (i.e., immune complex deposits) distributed over the GBM.

Treatment—Courses of treatment for membranous nephropathy are controversial.

Complications and Prognosis—Predictors of clinical expression for end-stage renal disease (ESRD) include:

- High levels of proteinuria or renal insufficiency at presentation
- Older age
- Male gender
- Histologic findings of glomerulosclerosis
- Tubulointerstitial inflammation

Focal Segmental Glomerulosclerosis

Focal segmental glomerulosclerosis (FSGS) is the final common pathway of **many forms of glomerular injury**. This is a primary or secondary process to another disease such as:

- HIV
- Infection
- Hypertension
- Vesicoureteral reflux
- Obesity
- Sickle cell disease
- Heroin abuse

Nephrotic syndrome is also present with microscopic hematuria, hypertension, and progressive renal failure.

Biopsy—Segmental glomerular collapse with some focal glomeruli involved.

Treatment—FSGS is treated with a course of prednisone for 3–4 months. ACE inhibitors are renal protective and reduce proteinuria.

Complications and Prognosis—Dictated by degree of proteinuria and serum creatinine at presentation, as well as extent of interstitial fibrosis on biopsy. More than 50% of patients who present with nephrotic-range proteinuria will reach ESRD in 6–8 years. Those who achieve remission usually do well.

NEPHRITIC SYNDROME

Postinfectious Glomerulonephritis

This includes **poststreptococcal (PSGN)** but also other infections. This is, typically, a **disease of childhood**, especially **PSGN**. Alcoholics, diabetics, and IV drug users are also commonly affected.

***Clinical Features*—**PSGN occurs **10–14 days after infection with group A hemolytic streptococcal pharyngitis or impetigo**. Symptoms include hematuria, edema, and hypertension. Proteinuria can also occur. **Antibody and antigen complexes are deposited in the glomeruli**, leading to complement activation, and recruitment of neutrophils and monocyte or macrophages, leading to inflammation.

Nephritogenic antigens responsible for PSGN:

- Nephritis-associated plasmin receptor (NAPir)
  - Glycolytic enzyme with GADPH activity
- Streptococcal pyogenic exotoxin B (SPEB)
  - Cationic cysteine proteinase

***Diagnosis*—Low C3** with evidence of **circulating immune complexes** as well as cryoglobulins, rheumatoid factor, and elevated IgG. Based on combination of **antecedent streptococcal infection** or **positive antistreptolysin O** or **anti-DNAse B titers**. Biopsy is only recommended when diagnosis is in question.

***Treatment*—**In adult cases of postinfectious glomerulonephritis, antibiotics and supportive therapy are prescribed. In children, treatment is self-limited.

IgA Nephropathy

This is the **most common GN in the world.** It may also be the renal-limited form of Henoch-Schönlein purpura.

***Clinical Features*—**Presents with **asymptomatic microscopic or macroscopic hematuria**. Nephrotic presentation and acute renal failure is uncommon. Renal biopsies are only recommended for patients with progressive renal insufficiency or in cases where protein is greater than 1 g/day.

***Biopsy*—**Mesangial IgA staining on immunofluorescence**. Elevated circulating polymeric IgA** and **complexes with IgA** in the serum.

***Treatment*—**Treatment of IgA nephropathy is limited to patients with abnormal renal function. In those with mild renal disease, the physician should monitor closely and control BP with ACE inhibitor or ARB (angiotensin receptor blocker). If more than 1 g/day of proteinuria, then prednisone is indicated. Fish oil may be of some benefit. If there is hyperlipidemia and renal disease statins may be beneficial.

***Complications and Prognosis*—**ESRD in 20–30% of patients after 20 years of age. Predictors of poor outcome are hypertension, early age of onset, proteinuria > 1 g/day, and renal insufficiency at diagnosis.

Henoch-Schönlein Purpura

Henoch-Schönlein purpura (HSP) is a multisystem disorder that is characterized **by deposition of IgA immune complexes in affected organs**. It is associated with drug sensitivities. Typically, the disorder affects **children younger than 10 years of age**, with **male predominance**, following an **upper respiratory tract infection**. Manifestations include:

- Abdominal pain
- Arthralgias
- Purpuric, lower-extremity rash (Figure 8-10)
- Hematuria

Renal biopsy findings are indistinguishable from IgA nephropathy. Renal manifestations are generally self-limiting. Progressive renal disease (as evidenced by crescent changes) and renal failure should be treated with IVIG.

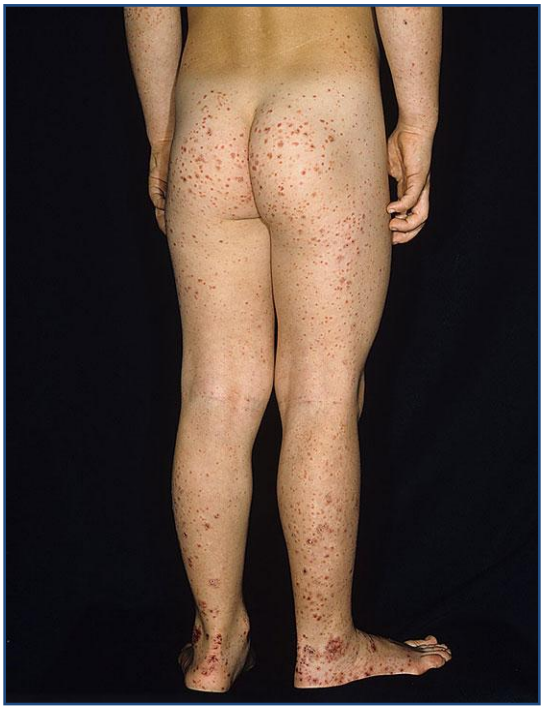


Figure 8-10. Purpura. Note the classic lower extremity distribution of purpura seen in HSP.
(Reproduced, with permission, from Wikimedia Commons.)

Membranoproliferative Glomerulonephritis (MPGN)

This kidney disorder can occur in conjunction with other diseases or it is a renal-limited, idiopathic disease. It can also occur after viral upper respiratory infections.

- **Secondary MPGN**: The most common cause of secondary MPGN is **essential mixed cryoglobulinemia,** which is **associated with hepatitis C**. Monoclonal IgM, possessing RF activity, is a cryoglobulin. Pathogenesis related to deposits of circulating immune complexes with complement activation or monoclonal IgM recognizing endogenous glomerular proteins.
- **Primary MPGN:** Variable amounts of microscopic hematuria, proteinuria, hypertension, hypocomplementemia, and renal insufficiency.

***Treatment*—**For **primary MPGN,** corticosteroids are given; treatment is more successful in children than adults. Long-term treatment with antiplatelet agents slows progression of the disease in adults. For **secondary MPGN,** IFNα is given; and, ribavirin is used for hepatitis C. **Note:** Do not use ribavirin if creatinine clearance is less than 50 mL/minute. (See Chapter 9.)

***Complications and Prognosis*—**Fifty percent of patients will progress to ESRD within 10 years of diagnosis. Risk factors for progression are hypertension and nephrotic syndrome.

Rapidly Progressive Glomerulonephritis (RPGN)

RPGN is characterized by crescentic GN associated with focal necrotizing lesions in the glomeruli and small vessels. Clinically, there is rapidly deteriorating renal function (i.e., days to weeks) that can be caused by a number of conditions. **Pauci-immune GN** is the most common cause of RPGN **in adults**. **Immune-complex–mediated GN** is the most common cause **in children**.

Antiglomerular Basement Membrane (Anti-GBM) Disease

Anti-GBM disease presents as a disease limited to RPGN or the classic pulmonary-renal vasculitic syndrome known as **Goodpasture's syndrome**. It has a bimodal incidence:

- First peak preferentially affects **young men** who **smoke (classic Goodpasture syndrome).**
- Second peak affects **women in their sixth or seventh** decade and is characterized by renal-limited disease.

Flash Card Q23

What key immunologic process causes MPGN?

Presentation—Acute nephritis with crescent formation and rapid renal failure. Abrupt onset of oliguria or anuria as well as hematuria and anemia. Pulmonary hemorrhage occurs in some patients with smoking or lung injury.

Renal Biopsy—Crescents over more than 50% of glomeruli and immunofluorescence of diseased glomeruli reveals staining of IgG along GBM (linear distribution).

Diagnosis—Confirmed by detection of circulating **antibodies directed against α3 chain of type IV collagen.** One third of patients have positive perinuclear antineutrophil cytoplasmic antibodies (pANCA) and cytoplasmic antineutrophil cytoplasmic antibodies (cANCA). Patients with these circulating Abs have milder disease.

Pauci-Immune Crescentic GN

This is the most common cause of RPGN in adults. Pauci-immune indicates the lack of binding of antibody or complement to the affected glomeruli.

Classification—Primary pauci-immune crescentic GN is secondary to vasculitis, including Wegener's granulomatosis, microscopic polyangiitis, and Churg-Strauss syndrome. Related to activated T lymphocytes and autoantibodies.
Pauci-immune crescentic GN is associated with the use of:

- Propylthiouracil
- Hydralazine
- Penicillamine
- Minocycline

One third of patients will have renal-limited disease and the rest will have associated systemic small-vessel vasculitis symptoms. The disease is often preceded by nonspecific symptoms of a flu-like prodrome followed by myalgias, arthralgias, and fatigue. Relapsing disease is common.

Diagnosis—Based on clinical presentation, renal pathology, and serologic findings. Biopsy characterized by crescent-shaped GN with focal necrotizing lesions in glomeruli and small vessels.

- 80–85% of patients present positive ANCA, especially in patients who are older than 50 years of age and white.

Flash Card A23

IgM immune complexes deposit on glomerular proteins and activate complement or via direct monoclonal IgM binding to glomerular proteins.

Immune-Complex–Mediated RPGN

This accounts for most cases of RPGN in children. **Hemolytic-uremic syndrome (HUS)** is the primary cause, characterized by microangiopathic hemolytic anemia, thrombocytopenia, and renal failure.

Immunologic Skin Disease

Immunologic skin diseases are groups of skin conditions induced by autoantibodies to different parts of unique skin structures. Important target skin structures include the following (Figure 8-11):

- **Desmogleins-1 and -3** in desmosome for **pemphigus**
- **BP Ags (BP180 and BP230)** in hemidesmosome/lamina lucida for **pemphigoid and linear IgA bullous dermatosis**
- **Type VII collagen** or anchoring fibrils for **epidermolysis bullosa acquisita**

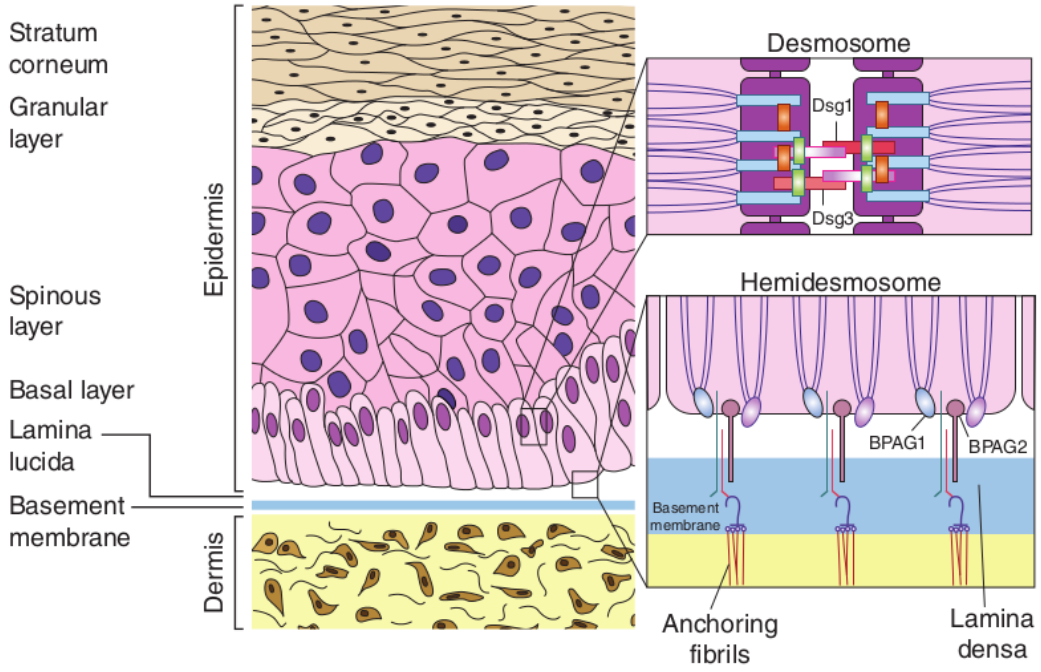


Figure 8-11. Skin structures.
(Abbreviations: Dsg1, Desmoglein-1; Dsg3; Desmoglein-3; BPAG, bullous pemphigoid antigen.)

Flash Card Q24

A 10-year-old girl presents to the emergency department with petechial rash on her extremities, bloody diarrhea, and decreasing urine output after consuming undercooked hamburger. Preliminary lab findings reveal Hgb 7, platelets 40,000, and schistocytes on peripheral smear. What is the most likely diagnosis?

Table 8-38. Immunologic Skin Diseases

Disease	Type	Clinical Signs	Target	Immunopathology	Treatment
Pemphigus	Pemphigus vulgaris	Flaccid bullae on noninflamed skin Nikolsky's sign Found on scalp, chest, oral mucosa, intertriginous areas	IgG epithelial surface Desmoglein-3 +/– desmosome	Epidermal IgG/C3	Prednisone Immuno-suppressives +/–
	Pemphigus foliaceous		Desmoglein-1 +/– desmosome	Epidermal IgG/C3	Steroids
	IgA pemphigus		IgA epith surface Desmocollin-1	Epidermal IgA	Steroids
Pemphigoid	Bullous pemphigoid	Tense bullae on urticarial base Pruritus Flexural areas Elderly	IgG basement membrane zone (BMZ) BP180 BP230	IgG/C3 (BMZ)	Steroids Immuno-suppressives +/–
	Herpes gestationis	Urticaria Blistering Periumbilical area During or immediately postpartum May flare w OCP/menses	IgG BP180 BMZ	Linear BMZ C3	Steroids Antihistamines
Others	Epidermo-lysis bullosa acquisita	Areas of trauma Oral mucosa	Type VII collagen	Linear BMZ IgG/C3 Linear IgA/IgM	Colchicine or dapsone + steroids Immuno-suppressives +/ – for refractory cases
	Dermatitis herpetiformis	Small grouped vesicles LE/buttocks Intense pruritus Associated with celiac disease	Endomysial IgA Transglutaminase IgA Epidermal transglutaminase	Granular BMZ IgA	Eliminate gluten from diet

Abbreviation: BMZ, basement membrane zone; Ig, immunoglobulin; LE, lower extremity; OCP, oral contraceptive pills.

Flash Card A24

Hemolytic-uremic syndrome

PEMPHIGUS

Pemphigus Vulgaris

Presentation

- Flaccid bullae on noninflamed skin
- Crusting
- Nikolsky's sign—application of pressure along one edge of the blister will extend it further to uninvolved skin
- Commonly found on:
  - Scalp
  - Chest
  - Intertriginous areas
  - Oral mucosa
- **Severe morbidity and death** as a result of skin loss, oropharyngeal ulcerations, and sepsis

***Serum Autoantibodies*—IgG on epithelial surface**. Target protein and structure: **Desmoglein-3 and/or desmosome**.

***Tissue Immunofluorescence*—**Epidermal IgG and C3 cell surface staining (Figure 8-12).

***Treatment*—**Pemphigus vulgaris is treated with prednisone, with or without other immunosuppressives.

Mnemonic

The key features of pemphigus:

DEsMO (Desmosome) = **DEMO**.

DEMO is on the surface (of skin); so, when DEMO is affected, **the surface of skin** is damaged, leading to flaccid bullae.

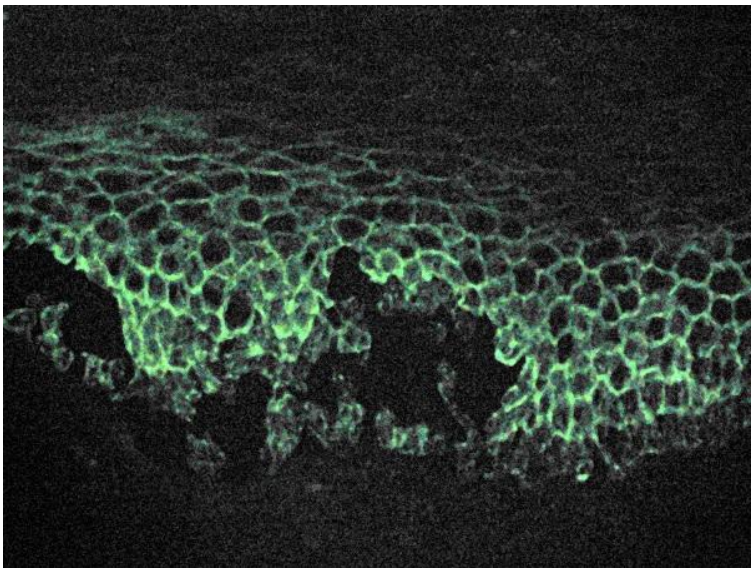


Figure 8-12. Pemphigus vulgaris.
(Reproduced, with permission, from Wikimedia Commons.)

Flash Card Q25

A 70-year-old woman presents with a pruritic rash over her upper extremities with what appear to be tense, fluid-filled blisters. What is the diagnosis?

PEMPHIGOID

Bullous Pemphigoid

***Clinical Presentations*—Tense bullae**, often on **urticarial base** and on **flexural areas**, with prominent **pruritus** (Figure 8-13). Affects **elderly** patients.

***Serum Autoantibodies*—**IgG BMZ and epidermal. IgE antibody to BP180 present in 86% patients and total IgE levels correlate with disease activity. Target protein and structure: **BP180**, **BP230** or **hemidesmosome,** and lamina lucida.

***Tissue Immunofluorescence*—**Linear BMZ IgG and C3.

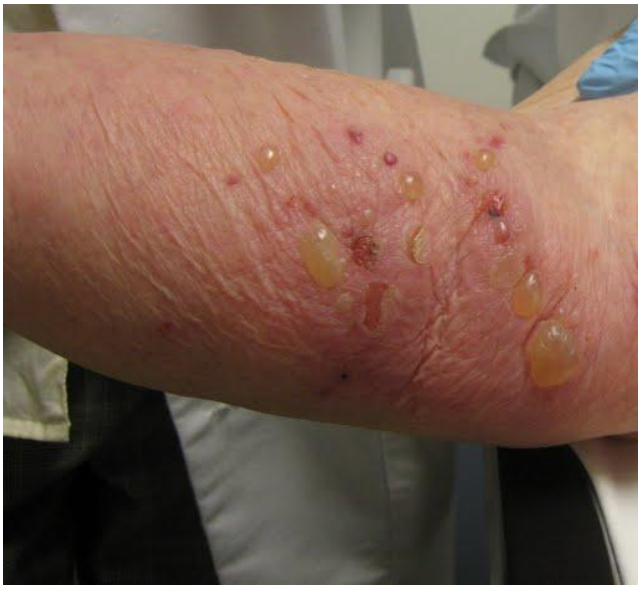


Figure 8-13. Bullous pemphigoid.
(Reproduced, with permission, from USMLERx.com.)

LUPUS ERYTHEMATOSUS (LE)

***Tissue Immunofluorescence*—**Granular immune deposits at BMZ in both involved and uninvolved skin (lupus band), IgM = C3 >IgG >IgA, where two or more required. Refer to Tables 8-25 and 8-29 for antigen testing in these conditions.

Flash Card A25

Bullous pemphigoid. Tends to occur in the elderly, is pruritic, and occurs in flexural areas

IMMUNOLOGIC EYE DISEASES

Ocular Cicatricial Pemphigoid

***Clinical Features*—**This is a **sight-threatening,** chronic inflammation and/or scarring of the conjunctiva that occurs in systemic pemphigoid. It occurs mostly in older women (i.e., 60–70 years of age). *Refer to ophthalmologist.*

***Symptoms and Signs*—**Relapsing conjunctivitis with:

- Severe conjunctival redness
- Mucus discharge
- Eyelid and eyelash in-turning
- Breakdown of corneal and conjunctival epithelium

Fornix is characteristically shortened.

***Treatment*—**Diagnosis requires conjunctival biopsy. Mild disease responds to dapsone, and severe disease usually requires systemic steroids or cytotoxic therapy.

Peripheral Ulcerative Keratitis

***Clinical Features*—**Peripheral ulcerative keratitis involves any part of the limbal structure and is divided into **two types**: mild and necrotizing (Mooren's ulcer). Its mild form is non-sight-threatening and occurs in both genders at any age (i.e., *Staphylococcal blepharitis*). Mooren's ulcer is idiopathic, **vision-threatening**, and occurs in adults unilaterally (worldwide) or bilaterally (i.e., in African men). *Refer to ophthalmologist ASAP.*

Symptoms and Signs

Mild:

- Worsening pain
- Photophobia
- Redness
- Tearing

Necrotizing:

- As above, plus circumferential destruction of cornea

***Treatment*—**For mild cases of peripheral ulcerative keratis: Topical antibiotics and topical steroids. For necrotizing cases (i.e., Mooren's ulcer): exclude infection

Flash Card Q26

What skin condition is caused by IgG to desmoglein-3?

Flash Card Q27

The typical course of which skin condition follows the disease activity?

(i.e., HSV) and systemic disease (i.e., RA), and treat with immunosuppression (i.e., steroids); however, surgery may be needed. Table 8-39 summarizes the key features of episcleritis and scleritis (Figure 8-14).

Table 8-39. Episcleritis Versus Scleritis

	Episcleritis	Scleritis
Clinical features	Benign inflammation superficial episcleral/conjunctival vascular plexus Predominantly occurs in women Peak age 40s	Sight-threatening inflammation of sclera Predominantly occurs in women Peak age: 40s 40% associated with RA
Signs/symptoms	Rapid onset conjunctival redness May persist months/days Minimal pain	Anterior: Eye redness, tearing, photophobia, boring pain
		Posterior: Pain on eye movement; decreased vision
Treatment	Cool compresses Natural tears Topical NSAIDs/steroids	NSAIDs Steroids MTX,immunosuppressants
Ophthalmologic emergency	No	Yes

Abbreviations: MTX, methotrexate; RA, rheumatoid arthritis

Key Fact

Just one drop of 10% phenylephrine will blanch episcleral redness within 20 minutes, but true scleritis will persist.

Figure 8-14. Scleritis. Note the injection of scleral vessels and violaceous hue (can be seen better with natural sunlight).
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Flash Card A26

Pemphigus vulgaris

Flash Card A27

Dermatitis herpetiformis. The rash typically flares/remits with celiac disease activity. Strict gluten elimination from the diet alleviates the rash and symptoms.

Uveitis

Clinical Features—A broad term for intraocular inflammation, in which 60% is idiopathic and 40% is associated with a systemic disease. It is **classified by location**: (1) **anterior uveitis** is in front of the lens (70%); (2) **posterior uveitis** involves the retina, choroid, or vasculature (10%); and (3) **intermediate uveitis** is in the remaining, middle portion of the eye (20%) (Table 8-40).

Table 8-40. Uveitis

	Anterior	Intermediate	Posterior
Clinical Features	In front of lens	Middle of eye	Retina, choroid, vessels
Signs/ Symptoms	Sudden pain Redness Photophobia Vision change variable Miotic pupil	Bilateral blurry vision Floaters	Pain Redness Photophobia Vision loss
Treatment	Topical steroid	Periocular steroid	Oral steroid or Immunosuppressives

INFLAMMATORY GASTROINTESTINAL DISEASES

Autoimmune Gastritis (Pernicious Anemia)

Presentation—Autoimmune gastritis is associated with other autoimmune diseases (e.g., autoimmune thyroid disease, vitiligo, type I DM, and Graves' disease).

Epidemiology—This disease occurs in more women than men (3:1). **It is the most common cause of vitamin B_{12} deficiency**.

Flash Card Q28

This eye condition results in inflammation of the eye, photophobia, tearing, and boring eye pain. Which condition does this occur in?

Flash Card Q29

Eye pain, redness, and miosis of the pupil is seen in which condition?

Pathogenesis—Gastric atrophy and metaplasia are the main pathologic findings. **Autoantibodies to parietal cell and intrinsic factor** are involved in the disease process. Major molecular target of antibodies are the **H+ and K+ATPase**. CD4+ T lymphocytes, which recognize a subunit of the ATPase, mediate the disease; this process is usually suppressed by Tr1 cells. Lymphoid infiltration of mucosa from the gastric body eventually leads to atrophy of the gastric parietal cell and loss of intrinsic factor production by zygomatic cells. This further leads to **B_{12} malabsorption**. Intrinsic factor (IF) antibody may also block the binding of B_{12}.

Genetics—HLA-B8 or -DR3. Autosomal dominant.

***Diagnosis—*Low serum B_{12}** during evaluation for **anemia** or **neurologic symptoms**. Antiparietal cell antibody is suggestive but not specific. **Multipart Schilling test** can confirm diagnosis.

Treatment—The treatment course for autoimmune gastritis is lifelong vitamin B_{12} replacement, typically given as an intramuscular injection.

Prognosis—Left untreated, autoimmune gastritis leads to neurologic damage.

Celiac Disease

Celiac disease is a disorder of the small intestine with chronic inflammation of mucosa; it leads to atrophy of the intestinal villi **with sensitivity to gliadin** found in wheat, barley, and rye, with or without oat.

Genetics—Most common in patients of northern European descent (0.3% incidence); and, absent among Asian and African populations. Strong genetic linkage **with HLA DQ2** (95% patients) and DQ8; however, most people with DQ2 and DQ8 do not develop celiac disease.

Presentation—Symptoms, when present, include:

- Diarrhea
- Flatulence
- Abdominal cramping
- Bloating
- Weight loss (this and the preceding all attributable to malabsorption)
- Fatigue
- Nutritional deficiency
- Anemia
- Osteoporosis

Flash Card A28

Scleritis. This is a sight threatening condition

Diagnosis—Diagnosed by biopsy evidence of small-bowel villous atrophy, with **resolution on gluten-free diet**.

Flash Card A29

Anterior uveitis

- The detection of **IgA autoantibodies** to either **endomysium or tissue transglutaminase (tTG)** are most sensitive and specific methods. Currently, **IgA anti-tTG** is the test of choice, because it is more cost-effective, with high sensitivity and specificity.
- **IgA deficiency can lead to false-negative results**.
- Serum IgA antigliadin antibodies have 80–90% sensitivity, but low specificity (high false-positive). In patients with known IgA deficiency, the IgG anti-tTG test should be used.
- IgA endomysial antibodies (EMA), IgA tTG, and IgA anti-gliadin antibodies (AGA) levels fall with treatment and may be used as noninvasive markers of response to gluten-free diet.
- IgG antibodies against deamidated gliadin peptides (IgG-anti-dGli) have been shown to be sensitive for diagnosis of celiac disease in IgA-deficient patients.

Biopsies—Characteristic histopathology of small intestine that includes blunting of villi with crypt hyperplasia and expansion of lamina propria mononuclear cells (i.e., villous atrophy). Granular IgA and complement deposits can be seen at the dermal-epidermal junction.

Associations—Celiac disease is associated with dermatitis herpetiformis, IgA deficiency, autoimmune disorders, and malignant disease.

Dermatitis Herpetiformis—Characterized by pruritic, vesicular (excoriated) lesions on the extensor surfaces of the extremities, trunk, buttocks, scalp, and neck.

Treatment—Dermatitis herpetiformis responds slowly to withdrawal of gluten from the diet. It is also treated with dapsone.

Flash Card Q30

Which HLA type is strongly associated with celiac disease?

Eosinophilic Gastrointestinal Diseases (EGID, Primary)

A group of gastrointestinal diseases characterized by **abnormal infiltration of eosinophils** in the **absence of known causes of eosinophilia** (e.g., drug reaction, parasitic infection, and malignancy, etc.), including:

- Eosinophilic esophagitis
- Eosinophilic gastritis
- Eosinophilic enteritis
- Eosinophilic colitis
- Eosinophilic gastroenteritis (involving a combination of gastrointestinal segments)

Flash Card Q31

Which eye condition results in inflammation of the eye, photophobia, tearing, and boring eye pain?

Flash Card Q32

Which antibody test has been shown to be sensitive for diagnosis of celiac disease in IgA-deficient patients?

Eosinophilic Esophagitis (EoE)

Genetics—The condition appears to arise from the interplay of genetic and environmental factors. Familial form is seen in 10% patients, with a sibling risk ratio of 50-fold.

Pathogenesis—Primarily T_h2-mediated mechanism.

- Studies strongly implicate eotaxin-3 in the pathophysiology of EoE, and the overexpression of eotaxin-3 could be a biological marker for this condition.
- IL-5 is required for eosinophilic infiltration of esophagus, whereas IL-13 induces eotaxin-3 expression.

Presentation—Most common symptoms include:

- Failure to thrive, feeding disorders in children
- Abdominal pain
- Irritability
- Gastric dysmotility
- Vomiting
- Diarrhea
- Dysphagia
- Food impaction

Symptoms typically occur independently of peripheral blood eosinophilia (more than 50% of the time). But, some patients with EGID (typically those with eosinophilic gastritis) have substantial peripheral blood eosinophilia, meeting the diagnostic criteria for the idiopathic hypereosinophilic syndrome (HES).

Diagnosis—EoE is a clinicopathologic disease. Clinically, EoE is characterized by symptoms related to esophageal dysfunction. Pathologically, 1 or more biopsy specimens must show eosinophil-predominant inflammation. With few exceptions, 15 eosinophils/hpf (peak value) is considered a minimum threshold for a diagnosis of EoE. For optimal pathologic evaluation, multiple biopsy specimens from the proximal and distal esophagus should be obtained and evaluated for a variety of pathologic features:

Flash Card A30

HLA DQ2

- Strictures
- Mucosal rings
- Furrowing
- Ulcerations
- Whitish papules
- Polyps

Flash Card A31

Scleritis. This is a sight-threatening condition.

Gastroesophageal reflux disease (GERD) and EoE may overlap. **The presence of GERD does not exclude the diagnosis of EoE**. The majority of EoE patients have evidence of food and aeroallergen hypersensitivity as defined by skin prick and/or measurement of allergen-specific IgE antibodies. A subgroup of patients

Flash Card A32

IgG antibodies against deamidated gliadin peptides

has been recognized to have (1) a typical EoE symptom presentation, (2) have had GERD diagnostically excluded, and (3) demonstrated a clinicopathologic response to proton pump inhibitors (PPIs). Terms used to describe these patients include PPI-responsive esophageal eosinophilia and PPI-responsive EoE.

Treatment—A trial of specific food antigen and, perhaps, aeroallergen avoidance is often indicated for patients with atopic EoE, and, if unsatisfactory or practically difficult (e.g., when patients are sensitized to many allergens), a diet consisting of an **elemental formula** is advocated.

A recent study showed that an elimination diet based on skin prick test/atopy patch test (SPT/APT) results leads to resolution of esophageal eosinophilia in a similar proportion of patients to empiric removal of foods but required that fewer foods be removed. The most common definitive foods identified were milk, egg, wheat, and soy. Another study recently showed that an empiric six-food (cereals, milk, eggs, fish/seafood, legumes/peanuts, and soy) elimination diet effectively induced remission of active adult EoE, which was maintained for up to 3 years.

Systemic steroids are used for acute exacerbations, whereas **topical steroids** are used to provide long-term control. Swallowed fluticasone and viscous budesonide have been shown in studies to be effective in inducing clinicopathologic remission in EoE. The patient is instructed to swallow the dose to promote deposition on the esophageal mucosa. Even if GERD is not present, neutralization of gastric acidity, with PPIs, may improve symptoms and the degree of esophageal pathology.

Autoimmune Hepatitis

Autoimmune hepatitis occurs in greater numbers of women than in men. Incidence is 17/100,000 in Northern European and/or North American white populations.

Autoantibody Specificity

Type I (Classic): Presents at any age and is the most common type. Autoantibodies are for:

- ANA
- ss- or ds-DNA
- *Saccharomyces cerevisiae*
- p-ANCA (myeloid 50-kD nuclear envelope antigen)
- **Smooth muscle**
- **Actin**
- **Soluble liver or pancreas Ag**

Antismooth-muscle antibodies higher than 1:320 are reflective of the more specific antiactin antibodies.

Flash Card Q33

What is the antibody most specific for diagnosis of autoimmune hepatitis type I?

Flash Card Q34

What is the antibody specific for diagnosis of autoimmune hepatitis type II?

Type II: Presents at any age. Autoantibodies are for:

- **Antiliver-kidney-microsomes-1, (ALKM-1)**
- **Liver-cytosol antigen (ALC-1 or LC1)**
- Rare ANA
- +/– Soluble liver or pancreas Ag
- Autoantibody to cytochrome p450D6 (CYP2D6)

There is some overlap with primary biliary cirrhosis (e.g., mitochondria).

Genetics—

- HLA DR3: Early-onset disease
- HLA DR4: Late-onset disease
- Autoantibodies against asialoglycoprotein receptor (ASPGR) protein seen in periportal hepatocytes

Presentation

- Easy fatigability
- Jaundice
- Dark urine
- Abdominal pain
- Anorexia
- Amenorrhea
- Delayed menarche
- Hepatosplenomegaly (HSM)
- Spider nevi
- Cushingoid features

Laboratory Findings—Aminotransferase elevations more striking than those of bilirubin and alkaline phosphatase. Hypergammaglobulinemia may reflect high autoantibody levels.

Treatment—Treatment of autoimmune hepatitis includes corticosteroids and immunosuppressive drugs.

Primary Biliary Cirrhosis *(PBC)*

Flash Card A33

Antibody against soluble liver antigen.

PBC is the chronic inflammation of intrahepatic ducts, which progresses, ultimately, to the disappearance of ducts. This autoimmune disease leads to:

- Chronic cholestasis
- Hepatic fibrosis
- Cirrhosis
- Liver failure

Flash Card A34

ALKM-1

Epidemiology—PBC is more prevalent in women than men. Patients experience **severe pruritus**. **Middle-aged women** (90% of patients) in their fifth and sixth decades of life are most commonly affected. Prevalence 2.3–14.4 / 100,000 worldwide.

***Genetics—*HLA-A*0201** and HLA-DR8. Increased HLA-DR on hepatocytes.

Immune Mechanism—A triggering event initiates autoimmune attack against bile duct cells in the setting of genetic susceptibility. Appears to be a CD4+ and CD8+ T-cell mediated process, against specific epitopes on bile duct cells.

Specificity—Antimitochondrial antibody (four subtypes and pyruvate dehydrogenase); 95% of patients have increased titer, which can be IgG, IgM, or IgA, although IgG is primary. Antibody titers do not correlate with severity or progression of disease.

Presentation—Insidious onset, with:
- Pruritus
- Fatigue
- Increased skin pigmentation (secondary to melanin deposits)
- Excoriations
- Xanthomata
- Xanthelasma
- Spider telangiectasias

Late in disease course, the following are also found:
- Jaundice
- Petechia
- Purpura
- Hepatic encephalopathy

Treatment—Supportive. Treat vitamin deficiencies and symptoms of pruritus. Steroids exacerbate metabolic bone disease. Ursodeoxycholic acid can be very helpful. Liver transplant may be required in severe cases.

Primary Sclerosing Cholangitis (PSC)

PSC is characterized by inflammation, fibrosis, and the stricture of bile ducts, which affect the intrahepatic and extrahepatic ducts. PSC is more prevalent in men than women (2:1). Ulcerative colitis (UC) presents in 50–90% patients (closer to 90%).

Genetics—HLA-B8 and/or HLA-DR3, and DRw52 (infrequent in UC without PSC).

Flash Card Q35

What autoantibodies are seen in type 1 and type 2 autoimmune hepatitis?

Flash Card Q36

Which HLA type is associated with primary biliary cirrhosis?

Immune Mechanism—Fibrous, obliterative process in bile ducts, and CD8 T cells predominate. Increased CD4 T cells in liver and bile duct epithelium expresses aberrant MHC class II and intercellular adhesion molecule (ICAM-1).

Autoantibody Specificity—The most common are **antismooth-muscle** antibodies and ANA, which are found in approximately 75% of patients.

Presentation

- Fatigue
- Pruritus
- Hyperpigmentation
- Xanthelasma
- Jaundice
- Recurrent fever
- Pain

Liver disease is progressive and leads to cirrhosis; then, risk of cholangiocarcinoma increases.

Diagnosis—Endoscopic retrograde cholangiopancreatography (ERCP).

Treatment—Includes immunosuppressive and anti-inflammatory agents. Endoscopic therapy and liver transplant is the treatment of choice for the advanced disease.

Inflammatory Bowel Disease (IBD)

IBD is an idiopathic inflammatory condition of the intestine, which has three presentations: ulcerative colitis (UC), Crohn's disease, and indeterminate colitis.

Epidemiology—Five to 10 per 100,000, with the peak age of onset between 15–30 years of age. The highest rates are in Europe, North America, and other parts of the developed world. Rates are particularly high among **Ashkenazi Jews** and **African Americans**. Smoking is associated with an increased risk of Crohn's disease. Smoking cessation in patients with ulcerative colitis is associated with an increase in the disease activity and hospitalization. The basis for this difference is unknown.

Genetics—More important in Crohn's disease than in ulcerative colitis. **Mutation of the NOD gene**, which is involved in bacterial recognition, is a representative defect **(NOD2/CARD15);** this gene presents in 20–50% of patients with Crohn's disease. This mutation is also associated with Blau's syndrome. A gene associated with autophagy, **ATG16L1,** has also been associated with Crohn's disease. Recent studies have linked IL-10 mutations with IBD.

Flash Card A35

Antibodies in type 1: Antiactin, antiliver (most specific), antismooth-muscle.
Autoantibodies in type 2: ALKM-1, CYP2D6

Flash Card A36

HLA-A*0201

Presentation—Abdominal pain, diarrhea, and weight loss are most common. Also present are:

- Mouth ulcers
- Skin lesions
- Perianal lesions
- Fever

Additional GI features include arthralgias and arthritis, including sacroiliitis (UC + HLA B27).

Diagnosis—Determine disease activity with CBC and ESR. Rule out enteric infection with stool ova and parasite exam, culture, and *Clostridium difficile* toxin. Confirm the diagnosis with biopsy. IBD panel can also be useful.

- **IBD Panel for ulcerative colitis:** ANCA-positive and ASCA-negative = 60% sensitive and 95% specific.
- **IBD Panel for Crohn's disease:** ANCA-negative and anti-*Saccharomyces cerevisiae* assay (ASCA)-positive = 50% sensitive and 95% specific.

Biopsy

- **Ulcerative colitis:** Mucosal inflammation and superficial cryptitis, intestinal involvement limited to the colon.
- **Crohn's disease:** Mucosal inflammation anywhere along the GI tract, with preponderance of ileum. Evidence of noncaseating granulomas, skip lesions, transmural involvement, terminal ileal narrowing.

Complications

- **Intestinal**: Obstruction, fistula, and abscess
- **Extraintestinal**:
  - Eye: Uveitis and episcleritis
  - Arthritis: Large joints more than small joints
  - Liver: Sclerosing cholangitis

Treatment—Anti-TNFα antibodies (infliximab and adalimumab) are approved for treatment.

Flash Card Q37

Fingolimod is a drug recently approved for treating multiple sclerosis. What is its mechanism of action?

IMMUNOLOGIC NEUROPATHIES

See Tables 8-41, 8-42, and 8-43 for discussions of immunologic neuropathies.

Table 8-41. Immunologic Neuropathies of CNS

Disorder	Epidemiology	AutoAb	Pathogenesis	Clinical Presentation	Diagnosis	Treatment	Other Associations
MS	F > M; 20–40 years of age; White ; Colder temps	NONE	Immune-mediated destruction of CNS myelin sheath (MBP) by T lymphocytes, with increased IL-12, IL-18, IFNγ	Limb weakness, optic neuritis, ophthalmoplegia, sensory symptoms, and gait disturbance	Neurologic deficits with ≥2 MRI findings (lesions in white matter)	Steroids, IFNβ, glatiramer, and natalizumab (anti-VLA4 mAb). **Fingolimod**, a sphingosine-1 phosphate receptor modulator is approved for use in MS	HLA-DR2-associated; not autoimmune disease, but it is immune-mediated attack by myelin-specific T lymphocytes
ADEM	Children and adults affected; Seasonal peak in winter		Clinically similar to MS	Fever HA, and meningeal signs may be seen first	No specific diagnostic testing.	None, but steroids may help	Clinically similar to MS, but monophasic or nonprogressive Can be associated with measles infection
Stiff-person syndrome	F > M	Anti-GAD and anti-amphiphysin	Excessive firing of the motor unit Association with thymoma, vitiligo, DM type 1	Lumbar and thoracic muscle spasms, progressive axial stiffness, and rigidity	EMG shows continuous motor unit activity after benzodiazepine administration	Diazepam, IVIG; and steroids not shown to be effective	**Anti-GAD 100-500× greater than in type I DM**. Antiamphiphysin associated with malignancy

Abbreviations: ADEM, acute disseminated encephalomyelitis; AutoAb, autoantibodies; DM, diabetes mellitus; EMG, electromyelography; GAD, glutamic acid decarboxylase; HA, hypersensitivity angiitis; HLA, human leukocyte antigen; IFN, interferon; IL, interleukin; IVIG, intravenous immunoglobulin; MBS, myelin basic protein; MS, multiple sclerosis;

Flash Card A37

Sphingosine-1-phosphate receptor modulator

Table 8-42. Immunologic Neuropathies of Peripheral Nerves

Disorder	Epidemiology	AutoAb	Pathogenesis	Clinical Presentation	Diagnosis	Treatment	Other Associations
GBS[a]	Incidence increases with age	Anti-GM1, GD1a, GD1b, GT1a, and GaINAc-GD1a	Molecular mimicry causes formation of AutoAb to myelin glycoproteins Demyelination due to complement activation	Symptoms are symmetrical and start peripherally in all forms	Increase in CSF protein, with normal cellularity	Steroids are **not** beneficial in GBS	May be associated with autonomic dysfunction
AIDP	M > F			Acute flaccid weakness and sensory loss		IVIG and plasmapheresis	97% of GBS cases are AIDP; 60% preceded by illness
AMAN/ AMSAN				Acute flaccid weakness, but no sensory loss in AMAN		IVIG and plasmapheresis	Association with preceding *Campylobacter jejuni* infection More frequent in China, Japan, Mexico, Korea, and India
Miller-Fisher syndrome		**Anti-GQ1b**		Acute ataxia, ophthalmoparesis, and areflexia		IVIG and plasmapheresis	Cranial nerve involvement; nerve conduction velocities usually normal
CIDP	F > M; 30–50 years of age.	Antiganglioside (LM1, GM1), IgM, and IgG	Macrophage-mediated segmental demyelination	**Slow onset** of weakness, hyporeflexia, and sensory loss		Steroids; Consider IVIG and plasmapheresis	Antecedents are much less common than GBS "Onion-bulb" formation suggests repeated myelination and demyelination

Abbreviations: AIDP, acute inflammatory demyelinating polyneuropathy; AMAN/AMSAN, acute motor axonal neuropathy / acute motor and sensory axonal neuropathy; AutoAb, autoantibodies; CIDP, chronic inflammatory polyradiculoneuropathy; CSF, cerebrospinal fluid; GBS, Guillain-Barré syndrome;
[a]Guillain-Barré syndrome is an acquired inflammatory peripheral neuropathy that can be further subdivided into AIDP, AMAN/AMSAN, and Miller-Fisher syndrome.

Flash Card Q38

A 33-year-old man presents with diplopia and balance problems. Exam reveals absent reflexes, ataxia, and ophthalmoplegia. What autoantibody would confirm your suspicion of Miller-Fisher syndrome?

Table 8-43. Immunologic Neuropathies of the Neuromuscular Junction

Disorder	Epidemiology	AutoAb	Pathogenesis	Clinical Presentation	Diagnosis	Treatment	Other Associations
MG	F; 20–40 years of age; and, older patients (M = F).	**Anti-AChR**	T–lymphocyte-dependent, antibody-mediated damage of AChR HLA-B8, DRw3, DQw2	Weakness of ocular, bulbar, limb, and respiratory muscles; also fatigue	AChR–antibody-positive and decremental EMG	Acetylcholine esterase inhibitors (pyridostigmine); consider thymectomy	HLA-B8/DR3 association **Associated with thymoma / thymic hyperplasia** in younger patients Thymectomy often associated with improved of disease Symptoms worsen at end of day
Lambert-Eaton syndrome		Anti-VGCC	Crosslinking VGCC on the surface of the presynapse leads to clustering, internalization, and reduced number of ACh vesicles released by each nerve impulse	Difficulty walking; weakness affects mostly proximal muscles; and, autonomic symptoms	Positive anti-VGCC	IVIG and plasmapheresis	Paraneoplastic form can be seen with lung cancer and lymphoma Nonparaneoplastic form associates with HLA-A1, -B8, and -DR3, as in MG, and improved strength with repetitive muscle contraction

Abbreviations: AutoAb, autoantibodies; Ach, acetylcholine; AChR, acetylcholine receptor; EMG, electromyelography; IVIG, intravenous immunoglobulin; MG, myasthenia gravis; VGCC, voltage-gated calcium channel.

Flash Card A38

Anti-GQ1b

Hypereosinophilic Syndromes (HES)

These consist of a group of leukoproliferative disorders, which are characterized by the following:

- M**arked peripheral eosinophilia (>1500/mm^3)**
- **Evidence of end-organ dysfunction**
- **Absence of other causes of eosinophilia**, including parasitic, drug reaction, neoplasm, or allergic disorders

Epidemiology—HES are more prevalent in men than women (9:1), often occur between 20–50 years of age, but may also develop in children.

Presentation—The onset is often insidious, eosinophilia may be detected incidentally, or initial manifestations may be due to sudden cardiac or neurologic complications.
Based on the findings of a recent retrospective multicenter trial the incidence of organ involvement at presentation was dermatologic (37%), pulmonary (25%), gastrointestinal (14%), and cardiac (5%).

Cardiac disease is a major cause of morbidity and mortality in these patients. It can occur whether eosinophilia is due to HES or another etiology. For example, Loeffler's endocarditis is a form of HES; however, it can also be induced by parasitic infection (usually ascariasis).

Subtypes

Myeloproliferative—Display features of myeloproliferative disorders such as **elevated B_{12} level** (>1000 pg/mL), splenomegaly, cytogenic abnormality, myelofibrosis, anemia, myeloid dysplasia.

May be prone to develop cardiac endomyocardial fibrosis and thrombosis.

There are three myeloproliferative HES variants:

- FIP1L1/PDGFRA-associated HES; **FIP1L1 or PDGFRA mutation positive** by reverse transcriptase polymerase chain reaction (RT-PCR) or fluorescent in situ hybridization (FISH); **serum tryptase increased** (≥12 ng/mL); almost exclusively in men, 20–40 years of age; most responsive to **imatinib,** a tyrosine kinase inhibitor). FIP1L1 mutations (in chromosome 4) lead to a constitutively activated fusion tyrosine kinase
- Myeloproliferative with **negative FIP1L1/PDGFRA mutations**
- Chronic eosinophilic leukemia

Flash Card Q39

Prior to initiating systemic corticosteroids for suspected HES, what condition should be ruled out, especially in individuals from developing countries?

Flash Card Q40

Name two primary immunodeficiencies associated with hypereosinophilia.

Flash Card Q41

What mutation in HES patients predicts good response to imatinib?

Lymphocytic—Clonal expansions of lymphocyte populations with aberrant phenotypes (like CD4+CD3– T_h2-like lymphocytes), and skin manifestations predominate. **No response to imatinib**. May progress to lymphoma. Less risk for cardiac disease; however, more prone to associated cutaneous manifestations, such as angioedema or urticarial lesions, or erythematous, pruritic papules, and nodules.

Familial—Autosomal dominant transmittal with family history of persistent eosinophilia and unknown causes.

Other Subtypes

- Primary EGID
- Eosinophilic pneumonia
- Churg-Strauss syndrome
- Systemic mastocytosis
- IBD
- Adrenal insufficiency
- Atheroembolic disease and HIV. Primary immunodeficiencies associated with eosinophilia: Omenn's syndrome and HIES. Gleich's syndrome: Episodic angioedema with eosinopholia

Treatment—No therapy is indicated for patients with eosinophilia without organ involvement, which usually has a benign course.

- Monitoring for end-organ damage with blood chemistries, echocardiogram and pulmonary function tests (PFTs)
- All patients with aggressive disease should undergo HLA typing in preparation for possible hematopoietic cell transplantation.
- Prednisone: Usual initial therapy (1 mg/kg/day)
- Treatment is urgently indicated in myeloproliferative variant with **FIPL1/PDGFRA mutation with tyrosine kinase inhibitor: Imatinib.** Patients with cardiac disease should receive glucocorticoids along with imatinib to prevent cardiac disease.
- Other options include: Hydroxyurea, IFNα, anti-IL5 monoclonal antibodies, cyclosporine A, vincristine.

Flash Card A39

Strongyloidosis

Prognosis—Better prognosis predictors:

- Prolonged eosinopenia with prednisone
- Absence of myeloproliferative findings (i.e., elevated serum B_{12} levels, elevated leukocyte alkaline phosphatase [LAP] scores, splenomegaly)
- Presence of angioedema

Flash Card A40

Omenn's syndrome, HIES

Flash Card A41

FIP1L1/PDGFRA fusion gene

LEUKEMIA, LYMPHOMA, AND MYELOMAS

Table 8-44 shows the epidemiologic, pathologic, and most common features associated with hematologic cancers.

Table 8-44. Most Common Features of Hematologic Cancers

Leukemias	Epidemiology	Pathology	Associated Features
Acute lymphocytic leukemia	Most common malignancy diagnosed in childhood Peaks at 2–5 years of age	Lymphoid progenitors undergo dysregulated proliferation	Increased risk seen in patients with Down syndrome and Wiskott-Aldrich syndrome
Acute myeloid leukemia	Prevalence increases with age, with median age of onset70 years M > F.	Myeloblast proliferation (>20% in bone marrow)	Increased risk seen in patients with Bloom's syndrome, Down's syndrome, congenital neutropenia, and Fanconi's anemia
Chronic lymphocytic leukemia	Most common adult leukemia in US Usually affects patients 60 years of age M > F	Clonal B lymphocytes are arrested in the B-lymphocyte differentiation pathway Cells express low levels of surface immunoglobulins	Associated with overexpression of proto-oncogene Bcl-2 Hypogammaglobulinemia can be treated with IVIG; and More than 50% of cases associated with 13q deletion

HODGKIN'S LYMPHOMA

Epidemiology

- Greater prevalence in men than women
- Bimodal distribution ages (1) between 15–34 years of age, and (2) older than 55 years of age

Pathology—Reed-Sternberg cell (B-lymphocyte origin) derived from lymph node germinal centers, but no longer able to express antibodies (Figure 8-15).

Associated Features

- May be associated with HIV or EBV infections
- Positive family history in 1% of cases

Flash Card Q42

The incidence of which lymphoma is very high in patients with X-linked lymphoproliferative syndrome (XLP)?

Presentation

- Asymptomatic lymphadenopathy
- Mediastinal mass
- Systemic (B) symptoms: Fever, night sweats, weight loss
- Pruritus may be an early symptom, sometimes preceding diagnosis by weeks, or even a year

NON-HODGKIN's LYMPHOMA (NHL)

NHLs are a diverse group of blood cancers that include any kind of lymphoma except Hodgkin's lymphoma. Types of NHL vary significantly in their severity from indolent to very aggressive (Table 8-45).

Presentation

- Clinical presentation of NHL varies tremendously depending on the type of lymphoma and the areas of involvement.
- Aggressive lymphomas commonly present acutely or subacutely with a rapidly growing mass, systemic B symptoms, elevated levels of serum LDH and uric acid.
- Indolent lymphomas are often insidious, presenting only with slow-growing lymphadenopathy, hepatomegaly, splenomegaly, or cytopenias.
- Exaggerated (hypersensitivity) reactions to insect stings or bites, especially mosquito bites, may be noted in some patients with NHL.
- There is an association between acquired angioedema (AAE), and NHL and lymphoproliferative disorders.

Figure 8-15. Reed-Sternberg cell.
(Reproduced, with permission, from the National Cancer Institute.)

Flash Card A42

Ileocecal B-cell lymphoma

Table 8-45. Comparison of NHL types

Non-Hodgkin's Lymphomas	Pathology	Associated Infections	Genetics
Burkitt's lymphoma	Highly aggressive type of B-cell lymphoma	EBV (sporadic)	Associated with c-*myc* gene translocation, found at 8q24
Splenic marginal zone B-cell lymphoma	Postgerminal center B lymphocyte with an unknown degree of differentiation Represents <1% of all NHL	Hepatitis C	Deletion of 7q21-32 is seen in 40% of cases.
MALToma	Extranodal manifestations of marginal-zone lymphomas	70% of gastric MALTomas associated with *Helicobacter pylori* infection	Associated with trisomy 3 or t(11;18)

Abbreviations: EBV, Epstein-Barr virus; MALToma, mucosa-associated lymphoid tissue lymphoma.

MYELOMAS

Serum protein electrophoresis (SPEP) and immunofixation are critical to the diagnosis and management of myelomas (Figure 8-16). A brief overview of these diagnostic modalities is presented as follows.

Indications for SPEP:

- Unexplained anemia, back pain, weakness, or fatigue
- Osteopenia, osteolytic lesions, or spontaneous fractures
- Renal insufficiency with a bland urine sediment
- Heavy proteinuria in a patient older than age 40
- Hypercalcemia
- Hypergammaglobulinemia
- Immunoglobulin deficiency
- Bence Jones proteinuria
- Unexplained peripheral neuropathy
- Recurrent infections
- Elevated ESR or serum viscosity

Drawbacks of SPEP:

- Not as sensitive when M-proteins are small
- If an M-protein is present, the immunoglobulin heavy- and light-chain class cannot be determined from the SPEP

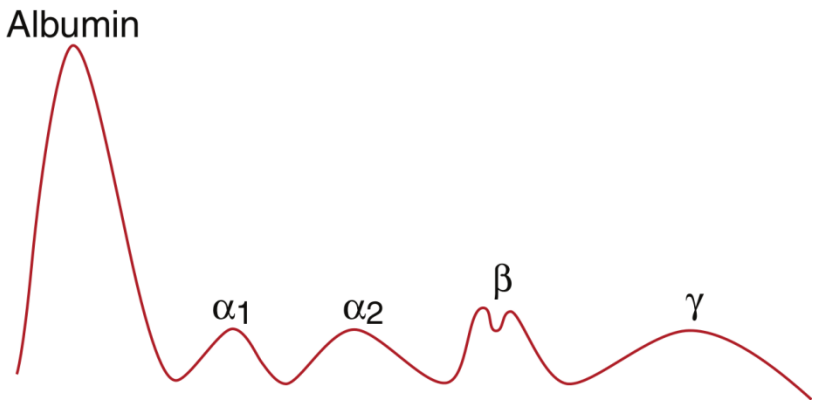


Figure 8-16. Normal pattern for serum protein electrophoresis.

Serum immunofixation must be performed in order to ascertain the presence of an M-protein and to determine its type.

Serum immunofixation determines the heavy- and light-chain type of the monoclonal protein. Immunofixation will detect a serum M-protein at a concentration of at least 0.02 g/dL and a urine M-protein at a concentration of at a cog/dL . It should be done in conjunction with electrophoresis.

A-1 globulin= α_1-antitrypsin, thyroid-binding globulin, transcortin
A-2 globulin= haptoglobin, ceruloplasmin
B-1= transferrin
B-2= β-lipoprotein, some IgGAM, complement
Between B and γ= C-reactive protein, fibrinogen
Γ= immunoglobulins

Monoclonal Gammopathy of Unknown Significance (MGUS)

MGUS involves the proliferation of bone marrow plasma cells derived from a single abnormal clone that involves less than 10% of the bone marrow. MGUS is believed to be a premyeloma condition; 30–40% of cases may progress to myeloma at a rate of 1–2% per year. The incidence of MGUS increases with age, with mean diagnosis at 70 years of age. There are no specific findings on physical examination; and, the disease is often diagnosed during evaluation for an unrelated problem.

Diagnosis is made by observing <3 g/dL of monoclonal paraprotein spike on SPEP; <10% of bone marrow involvement; and, no myeloma-related organ or tissue impairment (Figure 8-17).

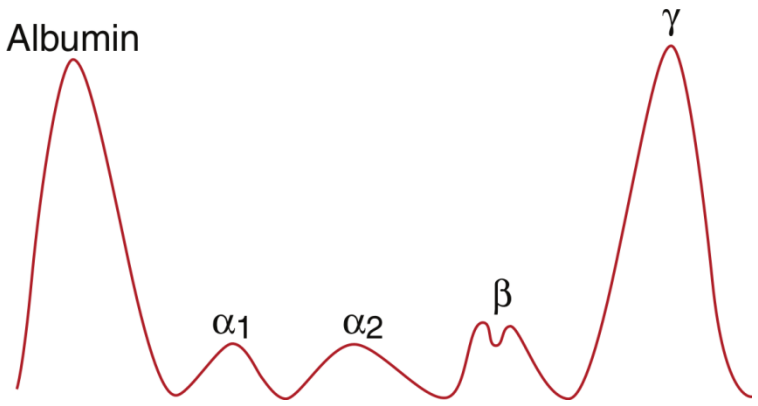


Figure 8-17. SPEP seen in MGUS.

Multiple Myeloma (MM)

MM is the proliferation of bone marrow plasma cells derived from a single, abnormal clone that involves >10% of the bone marrow and the large bones of the body (e.g., skull, vertebrae, and hips).

Genetics—Fifty-percent are associated with Cs 14 abnormality.

Epidemiology—Affects more men than women (3:2), and usually patients older than 65 years of age.

Presentation—Tetrad of symptoms: Hypercalcemia, renal failure, anemia, and bone lesions.

Diagnosis—Observation of >3g/dL monoclonal paraprotein spike on SPEP; >10% of clonal plasma cells on bone marrow biopsy; and, evidence of myeloma-related organ or tissue impairment.

Mnemonic

Symptoms of Multiple Myeloma: **CRAB**

Calcium (↑)
Renal failure
Anemia
Bone lesions

Waldenström's Macroglobulinemia

Malignant proliferation of terminally differentiated B lymphocytes resulting in high levels of IgM. Associated with chromosome 6 abnormalities. Associated with history of autoimmune diseases with:

- Autoantibodies
- Hepatitis
- HIV
- Rickettsiosis

Epidemiology—Waldenström's macroglobulinemia affects more men than women, with the median age at diagnosis 64 years.

Presentation

- Pallor
- Oronasal bleeding
- Organomegaly
- Constitutional symptoms
- Hyperviscosity
- Abnormal coagulation
- Sensorimotor peripheral neuropathy

Diagnosis—SPEP showing an M component with β-to-γ mobility is highly suggestive of Waldenström's macroglobulinemia. High-resolution electrophoresis and immunofixation to characterize the monoclonal IgM paraprotein; the light chain of the monoclonal protein is usually the κ light chain. Bone marrow biopsy shows malignant cells.

Solitary Plasmacytoma

Discrete solitary mass of neoplastic monoclonal plasma cells in bone (medullary) or soft tissue (extramedullary). Increased risk of progression to multiple myeloma; more prevalent in men than women; median age at diagnosis is 55 years.

Presentation—Pain at the site of the skeletal lesion is secondary to bone destruction by infiltrating plasma cells. Monoclonal paraprotein spike on SPEP and lytic lesions seen on radiograph.

Polyneuropathy, Organomegaly, Endocrinopathy, Monoclonal Protein, Skin Abnormalities (POEMS) Syndrome

Monoclonal plasma cell disorder, peripheral neuropathy, and one or more of the following seven features:

- Osteosclerotic myeloma
- Castleman's disease (giant cell lymph node hyperplasia, angiofollicular lymph node hyperplasia. HHV-8 is implicated in the pathogenesis of Castleman's disease))
- Organomegaly
- Endocrinopathy (excluding DM or hypothyroidism)
- Edema
- Typical skin changes
- Papilledema

Almost all patients with POEMS syndrome have either osteosclerotic myeloma or Castleman's disease. Chronic overproduction of proinflammatory and other cytokines appear to be a major feature of this disorder (IL-1β, TNFα, IL-6, and vascular endothelial growth factor [VEGF]). Median age at diagnosis is 51 years. Affects more men than women.

Diagnosis—Monoclonal paraprotein spike on SPEP; immunohistochemical staining of biopsy specimens almost always shows the λ chain; and bone marrow examination is most often nondiagnostic.

Heavy-Chain Disease (α, γ, and μ)

Heavy-chain disease is a rare B-lymphocyte proliferative disorder. It is characterized by the secretion of incomplete immunoglobulin heavy chains, in which the deletion in an amino acid sequence results in the inability of the heavy chain to form disulfide bonds with the light chains.

Alpha (α)—This is the most commonly reported heavy-chain disease. Median age at diagnosis is 30–40 years, and patients are mostly of Middle Eastern or Mediterranean ancestry.

- **Presentation:** Severe diarrhea and weight loss from second-degree infiltration of the intestinal tract wall by cancerous plasma cells, which can lead to malabsorption.

Gamma (γ)—Median age at onset is 60 years; more prevalent in men than women. Up to one third of patients with γ heavy-chain disease have an associated autoimmune disorder (e.g., rheumatoid arthritis, Sjögren's syndrome, lupus erythematosus, or autoimmune hemolytic anemia).

- **Presentation:** lymphoma-like illness with lymphadenopathy, hepatosplenomegaly, and sometimes B symptoms.
- **Diagnosis:** SPEP shows a broad-based band that migrates to the β region. Immunoselection combined with immunophoresis is the most reliable test in the detection of γ heavy-chain disease. Bone marrow biopsy and aspirate findings may be normal; however, they often reveal increased numbers of plasma cells, lymphocytes, and large immunoblasts. Eosinophilia is also common.

Mu (μ) —This is the rarest of the heavy-chain diseases. No age or gender predilection.

- **Presentation:**
  - Splenomegaly
  - Hepatomegaly
  - Pallor (if significant anemia is present)
  - Lymphadenopathy.

Flash Card Q43

Which viral infection is associated with cryoglobulinemia, and with what types?

  - Most distinctive symptom is palatal edema that is secondary to nodal involvement of Waldeyer's ring.
- **Diagnosis:** Anomalous serum M component that reacts with anti-IgG but not antilight-chain reagents. Bone marrow examination is usually nondiagnostic.

Cryoglobulinemia

Cryoglobulins are single or mixed immunoglobulins that undergo reversible precipitation at low temperatures. It occurs more often in women than men (3:1). The mean age reported is 42–52 years.

Types

Key Fact

With cryoglobulins, RF is positive in types II and III.

- Type I: Results of a monoclonal immunoglobulin IgM > IgG, IgA, or light chains
- Types II and III: Contain rheumatoid factors (RFs) usually IgM, and which form complexes with the fragmented, crystallizable (Fc) portion of the polyclonal IgG
  - Monoclonal RF in type II
  - Polyclonal RF in type III
  - Types II and III represent 80% of all cryoglobulins

Presentation

- **Type I:**
  - Related to hyperviscosity and thrombosis (acrocyanosis, retinal hemorrhage, severe Raynaud's phenomenon with digital ulceration, livedo reticularis purpura, and arterial thrombosis).
- **Type II and III:**
  - Joint involvement (usually, arthralgias in the proximal interphalangeal PIP joints, metacarpophalangeal MCP joints, knees, and ankles)
  - Fatigue
  - Myalgias
  - Renal immune-complex disease
  - Cutaneous vasculitis
  - Peripheral neuropathy

Key Fact

Type I cryoglobulinemia is associated with plasma cell dyscrasias and multiple myeloma. Type II and III cryoglobulinemias are associated with hepatitis C infection.

Diagnosis

- **Evaluation of serum cryoglobulins:** Blood specimen must be obtained in warm tubes (37°C, 98.6°F) in the absence of anticoagulants. Allow the blood sample to clot before removal of serum with centrifugation at 37°C (98.6°F).
- Type I tends to precipitate within the first 24 hours (at concentrations >5 mg/mL).
- Type III cryoglobulins may require seven days to precipitate a small sample <1 mg/mL).

Flash Card A43

Hepatitis C virus, with types II and III

GRANULOMATOUS DISEASES

GRANULOMA

Granulomas form either in response to persistent antigen exposure or from the presence of undigestible particulate antigen. Most consist of organized T and B lymphocytes surrounded by **histiocytes** (macrophages with indistinct boundaries). And, if caused by infectious etiology, large, multinucleated giant cells are usually present at the granuloma center. One way to categorize granulomatous disease is by the T-lymphocyte phenotype (i.e., T_h1 versus T_h2; see Table 8-46).

After activation, macrophages secrete molecules that promote granuloma formation, including ACE, 1, 25-dihydroxyvitamin D_3 (1, 25-$[OH]_2D_3$), osteoporin, and TNFα.

***Laboratory Findings*—ACE** is a marker for the presence of granulomatous disease and its activity; however, it lacks disease specificity and has limited diagnostic and therapeutic utility. **High serum levels of $1,25(OH)_2D_3$ are a common feature. Osteopontin** is secreted by many granuloma cells that are implicated in the regulation of chemotaxis, T-lymphocyte development, and cell adhesion, which are all essential elements to granuloma formation. **TNFα** is also critical to granuloma development and plays an important role in containing the prototypical granuloma-inducing infectious agent, *Mycobacterium tuberculosis*.

Table 8-46. Granulomatous Diseases: T_h1-Dominated and T_h2-Dominated

T_h1-Dominated	T_h2-Dominated
Tuberculosis	Churg-Strauss syndrome
Sarcoidosis	Lepromatous leprosy
Berylliosis	
Hypersensitivity pneumonitis	
Wegener's granulomatosis	
Tuberculoid leprosy	

Flash Card Q44

What is Lofgren's syndrome?

Flash Card Q45

What is the key difference between sarcoidosis and hypersensitivity pneumonitis, in terms of T-cell counts on BAL?

Causes of Granulomatous Diseases

- Infectious: Tuberculosis and atypical mycobacterium, fungal infections such as histoplasmosis, blastomycosis, coccidioidomycosis
- Immune dysregulation:
  - Wegener's granulomatosis
  - Churg-Strauss syndrome
  - Sarcoidosis
  - Chronic granulomatous disease (CGD)
  - Takayasu's arteritis
  - Crohn's disease
  - Primary biliary cirrhosis
  - Patients with common-variable immune deficiency (CVID) may develop noncaseating granulomas in the lymphoid or solid organs
- Environmental exposures: Hypersensitivity pneumonitis, berylliosis, and silicosis

Sarcoidosis

Sarcoidosis is a disease of unknown etiology. It is characterized by the presence of multiple, noncaseating granulomas that results in multiple organ dysfunction; it involves the lung, especially. The high prevalence of hilar lymphadenopathy and asymptomatic disease in the African-American population clearly suggests a genetic basis.

Pulmonary function testing usually shows restriction. However, sarcoidosis is one of a handful of lung diseases that can have mixed-obstructive and restrictive physiology.

Sarcoidosis is characterized histologically by the presence of perilymphatic, **noncaseating granulomas,** with an abundant rim of perigranulomatous lymphocytes. The perigranulomatous lymphocyte population contains both CD4+ and CD8+ T lymphocytes; and, intracellular cytokine-staining profiles demonstrate a **T_h1** (IFNγ)-**predominant cytokine pattern**.

Flash Card A44

Triad of hilar adenopathy, acute polyarthritis and erythema nodosum

Diagnosis—Diagnosis is made based on finding this characteristic pathology in the lung, or perhaps other tissues, in the absence of known granuloma-inducing agents. Increased serum ACE and vitamin D levels are often detected, although they are not specific. Bronchoalveolar lavage (BAL) fluid shows elevated CD4 to CD8 ratio (compare that with hypersensitivity pneumonitis in which the ratio is decreased).

Flash Card A45

CD4/CD8 count is elevated in sarcoidosis, decreased in hypersensitivity pneumonitis

Treatment—Oral corticosteroids are typically given for symptomatic disease, but probably do not improve long-term outcome. Infliximab (anti-TNFα mAb) has

been used successfully as a treatment for refractory sarcoidosis, but serious adverse effects might preclude its widespread use.

Berylliosis

Berylliosis is clinically identical to sarcoidosis; however, it results from environmental exposure to beryllium, often in an occupational setting. Like sarcoidosis, berylliosis is associated with a broad spectrum of lung abnormalities, ranging from patchy radiographic opacities to diffuse interstitial lung disease. Clustering of cases of hypersensitivity pneumonitis and berylliosis within patients bearing certain MHC and TNFα polymorphisms suggests a common pathogenetic link between these distinct lung diseases.

Treatment—Treatment of berylliosis centers on removal of the patient from exposure to the offending metal. Corticosteroids are commonly used as adjunctive therapy; but, randomized efficacy studies have been inconclusive. Their use remains controversial.

Wegener's Granulomatosis (Granulomatosis with Polyangiitis)

A form of vasculitis that affects small to medium blood vessels. Granulomatous inflammation of the respiratory tract. It affects women as much as men

Presentation

- Sinuses are involved in 95% of patients.
- Lungs are affected in 85% of patients with nodules, cavitary lesions, and pulmonary hemorrhage.
- Kidneys are affected in 80% cases and are affected at the time of diagnosis in 20% cases. Typically asymptomatic, but can lead to rapidly progressive renal failure. Detected by active urine segment.
- But other organs such as the eye and skin are also frequently involved.

Diagnosis—Presence of c-ANCA (by immunofluorescence) with proteinase-3 (PR3) specificity by ELISA is most specific, although p-ANCA can also be seen. In the absence of classic organ involvement in c-ANCA-positive patients, the diagnosis of Wegener's granulomatosis is established by tissue biopsy. Kidney biopsy specimens show pauci-immune glomerulonephritis (focal, segmental, crescentic).

Treatment—For severe disease, prednisone + cyclophosphamide or prednisone + rituximab, transition to azathioprine or mycophenolate, once controlled. For nonsevere disease, prednisone + methotrexate.

Flash Card Q46

What type of ANCA antibody is found in 75–90% of patients with granulomatosis with polyangiitis?

Mortality is usually from pulmonary or renal failure.

Eosinophilic Granuloma of the Lung (Histiocytosis X)

Associated with pulmonary interstitial fibrosis, the most common symptom is nonproductive cough.

Diagnosis—The diagnosis is made by lung biopsy and high-resolution CT scanning with pathognomonic cysts and nodules. The major cells in lesions are histiocytosis X cells, which are of Langerhans cell origin, and are positive for CD1 and HLA-DR.

AMYLOIDOSIS

Amyloidosis is a group of disorders associated with deposits of amyloid proteins in various organs. Symptoms vary widely depending on where in the body amyloid deposits accumulate, with most commonly affected organs being the kidneys, heart, and liver (Table 8-47).

Table 8-47. Common Clinical Manifestations of Amyloidosis

System	Clinical Manifestations
Renal	Proteinuria, nephrotic syndrome
Cardiac	Cardiomyopathy leading to heart failure
Hepatic and splenic	Hepatosplenomegaly
Neurologic	Mixed sensory and motor peripheral neuropathy, autonomic neuropathy, or stroke
Musculoskeletal	Macroglossia (pseudohypertrophy)
Hematologic	Bleeding diathesis
Pulmonary	Tracheobronchial infiltration, persistent pleural effusions, parenchymal nodules
Skin	Waxy thickening, easy bruising (ecchymoses), subcutaneous nodules or plaques

Flash Card A46

c-ANCA (anti-proteinase 3)

Primary Amyloidosis (AL)

This plasma cell dyscrasia is the **most common form** in developed countries and involves deposits of **immunoglobulin light chains.** It may occur alone or associated with multiple myeloma, Waldenström's macroglobulinemia or non-Hodgkin's lymphoma. Immunoglobulin is detectable in the serum or monoclonal light chains in the urine in approximately 80% of cases. Patients tend to be 60 years of age or older with nonspecific symptoms such as fatigue and weight loss.

Secondary Amyloidosis (AA)

This may occur at any age in patients with chronic inflammatory or infectious conditions, including RA, IBD, and various periodic fever syndromes (including familial Mediterranean fever [FMF] and cryopyrin-associated periodic syndromes [CAPS, i.e., familial cold autoinflammatory syndrome [FCAS] and Muckle-Wells syndrome]MWS]) and involves deposits of the acute-phase reactant and serum amyloid A (AA) protein in tissues.

Definitive Diagnosis—Congo-red-positive deposits in tissue/fat pad biopsy as seen in Figure 8-18. The sensitivity of the abdominal fat pad aspiration technique is around 80%, but its specificity is near 100%.

Treatment—Treatment for primary amyloidosis involves melphalan and prednisone and may include stem cell transplantation. Treatment for patients with secondary amyloidosis should focus on resolving the chronic inflammatory state. Colchicine helps treat amyloidosis in patients with FMF. Liver transplantation may be effective in certain cases of the hereditary amyloidoses.

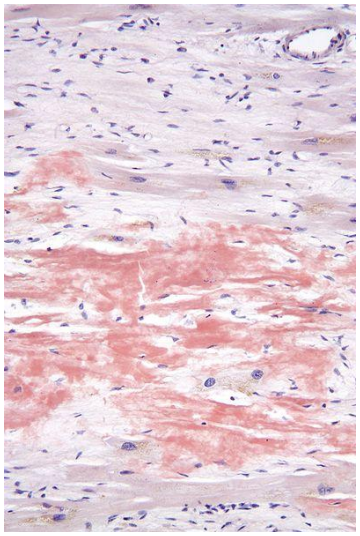


Figure 8-18. Cardiac amyloidosis**.** Micrograph showing amyloid deposition (red fluffy material) in cardiac tissue. Amyloid will show "apple-green" birefringence when stained with Congo red and viewed under polarized light.
(Reproduced, with permission, from Wikimedia Commons.)

Flash Card Q47

A 16-year-old woman with a history of progressive hearing loss presents with recurrent episodes of fever, diffuse urticaria, and diffuse joint pains that have occurred every 3 weeks for the past year, triggered by cold temperatures. Laboratory studies reveal proteinuria and kidney biopsy reveals AA deposits. What is the diagnosis?

Flash Card Q48

An 8-year-old boy of Ashkenazi Jewish descent presents with intermittent fevers, recurrent attacks of abdominal pain, and arthritis in the right knee. An extensive work-up ruled out infectious sources and showed proteinuria on urinalysis. Subsequent bone marrow biopsy revealed AA deposits. What is the diagnosis?

MASTOCYTOSIS

Key Fact

Darier's sign is urticaria and erythema observed on and around a macule after stroking the lesion.

Diffuse cutaneous mastocytosis (DCM) typically is a pediatric skin mastocytosis variant and usually resolves without progression to systemic mastocytosis.

Flash Card A47

Muckle-Wells syndrome is a rare autosomal dominant disease that causes sensorineural deafness, recurrent hives, and can lead to secondary amyloidosis with nephropathy. Cold temperatures may precipitate attacks. Patients have a mutation in NLRP3.

Flash Card A48

Familial Mediterranean fever. 90% of patients with FMF, which is common in individuals off Turkish or Ashkenazi Jewish descent, present before age 20 with symptoms of intermittent fevers, serositis causing abdominal pain or pleuritis, monoarthritis, and AA deposition in the kidney, spleen, liver, and gut. Symptoms typically respond to colchicine.

Disease caused by clonal proliferation of mast cells within tissues that can occur at any age. The true prevalence is unknown, and familial occurrence is unusual. It is classified into disease variants based on clinical presentation, pathologic finding, and prognosis (see Tables 8-48 and 8-49).

Table 8-48. Cutaneous Mastocytosis (CM)

Classification	Clinical Presentation
Urticaria pigmentosa (UP)	**Discrete** yellow-brown macular-papular or nodular plaque-like lesions with characteristic Darier's sign
Diffuse cutaneous mastocytosis (DCM)	**Diffuse** yellow-brown thickened skin; no discrete lesions; usually occurs in patients younger than 3 years of age
Mastocytoma of skin	**Solitary** reddish brown skin lesion that usually presents in first 3 months of life and frequently resolves spontaneously
Telangiectasia macularis eruptiva perstans	Macular telangiectasias characterized by increased mast cells around dilated capillaries and venules. Typically occurs in adults.

Table 8-49. Classification of Systemic Mastocytosis (SM)

Indolent systemic mastocytosis (ISM): most common form of SM; slow progression and good prognosis Rare provisional subvariants of ISM: Smoldering SM: high mast cell burden (>30% bone marrow space are MC, High tryptase >200 ng/mL) Isolated bone marrow mastocytosis—bone marrow involvement without skin involvement
Systemic mastocytosis with associated hematologic nonmast cell lineage disease (SM-AHNMD)
Aggressive systemic mastocytosis (ASM): Aggressive tissue infiltration causing hepatic fibrosis, portal hypertension, malabsorption, or cytopenia
Mast cell leukemia (MCL): Rare; >10% immature MC in peripheral blood or >20% immature MC in marrow
Mast cell sarcoma (MCS): Reported in tibia, skull, and other bones
Extracutaneous mastocytoma

Pathogenesis

Molecular Pathogenesis of Systemic Mastocytosis–Activating Mutations in c-kit—Activating mutations in c-*kit* (e.g., KIT D816V) can lead to increased number of mast cells due to constitutive activation of KIT tyrosine kinase signaling and aberrant expression of antiapoptotic proteins (e.g., Bcl-XL & Bcl-2).

Key Fact

Stem cell factor (SCF) is required for mast cell survival and is the ligand for **c-KIT (CD117).**

Clinical Features

Symptoms (Table 8-50) are caused by infiltration of mast cells in the target organs and/or the release of mast cell mediators during mast cell activation (e.g., histamine, tryptase, chymase, and other mediators).

Bone Marrow—The most common site of mast cell infiltration and most useful biopsy site for making diagnosis. Mast cells are identified by immunohistochemical staining for CD117 (c-*kit*) and coexpression of CD2 and/or CD25.

Key Fact

The following medications may induce symptoms in patients with mastocytosis and should be avoided (unless specifically tolerated by any individual patient):

Alcohol, amphotericin B, dextran, dextromethorphan, IV vancomycin, α-blocker, β-blockers, quinine, polymyxin B, opioids (fentanyl and tramadol usually tolerated), aspirin and NSAIDs, muscle relaxants (succinylcholine and atracurium; pancuronium, and vecuronium usually tolerated), and local anesthetics (benzocaine and procaine).

Diagnostic Criteria

Cutaneous Mastocytosis (CM)—Requires presence of at least one of the following:

- Focal dense mast cell infiltrates (>15 mast cells per cluster) or diffuse mast cell infiltrates (>20 mast cells per high-powered field) on skin biopsy
- c-KIT D816V mutation

Systemic Mastocytosis (SM)—See Table 8-51.

Table 8-50. Other Symptoms of Mastocytosis

System	Clinical Manifestations
Cutaneous	Urticaria pigmentosa, flushing, pruritis
Gastrointestinal	Abdominal pain, diarrhea, nausea, vomiting, and peptic ulcer disease
Musculoskeletal	Musculoskeletal and bone pain, osteopenia, osteoporosis, and pathologic fractures
Cardiovascular	Anaphylaxis
Neuropsychiatric	Decreased attention span, memory impairment, and irritability reported

Key Fact

Obtain baseline tryptase values for a patient who presents with severe anaphylaxis following a Hymenoptera sting to screen for mastocytosis.

Table 8-51. Systemic Mastocytosis—World Health Organization (WHO) Diagnostic Criteria

Major Criteria
Biopsy[a] with multifocal, dense infiltrates of mast cells (>15 mast cells in aggregates)
Minor Criteria
Biopsy[a] with more than 25% of mast cells having spindle-shaped or atypical morphology
Detection of c-*kit D816V* mutation[a]
Expression of CD2 and/or CD25 on CD117+ mast cells
Total serum tryptase > 20 ng/mL

[a]In bone marrow or other extracutaneous organ.

Key Fact

One major and one minor, or three minor criteria are required to diagnose systemic mastocytosis.

Mast Cell Activation Syndrome (MCAS)—This is a proposed diagnosis describing patients who have a variety of symptoms suggestive of mast cell activation, **but fail to meet the WHO criteria for diagnosis of SM**, and for whom all other relevant differential diagnoses have been excluded. Patients may have recurrent episodes of mast cell activation symptoms, such as recurrent flushing, gastrointestinal cramping, and hypotension, and may exhibit hypotensive reactions to Hymenoptera stings similar to patients with systemic mastocytosis. However, they lack urticaria pigmentosa and may demonstrate baseline serum tryptase values that are normal or mildly increased. This diagnosis may be suggested when serum tryptase or other laboratory tests for mast cell mediators are elevated from baseline in conjunction with a "flare" of symptoms.

Monoclonal Mast Cell Activation Syndrome (MMAS)—Distinguished from MCAS by the presence of the D816V c-KIT mutation, but does not meet full WHO criteria for SM.

Treatment

Symptomatic treatment includes the following:

- Avoidance of triggers, which may include alcohol, NSAIDs, narcotics, intense exercise, and stinging insects
- **First- and second-generation antihistamines:** Reduce pruritus flushing, and headaches
- **H_2 antagonist:** Add-on therapy for pruritus, flushing, and headaches
- **Disodium cromoglycate:** May be useful in alleviating GI complaints
- **Epinephrine:** Used to treat episodes of hypotension
- **Topical steroids:** Used to treat urticaria pigmentosa (UP) or DCM
- **Cytoreductive treatment:** imatinib mesylate (Gleevec)—tyrosine kinase inhibitor used in patients with aggressive systemic mastocytosis **without D816V c-KIT mutation**

- **DEXA scanning and calcium supplementation:** Monitoring and treatment of osteoporosis
- **Associated hematologic disorders existing concomitantly with SM:** Managed according to their specific hematologic abnormalities

Prognosis

Variables associated with poor prognosis include:

- Male gender
- Absence of cutaneous symptoms
- Presence of constitutional symptoms
- Anemia or thrombocytopenia
- Abnormal liver function tests (LFTs)
- Bilobed mast cell nuclei
- Low percentage of fat cells in bone marrow biopsy, or
- An associated hematologic malignancy

IMMUNOHEMATOLOGIC DISEASE

The predominant antibody involved in immunohematologic disease is IgG, which opsonizes blood cells, resulting in extravascular clearance by Fc receptors mainly in the spleen. Two IgG molecules can also activate complement, enhancing cell clearance via complement receptors. Hemolytic reactions affecting IgM also involve complement fixation; only one IgM molecule is required to fix complement. Reticuloendothelial cells (in spleen) lack IgM receptors.

Transfusion Reactions

Acute Hemolytic Transfusion Reactions (HTRs)—HTRs are most commonly due to transfusion of ABO-incompatible blood. This leads to the formation of IgM antibody:RBC antigen complexes, which induce complement-mediated lysis, activate the coagulation cascade, and release proinflammatory cytokines (i.e., TNFα). Clinically, the classic presentation includes fever, flank pain, and red-brown urine (hemoglobinuria), and if the problem is unrecognized, the patient may progress to disseminated intravascular coagulation (DIC) and a shock-like state. Laboratory findings include hemoglobinuria, elevated plasma free hemoglobin, and a positive direct antiglobulin test (DAT).

Flash Card Q49

What is most common activating point mutation in c-*kit* associated with systemic mastocytosis?

Delayed HTRs—Delayed HTRs result from exposure to minor blood group antigens such as Rh, Kell, Kidd, or Duffy blood groups. These are clinically milder reactions than acute HTRs, and present 5–10 days after transfusion with fever, jaundice, and a gradual decline in hemoglobin. Additional laboratory findings include an increased lactate dehydrogenase (LDH), elevated indirect bilirubin, and a positive DAT.

Nonhemolytic Febrile Reactions—The most common cause of fever after blood transfusion. They are more commonly seen after platelet transfusions and are more common in women secondary to pregnancy-related allosensitization. They are diagnosed after a temperature increase of more than 1°C (33.8°F). Cytokines (TNFα and IL-1β) play a role in the etiology, and cytokine levels increase over time during storage. Reactions can be prevented by prestorage removal of leukocytes (leukoreduction).

Urticarial (Allergic) Reactions—Urticarial reactions occur during 1–3% of fresh frozen plasma and platelet transfusions and are due to IgE-mediated hypersensitivity to plasma proteins. Patients present with urticaria and pruritus. Treatment includes temporarily stopping the transfusion and administering antihistamines. Prevention includes washing future blood products.

Anaphylactic Reactions—Occur rarely in IgA-deficient patients who have anti-IgA antibodies. Prevent using twice-washed RBCs or products from known IgA-deficient donors. May also occur via an IgE-mediated mechanism.

Graft-Versus-Host Disease (GVHD)—A highly lethal complication of transfusion after bone marrow transplantation. Donor T lymphocytes react against host histoincompatible tissues (e.g., skin, GI, liver, and bone marrow). There is a high fatality rate secondary to marrow aplasia. GVHD prevention includes γ irradiation of cellular blood products.

Transfusion-Related Acute Lung Injury (TRALI)—Should be suspected when patients present with severe respiratory distress 1–4 hours after transfusion. TRALI results when donor leukocyte antibodies are directed at recipient neutrophil antigens or HLA determinants. C5a is released, resulting in pulmonary leukosequestration. Neutrophil sequestration and priming is followed by neutrophil activation and release of a variety of neutrophil mediators. Treatment includes intensive respiratory support (ventilation) and high-dose corticosteroids, leading to resolution in 24–48 hours.

Flash Card A49

The most common mutation in c-*kit* is at codon 816 with a substitution of valine for aspartic acid (Asp816Val or D816V). Other mutations have been identified but are uncommon.

Autoimmune Hemolytic Anemia

Criteria for Autoimmune Hemolytic Anemia (AIHA)—Criteria for AIHA consist of the following:

- Positive DAT or indirect antiglobulin test (IAT)

- Evidence of hemolysis (i.e., anemia with reticulocytosis, jaundice, hyperbilirubinemia (indirect), high levels of low-density lipoprotein (LDH), or a low haptoglobin)

Warm-Reactive AIHA—Caused by polyclonal IgG against RBCs. The RBCs attach to Fc receptors on macrophages in the spleen and are removed by phagocytosis. There are primary (idiopathic) or secondary (SLE or chronic lymphocytic leukemia, CLL) causes, and patients have a positive DAT.

Cold-Reactive AIHA—Occurs when IgM binds to I antigen (polysaccharide on all RBCs) at temperatures less than 37°C or 98.6°F (i.e., not at room temperature). This activates complement. Idiopathic cases are seen in the elderly, who present with acrocyanosis and have a monoclonal spike. Cold AIHA can also present secondary to *Mycoplasma* infection or mononucleosis. Disease is worsened by cold exposure.

Paroxysmal Cold Hemoglobinuria—A cold-reacting biphasic, anti-RBC IgG antibody (also known as Donath-Landsteiner antibody) that is usually against the P antigen on RBC.

Paroxysmal Nocturnal Hemoglobinuria—Loss of phosphatidyl-inositol-glycerol (PIG)-linkage proteins on surface of RBCs, including CD59 and CD55 (DAF, decay-accelerating factor). Patients present with hepatic vein thrombosis; and, diagnosis is made via flow cytometry.

Hemolytic Disease of the Newborn

- Rh disease is the most common. Prophylaxis of RhD– women with anti-D immune globulin at 28 weeks' gestation and within 72 hours after delivery prevents sensitization
- ABO incompatibility: Mom is O and baby is A, B, or AB; 10% of pregnancies are a "set up" for this
- Minor blood group incompatibilities, which include D, Kell, and Duffy

Immune Neutropenia

Neonatal Alloimmune Neutropenia—Occurs when fetal neutrophil antigens (NA1 and NB1) provoke formation of maternal IgG that crosses the placenta to destroy fetal neutrophils. Firstborns are commonly affected.

Primary Autoimmune Neutropenia—Occurs in six-to 24-month-olds and is usually benign, presenting with minor infections (e.g., skin abscesses, URIs, and otitis media). Remission is typically seen by age of two to four years (90%). Diagnosis is clinical; can order antineutrophil antibodies. Neutropenia is severe, and neutrophil percentage is generally less than 5%.

Immune Thrombocytopenia

Neonatal Alloimmune Thrombocytopenia (NAIT)—NAIT occurs when maternal antibodies against an inherited fetal platelet antigen from the father (e.g., $P1^{A1}$ (HPA-1) – the most common antigen in whites) cross the placenta and destroy fetal platelets. The typical presentation is of a first born child with neonatal thrombocytopenia; infant may develop intracranial hemorrhage. Postnatal treatment involves maternal (platelet antigen-negative) platelet transfusion to infant.

Immune Thrombocytopenic Purpura (ITP)—ITP is a clinical diagnosis. Patients present with petechiae, purpura, and ecchymoses, with isolated severe thrombocytopenia; otherwise, normal CBC. Treatment indicated under platelet counts of 20,000–30,000/μL with IVIG 0.5–1 g/kg, anti-D 50–75 μg/kg and/or prednisone 1–2 mg/kg; 80% improve within 1 year. The most severe complication is intracranial hemorrhage.

Key Fact

Evans' syndrome is the simultaneous or sequential development of ITP and AIHA with a positive DAT in the absence of a known underlying etiology. Some patients may also have autoimmune neutropenia. A majority of patients with Evans' syndrome have a chronic relapsing course despite treatment with steroids, IVIG, and/or other immuno-suppressive agents. Evans' syndrome has been associated with primary immune deficiencies such as CVID or ALPS.

Drug-Induced Immune Thrombocytopenia

Heparin-Induced Thrombocytopenia (HIT)—Occurs when IgG Fab binds to the complex between heparin and platelet factor 4. The IgG Fc binds to platelet FcIIa, triggering platelet activation and consumption. Five percent of patients treated with unfractionated heparin for 5–14 days are affected, and they present with thrombocytopenia and/or thrombosis. Lower incidence with low-molecular-weight heparin, but crossreactivity is possible.

Mnemonic

Clinical features of TTP:
FAT RN

Fever
Anemia,
microangiopathic
hemolytic
Thrombocytopenia
Renal failure
Neurologic changes

Quinine-Induced—Thrombocytopenia occurs when IgG Fab binds to drug-dependent antigens localized to glycoproteins IIb/IIIa and Ib/IX on platelets.

Posttransfusion Purpura—Occurs 7–10 days after RBC transfusion. $P1^{A1-}$ women develop antibodies to transfused $P1^{A1+}$ cells.

Thrombotic Thrombocytopenic Purpura (TTP)—Involves an autoantibody against von Willebrand factor-cleaving protease (ADAMTS13).

Autoantibodies Versus Clotting Factors

Lupus Anticoagulant or Antiphospholipid Antibody—Can be primary (antiphospholipid syndrome (APS) or secondary to autoimmune diseases such as SLE. In these cases, it is associated with a high risk of thrombosis (30–40%) and recurrent, spontaneous abortions. The lupus anticoagulant is an IgG or IgM antibody that binds to anionic phospholipids that have formed complexes with proteins, leading to prolongation of the activated partial thromboplastin time (aPTT).

Factor VIII Antibodies—Inhibitory IgG antibody to factor VIII are found in 15–35% of severe hemophilia patients and can prevent hemostasis, leading to major bleeding complications. Why some patients develop inhibitors is poorly understood. Immune tolerance can be achieved in approximately 70% of patients who receive regular and prolonged infusions of fVIII with or without immune modulation.

CYSTIC FIBROSIS

Epidemiology

Table 8-52 lists the incidence of cystic fibrosis (CS) across some US ethnic groups.

Genetics

Develops from a mutation in **cystic fibrosis transmembrane conductance regulator (CFTR)**, an apical membrane-bound protein; there may be complete or partial loss of CFTR protein. Phenylalanine 508 (ΔF508) is absent from CFTR in 73% of patients. The end result is a decrease in chloride transport and thickening of all secretions; partial CFTR mutations exhibit milder disease.

Clinical Features

Sinuses—Chronic sinusitis: bilateral, can be associated with hypoplasia of frontal and sphenoid sinuses.
Pathogens include *Pseudomonas aeruginosa, Staphylococcus aureus, Haemophilus influenzae,* and anaerobes.

- Allergic fungal sinusitis: Most commonly *Aspergillus, Curvularia* sp.
- Nasal polyps: Incidence up to 48% of children with CF (compared with 0.1% in healthy children).

Flash Card Q50

Name three autoantibodies associated with SLE.

Table 8-52. Incidences of Cystic Fibrosis in Whites, African-Americans, and Native Americans

	Whites	African Americans	Native Americans
Incidence	1/2000	1/17,000	1/80,000
Median survival is 32 years in all groups			

Flash Card Q51

What are two treatment options besides desmopressin or aminocaproic acid for a hemophilia patient with factor VIII antibody who does not respond to factor VIII replacement?

Pulmonary

- Recurrent pneumonias: Particularly *P. aeruginosa, S. aureus* pathogens.
- Allergic bronchopulmonary aspergillosis (ABPA): Up to 15% prevalence in CF patients. Pathogens include *Aspergillus fumigatus, A. flavus, Candida, Penicillium, Curvularia.*

Diagnostic criteria for ABPA in CF include the following:

- Acute or subacute clinical deterioration
- **Total serum IgE > 2400 ng/mL (or >1000 IU/mL)**
- Skin test reactivity or specific IgE for *A. fumigatus*
- Precipitating or IgG antibodies to *A. fumigatus*
- New infiltrates on chest radiograph or CT findings (bronchiectasis)
- Bronchiectasis

Gastrointestinal

- Failure to thrive
- Malabsorption
- Diarrhea
- Meconium ileus
- Small-bowel obstruction
- Focal biliary cirrhosis
- Pancreatic exocrine insufficiency
- Pancreatitis

Reproductive—Obstructive azoospermia.

Skin—Dehydration due to excessive salt loss.

Musculoskeletal—Reduced bone mineral content, digital clubbing, and hypertrophic osteoarthropathy.

Renal—Nephrolithiasis and nephrocalcinosis.

Flash Card A50

anti-dsDNA—specific for SLE; associated with glomerulonephritis; may fall to normal when disease is quiescent

anti-Smith (anti-Sm)—high specificity, remains high in remission

anti-U1 RNP—common in all mixed connective tissue disorders; low specificity

Flash Card A51

Recombinant activated factor VII (rfVIIa; Novoseven) is produced using baby hamster kidney (BHK) cells expressing the cloned human factor VII gene and facilitates hemostasis by activating factor X directly on the platelet surface. FEIBA VH is a plasma-derived vapor-heated concentrate of vitamin K-dependent clotting factors (factors II, VII, IX, X)

Diagnosis

The diagnosis of CF requires symptoms consistent with CF in at least one organ (or positive newborn screen) **and** evidence of CFTR dysfunction (via elevated sweat chloride, presence of two mutations in CFTR, or abnormal nasal potential difference).

Laboratory Tests

- **Newborn screening program:** Measures immunoreactive trypsinogen, which can be falsely positive
- **Sweat chloride test**

  - Normal: <40 mEq/L
  - Borderline: 40–60 mEq/L (repeat)
  - **Elevated: >60 Eq/L** (**Note: Diagnostic in children**)
  - Sometimes false-negatives, if patient presents with edema or hypoproteinemia; false-positives if there is malnutrition and eczema
- **Molecular diagnosis:** CFTR gene mutation
- **Nasal potential difference measurement**

Treatment

Sinuses—Functional endoscopic sinus surgery (FESS), if severe sinusitis and/or nasal polyps or before lung transplant.

Pulmonary

- Chest physiotherapy
- Physical training
- Recombinant human deoxyribonuclease (dornase alfa-inhaled): Reduces sputum viscosity and need for antibiotics
- Nebulized hypertonic saline: Improves mucociliary clearance
- Antibiotics for pneumonia:
  - Inpatient: Combination of β-lactam and aminoglycoside given IV
  - Outpatient: Oral quinolones
  - Prophylactic nebulized tobramycin to reduce colonization with *P. aeruginosa*
- Oxygen therapy, if advanced disease
- Lung transplant: Double-lung or heart-lung transplantation; 2-year survival rate at 50–60%.

Gastrointestinal

- Supplemental pancreatic enzymes
- Fat-soluble vitamins
- High-calorie diet and/or tube feedings

REPRODUCTION AND THE IMMUNE SYSTEM

Fertilization

Achieved following successful fusion of gametes that forms embryo. Despite repetitive exposure to "foreign" spermatozoa, the majority of women do not mount an immune response to sperm antigen.

Flash Card Q52

Nasal polyps associated with CF are distinct from nasal polyps associated with asthma due to increased numbers of which of the following?

A. Mast cells
B. Monocytes
C. Eosinophils
D. Neutrophils

Pregnancy

Women develop a tolerance to fetal tissue expressing maternal (self) and paternal (nonself) genes. An immunologically safe environment is created by diminishing adaptive immune responses while preserving innate immunity.

Placental and Extraplacental Membranes

Key Fact

Placental and fetal membranes are directly exposed to maternal blood and tissues.

Fetal Trophoblast Cells—Cells that surround the embryo throughout pregnancy; they seclude and protect it from the maternal immune system.

Placental Membranes—Protect themselves from maternal immune cell attack through various mechanisms, including unique expression of HLA molecules that are thought to interact with LIRs (leukocyte inhibitory receptors) on NK cells (Table 8-53).

Immune System and the Pregnant Uterus

Lymphocyte Populations—T and B lymphocytes decrease in the pregnant uterus, whereas the number of maternal NK cells and macrophages increases. Uterine macrophages help prevent uterine infections in pregnant women, and uterine NK cells (u-NK) have a higher CD56 expression than peripheral NK cells and are critical in remodeling spiral arteries for normal placentation. The presence of γ/Δ T lymphocytes and double-negative T lymphocytes is also seen, but their roles are unclear.

Table 8-53. Placental Membrane-Specific Protective Mechanisms

Immune Molecule		Altered Expression
HLA molecules	Class I	Absence of HLA-A and HLA-B class Ia antigens Predominate expression of HLA class Ib molecules (HLA-E, HLA-F, and HLA-G)
	Class II	Repressed expression of MHC class II genes that encode paternally derived foreign MHC
Soluble immunomodulators		Synthesis of immunosuppressive molecules and anti-inflammatory cytokines (e.g., prostaglandin E2, IL-10, and IL-4)
Complement proteins		Increased expression of complement regulatory proteins (CD46 and DAF) that prevent activation of complement by maternal antipaternal antibodies

Abbreviations: DAF, decay-accelerating factor; HLA, human leukocyte antigen; MHC, major histocompatibility class.

Flash Card A52

D. Neutrophils. Among inflammatory cells, EG2+ (activated) eosinophils are a prominent and characteristic feature in about 80% of polyps with asthma and allergic inflammation, whereas lymphocytes and neutrophils are the predominant cells in CF and in primary ciliary dyskinesia.

Soluble Agents—Induction of progesterone, prostaglandins, and cytokines is seen in the pregnant uterus. Progesterone alters the T_h1/T_h2 balance and inhibits production of TNFα in macrophages. PGE_2 contributes to poor lymphocyte proliferation, and the production of T_h2-type cytokines drives antibody-mediated responses in preference to cell-mediated immune responses.

Neonatal Immunity

Humoral Immunity—Consists of maternally produced IgG antibodies that are transported across the placenta into fetal circulation, as well as of maternal IgA and IgG antibodies in breast milk transported across the neonatal intestinal epithelium after ingestion.

Cell-Mediated Immunity (CMI)—Neonates have both qualitative and quantitative differences in CMI compared with adults. In general, they have lower numbers of antigen-specific T-lymphocyte precursor and T-lymphocyte subsets as well as a decreased cytokine response to mitogen exposure.

Maternal Immune Response

Humoral Immunity—B-lymphocyte immunity is maintained and immunoglobulin levels are unchanged throughout pregnancy.

Regulatory T Cells—Enhanced tolerance has been seen in pregnancy due to increased numbers of IL-10-producing Treg cells, which are made in response to the exposure of fetal cells expressing paternal antigens.

Infertility and Spontaneous Abortions

Antisperm Antibodies—Can occur in males following disruption of blood-testis barrier (e.g., trauma) and in women following oral-genital intercourse, and, more rarely, through vaginal inoculation. Infertility secondary to this can be treated with intrauterine insemination, steroid treatments, or in vitro fertilization (IVF).

Allergic reactions to homologous proteins in seminal plasma (HSP) are seen exclusively in women and present as systemic or localized vaginal reactions during or following vaginal sexual intercourse. Condom use can prevent reactions. Skin testing to HSP can be performed once the partner has been evaluated for infectious disease. Desensitization through vaginal or subcutaneous immunotherapy has been successful.

Autoimmune Disease of Testis or Ovaries—A known cause of infertility that can be due to ovarian antibodies that cause premature ovarian failure, or by

Key Fact

Maternal IgG is transferred transplacentally to the fetus during the third trimester. Full-term infants often have IgG levels equal or greater to maternal IgG level. After birth, levels of maternally derived IgG rapidly decline, reaching a nadir of approximately 400 mg/dL at 3–6 months. At the same time, the infant's own production of IgG is not fully developed. This sequence of events represents normal "physiologic" hypogammaglobulinemia.

Key Fact

Adults can also express FcRn, which functions to protect IgG antibodies from catabolism; this explains the long half-life of this class of antibody in the serum.

Key Fact

There is little to no depression of the maternal immune response during pregnancy.

Key Fact

Between 1–12% of fertile women make antisperm antibodies.

Flash Card Q53

Which neonatal receptor allows the transfer of maternal IgG across the placenta and neonatal intestinal epithelium?

Flash Card Q54

What treatment options are available for pregnant women with APS?

autoimmune orchitis and oophoritis as components of human polyendocrine autoimmune syndromes.

Antiphospholipid Syndrome (APS)—Characterized by:

- Recurrent pregnancy losses
- Prolonged bleeding times
- History of hypercoagulability
- False-positive Venereal Disease Research Laboratory (VDRL) test

Diagnosis: The previous symptoms in a patient with laboratory evidence of antiphospholipid antibodies (aPL) is necessary for diagnosis. aPL are directed against serum proteins bound to anionic phospholipids and may be detected as: IgG or IgM antibodies to anticardiolipin (aCL), anti-ß2-glycoprotein I (anti-β2GPI) or lupus anticoagulant (LA) testing (↑aPTT, ↑ dilute Russell viper venom time (dRVVT)). The antibodies interfere with the synthesis of prostaglandins and prostacyclins that predispose a woman to placental insufficiency and pregnancy loss. Confirmatory aPL testing after at least 12 weeks from initial testing to confirm persistence of the aCL, anti-β2GPI, or LA test is required to satisfy laboratory criteria for APS.

Key Fact

Two percent of women test positive for aPL; however, this is not clinically significant in women with no history of recurrent pregnancy losses.

IMMUNOLOGIC ASPECTS OF INFECTIOUS DISEASES

LYME DISEASE

Lyme disease is the most common vector-borne disease in the United States and may be classified into acute localized, acute disseminated, and late disease. Laboratory testing is usually performed in patients with confusing presentations. Cultivation of *Borrelia burgdorferi* is definitive, but 2–8 weeks is required to grow the organism and it is not available in many clinical laboratories. Therefore, serologic tests are important to validate the presence of *B. burgdorferi*.

Flash Card A53

The FcRn receptor is an IgG-specific receptor, resembling a class I MHC molecule, which allows transfer of maternal IgG across the placenta and neonatal intestinal epithelium.

Serologic Testing

In asymptomatic patients and patients with nonpecific symptoms, serologic screening (ELISA or immunofluorescence assay [IFA]) is not useful because of low-positive and low-negative predictive values, which are not helpful in guiding therapy. In endemic regions, erythema migrans (with or without a history of tick bite) is sufficient to treat empirically without laboratory confirmation. In symptomatic patients, a two-tier serologic diagnosis is commonly utilized; ELISA

Flash Card A54

Low-dose aspirin, unfractionated heparin, or low-molecular-weight heparin (LMWH)

or IFA is initially performed to support the diagnosis. In patients with an indeterminate or positive ELISA or IFA, a Western blot is performed on the same serum to look for IgM and IgG in early disease and IgG in late disease. If ELISA or IFA are negative, no further work-up is indicated.

Within 1–2 weeks of onset of infection, IgM antibodies to *B. burgdorferi* appear, and in 4–6 weeks, IgG antibodies are present. Seroreactivity, however, may persist for months after antibiotic treatment of early infection and for years after treatment of late infection.

The **variable major protein-like sequence-expressed (VlsE)** sixth invariant region (C6) peptide ELISA, a one-step test that measures IgG, has been shown to be as sensitive and nearly as specific as two-tier serologic testing for the diagnosis of Lyme disease.

Polymerase Chain Reaction (PCR)

PCR may be used to identify *B. burgdorferi* DNA in CSF and synovial fluid, but it has many limitations; it has low sensitivity, false-positive results are common, and the test does not differentiate between active infection and the presence of remnant DNA.

IgM Capture Assay

During the initial phase of localized disease, serologic tests may be negative because specific serum antibodies to *B. burgdorferi* may be trapped in immune complexes that render them undetectable by standard assays. An **IgM capture assay (EMIBA)**, which measures immune complex-derived IgM antibodies, is a promising new assay for serologic confirmation of early and active Lyme disease; however, this technique needs to be standardized.

T-Lymphocyte Assays

T-lymphocyte proliferative responses to borrelial antigens have been detected in blood, synovial, fluid, and CSF samples; however, the test remains difficult to perform and interpret, thus it is not recommended. Although, detection of B-lymphocyte chemokine chemoattractant (CXCL13) in CSF as a marker for Lyme neuroborreliosis appears promising, it requires validation and standardization before it is recommended as a diagnostic technique.

TUBERCULOSIS

The innate and adaptive arms of the immune system are known to work in concert to prevent tuberculosis (TB) infection.

Innate Immunity

Upon entry into the body, the bacilli (*Mycobacterium tuberculosis*) are phagocytosed by macrophages and neutrophils; however, the organism has evolved mechanisms to evade intracellular killing. These mechanisms include resistance to reactive oxygen species (ROS), inhibition of phagosome-lysosome fusion, and inhibition of phagosome acidification. The mechanisms that lead to mycobacterial resistance to ROS include:

- Scavenging of oxygen intermediates by lipoarabinomannan (LAM)
- Reactive oxygen intermediates not being stimulated in macrophages when the bacilli are phagocytosed via complement receptors, CR1 and CR3
- Cyclopropanated mycolic acids in bacterial cell walls, which assist in resisting the actions of hydrogen peroxide

The bacilli prevent acidification by selectively excluding the proton ATPase from the phagosome.

Adaptive Immunity

Humoral immunity has not been conclusively proven to provide defense from *M. tuberculosis*, where cell-mediated immune responses play a more critical role.

CD4+ and CD8+ T lymphocytes have been shown to play a protective role. CD4+ T lymphocytes produce IFNγ, which activates macrophages. This plays an important role in preventing infection during the early phase of *M. tuberculosis* infection. The mycobacterial killing by CD8+ T lymphocytes and NK cells has also been reported and is caused by granulysin, a protein present in the granules of human CTLs and NK cells.

Interferon gamma release assay (IGRA) T lymphocytes that specifically recognize mycobacterial antigens appear within 2–6 weeks of infection, around the same time that the tuberculin skin test (TST) becomes positive. IGRAs may detect the presence of TB. This is an ELISA-based assay that measures the amount of IFNγ released from T lymphocytes when they are stimulated by tuberculin antigens, such as early secretory antigenic target 6 (ESAT-6) and culture filtrate protein (CFP-10). These antigens are absent in the vaccine strain bacille Calmette-Guérin (BCG) and in *Mycobacterium bovis*. Peripheral blood samples for this testing

must be processed within 12 hours to ensure the viability of the leukocytes. Such IGRAs, unlike the TST, are not affected by prior BCG vaccination and, thus, do not give false-positive results. This test may also be useful for those who receive serial examination for TB, such as health care workers. However, the assay has limited data in certain populations such as those younger than 17 years of age, persons recently exposed to TB, immunocompromised populations, and those with other disorders (e.g., diabetes and chronic renal failure).

Natural human immunity to the mycobacteria group, including *Mycobacterium tuberculosis*, BCG (and/or *Salmonella* sp.), relies on the functional IL-12/23-IFNγ integrity of macrophages (monocyte/dendritic cell) connecting to T-lymphocyte/NK cells.

Increased mycobacterial infections are a characteristic of these primary immune deficiencies:

- IL-12p40, IL-12R-β1, and IL-23 β chain receptor deficiencies
- IFNγR1, IFNγR2 receptor deficiency
- STAT-1 deficiency
- CYBB mutation
- AR-Hyper IgE (TYK 2 deficiency)
- NEMO mutations (which impair CD40-dependent IL-12 production)
- Interferon regulatory factor 8 (IRF8)

Granuloma Formation

Granuloma formation plays an important role in controlling infection, and TNFα plays an essential role in the formation of a granuloma. The reactivation of latent TB in individuals treated with anti-TNFα antibody (e.g., infliximab) and TNFα receptor antibody (e.g., etanercept) provides evidence that TNFα plays a role in controlling mycobacterial infection.

LEPROSY

Leprosy Classification

The World Health Organization (WHO) has based its classification of leprosy on the number of skin lesions and smears of skin lesions (Table 8-54). Skin smears were originally used to distinguish between paucibacillary (PB) and multibacillary (MB) leprosy. However, skin smears are not always available, and their reliability is often doubtful, so most leprosy programs classify and choose the appropriate regimen for a particular patient using clinical criteria. In between the two ends of the spectrum of *M. leprae* infection, PB and MB, exist borderline diseases such as borderline tuberculoid (BT), midborderline (BB), and borderline lepromatous (BL), which are often referred to as “unstable diseases.”

Table 8-54. Leprosy Classification—World Health Organization

Classification	Number of Skin Lesions/ Nerves Involved	Treatment (Adult)
Paucibacillary single-lesion leprosy	1	One-time dose: Rifampicin 600 mg + Ofloxacin 400 mg + Minocycline 100 mg
Paucibacillary leprosy (PB)	2-5	12-month treatment regimen: Dapsone 100 mg/day Rifampicin 600 mg q month
Multibacillary leprosy (MB)	>5	12-month treatment regimen: Dapsone 100 mg/day Rifampicin 600 mg/month Clofazimine 50mg/day and 300 mg/month
Children: Age 5–14 years of age receive adjusted doses of treatment regimen.		

Genetics and Leprosy

Familial clustering and high concordance rates in identical twins support the role of genetic predisposition. PB is often linked to HLA-DR3, and MB is associated with HLA-DQ1. Studies have shown that the NRAMP1 gene is a leprosy susceptibility locus. (Murine homologue Nramp1 controls innate resistance to *Mycobacterium lepraemurium.*)

Immunologic Reactions

Type I, or Reversal Reaction—Borderline leprosy cases may be immunologically unstable; the disease may shift from BT to BL without treatment and may oscillate from BL back to BT with treatment. The reversal of the disease toward BT with treatment may result in a delayed-type hypersensitivity reaction, the reversal, or type 1, reaction. This reaction leads to additional motor and sensory loss, along with erythema and edema of preexisting skin lesions. The type I reaction is commonly associated with neuritis and, occasionally, with skin ulceration.

Type II, or Erythema Nodosum Leprosum (ENL)—Type II, or ENL, an immune complex disorder, may occur during treatment of MB or BT; it may also occur before or after completion of therapy. The disorder is characterized by fever and erythematous tender nodules; and, it may be associated with:

- Neuritis
- Edema
- Arthralgias

- Leukocytosis
- Iridocyclitis
- Pretibial periostitis
- Orchitis
- Nephritis

TNFα may contribute to the pathogenesis.

Alterations in immune responses have been shown to occur during pregnancy. Women with leprosy who get pregnant are more prone to develop type I and type II reactions, and a relapse of their disease. A type II reaction may occur during pregnancy, especially during the third trimester; type I reactions are known to occur during puerperium.

It is commonly reported that treatment of rheumatologic diseases with TNF antagonists may lead to TB. Though uncommon, it has been documented in case reports that leprosy may develop during treatment with infliximab, a chimeric IgG1 monoclonal antibody to TNF; in these reports, discontinuation of infliximab resulted in type 1 reactions.

HEPATITIS

Hepatitis A Virus (HAV)

HAV infection is usually subclinical in children, and infection in adults may varies from a mild flu-like illness to fulminant hepatitis.

The typical clinical presentation of HAV infection may be confirmed by detection of serum anti-HAV antibodies. Serum IgM HAV antibodies are usually detected at the onset of symptoms and remain positive for 4–6 months after exposure. The antibodies may persist for 12–14 months in individuals with a relapsing or protracted disease course. Serum IgG HAV antibodies may be detected in early convalescence and may remain positive for decades. The HAV and HAV RNA may also be detected to confirm the diagnosis, but serologic detection of antibodies is simple and easier.

Hepatitis B Virus (HBV)

Surface Antigen and Antibody—Radioimmunoassay (RIA) and enzyme-linked immunoassay (EIA) are generally used to detect serum hepatitis B surface antigen (HB_sAg), the serologic hallmark of an HBV infection. It may be detected 1–10 weeks after exposure. In the subset, those who recover from infection, HBsAg is usually undetectable in 4–6 months. Detection of HBsAg after 6 months usually denotes chronic infection. HBsAg usually disappears over time and is followed by

the appearance of hepatitis B surface antibody (HBsAb), which persists for life in most patients. In a subset, for a short time ("the window period"), neither the surface antigen nor antibody may be detectable; during this period, serologic diagnosis is made by detecting IgM antibodies against the hepatitis B core antigen (HBcAg). A small number of individuals may have detectable HBsAg and HBsAb at the same time and are termed "carriers" of the hepatitis B virus.

Core Antigen and Antibody—HBcAg is an intracellular protein, which is expressed in infected hepatocytes and is undetectable in serum. The detection of IgM HBcAb suggests an acute HBV infection. In a small number of cases, HBcAb may remain detectable for up to 2 years following an acute infection. It may also be detected during acute exacerbations of chronic hepatitis B infection. In those who recover from the acute infection, IgG anti-HBcAb and anti-HBsAb anti-HBs may be detected; individuals with chronic HBV infection usually have detectable HBsAg and IgG HBcAb. The isolated detection of HBcAb may occur during the "window period," or after many years of recovery from an acute HBV infection, or after many years of ongoing chronic HBV infection.

E Antigen and Antibody—The presence of serum HBeAg HeAg usually denotes active liver disease and ongoing HBV replication. It is associated with high levels of serum HBV DNA and signifies increased infectivity. In most, the seroconversion from HBeAg to anti-HBeAb is usually associated with remission of active liver disease and a decrease in serum HBV DNA.

Acute, Past, and Chronic HBV Infection, Carrier, and Vaccination

Acute HBV Infection—HBsAg and IgM HBcAB are usually detected in serum. HBeAg and HBV DNA, the markers of active HBV replication, are present. Recovery is accompanied by disappearance of HBV DNA, HBeAg to HBeAb seroconversion, and, finally, HBsAg to HBsA seroconversion.

Past HBV Infection—Serologic diagnosis may be made by detection of HBsAb and IgG HBcAb.

Chronic HBV Infection—Detection of HBsAg after 6 months of an acute infection denotes chronic HBV infection. All patients with chronic HBV must be monitored closely for progression of liver disease with HBV DNA titers and ALT levels.

Undetectable HBeAg with normal serum ALT levels and low or undetectable HBV DNA titers denote an inactive HBV carrier. Vaccination is evidenced by the presence of HBsAb only.

Table 8-55 offers a summary of the spectrum of antigen and antibodies in HBV infection.

Table 8-55. Spectrum of Antigen and Antibodies in Acute and Chronic Hepatitis B Virus (HBV) Infection and in a Vaccinated Individual

	HBsAg	HBeAg	IgM HBcAb	IgG HBcAb	HBsAb	HBeAb	HBV DNA
Acute infection							
Early period	+	+	+				++
Window period			+				+
Recovery period				+	+	+	±
Chronic infection							
Early replicative period	+	+		+			+++
Nonreplicative period	+			+		+	±
Acute exacerbation	+	±	+	+			+
Vaccinated individual					+		

Hepatitis C (HCV)

Diagnostic tests for HCV infection may be serologic assays, which detect antibody to HCV, and molecular assays, which detect or quantify HCV RNA. Testing for HCV infection must be routinely undertaken in individuals who have:

- History of illicit injection drug use
- Received clotting factors that were manufactured prior to 1987
- Received blood/organ transplant before July 1992
- Received chronic hemodialysis
- HIV infection
- Children born to mothers with HCV

The commonly used screening assay for HCV infection utilizes a second-generation EIA that detects antibodies to recombinant antigens from the core (C22) and nonstructural regions 3 (C33), and 4 (C100) of HCV. A third-generation EIA, which detects antibodies to an additional antigen from the nonstructural region (NS5), is being used in some institutions.

Molecular Testing and HCV Genotyping

Molecular-testing methods consist of nucleic acid tests (NAT), which detect HCV RNA and may be divided into qualitative and quantitative assays.

Qualitative Assays—Must have a lower limit of detection of less than 50 IU/mL of HCV RNA. They provide confirmation of HCV infection and may be used to evaluate sustained virologic responses (SVR) in patients undergoing antiviral therapy.

Quantitative Assays—Consist of real-time PCR-based methods. Pretreatment quantitative assays are utilized to measure baseline HCV viral load; and, during therapy, to evaluate for early virologic response (EVR). EVR is defined as ≥ a 2-log decline in HCV RNA from baseline to week 12 of treatment. If the patient does not achieve EVR, it most likely represents failure of therapy and treatment is stopped. Rapid virologic response (RVR), which is defined as an undetectable HCV RNA by week 4 of treatment is predictive of SVR. Genotyping is essential prior to therapy since duration of therapy and dose of ribavarin is dictated by the HCV genotype. The commonly used method of genotyping is the line probe assay.

SYPHILIS

Testing for syphilis (*Treponema pallidum* infection) is usually performed when the disease is suspected clinically, for screening in a high-risk population, and routinely during antenatal visits. Laboratory diagnosis of syphilis may be done by the commonly used serologic methods, or by direct visualization techniques, which are less commonly used.

Serologic Tests

These test commonly involve an initial nonspecific nontreponemal antibody test for primary screening, which is followed by a more specific treponemal test for diagnostic confirmation. A clinical suspicion with positive serology provides presumptive diagnosis. Direct visualization techniques via darkfield microscopy and direct fluorescent antibody (DFA) provide definitive diagnosis.

Nontreponemal tests evaluate the reactivity of the patient's serum to a cardiolipin-cholesterol-lecithin antigen. Nontreponemal tests include the Venereal Disease Research Laboratory (VDRL), rapid plasma reagin (RPR), and toluidine red unheated serum test (TRUST). These tests are semiquantitative and reported as a titer. For example a result of 1:40 indicates the most dilute serum at which the antibody was detectable. A reactive nontreponemal test must be confirmed by a treponemal test, which includes:

- The fluorescent treponemal antibody absorption (FTA-ABS)
- Microhemagglutination test for antibodies to *T. pallidum* (MHA-TP)
- *Treponema pallidum* particle agglutination assay (TP-PA)

- *Treponema pallidum* enzyme immunoassay (TP-EIA), which is most favored

These tests are based on the detection of antibodies directed against specific treponemal antigens and are qualitative (i.e., reported as positive or negative).

Interpretation of Serologic Tests

A reactive nontreponemal and treponemal test denotes active syphilis infection, unless the patient has a history of prior treatment for syphilis. An asymptomatic person with reactive nontreponemal and treponemal tests denotes "latent" syphilis. Diagnosis of a new infection in a patient treated in the past for syphilis infection is made by quantitative testing on a RPR test, which reveals a fourfold or greater increase in antibody titer than that present during the past treated infection.

False-positive nontreponemal tests may occur during:

- Pregnancy
- Intravenous drug use
- Tuberculosis and rickettsial infection
- Nonsyphilis treponemal infection
- Endocarditis
- HIV infection

False-negative tests are usually encountered in two situations: (1) when the test is performed prior to the formation of antibodies to syphilis or (2) due to a prozone reaction. The latter instance is seen in situations such as secondary syphilis or HIV coinfection, when very high antibody titers (antibody excess) are present that interfere with the formation and thus visualization of antigen-antibody complexes. If this situation is suspected, further dilutions of the sample should be done, which allows for the zone of equivalence to be reached.

Flash Card Q55

What are the key clinical symptoms of a patient with primary, secondary, tertiary syphilis?

Flash Card A55

Primary: Small painless chancre (ulcer) on genitals, mouth, skin or rectum which resolves in 3–6 weeks.

Secondary: Skin rash on palms and soles, condylomata lata (moist warty patches) in genitals or skin folds. Other symptoms, such as fever, general ill feeling, loss of appetite, muscle aches, joint pain, enlarged lymph nodes, vision changes, and hair loss may occur.

Tertiary: Symptoms vary widely depending on organ system involved. May include cardiovascular syphilis (aortic aneurysm or valve disease), neurosyphilis, or tumors of skin, bones or liver (gumma).

9 Pharmacology and Therapeutics

ALLERGEN AVOIDANCE

Outdoor Allergens

Grass, tree, and weed pollens are difficult to avoid during pollen season. Some recommendations for avoiding outdoor pollen include the following:

- Avoid outdoor activities in early morning (pollination peaks from 5–10 AM.).
- Stay indoors during humid and/or windy days.
- Do not mow grass (grass or mold) or rake leaves (mold).
- Do not hang sheets or clothing outside to dry.
- Wear protective masks.

Indoor Allergens

Dust Mite (Der p1, Der f1)—Highest exposure to dust mites (*Dermatophagoides* spp.) occurs in the bedroom (i.e., mattresses, stuffed animals, carpeting). Higher contamination is found in areas with high humidity (≥ 55%) and low altitude. A **significant** decrease in allergen levels of less than 6 months improves bronchial hyper-responsiveness and symptoms of atopic dermatitis.

Methods of avoidance:

- Impermeable covers (i.e., pore size ≤ 10 μm) for mattress, pillows, and box springs
- Weekly washing of bed and pillow coverings in hot water (54.4°C, or 130°F), and dry bedding on high heat
- Remove stuffed animals or periodically place them in plastic bags in the freezer overnight
- Remove carpet removal and replace with hard flooring
    - Alternative is benzyl benzoate or tannic acid applied to carpet (temporary)
    - Vacuuming weekly using high-efficiency particulate air (HEPA) filter or double-thickness bag
- Remove upholstered furniture or change to vinyl or leather
- Decrease indoor humidity levels to less than 50% (unproven)

Flash Card Q1

Which type of allergen is most likely to be found in undisturbed air in homes?

Cat (Fel d 1) and Dog (Can f 1) Allergens—Generally, removal of pet from home followed by thoroughly vacuuming, cleaning, and/or washing all surfaces is best.

Alternatives to elimination (all unproven):

- Restrict pet from bedroom
- Use allergen-proof bed covers
- Use HEPA filter
- Wash animal at least twice weekly
- Remove carpet from bedroom and remove upholstered furniture
- For cats, higher Fel d1 in males of the species; **allergen remains for up to 4–6 months after cat removal**

Other pets known to cause symptoms include:

- Rodents (rats, mice, guinea pigs, gerbils and/or hamsters, and rabbits)
- Rats and/or mice can be problems for laboratory workers and in the inner city
- Birds and other exotic pets

Cockroach (Bla g 1-4, Per a 1)—Methods of avoidance:

- Poison baits with hydramethylnon or boric acid (avoid organophosphates), with second application in 1–2 weeks
- Comprehensive housecleaning, focusing on countertops or surfaces and carpeting
- Eliminating food sources in kitchen and/or dining areas
- Sealing access points to the house
- Takes 6 months for 80–90% reduction
- Insecticides and HEPA filters generally ineffective

Fungal Proteins (Various)—Generally, damp environments carry more mold and bacteria, but there is no clear relationship of indoor spore counts to symptoms.

Methods of avoidance:

- Eliminate excess moisture (e.g., water leaks from plumbing, dishwashers, and/or hot water heaters)
- Limit relative humidity to less than or equal to 40%
- Increase ventilation and/or exhaust fans
- Replace carpet
- Use HEPA filters

Flash Card A1

Cat, dog

Table 9-1 provides an overview of common indoor allergens.

Table 9-1. Common Indoor Allergens

Allergen	Allergenic Protein	Location	Risk Factors	Avoidance Methods
Dust mite	Der f 1 and Der p 1	Carpet, bedding (mattress/pillow/box springs), and upholstery	Humidity, older homes, single family dwelling and absence of AC	Impermeable covers, hot-water washing of linens, and carpet removal
Dog	Can f 1 and albumin	Carpet and bedding	Depends on the breed and/or animal	Removal, impermeable covers, HEPA filter, and carpet removal
Cat	Fel d 1	Carpet and bedding	Depends on the breed and/or animal	Removal, impermeable covers, HEPA filter, and carpet removal
Cockroach	Bla g 1-4 and Per a 1	Kitchen and dining	Humidity and open food sources	Extermination and gel baits.
Fungus	Various	Bathrooms and/or kitchens, and areas of water damage	Humidity, water damage, and leaky plumbing	Eliminate water source, roof inspection, and carpet removal

Abbreviations: AC, air-conditioning; HEPA, high-efficiency particulate air.

IMMUNOTHERAPY

General Principles

Allergen immunotherapy (AIT) is an effective treatment for allergic rhinitis/conjunctivitis, allergic asthma, atopic dermatitis, and stinging-insect hypersensitivity. Patients selected for such therapy should demonstrate specific IgE antibodies to relevant allergens to which they are allergic, either by skin testing or appropriate in vitro tests. AIT is **not** for food, latex, or drug allergy and is not usually prescribed for very young or elderly patients. Aeroallergen immunotherapy can usually be stopped after 3–5 years of successful therapy; but, with severe stinging-insect hypersensitivity, AIT may be used indefinitely.

Studies Demonstrating Efficacy of AIT

Efficacy has been demonstrated in double-blind placebo-controlled clinical (DBPC) trials for allergic rhinitis and/or conjunctivitis, atopic dermatitis (AD)

and allergic asthma, in both adults and children; and, for allergy caused by pollens, certain molds (*Alternaria*, *Cladosporium*), animal allergens (dog, cat), dust mites, cockroaches. Efficacy has also been demonstrated in stinging-insect hypersensitivity caused by Hymenoptera and imported fire ant. AIT has been shown to reduce symptoms of allergic rhinoconjunctivitis AD and asthma, and large meta-analyses have shown a decreased need for medication after AIT. Regarding asthma, AIT also decreases bronchial responsiveness, but does not affect pulmonary function results. It can prevent or delay the onset of asthma in patients with allergic rhinitis. It is best to measure clinical outcomes to monitor patient response to AIT; repeated in vivo or in vitro testing is unnecessary.

Potential Mechanisms and Immunologic Changes Associated with Immunotherapy

- Modified allergic response to allergens over time
- Increase in IgG blocking antibody (initially IgG1, later IgG4)
- Increased numbers of $CD4^{+}CD25^{+}$ regulatory T lymphocytes and % of CD8+ T cells
- Initial **increase** (months), then a steady **decrease** (years) in allergen-specific IgE
- Blunted seasonal rise in allergen-specific IgE
- Decrease in low-affinity FcεRI and FcεRII (CD23)
- Increased IgA in respiratory secretions
- Reduction in basophil hyper-reactivity
- Increased interferon (IFN)-γ/interleukin (IL)-4 ratio and secretion of IL-10 and transforming growth factor (TGF)-β
- Decreased number of eosinophils, basophils, and mast cells in nose/lung
- Shift of T_h2 cytokines (IL-4, IL-5, and IL-13) to T_h1 cytokines (IFNγ, TGFβ, and IL-10)

Clinical Aspects of Allergen Immunotherapy

Selection of Allergens and Nomenclature—Selection of allergens for a particular patient depends on the patient's history, the local aerobiology in the patient's indoor and/or outdoor environment, and the patient's **serum-specific IgE and**/or skin tests results. The highest concentration of an extract, termed **maintenance vial**, is represented by 1:1 vol/vol and labeled "red vial" (see Table 9-2 for further dilutions). The starting dose for build-up is usually a 1000– or 10,000-fold dilution of the maintenance concentrate.

Table 9-2. Nomenclature for Labeling Dilutions from the Maintenance Concentrate

Dilution from Maintenance	Volume/Volume Ratio	Weight/Volume Ratio	Vial Color
10,000-fold	1:10,000	1:1,000,000	Silver
1000-fold	1:1000	1:100,000	Green
100-fold	1:100	1:10,000	Blue
10-fold	1:10	1:1000	Yellow
Maintenance concentrate	1:1	1:100	Red

Cross Reactivity—Selecting the most locally prevalent allergen from cross-reacting families of allergens permits the addition of optimal doses of clinically relevant allergens to a vaccine (Table 9-3).

Mixing of Allergens Containing Proteolytic Enzymes—**Mold and cockroach extracts contain proteolytic enzymes that degrade pollen**, reducing IgE-binding affinity. Therefore, these extracts should remain in separate vials; but, they can be mixed. Dust mite (in 50% glycerin) is safe to mix with pollen, dog or cat, cockroach, and mold extracts. Ragweed and **cat extracts** in 50% glycerin resist degradation when mixed with high-protease extracts.

Table 9-3. Cross-Reactivity of Allergen Extracts

Weeds	Grasses	Trees
Ambrosia sp. (short/giant/false/western ragweed)	*Festuca* sp. (meadow fescue, timothy, rye, Kentucky blue, orchard, and red top)	Cupressaceae family (juniper, cedar, and cypress)
Artemisia sp. (sage/wormwood/mugwort)		Betulaceae family (birch, alder, hazel, and hornbeam)
Chenopod sp. (Russian thistle/lambs quarter/burning bush)		Fagaceae sp. (oak, beech, and chestnut)
Amaranthus sp. (pigweed/red root pigweed)		Oleaceae family (ash, olive, and privet)
Atriplex sp. (saltbush/wingscale)		*Populus* sp. (cottonwood, poplar, and aspen)

Flash Card Q2

Which allergen extracts are standardized?

Venoms contain proteases that may degrade each other and, therefore, should be separated. An exception are vespid venoms (hornets or yellow jackets), which can be mixed.

***Doses and Dosing Schedules*—Effective maintenance doses for most inhalant allergens are between 5-20 µg** of the major allergen per 0.5 mL maintenance dose. Effective doses in biologic allergy units (BAU) or arbitrary units (AU) are 1000–4000, depending on the allergen. Table 9-4 includes clinically effective maintenance doses for standardized aeroallergens, based on efficacy studies.

Adverse Reactions

***Local Reactions*—**Consist of redness and/or swelling (dime- to quarter-sized), which may cause local discomfort. They do not appear to increase risk for systemic reactions. Frequent large local reactions (i.e., larger in diameter than 25 mm) may increase risk for future systemic reaction.

***Systemic Reactions*—**Risk in approximately 1/2000 injections; most reactions begin within 30 minutes. There is a greater risk of fatal reactions in patients taking β blockers. Risk of fatal reaction is 1 in 2.5 million.

Table 9-4. Probable Effective Maintenance Doses for Allergen Immunotherapy

Allergen	Labeled Extract Potency	Effective Dose of Major Allergen	Effective Biologic (BAU) Allergy Units (AU)
Grass	10,000–100,000 BAU/mL	15 µg of various	1000–4000 BAU
Short ragweed	1:10–1:20 w/v; 100,000 AU/mL	6–12 µg	1000–4000 AU
Cat	5000–10,000 BAU/mL	11–17µg	1000–4000 BAU
Dog, nonstandardized	1:10–1:100 w/v	15 µg	
Dust mite (Der f1 and Der p1)	3000, 5000, 10,000, and 30,000 AU/mL	7–12 µg	500–2000 AU
Other nonstandardized extracts	1:10–1:40 w/v or 10,000–40,000 PNU/mL	Highest tolerated dose	
Bermuda	10,000 BAU/mL		300–1500 BAU

Flash Card A2

Grass (i.e., northern grasses and Bermuda), ragweed, cat, dust mites

Greater risk of systemic reactions in the following:

- Taking β-blocker medications may increase risk for more serious reactions
- During "priming" of pollen season
- During build-up phase
- During accelerated or rushed protocols
- First injection from new vials
- Unstable asthma
- Dosing error
- History of previous systemic reaction

Contraindications

Relative contraindications to starting immunotherapy:

- Pregnancy (OK to continue without increasing dose if patient becomes pregnant after starting)
- Serious immunodeficiency
- Malignancy
- Uncontrolled asthma
- Significant cardiovascular disease
- Children younger than age 5 years
- Systemic mastocytosis

Sublingual Immunotherapy

Sublingual immunotherapy (SLIT) is currently undergoing clinical trials in US. It has been used clinically in Europe for years with no fatalities; and, there is an improved rate of systemic reactions versus in subcutaneous immunotherapy (SCIT). SLIT is shown to be clinically effective in monotherapy with grass, ragweed, birch, cat, *Parietaria*, and dust mite; however, is has less robust efficacy than SCIT. Efficacy data in polysensitized individuals is lacking.

HISTAMINE ANTAGONISTS

Flash Card Q3

What is the probable effective dose in biologic allergy units (BAU) for cat immunotherapy?

Histamine is formed by histidine decarboxylase (an enzyme expressed in cells throughout the body) acting on L-histidine. Histamine is released by mast cells

and basophils as a preformed mediator (typically, after cross-linkage of surface IgE bound to FcεR1 in allergic individuals) along with other mediators (i.e., preformed: tryptase; newly generated: leukotrienes and prostaglandins). See Table 9-5 for different types of histamine receptors.

Table 9-5. Four Types of Histamine Receptors

	H_1	H_2	H_3	H_4
Receptor expression	Widespread: Nerve cells, airway smooth muscle, vascular smooth muscle, endothelial cells, epithelial cells, neutrophils, eosinophils, monocytes, macrophages, dendritic cells, T lymphocytes, B lymphocytes, hepatocytes, and chondrocytes	Widespread: Same as H_1 except **not on macrophages**	Histaminergic neurons (presynaptic), eosinophils, dendritic cells, and monocytes	Bone marrow and peripheral hematopoietic cells, eosinophils, neutrophils, dendritic cells, T lymphocytes, basophils, and mast cells
General function	Increase pruritus, pain, vasodilation, vascular permeability, hypotension, flushing, headache, tachycardia, bronchoconstriction, airway vagal afferent nerve and cough receptor stimulation, and decreased atrioventricular node conduction time	Increases gastric acid secretion, vascular permeability, hypotension, flushing, headache, tachycardia, chronotropic and inotropic activity, bronchodilation, and airway mucus production	Increased pruritus and nasal congestion. Prevent excessive bronchoconstriction	Increased pruritus and nasal congestion, and differentiation of myeloblasts and promyelocytes
Function in central nervous system	Sleep or wake, food intake, thermal regulation, memory, and learning	Neuroendocrine	Decreased histamine, dopamine, serotonin, norepinephrine, and acetylcholine release	Not completely defined at this time

Flash Card A3

1000–4000 BAU

Antihistamines as Inverse Agonists

Histamine receptors are G protein-coupled, seven-helical, transmembrane molecules with constitutive activity. Equilibrium exists between the active and inactive states of the receptor. When histamine binds to its receptor, it stabilizes the receptor in its active form; thus, shifting the equilibrium to the active state. By contrast, when an H_1-antihistamine binds to this receptor, it stabilizes the inactive form of the receptor; thereby, shifting the equilibrium to the inactive state (Figure 9-1).

Antiallergic and Anti-Inflammatory Effects of H_1-Antihistamines

- **Antiallergic:** Inhibit release of mast cell and basophil mediators (mediated through inhibition of calcium ion channels). Pretreatment of an allergen challenge with an oral H_1-antihistamine reduces the early response in the nose, conjunctiva, skin, and lower airways (fewer adhesion molecules, eosinophils, neutrophils, cytokines, histamine, leukotrienes, and prostaglandins in lavage fluid).
- **Anti-inflammatory:** Inhibit expression of cell adhesion molecules and eosinophil chemotaxis (via downregulation of nuclear factor-κB). The anti-inflammatory effects are weak compared with those of corticosteroids.

Metabolism and Pharmacokinetics/Pharmacodynamics

First-generation antihistamines and some second-generation antihistamines are metabolized in the liver by the cytochrome P450 system. (Be familiar with Table 9-6).Greater than 97% of histamine is metabolized by two major pathways before excretion: (1) histamine *N*-methyltransferase and (2) diamine oxidase.

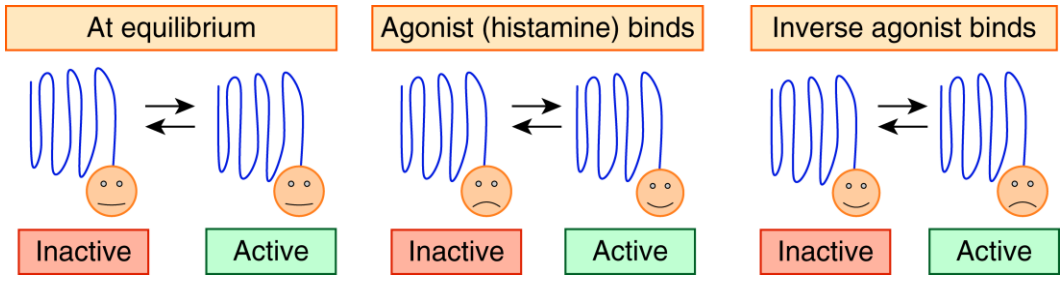


Figure 9-1. Histamine receptor.

Table 9-6. Antihistamines in Adults

	Onset of Action Based on Wheal-and-Flare Studies(n Hr)	Half-Life (*t* ½, in Hr)	Conditions That May Require Dose Adjustment
First-generation antihistamines			
Chlorpheniramine	3	27.9 ± 8.7	
Diphenhydramine	2	9.2 ±2.5	Hepatic impairment
Doxepin		13	Hepatic impairment
Hydroxyzine	2	20 ± 4.1	Hepatic impairment
Second-generation antihistamines			
Cetirizine (a metabolite of hydroxyzine) cleared through urine	1	6.5–10	Renal and hepatic impairment
Desloratadine (metabolite of loratadine)	2	27	Renal and hepatic impairment
Fexofenadine *cleared through feces.	2	14.4	Renal impairment
Levocetirizine (enantiomer of cetirizine)	1	7 $\pm$ 1.5	Renal and hepatic impairment
Loratadine	2	7.8 $\pm$ 4.2	Hepatic impairment
Topical antihistamines			
Azelastine	0.5	22–27.6	N/A
Emedastine	0.25	7	
Epinastine	0.1	6.5	
Ketotifen	0.25	20–22	
Levocabastine	0.25	35–40	
Olopatadine	0.25	7.1–9.4	

Adverse Effects

Central Nervous System—First-generation H_1-antihistamines block histamine's effects in the central nervous system because they can cross the blood-brain barrier, accounting for increased sedation. Second-generation antihistamines penetrate the blood-brain barrier poorly.

Cardiac—Although uncommon, first-generation H_1-antihistamines may cause a dose-related prolonged QT interval via antimuscarinic and anti-α-adrenergic effects. Blockade of the rapid component of the delayed rectifier potassium

current (IKr) and prolongation of the QT interval may result in torsades de pointes.

Potential adverse effects of H_1 antihistamines due to effects on receptors and ion channels:

- H_1 receptor: **Increased sedation**, **increased appetite**, **decreased cognitive and psychomotor performance**, and decreased central nervous system neurotransmission
- Muscarinic receptor: **Dry mouth**, **urinary retention**, sinus tachycardia
- α-Adrenergic receptor: Hypotension, dizziness, and reflex tachycardia
- Serotonin receptor: Increased appetite
- Ion channels: Prolonged QT intervals

Efficacy of H_1 Antihistamines

***Allergic Rhinoconjunctivitis*—**Relieve nasal itching, sneezing, rhinorrhea, conjunctival itching, watering, redness, and itching of the palate throat and ears. Topical administration to the nose or eyes has more rapid onset of action but requires multiple doses per day.

***Other Airway Disorders*—**Published evidence does not support efficacy in respiratory tract infections, otitis media, sinusitis, or asthma. However, according to the "united airway hypothesis," treatment of rhinitis may reduce symptoms in lower respiratory tract.

***Urticaria*—**H_1-antihistamines decrease itching and reduce wheal size, number, and duration. May be effective for physical urticarias, but not urticarial vasculitis or hereditary angioedema.

***Other Allergic Conditions*—**Ancillary treatment for anaphylaxis, but they do **not** replace epinephrine.

Flash Card Q4

Which second-generation antihistamines are safest to use in pregnancy?

THEOPHYLLINE

General Considerations

Flash Card Q5

Which of the following antihistamines has the longest half-life: loratadine, cetirizine, fexofenadine, hydroxyzine?

Theophylline is a member of methylxanthine family (caffeine is also in this family) with a **narrow therapeutic range**. It is used for treatment of asthma and chronic obstructive pulmonary disease. Since β_2 agonists are more effective as

bronchodilators and inhaled corticosteroids have a superior anti-inflammatory effect, the use of theophylline has decreased.

- Asthma: 2007 NHLBI guidelines list theophylline as an alternative agent to be considered when asthma in patients (younger than 5 years old only) is not controlled with low-dose inhaled corticosteroids. Slow-release theophylline may be helpful for nocturnal asthma symptoms.
- COPD: Theophylline is still used, although inhaled anticholinergics and β_2 agonists are preferred.

Mechanism

Theophylline inhibits phosphodiesterase (PDE), resulting in elevated cyclic adenosine momophosphate (cAMP) (Figure 9-2), and also acts as an adenosine receptor antagonist. Adenosine is thought to cause histamine and leukotriene release from mast cells, resulting in bronchoconstriction. Adenosine antagonism is responsible for the toxic effects of theophylline at high concentrations (i.e., cardiac arrhythmias, seizures)

Clinical Effects

Bronchodilation—Produces acute bronchodilator response above plasma concentration of 10 mg/L (therapeutic range 5–15 mg/L). Directly relaxes airway smooth muscle and reverses effects of bronchoconstrictor agonists. The degree of PDE inhibition is very small (i.e., 5–10%) at concentrations of theophylline that are therapeutically relevant and may account for the frequent side effects of nausea, vomiting, and headache.

Flash Card A4

Cetirizine, levocetirizine, and loratadine (category B)

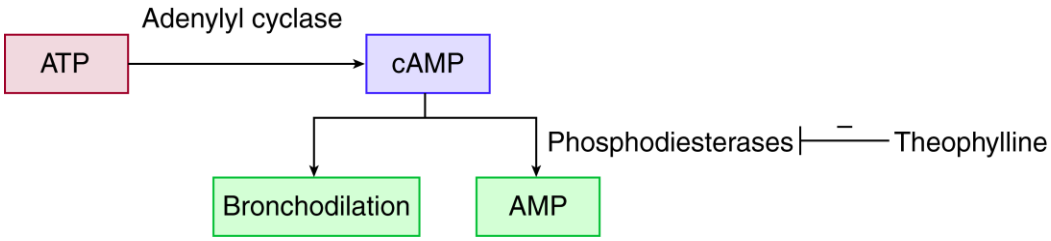


Figure 9-2. Theophylline inhibits phosphodiesterase resulting in elevated cAMP.
Abbreviations: AMP, adenosine monophosphate; ATP, adenosine triphosphate; cAMP, cyclic adenosine monophosphate.

Flash Card A5

Hydroxyzine

Anti-inflammatory Effects—Intravenous theophylline inhibits the late response to allergen challenge, and airway influx of neutrophils and eosinophils. Other anti-inflammatory actions of theophylline include: increase in IL-10, increased histone deacetylase activity (suppressing inflammatory cytokine gene expression), prevents nuclear translocation of nuclear factor kappa B (NFκβ), and increased apoptosis of inflammatory cells

Other—May help with mucociliary clearance and improve respiratory muscle strength.

Factors That Affect Clearance

Theophylline is metabolized in the liver by cytochrome P450 microsomal enzymes, and other medications affecting this enzyme may alter theophylline levels. See Table 9-7 for factors affecting clearance of theophylline.

Adverse Effects

Most common adverse effects are headache, nausea, vomiting, abdominal discomfort, and restlessness. Patient may also have increased gastric acid secretion, reflux, and diuresis. There is potential for significant toxicity (e.g., convulsions, cardiac arrhythmias, and death) at high concentrations. **Adverse effects tend to occur at plasma levels greater than 20 mg/L.**

Table 9-7. Factors That Lead to Increased and Decreased Clearance of Theophylline

Decreased Clearance (↑ Serum Level)	Increased Clearance (↓ Serum Level)
Macrolide antibiotics	Carbamazepine, phenobarbital, and phenytoin
Cimetidine (but **not** ranitidine)	Rifampin
Ciprofloxacin	Ethanol
Verapamil	Smoking tobacco and marijuana
Zileuton	High-protein and low-carbohydrate diet
Allopurinol	Younger children have greater metabolism than older children
Congestive heart failure	
Liver disease	
Viral infection	
High-carbohydrate diet	
Older age	

Flash Card Q6

A high-protein, low-carbohydrate diet has what effect on theophylline clearance?

Dosage

Based on body weight. Check serum level after 3 days of maximum dose. Peak serum level occurs 8–13 hours after sustained-release preparations. Target level of 5–15 μL/mL.

β AGONISTS AND BLOCKERS

β AGONISTS

Mechanism

Key Fact

Albuterol binds to GPCR and increases cAMP.

β Agonists bind to G protein-coupled receptors (GPCR) and activate adenylyl cyclase, resulting in increased cAMP. This activates protein kinase A, leading to phosphorylation and muscle relaxation.

There are three types of β receptors:

- B1: Heart
- B2: Lung (smooth muscle, epithelium, and alveoli), inflammatory cells (mast cells, eosinophils, lymphocytes, and neutrophils)
- B3: Adipose tissue

Modifications of β-agonist structure affect its function. Increasing the bulk of the side chain results in more selectivity for the β_2-receptor, prolongs bronchodilator action and protects from catechol-*O*-methyltransferase (COMT). Also, increasing the size of the terminal amino group protects the drug from degradation by monoamine oxidase (MAO). Salmeterol and formoterol have long lipophilic side chains that allow entry into the plasma membrane and slow release (Table 9-8).

Beta agonists have the following multiple **nonbronchodilator actions**:

Flash Card A6

Increased clearance (↓ level)

- Increased mucociliary clearance (increased chloride ion and H_2O secretion, and increased ciliary beat frequency)
- Protect epithelium against bacteria
- Suppress microvascular permeability
- Inhibit cholinergic neurotransmission

Table 9-8. Comparing the Onset and Duration of Action of β2 Agonists

Types of β Agonists	Onset of Action (min)	Duration of Action (hr)
SABAs (i.e., albuterol, terbutaline, pirbuterol, levalbuterol)	2–4	4–6
LABAs (i.e., formoterol, salmaterol)	Formoterol (2–3) Salmeterol (30)	> 12
Ultra long-acting β agonists (i.e., carmoterol, indacaterol)	< 5	≥24

Abbreviations: LABA, long-acting β agonist; SABA, short-acting β_2 agonist.

- Inhibit mediator release from mast cells and basophils
- Inhibit function of eosinophils, antigen-presenting cells, T lymphocytes, and epithelial cells
- Prime glucocorticoid receptor by mitogen-activated protein (MAP) kinases, leading to enhanced nuclear translocation
- BAGS study: Regular β agonist use was found to be neither harmful nor beneficial compared with prn use
- SOCS study: Salmeterol monotherapy ineffective
- SLIC study: Patients with moderate asthma achieving control with addition of long-acting β agonist (LABA) to inhaled corticosteroid (ICS) could have 50% ICS dose reduction but not ICS removal.
- LABA versus theophylline: Salmeterol with increased morning peak expiratory flow (PEF), symptom improvement, and rescue-free days.
- LABA versus leukotriene modifiers: Adding LABA to ICS shows greater improvement in lung function and symptoms versus adding LTRA to ICS.
- A maximum of 12–14% of a measured-dose inhaler (MDI) dose is deposited in the lungs.
- IM epinephrine has quicker onset than SQ; vastus lateralis is quicker than deltoid.

Adverse Effects

Pharmacologically-mediated adverse effects include:

- Tremor (stimulation of β_2 in skeletal muscles)
- Tachycardia

Flash Card Q7

Long lipophilic side chains of β agonists, have what effect on bronchodilation?

- Prolonged QTc /arrhythmias
- Hyperglycemia
- Hypokalemia
- Hypomagnesemia. Transient increased hypoxia

Paradoxical bronchoconstriction can occur with first use of new MDI. The Salmeterol Multicenter Asthma Research Trial (SMART) suggested that asthmatic patients receiving salmeterol were at an increase for fatal asthma events. However, the risk appears to be limited to asthmatics taking salmeterol alone and does not apply to the concurrent use of an inhalational corticosteroid. Findings from the study discourage the use of LABAs as monotherapy in asthmatics. Polymorphisms of the β_2-adrenergic receptor (ADRB2) results in agonist receptor downregulation, which induces resistance to the smooth-muscle relaxing effect of β_2 agonists. Regular use of albuterol is associated with worsening lung function in patients with this mutation.

β2-Receptor Desensitization Versus Tachyphylaxis

Desensitization (autoregulatory and safety valves), decrease duration of bronchodilation (not peak), prevents overstimulation, and occurs over days to weeks. There are three mechanisms for this: (1) receptors uncouple from adenylate cyclase, then (2) internalize, and then (3) become phosphorylated. Systemic steroids can reverse downregulation.

Tachyphylaxis occurs rapidly. Ephedrine displaces norepinephrine from sympathetic nerve endings.

Loss of bronchoprotection to stimuli may occur with chronic use (i.e., exercise), as the protection by the first dose of a β agonist will not be replicated during the course of regular β-agonist therapy (protection reduced but not lost).

β-BLOCKERS

Nonselective β blockers ($\beta_1 = \beta_2$) include propranolol, timolol, pindolol, nadolol, and labetalol (also blocks α_1-receptors). They are associated with a blunted response to β_2-agonists and thus may increase the patient's risk during an asthma exacerbation.

Flash Card A7

Prolongs bronchodilation

Cardioselective β blockers ($\beta_1 > \beta_2$), including metoprolol and atenolol, have greater than 20 times more affinity for β_1-receptors and pose less risk for bronchoconstriction or loss of asthma control.

Inhalant allergen immunotherapy is a relative contraindication for patients on a β blocker.
Venom immunotherapy is not an absolute contraindication if taking a β blocker.

In anaphylaxis, use glucagon to overcome β blockade if the patient is unresponsive to epinephrine.

LEUKOTRIENE PATHWAY MODULATORS

Leukotriene Pathway

- Leukotrienes are formed by arachidonic acid metabolism through lipoxygenase (LO) pathway (Figure 9-3).
- Two major enzymes, 5-lipoxygenase (5-LO) and 15-lipoxygenase (15-LO) are involved. 5-LO catalyzes the formation of an unstable intermediate leukotriene, A4 (LTA4). LTA4 hydrolase, expressed mostly by neutrophils, converts LTA4 to the leukocyte chemoattractant substance LTB4, which upregulates CD11b/CD18 and enhances neutrophil chemotaxis.
- The enzyme leukotriene C4 synthase converts LTA4 to cysteinyl leukotrienes (cysLTs) LTC4, LTD4, and LTE4. This enzyme is expressed in eosinophils, basophils, macrophages, and mast cells.
- The other major enzyme, 15-LO, leads to the formation of lipoxins, a group of mediators with anti-inflammatory functions.

Pharmacologic Targets

Antileukotrienes include the following:
- 5-Lipoxygenase inhibitor
- CysLT receptor antagonists

There are two types of leukotriene receptors: cysLT1 and cysLT2.

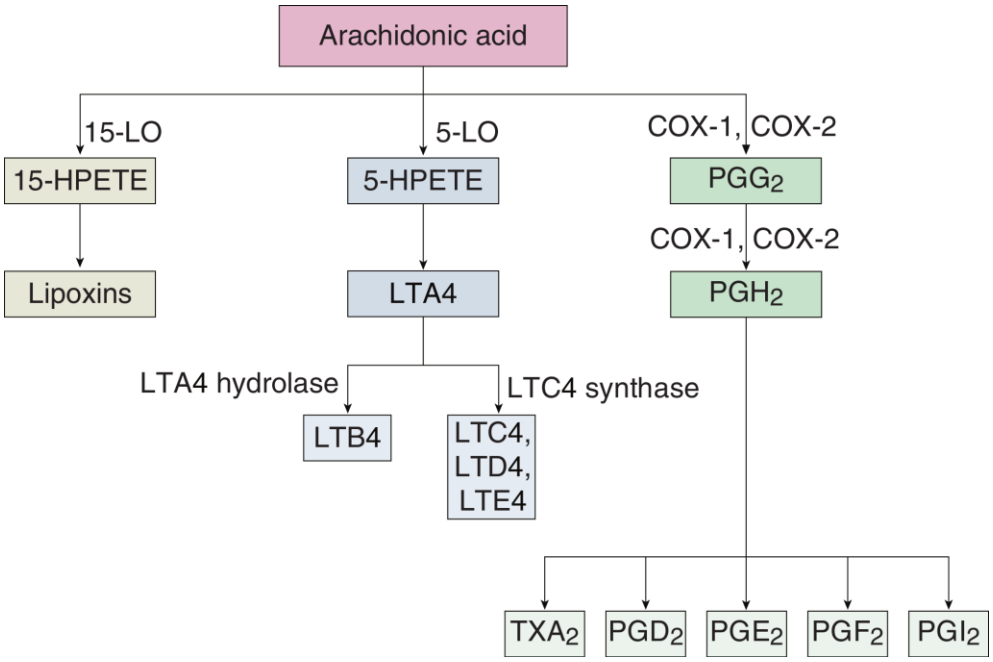


Figure 9-3. Metabolism of arachidonic acid by the cyclooxygenase and lipoxygenase (LO) enzymatic pathways.
Abbreviations: COX, cyclooxygenase; 5-HETE, 5-hydroxyeicosatetraenoic acid; 5-HPETE, 5-hydroperoxyeicosatetraenoic acid; LT, leukotriene; PG, prostaglandin; PGI_2, prostacyclin; TX, thromboxane.

- **CysLT1 receptors** are expressed on inflammatory cells, including eosinophils, mast cells, lymphocytes, macrophages and neutrophils as well as on bronchial epithelium and muscles. These receptors mediate proasthmatic actions such as sustained bronchoconstriction, mucus secretion, and increased vascular permeability. (Receptor affinity: LTD4 > LTC4 > LTE4.)
- **CysLT2 receptors** do not have a known role in bronchoconstriction, although they may contribute to edema formation. They appear important in activation of endothelial cells. (Receptor affinity: LTC4 = LTD4 > LTE4.)

***CysLT1 Receptor Antagonist*—**Leukotriene receptor antagonists block the cysLT1 receptor only. They include montelukast (has highest binding affinity), pranlukast, and zafirlukast.

Leukotrienes in Asthma Pathogenesis

Cys-LTs are produced within minutes of exposure to an allergen, and these cytokines further upregulate expression of enzymes involved in their synthesis. Asthmatics produce higher baseline levels of cys-LTs, and levels of sputum cys-LTs correlate with asthma severity in adults. High levels of cys-LTs are present in patients with aspirin-sensitive asthma.

Effects of cys-LTs include smooth muscle contraction and bronchoconstriction (much more potent than histamine); they increase permeability that leads to bronchovascular leakage and mucous gland secretion, leukocyte chemotaxis, stimulation of airway smooth muscle cells, and fibroblasts proliferation, suggesting a possible role in airway remodeling.

Clinical Uses of Leukotriene Modulators

- Exercise-induced bronchospasm
- Allergic asthma and allergic rhinitis
- Aspirin-exacerbated respiratory disease
  - Leukotriene antagonists attenuate **both** early- and late-phase bronchoconstrictor responses. Antileukotriene therapy leads to (1) improvement in forced expiratory volume in the first second (FEV_1), (2) less need for rescue β-agonist therapy, and (3) a decrease in asthma exacerbations. However, antileukotrienes are generally less efficacious than corticosteroids.
  - Leukotriene antagonists are recommended prior to aspirin desensitization.
- Leukotriene modifiers have some anti-inflammatory effects, including reduced numbers of circulating and sputum eosinophils, exhaled nitric oxide, and bronchial hyper-responsiveness
- No reports of tolerance (tachyphylaxis) to antileukotrienes

Montelukast and Zafirlukast

Montelukast and zafirlukast are selective leukotriene-receptor antagonists that inhibit the cysLT1 receptor. Pregnancy class B.

Adverse Effects—Anaphylaxis, angioedema, dizziness, dyspepsia, muscle weakness, and **elevated transaminases**. Few instances of suicidal thinking, suicide, and behavioral or mood changes have been noticed among patients taking montelukast. Montelukast generally has no significant drug interactions. Zafirlukast absorption is decreased by food. It can also interact with warfarin, leading to an increased prothrombin time. Rare association with onset of Churg-Strauss vasculitis; although, this could be due to unmasking with corticosteroid taper.

Zileuton

A 5-LO inhibitor that blocks the formation of LTB4 and cys-LTs. It is approved for prophylaxis and treatment of asthma. Pregnancy class C.

Flash Card Q8

LTB4 is a potent chemoattractant for which types of cells?

Adverse Effects—Headache, dyspepsia, myalgias, leukopenia, and **elevated transaminases**. Use in patients with a history of liver disease or substantial alcohol consumption should be avoided. ALT should be monitored every 3 months. Zileuton inhibits cytochrome CYP1A2 and, therefore, affects the levels of certain coadministered drugs. For example it can lead to decreased clearance (i.e., increased serum levels) of theophylline, warfarin, and propranolol; therefore, decrease dose of these medications while on zileuton.

MAST CELL STABILIZERS

Physiochemical Properties

Mast cell stabilizers have negligible fat solubility and are totally ionized at physiologic pH, making them unable to enter the cell. They are well absorbed in the lung, are not metabolized, and are excreted unchanged in bile and urine. They are available in many different preparations.

Pharmacokinetics

Cromolyn Sodium—Approximately 8% of each inhaled dose reaches the lungs and is absorbed, while more than 1% of each oral dose is absorbed. Half-life elimination of the drug is 1.5 hours, and peak serum concentrations are reached in about 15 minutes.

Nedocromil Sodium—Inhaled formulation therapeutic effect is seen in 2 hours, but full therapeutic effect may not occur until 1 week or more of therapy. The drug has very low systemic absorption rates and has a half-life of 1.5–3.0 hours.

Mechanism of Action

Inhibits IgE-mediated calcium channel activation, which functions to maintain elevated intracellular calcium necessary for degranulation of mast cells. Inhibits chloride transport and Cl^- channels, which indirectly lower intracellular calcium levels. Prevents the mast cell release of histamine and leukotrienes. Blocks activation of eosinophils. Inhibits neutrophil activation, chemotaxis, and mediator release. Inhibits IgE production.

Flash Card A8

Neutrophils and eosinophils

Bronchoprotection

Prevents or attenuates the **early- and late-asthmatic reaction** to bronchial challenges. Provides protection against exercise and cold air challenges as well as irritants (i.e., sulfur dioxide and bradykinin). Provides protection against adenosine, saline, and mannitol challenges. Does not offer protection for methacholine or histamine challenges.

Common Clinical Applications

Asthma—Effective asthma treatment in both adults and children. Optimal effects seen in patients using bronchodilators alone and corticosteroid-sparing effect can be seen. Not effective for immediate relief of symptoms in acute asthma attacks. Must be used at regular interval for 2–4 weeks to be effective. No longer available.

Allergic Eye Disease—Effective in relieving eye symptoms in acute or chronic allergic conjunctivitis, vernal keratoconjuctivitis, and giant papillary conjunctivitis.

Nasal Disease—Effective in relieving nasal symptoms in allergic and nonallergic rhinitis.

Systemic Mastocytosis—Useful in symptomatic treatment of GI symptoms.

Safety and Toxicity

Excellent safety profile. Little to no systemic absorption. Most common adverse effect is unpleasant taste in mouth. Can be used in pregnancy (category B).

Flash Card Q9

Mast cell stabilizers attenuate which phase of an allergic response?

ANTICHOLINERGICS

Increased parasympathetic tone in asthma or chronic obstructive pulmonary disease (COPD) releases acetylcholine, which triggers bronchoconstriction, mucus secretion, and bronchial vasodilation. Acetylcholine induces bronchoconstriction by stimulating muscarinic receptors.

Flash Card Q10

Smooth muscle contraction and bronchoconstriction are primarily mediated by which M receptors?

Muscarinic Receptors

G protein-coupled acetylcholine receptors found in the cell membranes of neurons and other cells.

Three Types

- **M1**: Present on eosinophils from sputum of COPD patients
- **M2**: **Inhibitory receptor on parasympathetic nerves**. Decreases acetylcholine release; dysfunctional M2 causes **increased** acetylcholine
- **M3**: **Primary mediator of smooth muscle contraction in human airways**
  - Density greatest in smooth muscle of bronchi > trachea > alveoli >> airway epithelium
  - Increases intracellular Ca^{2+}, causing bronchoconstriction
  - Linked to smooth muscle proliferation
  - Many antihistamines also have anticholinergic effects, through M3 receptor

Causes of Vagally Mediated Bronchoconstriction

- **Irritants:** Sulfur dioxide**,** dust**,** citric acid**,** exercise, and cold air
- **Viruses:** Cause loss of inhibitory M2 receptors on parasympathetic nerves, leading to hyperreactivity
- **Allergen exposure:** Blocked by anticholinergic drugs (in mice)

Anticholinergic Drugs

***Atropine*—**Hallucinogenic, with numerous adverse effects. Toxicity limits its use for airway disease.

***Ipratropium Bromide (18 µg/puff)*—**Quaternary ammonium, first anticholinergic bronchodilator for COPD. Provides equal bronchodilation to β agonists in COPD patients. 7.6 minutes to 50% max bronchodilation. Blocks M2 = M3. May cause paradoxical bronchoconstriction (blocking M2 receptors leads to ↑ acetylcholine release from vagus which may ↑ bronchoconstriction).

Flash Card A9

Early and late phase

- **Role in asthma:** Recommended in 2007 Expert Panel Report 3 in combination with β agonists in moderate-to-severe asthma exacerbations; synergistic with β agonists, **and decreases hospitalization rate**. Recommended for patients with β-agonist adverse effects (e.g., palpitations or tachycardia), and drug of choice for β-agonist-induced bronchospasm.
- **Role in COPD:** Seventy-three percent fewer hospital days, and fewer and shorter exacerbations when treated with combined therapy (compared with a β agonist alone).

Flash Card A10

M3 receptors

- **Role in rhinitis:** Effective inallergic, nonallergic, gustatory, and infectious; decreases contralateral responses in unilateral histamine or cold air nasal challenges.

Tiotropium bromide (18 µg/cap DPI)—Structurally related to ipratropium, it is a potent inhibitor of M3 = M2; but, it dissociates from M2 10 times faster than M3. Dissociates from M1 and M3 100 times more slowly than ipratropium. Long duration of action, 9 hours; 34.8 minutes to 50% maximum bronchodilation.

- **Role in asthma:** May have a role in patients with β-agonist-resistance. Tiotropium shown to improve asthma when added to ICS alone or ICS/LABA.
- **Role in COPD:** Greater improvement in trough FEV_1 versus ipratropium; reduced dyspnea scores, use of β agonist, and decreased airway resistance; no clear benefit in acute exacerbations.

CORTICOSTEROIDS

CORTISOL

Cortisol peak levels are in the early morning, whereas the lowest levels are in early evening. The levels are increased by stress, IL-1, IL-2, IL-6, and tumor necrosis factor alpha (TNFα). Ninety percent of cortisol is protein-bound; cortisol binds to transcortin with high affinity and to albumin with low affinity.

Key Fact

Steroids inhibit the late-phase response of an antigen challenge, but NOT the early phase.

Mechanism

Steroids bind to a glucocorticoid (GC) receptor. There are two types of GC receptors, GRα and GRβ. Free steroid (i.e., unbound) binds to the GC receptor in cytoplasm. After binding with the receptor, heat shock proteins dissociate from the receptor, the receptor is phosphorylated, and then the steroid-receptor complex translocates into the nucleus.
Once in the nucleus, the steroid has the following multiple effects on gene regulation:

- Target gene activation via binding to a positive glucocorticoid response element (GRE)
- Target gene repression via binding to a negative GRE
- Indirect gene repression via interference with transcription activating factors (NFκB). There is also competition between GC receptor and transcription

Flash Card Q11

Which medications decrease glucocorticoid metabolism?

Mnemonic

Adverse effects of steroids: **STEROID**

Stunt growth, subcapsular posterior cataracts, and steroid myopathy
Thrush
Eyes (increased intraocular pressure, cataracts, and glaucoma), endocrine (diabetes)
Rage (psychiatric changes), raises blood pressure (hypertension)
Osteopenia or osteoporosis and obesity
Immunosuppression and increased weight
Dysphonia, diabetes, and dermatologic effects

factors on the surface of integrator proteins, such as CREB-binding protein (CBP/p300). CBP has associated histone acetyltransferase (HAT) activity, which leads to DNA unwinding and access to transcription factors. Steroids inhibit HAT activity and recruit histone deacetylases (HDACs), which oppose HAT activity.

- Induction of transcription factor inhibitors (i.e., GILZ, IκBα) which interfere with NFκB
- Destabilize target gene mRNA

Oral steroids cause the following changes in inflammatory cells:

- T and B lymphocytes: Decreases T lymphocytes > B lymphocytes; decreases CD4+ > CD8+ cells; upregulates CXCR4; and slight decrease in IgG and IgM
- Increases neutrophils
- Decrease eosinophils, basophils, and monocytes
- Spares innate immunity (Toll-like receptor [TLR], complement, and collectins)

Steroids reverse reduced β-adrenergic responsiveness and increase β-receptor numbers.

Adverse Effects

Steroids can adversely affect virtually any organ in the body, including the bone (osteopenia and osteoporosis), skin (acne), eyes (posterior cataracts), and central nervous system (rage and depression). This necessitates constant monitoring for adverse effects and consideration of periodic dual-energy X-ray absorptiometry (DEXA) scans and ophthalmologic examinations for those on high daily doses (oral or inhaled).

Drug Interactions—Barbiturates increase steroid metabolism. Troleandomycin prolongs steroid metabolism and half-life. Protease inhibitors (particularly ritonavir) may result in high systemic concentrations when coadministered with inhaled corticosteroids (particularly fluticasone), causing Cushing's syndrome and adrenal suppression.

Clinical Uses

Asthma—Most newer inhaled corticosteroid (ICS) products have low oral bioavailability due to extensive hepatic first-pass metabolism. ICS have not been shown to prevent progressive loss of lung function, affect natural history, or to work well during exacerbations. Relative binding affinity for GC receptor: mometasone > fluticasone > budesonide > triaminoclone. Relative anti-inflammatory potency: mometasone = fluticasone > budesonide =

Flash Card A11

Ketoconazole, oral contraceptives, and macrolides (i.e., troleandomycin)

beclomethasone > triamcinolone. Multiple GC receptor isoforms exist, but steroid resistance is mediated by overexpression of GC receptor β. It does not bind to GC; rather, it inhibits the glucocorticoid response elements (responsible for synthesis of anti-inflammatory proteins) via a dominant negative effect. Increased GC receptor β expression associated with fatal asthma and nocturnal asthma.

IMMUNOMODULATORS AND IMMUNOSUPPRESSIVES

BIOLOGIC IMMUNOMODULATORS

Anti-IgE Therapy (Omalizumab [Xolair])

Mechanism of Action—Omalizumab is a humanized monoclonal antibody that binds to the **CH3 domain (FC portion)** of the IgE molecule. This prevents free IgE from binding to the FcεRI on mast cells and basophils. It is an IgG_1 molecule composed of 95% human and 5% murine sequence. It binds to IgE, typically forming **trimers** (i.e., two IgE molecules and one omalizumab) that are cleared by the reticuloendothelial system. It only binds soluble IgE; therefore, it cannot cause degranulation of effector cells. Omalizumab increases **total IgE** (as complexes are eliminated slowly), but decreases **free IgE**.

Key Fact

FcεRI on mast cells and basophils has four chains: **αβγ2** versus other cells that have only three chains: **αγ2** (**Note:** No β chain). IgE binds to the **α chain** of the receptor.

Omalizumab will decrease:

- Free serum IgE and eosinophils (serum, sputum and bronchial biopsies)
- Expression of FcεRI on effector cells (mast cells, basophils, dendritic cells, monocytes)
- Circulating IL-13 and fractional exhaled nitric oxide (FeNO)
- Mediator release from mast cells and basophils
- B lymphocytes

Dosing—Dose is dependent on patient's body weight and total IgE (between 30–700 IU). It is administered every 2–4 weeks subcutaneously and is approved for individuals 12 years of age and older with moderate-to-severe allergic asthma. Trial of at least 12 weeks is needed to assess clinical improvement.

Safety—Most common adverse events include local reaction at the injection site, upper respiratory infection, headache. Anaphylaxis (0.2% incidence) can occur within 2 hours of injection, though delayed reactions (2–24 hr later) have been reported.

Flash Card Q12

What is the incidence of anaphylaxis associated with omalizumab?

Omalizumab and Allergic Disease

- Asthma: Compared with placebo, omalizumab was able to reduce asthma exacerbations, decrease systemic steroid use, decrease rescue medication use, decease hospitalizations and emergency department visits, and improve quality of life.
- Allergic Rhinitis: Compared with placebo, omalizumab decreased daily symptoms, decreased rescue medication use, decreased missed school or work days, and improved quality of life.
- Food Allergy: Study of a different humanized IgG1 monoclonal antibody (TNX-901) against IgE was studied in patients allergic to peanuts. It was able to increase the threshold of peanut tolerability from 0.5–1.5 peanuts to 9 peanuts in some patients. However, 25% of patients had no improvement. This has not been evaluated with omalizumab.
- Atopic Dermatitis: Omalizumab may be effective for atopic dermatitis, but high baseline IgE levels may be a limiting factor.
- Chronic Urticaria: **Omalizumab has shown to improve symptoms and signs of chronic idiopathic urticaria in patients who had remained symptomatic despite the use of approved doses of H antihistamines.**

Many biologic immunomodulators, including monoclonal antibodies and fusion proteins, are used in the treatment of cancers and autoimmune diseases (Tables 9-9 and 9-10).

Table 9-9. Other Therapeutic Antibodies

Generic Name	Brand Name	Molecular Target	Molecular Structure	Indication
Alemtuzumab	Campath	CD52 (cell surface glycoprotein)	Humanized IgG1κ and recombinant	Chronic B-lymphocyte leukemia (B-CLL)
Cetuximab	Erbitux	EGF (epidermal growth factor) receptor	Chimeric monoclonal antibody	Metastatic colorectal cancer, and head and neck cancer
Eculizumab	Soliris	Complement protein C5	Humanized	Paroxysmal nocturnal hemoglobinuria
Efalizumab	Raptiva	CD11a (α-subunit of leukocyte function antigen-1 [LFA-1])	Humanized IgG1κ and recombinant	Chronic and moderate to severe plaque psoriasis
Palivizumab	Synagis	respiratory syncytial virus fusion (RSV F) protein	Humanized IgG1κ	Prevention of serious lower respiratory tract disease caused by RSV in pediatric patients at high risk
Canakinumab	Ilaris	IL-1 beta	Humanized IgG1κ and recombinant	Cryopyrin-associated periodic syndromes (CAPS)

Flash Card A12

0.2%

Table 9-10. Therapeutic Fusion Proteins

Generic Name	Brand Name	Molecular Target	Molecular Structure	Indication
Abatacept	Orencia	B7-1 (CD80), B7-2 (CD86)	CTLA4-human IgG1 fusion protein	Rheumatoid arthritis and juvenile rheumatoid arthritis
Alefacept	Amevive	CD2	LFA-3-IgG1 Fc fusion protein	Moderate-to-severe, and chronic plaque psoriasis

Abbreviations: CTLA, cytotoxic T-lymphocyte antigen LFA, leukocyte function-associated antigen.

IMMUNOSUPPRESSIVES

Methotrexate (MTX)

Pharmacology/Metabolism—MTX is a drug and prodrug that is structurally similar to folic acid. It is administered orally or parenterally with a half-life of 8 hours and elimination via renal clearance. Widely used in rheumatoid arthritis and other autoimmune diseases.

Immunologic Effects—Inhibits cell replication (i.e., lymphocyte division).

Adverse Effects—MTX is a teratogen and contraindicated in pregnancy. Many of its adverse effects can be reduced by decreasing the dose or supplementing with folic acid. Common adverse effects include oral ulcerations, nausea or diarrhea and postdose arthralgia, and fatigue. Less common effects include abnormal liver function tests (LFTs), myelosuppression, and skin rash. Rare events: Myelosuppression, hepatic fibrosis, pulmonary toxicity, Epstein-Barr virus (EBV)-associated lymphoma, alopecia, and increased risk of infections.

Use in Allergic Disease—In corticosteroid-dependent asthma, there have been conflicting reports of MTX having modest corticosteroid-sparing effect.

Azathioprine (Imuran, Azasan)

Pharmacology/Metabolism—A systemic immunosuppressive prodrug that inhibits purine nucleotide synthesis and metabolism. Available in oral form.

Adverse Effects—Myelosuppression, GI disturbance, hepatotoxicity, susceptibility to infection and, risk of skin cancer.

Flash Card Q13

Cyclosporine (CsA) and tacrolimus function by binding to which type of protein(s)?

Use in Allergic Disease—Shown to be effective in severe atopic dermatitis. Has slow onset of action and needs a trial for several months to see improvement.

Mycophenolate Mofetil (CellCept)

Pharmacology/Metabolism—A systemic immunosuppressive agent that affects purine nucleotide synthesis and metabolism. Used in organ transplantation.

Adverse Effects—Diarrhea, vomiting, hepatotoxicity, and myleosuppression (↑ risk of sepsis and opportunistic infections).

Use in Allergic Disease—Shown to be effective in severe/recalcitrant atopic dermatitis, with responders showing lasting remission. Onset of action slow and benefit may not be apparent for several months.

Immunophilin-Binding Agents and Calcineurin Inhibitors (Cyclosporine, Tacrolimus, and Pimecrolimus)

Pharmacology/Metabolism—Cyclosporine A(CsA) has immunosuppressive properties of inhibiting T_h-lymphocyte function. Tacrolimus and pimecrolimus, though structurally different, have the same mechanism of action. Cyclosporine and tacrolimus are both used in organ transplantation and for autoimmune disease; they are eliminated via P450 system. Topical tacrolimus and pimecrolimus approved for treatment of atopic dermatitis for patients 2 years of age and older.

Immunologic Effect—These agents bind to cytosolic proteins called immunophilins: CsA binds to cyclophilin, and tacrolimus binds FK-binding protein. This results in inhibition of various transcriptional regulatory factors, such as nuclear factor of activated T lymphocytes (NFAT), which encodes cytokines that are critical for T lymphocyte function, such as IL-2.

Adverse Effects—Common adverse effects include nephrotoxicity (most common to CsA and tacrolimus), hypertension (less frequent tacrolimus), headache, hypertrichosis, and gingival hypertrophy. Rare events include increased risk of infection and lymphoproliferative disease. Topical agents may cause local burning on application.

Use in Allergic Disease—Cyclosporin A remains an investigational treatment in asthma and has been used in refractory cases of chronic urticaria. Cyclosporine A has not been effective topically for atopic dermatitis (AD), but studies of oral formulations have shown improvement in the extent and severity of AD, although toxicity remains a concern.

Flash Card A13

Immunophilins (CsA binds to cyclophilin and tacrolimus binds FK-binding protein)

DNA-BASED THERAPIES

DNA Vaccines

Immune Response—DNA vaccines are circular extrachromosomal pieces of plasmid DNA that can be modified to carry genes of interest in antigen-coding region, such as dust mite allergen for allergen immunotherapy. The subcutaneous injected DNA vaccine is taken up by dendritic or somatic cells to mediate immune responses. Somatic cells (i.e., muscle cells) serve as reservoirs of antigen and function in cross priming (transfer of protein to professional antigen-presenting cells).

Potential in Allergic Disease—DNA vaccines were studied extensively in mice and showed protection against specific allergen challenge and prevention of anaphylactic reaction to peanut. DNA vaccines possibly generate humoral and cellular immune responses to allergens. There are safety concerns regarding risk of malignancy and risk of triggering autoimmune response.

CpG DNA Therapy

Immune Response—Cytosine phosphorothioate-linked guanosine (CpG)-rich immunostimulatory DNA was shown to inhibit T_h2 cytokine responses to antigen in the mouse model via TLR9. Linking of protein antigen to CpG DNA increases the likelihood of its delivery to the same antigen-presenting cell (APC), amplifying T_h1 response.

Potential in Allergic Disease—The advantage of using conjugated CpG DNA therapy versus traditional IT is the ability to reach the target dose faster without inducing a systemic allergic reaction. Human pilot studies with ragweed protein CpG DNA conjugates showed that only six weekly injections reduced rhinitis scores in individuals with AR to ragweed, even through the patient had no additional IT in the second season. However, the results of a large multicenter study were less encouraging.

Oligodeoxynucleotide

Immune Response—Antisense oligodeoxynucleotide (ODN) therapy prevents the translation of messenger RNA into protein.

Potential in Allergic Disease—ODN therapy can be targeted against gene products important in asthma and allergy, such as adenosine-1, IL-5 receptor, IL-4, or GATA-3. Examples in mouse models demonstrate that GATA-3 antisense

inhibits eosinophilic inflammation, airway hyperreactivity, and cytokine expression.

Small Interfering RNAs

Immune Response—Small interfering RNAs (siRNAs) are small double-stranded RNAs that are complementary to specific single-stranded RNAs, which act to destroy the specific target RNA (gene silencing). May lead to a new class of drugs that switch off unwanted gene expression in disease.

Potential in Allergic Disease—Fomivirsen (Vitravene) has been approved in the treatment of cytomegalovirus (CMV) retinitis in patients with AIDS. No antisense therapy is approved for the treatment of allergic disease at this time.

IMMUNOGLOBULIN REPLACEMENT THERAPY

Immunoglobulin products contain pooled IgG from plasma of approximately 15,000–60,000 donors. These large donor numbers create a significant amount of antibody diversity for uses as passive immunization and immunomodulation in various conditions (Table 9-11).

Indications

FDA approved indications include:

- Primary immunodeficiencies
- B-lymphocyte chronic lymphocytic leukemia (CLL)
- Kawasaki's disease
- Following stem cell transplantation with B-lymphocyte nonengraftment
- Pediatric HIV infection (recurrent bacterial infections)
- Immune thrombocytopenic purpura (ITP)
- Chronic inflammatory demyelinating polyneuropathy (CIDP)
- Bone marrow transplantation

Off-label uses include toxic epidermal necrolysis (TEN), autoimmune, and neurologic diseases.

Table 9-11. Uses of Immunoglobulin Therapy

Condition	Definitely Beneficial	Probably Beneficial	Might Be Beneficial	Unlikely to Be Beneficial
Immune deficiency	PID	Pediatric HIV B lymphocyte CLL Specific Ab deficiency		Isolated IgA deficiency Isolated IgG4 deficiency
Autoimmune	ITP Graves' ophthalmopathy	Dermatomyositis Polymyositis Autoimmune uveitis	SLE Severe RA Vasculitis Autoimmune diabetes Autoimmune cytopenias	Inclusion body myositis APS in pregnancy
Neurologic	CIDP Guillain-Barré syndrome Multifocal motor neuropathy	Myasthenia gravis Lambert-Eaton syndrome	MS PANDAS Childhood epilepsy	ALS Autism
Infectious	CMV pneumonitis[a]	Neonatal sepsis Staph TSS Rotaviral entero-colotis[b]	RSV pneumonitis[b] Pseudomembranous colitis	Acute rheumatic fever
Dermatologic		TEN and SJS	Autoimmune bullous diseases Chronic urticaria	Atopic dermatitis
Other	Kawasaki's disease		HSCT Severe persistent steroid-dependent asthma	HSCT (chronic GVHD prevention) Nonsteroid-dependent asthma Miscarriage Dilated cardiomyopathy

[a] In immunosuppressed patients, [b]Orally administered Igs.

Abbreviations: AB, antibody; ALS, amyotrophic lateral sclerosis; CIDP, chronic inflammatory demyelinating polyneuropathy; CLL, chronic lymphocytic leukemia; GVHD, graft-versus-host disease; HSCT, hematopoietic stem cell transplant; ITP, idiopathic thrombocytopenic purpura; MS, multiple sclerosis; PANDAS, pediatric autoimmune neuropsychiatric disorder associated with streptococcal infections; PID, primary immunodeficiency; RA, rheumatoid arthritis; SJS, Stevens-Johnson syndrome; TSS, toxic shock syndrome; TEN, toxic epidermal necrolysis..

Immunoglobulin Replacement Manufacturing

Isolation of immunoglobulin (Ig) from plasma by ethanol fractionation. Most commonly used method is **modified Cohn-Oncley cold ethanol fractionation**. This step is also effective for removal of prion proteins. Bacteria are usually removed through filtration during final steps of plasma preparation.

Filtration of antibody aggregates and viruses. Commonly used methods are nanofiltration, treatment with pepsin, ultracentrifugation, and low pH. Nanofiltration removes particles and viruses as small as 20 nm.

Stabilization to prevent aggregation of Ig molecules. Commonly used stabilizers are albumin, glycine, proline, polyethylene glycol, D-mannitol, D-sorbitol, sucrose, glucose, or maltose. Lower pH (range = 4.5–5.0); also preserves the therapeutic activity of Ig and minimizes formation of aggregates, dimers, and fragments.

Viral Inactivation. Many enveloped viruses can be inactivated with chemical methods, such as treatment with solvent or detergent, caprylate precipitation, or incubation at low pH. Physical methods are required to remove nonenveloped viruses. Samples are pasteurization, column chromatography, and nanofiltration.

Ingredients and Physicochemical Characteristics

Commercial Ig products contain monomeric IgG (>95%) and small amounts of IgM and IgA. Products containing low amounts of IgA may be beneficial in patients with IgA deficiency Serum half-life is 3–4 weeks, but when changing the dose, several infusions may be needed to equilibrate to a new trough.

Dosing

The starting dose of intravenous immunoglobulin (IVIG) replacement therapy in primary immune deficiency (PID) management is 400–600 mg/kg every 3–4 weeks. For subcutaneous immunoglobulin (SCIG) it is 100 mg/kg/week. Although guidelines for dosing are available, for select patients continuing to have recurrent infections, the dose of IVIG may need to be increased above the recommended therapeutic range. For immunomodulation, as in management of Kawasaki's disease, and various autoimmune and neurologic diseases, higher dosing (2 g/kg) is recommended every 3–4 weeks.

Forms

Ig products are available in lyophilized powder or liquid from 3–20% and can be administered via IV, IM, or subcutaneous (SC) infusions. SCIG can be used as an alternative for IVIG, and is done by the patient as a self-infusion at home. It provides a more stable trough Ig level since it is given weekly. However, it is difficult to give large amounts of Ig by this route. SCIG appears to have fewer systemic adverse events, perhaps due to slower administration and absorption.
Specific (hyperimmune) globulins are prepared from select donors who have high titers of the desired antibody, either naturally acquired or stimulated by

immunization. They are used to transfer passive immunity for postexposure prophylaxis. Available hyperimmune globulins include Ig to hepatitis B, rabies, tetanus, varicella-zoster, vaccinia, CMV, and RSV.

Immunomodulating Effects of Ig Therapy

- **Fc Receptor Blockade**
  - Antibody-dependent cell-mediated cytotoxicity (ADCC) inhibition by blockade of FcγRIII (CD16) on natural killer (NK) cells
  - Inhibition of antibody production by blockade of FcγRIIb (CD32) on B lymphocyte
  - In ITP, IVIG blocks membrane Fc receptors on phagocytic cells in spleen and liver
- Sialyated IgG fraction mediates immunosuppressive activity
  - Terminal α-2,6-linked sialic acid residues on the N-linked glycans of the IgG Fc domain have been shown to confer immunosuppressive properties
- **Neutralization** of superantigens, toxins, and autoantibodies by anti-idiotype antibodies
- **Inhibition of cytokine** production or action
- **Inhibition of complement** uptake and elimination of immune complexes, which deposit in tissues
- **Other unknown mechanism:** In toxic epidermal necrolysis (TEN), IVIG inhibits Fas-mediated apoptosis on keratinocytes

Key Fact

Most reactions from IVIG are mild and self-limited. Systemic reactions are less common with SCIG than with IVIG.

IVIG not indicated for: selective IgA deficiency, IgG due to protein-losing states, IgG subclass deficiency (unless Ab deficiency coexists) or transient hypogammaglobuline mia of infancy.

Safety

The capacity for viral and prion elimination during IVIG production over the past decade is considered sufficient to prevent pathogen transmission. Since the mid-1990s, no transmission of the infectious disease has been reported from US licensed Ig products.

Adverse Reactions

Mild reactions during or after infusion are common and usually self-limited. Headache, fatigue, fever, chills, nausea, vomiting, and myalgias can occur from IgG aggregates or immune complex formation of Ig and antigens. Reactions usually are infusion rate-dependent and occur during initial doses of treatment and improve over time. Adverse events from SCIG are milder and mostly involve injection-site reactions.

Flash Card Q14

What is a potential cause of renal insufficiency when treating patients with IVIG?

Aseptic meningitis (history of migraine is predisposing factor) is less common and associated with large doses, rapid infusions, and the treatment of patients with autoimmune disorders or inflammatory disease.

Anaphylaxis may occur in patients with IgE antibodies to IgA, such as in selective IgA deficiency. Preventive measures include using products with lowest IgA content and premedication with antihistamines or corticosteroids.

Acute renal failure is rare; use of **sucrose** as a stabilizer has been most strongly associated. Those receiving higher (immunomodulatory) doses are at greater risk.

Other rare adverse effects include thrombotic events, stroke, myocardial infarction, hemolytic anemia, osmotic nephrosis, and transfusion-related acute lung injury (TRALI). These could be secondary to high osmolality or in patients with underlying cardiovascular or renal disease.

Management of Infusion Reactions

Mild reactions can be prevented by pretreatment with aspirin, acetaminophen, diphenhydramine or hydrocortisone, and slow infusion rate.

If **severe reactions** occur, consider reducing rate or volume, using different product, or changing to SCIG. Epinephrine and other means of treating acute reactions should be available.

Precaution

Live vaccines may have diminished immunogenicity when given shortly before or during several months after receipt of Ig products. Suggested intervals of live vaccines vary from 3–11 months after Ig, depending on Ig doses, products, and indications for use.

Use low IgA-containing products in patients with low IgA level.

CYTOKINE AND CYTOKINE RECEPTOR-MEDIATED THERAPY

Flash Card A14

Hyperosmolar renal damage induced by breakdown of sucrose (used as stabilizer in IVIG preparations)

The therapeutic administration of purified recombinant cytokines and the modulation of cytokine action are being used in a wide range of infectious and

autoimmune diseases, acquired immunodeficiency syndrome, and neoplasia. Given the large number of cytokines, relatively few have been used clinically to date.

CYTOKINES AS THERAPEUTIC AGENTS

Interferons

The term "interferon" is derived from their ability to *interfere* with viral infection. Interferons exert their effects by binding to membrane receptors, which initiates the activation of Janus kinase (JAK)-signal transducer and activator of transcription (STAT)-signaling pathways leading to gene transcription. The gene products formed are responsible for the antiviral, antiproliferative, and immunomodulatory effects of interferons. There are several type I interferon products (IFNα, IFNβ) currently approved for the treatment of chronic viral infections, autoimmune disease, and several malignancies. IFNγ, the only type II interferon, has significantly fewer antiviral properties than the type I interferons. It is currently approved for use in chronic granulomatous disease and malignant osteopetrosis.

A summary of the various interferon products currently available for use are presented in Table 9-12.

Interleukin-2

Interleukin-2 (IL-2), also known as T-lymphocyte growth factor, is an immunomodulator whose major function is activation of T lymphocytes and NK cells, enhancing their antitumor activity. IL-2 also stimulates the differentiation of regulatory T lymphocytes, which are of significance to the control of inflammatory responses. A recombinant form of IL-2 has been approved for clinical use (Table 9-13).

Interleukin-11

Interleukin-11 (IL-11), also known as thrombopoietic growth factor, directly stimulates the proliferation of hematopoietic stem cells and megakaryocyte progenitor cells and induces megakaryocyte maturation, resulting in increased platelet production. A recombinant form of IL-11 has been approved for clinical use (Table 9-12).

Table 9-12. Summary of Cytokine Therapies Approved for Clinical Use

Drug		Approved Indication	Adverse Effects	Precautions
Generic	**Trade**			
Recombinant IFNα-2a	Roferon-A	Chronic hepatitis C Hairy cell leukemia Chronic myeloid leukemia	Fever and "flu-like" symptoms Cytopenias Hepatic dysfunction Neuropsychiatric symptoms Cerebrovascular events Hypersensitivity reactions GI hemorrhage Hyperglycemia Pulmonary disorders Ophthalmologic disorders Pancreatitis Peripheral neuropathy	Caution in those using α-interferons with cardiovascular disease, thyroid dysfunction, coagulation disorders or severe myelosuppression Patients should receive an eye exam at baseline and periodic exams for those with preexisting ophthalmologic disorders
Recombinant IFNα-2b	Intron-A	Hairy cell leukemia Kaposi's sarcoma Chronic hepatitis B or C Malignant melanoma Follicular lymphoma Condylomata acuminate		
IFNα con-1	Infergen	Hepatitis C		
IFNα-n3 leukocyte derived	Alferon-N	HPV genital warts		
Pegylated IFNα-2a	Pegasys	Chronic hepatitis B or C		
Pegylated recombinant IFNα-2b	PEG Intron	Hepatitis C		
IFNβ-1a	Avonex/ Rebif	Relapsing multiple sclerosis	"Flu-like" symptoms Headache Depression Hepatotoxicity ↑ Risk of seizures	Caution in patients with pre-existing: • Seizure disorders • Cardiac disease Monitor for auto-immune disorders (ITP, hepatitis)
IFNβ-1b	Betaseron	Early or relapsing multiple sclerosis		Injection site necrosis reported
Bioengineered IFNγ-1b	Actimmune	Chronic granulomatous disease and malignant osteopetrosis	Fever Headache Hepatotoxicity	Caution in those with pre-existing cardiac conditions, seizure disorders, and myelosuppression.
Recombinant IL-2 or Aldesleukin	Proleukin	Metastatic renal cell carcinoma and metastatic melanoma	Hypotension Diarrhea Oliguria Chills Vomiting Cytopenias BLACK BOX WARNING Capillary leak syndrome Impaired neutrophil function (↑ risk infection) Coma (see warning)	Caution in those who have a history of cardiac or pulmonary disease. **Discontinue in patients developing lethargy and somnolence** Patients should have normal cardiac, pulmonary, hepatic, and CNS function at the start of therapy

Table 9-12. Summary of Cytokine Therapies Approved for Clinical Use, cont.

Drug		Approved Indication	Adverse Effects	Precautions
Generic	Trade			
Recombinant IL-11 or Oprelvekin	Neumega	Chemotherapy-induced thrombocytopenia in adult patients with nonmyeloid malignancies	Vomiting Edema Neutropenic fever Hypersensitivity reactions CV abnormalities (Afib) Pleural effusion Papilledema	Increased toxicity following myeloablative therapy. Anemia (will resolve after treatment)

Abbreviation: Afib, atrial fibrillation; CV, cardiovascular; ITP, idiopathic thrombocytopenic purpura.

Hematopoietic Cytokines

Hematopoietic cytokines stimulate growth and differentiation of various elements of bone marrow. Three cytokines: erythropoietin, granulocyte colony-stimulating factor (G-CSF), and granulocyte-macrophage colony-stimulating factor (GM-CSF), have now been in routine clinical use to stimulate cell production (Table 9-13). A trial of recombinant GM-CSF for use in pulmonary alveolar proteinosis is currently in progress.

Table 9-13. Summary of Hematopoietic Cytokine Therapies Approved for Clinical Use

Drug		Approved Indication	Adverse Effects
Generic	Trade		
Sargramostim (Recombinant GM-CSF)	Leukine	Acute myelogenous leukemia and bone marrow or stem cell transplants	Bone pain, malaise, fever, diarrhea, edema, and rash
Filgrastim (GM-CSF)	Neupogen	Acute myelogenous leukemia, bone marrow or stem cell transplants, and severe chronic neutropenia	Skeletal muscle pain, bone pain, alopecia, fever, rash, and injection site reaction
Pegfilgrastim (Pegylated GM-CSF)	Neulasta	Nonmyeloid malignancies	Bone pain, myalgia, alopecia, fever, rash, and edema
Epoetin alpha	Epogen, Procrit	Anemia due to chronic renal failure, HIV-infected patients, chemotherapy, and primary bone marrow disorders	Hypertension, thrombotic complication, and allergic reactions
Darbepoetin alpha	Aranesp	Anemia due to chronic renal failure, HIV-infected patients, chemotherapy, and primary bone marrow disorders	Mild transient injection site pain, and hypertension, thrombotic complication, and allergic reactions

Abbreviation: GM-CSF, granulocyte–monocyte colony-stimulating factor.

ANTICYTOKINES AS THERAPEUTIC AGENTS

Tumor Necrosis Factor-Alpha Inhibitors

Tumor necrosis factor-alpha (TNFα) plays a central role in inflammatory arthritides, including rheumatoid arthritis (RA), psoriatic arthritis (PsA), and ankylosing spondylitis (AS). Currently available anti-TNFα strategies involve either administration of anti-TNFα antibody or soluble TNF receptor to bind circulating TNFα before it binds to receptors on nearby cells, thus preventing initiation of apoptosis or an inflammatory response. There are five currently available TNF inhibitors (Table 9-14).

Table 9-14. Comparison of Tumor Necrosis Factor-Alpha Inhibitors

	Infliximab	Etanercept	Adalimumab	Certolizumab pegol	Golimumab
Trade name	Remicade	Enbrel	Humira	Cimzia	Simponi
Structure	Chimeric (25% mouse/ 75% human) IgG1κ monoclonal antibody	Recombinant dimeric fusion protein made of two soluble p75 TNF receptors (CD120b) and constant Fc portion of human IgG1	Fully human anti-TNFα monoclonal IgG1 antibody	Polyethylene-glycolated humanized Fab' fragment of a human anti-TNF α antibody	Human IgG1κ monoclonal antibody specific for human TNFα
Binds To	TNFα	TNFα and TNFβ (lymphotoxin α)	TNFα	TNFα	TNFα. (Binds both soluble and transmembrane bioactive forms)
Approved Indications	RA, AS, Crohn's disease, ulcerative colitis, PsA, and plaque psoriasis	RA, AS, PsA, plaque psoriasis, and juvenile idiopathic arthritis	RA, AS, Crohn's disease, PsA, severe plaque psoriasis, juvenile idiopathic arthritis	RA and Crohn's disease	RA, AS, and PsA.
Adverse Effects	Acute infusion reactions: fever, chills, pruritus, chest pain, dyspnea, flushing, urticaria, hypersensitivity reactions, serious infections, TB, sepsis, and hepatosplenic T-cell lymphomas	Mild injection site reactions, serious infections, TB, sepsis, neurologic events, hematologic events, and malignancies	Injection site reactions, upper respiratory infections (sinus infections), headache, rash, nausea, serious infections, TB, sepsis, malignancies, anaphylaxis, hepatitis B virus reactivation, demyelinating disease, cytopenias, heart failure, and "lupus-like" syndrome	Serious infections, malignancies, and heart failure	Upper respiratory infections, nasopharyngitis, serious infections, TB, invasive fungal infections, hepatitis B virus reactivation, malignancies (lymphoma), heart failure, demyelinating disease, and cytopenias
Precautions	**Perform purified protein derivative (TB) testing prior to initiation of therapy and annually (risk of reactivation)** Safety measures should also be taken in monitoring those with heart failure using this class of medication. Monitor for the development of autoantibodies and increases in liver function test results. Vaccination with live vaccines is not recommended while patients are receiving TNF inhibitors.				

Abbreviations: AS, ankylosing spondylitis; PsA, psoriatic arthritis; RA, rheumatoid arthritis; TB, tuberculosis.

Flash Card Q15

What TNF antagonist binds both TNFα and TNFβ?

Interleukin-1 Inhibitors

IL-1 production is induced by a variety of agents that stimulate molecular pattern receptors, and it activates T lymphocytes by enhancing the production of IL-2 and the expression of IL-2 receptors. IL-1 receptor antagonist (IL-1Ra) is a glycoprotein produced by macrophages that competitively inhibits IL-1α and IL-1β binding to IL-1 receptors (IL-1R). A biologically active engineered product of IL-1Ra has been developed for clinical use in autoimmune disease and infection. Another product, IL-1 Trap, is a long-acting IL-1 inhibitor that is a fully dimeric fusion molecule. It comprises the extracellular component of the IL-1 receptor (IL-1 receptor type I and IL-1 receptor accessory protein) and the Fc portion of IgG1, which binds circulating IL-1β and IL-1α with very high affinity and prevents its interaction with cell surface receptors. Recently, a human monoclonal antibody has been produced that targets IL-1β. These different products are summarized in Table 9-15.

Interleukin-2 Inhibitors

Interleukin-2 receptor (IL-2R) is expressed on the surface of activated T lymphocytes and B lymphocytes, and it is involved in their proliferation. Therapies have evolved to curb IL-2Rα (CD25) over activity through monoclonal antibody directed against the alpha chain of IL-2 receptor (Table 9-15). Other approaches to IL-2R-targeted treatment of various cancers are under study and include use of ligand-toxin fusion protein and immunotoxins.

Interleukin-6 Inhibitors

Interleukin-6 (IL-6) has the ability to activate T lymphocytes, B lymphocytes, macrophages, and osteoclasts and is a mediator of the hepatic acute-phase response. A recombinant humanized IL-6 receptor monoclonal antibody of the IgG1 subclass is currently pending approval in the US. The approved forms of this product are listed in Table 9-15.

Interleukin-12 and -23 Inhibitor

Interleukin 12 has the ability to activate IFNγ production by NK cells and T cells. It also enhances NK cell and cytotoxic T-lymphocyte (CTL)-mediated toxicity as well as T_h1 cell differentiation. It is secreted by both dendritic cells and macrophages. IL-23 is involved in the development of T_h17 cells. Recently, a new human IgG1k monoclonal antibody against the p40 subunit of IL-12 and IL-23 has been developed, and is listed in Table 9-15.

Flash Card A15

Etanercept (Enbrel)

Janus Kinase 3 (JAK3) Inhibitors

JAK3 is a tyrosine kinase that belongs to the Janus family of kinases. Also in this family are JAK1, JAK2, and TYK2. JAK3 is located in the cytosol and is associated with cytokine receptors. Cytokine receptors lack signaling ability and rely on JAKs to initiate signaling upon binding of their ligands. JAK3 is predominately expressed in hematopoietic cells (NK, T, and B cells) and interacts with members of the signal transduction and activators of transcription (STAT) family in order to propagate signals. Recently, an inhibitor of JAK3 has been approved for use in RA (Table 9-15).

Table 9-15. Anticytokines as Therapeutic Agents

Generic Drug	Trade Name	Approved Indication	Adverse Effects	Precautions
Anakinra (recombinant IL-1Ra)	Kineret	Rheumatoid arthritis and sepsis	Injection site reactions, pneumonia, serious infections, hypersensitivity reactions	Live vaccines should not be given while on therapy
Rilonacept (IL-1 Trap)	Arcalyst	Cryopyrin-associated periodic syndromes (CAPS): familial cold autoinflammatory syndrome, Muckle-Wells syndrome, NOMID	Injection-site reactions, increased risk of infection infections, hypersensitivity reactions, risk of reactivation of TB or new opportunistic infections, and may result in an increase in the risk of malignancies	Evaluate for latent TB before initiating therapy. Live vaccines should not be given concurrently Taking with TNF inhibitors not recommended (may increase the risk of serious infections) Monitor lipid profile
Canakinumab (human IgG1/κ against IL-1β)	Ilaris			
Basiliximab (chimeric IL-2Rα)	Simulect	Prophylaxis of acute organ rejection in renal transplant patients	Nausea, constipation, abdominal pain, urinary tract infection, upper respiratory tract infection, and hypersensitivity including anaphylaxis.	
Daclizumab (humanized IL-2Rα)	Zenapax	Prophylaxis of acute organ rejection in renal transplant patients	Nausea, constipation, headache, hypersensitivity including anaphylaxis, and hyperglycemia.	

Table 9-15. Anticytokines as Therapeutic Agents, cont.

Generic Drug	Trade Name	Approved Indication	Adverse Effects	Precautions
Tocilizumab (humanized IL-6Rα)	Actemra	Rheumatoid arthritis, Castleman's disease	Infusion reactions, serious infections, anaphylactic shock or an anaphylactoid reaction, pleurisy, abnormal lipid parameters, and cardiac abnormalities	Careful administration in those with concurrent active infection or suspected infection, previous tuberculosis, patients in an immune compromised state, or patients with an intestinal diverticulum No live vaccines during treatment Lipid tests, LFTs, and cardiac function should be monitored periodically
Ustekinumab (human IgG1/κ against p40 subunit of IL-12 and IL-23)	Stelara	Plaque psoriasis	Increased infections Increased risk of malignancy	Evaluate for TB prior to starting therapy No live vaccines during treatment
Tofacitinib (inhibitor of JAK3)	Xeljanz	Rheumatoid arthritis	Increased infections Abnormal lipid parameters Increased risk of malignancy	Evaluate for TB prior to starting therapy Monitor neutrophil and lymphocyte counts, hemoglobin level, LFTs, and lipid levels

Abbreviations: LFTs, liver functions tests; NOMID, neonatal onset multisystem inflammatory disease; TB, tuberculosis; TNF, tumor necrosis factor.

Chemokines

Chemokines function as regulatory molecules in leukocyte maturation, traffic, homing of lymphocytes, and in the development of lymphoid tissues. In addition to these roles, they are also critical in the pathogenesis of allergic responses, infectious and autoimmune diseases, angiogenesis, inflammation, tumor growth, and hematopoietic development. Specific therapies for several diseases, based on chemokine receptor antagonists, are being developed.

Other Cytokines of Possible Importance

A number of cytokine therapies are in clinical development. Many cytokines, including IL-5, IL-6, IL-7, IL-8, IL-10, IL-13, IL-15, IL-18, and IL-21 have been used experimentally in animals; these are in clinical trials for various conditions, but have not yet reached routine clinical use. A registry of clinical trials using cytokine and cytokine receptor-mediated therapies can be found on the National Institutes of Health clinical trials website.

CELLULAR IMMUNE RECONSTITUTION

Stem cell transplantation (SCT) is used to replace defective, absent, or malignant cells in an affected host, with pluripotent hematopoietic stem cells from a healthy donor (see sections on PID and stem cell transplantation in Chapter 8). Cellular immune reconstitution is a complex process that is influenced by many variables. This section reviews the most generally accepted aspects of immune reconstitution, with a focus on immune reconstitution after SCT for PID.

Factors That Affect Cellular Immune Reconstitution

Numerous elements contribute to success following hematopoietic stem cell transplant (HSCT), as shown in the following lists.

HSC Factors

- Human leukocyte antigen (HLA) compatibility
- Related vs. unrelated donor
- Source: Cord, peripheral, bone marrow
- T-cell depletion vs. CD34+ selection

Patient Factors

- Age
- Type of gene defect
- Pretransplant infection (CMV, EBV)
- Organ function damage
- +/– Myeloablative conditioning
- Use of lineage-specific mAbs
- +/– GVHD prophylaxis

Post-Transplant Factors

- Degree of chimerism
- Use of booster HSCT
- Persistence of immune reconstitution
- Effects of myeloablative chemotherapy (early/late)
- Chronic infections
- Chronic GVHD
- Autoimmunity
- Malignancies
- Neuropsychological development and quality of life

Donor type and HLA matching have a profound effect on immune reconstitution and engraftment. (See section on stem cell transplantation in Chapter 8). Although

Key Fact

Factors associated with improved **survival** after SCT include the following:

- Younger age (<3.5 months for SCID, < 5 years for chronic granulomatous disease (CGD), Wiskott-Aldrich syndrome (WAS)
- SCID phenotype with absent NK cells (i.e., XL-SCID, ADA deficiency, Jak3)
- HLA-identical stem cell donor
- No pretransplant conditioning for SCID
- Larger dose of CD34+ stem cells, and a lower dose of mature T lymphocytes
- Absence of viral or fungal infections at the time of transplantation

Key Fact

The defects related to Wiskott-Aldrich syndrome protein (WASP) expression (i.e., cell migration, immune synapse stability, and susceptibility to infection) may be overcome by partial chimera in immune cell lines. However, in order to achieve resolution of the autoimmune manifestation of the disease, a full donor chimera is necessary.

Post-transplant function of B cells is more likely when the patient has genetically normal B cells prior to transplant (i.e., IL-7R mutations).

inconceivable in an immunocompetent host, such as a patient with cancer or a non-T–lymphocyte primary immunodeficiency, T–lymphocyte-depleted haploid-identical related (parental) donors can be used in patients with no innate T-lymphocyte function, such as SCID. Successful SCT has been reported using a wide variety of related and unrelated donor types, including HLA-identical siblings, T–lymphocyte-depleted haploidentical related, matched unrelated, and matched related and unrelated cord blood transplants. Survival after transplant is dependent on both HLA matching and source of the donor. HLA-identical related donation has the highest survival, followed by HLA-identical unrelated, and then HLA-mismatched related. Marrow from a matched related sibling confers immune function to the patient within 2 weeks since both stem cells and activated mature immune cells are transferred to the patient and are the best tolerated by the host. Table 9-16 differentiates between the various sources of HCST and some of the primary differences associated with them.

Conditioning regimes, prior to bone marrow transplantation in PID, range from full myeloablation to no pretransplant conditioning. Although the intensity of the pretransplant conditioning is directed by the underlying immune defect, the type of conditioning can affect post-transplantation immune reconstitution (Table 9.17).

For many PIDs, the use of "reduced" or "minimal" intensity regimens are being employed to reduce the adverse complications associated with myeloablative regimens. Some examples of myeloablative versus newer reduced and minimal intensity regimens are the following:

- Myeloablative
  - Total body irradiation (TBI) + cyclophosphamide
  - Busulfan (high dose) + cyclophosphamide
  - Treosulfan + cyclophosphamide
- Reduced Intensity (can be given with or without a donor lymphocyte infusion)
  - Fludarabine + treosulfan
  - Fludarabine + melphalan
  - Fludarabine or cyclophosphamide + busulfan (low dose)
- Minimal Intensity (+/– alemtuzumab [Campath] or antithymocyte globulin [ATG])
  - Fludarabine + cyclophosphamide

Many adverse effects are associated with myeloablative therapies. Some of the primary organs affected by select drugs are the following:

- Radiation: Lungs, heart
- Cyclophosphamide: Heart
- Busulfan/treosulfan: Lungs, gastrointestinal
- Fludarabine: Lungs, gastrointestinal
- Melphalan: Lungs, gastrointestinal

Table 9.16. Hematopoietic Cell Sources for Stem Cell Transplant

Characteristic	Bone Marrow (BM)	Peripheral Blood Stem Cells (PBSC)	Umbilical Cord Blood
Sources (donor)	Matched related Mismatched related Matched unrelated	Mismatched related	Matched related or unrelated
How/where obtained	From large bone of donor (i.e., pelvis) under general anesthesia	Given hematopoietic growth factors (G-CSF, GM-CSF) to allow release of stem cells into peripheral blood. Collected through apheresis	From UC blood (~ 50 mL)
HLA matching:	Higher match = better outcomes		More permissive matching than marrow or PBSC
Minimum requirements for unrelated donors (NMDP)	6 of 8 HLA match		4 of 6 HLA match
Time to neutrophil engraftment	Medium	Fastest	Slowest
CD34+ cell dose	Adequate	Good	Low
T-cell content	Low	High	Low, functionally immature
Time to identify and collect cells from unrelated donors	~2 months		~1 month
Risk of acute GVHD in recipient	Medium	Highest	Lowest
Risk of chronic GVHD in recipient	Medium	Highest	Lowest
Second donation (to salvage graft failure or relapse)	Potentially available (donor availability)		Not available (though may obtain second unit)

Key Fact

The alleles used in typical eight-allele HLA matching are HLA A, -B, -C, and -DRB1. For umbilical cord transplants (six vs. eight alleles used), they are HLA-A, -B, and -DRB1.

The target dose of CD34+ stem cells is approximately 2 × 108 cells/kg.

Flash Card Q16

What type of donor can be used in immune deficiencies with no T cell function (i.e., SCID) that can NOT be used in any other type of immune deficiency?

Flash Card Q17

What type of conditioning regimen(s) will lead to mixed-chimerism of donor and host cells?

Table 9.17: Need for Conditioning in Select Primary Immunodeficiencies Based on Donor Type

PID		Matched Related Donor	Unrelated/ Mismatched Donor
B+ SCID (XL-SCID, Jak3, IL-7Rα, CD3 chain, CD45)		No	Possibly
B- SCID (ADA, reticular dysgenesis)		No	Yes
Other SCID variants	Omenn's syndrome	Yes	Yes
	PNP	Yes	Yes
	Artemis	Reduced intensity	Reduced intensity
	DNA ligase IV deficiency	Reduced intensity	Reduced intensity
	ZAP-70	No	No
Complete DiGeorge syndrome		No	No
Chronic granulomatous disease		Yes	Yes
Hyper-IgM syndrome (CD40L or CD40)		Yes	Yes
Other: Wiskott-Aldrich syndrome (<5 years old) X-linked proliferative disease (XLP) Leukocyte adhesion deficiency (LAD) MHC II deficiency Chediak-Higashi syndrome Griscelli's syndrome Hemophagocytic lymphohistiocytosis (HLH)		Yes	Yes

Flash Card A16

T–lymphocyte-depleted haploidentical related (i.e., parental donors)

Graft-Versus-Host Disease (GVHD) can affect the overall survival of the graft and the morbidity and mortality of the host.
Factors reducing the risk of GVHD include:

- Prophylaxis with immune suppressive drugs (i.e., steroids and calcineurin inhibitors)
- Selective depletion of alloreactive T lymphocytes from donor grafts
- Using umbilical cord blood as a source
- Choosing more closely related HLA-matched donors

Flash Card A17

Nonmyeloablative or reduced-intensity conditioning. (Myeloablative conditioning leads to higher rate of donor T-cell and B-cell engraftment)

When GVHD develops, the patient must be treated with immunosuppressive therapy. Although they suppress the pathologic activated donor T lymphocytes,

these drugs also theoretically suppress the development of the therapeutic donor stem cells in the host. Standard treatment regimens make use of corticosteroids in addition to cyclosporin or tacrolimus, though patients only have a 50% response rate. Newer agents being used include:

- Monoclonal antibodies (anti-CD3, anti-CD5, and anti-IL-2)
- Mycophenolate mofetil (CellCept)
- Alemtuzumab (Campath)
- ATG
- Sirolimus

Time to Engraftment

After HSCT:

- T lymphocytes will be of donor origin (chimerism can occur in patients with pretransplant T-cell function)
- Other cell lines (B cells, dendritic cells (DCs), neutrophils, platelets, RBCs) can be of donor origin (if myeloablative regimen) or chimeric (if reduced intensity regimen).

Key times in immune reconstitution (Innate→T lymphocytes→ B lymphocytes):

- Innate immunity recovers quickly (unless GVHD)
    - Neutrophils in first few weeks
    - NK cells by 1 month
    - Donor DCs seen later (host DCs can persist up to 1 year)
- By 3 months: T lymphocytes should be present in circulation, some B lymphocytes
    - Patients vulnerable to opportunistic infections prior to this
    - CD8 cells recover prior to CD4 cells (leads to CD4:CD8 ratio of <1)
- Between 1–2 years: Peak thymic T-cell output
- Between 2–3 years: B-lymphocyte function (if at all)
- Functional reconstitution can take up to 24 months

Monitoring should include various studies (Table 9-18).

Flash Card Q18

What two SCID types should NOT undergo myeloablative conditioning, regardless of donor type?

Flash Card Q19

Which mitogen assesses both T *and* B lymphocyte function?

Flash Card Q20

What mitogen bypasses the T-cell receptor?

Table 9-18. Monitoring Reconstitution: Quality Not Quantity

Cell Type	Assay	Measure
T lymphocytes	Flow cytometry	Quantification of T-lymphocyte numbers and phenotypes (e.g., CD3, CD4, CD8, CD45RA/RO)
	CDR3 spectratyping	TCR repertoire diversity, clonality, thymic recovery
	TREC	Naïve T-lymphocyte production, thymic output
	Mitogen or antigen stimulation (PHA, ConA, PWM, tetanus, *Candida*)	Qualitative T-lymphocyte function
	Tetramers	HLA class I or II antigen-specific function
	DTH	T lymphocyte function
	Cytotoxic assays	Cytotoxic T-lymphocyte lytic function
	Mixed lymph proliferation	Donor/recipient in vitro reactivity
B lymphocytes	Flow cytometry	CD19, CD21, CD27 (memory)
	B-lymphocyte excision circles	Class switching
	Serology	Quantitative antibodies and functional responses
NK cells	Flow cytometry	CD16, CD56
	Cytotoxic assay	Lytic function
Neutrophil	DHR	Oxidative burst (CGD)
	Flow cytometry	CD18 (LAD Type I)

Abbreviations: CDG, chronic granulomatous disease; CDR3, compliment determining region 3; DHR, dihydrorhodamine; DTH, delayed type hypersensitivity; HLA, human leukocyte antigen; MLR, mixed leukocyte reaction; PHA, phytohemagglutinin; PWM, pokeweed mitogen;. TREC, T lymphocyte receptor excision circle.

Key Fact

Children with SCID should never receive live viral vaccinations, even after SCT because there have been reports of SCID patients with apparently normal T-lymphocyte function succumbing to varicella after vaccination. Instead, acyclovir prophylaxis should be given after exposure or the patient should be admitted for IV acyclovir treatment if symptomatic.

Flash Card A18

Artemis and DNA ligase 4 deficiency. A full myeloablative regimen would kill these patients as they lack DNA repair capabilities. In some circumstances a reduced-intensity conditioning regimen can help increase survival of transplant

Special Considerations: Vaccinations Post-SCT

Flash Card A19

Pokeweed mitogen (PWM)

Patients who receive stem cell transplantation after myeloablative treatment for malignancy should be reimmunized as though they had a naïve immune system post-transplantation. Table 9-19 gives an example of a reimmunization schedule following SCT for otherwise healthy individuals.

Flash Card A20

Phorbol myristate acetate (PMA)

Vaccines that are NOT recommended for use in post-HCT patients include bacille Calmette-Guérin (BCG), oral polio, intranasal influenza, cholera, typhoid (oral or intramuscular), rotavirus, yellow fever, or varicella-zoster.

Table 9-19. Vaccination Recommendations After HCT (Autologous and Allogenic)

Immunization	Time After HCT (months)	Doses*	Notes
Tetanus, diphtheria, acellular pertussis	6–12	3	May consider additional dose for older children/adults DTaP preferred (use Tdap in adults)
Inactivated polio	6–12	3	
Pneumococcal	3–6	3–4	Give three PCV13 doses, consider PPSV23 to broaden immune response
Haemophilus influenzae B	6–12	3	
Influenza (seasonal)	4–6	1–2	For children < 9 years old, two doses yearly between transplant and 9 years of age
Hepatitis B	6–12	3	
Hepatitis A	12	2	6 months between doses
Meningococcal	6–12	1	
Measles, mumps, rubella (MMR)	24	1	Contraindicated in those with active graft-vs.-host disease (GVHD) or who are immunosuppressed
Varicella	N/A	N/A	Contraindicated at this time

[a] No specific recommendation on intervals between doses (at least 1 month).
Adapted from Centers for Disease Control and Prevention vaccines website. See hemato cell transplants.

IMMUNOPROPHYLAXIS VACCINE

Primary and secondary immunodeficiencies have specific vaccine contraindications and risk-specific recommendations (Table 9-20).

Table 9-20. Vaccine Contraindications and Recommendations for Primary and Secondary Immunodeficiencies

Category	Specific Immunodeficiency	Contraindicated	Risk-Specific Recommendations
Primary immunodeficiencies			
B lymphocyte	Severe (XLA and CVID)	Most live vaccines (OPV, smallpox, live influenza, BCG, and live oral typhoid)	Pneumococcal Influenza Consider measles and varicella IVIG can interfere with response to measles
	Less severe (IgA and IgG subclass)	None	Pneumococcal Influenza All vaccines probably effective
T lymphocyte	Complete defects (SCID)	All live vaccines	Vaccines may be ineffective
	Partial defects (AT, WAS, and majority of DiGeorge abnormalities)	All live vaccines	Vaccine effectiveness dependent upon degree of immunosuppression
Complement	Early or late	None	Pneumococcal Meningococcal Influenza (All effective)
Phagocyte	CGD and LAD	Live bacterial	Pneumococcal Influenza Inactivated and live viral vaccines likely safe and effective
Secondary immunodeficiencies			
HIV		OPV, Smallpox, BCG, LAIV, Withhold MMR and varicella if severely immunocompromised	Influenza Pneumococcal Hib Meningococcal MMR okay if asymptomatic or mildly symptomatic and CD4+>15% for age
Malignancy		Live vaccines contraindicated depending on level of immunosuppression; inactivated may not be as effective	
Steroids		If <2 weeks; okay to give all vaccines If > 20 mg/day for ≥ 2 weeks: wait one month after stopping steroid	

Table 9-20. Vaccine Contraindications and Recommendations for Primary and Secondary Immunodeficiencies, cont.

Category	Specific Immunodeficiency	Contraindicated	Risk-Specific Recommendations
Secondary immunodeficiencies			
Other immunosuppressive drugs		Inactivated: okay to give vaccine (may not be effective) Live: avoidance Anti-TNF agents may re-activate latent TB	
Chemotherapy		Inactivated: may be ineffective (need readministration) Live: wait three months after chemotherapy stopped	
Transplant (hematopoietic stem cell transplant)		Antibody titers decline one to four years after transplant; Should be revaccinated routinely after transplant; (see individual recommendations in Table 9-19)	
IVIG		HIV patients on IVIG: Give live vaccine two weeks before next scheduled IVIG dose, although optimal response may not occur Patients may not respond to MMR and Varicella if on IVIG due to passively-acquired antibody; Regarding live vaccines (MMR, Varicella) and IVIG: If IVIG given first, wait 8 to 11 months to give live vaccine (depends on IVIG dose and indication); If live vaccine given first, wait two weeks to give IVIG	
Pregnancy		Contraindicated: live vaccines Okay: Td/Tdap, influenza (inactivated), IPV, HepB, HepA, and meningococcal	
Breast feeding		Only contraindication is smallpox	
Immunosuppression in household		Only contraindication is smallpox	
Preterm birth		Vaccinate on same schedule as full term children; Only exception: HepB at birth has decreased seroconversion if weight is ,< 2 kg	
Asplenia		None contraindicated; Vaccinate against encapsulated bacteria (Pneumococcal, meningococcal, and Hib); Try to give 2 weeks before elective splenectomy	
Chronic renal failure		LAIV contraindicated (should get inactivated influenza); Recommended: Pneumococcal and HepB	

Abbreviations: AT, ataxia telangiectasia; Hib, *Haemophilus influenzae* type B; HepA, hepatitis A; HepB, hepatitis B; IPV, inactivated polio vaccine; IVIG, intravenous immunoglobulin; LAIV, live attenuated intranasal vaccine; MMR, measles, mumps, rubella; OPV, oral polio vaccine; Td/Tdap, tetanus, diphtheria, pertussis WAS, Wiskott-Aldrich syndrome.

Flash Card Q21

How long after administering a live vaccine should you wait to give a patient an IVIG infusion?

Flash Card Q22

What two vaccines should be administered during pregnancy, if patient is not up to date?

APHERESIS

Apheresis can be defined as the act of running donor blood through a filtering machine that removes a particular constituent and returns the rest of the unfiltered segment back to the donor. It is invasive, must be performed often, and can result in minor volume losses that would need to be replaced. The following are the four different types of apheresis:

- **Plasmapheresis:** Useful for collecting fresh frozen plasma (FFP), immune globulin, WBC and RBC antibodies. Used to treat Guillain-Barré syndrome, lupus, hyperviscosity syndromes (cryoglobulinemia, paraproteinemia, Waldenström's macroglobulinemia), and thrombotic thrombocytopenic purpura.
    - **Intravenous Immunoglobulin (IVIG):** Pooled IgG that is collected from various donors via plasmapheresis, used to treat a whole host of immune deficiency states, confers passive immunity, and reduces infection by trying to maintain a steady state of antibody in the patient. It will be discussed fully in another section.
- **Thrombapheresis:** Useful for collecting platelets, which can be used to treat thrombocytopenia.
- **Leukapheresis:** Useful for collecting neutrophils, eosinophils, and basophils. Commonly used to treat leukemia, as well as autoimmune diseases, such as ulcerative colitis and rheumatoid arthritis. Granulocyte infusions can be used in the treatment of severe infections in CGD (i.e., aspergillosis).
- **Stem Cell Harvesting:** Useful for collecting bone marrow stem cells to be used in stem cell transplantation.

ANTI-INFLAMMATORY AGENTS

CYCLOOXYGENASE (COX) PATHWAY

Flash Card A21

2 weeks

Cycloxygenase-1 (**COX-1**) is constitutively expressed in most tissues and involved in regulating normal cellular processes, such as gastric cytoprotection, vascular homeostasis, platelet aggregation, and kidney function.

Flash Card A22

Tdap, and influenza (inactivated)

Cycloxygenase-2 (**COX-2**) is usually undetectable in most tissues and is inducible during inflammatory states. COX-2 expression is inhibited by glucocorticoids, which may contribute to their significant anti-inflammatory effects.

A splice variant derived from the COX-1 gene has been described as **COX-3,** which appears to be expressed at a high level in the central nervous system.

NONSTEROIDAL ANTI-INFLAMMATORY DRUGS (NSAIDS)

The primary effect of NSAIDs is to inhibit cyclooxygenase, thereby impairing the ultimate transformation of arachidonic acid to prostaglandins, prostacyclin, and thromboxanes (Figure 9-3).

Key Fact

COX-2 inhibition: Anti-inflammatory effects of NSAIDS

COX-1 inhibition: Undesirable adverse effects (see under Pathogenesis)

AERD patients typically tolerate acetaminophen at doses < 1000 mg

Nonselective NSAIDS include the following:

- Propionic acid derivatives (ibuprofen, naproxen, and ketoprofen)
- Acetic acid derivatives (indomethacin and diclofenac)
- Enolic acid derivatives (piroxicam and meloxicam)
- Fenamic acid derivatives (mefenamic acid)

Weak inhibitors of COX-1 (usually well tolerated in aspirin hypersensitivity) include: acetaminophen, salsalate, azapropazone, choline magnesium trisalicylate, and diflunisal.

Selective COX-2 inhibitors include celecoxib, etoricoxib, lumiracoxib, parecoxib, rofecoxib, and valdecoxib. These are generally well tolerated in patients with aspirin sensitivity.

NSAIDS have also been demonstrated in vitro to inhibit NFκB-dependent transcription, leading to inhibition of inducible nitric oxide synthetase (iNOS). Induced nitric oxide synthetase produces large amounts of NO, leading to increased inflammation, including vasocongestion, cytotoxicity, and vascular permeability.

Adverse Effects

All NSAIDs are absorbed completely, have negligible first-pass hepatic metabolism, and are tightly bound to albumin. The patients with low albumin (liver disease) have higher free serum concentration of the drug. Common adverse effects include gastrointestinal toxicity, including dyspepsia, peptic ulcer disease, and bleeding. Other adverse effects can include acute renal failure due to renal vasoconstriction or interstitial nephritis, worsening of underlying hypertension, increased liver transaminases, anaphylaxis (IgE-mediated reaction), TEN or Stevens-Johnson syndrome, and aspirin-exacerbated respiratory disease (AERD).

HYPERSENSITIVITY TO ASPIRIN AND NSAIDS

The classic aspirin triad (aspirin sensitivity, asthma, and nasal polyps) was described by Samter and Beers in 1968 (a.k.a "Samter's triad"). The prevalence of AERD, a tetrad consisting of Samter's triad in addition to chronic rhinosinusitis, is approximately 5–10% in asthma patients.

Pathogenesis

This is a non-IgE-mediated reaction. There is an increased expression of leukotriene C4 synthase as well as enhanced expression of cysLT1 receptor in patients with ASA and/or NSAID hypersensitivity. Blocking the cyclooxygenase pathway by COX-1 inhibitors leads to the overproduction of leukotrienes by the lipoxygenase pathway. Increased LTD4 causes acute bronchoconstriction and increased vascular permeability. Urine LTE4 can be measured to reflect the increased production of cysLTs. Levels of prostaglandin E2 (PGE_2) is decreased by COX-1 inhibition. **PGE_2 is an inhibitor of 5-lipoxygenase pathway**; thus, selective COX-2 inhibitors are generally tolerated in these patients.

Presentation

Age of presentation is usually between second and forth decade. Majority of patients initially develop refractory rhinitis, followed by anosmia and nasal polyposis. The "classic" adverse reaction to aspirin includes bronchospasm of varying severity, usually accompanied by profuse rhinorrhea, and nasal congestion. Some patients experience skin rash, and erythema of the head and neck.

Diagnosis and Treatment

The definite diagnosis of AERD or aspirin-induced asthma can only be established through aspirin provocation challenges. There are four types of aspirin provocations: oral, inhalation (bronchial), nasal, and intravenous. Certain asthma medications, such as SABAs, should be withdrawn before the aspirin challenge.

Oral Aspirin Challenge/Desensitization

Assessment (1 week prior to procedure): FEV_1 > 70% predicted (if low, consider systemic steroid burst); continue treatment with ICS and/or LABA. **Start leukotriene inhibitor** (e.g., 10 mg montelukast).

Procedure: Should be done in a monitored environment. Several protocols are available; two of which are outlined in Table 9-21. When performing the

Table 9-21. Aspirin Oral Challenge/Desensitization Protocols for AERD

Protocol 1		Protocol 2	
Time (hr)	**Aspirin Dose (mg)**	**Time (min)**	**Aspirin Dose (mg)**
Day 1: Onset	30	0	20.25
Day 1: 3	60	90	40.5
Day 1: 6	100	180	81
Day 2: Onset	150	270	162.5
Day 2: 3	325	360	325.00
Day 2: 6	650		

challenge, reactions typically will occur with **low** doses (20–101 mg). These reactions should be treated before continuing protocol. After patient is stabilized, the provoking dose should be repeated. A persistent decrease in $FEV_1 \geq 15\%$ for more than 3 hours is an indication to discontinue desensitization for the day. Typical protocol will take 2 days (even with protocol 2).

Key Fact

Reactions to aspirin during an oral desensitization protocol typically occur at lower doses (e.g., ~40 mg).

Postprocedure: The patient will take the daily dose (usually 650 mg twice daily) indefinitely, though the patient can sometimes be weaned to as low as 325 mg once or twice daily, based on control of symptoms. Patients should not be weaned to lower than 325 mg, however, as they may lose desensitized state against respiratory symptoms. This procedure can have beneficial effects on asthma (decreased hospitalizations and need for systemic steroids), sinusitis, and the recurrence of nasal polyps (though NOT the size). The desensitized state only lasts as long as daily administration is continued. If a dose is missed, the refractory period may last 48–72 hours.

SURGICAL INTERVENTIONS WITH SINUSES OR MIDDLE EAR

Sinus Interventions

Indications

- Complications of sinusitis (meningitis, brain abscess, cavernous sinus thrombosis, and Pott's tumor)
- Recurrent or persistent infectious sinusitis refractory to medical management after failing multiple rounds of antibiotics with adequate antimicrobial coverage

Flash Card Q23

What arachidonic acid derivatives contribute to the clinical manifestations of AERD?

Flash Card Q24

What treatment should be initiated prior to performing aspirin desensitization?

- Obstructive nasal polyps despite an adequate trial of medical management (surgical cure is an exception; usually combined medical and surgical approaches are required)
- Anatomic blockage thought to contribute to infection, such as ostiomeatal unit blockage, adenoidal hypertrophy (especially in children), and nasal septal deviation thought to contribute to nasal blockage or recurrent sinusitis
- Biopsy for evaluation of possible Wegener's granulomatosis, tumor, ciliary dyskinesia, and fungal infection
- Maxillary antral puncture required for culture and guiding antibiotic treatment

Functional endoscopic sinus surgery (FESS) is now the surgical standard of care. FESS restores adequate sinus drainage by establishing patency of the ostiomeatal complex and provides at least moderate improvement of symptoms in up to 90% of properly selected patients.

FESS typically involves maxillary antrostomies, opening of the sinus ostia, and removing the uncinate process. Radical ethmoidectomy may be done to eradicate severe nasal polyps. Complications of FESS include bleeding, orbital trauma, cerebrospinal fluid rhinorrhea, secondary infections, and mucoceles. Frontal and sphenoid sinus surgeries are associated with an increased complication rate due to their locations.

Middle Ear Interventions

Tympanocentesis: May provide rapid relief from pain in severe acute otitis media (AOM). May serve as a means of obtaining cultures in chronic or recurrent otitis.
Tympanostomy tubes result in a 62% relative decrease in otitis media with effusion (OME) in the subsequent year (absolute decrease of 128 effusion days per child in the subsequent year).

Indications

- OME with structural damage
- Recurrent OME (three or more episodes in 6 months or four or more in a year) or persistent OME for at least 3 months (bilateral) or at least 6 months (unilateral)
- Hearing loss of 40 dB or greater in better ear (moderate hearing loss)
- Hearing loss of 21–39 dB (mild hearing loss) in better ear if comorbidities increase risk of developmental delay (e.g., mental retardation or cleft palate)
- Repeat tympanostomy tubes should include adenoidectomy and myringotomy in children older than 4 years of age according to American Academy of Pediatrics

Flash Card A23

Decreased PGE_2 (inhibits the 5-LPO pathway), and *increased* LTD4 (bronchoconstriction)

Flash Card A24

Antileukotriene therapy

CONTROVERSIAL TREATMENTS

Neutralization Therapy

After test doses of various agents (chemicals, allergens, food extracts, or other) are administered by various routes (intradermal, subcutaneous, or sublingual), patients self-administer these "neutralizing" substances for a variety of atopic and nonatopic conditions. This is not standardized, has no established protocols, and no convincing ramdomized controlled trials (RCTs) support this therapy.

Enzyme-Potentiated Desensitization (EPD)

A very low dose of allergen (equivalent to that used in prick testing) is administered in combination with β-glucuronidase to treat a variety of atopic and nonatopic conditions. Beta-glucuronidase is theorized to activate CD8 lymphocytes, thereby suppressing the immunogenicity of the allergen. Some small trials have suggested some clinical improvements, but this is still experimental.

Detoxification

Detoxification procedures, including but not limited to exercise, sauna/ or sweat spas, vitamins, minerals, oils, nutritional therapy, and colon cleansing or enemas, eliminate "toxic chemicals" from the body. Methods are anecdotal with no sound evidence for "immunotoxicity" as a plausible cause of atopic disease.

Autogenous Urine Therapy

The ingestion of one's own urine for supposed health benefits has occurred throughout various cultures and times. Proposed presence of a substance known as "proteose" in urine is supposed to have allergen-specific properties. The patient's own urine is prepared then injected SC for treatment of a variety of allergic diseases. There is no rational basis for efficacy and, theoretically, it has potential for harm (autoantibody production).

Homeopathy

Plant or animal extract ingestion (e.g., bee-transported pollen in honey) of very small doses of allergens are claimed to promote tolerance. Not standardized.

There are both positive and negative trials, but meta-analysis failed to support any benefit.

Acupuncture, Relaxation, Hypnosis, and Biofeedback

Although there are mixed reports of the efficacy of acupuncture for allergy symptoms, most of the positive trials are methodologically flawed. Rigorous trials have failed to support any of these methods over placebo.

Candida Hypersensitivity Syndrome (Yeast Hypersensitivity Syndrome)

Various allergic and nonallergic ailments are hypothesized to be secondary to sensitivity to a toxin released from *Candida albicans*. Diagnosis is based on history. Treatment is based on dietary changes, possibly antifungal therapy. There are no RCTs, and some elements of treatment have the potential for harm (e.g., liver or kidney toxicity from antifungals).

CARDIOPULMONARY RESUSCITATION

Basic "Code" Approach: Remember CAB

In 2010, the American Heart Associated recommended a change from the previous A-B-C stepwise progression, to that of C-A-B. This places an emphasis on performing chest compressions first. The new recommendations are as follows:

- Call 911 or ask someone else to do so; have them get automated external defibrillator (AED) (if available). If you are the sole rescuer, do not leave to call 911 and get AED initially. Wait until after five cycles of CPR (or 2 minutes).
- Try to get the person to respond; if they don't, roll the person on his or her back.
- **C** = Start chest compressions. The proper technique involves placing the heel of your hand on the center of the victim's chest with the other hand on top of the first with fingers interlaced. Each press should compress the chest at least 2 inches in adults and children and 1.5 inches in infants. Rate should be 100 compressions per minutes (same rhythm as "Stayin' Alive," by the Bee Gees)
- **A** = if trained in CPR, may proceed to open airway after first 30 compressions (head tilt-chin lift; use jaw thrust if suspect cervical trauma).

- **B =** assess breathing; if inadequate effort or no spontaneous respirations, start rescue breathing with two, 1-second rescue breaths while watching for chest to rise.
- **D =** disability; useful to remember **defibrillator** (this should be part of initial response if person unconscious).
- Continue compressions and breaths (30 compressions, 2 breaths for all persons) until help arrives (see differences for children below).

Differences Between Adult Versus Child or Infant CPR

- Child victim (≤8 years old), two rescuers = use **15:2 compression to breath ratio**. **Pediatric arrests are much more commonly pulmonary in nature.**
- AED use not recommended for infants (<1 year of age).

When a Secure Airway is Obtained (i.e., Intubation)

- No longer cycle chest compressions and breaths. Instead, give 12 breaths per minute and 100 chest compressions per minute simultaneously.

Choking in a Responsive Patient

- Adults and children: Abdominal thrusts (Heimlich maneuver)
- Infants (< 1 year of age): alternate five back blows and five chest thrusts
- Choking in an unresponsive patient signals need to start CPR

Defibrillation

- The most effective treatment for ventricular fibrillation and the most common cardiac arrest rhythms
- The probability of successful defibrillation decreases rapidly over time

Code Situations and Anaphylaxis

- Do not forget that, in cases of anaphylaxis, **epinephrine is both the drug of choice and the initial medication**. Even if the patient is on a β blocker, the **first** drug given is epinephrine (0.01 mg/kg SC/IM of 1:1000 or 0.01 mg/kg IV of 1:10,000 concentration).
- Glucagon may be given if patient is on a β blocker and does not respond to epinephrine.

Flash Card Q25

What is the rate of compression when performing CPR?

- **Do not forget adequate volume resuscitation and supplemental oxygen.**
- May also use H_1 and H_2 blockade and steroids.
- Place patient supine to facilitate passive venous return to heart.

DERMATOLOGIC AND OPTHALMIC TREATMENTS

ATOPIC DERMATITIS

Prevention

Identify triggers and avoid irritants such as detergents, soaps, chemicals, pollutants, abrasive materials, extreme temperatures, and humidity.

Treatment

Hydration—Soak skin in warm water for 10 minutes. This is important because of decreased water-binding capacity secondary to decreased ceramide levels in AD skin.

Moisturizers—Emollients restore and preserve stratum corneum layer. Water content: lotions > creams > ointments.

Corticosteroids—Topical: Use most effective lowest potency formulation (Table 9-22). Ointments are the most occlusive and, therefore, provide better delivery of medication. Thirty grams is needed to cover the entire body of an average adult. Adverse effects: Thinning of the skin, telangiectasias, bruising, hypopigmentation, acne, striae, and secondary infections. Use only low-potency steroids on the face or groin. Oral steroids should be avoided when possible; if necessary, can give a short course with taper.

Topical Calcineurin Inhibitors—Available preparations: Tacrolimus 0.03% and 0.1%, and pimecrolimus 1%. Black box warning for lack of long-term safety data; therefore, used as second-line treatment only for those older than 2 years of age. Avoid topical calcineurin inhibitors with occlusive dressings. Adverse effects: Burning or stinging sensation.

Tar Preparations—Inhibit the influx of proinflammatory cells and expression of adhesion molecules in response to allergen. Avoid use on acutely inflamed skin. Adverse effects: Rare, but include photosensitivity and pustular folliculitis.

Flash Card A25

100/min

Table 9-22. Topical Steroids Arranged by Potency

Potency (Class)	Topical Steroid
Very low (VII)	Hydrocortisone 1%, 2.5%
Low (VI)	Alclometasone dipropionate, desonide, fluocinolone, hydrocortisone butyrate 0.1% (C)
Medium (IV-V)	Betamethasone valerate, Desoximetasone 0.05% (C), fluocinolone acetonide, fluticasone propionate 0.05% (C), hydrocortisone butyrate, hydrocortisone valerate, mometasone furoate, triamcinolone acetonide 0.25–0.1%
Med-high (III)	Amcinonide (C), fluticasone propionate 0.005% (O), Triamcinolone acetonide 0.5% (C, O)
High (II)	Amcinonide (O), betamethasone dipropionate, desoximetasone, fluocinonide, halcinonide
Very high (I)	Clobetasol propionate, diflorasone diacetate, halobetasol propionate

Abbreviations: C, cream, O, ointment. As a general rule, potency is as follows: ointment > cream > lotion.

Wet Dressings—Cool the skin and act as a barrier to trauma; improve penetration of topical corticosteroids. Adverse effects: Chilling, maceration of the skin, and secondary infections.

Anti-Infective Therapy—May be indicated for secondary infections. Semisynthetic penicillins or first- and second-generation cephalosporins. Avoid maintenance antibiotic therapy. Topical antibiotic may be effective for treating localized areas of infection. Can use mupirocin intranasally to reduce nasal carriage of *Staphylococcus aureus.* Oral acyclovir for disseminated eczema herpeticum. Recurrent cutaneous herpetic infections can be treated with daily prophylactic acyclovir.

Antipruritic Agents—Antihistamines (first and second generations). Doxepin has both H_1- and H_2-receptor-binding affinity and a long half-life. Avoid topical antihistamines and anesthetics because of potential for sensitization.

Treatment of Recalcitrant Disease

Hospitalization—Removes patient from environmental allergens or stressors; may be necessary for those with severe disseminated disease.

Cyclosporin A—Results in decreased transcription of a number or proinflammatory cytokines; long-term treatment resulted in improvements in disease activity. There is concern for irreversible nephrotoxicity with extended treatment.

Phototherapy or Photochemotherapy—Decreases expression of activation markers on CLA+ T cells. Available forms: UVB, narrow-band UVB, and high-dose UVA1. High-dose UVA1 decreases dermal IgE-binding cells, including mast cells and dendritic cells; also downregulates proinflammatory cytokines and induces apoptosis of skin infiltrating CD4 T lymphocytes.

Recombinant Human Interferon-γ—Suppresses IgE synthesis and inhibits T_h2 cell differentiation; results in reduced clinical severity and decreased total circulating eosinophil counts in patient with AD.

Azathioprine—Systemic immunosuppressive agent affecting purine nucleotide synthesis and metabolism. Adverse effects include myelosuppression, hepatotoxicity, GI disturbances, increased susceptibility for infections, and risk of developing skin cancer

Ophthalmic Treatments

Many different medications/combinations can be used in the treatment of ocular allergies. They are listed in Table 9-23.

Table 9-23. Ophthalmic Treatments

Drug	Available Preparations	Mechanism of Action	Side Effects
Vasoconstrictors	Tetrahydrozoline Naphazoline	α Agonist: Constrict conjunctival blood vessels	Rebound hyperemia if discontinued after prolonged or excessive use
H_1-antihistamines	First-generation: Pheniramine, antazoline, levocabastine Second-generation: Emedastine	H_1-receptor antagonists; do not prevent histamine release	First-generation: CNS Second-generation: Headache and dry eye
Mast cell stabilizers	Cromolyn, lodoxamide Nedocromil Pemirolast	Inhibit degranulation of activated mast cells; most effective when instituted before or soon after the onset of clinical symptoms	Burning, dry eyes, and eye swelling
Vasoconstrictor or antihistamines	Naphazoline + antazoline Naphazoline + pheniramine	Constrict blood vessels and inhibit binding of circulating histamine	Rebound hyperemia
Antihistamine and mast cell stabilizers	Olopatadine, ketotifen, azelastine, alcaftadine, and epinastine	Antagonize H_1 receptors, inhibit histamine release from mast cells	Headaches and eye swelling

Table 9-23. Ophthalmic Treatments, cont.

Drug	Available Preparations	Mechanism of Action	Side Effects
NSAIDs	Ketorolac	Inhibit prostaglandin and thromboxane synthesis	Ocular burning, stinging, conjunctival hyperemia, and corneal infiltrates
Corticosteroids	Loteprednol etabonate Dexamethasone sodium phosphate	Induce lipocortins, which inhibit the release of arachidonic acid (precursor to PGs and leukotrienes).	Glaucoma, cataracts; check IOP 3 months after starting treatment, then annually

Abbreviations: CNS, central nervous system; IOP, intraocular pressure; PGs, prostaglandins.

GENE THERAPY IN PID

- Refers to treatment resulting from insertion of a gene into somatic cells. The transferred normal gene will express a normal product, thus correcting the previously manifested defect.
- Current technology, regulation, and predictable success are focused on lethal single-gene defects.
- Primary immune deficiency encompasses many disorders, and the hematopoietic origin of the immune system provides an attractive platform for gene therapy.
- Gene therapy targeting hematopoietically immature cells allows the gene copies to be carried throughout the cell lineages and passed to subsequent generations.
- Gene therapy has been used successfully in correcting the genetic defect in X-linked, adenosine deaminase (ADA) deficiency SCID, and Wiskott-Aldrich syndrome.
- Active areas of research for future use of gene therapy in PIDs include other forms of single-gene defect: SCID, CGD, LAD, and others.

Obstacles:

- The major obstacle to widespread use of gene therapy is insertional mutagenesis. Of particular concern is variant vector/gene integration into unintended areas of DNA. In the case of X-linked SCID and WAS gene therapy trials, development of leukemia has occurred. The concept of gene therapy has been proven, and now the obstacle lies in vector engineering and the safety of gene therapy. Examples of viral vectors are listed in Table 9-24.
- Isolation of appropriate and specific cell populations.
- Efficiency of transducing genes into target cells

- Correction of defects in which proper regulation of gene expression is required.

Viral Vectors

Table 9-24 summarizes the advantages and disadvantages of viral vectors.

RNA Interference (RNAi)/Post Transcriptional Gene Silencing (PTGS)

RNAi uses antisense oligonucleotides to downregulate the expression of specific mRNA molecules. This is mediated through the endogenous enzyme "Dicer," which cleaves long double-stranded RNA (dsRNA) molecules into short double-stranded fragments called small interfering RNAS (siRNA). The siRNAs are unwound into guide ssRNA molecules (a guide strand and passenger strand). The guide strand is incorporated into the RNA-induced silencing complex (RISC), which leads to gene silencing.

Table 9-24. Use of Viral Vectors

Viral Family	Advantages	Disadvantages
Retroviruses (gamma-retroviruses)	Integrated genes are transferred to progeny cells	Potential for insertional mutagenesis, only shows expression in actively dividing cells
Lentiviruses (subclass of retrovirus)	Can integrate into genome of nondividing cells Lower tendency to integrate into places than gamma-retroviruses	Theoretical potential for insertional mutagenesis
Adenoviruses	Generates high levels of transgene expression	Immune response to viral antigens may lead to destruction of infected cells
Adenoassociated viruses (Parvoviridae)	Does not induce immune responses against infected cells	Can only carry transgenes of small sizes; large-scale production difficult
HSV	Can carry large transgenes and infects broad range of cell types	Residual neuronal cytotoxicity

Abbreviation: HSV, herpes simplex virus.

10 Specific Diagnostic Modalities

SKIN TESTS (IMMUNOASSAY FOR TOTAL AND SPECIFIC IgE)

TOTAL IgE

Measurement of total IgE has limited value in the diagnosis of atopy. IgE is elevated in some immunodeficiencies and other disease states (Table 10-1).

Hyper IgE Conditions

T able 10-1 summarizes the conditions associated with a high total IgE**.**

Table 10-1. Disease States Associated with a High Total IgE Level and Immunodeficiencies Associated with an Elevated Total IgE Level

Conditions with High Total IgE	Immunodeficiency Plus Increased Total IgE
Atopy (atopic dermatitis, AR, asthma, and allergic fungal sinusitis)	Wiskott-Aldrich syndrome
Infections (parasites, ABPA, HIV, TB, EBV, CMV, viral respiratory infections)	Hyper IgE syndrome
Cutaneous (alopecia areata, bullous pemphigoid, chronic acral dermatitis, streptococcal erythema nodosum)	Omenn's syndrome
Oncologic (IgE myeloma and Hodgkin's lymphoma)	Di George's syndrome
Kimura's disease (Asian men in third decade, and lymphadenopathy or adenitis of face and neck)	Netherton's syndrome
Churg-Strauss syndrome	Nezelof's syndrome (cellular immunodeficiency with immunoglobulins)
Other: Drug-induced interstitial nephritis, cystic fibrosis, Kawasaki's disease, burns, cigarette smoking	Selective IgA deficiency
	DOCK8 deficiency
	AIDS
	GVHD and bone marrow transplantation

Abbreviations: ABPA, allergic bronchopulmonary aspergillosis; AR, allergic rhinitis; CMV, cytomegalovirus; EBV, Epstein-Barr virus; GVHD, graft-versus-host disease; TB, tuberculosis.

Flash Card Q1

Where are the highest concentrations of IgE-producing plasma cells?

Measurement of Total IgE

- Nephelometry or immunoassay
- 1 IU/mL = 2.44 ng/mL Standardized vs. World Health Organization reference IgE serum

Clinical Features Regarding Total IgE

- Generally nondiagnostic of allergic disease due to wide ranges (Atopic diseases: Atopic dermatitis: mean 978 IU/L; range 1.3–65,208) and wide overlap in the IgE distribution of atopic and nonatopic populations
- Can be utilized as a screen for allergic bronchopulmonary aspergillosis (ABPA) (>417 IU/mL) or to evaluate a patient for omalizumab candidacy (indicated for moderate-to-severe persistent allergic asthma not controlled with standard therapy if total IgE 30–700 IU/L)
- Hyper-IgE syndrome (ranges ~1000–50,000 IU/mL)
- IgE does not cross the placenta; undetectable at birth. Reaches a peak between 10–15 years of age and then declines through adulthood. Rises at a rate slower than IgG and comparable to IgA
- Total IgE levels tend to be higher in men than women (not in children), higher in blacks than whites, and higher 4–6 weeks after the pollen season
- Decreased IgE seen in familial IgE deficiency and recurrent sinopulmonary infections, HTLV-1 infections, primary biliary cirrhosis

Key Fact

Polymorphisms of CCL11 (eotaxin) are associated with serum total IgE; increased in blacks, decreased in whites.

SPECIFIC IgE TESTING

Detection of serum-specific IgE—sensitization—does not equal allergic disease. Sensitization occurs in 8–30% of the population when using a standard panel of aeroallergens. Thirty percent to 60% of sensitized individuals may develop future clinically relevant allergic symptoms.

Skin Testing

Skin Prick Testing (SPT)—See Table 10-2 for skin test for early- and late-phase mediators.

Flash Card A1

Tonsils and adenoids

Table 10-2. Early- and Late-Phase Mediators

Early-phase Mediators: Immediate Reaction	Late-phase Mediators: 1–2 hr, Peaks 6–12 hr, Resolves 24–48 hr[a]
Histamine	CD4+ T cells
Tryptase	Eosinophils
Substance P	
Calcitonin gene-related peptide	

[a]Late-phase reaction does not predict symptoms on exposure and is not used in the diagnosis of IgE-mediated disease.

See Table 10-3 for factors that affect skin tests.

Table 10-3. Factors Affecting Skin Tests

Factor	Affect
Skin reactivity of test site (Figure 10-1)	Mid (1) and upper back (1) > lower back (2) > forearm (antecubital fossa [3] > wrist [4]) Place tests at least >2 cm apart
Age	Infants have a flare but smaller wheal The elderly have smaller wheal and flare; highest in second and third decade of life
Race	Wheal is greater in nonatopic blacks
Gender	No difference
Season variations	Increase peaks after pollen season
Some chronic conditions	Eczema, chronic renal failure, hemodialysis, cancer, spinal cord injuries, peripheral nerve abnormalities may diminish skin reactivity
Presence of dermatographism	RSV infection can increase reactivity
Use of medications	Antihistamines (1st-generation H_1 antihistamines have a greater suppressive effect than 2nd-generation; H_2 blockers and nasal antihistamines have more limited effects on skin testing) Tricyclic antidepressants, phenothiazines, and omalizumab (for up to 6 months) also suppress skin tests (Medications not affecting the skin test include oral (short-term use), nasal, or inhaled corticosteroids; β agonists; theophylline; leukotriene antagonists; and cromolyn.
Type of device	Multiheaded devices produce more false negatives than single-headed devices

Abbreviation: RSV, respiratory syncytial virus.

Flash Card Q2

Which part of the back (upper or lower) has greater skin reactivity for skin testing?

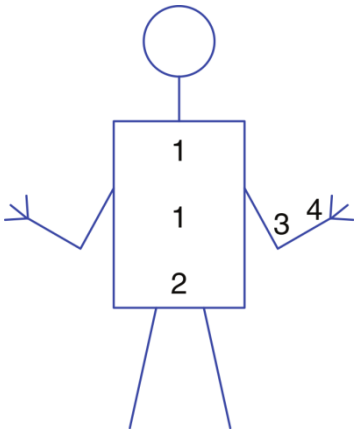


Figure 10-1. Skin reactivity of test sites: Mid (1) and upper back (1) > lower back (2) > forearm (antecubital fossa) (3) > wrist (4).

Diagnostic Characteristics—For inhalant allergy, the sensitivity and specificity of the SPT are approximately 80–85% compared with nasal provocation challenges, lower for molds. For food allergy, the SPT is generally sensitive (30–70%), with high rates of false positives (30–70% specific). When skin testing for allergies to fruits and vegetables, use of fresh foods increases sensitivity to 90%.

Limitations of Skin Prick Tests. False-positive SPT results to tree pollen can be seen in patients allergic to honeybee stings due to cross-reactive carbohydrate determinants in honeybee venom.

False-negative SPT can be seen if there has been an episode of anaphylaxis in the previous month. False-negative skin tests can also be caused by low potency of extracts, reduced reactivity of skin in children and elderly, improper technique, and UV exposure.

Patients at higher risk for systemic reaction to skin testing include those with poorly controlled asthma and reduced lung function, and clinical histories of severe reactions to minute amounts of allergen.

Intradermal Tests (IDST)

- IDST should be performed with a 100- to 1000-fold dilution of the SPT concentration. IDST involves injection of 0.02–0.1 mL of allergen with a 26- or 27-gauge needle.
- IDST are more sensitive but less specific than the SPT (high false-positive rate).

Flash Card A2

Upper back

- SPTs need to be performed before IDSTs for safety reasons since fatalities have been reported when intradermals are performed without a preceding SPT.
- IDSTs are utilized to perform the IDEAL50 (intradermal dilution equals 50 mm), which is used for the standardization of extracts. In the IDEAL50, erythema (**not** wheal) is measured.
- Any reaction larger than the negative control may indicate the presence of specific IgE antibody, though small positive reactions may not be clinically significant.

Clinical Utility

- **Venom hypersensitivity:** Testing done up to 1 µg/mL. False-positive rates increase above this concentration.
  - Intradermals especially important for diagnosing venom hypersensitivity as failure to identify can lead to life-threatening consequences.
- **Drug hypersensitivity:** IDST is useful for penicillin, chemotherapeutic agents, muscle relaxants, insulin, and heparin skin testing. Value of drug skin testing to other agents is variable as (1) patients may be allergic to a metabolite (2) lack of standardization, (3) non-IgE mediated mechanism may be involved
- **Specific inhalant allergens:** IDST is not typically beneficial for cat or grass; but, may be more helpful for weaker nonstandardized inhalant allergens.
- Intradermal testing is **not** used in the diagnosis of food or latex allergy due to a high rate of systemic reactions
- **Systemic reaction rate:** <0.02–3.6% in prospective studies, fatality exceedingly rare, nearly always with intradermal without prior SPT
- For occupational sensitizers, skin tests are often unreliable with the exception of high-molecular-weight (HMW) compounds such as latex, enzymes, and flour.

> ***Key Fact***
>
> Venom intradermal skin tests are performed up to 1 µg/mL. Do not include any positive flying Hymenoptera skin tests at 10 µg/mL in venom immunotherapy prescriptions.

Serum-Specific IgE

Methods

- RAST: Cyanogen bromide-activated paper disc allergosorbent, bound IgE detected with radiolabeled antihuman IgE Fc, semiquantitative, four to six classes
- ELISA, chemiluminescence, or fluoroenzymatic detection systems:
  - Quantitative, more sensitive than RAST
  - ImmunoCAP system uses encapsulated hydrophilic carrier polymer that is configured into the shape of a small cup (and called a CAP); it has allergen covalently coupled
  - Another third-generation assay (DPC) labels allergenic protein with biotin
  - Combine this improved binding with fluoroenzymatic or chemiluminescence detection systems
  - Calibrated against WHO human-IgE reference, reported in units (kIU/L)

- Performance characteristics versus SPT: Generally less sensitive

Benefits

- Use in severe AD and dermatographism
- Use in highly sensitive patient/recent anaphylaxis (no risk for reaction)
- Use in patients with poor pulmonary function
- Use in patients unable to be weaned from antihistamines
- High positive predictive values in children can be achieved for some of the major food allergens

Limitations

Overall, sensitivity ranges 60–95% and specificity 30–95%

Greater than 90% sensitivity, specificity, and predictive values have been obtained with pollens of common grasses and trees, dust mites, and cat allergens. Less for venoms, foods, weed pollens, latex, drugs, dogs and molds.

- False positives
  - Nonspecific binding to glycoepitopes
  - 20–30% of patients allergic to pollen will have positive food-specific IgE without reporting symptoms to foods
  - Wheat epitopes cross-react with grass epitopes
  - Use bromelain (protein digestive enzyme) to evaluate and clarify
- False negatives
  - Food: Approximately 20% of patients with egg- or peanut-specific IgE < 0.35 kIU/L have clinical reactions (most have positive skin tests)
  - Venom: Insect allergic patients with negative skin test have serum-specific IgE in 15–20% of cases
  - Latex: Sensitivity of only 80% and specificity of 95%
  - Penicillin G, penicillin V, penicilloyl, amoxicillin, and ampicillin are commercially available; sensitivity of penicillin-specific IgE only ~45%
  - Component-resolved diagnostics utilize purified native or recombinant allergens to detect IgE sensitivity to individual allergen molecules. Can identify patients with clinical allergy as opposed to patients who are merely sensitized but tolerant. Best studied for peanut: Ara h 8 peanut—Bet V 1 family (birch)—labile—oropharyngeal symptoms
  - Ara h 9 peanut and Pru p 3 peach—lipid transfer protein—stable—mixed—can have severe reactions (more in Europe)
  - Ara h 2 > 3 and 1 peanut—seed storage proteins—stable—anaphylaxis
  - Ara h 6 is homologous to Ara h 2

NASAL PROVOCATION

Nasal provocation is accepted as a generic model for the human allergic response and is used to study the pathophysiology of allergic and nonallergic rhinitis.

Indications

- Used mainly as a research tool in USA and Great Britain
- Study early- and late-phase changes in mucosa and nasal secretions
- Look at response to individual mediators or cytokines, assess the relationship between allergic rhinitis and other illnesses (asthma, conjunctivitis, and otitis media)
- Test therapeutic agents

Also can be clinically diagnostic for the following:

- Identify relevant allergens and confirm nasal reactivity (e.g., strong history despite negative skin testing and serum IgE testing)
- Induction of ocular or bronchial symptoms (may be safer than bronchial challenge for allergic asthma)
- Diagnosis of aspirin-exacerbated respiratory disease with nasal ketorolac
- Occupational allergens (e.g., baker's yeast, carpenter's saw dusts, or latex)
- Food induced rhinorrhea

Methods

Note the overlap with bronchial provocation methods.
Table 10-4 shows details about necessary washout periods of drugs that may interfere with nasal provocation testing. This should be compared with drugs that interfere with bronchoprovocation (see Bronchial Provocation section and Table 10-5).

Table 10-4. Drugs That Inhibit the Nasal Response and Washout Periods

Medication	Washout Period (Days)	Inhibits Early or Late Response
α-Adrenergic agonist, nasal and oral	1	Neither
Anticholinergics	?	Early
Antihistamine, nasal	3	Early
Antihistamine, oral	3–10	Early
Chromones, nasal	3	Early and late
Corticosteroid, nasal	7	Early (with prolonged treatment) and late
Corticosteroid, oral	7	Early and late
Leukotriene receptor antagonists	7	Inconsistent results (zileuton)

Allergen Provocation

Produces early and late responses. Only aqueous allergen extracts should be used because glycerinated extracts can cause significant irritant effects.

- Techniques:
  - Application to the entire nasal cavity (metered-dose pump spray, spray bottle, nasal pool device, or dropper)
  - Application to a very small area of the mucosa (paper disc or cotton swab)
  - Allergen exposure chamber (more natural level of exposure)
  - Unilateral provocation, allows distinction between the direct and indirect effects of exposure. Nasal secretions increase bilaterally, but nasal airway resistance increases only in the challenged nostril. Unilateral allergen challenge results in acute increase of prostaglandin D2 and histamine in the contralateral side (reflex activation of mast cells)

Physical and Irritant Stimuli

- **Cold and dry air:** Symptoms associated with sensorineural activation and with mast cell mediator release. Can help differentiate nonallergic, noninfectious rhinitis from healthy controls.
- **Hyperosmolar solutions:** Either sodium chloride or mannitol activate mast cells in a nonantigenic fashion that can mimic effects of cold air.
- **Capsaicin:** Acts on the vanilloid receptor TRPV1 located on unmyelinated, slow-conducting sensory nerve fibers. Unilateral provocation results in

bilateral secretory response. **Nasal responsiveness is increased in allergic rhinitis, but not in nonallergic rhinitis.**

- **Air pollutants and other irritants** (e.g., tobacco, volatile organic compounds, cleaning products)**:** Provocation agent is in gaseous form; however, the settings to generate various dosages can be complicated. Passive tobacco exposure has been shown to potentiate the inflammatory response of nasal allergen provocation.

Biochemical Stimuli

Biochemical stimuli have limited utility due to considerable overlap in response between allergic and nonallergic rhinitis.

- **Histamine:** The most common stimulus used. It mimics symptoms of an acute allergic response to an allergen challenge; however, it does **not** induce late inflammatory events. Most effects are mediated by the H_1 receptor, but the H_2 receptor plays a role in vascular congestion.
- **Methacholine:** Stimulates nasal muscarinic receptors and induces a secretory response. Unlike histamine, **methacholine does not induce nasal reflexes**, therefore it is useful to assess responsiveness of secretory glands. Unilateral challenge produces only ipsilateral response.
- **Adenosine:** Causes mast cell degranulation and sensory nerve stimulus.
- **Neuropeptide:** Looks at the role of the nervous system in the dysfunction of the nose (substance P, neurokinin A, serotonin, calcitonin gene-related peptide, bombesin, and neuropeptide Y).

Outcomes Measured

Symptoms include lacrimation, sneezing, pruritus, rhinorrhea, posterior nasal drainage, congestion.

Except for sneezing, the other measures are very subjective.

Changes in nasal patency include the following:

- **Nasal peak flow:** Quick and inexpensive, but effort-dependent.
- **Nasal airway resistance:**
  - Rhinomanometry is the standard technique for measuring nasal airway resistance. Nasal airway resistance does **not** correlate well with subjective sense of nasal congestion. This may be due to effect of nasal valve on determining airway resistance but not contributing as much to sensation of congestion.
  - Location of greatest nasal resistance is the nasal valve.

Refer to section on “nasal smears” (Chapter 7, Hypersensitivity Disorders) for further details on analysis of nasal secretions.

Mucosal tissue changes should only be performed by experienced physicians due to possible complications.

Contraindications to Nasal Provocation

Relevant contraindications are as follows:

- Acute inflammatory conditions in the nose or the paranasal sinuses
- Poorly controlled asthma
- History of systemic allergic reactions to immunotherapy or to skin testing
- History of oral or oropharyngeal angioedema
- Receiving medications that could theoretically increase the risk of lower airway or systemic reactions, or that could interfere with their treatment (e.g., ACE-inhibitors or β-adrenergic blockers)
- Pregnancy

PULMONARY FUNCTION TESTS (PFT)

General Considerations

Airflow limitation is measured by spirometry. Expiratory flow is affected by airway caliber, airway wall compliance, and elastic recoil. **The forced expiratory volume in 1 second (FEV_1) is the most reproducible pulmonary function test.** Lung volume is related to body size, with standing height being the most important correlating variable. Reference equations compare patient data to reference subjects of similar sex, age, height, and ethnicity.

Figure 10-2 shows typical spirometric flow-volume patterns.

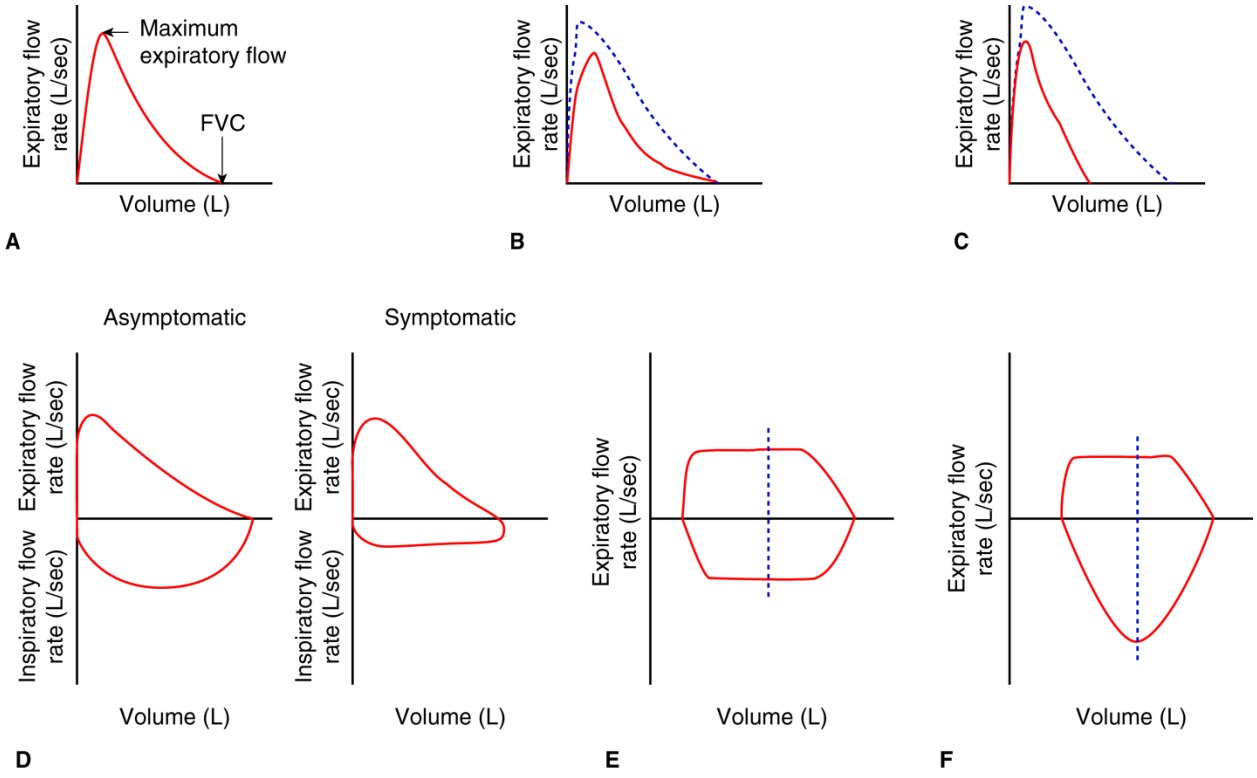


Figure 10-2. Types of ventilatory defects**.** (A) Normal. (B) Obstructive abnormalities. Reduced FEV_1 /FVC. May also see a reduction in mean expiratory flow between 25% and 75% (FEF 25–75), although this is considered a less specific indication of obstruction. (C) Restrictive abnormalities. Defined by reduced TLC. May have normal or increased FEV_1/FVC. Restrictive lung disease cannot be diagnosed by spirometry alone as FVC and TLC may be poorly correlated. Lung volume measurement is required for confirmation with plethysmography or other such method. (D) Vocal cord dysfunction. A variable extrathoracic obstruction with truncation of inspiratory loop seen when patient is symptomatic. (E) Fixed upper airway obstruction. Flattening of both inspiratory and expiratory loop. Examples include tracheal stenosis and goiters. (F) Variable intrathoracic obstruction. Flattening of expiratory loop; an example is tracheomalacia.
Abbreviations: FEF, forced expiratory flow; FEV, forced expiratory volume; FVC, forced vital capacity; TLC, total lung capacity.)

Interpretation of PFTs

- Was it a good test? Look at effort. Exhalation for at least 6 seconds in adults [3 seconds in children] or reached plateau for at least 1 second on the volume-time curve.
- Must have three reproducible efforts with the largest volumes of FEV_1 and FVC within 150 mL of each other.
- Look at flow-volume loop.

Key Fact

A significant response to a bronchodilator in adults is an increase in FEV_1 of 12% **and** at least 200 mL.

- Assess FEV_1, FVC, and FEV_1/FVC for airflow limitation (also TLC and diffusion lung capacity for carbon monoxide [DLCO] if needed). See Figure 10-3.
- For FEV_1/FVC ratio, normal ratio varies by age: 8–19 years old: 85%; 20–39 years old: 80%; 40–59 years old: 75%; and, 60–80 years old: 70%.
- Patients should withhold short-acting β agonist (SABA) × 4 hours, long-acting β agonist (LABA) × 12 hours, tiotropium × 24 hours.

PFTs in infants and young children:

- Spirometry recommended for children older than 4 years old; most can perform by age 6 years
- Other options for young children: whole-body plethysmography (respiratory system resistance [Rrs]), interrupter technique (Rint), or forced oscillation technique (measures resistance and reactance)
- For infants: Partial flow volume curves, resistance, compliance, functional residual capacity (FRC); require sedation

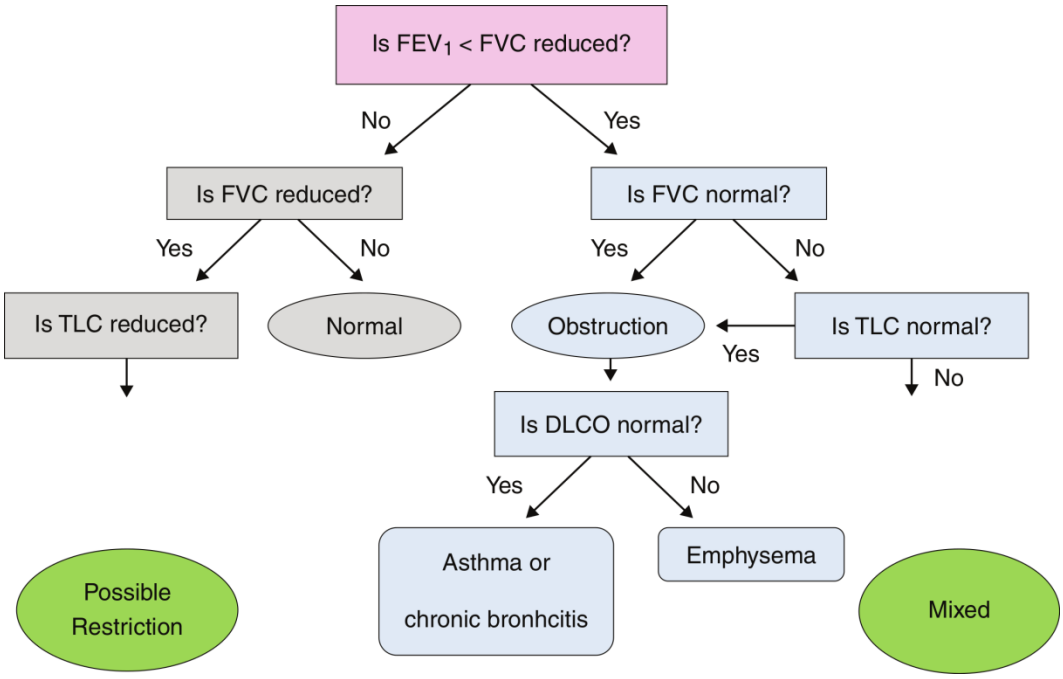


Figure 10-3. Algorithm for interpretation of PFTs.
Abbreviations: DLCO, diffusing capacity of carbon monoxide; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; TLC, total lung capacity.

BRONCHIAL PROVOCATION

Bronchial Provocation Challenge Methods

Bronchial provocation is accomplished using the following methods:

- **Dosimeter:** Five inspiratory (TLC) breaths from a breath-activated dosimeter
- **Tidal breathing:** Two-minute tidal breathing from a jet nebulizer. Preferred method in children

These methods are considered equivalent; however, tidal breathing exposes patient to twice the volume of aerosol at each concentration.

Key Fact

Severity of airway hyper-responsiveness (AHR) does not correlate with severity of asthma.

Key Fact

Nonselective or nonallergic versus selective or allergic agents: Nonselective agents have the potential to produce bronchoconstriction in **all** asthmatics, and they do not purge any specific triggers. Selective testing looks at specific triggers in susceptible asthmatics.

Direct versus indirect testing: Direct testing acts on the airway smooth muscle receptors (muscarinic and histamine) directly, but not by inducing mediator release. If mediator release is necessary, then it is an indirect test (see Table 10-5).

Table 10-5. Bronchoprovocation Testing Categories

Nonselective	Selective
Direct Histamine Methacholine Prostaglandins Leukotrienes	**Immunologic** Allergen Occupational
Indirect (osmotic) Exercise EVH Cold air Nonisotonic aerosols (hypertonic saline) Mannitol **Indirect (nonosmotic)** AMP Propranolol Bradykinin	**Nonimmunologic** ASA NSAIDs Food additives

Abbreviations: ASA, aspirin; EVH, eucapnic voluntary hyperpnea (hyperventilation); NSAIDs, nonsteroidal anti-inflammatory drugs.

Direct Testing

Direct testing causes bronchoconstriction by **directly** activating contraction of smooth muscle cells after binding to receptors. It is highly sensitive and functions best when trying to **exclude** asthma with reasonable certainty. Direct testing has low specificity.

Methacholine Challenge Test (MCT)—analog of acetylcholine.

Important caveats for interpretation:

- Requires normal expiratory flow rates (such as FEV_1).
- Symptoms must be clinically current (in past few days).

Flash Card Q3

What conditions can cause a false-positive AHR?

Key Fact

A negative MCT or histamine challenge in the winter does **not** exclude seasonal pollen-induced asthma.

- Five percent to 15% of normal population can have a false-positive MCT.

Ipratropium bromide blocks methacholine secondary to anticholinergic mechanism of action. Effects of nonrespiratory medication with anticholinergic include β_2 agonists, antimuscarinics, some antidepressants, and antipsychotics.

Histamine—Correlates significantly with MCT results (Table 10-6). Exercise symptoms correlate better with MCT than histamine challenge.

Indirect Testing

Challenge of choice when exercise-induced bronchospasm (EIB) is suggested, or when attempting to differentiate asthma from chronic obstructive pulmonary disease (COPD). The response to inhaled corticosteroids is assessed with indirect tests. Also see Table 10-7.

Table 10-6. Interpretation of Methacholine Challenge Test Results

PC20 or Dose	Results
>16 mg/mL	Normal
4–16 mg/mL	Borderline
1–4 mg/mL	Mild AHR
0.25-1 mg/mL	Moderate AHR
< 0.25 mg/mL	Severe AHR

Abbreviations: AHR, airway hyper-responsiveness PC 20, provocation concentration (or dose) producing a 20% fall in FEV_1.

Flash Card A3

Allergic rhinitis, chronic obstructive pulmonary disease (COPD), congestive heart failure (CHF), cystic fibrosis (CF), siblings of asthmatics, upper respiratory infection (URI), and smoking

Table 10-7. Comparison of Methods

Method	Advantage	Disadvantage
Methacholine or histamine	Universally accepted	Not specific for asthma
Exercise	Highly specific for asthma	Lacks sensitivity; equipment
Cold air	Highly specific for asthma	Lacks sensitivity; equipment
Hypotonic	Highly specific for asthma	Lacks sensitivity
Hypertonic	Can do a dose response	Lacks sensitivity
EVH or hyperventilation	Highly specific for asthma	Lacks sensitivity

Abbreviation: EVH, eucapnic voluntary hyperpnea (hyperventilation);

Methods of Indirect Testing

In general, these testing methods have high specificity and low sensitivity.

- Exercise
  - Near-maximal exercise for 6 minutes breathing dry cool air, relative humidity less than 50% and less than 25°C through the mouth
  - Looking for fall in FEV_1 of 15%
- EVH or hyperventilation
  - Inhale dry air with 5% CO_2 for 6 minutes
  - Ten percent reduction considered positive
  - Similar to exercise challenge and requires less equipment. Can also be done when patient unable to exercise
- Nonisotonic aerosols
  - Hypertonic saline (4.5%) most common, but distilled water can also be used
  - Doubling dose achieved by doubling amount of time
  - Can also be used to induce sputum for inflammatory analysis. Pretreat asthmatic patients with bronchodilator (if only using for this purpose) and not to measure AHR.
- Cold air
  - Considered a modified-EVH challenge using cold, dry air
- Mannitol (dry powder)
  - Provides osmotic challenge to the airway mucosa, causing release of mast cell mediators
  - Mast cell mediators (e.g., prostaglandin 2 [PDG_2], leukotriene E4) cause bronchoconstriction
  - Rapidly inhaled in progressively increasing doses (0, 5, 10, 20, 40, 80, 160, 160, 160 mg). FEV_1 measured at baseline and 1 minute after each dose. If the FEV_1 decreases by 10% after a dose then that dose is repeated.

  - Fifteen percent fall in FEV_1 at a total cumulative dose of ≤ 635 mg (known as the provocative dose, or PD 15) is considered a positive response
  - Premeasured doses more convenient
  - Cough common side effect
- Adenosine or AMP
  - Causes bronchoconstriction through release of mediators from mast cells
  - AMP constriction 80% inhibited by terfenadine and astemizole
  - AMP constriction attenuated by cromolyn and nedocromil
  - Allergen avoidance reduces bronchial hyper-responsiveness (BHR) to AMP but not MCT
- Allergen
  - Induces bronchial smooth muscle constriction indirectly as a direct result of mast cell activation in the lung.
  - The best quality and best standardized allergen extracts should be used; and allergens for inhalation should be aqueous and not glycerinated (due to irritant effect).
  - Just as is seen in nasal provocation testing, some medications can interfere with the early and late airway response and have variable washout periods (Table 10-8).

Key Fact

Methach**O**line has a high sensitivity so is most useful to rule **O**ut asthma. Mann**I**tol has a high specificity, so if positive helps to rule-**I**n asthma, particularly exercise-induced.

Airway Responses to Allergen

Table 10-8. Drugs That Inhibit the Early and Late Asthmatic Response(LAR) to Allergens

Medication	Washout Period (Hours)	Inhibits EAR	Inhibits LAR
β_2-Adrenergic agonist	6–8	Yes	No
LABA	24	Yes	Masks
Anticholinergics	8–48	Yes	Yes
Antihistamine	48	Yes	No
Chromones	48	Yes	Yes
Inhaled corticosteroid, single dose	0	No	Yes (single dose after EAR)
Inhaled corticosteroid, regular use	7	Yes	Yes
Leukotriene receptor antagonists	7	Yes	Yes
Xanthenes		Yes (dose-related)	
Anti-IgE	?	Yes	Yes
Furosemide		Yes	Yes
Tricyclics		Yes	??

Abbreviations: EAR, early asthmatic response, LABA, long-acting β agonist; LAR, late asthmatic response.

Allergen-Induced AHR

Correlates with both late asthmatic response (LAR) and allergen-induced sputum eosinophilia. Direct AHR testing (MCT and histamine) is increased after allergen exposure.

Allergen-Induced Increase in (Eosinophilic) Airway Inflammation

Correlated to LAR and suspected to be causally related to AHR. Eosinophils, mast cells, and basophils are increased in sputum after allergen exposure.

Key Fact

The late sequelae are clinically more important than the early asthmatic response (EAR).

NASAL AND SPUTUM SMEARS

Nasal Smears (Table 10-9)

- Used for detection of nasal eosinophilia
- Most useful when the diagnosis of allergic rhinitis is in question (e.g., positive history, negative skin test)
- Collecting methods: Blown secretions, nasal lavage, nasal swab, nasal brushing

Sputum Smears

A sputum sample is material expelled from the respiratory passages (lungs, bronchi, and trachea) taken for laboratory analysis. It is **not** saliva. Bad sputum smears can lead to false results. See Table 10-10.

Table 10-9. Nasal Smears: The Quick and Dirty

If the dominant cell type seen is:	Then think of these:
Eosinophil	Allergic (>10% eosinophils) → if skin test negative, may prompt nasal or conjunctival challenge ASA-induced Nonallergic eosinophilic rhinitis (NARES) (>5–>20% eosinophils) → negative allergen challenge
Neutrophil	Irritant and nasopharyngitis or sinusitis (infectious—bacterial > viral) (low specificity)

Abbreviation: ASA, aspirin.

Table 10-10. Comparison of Asthma and Bronchitis Sputum

Possible Sputum Findings	Description	Asthma	Bronchitis
Charcot-Leyden crystals	Abnormal bipyramidal crystals of eosinophil lysophospholipase and metachromatic cells	Yes	No
Curschmann's spirals	Corkscrew-shaped twists of condensed mucus	Yes	No
Creola bodies	Multiple clumps of sloughed surface epithelial cells	Yes	No
Eosinophilia		Yes—mild and severe	No
Neutrophilia		Yes (more likely during exacerbation following viral URI or in severe asthmatics)	Yes
ECP and MBP		Yes	No

Abbreviations: ECP, eosinophilic cationic protein; MBP, major basic protein; URI, upper respiratory infection.

- **Sputum eosinophilia:** Can be seen in absence of AHR, but is also associated with development of persistent airflow limitation in adult asthmatics.
  - Eosinophil counts are increased in sputum of both mild and severe asthma; IL-5 is highest in mild asthma, but eosinophilic cationic protein (ECP) is highest in severe asthma.

MUCOCILIARY FUNCTION

Airway Mucus

Airway mucus is an important component of the innate immune system and consists of the following:

- **"Sol" layer:** Watery mixture in direct contact with airway epithelial cells. The cilia beat freely in the sol layer.
- **"Gel" layer:** Elastic layer in direct contact with inhaled air.
- **Protein constituents:** Mucin glycoproteins, proteoglycans, and a variety of other proteins important for host airway defenses.
  - **Mucin glycoproteins** are encoded by the mucin (MUC) genes. Their carbohydrate components bind surface adhesins or hemagglutinins on microorganisms, making it an important part of host defense and clearance. **The mucin gene (MUC5AC) expression is increased in asthmatics compared to normal controls.**

Mucociliary Clearance

- Coordinated activity of ciliated airway epithelial cells, resulting in movement of lung secretions toward the head
- Key cells:
  - **Goblet cell** mucus secretion appears to occur in response to environmental stimuli (e.g., smoke, ammonia, and nitric oxide) and inflammatory mediators (i.e., histamine, leukotrienes, and neutrophil elastase). Hyperplasia and metaplasia of goblet cells play important role in mucus hypersecretion.
  - **Clara cells** are nonciliated secretory cells, which are abundant in terminal bronchioles. May be precursor to goblet cell.
  - **Submucosal glands** contain short ciliated duct, a nonciliated collecting duct and secretory tubules lined by mucous and serous cells. Mucous cells produce more viscous secretion than serous cells. In response to sulfur dioxide or tobacco smoke, mucous cells form from serous cells.
- Requires optimal properties of mucus and normal functioning cilia

Nasal Mucociliary Clearance

- Measures of mucociliary clearance:
  - Aerosol of colloid albumin tagged with technetium-99m (^{99m}Tc), radioactivity within lungs measured for 2 hours and again at 24 hours
  - Saccharin deposited in inferior turbinate, measure time for taste (more simple, rapid, less reliable)
- If abnormal: Nasal brushing from inferior turbinate, microscope attached to a photometric cell to detect frequency of beating cilia (normal range 12–15 Hz)

Ciliary Structure (Figure10-4)

- Shaft anchored to the cytoplasm containing longitudinal fibrils composed of nine outer pairs of microtubules (doublets) and two central microtubules
- Microtubules composed of tubulin
- Dynein arms join adjacent doublets. Normal cilium shows an inner microtubule pair connected by radial spokes to nine outer microtubule doublets, which are connected by nexin. Outer doublets each carry an outer and inner dynein arm

Flash Card Q4

What is the name given to multiple clumps of sloughed surface epithelial cells that may be seen in asthmatics?

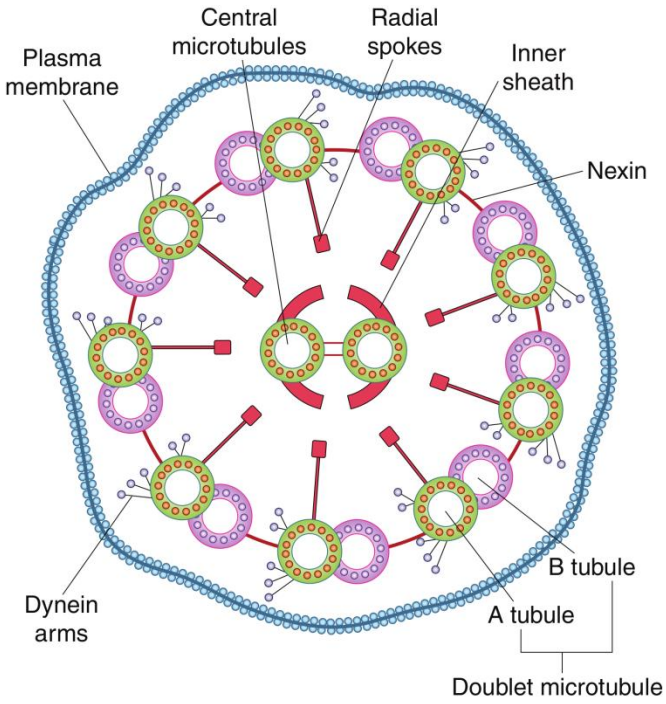


Figure 10-4. Ciliary structure.

DISORDERS OF MUCOCILIARY FUNCTION

Mucociliary Clearance and the Immune System

Infection and irritants can increase both mucus production and mucociliary clearance to augment airway defenses. When either is disturbed, such as in cases of recurrent otitis, sinusitis, rhinitis, bronchitis, and bronchiectasis, infections and irritants can have more significant effects. A comparison of mucus production and ciliary clearance is seen in Table 10-11.

Flash Card A4

Creola bodies

Table 10-11. Effects of Impaired Mucus Production or Ciliary Clearance

Mucus Production	Ciliary Clearance	Results in:	Example	Notes
High	Normal	Expectoration (overflow)	Asthma	↑ Expression mucin gene *MUC5AC* Mucus accumulation contributes to fatal attacks Mucin glycoproteins predominant proteins; ↑ albumin and DNA
Normal	Impaired/Low	Sludging	Primary ciliary dyskinesia (Kartagener's syndrome: Situs inversus in 50% of patients)	Lack of dynein arms, defective ciliary spokes (loss of synchronized ciliary movement)
Abnormal quality	Normal	Accumulation	Cystic fibrosis	*CFTR* gene defect causes ↓ serous cell secretion of water, producing thick immobile mucus

Key Fact

Primary ciliary dyskinesia (PCD) patients have fewer airway infections than those with cystic fibrosis (CF). This suggests that, in mucus hypersecretory states with chronic bacterial infections, the altered mucus plays a larger role in disease progression than ciliary movement.

SEROLOGIC TESTS

MEASUREMENT OF ANTIGENS AND ANTIBODIES

Serum Immunoglobulins (Igs)

Methods include radial immunodiffusion (RID), nephelometry, and turbidimetry.

Rate nephelometry is the most common method used for quantifying IgG, IgA, IgM, and inflammatory markers in serum (e.g., C-reactive protein [CRP], haptoglobin, C3, and C4). It is based on light-scatter measurement, which depends on the formation of antibody-antigen complexes in the test solution. It is done by adding variable concentrations of antigen to a fixed amount of antibody in a spectrophotometric reaction cell.

Turbidimetry measures the decrease in amount of light penetrance, which correlates with analyte (Ig) concentration. Its application is similar to nephelometry.

Due to the small serum concentrations of IgE and IgD, they are measured using an enzyme-linked immunosorbent assay (ELISA) or radioimmunoassay (RIA).

Flash Card Q5

In which condition is there a lack of dynein arms and loss of ciliary spokes?

ELISA—Method of detecting antibody (Ab) or antigen (Ag). Sandwich ELISA is a type of ELISA technique in which the antigen or antibody in question is sandwiched between two proteins, either an Ag and Ab or an Ab and Ab. The steps of a sandwich ELISA (Figure 10-5) are as follows:

- Plate is coated with a capture Ab.
- Sample is added, and any Ag present binds to capture Ab.
- Detecting Ab is added and binds to Ag.
- Enzyme-linked secondary Ab is added and binds to detecting Ab.
- Substrate is added, and it is converted by enzyme to detectable form that is measured with spectrophotometry for quantification.

Examples of a sandwich ELISA include ELISA screening test for anti-HIV antibodies, total IgE measurement, and C1q and C1 inhibitor levels.

Serum Protein Electrophoresis (SPEP)—Separates proteins using an electric field on agarose gel. SPEP measures the general distribution of serum proteins (Figure 10-6). The two main fractions of serum proteins are albumin and globulins. These are further subdivided as follows:

- **Albumin:** The major fraction, comprising more than half of serum proteins
- **A fraction:** These bands can be increased by acute-phase reactants:
  - A-1 globulin fraction includes α_1-antitrypsin, thyroid-binding globulin, transcortin, and HDL cholesterol.
  - A-2 globulin fraction includes ceruloplasmin, α_2-macroglobulin, and haptoglobulin.
- **B fraction:** IgA, IgM, sometimes IgG, and complement proteins can be identified in the β fraction. CRP is found between the β and γ components.
  - B-1 fraction composed mostly of transferrin
  - B-2 fraction contains β-lipoproteins

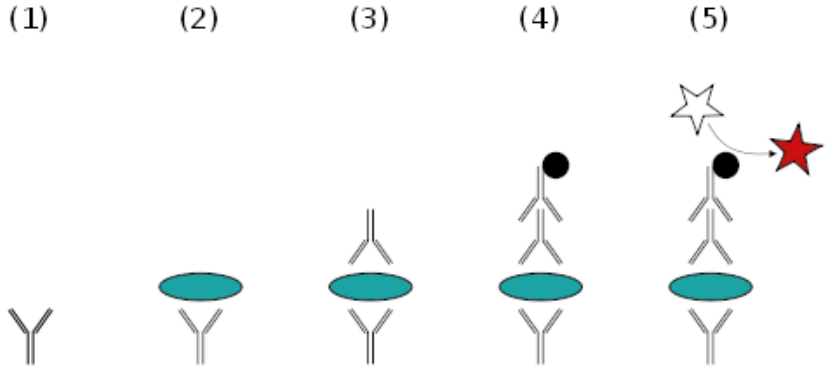


Figure 10-5. Sandwich ELISA.
(Reproduced, with permission, from Wikimedia Commons.)

Flash Card A5
Primary ciliary dyskinesia (PCD)

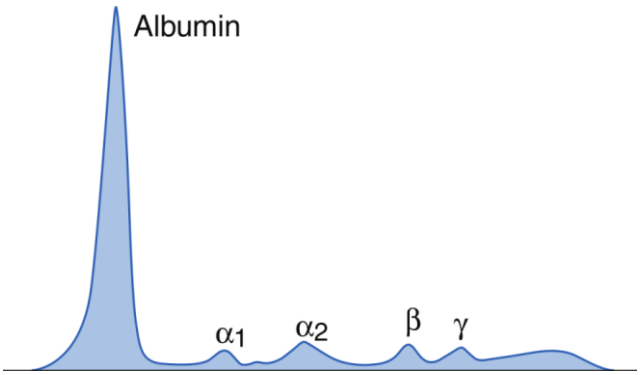


Figure 10-6. Normal serum protein electrophoresis.

- **γ Fraction:** Much of the clinical interest is focused in this since immunoglobulins migrate to this region. Thus, SPEPs (and electrophoresis of urine samples [UPEPs]) are often used to screen for conditions such as multiple myeloma (MM), Waldenström's macroglobulinemia, and amyloidosis, which cause abnormalities in this region (Figures 10-7 and 10-8).

Immunofixation Electrophoresis (IFE)—Useful for the identification and quantification of monoclonal paraproteins seen on an SPEP. This procedure identifies which immunoglobulin isotype causes an "M" spike when it is present. It can also determine which light chain (i.e., κ or λ) is present. After the SPEP is run and the monoclonal bands are identified, they are exposed to specific antiserum to detect specific antibodies (Figure10-9).

Western Blot—Modified ELISA. Proteins to be assayed are separated by electrophoresis and then transferred to nitrocellulose membrane. Serum with antibodies is added, and excess antibody is washed off. Enzyme-conjugated antiantibody is added, and again excess antibody is washed off. Finally, a substrate to the enzyme is added, and the color or chemiluminescence is detected. **One use of this test is to confirm HIV infection**.

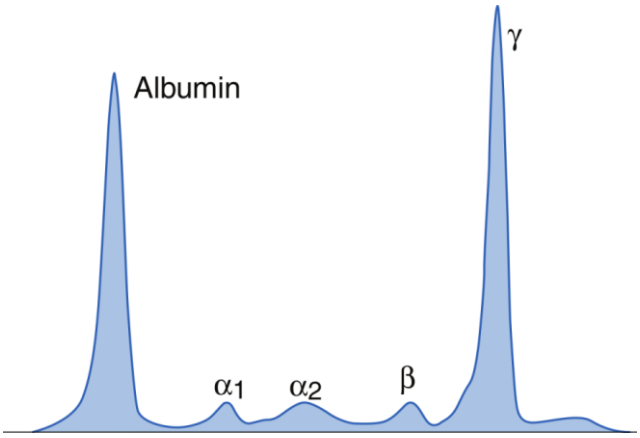


Figure 10-7. M-shaped spike serum protein electrophoresis.

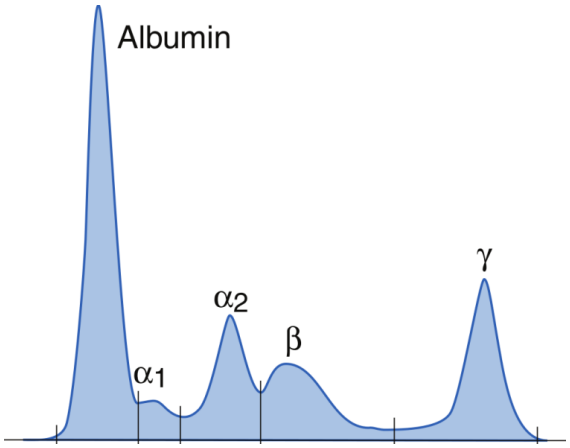


Figure 10-8. Serum protein electrophoresis showing γ- and α2 spike.

Key Fact

Antinuclear antibody (ANA) is a sensitive but not specific test. It is not a good screening test in the absence of significant clinical suspicion (high false-positive rate). But, in high titers, ANA may point toward an autoimmune disorder.

Allergen-Specific IgE Measurement—Most often done as an automated fluoroenzyme immunoassay (indirect ELISA). Allergen is bound covalently to a solid phase, which binds all allergen-specific IgE. An enzyme labeled "anti-IgE" is added and incubated with developing agent, and the fluorescence is then measured.

Radioallergosorbent test (RAST)—Radioimmunoassay for detecting antibodies against antigens (allergens) adsorbed on solid-phase support. **Note:** This is an older method, using radioisotopes to detect specific IgE. It is no longer in common clinical use; thus, the term "RAST" is obsolete.

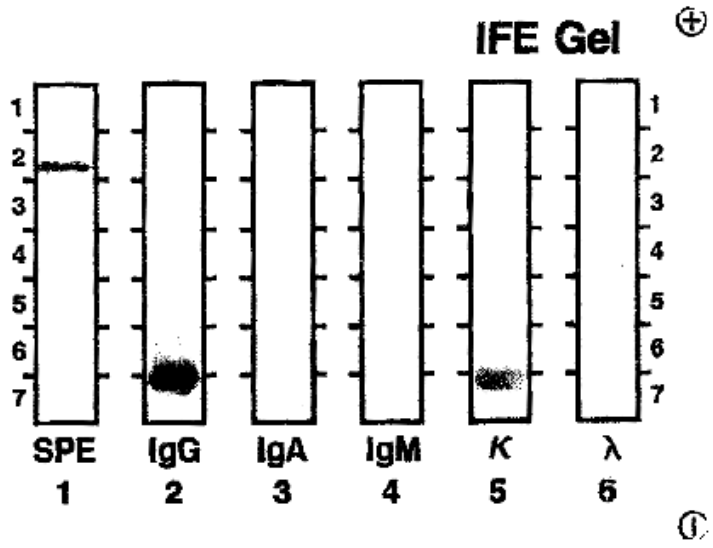


Figure 10-9. Immunofixation electrophoresis showing IgG and κ bands.

Functional Complement Tests

CH50—Function of classical pathway is measured using sheep erythrocytes (E) plus rabbit antibody (A). A serial dilution of patient serum is mixed with EA suspension, which results in lysis of RBC and release of hemoglobin. This is measured by spectrophotometer. The dilution of serum to lyse 50% of RBCs of EA cells is the quantitative CH50 unit.

CH50 measures functional activity of the components of the **classical complement pathway**. This includes C1qrs, C2, C4, C3, C5, C6, C7, C8, and C9.

AH50—Measures functional activity of the **alternative complement pathway**. Thus is a screen for deficiencies in factors D, B, C3 properdin, C5, C6, C7, C8, and C9.

SEROLOGIC TESTS FOR AUTOIMMUNITY

Serologic tests are an important tool in the diagnosis of autoimmune conditions (Table 10-12).

Table 10-12. Serologic Tests in the Diagnosis of Autoimmune Conditions

Disease	Autoantibody
Systemic autoimmune disorders	
Neonatal lupus	Anti-SSA or Ro and anti-SSB or La
Systemic lupus erythematosus (SLE)	**ANA, anti-dsDNA, and anti-Smith**
Drug-induced lupus	Antihistone
Rheumatoid arthritis	**RF and anti-CCP**
Juvenile inflammatory arthritis	RF and ANA
Antiphospholipid syndrome	Lupus anticoagulant, anticardiolipin IgG IgM, anti-b2GPI, and ANA
Sjögren's syndrome	ANA, anti-SSA, and anti-SSB
Systemic sclerosis (diffuse)	Anticentromere, antitopoisomerase I, anti-RNA polymerase I or III, and antifibrillarin
CREST (limited scleroderma)	Anticentromere
Polymyositis	ANA, anti-Jo 1, antisignal-recognition particle antibodies, and anti-PM-Scl antibodies
Dermatomyositis	ANA, anti-Jo 1, antisignal-recognition particle antibodies, and anti-PM-Scl antibodies

Flash Card Q6

The disease granulomatosis with polyangiitis is also known as what?

Table 10-12. Serologic Tests in the Diagnosis of Autoimmune Conditions, cont.

Disease	Autoantibody
Immunologic GI disorders	
Autoimmune hepatitis-1 (AIH)	ANA, SMA(anti-actin), and ANCA(p-ANCA)
Autoimmune hepatitis-2	Anti-LKM-1(liver kidney microsomes)
Primary biliary cirrhosis (PBC)	Antimitochondrial antibodies (AMA, very specific), anti-M2-subset of AMA, anti-sp100, and anti-PML(promyelocytic leukemia)
Primary sclerosing cholangitis	p-ANCA, SMA, AMA, and ANA
Crohn's disease	Anti-*Saccharomyces cerevisiae* (ASCA)
Ulcerative colitis	p-ANCA
Immunologic renal disease	
Granulomatosis with polyangiitis (GPA) (Wegener's granulomatosis)	c-ANCA (proteinase 3 = PR3 and sensitivity 90%), p-ANCA (myeloperoxidase = MPO) can also be positive
Goodpasture’s syndrome	Antiglomerular basement membrane (GBM) and ANCA
Immunologic neuropathies	
Guillain-Barré syndrome	Antiganglioside antibodies
Myasthenia gravis	Antiacetylcholine receptor
Chronic inflammatory demyelinating polyneuropathy	Antiganglioside antibodies
Vasculitis	
Hypocomplementemic urticarial vasculitis	ANA (low c3 and c4)
Hemolytic uremic syndrome	Factor H autoantibodies
Polyarteritis nodosa	No identified autoantibody. Presence of increased immunoglobulins.
Eosinophilic granulomatosis with polyangiitis (EGPA) (Churg-Strauss syndrome)	p-ANCA (MPO)
Microscopic polyangiitis	p-ANCA (MPO) in 60% of patients and c-ANCA (PR3) in 40%
Autoimmune endocrine disorders	
Diabetes mellitus type 1	Islet cell antibodies, insulin autoantibodies, and anti-glutamic acid decarboxylase (GAD)
Grave's disease	Thyroid peroxidase antibody (TPO), thyroid receptor antibody (TRSAb), and thyroid-stimulating immunoglobulin (TSI)
Hashimoto's thyroiditis	TPO and anti-thyroglobulin antibody
Addison's disease (chronic adrenal insufficiency)	Antiadrenal cytoplasmic antibodies(CYP21)-cytoplasmic antibody

Flash Card A6

Wegener's granulomatosis

Table 10-12. Serologic Tests in the Diagnosis of Autoimmune Conditions, cont.

Disease	Autoantibody
Immune hematologic disorders	
Autoimmune hemolytic anemia	Positive direct antiglobulin (Coombs' test)
Paroxysmal cold hemoglobinuria (PCH)	Ig antibody against P antigen (anti-P)
Autoimmune thrombocytopenia	Antiplatelet antibodies
Autoimmune neutropenia	Antineutrophil antibody-HNA-1 or HNA-2
Neonatal autoimmune neutropenia (NAIN)	Anti-HNA-1a and anti-HNA-1b-antineutrophil antibody
Immune skin disorders	
Pemphigus vulgaris	Antidesmoglein-3 antibodies
Pemphigus foliaceous	Antidesmoglein-1 antibodies
Linear IgA disease	IgA antibody against basement membrane zone (BMZ)
Pemphigoid	IgG antibody against BMZ—BP230 and BP180, and hemidesmosomal antigens
Dermatitis herpetiformis	BMZ IgA autoantibodies
Epidermolysis bullosa acquisita	Anticollagen type VII

Abbreviations: ANA, antinuclear antibody; c-ANCA, cytoplasmic antineutrophil cytoplasmic antibodies; p-ANCA, perinuclear antineutrophil cytoplasmic antibodies.

MOLECULAR DIAGNOSTICS AND TISSUE TYPING

MOLECULAR TECHNIQUES

Polymerase Chain Reaction (PCR)

PCR is an enzyme-driven technique for rapidly amplifying short regions of DNA in vitro. It relies on knowing at least partial sequences of the target DNA and uses this knowledge to design oligonucleotide primers that hybridize to the target DNA sequence.

Flash Card Q7

What autoantibody is associated with pemphigus vulgaris?

Key Fact

In patients with defects in antibody production (e.g., common variable immunodeficiency [CVID], X-linked agammaglobulinemia [XLA]) or on intravenous immunoglobulin (IVIG) replacement, antibody testing for infections is unreliable, and direct PCR methods are preferred.

Examples of PCR assays used for pathogen detection approved by the FDA:

- Human immunodeficiency virus (HIV)
- Hepatitis C virus (HCV)
- Cytomegalovirus (CMV)
- Groups A and B *Streptococcus*
- *Mycobacterium tuberculosis*
- *Chlamydia trachomatis*
- *Neisseria gonorrhoeae*

Over the past two decades, variations in the PCR technique have been developed that have increased its versatility, including the following.

Reverse Transcriptase PCR (RT-PCR)—Used to generate copies of RNA sequences. The RNA sequence is first converted to a double-stranded complementary DNA (cDNA) utilizing a viral reverse transcriptase enzyme. A large number of copies of the newly formed DNA are then generated as described earlier. RT-PCR may be used for detection of RNA viruses such as HIV and hepatitis C.

Quantitative PCR (qPCR) or Real-Time PCR—qPCR may be used to quantify residual disease following bone marrow transplantation or after therapy for chronic myelogenous leukemia (CML).

Sequencing-Based Technologies

The original goal of DNA-sequencing technology was genomic sequencing. It has also been used to identify organisms that cannot be cultured or those that are difficult to identify using conventional lab techniques. The Sanger technique was the most often used DNA-sequencing technique. However, in recent years, there has been a shift from the automated Sanger technique, now considered first generation, to newer methods that are often referred to as **next-generation sequencing**. Next-generation sequencing technologies may be utilized for:

- Genome-wide characterization and profiling of mRNAs
- Transcription factor regions
- Structure of chromatin
- DNA methylation patterns
- Metagenetics

Flash Card A7

Antidesmoglein-3 antibodies

Direct Hybridization Assays

Table 10-13 summarizes the four direct hybridization assays.

Southern Blot—Technique used to detect translocations, deletions and duplications of relatively large areas of the genome such as whole chromosome arms.

- DNA is extracted from tissue, purified and enzymatically fragmented by appropriate restriction endonucleases.
- The fragmented strands are electrophoresed on an agarose gel according to size.
- DNA fragments are blotted onto a nylon filter and immobilized/fixed on the membrane.
- DNA sequences are then hybridized with a probe (radiolabeled), single-stranded DNA sequence complimentary to the blot-transferred DNA sequence.
- The pattern of hybridization is then visualized on radiographic film.

Northern Blot—Technique used to detect specific RNA sequences and their size, and the expression profiles of tissue-specific genes. Although highly specific, the Northern blot is semiquantitative and rarely used in clinical applications. The technique may be divided into three steps:

- Electrophoresis of RNA under denaturing conditions in an agarose or formaldehyde gel
- Transfer of RNA from the gel to a nylon or nitrocellulose membrane
- Hybridization and analysis of the RNA sequences of interest using a labeled DNA or RNA probe

Table 10-13. Direct Hybridization Assays

Technique	Mechanism	Example
Southern blot	DNA/DNA analysis	Gold standard for T-cell clonally in detection of lymphoma and leukemia
Northern blot	RNA/DNA analysis	Observe a particular gene's expression pattern between tissues, other techniques often used more often clinically
Western blot	Gel electrophoresis of proteins	Diagnosis of HIV infection and Lyme disease
Fluorescent in situ hybridization	Fluorescence-labeled oligonucleotide (DNA) probes	DiGeorge's syndrome (22q11.2)

Fluorescent in Situ Hybridization (FISH)—FISH is used to detect the presence or absence of specific DNA sequences (duplications, deletions, and translocations). FISH uses fluorescence-labeled oligonucleotide probes that attach to their complementary sequences in the DNA strand contained within the cells. The three most commonly used types of probes are (1) painting probes that identify an entire chromosome, (2) centromeric probes that recognize the centromeric regions, and (3) the allele-specific probe that adheres to a specific target allele sequence. The oligonucleotide-labeled DNA region may then be visualized under a florescence microscope.

FISH has advantages over conventional cytogenetics in the evaluation of individuals with chromosomal deletions and translocations, and in gene amplifications. Unlike conventional cytogenetic techniques, FISH may be performed on cells that are dividing (in metaphase) or resting (in interphase). Another advantage is that FISH may be performed on fresh frozen specimens, archival smears, and paraffin-embedded tissue sections. Diseases that may be diagnosed using FISH include:

- Prader-Willi syndrome (PWS)
- Angelman syndrome (AS)
- 22q11.2 deletion
- Cri-du-chat
- Down syndrome
- Acute lymphoblastic leukemia (ALL)

Array-Based Technologies

Array-based technologies may be macro- or microarrays. Microarrays are miniaturized versions of macroarrays, with spot sizes less than 200–300 μm in diameter. Microarray has evolved from RNA and DNA blot assays and ELISA. The principle behind microarrays is the hybridization of complementary nucleotides base pairs contained in two DNA strands. The first strand, the **probe**, of known DNA sequences is fixed to a support (e.g., glass, silicone, or polystyrene beads). The second DNA strand contains sequences that are labeled with fluorescent molecules or dyes for generation of a detectable signal. Microarrays may detect DNA or RNA, the latter usually as cDNA produced by reverse transcription.

Microarrays may be employed for gene expression profiling, such as finding the relative difference in gene expression between normal and cancer cells. Microarrays may be used to detect:

- Single-nucleotide polymorphisms (SNPs)
- Chromosomal abnormalities such as in Down syndrome
- Genotype mutations, which result in monogenetic diseases such as ataxic telangiectasia

TISSUE TYPING

Serologic, Cellular, and DNA Techniques in HLA Typing

A variety of techniques may be used for human leukocyte antigen (HLA) typing with the goal of increasing the success of human transplant. Thus far, three techniques have been used for HLA typing: serologic, cellular, and the currently more popular DNA techniques.

Serologic techniques consist of variations of complement-dependent cytotoxicity (CDC) assays. They are easy to perform, do not require expensive equipment, and produce reliable results. However, they require viable lymphocytes and a relatively large volume of blood.

Cellular typing methods such as mixed lymphocyte reaction (MLR) are labor-intensive, take a long time to perform, and usually employ radioactive isotopes with the consequent problems of radioactivity.

DNA techniques have superseded serologic and cellular techniques. They are more time-efficient and robust and have become the technique of choice in most laboratories. The currently used DNA technique for HLA typing has an element of PCR technique within it. The technique allows for more precise HLA typing than serologic or cellular methods. The reagents, the primers, probes, and thermostable DNA polymerases utilized in PCR-based HLA typing are usually standardized. Unlike serologic methods, PCR amplification allows for HLA typing of minute samples; and, viability of the cell or expression of relevant HLA antigen is not required.

Histocompatibility Testing (HCT)

Approximately 70% of individuals who require hematopoietic stem cell transplant (HSCT) may not have an available HLA-identical sibling donor. Current evidence validates the importance of matching an unrelated donor to the recipient for HLA alleles. The accepted criteria for a HCT from an unrelated donor are based on compatibility of **class I HLA A, B and, C, and class II DRB1 and DQB1 alleles**. Matched alleles at all five loci (10/10 matched “perfect match”) appear to reduce the risk of clinically severe acute graft-versus-host disease (GVHD). Amongst the classic major histocompatibilty complex (MHC) genes, HLA-DQB1 mismatching is usually considered permissible. Therefore, HCT outcome from 8/8 matched donor at HLA A, B, and C and DRB1 is associated with an equally good outcome as 10/10 matched.

Optimal matching criteria for solid-organ grafts differ from HCT. Matching for solid-organ grafts usually includes HLA-A, -B, and -DR. Donors and recipients matched at all three loci are termed 6/6. In renal grafts, the majority of HLA alloantibodies formed by renal graft recipients are directed toward cross-reactive antigen groups (CREGs), which are shared determinants between class I HLA antigens. Therefore, rather than matching for a unique variant of an allele, which is infrequently identified, the HLA class I molecules may be matched for CREGs.

Donors of heart, liver, lung, and pancreatic grafts are not usually matched for HLA type. However, HLA -A, -B, and -DR matching has a significant influence on the outcome of patients receiving their first heart transplant. Serum antibody screens may be performed to identify patients at risk for graft rejection.

HLA typing may also be used to monitor chimerism following allogenic stem cell transplantation, to determine clonality and characterize neoplastic disorders, and to establish gene mutation.

Panel Reactive Antibody

Preformed antibodies present in recipients against donor HLA antigens lead to hyperacute or accelerated acute antibody-mediated rejection. Therefore, it is essential to exclude these antibodies. These antibodies result from prior blood transfusions, previous grafts, or from sensitization during pregnancy. Their presence may be detected by testing the recipient's serum against cells that contain a panel of well-defined HLA antigens (direct complement-dependent cytotoxicity or antiglobulin-augmented cytotoxicity assays) or against solubilized HLA antigens attached to solid support (ELISA and microparticle-based flow cytometric assays). These tests estimate the panel reactive antibodies or the percentage of likely crossmatched incompatible donors (Table 10-14.)

Detection of these HLA antibodies is essential in renal transplant and is widely practiced in lung or heart transplant. However, they do not appear to be involved in the rejection of liver grafts. Their role in allogenic HCT is less clear.

Mixed Lymphocyte Reaction (MLR)

MLR is used to predict T-lymphocyte-mediated graft rejection of tissue allografts. It is an in vitro technique utilized to assay T-lymphocyte proliferation in response to foreign MHC molecules. The mononuclear leukocytes (T lymphocytes, B lymphocytes, natural killer (NK) cells, dendritic cells, and mononuclear phagocytes) obtained from peripheral blood of one individual are cultured with mononuclear leukocytes obtained from peripheral blood of another individual. In this culture, cells from both individuals may proliferate in response to each other's' HLA molecules. To circumvent this, a one-way mixed-lymphocyte

Table 10-14. The Perfect Donor

	HLA-A	HLA-B	HLA-C	HLA-DRB1	HLA-DQB1
Recipient	2501 6801	1801 4002	0308 1203	0407 0411	0301 0302
Donor 1	2501 6801	1801 4002	***0304*** 1203	0407 ***1501***	***0602*** 0302
Donor 2	2501 6801	1801 4002	***0304*** 1203	0407 ***1501***	***0602*** 0302
Donor 3	2501 6801	1801 4002	***0306*** 1203	0407 ***1501***	***0602*** 0302
Donor 4	2501 6801	1801 4002	0308 1203	0407 0411	0301 0302

Each four-digit number represents a locus. For example, the numbers 2501 and 6801 represent the two loci for HLA A. Donor 4 is a perfect match for the recipient because HLA-A, -B, -C, -DRB1, and -DQB1 are matched (i.e., 10/10). Donor 1, 2, and 3 are each matched at 7/10.
Abbreviation: HLA, human leukocyte antigen.

reaction is employed. In this technique, mononuclear leukocyte population from one individual is rendered mitotically inactive by γ-irradiation or mitomycin C treatment. These irradiated or treated cells now function as stimulators, and the unirradiated or untreated cells serve as the responder cell population. If the unirradiated cells contain alloreactive T lymphocytes, these will proliferate and differentiate into effector cells. The proliferated cells are mainly CD4+ T lymphocytes, which recognize **differences in MHC class II molecules.**

IMAGING

SINUSES

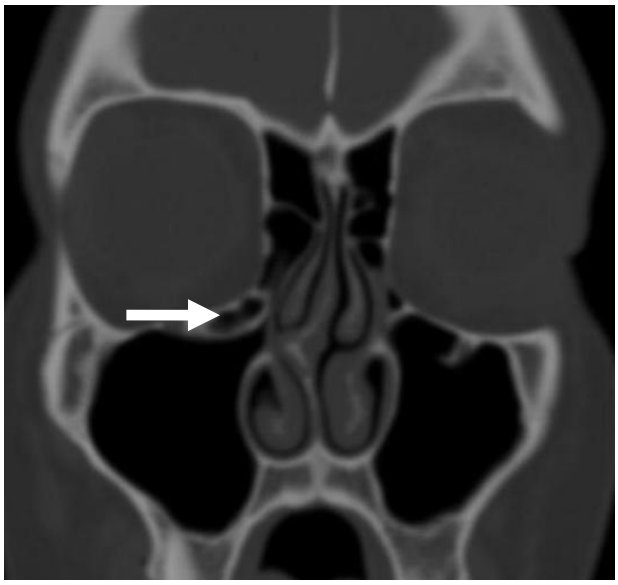
Radiographs

- Fast, relatively cheap
- Not reliable
- Can only reliably evaluate lower third of nasal cavity, maxillary, front, sphenoid, and posterior ethmoid sinuses
- Often obscured: Anterior ethmoid cells, upper two thirds of nasal cavity, middle meatus, frontal recess

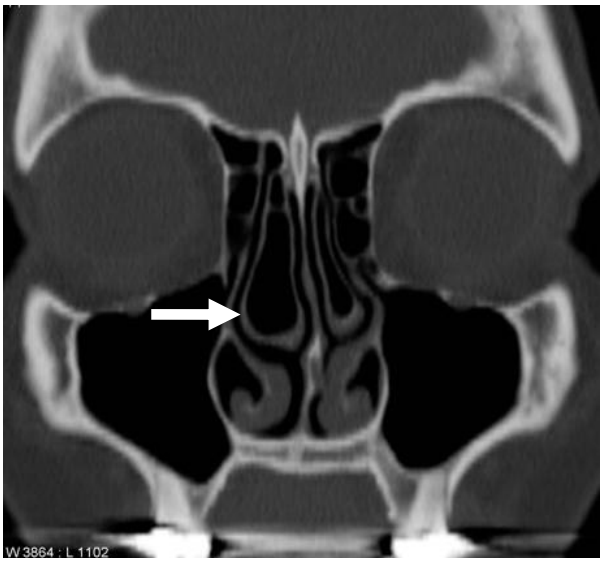
Computed Tomography (CT)

- CT allows for coronal, sagittal, and axial views
- Sphenoid sinus: Close proximity to carotid artery and optic nerve

The CT scans in Figures 10-10 and 10-11 show anatomic variants or abnormalities.

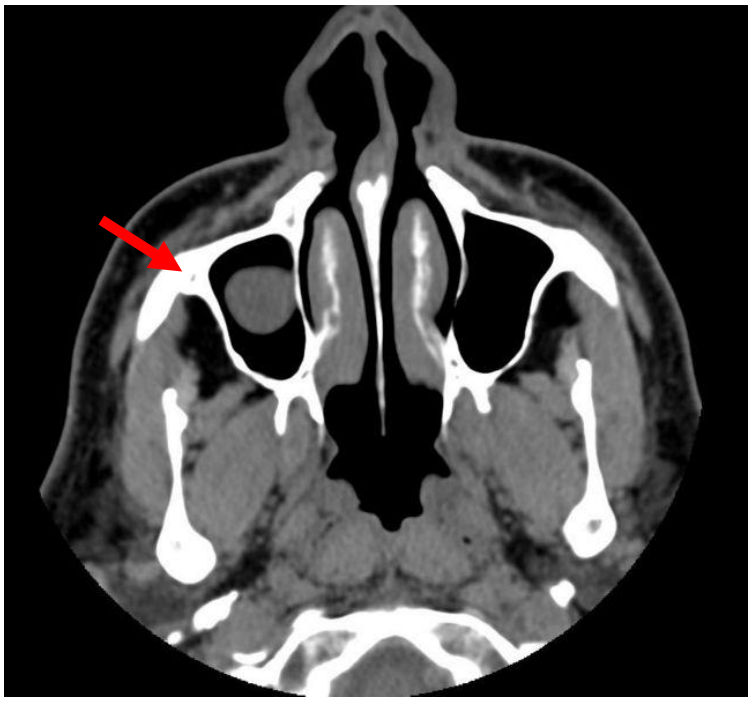


A



B

Figure 10-10. (A) CT scan of the Haller cell (arrow). Ethmoid cell that migrates to superior roof of maxillary sinus. (B) CT scan of concha bullosa (arrow). Ethmoid cell migrates into middle turbinate.
(Figure A reproduced, with permission, from Radswiki.Net.; Figure B reproduced, with permission, from Radiopaedia)



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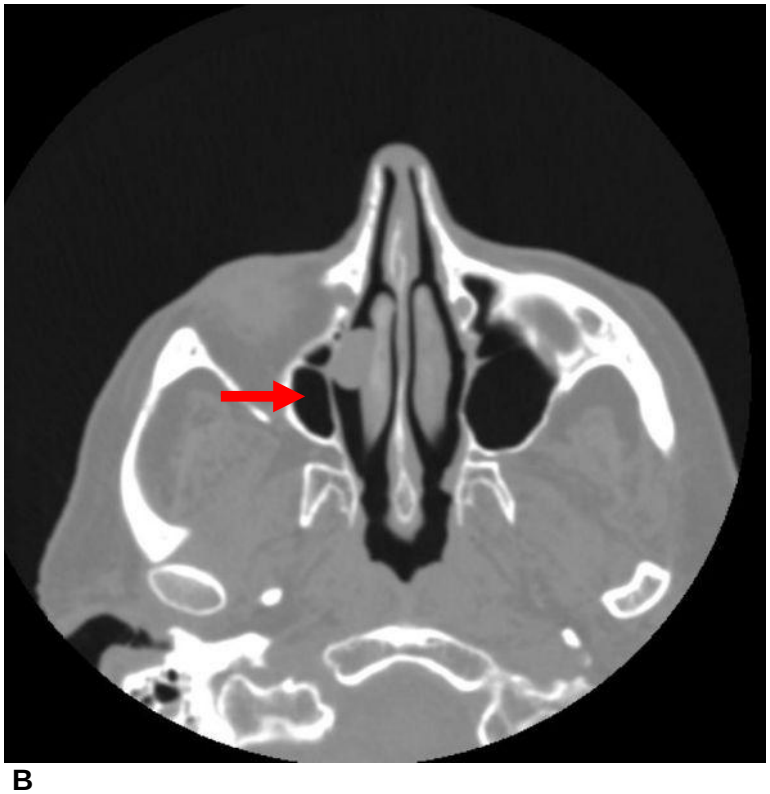

B

Figure 10-11. (A) CT scan shows mucous retention cysts (arrow). (B) CT scan shows nasal polyps (arrow).
(Reproduced, with permission, from Radswiki.net.)

Flash Card Q8

What is the commonly used term for a pneumatized middle turbinate?

Magnetic Resonance Imaging (MRI)

MRI have the advantage of having no radiation and providing improved soft tissue contrast.

***Lung Diseases*—**Table 10-15 describes common immunologic lung diseases and their classic chest CT appearance.

Table 10-15. Immunologic Lung Diseases

Immunologic Lung Disease	Classic Chest CT Appearance
Wegener's granulomatosis	Multiple nodules without cavitation; peripheral wedge-shaped consolidation
Asthma	Bronchial wall thickening; air trapping on expiratory HRCT
Allergic bronchopulmonary aspergillosis (ABPA)	Central bronchiectasis (Figure 10-12)
Chronic eosinophilic pneumonia	Peripheral consolidation, ground-glass attenuation in middle and upper lung zones
Acute hypersensitivity pneumonitis	Centrilobular ground-glass and nodular opacities
Churg-Strauss syndrome	Peripheral consolidation or ground-glass attenuation; 30% pleural effusions
Granulomatous and lymphocytic interstitial lung disease (GLILD) in patients with CVID	Nodular opacities, linear and/or irregular opacities, pulmonary consolidations, ground-glass opacities, honeycombing, and bronchiectasis

Abbreviations: CVID, common variable immunodeficiency. HRCT, hematopoietic stem cell transplantation.

Flash Card A8

Concha bullosa

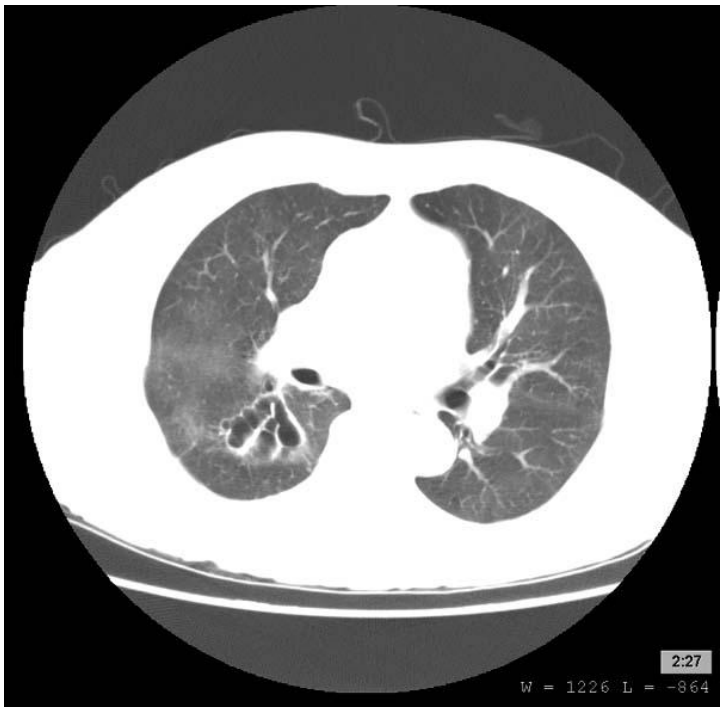

Figure 10-12. Allergic bronchopulmonary aspergillosis (ABPA) and central bronchiectasis.
(Reproduced, with permission , from Radswiki.net)

Miscellaneous Imaging Findings

- **Complete DiGeorge's syndrome** thymic shadow on chest film
- **Hyper-IgE syndrome** characteristic pneumatocele on chest film

Flash Card Q9

What radiographic finding is a diagnostic feature of ABPA?

Flash Card Q10

What characteristic radiographic finding might be seen in a patient with autosomal dominant hyper-IgE syndrome?

FLOW CYTOMETRY AND CELL SURFACE MARKERS

Flow cytometry is widely used as an important screening tool for the enumeration of cells expressing distinct cell surface molecules. These markers can identify the presence or absence of cellular populations and subpopulations through the binding of specific monoclonal antibodies carrying a fluorescent tag.

FLOW CYTOMETRY

Principle

Key Fact

Forward scatter: Size

Side scatter : Granularity and complexity

After being incubated with fluorochrome-labeled monoclonal antibodies, cells are suspended in a stream of fluid and passed one at a time though the light source, generating nonfluorescent scatter and fluorescent light emissions (Figure 10-13). All data from each cell can be collected simultaneously, both physical and chemical characteristics, up to several thousand particles per second. Two types of scatter—**forward** scatter (FSC), which detects **size,** and **side** scatter (SSC), which determines **granularity** and structural **complexity** of the cells—allow discrimination of lymphocytes, monocytes, and granulocyte (Figure 10-14). Fluorescent emissions are used to identify the subpopulation of interest based on expression of cellular markers or proteins.

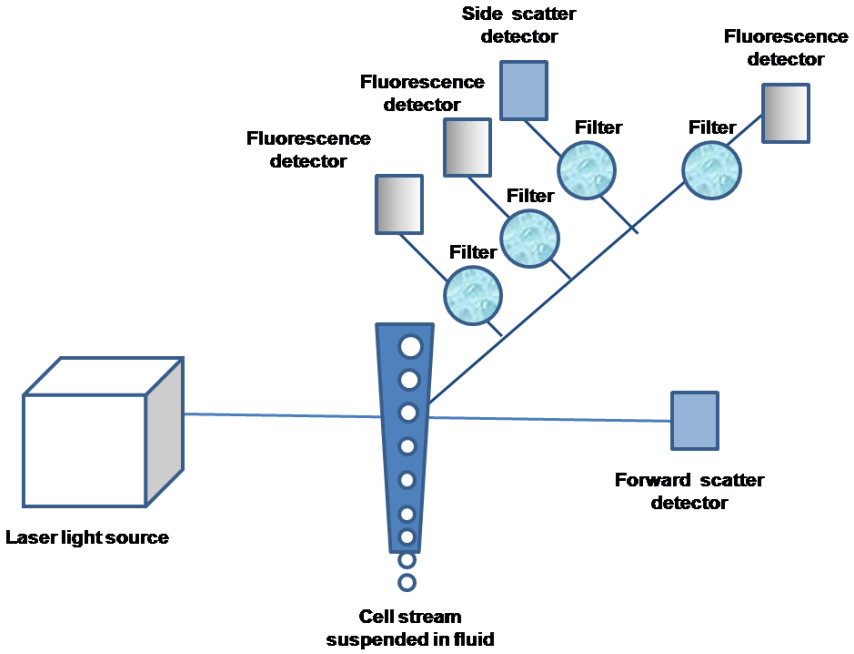

Figure 10-13. Flow cytometry schematic.

Flash Card A9

Central bronchiectasis

Flash Card A10

Pneumatocele

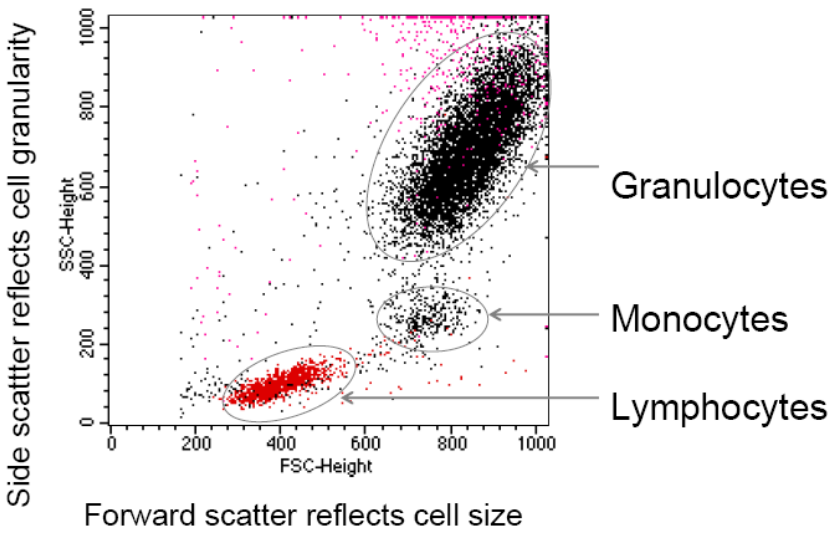

Figure 10-14. Flow cytometry pattern of peripheral whole blood.

Detection

Most extracellular surface proteins can be detected by flow cytometry (e.g., cluster of differentiation (CD) markers, Toll-like receptor (TLR) ligands, cell receptors). Intracellular flow cytometry can also be used to assess other cellular functions, such as neutrophil oxidative burst, calcium reflux, phosphorylation and cytokine production.

Data Display

Software collates and displays the aggregated data for analysis. Each cell, or event, is plotted based on scatter and fluorescent intensity. Data are commonly presented as dot plot, contour plot, and histogram and reported in percentages.

Clinical Applications

Flow cytometry is commonly used for diagnosing primary immunodeficiency disease (PID) and hematologic malignancies by studying blood or tissue samples. Examples include assessing cellular activation and intracellular cytokine production and for studying cell cycle and DNA ploidy. Although data from flow cytometry can be highly suggestive of the disease, most PIDs still require a molecular analysis to confirm the diagnosis.

CELL SURFACE MARKERS

Key Fact

B cells and NK cells are CD3–.

The CD system is commonly used as cell markers; this allows cells to be defined based on what molecules are present on their surface. These CD molecules often serve as functional receptors, ligands, or are involved in cell adhesion. We can identify each immune cell type using the combination of their CD markers. Commonly used markers are shown in Table 10-16.

Table 10-16. Commonly Used Lymphocyte Surface Markers

Cell Subpopulation	Surface Markers
Stem cell	CD34+
WBC	CD45+
Granulocyte	CD45+ CD15+
Monocyte	CD45+ CD14+
T cell	CD45+ CD2+ or CD3+
T helper	CD3+ CD4+
CTL	CD3+ CD8+
Treg	CD3+ CD25+ Foxp3+
B cell	CD45+ CD3– CD19+ or CD20+
Naïve mature B cell	CD19+ CD27– surface IgM (sIgM)+
Mature B cell	CD19+ CD20– sIgG+
Memory B cell	CD19+ CD27+ sIgM+
Switched memory B cell	CD19+ CD27+ sIgM–
	Note: Majority of B cells in CLL are CD5+
NK cell	CD45+ CD3– CD16+ or CD56+
NKT cell	CD45+ CD3+ CD16+ or CD56+
Platelet	CD45+ CD61+

Abbreviations: CLL, chronic lymphocytic leukemia; CTL, cytotoxic T lymphocyte; NK, natural killer cell; NKT, natural killer T cell.

Flow Cytometry Study in PID

Applications of flow cytometry for the study of PID are shown in these following samples (Figures 10-15 through 10-18).

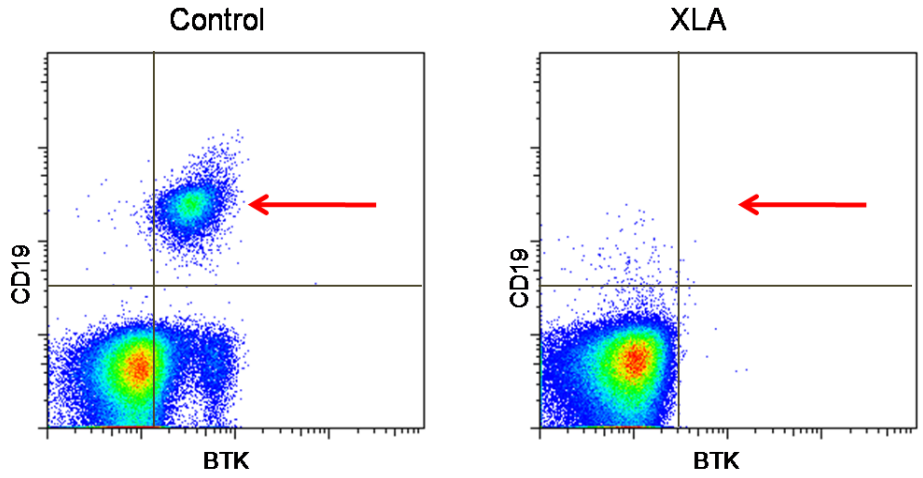

Figure 10-15. X-linked agammaglobulinemia (XLA). Low or absent of peripheral B cell and intracellular Bruton agammaglobulinemia tyrosine kinase (BTK) protein in a boy with panhypogammaglobulinemia suggests XLA. The definitive diagnosis is made by molecular analysis of BTK gene.
(Reproduced, with permission, from Dennis W. Schauer, Jr., Trivikram Dasu, PhD, James W. Verbsky, MD, PhD, Clinical Immunodiagnostic & Research Lab, Medical College of Wisconsin.)

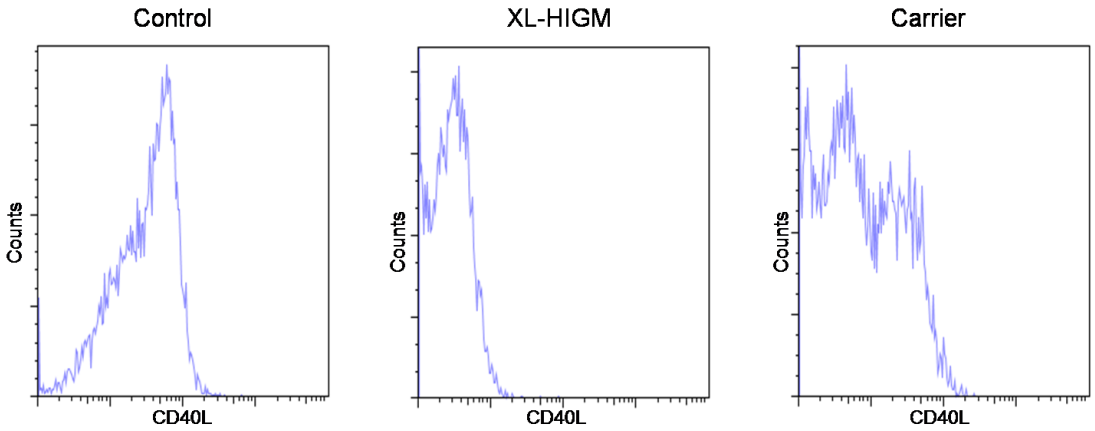

Figure 10-16 X-linked hyper-IgM syndrome. CD40L is normally present on the surface of activated CD4+ T lymphocytes and is absent in patients with X-linked hyper-IgM. The carrier has bimodal CD40L expression following stimulation.
(Reproduced, with permission, from Dennis W. Schauer, Jr., Trivikram Dasu, PhD, James W. Verbsky, MD, PhD, Clinical Immunodiagnostic & Research Lab, Medical College of Wisconsin.)

Flash Card Q11

What is a characteristic cell surface marker of memory B cells?

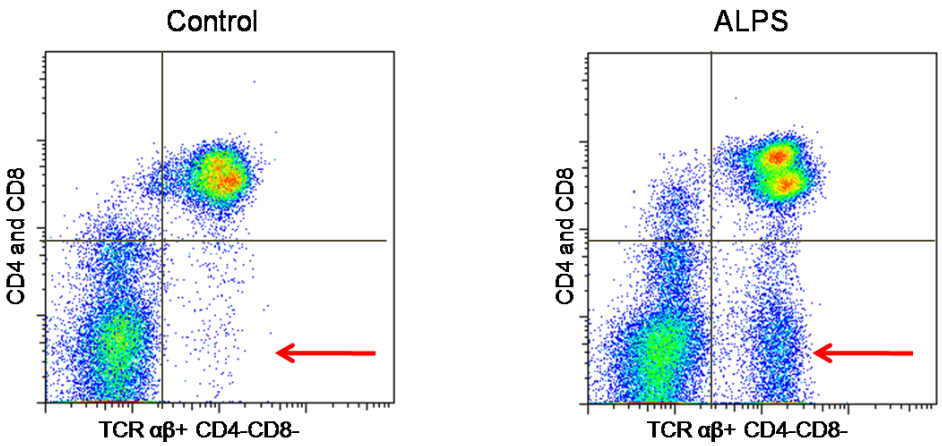

Figure 10-17. Autoimmune lymphoproliferative syndrome (ALPS). The flow cytometry study demonstrates a marked increase in αβ-double-negative (CD4–CD8–) T cells (DNT). Elevated αβ-DNT cells (≥1%) in blood or lymph node is one of the diagnostic features for ALPS.
(Reproduced, with permission, from Dennis W. Schauer, Jr., Trivikram Dasu, PhD, James W. Verbsky, MD, PhD, Clinical Immunodiagnostic & Research Lab, Medical College of Wisconsin.)

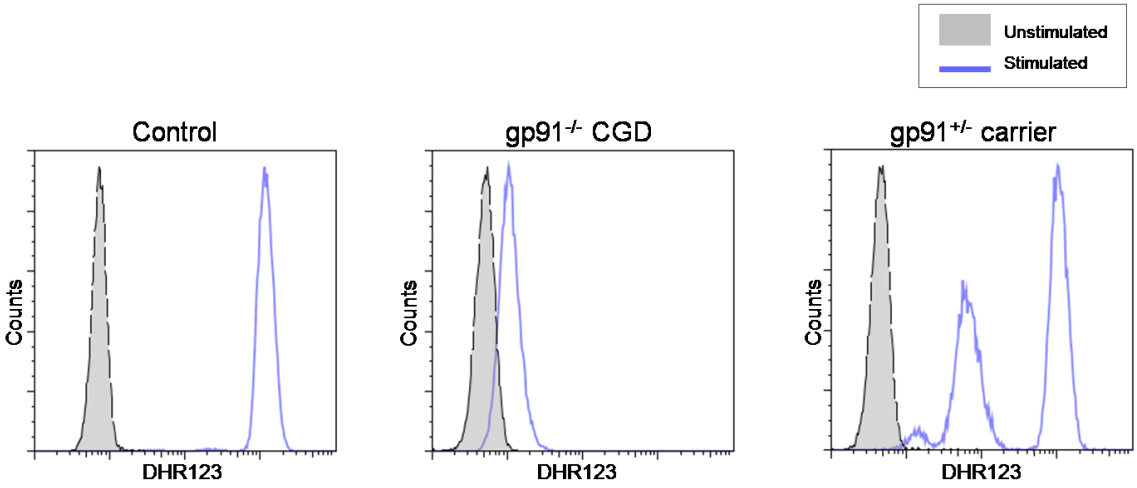

Figure 10-18. Chronic granulomatous disease (CGD). The activated phagocytes from the patient with gp91phox-deficient CGD (X-linked) cannot undergo an oxidative burst to generate superoxide radicals measured by dihydrorhodamine (DHR) 123 assay. Female carriers of this form of CGD will display a mosaicism with a bimodal DHR pattern of normal and abnormal oxidizing cells.
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Flash Card A11

CD27

CONTROVERSIAL TESTS

Skin End-Point Titration (Rinkel Method)

Employed for allergic rhinitis, the Rinkel method establishes a safe dose for starting immunotherapy (IT) by using increasing serial allergen concentrations injected intradermally, then measuring the wheal. "End-point" is the dose that causes ≥ 2 mm increase in wheal diameter that occurs with increasing five-fold concentrations (up to nine injections). This establishes the starting dose of IT; the maintenance dose is estimated to be between 25–50 times the end-point dose. May underestimate the safe starting doses and maintenance dose. Supported by case reports only, randomized controlled trial **(RCT) show that IT using this method is no more effective than placebo.**

Provocation-Neutralization

Similar to skin end-point titration, this uses test doses of various agents (chemicals, allergens, food extracts, or other), administered ID, SC, or SL. Subjective symptoms are recorded for 10 minutes following each dose. If there are no symptoms, increasing doses are given. If symptoms (provocation) occur, then lower doses are given until no symptoms (neutralization) occur. That dose is given as therapy/treatment. Not standardized and a double-blind placebo-controlled trial **(DBPCT) found this method to be equivalent to placebo.**

Cytotoxic Test and Antigen Leukocyte Cellular Antibody Test (ALCAT)

Based on claim that morphologic changes in leukocytes exposed to an allergen in vitro indicates allergy to that allergen. The ALCAT test is similar but measures changes in leukocyte cell volume. There are anecdotal reports regarding the utility of this test for guiding elimination diets in suspected food allergy. Not supported by any DBPCT.

Electrodermal Testing

Measures electrical impedance of the skin at designated acupuncture points in response to an electric current while foods or inhalant extracts are placed in contact with the circuit. A change in electrical impedance would be considered to have detected an allergy. DBPCT has shown that this method cannot independently distinguish atopic from nonatopic patients.

Flash Card Q12

What X-linked immune deficiency, along with carriers of the defect, can be identified by a dihydrorhodamine flow cytometric assay?

Applied Kinesiology

Allergens are placed in patient's hand while a technician assesses subjective muscle strength in the opposite arm. A decrease in muscle power purportedly indicates allergy. Not supported.

Controversial Uses for Immunologic Laboratory Tests

Total Serum IgE—This test can helpful in certain conditions (Job's syndrome/ABPA), but not in diagnosis of allergic rhinitis/food allergy/ Hymenoptera stings, because a **normal IgE does not necessarily exclude significant allergy, and somewhat high levels may also be seen in nonatopic patients**.

Serum-Specific IgG—Merely indicative of exposure and not causation, serum-specific IgG levels have no utility in diagnosing food or inhalant allergy.

Food Immune Complex Assay

Based on belief that food antigen-immune complexes may potentiate delayed symptoms of food intolerance (i.e., more than 2 hours after ingestion), this test assesses the presence of IgE or IgG immune complexes with food antigen. However, these complexes have never been clearly associated with allergy. Considered experimental.

DELAYED-TYPE HYPERSENSITIVITY (DTH) TESTING

DTH (type IV) testing serves as a screening functional assessment of the cellular immune system. DTH depends upon a T-cell response to an antigen previously encountered through ubiquitous exposure or vaccination (Table 10-17). These "recall antigens" are usually *Candida*, tetanus toxoid, or mumps. The tuberculin skin test (TST) is another standardized DTH test. However, currently the only FDA-approved tests for measuring DTH are the TST and *Candida. Trichophyton* may be used, and in endemic areas coccidioidin and histoplasmin may also be used.

Flash Card A12

Chronic granulomatous disease

DTH testing is performed by injecting a small amount of antigen intradermally and recording the amount of induration 48–72 hours later.

Table 10-17. Advantages and Disadvantages of Delayed-Type Hypersensitivity Testing

Advantage	Disadvantage
Quick	Not sensitive[b]
Inexpensive[a]	Not specific
Simple	Qualitative

[a] DTH would not be expected to be positive in unvaccinated patients (i.e., patients who have not yet received tetanus or mumps vaccines) or very young patients who haven't been sufficiently exposed to the antigen in the environment (i.e., *Candida*).
[b] Other conditions that may cause abnormal DTH test results by adversely affecting cellular immunity include malnutrition or uremia.

A negative reaction (absence of induration) may simply reflect a lack of exposure to the antigen, relatively weak nature of the antigen (e.g., *Candida*), or anergy.

- Anergy may be associated with primary or acquired cellular immunodeficiency, cancer, sarcoidosis, cytotoxic immunosuppressive therapy (e.g., steroids), severe atopic dermatitis, or recent/concurrent infection.
- Anergy also correlates with severity of cellular immunodeficiency; anergy is present in advanced HIV but not seropositive HIV with adequate CD4+ counts.
- Anergy without apparent clinical sequelae will be observed to DTH testing for a period of time after receiving the measles, mumps, rubella (MMR) vaccine, because a vigorous immune response to one antigen such as measles can temporarily suppress other DTH responses.

Pathophysiology

DTH results from the activation of specific T cells by antigen-presenting Langerhans cells in the skin. This T-cell activation leads to cellular infiltration with macrophages, monocytes, and lymphocyte in a nonspecific fashion. These cells then secrete inflammatory cytokines, tumor necrosis factor alpha (TNFα) and interferon gamma (IFNγ). Histamine and serotonin release result in increased vascular permeability; the leaky vasculature in combination with an upregulation in adhesion molecules results in additional cellular influx into the site of antigen exposure, which increases induration and erythema.

Clinical Use of DTH Testing

> ***Key Fact***
>
> A positive DTH test reflects prior exposure to the antigen in question and is typically reassuring if the DTH test is being done to assess competence of cellular immunity. A negative DRH test may simply reflect lack of exposure to the antigen or it may reflect anergy as a result of either primary or secondary cellular immunodeficiency.

Testing is typically done with 0.1 mL of a 1:10 to 1:100 concentration antigens on the volar surface of the forearm; several antigens may be placed at the same time. Positive tests are usually 5 mm or greater in induration when read at 48 or 72 hours.

A negative test to a recall antigen, which a patient is expected to have because of adequate exposure, should prompt further evaluation. Negative tests may also reflect antigen potency, or concentration, and technique of application. Seventy-five percent of healthy children from 1–3 years of age have a positive *Candida* DTH test results, and some infants as young as 3 months old have positive *Candida* DTH test results. Positive tetanus DTH usually requires the completion of several separate tetanus vaccinations in the series (three or more); about a third of patients have a positive DTH to tetanus after the first injection in the series only. Prior bacille Calmette-Guérin (BCG) vaccination can temporarily result in a positive TST, but this somewhat weak reaction fades after a few years.

Excessive DTH reactions may be seen in patients with rare mutations in IL-12, IL-12R, IFNγ, or IFNγR.

FOOD CHALLENGES

Definition

Controlled ingestion of certain foods known to contain allergens. Strict elimination of suspected food for 7–14 days is recommended before challenge. Food challenges are the gold standard for the diagnosis of food allergies in both IgE- and non-IgE-mediated food allergies, especially double-blind placebo-controlled food challenges (DBPCFC).

- Due to the risk of anaphylaxis in IgE-mediated allergy, food challenges are usually conducted in a physician's office or hospital.
- Blind food challenges involve masking the suspected allergen in a vehicle, giving it to the patient, and observing the patient for signs or symptoms of an allergic reaction.

Types of Challenges

Double-Blind, Placebo-Controlled Food Challenges (DBPCFC)—Both the patient and the tester are unaware of food being used in challenge. A negative DBPCFC must be confirmed by open feeding challenge under observation. Must do equal number of randomly alternating food allergens and placebo challenges.

- Start with 125–500 mg of lyophilized food (give in fasting state)
- Increase the dose every 15–60 minutes
- Clinical reactivity ruled out once 10 g of dry food ingested

Single Blind Challenges—The patient is unaware of food being used in the challenge, but the tester is aware. It may be useful to screen for suspected food allergies.

Open Challenges—Both the patient and tester are aware of food being used in the challenge. Most commonly performed in practice.

Diagnostic Decision Points

Indicate the likelihood that patient will "outgrow" the food allergy. As IgE increases, so does the risk of clinical reactions but the curve is distinct for each food and may vary with age and atopic disorder.

- Ten percent to 25% of those with negative serum-specific IgE may have clinical reactions. Therefore, if the in vitro test is negative but there is a high clinical suspicion for food allergy then an SPT and/or food challenge must be done.
- SPTs are generally more sensitive and may be positive despite undetectable specific serum IgE.
- Serum levels of food-specific IgE antibodies and SPT wheal sizes are not absolute indications or contraindications to performing an oral food challenge (OFC) and must be interpreted in the context of clinical history.

Specific Diagnostic Decision Points

All decision points in Table 10-18 indicate there is a >95% chance of a reaction upon food challenge, except for soy and wheat, for which there is a 75% chance. Table 10-19 shows levels for which there is a 50% chance of experiencing a negative challenge. These patients are thought to be the optimal candidates for an office-based OFC.

Table 10-18. Specific Diagnostic Decision Points for Food Challenges ~ 95% PPV

Allergen	Age (years)	Serum IgE (kUa/L)
Egg	≥2	7
	≤2	2
Milk	≥2	15
	≤2	5
Peanut		14
Fish		20
Soybean		30*
Wheat		26*
Tree nuts		~15

* 75% predictive value
Abbreviation: PPV, positive predicted value.

Table 10-19. Specific Diagnostic Decision Points for Food Challenges ~50% Negative

Allergen	SPT wheal (mm)	Serum Food IgE (kUa/L)
Egg	≤3	≤ 2
Milk		≤ 2
Peanut	≤3	≤ 2 with history of peanut reaction
		≤ 5 without history of peanut reaction

11 Allergens and Antigens

AEROBIOLOGY

Factors Influencing Clinical Significance of Aeroallergens

Thommen's Postulates of Allergenicity outline the following characteristics of clinically important pollen types:

- Pollen must be capable of eliciting an allergenic response (proteins and glycoproteins).
- Pollen must be **anemophilous** (wind-pollinated) and sufficiently buoyant to be carried long distances (e.g., hundreds of miles).
- Pollen must be produced in abundance, and the plant producing the pollen must be widely distributed.

Particle Size

- Pollens can range from 10 to >200 μm in size, with most significant aeroallergens ranging between 10–60 μm.
- Mold spores may be extremely small at about 1–2 μm or quite large at close to 100 μm.

Aeroallergen Occurrence

- **Seasonal** or occurring at predictable times of the year: tree, weed, and grass pollen
- **Perennial** or prevalent year round: dust mite, dog, cat, and mold spore aeroallergens

Dispersal of Pollen Aeroallergens

- **Anemophilous** (wind-pollinated)
  - Ten percent of all flowering plants, usually those with small, inconspicuous flowers
  - Usually plants with high pollen production
- **Entomophilous** (insect-pollinated)
  - Most common form of pollination; plants often have "flashy" flowers, with scent and nectar
  - Large sticky pollen grains; usually smaller amounts of pollen produced

Flash Card Q1

What are the three requirements of an aeroallergen (derived from Thommen's Postulates)?

Pollination and Plant Flower Characteristics

Pollen is the male gametophyte and analogous to sperm in humans. **Pollination** is the transfer of pollen from the anther sac (male portion) to the stigma (female portion) of the same or another flower.

- **Monoecious:** Plant species that have both male and female flowers on the **same** plant
  - These include **monoclinous** (perfect) flowers, which contain both male and female parts within the same flower, and **diclinous** flowers, in which male and female flowers are separate, but located on the same plant.
- **Dioecious:** Species that have male and female flowers on **different** plants. Thus, separate male and female plants

Sampling Methods and Detection of Aeroallergens

Sedimentation and gravitational sampling is the most elementary sampling method, relying on gravity; however, quantitative data for this method are difficult to obtain.

Durham Sampler—Microslides are thinly coated with adhesive and exposed for a 24-hour period.

- **Cons:** Biased toward larger particles. Cannot determine airborne concentration. Open culture plates can be exposed to weather
- **Pros:** Low cost, durable, and independent of power source

Settle Plates—Particles are allowed to settle onto an agar medium for a period of time, then incubated at the required temperature and examined or counted (e.g., bacteria and molds).

- **Cons**: Biased toward larger particles. Cannot determine airborne concentration. Generally for indoor use only
- **Pros**: Identifies viable airborne organisms (molds)

Impaction Volumetric Samplers

Rotorod—Employs rods that sweep through the air to collect particles on surfaces coated with an adhesive; then, a volumetric count is determined by dividing the number of particles collected by the volume of air sampled (Figure 11-1A).

- **Cons**: Not efficient for smaller particles (i.e., <10 μm) as the smaller particles seem to air-stream around the collecting rods
- **Pros**: Not significantly affected by wind; good for particles in the range of most airborne pollen; enables calculation of airborne particle concentration

Flash Card A1

Must be allergenic, buoyant, and present in significant concentration

Suction Samplers and Spore Traps—These samplers inhale air and particles at specific rates of flow through slits, vents, or holes.

Burkard Spore Trap (Hirst-Like)—Known amounts of air are drawn in through a sampling orifice, and a "tail" keeps the device oriented to the wind (Figure 11-1B.)

- **Pros**: More efficient than the Rotorod for collecting particles that are less than 10 μm; consistent flow speed of 10 L/min; samples can be taken over 7 days or over 24 hours
- **Cons**: More expensive than most other samplers. Affected by wind speed

Anderson Sieve Impinger (Multistage Cascade Sampler)—The sampler comprises a series of sieves or stages, each with up to 400 perforations, through which air is drawn at 1 ft^3/min. As air passes through stages, particles pass through progressively smaller holes and are separated into size-specific fractions. Used primarily for culture-based sampling of airborne fungi.

- **Pros**: Separates particles based on size; good for brief collection periods
- **Cons**: Expensive

Allergenco Air Sampler—Nonwind-oriented "grab type" suction sampler that collects samples on laboratory slides, according to a programmed cycle. Can sample over an entire weekend period. Primarily for indoor use.

A B

Figure 11-1. (A) Rotorod sampler and (B) Burkard spore trap.
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Flash Card Q2

Define monoecious and dioecious.

Flash Card Q3

Name an advantage and disadvantage of the Rotorod.

Other Sampling Devices

Liquid impingers draw air into liquid and particles become suspended in circulating fluid. They are used to collect bioaerosols that can be examined immunochemically. **Cyclonic collectors** are used for dust collection and separation. Particles are counted visually and may also be cultured for fungi. **Filtration samplers** are used to detect dust levels, endotoxins, antigens, fungi, and microorganisms. They basically suck particles through a filter, with a wide range of sampling rates from 2–1000 L/min.

Pollen and Mold Spore Counting and Identification

Basic Technique

- Sampling rod or slide surfaces are stained with Calberla's solution—a basic fuschin stain—then examined microscopically at 400× for pollen and 1000× for fungi
- Pollen is counted manually for the "raw count," then converted to grains per cubic meter, using a formula dependent on sampling methodology (i.e., sampler used, exposure time, volume of air sampled, etc.)

Pollen and Spore Identification

- Size and shape (most pollens are between 20–60 μm in size, with various shapes)
- Surface texture and sculpturing (i.e., smooth, spiny, warty or granular)
- Apertures (pores and furrows)
- Staining (characteristically, light pink to darker red)
- Unique exine (outer layer) or intine (inner layer) characteristics

Flash Card A2

Monoecious: Having both male and female flowers on the same plant
Dioecious: Having separate male and female plants

Flash Card A3

Advantages include ability to obtain quantitative results and this method is not significantly affected by wind
Disadvantage is its poor collection efficiency for particles <10 μm

See Figure 11-2 for pollen grain structure.

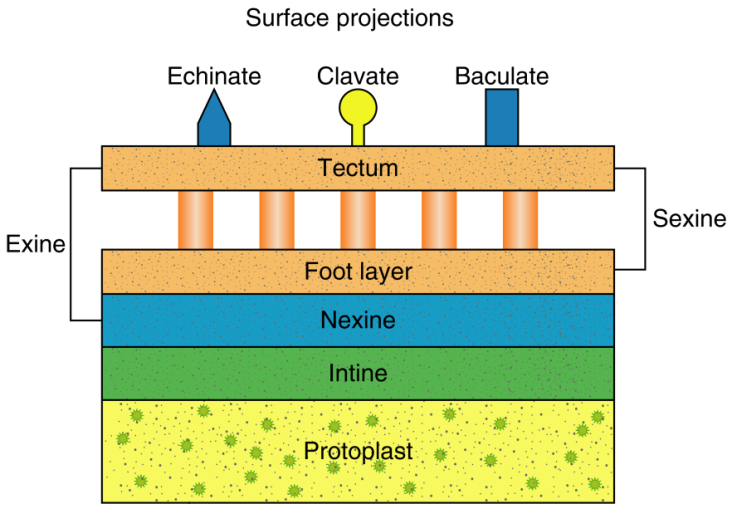

Figure 11-2. Pollen grain structure. (Reproduced, with permission, from Sue Kosisky, United States Army Centralized Allergen Extract Laboratory.)

POLLENS

GRASS POLLEN

Grass pollen looks the same, regardless of species: **monoporate**, relatively large (i.e., 20–45 µm), round pollen grain (Figure 11-3). It is slightly granular in appearance. The pore is surrounded by a thickened ring (annulus) and may have a cap (operculum).

Grass tends to pollinate during the late spring and summer months; but, in southern climates, may pollinate year-round.

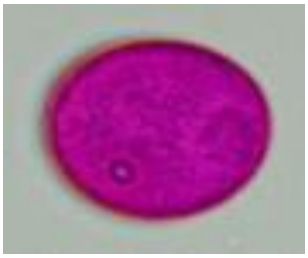

Figure 11-3. Grass pollen.
(Reproduced, with permission, from Sue Kosisky, United States Army Centralized Allergen Extract Laboratory.)

Southern Grasses

- **Chloridoideae:** Bermuda, lovegrass**,** and prairie grasses (e.g., salt, buffalo, and grama)
- **Panicoideae:** Bahia, Johnson, corn, and sugarcane

Northern Grasses (Pooideae)

Northern grasses include **timothy**, **orchard**, **rye**, **fescue**, **bluegrass**, redtop, sweet vernal, brome, velvet, and canary.

Table 11-1 lists southern and norther grasses and their allergens.

Mnemonic

To recall the three main allergenic southern grasses, remember **Bahia** is a coastal region of Brazil and, along with **Bermuda**, should conjure up images of the beach. For the third one, think of American blues great Robert **Johnson**, who hailed from the Mississippi delta.

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Flash Card Q4

Which of these grasses are most cross-reactive with timothy: bahia, Bermuda, bluegrass, Johnson, rye, fescue?

Table 11-1. Grass Pollen Allergens

Type of Grass	Common Name	Scientific Name	Allergen(s)
Southern grasses	Bahia	*Paspalum notatum*	Pas n 1, 13
	Bermuda	*Cynodon dactylon*	Cyn d 1-14
	Johnson	*Sorghum halepense*	Sor h 1-14
Northern grasses	Timothy	*Phleum pratense*	Phl p 1-14
	Rye	*Lolium perenne*	Lol p 1-14
	Bluegrass	*Poa pratensis*	Poa p 1-14

Key Fact

Fourteen grass allergen groups have been characterized, with as many as five of those being major allergens (Table 11-1). **Bermuda** is cross-reactive with other members of the subfamily Chloridoideae, but not with the other subfamilies. Bahia and Johnson grasses have limited cross-reactivity. Members of the subfamily Pooideae showed strong cross-allergenicity based on homology of three major allergens with possible unique allergens in **timothy** and **sweet vernal**.

Note: In a question about cross-reactivity of allergens, if two northern grasses are among the answer choices, those (e.g., timothy and sweet vernal) would be a good bet!

WEED POLLEN

This category of weeds refers to invasive plants that are neither trees nor grasses, which pollinate generally in the late summer and fall (Table 11-2). See Figure 11-4 for an overview of weed taxonomy, which may prove helpful for remembering cross-reactivity; however, it is not meant for memorization. The genus names are the most important.

Table 11-2. Weed Pollen Allergens

Common Name	Scientific Name	Allergen(s)
Ragweed	*Ambrosia artemisiifolia*	Amb a 1-10, profilin, and cystatin
Mugwort	*Artemisia vulgaris*	Art v 1-3 and profilin
Pellitory (Urticaceae)	*Parietaria* spp.	Par o 1 and 2

Flash Card A4

Bluegrass, rye, and fescue. The northern grasses are strongly cross-reactive with each other.

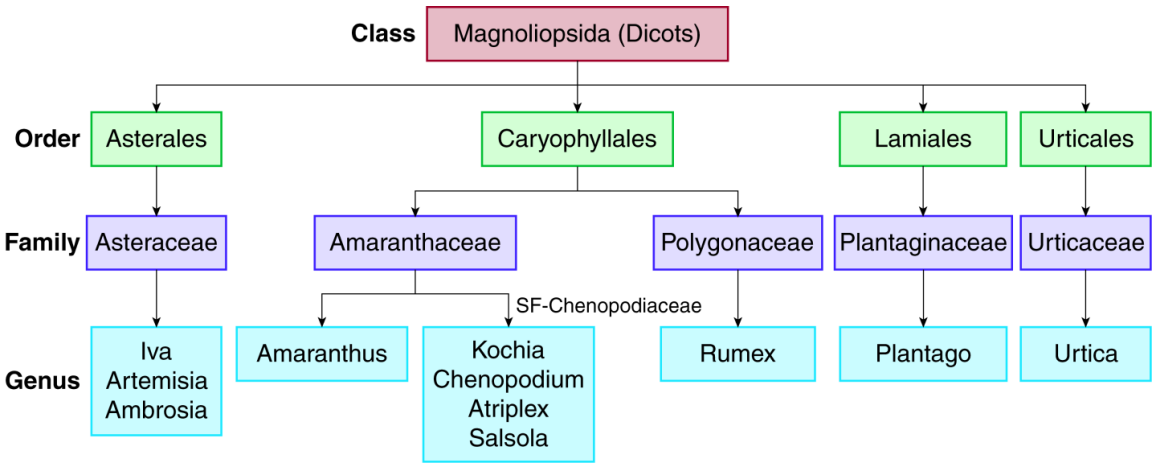

Figure 11-4. Weed taxonomy.

Chenopod and Amaranth

Pollen grains are 20–35 μm, spherical and periporate (like a golf ball), with a slightly granular-to-smooth surface (Figure 11-5). Chenopods and amaranths may have significant cross-allergenicity. **Pigweed or careless** (*Amaranthus*): Includes Palmer amaranth (careless weed), rough pigweed, and western water hemp.

- **Lambsquarter** (*Chenopodium*): Also known as "goosefoot" (chenopod) due to the shape of its leaves
- **Wingscale or saltbush** (*Atriplex*): Mostly found in the western US; seed pods use "wings" for dispersal
- **Burning bush** (*Kochia scoparia*): Named for its red leaves
- **Russian thistle** (*Salsola*): Probably the most important cause of hay fever among the chenopods

Figure 11-5. Chenopod pollen.
(Reproduced, with permission, from Sue Kosisky, United States Army Centralized Allergen Extract Laboratory.)

Composites (Asteraceae)

Ragweed (Ambrosia)

- Pollen grains are 15–25 μm and either tricolporate or tetracolporate, but this is overshadowed by the spiny exine. Furrows are short, only slightly longer than the pore (Figure 11-6).
- Allergenic species include giant (*Ambrosia trifida*), short (*Ambrosia artemisiifolia*), western (*Ambrosia psilotachya*), and false (*Ambrosia acanthicarpa*); strong cross-reactivity between species.
- Pollen-food associations include banana, cantaloupe, and watermelon.
- Prolific pollen producers, accounting for 75–90% of all pollen captured between August and October in some regions. (Its reputation is well deserved!)
- Ragweed season ends typically when frost prevents plants from flowering.

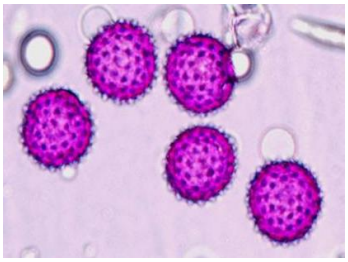

Figure 11-6. Ragweed pollen.
(Reproduced, with permission, from Sue Kosisky, United States Army Centralized Allergen Extract Laboratory.)

Sage and Mugwort (Artemisia)

- Grain is 20–30 μm, round to triangular in shape, and has three prominent furrows (Figure 11-7).
- Pollen food associations include mugwort-celery spice, mugwort-peach, and mugwort-mustard.

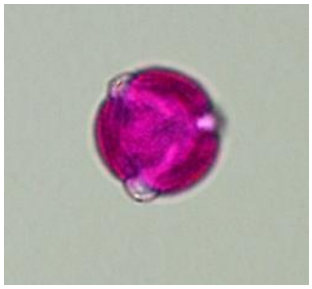

Figure 11-7. Sage pollen.
(Reproduced, with permission, from Sue Kosisky, United States Army Centralized Allergen Extract Laboratory.)

Cockleburs **(Xanthium)—**Grain is 25–30 μm, spheroidal, and similar to ragweed; however, it is larger in size with smaller, blunter spines (Figure 11-8).

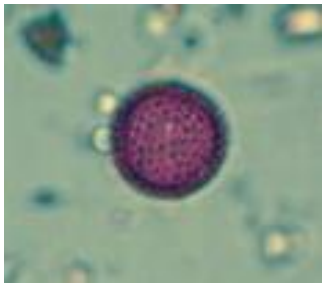

Figure 11-8. Cocklebur pollen.
(Reproduced, with permission, from Sue Kosisky, United States Army Centralized Allergen Extract Laboratory.)

***Marshelder* (Iva)**

- Rivals ragweed as an allergen in some parts of the Mississippi River Basin
- Differs from ragweed pollen due to long furrows

Nettle (Urticaceae)

- Species include the stinging nettle, common in North America, and wall pellitory (*Parietaria*), a similar weed that is an important aeroallergen in Europe.
- One of the smallest pollens (12–16 μm) with three to four pores (Figure 11-9).

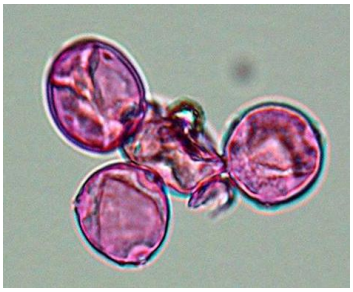

Figure 11-9. Nettle pollen.
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***Plantain* (Plantago)—**Pollen is large (i.e., 20–40 μm) and periporate (6–10 pores), with a distinctive pore cap (operculum) that gives it a "doughnut" appearance (Figure 11-10).

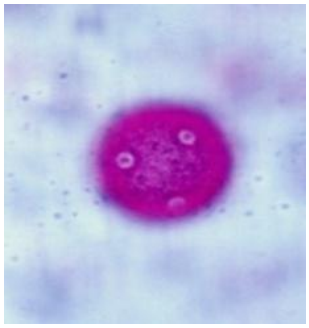

Figure 11-10. Plantain pollen.
(Reproduced, with permission, from Sue Kosisky, United States Army Centralized Allergen Extract Laboratory.)

***Dock or Sorrel* (Rumex)**

- This buckwheat genus includes curly dock and sheep sorrel.
- Pollen is round, 20–30 μm, and tricolporate, with characteristic starch inclusion granules. Furrows are long, almost reaching the poles (Figure 11-11).

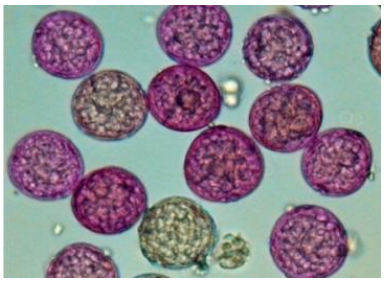

Figure 11-11. Dock or sorrel pollen.
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TREE POLLEN

In general, trees pollinate in the spring. A few **exceptions** include **three species of elm,** which pollinate in the fall, and the **mountain cedar,** which pollinates in midwinter.

Table 11-3 lists the common tree pollen allergens.

Ash, Olive, Privet, and Russian Olive (Oleaceae)

- The pollen of the ash family usually includes four- or five-sided grains, with furrows that suggest a square or pentagonal appearance (Figure 11-12).
- Ash trees are allergenically important in eastern and central North America; whereas, olives are found primarily in western North America and Europe (particularly, the Mediterranean).
- Strong cross-reactivity exists among family members.

Table 11-3. Tree Pollen Allergens

Common Name	Scientific Name (genera in italics)	Allergen(s)
Ash	*Fraxinus*	Fra a 1
Birch	Betulaceae	Bet v 1-7
Mountain cedar	*Juniperus*	Jun a 1-3
Oak	*Quercus*	Que a 1
Olive	Oleaceae	Ole e 1-8
Sycamore or plane tree	*Platanus*	Pla a 1

- Exine has net-like (reticulate) pattern. In ash, the net pattern is fine; but, in olive and privet, the net pattern is coarse and quite apparent.
- Olive and privet have three furrows.

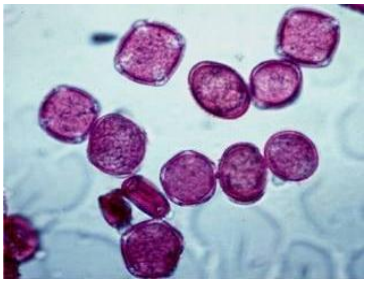


Figure 11-12. Ash pollen.
(Reproduced, with permission, from Sue Kosisky, United States Army Centralized Allergen Extract Laboratory.)

Birch, Alder, Hazelnut, and Hornbeam (Betulaceae)

- Three pores (triporate) protruding from the surface, each of which contains a collar (oncus). May appear like a lemon, if only two pores are visible (Figure 11-13).
- Alder is slightly different in appearance, with four to six pores.
- Pores protrude (aspidate).
- Foods known to cross-react with birch and cause oral allergy syndrome, include **apple**, almond, apricot, **carrot**, celery, cherry, coriander fennel, hazelnut, kiwi, nectarine, parsley, parsnip, pear, pepper, plum, potato, and walnut.
- Strong cross-reactivity within family, extending to the family Fagaceae.

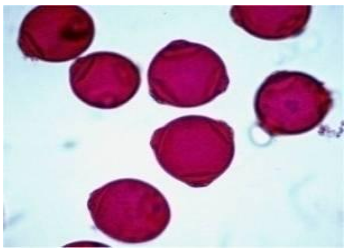


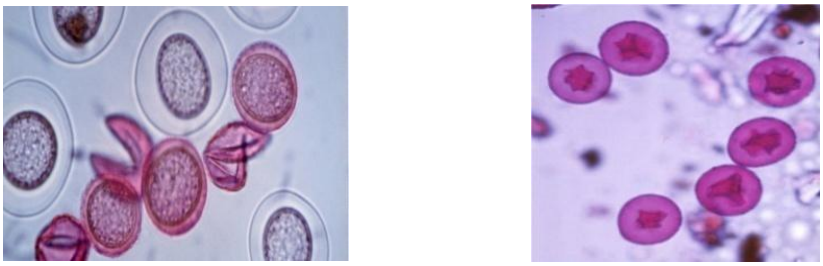
Figure 11-13. Birch pollen.
(Reproduced, with permission, from Sue Kosisky, United States Army Centralized Allergen Extract Laboratory.)

Flash Card Q5

Which pollen has been most implicated in pollen-food syndrome? What are the major allergens involved?

Cypress, Juniper, and Cedar (Cupressaceae)

- Thick intine with stellate cytoplasmic contents and an exine, which can break and look like Pac-Man (Figure 11-14).
- Mountain cedar (*Juniperus ashei*) pollinates **midwinter** in Texas, causing "cedar fever"; Eastern red cedar (*Juniperus virginiana*) is common in the eastern US and pollinates in the spring.
- Strong cross-reactivity within family; one member should suffice for treatment.



A **B**

Figure 11-14. (A) Mountain cedar pollen with disrupted exine; (B) intact mountain cedar pollen. (Reproduced, with permission, from Sue Kosisky, United States Army Centralized Allergen Extract Laboratory.)

Oak, Beech, and Chestnut (Fagaceae)

- Oak pollen is triangular-shaped with three germinal furrows that appear as white "pie slices" slightly protruding from the surface. Size, 25–35 μm (Figure 11-15).
- Common spring pollinator and major allergen throughout most of the US.
- In addition to cross-reactivity within Fagaceae, oak is also cross-reactive with birch and other members of the Betulaceae family.

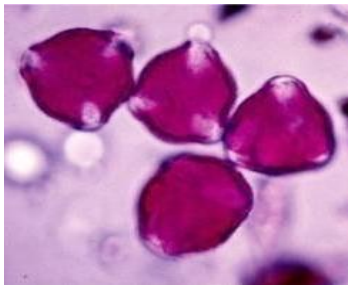


Figure 11-15. Oak pollen.
(Reproduced, with permission, from Sue Kosisky, United States Army Centralized Allergen Extract Laboratory.)

Flash Card A5

Betula (Birch). Bet v 1 and Bet v 2

Sycamore (Platanaceae)—Round grains, with three furrows and a thin exine that is finely reticulate (Figure 11-16).

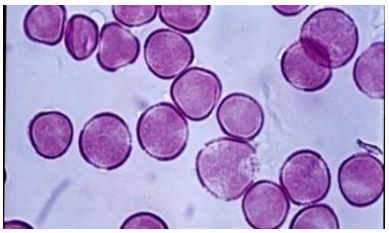


Figure 11-16. Sycamore pollen.
(Reproduced, with permission, from Sue Kosisky, United States Army Centralized Allergen Extract Laboratory.)

Pine (Pinaceae)

- Bladders give the impression of a child's Mickey Mouse cap (Figure 11-17).
- Large size (i.e., 50–100 μm) means that they are rarely implicated in allergy.

Figure 11-17. Pine pollen.
(Reproduced, with permission, from Sue Kosisky, United States Army Centralized Allergen Extract Laboratory.)

Maple and Box Elder (Aceraceae)

- Grains contain three furrows, like oak; but, are generally round and may have a "beach ball" appearance (Figure 11-18).
- Box elder is wind-pollinated; maple is primarily insect-pollinated

Flash Card Q6

A 21-year-old man in San Antonio, Texas, has rhinorrhea, nasal congestion, and conjunctivitis for several weeks each January for 3 years. He is asymptomatic the rest of the year. What is the most likely allergen?

Flash Card Q7

Lolium, Pinus, Platanus, Quercus, Urtica. Which has the smallest pollen? The largest pollen?

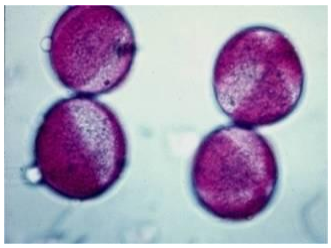


Figure 11-18. Maple pollen.
(Reproduced, with permission, from Sue Kosisky, United States Army Centralized Allergen Extract Laboratory.)

Elm (Ulmaceae)

- American elm pollinates in the spring, whereas several other elms pollinate in the **fall.**
- Four to seven (usually five) oval-shaped pores and may appear pentagonal. Outer surface appears wavy or undulating (Figure 11-19).
-

Figure 11-19. Elm pollen.
(Reproduced, with permission, from Sue Kosisky, United States Army Centralized Allergen Extract Laboratory.)

Flash Card A6

Juniperus ashei (mountain cedar) pollen

Flash Card A7

Smallest: *Urtica* (nettle) 12–14 µm; Largest: *Pinus* (pine) 50–100 µm.

Poplar, Willow, and Cottonwood (Salicaceae)

- Poplar and cottonwood have round grains, each with an outer surface that is granular and often appears "cracked" or "flaky" (Figure 11-20); but, there are **no furrows**. By contrast, willow has three furrows and a reticulate pattern on the pollen wall.
- Willows are entomophilous and not considered allergenically important; however, they are medically important since aspirin is made from their bark. Poplars are anemophilous and produce significant allergenic pollen throughout North America.

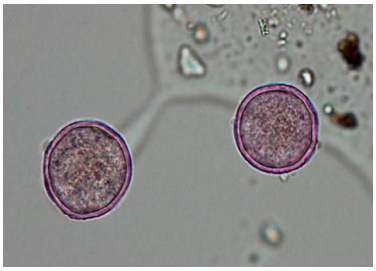


Figure 11-20. Cottonwood pollen. Smallest: *Urtica* (nettle) 12–14 µm; Largest: *Pinus* (pine) 50–100 µm.
(Reproduced, with permission, from Sue Kosisky, United States Army Centralized Allergen Extract Laboratory.)

Sweetgum (Hamamelidaceae)

- Periporate, with 12–20 pores per grain that often bulges, suggesting a "soccer ball" appearance.
- Sweetgum is the only species of allergenic importance in this family, which also includes witch hazel, and is found generally south of the Mason-Dixon line in the US (Figure 11-21).

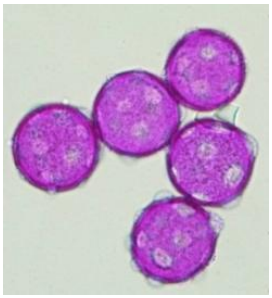


Figure 11-21. Sweetgum pollen.
(Reproduced, with permission, from Sue Kosisky, United States Army Centralized Allergen Extract Laboratory.)

Mulberry (Moraceae)

- Grains are small (i.e., 11–20 µm), thin-walled, and usually diporate with onci, giving the appearance of a light, pinkish lemon (Figure 11-22).
- Pores are slightly raised or aspirated ("shield-shaped").

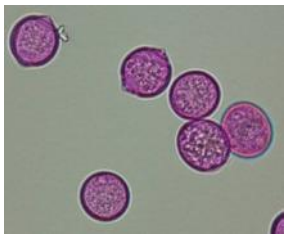


Figure 11-22. Mulberry pollen.
(Reproduced, with permission, from Sue Kosisky, United States Army Centralized Allergen Extract Laboratory.)

Flash Card Q8

Which of these trees is entomophilous?

A. Ash (Fraxinus)

B. Oak (Quercus)

C. Poplar (Populus)

D. Willow (Salix)

Walnut, Hickory, and Pecan (Juglandaceae)

- Walnut is periporate, with 9–15 slightly raised germinal pores; hickory and pecan are indistinguishable, with each containing three nonprotruding pores (Figure 11-23).
- The family Juglandaceae, derived from Latin meaning "Jove's nuts," contains both *Juglans* (walnut) and *Carya* (hickory) species. Allergenically important walnut species exist on both the west and east coasts of North America; but, the hickories are limited to the eastern US.
- Cross-reactivity exists between members.

Figure 11-23. (A) Walnut pollen; (B) Hickory pollen.
(Reproduced, with permission, from Sue Kosisky, United States Army Centralized Allergen Extract Laboratory.)

Mimosa, Acacia, Locust, and Mesquite (Leguminosae)

- Pollen grains may exist as monads or polyads, with the polyads usually having either 4 or 16 quadrangular grains in a group (Figure 11-24).
- These four are exceptions among leguminous plant species, which are usually nonallergenic. *Mimosa* and *Acacia* are cultivated as ornamental trees in tropical and subtropical regions. Mesquite is found primarily in the southwest US, where it was an important food source for early Native Americans.

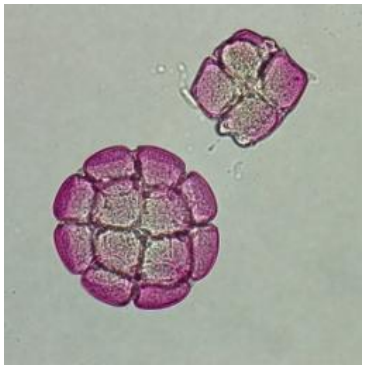


Figure 11-24. *Acacia* pollen.
(Reproduced, with permission, from Sue Kosisky, United States Army Centralized Allergen Extract Laboratory.)

Flash Card Q8

D. Willow (*Salix*) is entomophilous (insect-pollinated)

Environmental Control of Pollen

- Limit outdoor exposure during high pollen counts.
- Keep windows and doors closed.
- Use air conditioning with outdoor vents closed.
- Consider bathing following outdoor activity to reduce indoor contamination.
- Wash pets after they have been outdoors.

MOLDS AND FUNGI

Medical Importance of Fungi

Fungi can be saprophytic or exist as parasites to both humans and plants. They are implicated in a number of allergic diseases in humans, including allergic asthma, hypersensitivity pneumonitis (HP), rhinoconjunctivitis, and bronchopulmonary mycoses.

- **Immune Response:** Both conidia and spores are associated with IgE immediate-type hypersensitivity. Smaller spores that penetrate the smaller airways have been associated with delayed-type hypersensitivity. Respiratory allergy and atopic asthma may be caused by the inhalation of mold spores. Allergic fungal sinusitis is also due to mold spores.
- **Infection:** Includes cutaneous and respiratory infections. Important consideration in the immunocompromised patient. *Aspergilla*, *Candida*, and *Mucor* (in diabetics) are common agents.
- **Toxic Irritant:** Fungal metabolites and mycotoxins have numerous health effects for humans. Aflatoxins from *Aspergillus flavus*, for example, are known to be carcinogenic and mutagenic. The mycotoxin tenuazonic acid produced by *Alternaria alternata* has hepatotoxic and nephrotoxic health effects. Volatile organic compounds (VOCs) are released by various fungi growing on different substrates.

Spore Structure and Development

- Fungal organisms can be unicellular or multicellular (single-celled yeast forms to considerably branched hyphae).
- Fungal reproduction occurs by many forms, including budding, fission, fragmentation, and the production of spores.
- The size, shape, color, and characteristics of mold spores are important for the taxonomic classification of fungi.

Fungal Taxonomy

Fungi consist of a large group of complex and diverse organisms, making classification and identification very difficult. Fungi reproduce both sexually and asexually. Five major divisions include:

- **Ascomycota:** The largest phylum of fungi. Ascomycota produce sexual spores (ascospores) in "sacs" known as an **ascus**. It has multiple classes and includes most of the allergenic fungi.
- **Zygomycota:** Asexual spores are produced within sacs called sporangia. Found on soils and leaves, and damp interiors. *Mucor* and *Rhizopus* are prominent genera in this group.
- **Basidiomycota:** Includes puffballs, mushrooms, rust, and smuts. Produce basidiospores on the ends of protruding "pegs" or finger-like structures called **basidia.**
- **Deuteromycetes:** Also known as the **fungi imperfecti** and asexual spores. Conidial fungi with no identified sexual stage that constitutes the second largest group of fungi. Spores vary considerably in size, shape, color, and structure.
- **Oomycota:** Rarely reported as allergens. Water molds; the downy mildews are included.

Airborne Fungi of Allergenic Importance

Alternaria

- In air samples occur singularly or in chains.
- Club-shaped with multicellular beak, 20–75 μm (Figure 11-25).
- Prevalent outdoors as dry day mold spore.
- *Alternaria* sensitivity has been associated with severe asthma and life-threatening exacerbations as well as hay fever.
- Likes decaying plants; high prevalence in grain-growing areas.

Figure 11-25. *Alternaria*.
(Reproduced, with permission, from Sue Kosisky, United States Army Centralized Allergen Extract Laboratory.)

Cladosporium

- The most abundant spores in temperate areas of the world
- Asexual spore often noted in air sample in chains
- Individual conidia range from 6–25 μm in length
- Variations in shape, including hot dog, cylindrical, and spherical (Figure 11-26)
- Dry day spore; also prevalent indoors (due to high outdoor concentrations)

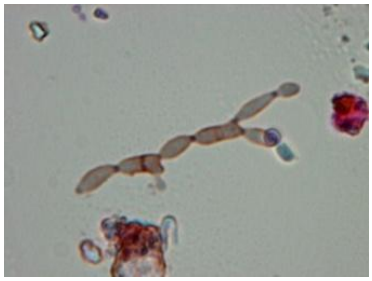


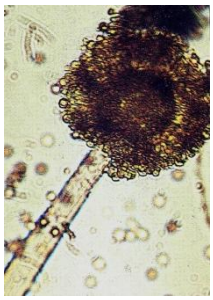
Figure 11-26. *Cladosporium*.
(Reproduced, with permission, from Sue Kosisky, United States Army Centralized Allergen Extract Laboratory.)

Aspergillus

- Common, indoor mold spore; asexual
- Spherical to oval spores, which can be single or in chains (Figure 11-27A)
- May produce mycotoxins and cause **allergic bronchopulmonary aspergillosis (ABPA)**

Penicillium

- Common, indoor mold spore
- Distinctive structure (conidiophore) looks like a paint brush, but only visible in culture (Figure 11-27C)
- May produce mycotoxins and cause **hypersensitivity pneumonitis (HP),** also called **extrinsic allergic alveolitis (EAA)**, in cheese workers



A

B

C

Figure 11-27. (A) *Aspergillus*; (B) aspergillum; and (C) *Penicillium*.
(Images A and C reproduced, with permission, from Sue Kosisky, United States Army Centralized Allergen Extract Laboratory ; image B reproduced, with permission, from Wikimedia Commons.)

Mnemonic

Aspergillus and ***Penicillium*** are often indistinguishable. However, *Aspergillus* was discovered by an Italian priest, who named it after the aspergillum, the implement used by clergy to sprinkle holy water (Figure 11-27B). *Penicillium* comes from the Latin *penicillus,* which means "paintbrush."

Flash Card Q9

What is the defining characteristic of the largest phylum of fungi, Ascomycota?

Flash Card Q10

Near-fatal asthma is associated with sensitivity to which mold?

Helminthosporium, Drechslera, Bipolaris, *and* Exserohilum *Group*

- Single, dry, day spores, with thick cell walls
- Variable in size: 15–180 μm long by 14–22 μm at broadest part, width-wise (Figure 11-28)
- Light to dark brown in color
- Implicated in allergic fungal sinusitis

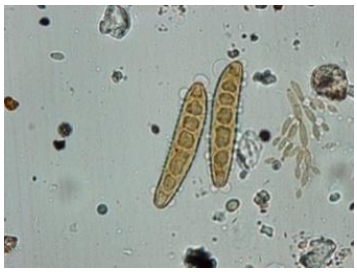


Figure 11-28. *Helminthosporium*.
(Reproduced, with permission, from Sue Kosisky, United States Army Centralized Allergen Extract Laboratory.)

Epicoccum

- Asexual, round, multicellular, and dry day spores
- Usually 15–25 μm, but can be as much as 50 μm (Figure 11-29)
- Dark, golden brown with warts on the surface

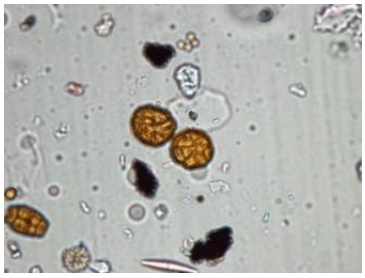


Figure 11-29. *Epicoccum*.
(Reproduced, with permission, from Sue Kosisky, United States Army Centralized Allergen Extract Laboratory.)

Flash Card A9

The production of an ascus (saclike structure containing sexual spores)

Fusarium

- Colorless, wet, day spore with three to seven transverse septa
- Spindle-shaped and curved, with tapered ends (Figure 11-30)
- Size: 20–50 μm by 3–5 μm.

Flash Card A10

Alternaria

Figure 11-30. *Fusarium*.
(Reproduced, with permission, from Sue Kosisky, United States Army Centralized Allergen Extract Laboratory.)

Ascomycota

- Rainy day sexual spore, with wide range of shape and size that forms in an ascus or sac; usually eight ascospores to an ascus (Figure 11-31)
- Single or multicelled; colorless to deeply pigmented

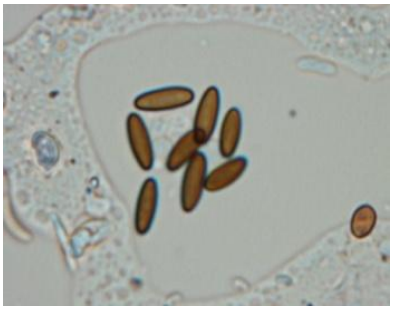


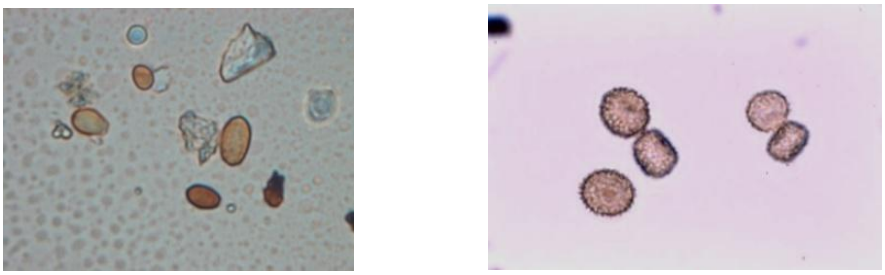
Figure 11-31. Ascomycota.
(Reproduced, with permission, from Sue Kosisky, United States Army Centralized Allergen Extract Laboratory.)

Basidiomycota

- Common mushroom, bracket fungi, and puffball spores
- Rainy day spores with wide range of shape, size, and color
- Always single-celled
- Range of 2–18 μm in size (Figure 11-32)

Smut spores and **rust spores** are two groups of plant pathogenic fungi in this phylum.

- **Smut spores** are especially abundant in agricultural areas; single-celled, globose to subglobose, 6–15 μm in diameter, and golden brown to dark brown; and, they may have a smooth, spiny, or reticulate wall.
- **Rust spores** are larger than smut spores (i.e., 20–30 μm), oval to diamond-shaped, and pale to deeply pigmented, with a smooth or spiny wall.



A B

Figure 11-32. (A) Basidiomycota and (B) smut spores.
(Reproduced, with permission, from Sue Kosisky, United States Army Centralized Allergen Extract Laboratory, and Estelle Levetin, University of Tulsa.)

Aureobasidium
- Common, indoor mold spore found on damp surfaces (e.g., in kitchens and bathrooms)
- Difficult to identify in outdoor air samples
- Colonizes paper and lumber

Zygomycetes
- Class of lower fungi with nonseptate hyphae; found indoors, in damp interiors
- Genera include *Mucor* and *Rhizopus*
- Abundant on leaf litter and decaying vegetation

Stachybotrys—"Black mold"; a toxin-producing mold that can be present indoors after water damage.

Yeasts
- Unicellular fungi that can be opportunistic pathogens (*Candida*)
- Found in water, soil, plants, and air

Indoor Versus Outdoor Mold

Common Indoor Spores
- *Aspergillus* and *Penicillium* (also *Rhizopus* and *Mucor*)
- Outdoor spores move readily indoors (*Cladosporium*)

Outdoor Spores
- Dry, day mold spores; prevalent on dry, sunny, windy days: *Alternaria, Cladosporium, Epicoccum* (**ACE**); also *Curvularia, Drechslera, Pithomyces, Botrytis,* and smut spores
- Rainy day mold spores: Ascospores, basidiospores, *Fusarium*
- Prevalent at night and in high humidity: Ascospores, basidiospores
- Outdoor molds usually present year-round, except with snow cover

Mnemonic

The most important indoor molds are *Aspergillus* and *Penicillium*. Abundant outdoor molds (*Cladosporium*) can also be found at high concentrations indoors.

Table 11-4. Mold Allergens

Common Name	Scientific Name	Allergen(s)
Alternaria	*Alternaria alternata*	Alt a 1-12
Cladosporium	*Cladosporium herbarum*	Cla h 1-12
Aspergillus	*Aspergillus fumigates*	Asp f 1-22
Penicillium	*Penicillium chrysogenum*	Pen ch 13, 18,20

See Table 11-4 for common molds and their allergens.

Environmental Control of Mold

- Dehumidify and eliminate dampness (relative humidity <50%).
- Avoid or use a mask while raking, mowing, and mulching.
- Disinfect bathroom and use fungicides (e.g., Lysol, Clorox, X14).
- Treat directly with bleach and clean with soapy water.
- Remove/replace contaminated porous materials (e.g., carpets).

INDOOR ALLERGENS

House dust is a combination of multiple allergens, including animal dander, insects, fungi, and even human proteins. House dust extracts have largely been replaced by more specific allergen extracts.

ANIMAL PROTEINS

Dust Mite

Species include the following:

- The pyroglyphid mites: ***Dermatophagoides pteronyssinus***, ***Dermatophagoides farinae***, and ***Euroglyphus maynei***.
  - The "*Dermatophagoides* twins" are the most common offenders in the US.
- ***Blomia tropicalis*** in tropical locales (e.g., Florida, Puerto Rico, and Brazil).
- Storage mites: ***Lepidoglyphus destructor*** and ***Tyrophagus putrescentiae***.

Flash Card Q11

What is usually the most abundant outdoor mold spore?

Dust mites are not capable of drinking liquids, so they are dependent entirely on ambient humidity for survival (i.e., they like relative humidity that is greater than 50%). Optimal growth temperatures are between 18–27°C (65–80°F).

Flash Card Q12

Which mold spores are higher during periods of rain?

Two major allergens have been identified:

- **Group 1** allergens (**Der p 1**, **Der f 1**):
  - Show homology with cysteine proteases and are highly cross-reactive
  - Present on fecal particles, which are similar in size to pollen grains (i.e., 10–35 μm)
- **Group 2** allergens (**Der p 2**, **Der f 2**):
  - Also highly cross-reactive
  - **Der p 10** is a tropomyosin and cross-reactive with other invertebrate tropomyosins (e.g., shrimp and limpet)
  - Individuals predisposed to atopy may be sensitized at mite levels of **2 μg/g** of dust

Environmental control measures for dust mite include the following:

- Dehumidifier (set at <45% relative humidity)
- Impermeable mattress and pillow covers
- Hardwood floors instead of carpets
- Vacuum weekly
- Wash bedding in hot water (i.e., >54°C or >130°F), and remove stuffed animals and similar toys
- Vinyl or leather furniture
- Acaricides (benzyl benzoate, tannic acid)

Key Fact

It can take 4–to 6 months after cat removal to significantly reduce cat allergen levels in the home. Additional aggressive control measures may reduce the allergen levels more quickly.

Isolated use of dust mite covers without other interventions is unlikely to be effective. Multiple interventions may be needed.

Cat

Fel d 1 is the only major allergen, primarily made in the skin, and homologous to uteroglobin. (About 80% of cat-sensitive individuals react to Fel d 1.) A level of Fel d 1 that is **greater than 2 μg/g** is associated with risk of sensitization; **greater than 10 μg/g** is a risk factor for asthma in sensitive individuals. **Fel d 2** (albumin) and **Fel d 3** are minor allergens.

Cat allergen is ubiquitous! It was found in over 90% of US homes, over half of which did not even have a resident cat, presumed to be carried on the clothing of cat owners who visit the home. It is carried by small vectors (e.g., <25 μm and some even <2.5 μm!) and **remains airborne**, **even in undisturbed conditions**.

Flash Card A11

Cladosporium

Environmental control measures for cat include the following:

- Cat removal is the only clearly effective avoidance or solution.
- Washing the cat once or twice weekly reduces allergen temporarily.

Flash Card A12

Fusarium species as well as Ascomycota and Basidiomycota.

- Aggressive cleaning of the home can reduce allergen levels more quickly. (The vacuum cleaner must be equipped with a good filter, provide increased ventilation or use high-efficiency particulate-absorbing (HEPA) to remove small airborne particles.)

Dog

Over 28 dog allergens have been detected, at least 5 of which are significant:

- **Can f 1**: Lipocalin and major allergen; found in saliva and hair/dander
- **Can f 2**: Lipocalin and minor allergen
- **Can f 3**: Dog serum albumin; cross-reactive with other mammalian albumins
- **Can f 4**: Lipocalin
- **Can f 5**: Kallikrein and major allergen; cross-reactive with human prostate-specific antigen (i.e., sensitization could increase risk of reacting to human seminal fluid)

Dog allergen is carried on larger vectors than those for a cat, so is not as easily airborne. Like cat allergen, it is also ubiquitous and can be found in public buildings as well as homes without dogs.

Key Fact

In addition to the dog allergens, the **lipocalin** (also known as **calycin**) family of proteins includes rat (Rat n 1), mouse (Mus m 1), horse (Equ c 1, Equ c 2), cow (Bos d 2, Bos d 5), cow's milk (β-lactoglobulin), rabbit (Ory c 1), and cockroach (Bla g 4). (But, not cat!)

Environmental control measures for dog include the following:

- Avoidance
- Washing the dog twice a week
- Wiping the dog down with a wet towel (water just as effective as commercially available "allergen removers")
- HEPA filtration may be effective

Cockroach

Species include the following:

- ***Blattella germanica*** (German cockroach): Most common in crowded North American cities
- ***Periplaneta americana*** (American cockroach)
- ***Blatta orientalis*** (Oriental cockroach)**:** Associated with water, especially leaking pipes

Flash Card Q13

What dust mite is unique (relatively) to Florida and Puerto Rico?

Allergens are from feces, saliva, or debris (frass) and include **Bla g 1** and **Bla g 2**, which are aspartic proteases. **Blag g 4** is a lipocalin family protein. **Per a 1** is cross-reactive with Bla g 1, and **Per a 7** is a tropomysin. Bla g 2 is typically measured in house dust and concentrations **greater than 10 µg/g** are associated with sensitization and disease (i.e., these levels are uncommon in suburban households). Allergens are not easily detected in undisturbed air; they are carried

Flash Card Q14

What is the source of Fel d 1 and Can f 1?

on large particles (e.g., dog and dust mites). Highest levels are detected in kitchens.

Environmental controls include:

- Poison bait
- Careful housekeeping to enclose all sources of food for the insects
- Cleaning to remove accumulated cockroach allergens
- Sealing all possible access points to the house.

Rodents (Mice, Rats, Gerbils, Hamsters)

Rodent infestation occurs in a wide range of environments, including homes, schools, and restaurants. Exposure to rodent allergens at concentrations greater than1.6 μg/g of dust is associated with sensitization, and may contribute to the development of asthma. The major allergens from the mouse and rat are Mus m 1 and Rat n 1. These are cross-reactive lipocalins excreted in the urine of male rodents. Rodent allergens are carried on small particles (like cat); so can remain airborne for some time.

Environmental controls should focus on sealing rodent entry points and removing their sources of food, water, and shelter.

Laboratory animal workers are at high risk of developing sensitization, especially in the first 3 years. Risk factors include an atopic background and the intensity of exposure.

Other Animals

Birds are not particularly allergenic, but IgE-mediated sensitivity can occur. Pigeon breeders and bird fanciers can develop hypersensitivity pneumonitis (HP), also called extrinsic allergic alveolitis (EAA), which is an IgG-mediated response to avian serum γ-globulin.

Horses, **cows**, and **rabbits** have all had both major and minor allergens characterized, and the major allergens all belong to the lipocalin family.

Table 11-5 lists of common indoor allergens.

Flash Card A13

Blomia tropicalis, found in tropical locales

Flash Card A14

Fel d 1—cat skin. Can f 1—dog saliva and hair/dander

Table 11-5. Indoor Allergens

Common Name	Scientific Name	Allergen(s)
Dust mites	*Dermatophagoides farinae*	Der f 1,2,3,7,10,11,14,15-17,18w
	Dermatophagoides pteronyssinus	Der p 1-11, 14, 20
	Euroglyphus maynei	Eur m 2,14
	Blomia tropicalis	Blo t 1-9, 13, 21
Storage mites	*Lepidoglyphus destructor*	Lep d 2,5,7,10,13
	Glycyphagus domesticus	Gly d 2
	Tyrophagus putrescentia	Tyr p 2,13
Animals		
Dog	*Canis familiaris*	Can f 1-4
Cat	*Felis domesticus*	Fel d 1-4, 5w-7w
Horse	*Equus caballus*	Equ ca 1-5
Guinea pig	*Cavia porcellus*	Cav p 1,2
Mouse urine	*Mus musculus*	Mus m 1
Rat urine	*Rattus norvegius*	Rat n 1
Insects		
German cockroach	*Blattella germanica*	Bla g 1-6
American cockroach	*Periplaneta americana*	Per a 1,3,7

POLLUTANTS

Air Quality Index (AQI)

The Air Quality Index (AQI) is an index for reporting air quality data to the general public. Values are standardized for ozone and particulate matter (PM), among others. Symptoms or disease correlate with exposure. The AQI is also known as the Air Pollution Index (API) and individual countries set their own relative values.

The United States Environmental Protection Agency (EPA) uses the AQI values shown in Table 11-6, which are relative to health concerns and categorized by color.

Flash Card Q15

What is the major mouse allergen and its source?

Table 11-6. Air Quality Index

Value	Color	Level of Health Concern	Meaning
0–50	Green	Good	Air quality is considered satisfactory and poses little or no risk
51–100	Yellow	Moderate	Air quality is acceptable; health concern for few people who are unusually sensitive to air pollution.
101–150	Orange	Unhealthy for Sensitive Groups	General public is likely fine, but members of sensitive groups may experience ill effects.
151–200	Red	Unhealthy	Everyone may experience adverse health effects; sensitive groups may experience more serious health effects.
201–300	Purple	Very Unhealthy	Health warnings of emergency conditions. Everyone is likely to be affected.
301–500	Maroon	Hazardous	Health alert: may cause serious adverse effects in anyone.

Outdoor Pollutants

***Nitrogen Dioxide (NO_2)*—**Precursor to photochemical smog, and found in urban and industrial regions. Is produced from the combustion of fossil fuels or natural gas. Associated with decreases in lung function, increased airway neutrophils, and proinflammatory cytokines. Increased allergen response is at 0.4 ppm.

***Ozone (O_3)*—**Byproduct of atmospheric reactions using nitric oxide (NO), VOCs, UV light; increased during summer and peaks in the afternoon. **Increases symptoms**, **hospitalization**, **and β agonist use in asthmatics**. **Major outdoor pollutant involved in asthma exacerbations**. Decreases lung function acutely and chronically; increases airway neutrophils, IL-6, IL-8, leukotrienes or prostaglandins, and potentiates allergen challenges.

***Sulfur Dioxide (SO_2)*—**Has been shown to decrease lung function in children; causes bronchospasm in asthmatics at 0.5 ppm, and reverses with β agonists.

***Particulate Matter (PM)*—**Composed of mineral dust, organic matter, and aerosolized byproducts of fossil fuel combustion; these are less than 10 μm in size.

***Diesel Exhaust*—**Contains both particulates and gases. Skews immune responses toward a T_h2 response.

Flash Card A15

Mus m 1, a lipocalin from male mouse urine

Indoor Pollutants

***Environmental Tobacco Smoke (ETS)*—**Increases indoor PM; **associated with recurrent otitis media**, **URI**, **LRI**, **wheezing**, **and cancer**. Increases T_h2 cytokines (IL-4, IL-10) and eosinophils as well as allergen sensitization.

***Endotoxin*—**Originates from gram-negative bacteria. Living with animals increases exposure. It is also increased in areas where tobacco smoking occurs.

***Nitrogen Dioxide (NO_2)*—**Effects as described earlier; comes from indoor natural gas appliances

***Other Irritants*—**Include agents from biomass burning (i.e., tobacco, wood and other biologic plant fuels), household chemicals, and dirty ventilation systems.

> ***Key Fact***
>
> Nitrogen dioxide is both an indoor and outdoor pollutant from burning fossil fuels, including natural gas.

Oxidant and Antioxidant Balance

Pollutants exert oxidant stress on airways; reactive oxygen species (ROS) activate nuclear factor kappa B (NFκB), AP-1 and other pathways. Studies show that asthmatics have decreased antioxidant levels at baseline (superoxide dismutases and glutathiones, predominantly). Antioxidant response element (ARE) genes neutralize ROS; genetic susceptibility data shows that some individuals have less ARE, less glutathione stores, and may be more susceptible to developing asthma.

STANDARDIZATION AND STABILITY OF ANTIGENS

Units of Potency

In the United States, allergen extracts can be licensed by the Food and Drug Administration (FDA), Center for Biologics Evaluation and Research (CBER) as either standardized or nonstandardized products.

Nonstandardized Units of Potency

- **Weight per Volume (W/V):** Based on the weight of raw source material extracted with a given volume of extracting fluid (e.g., 1 g of raw pollen and 10 mL of extracting fluid).
- **Protein Nitrogen Units/milliliter (PNU/mL):** Based on a determination of the protein nitrogen content per milliliter of the final product. One protein nitrogen unit equals 0.00001 mg of phosphotungstic acid precipitable protein nitrogen dissolved in 1 mL of antigen extract.

> ***Key Fact***
>
> There is no bioequivalent relationship between W/V, PNU/mL, allergy unit (AU) or bioequivalent allergy unit (BAU) products. For example, a 1:10 W/V extract product is not necessarily the same as a 20,000 PNU/mL or a 10,000 BAU/mL extract product.

Standardized Units of Potency—Standardization means that the potency of an allergen extract has been compared with an FDA reference standard.

- **Allergy unit per milliliter (AU/mL)** and **bioequivalent allergy unit per milliliter (BAU/mL)**: Initiated by FDA to reflect allergenic activity more accurately. FDA reference preparations are assigned BAU/mL units based on results of a direct test of allergenic activity, which is derived from an intradermal skin test endpoint titration in humans measuring erythema; namely, the **ID_{50}EAL method** developed by CBER, FDA. It can also be determined by radioallergosorbent test (RAST) or enzyme-linked immunosorbent assay (ELISA-inhibition in comparison with an FDA reference standard. The dust mite extract AU, originally defined by inhibition assay, is equivalent to its BAU.
- **Major allergen content:** Ragweed extract products are labeled with the amount of major allergen content (Amb a 1). 350 Amb a 1 units = 100,000 BAU.

  Venom products (freeze-dried): Standardized on the basis of venom protein content (enzymes hyaluronidase and phospholipase). Individual species: 100 µg/mL and mixed vespid 300 µg/mL. (Note: Available fire ant extracts are nonstandardized whole-body extracts).

Key Fact

Five types of standardized extracts are currently available in the US: Hymenoptera venom (labeled in µg of protein: hyaluronidase and phospholipase), dust mite (labeled in AU), cat, grass (both labeled in BAU), and short ragweed (labeled by major allergen: Amb a 1).

Extract Forms

Aqueous Extracts—Raw source material (i.e., pollen, dander, mold or fungi cultures, and dust) is added to an extracting fluid and prepared in saline or buffer solutions, with less than 50% glycerin. 0.4% phenol is added to prevent microbial growth.

Glycerinated—Raw material is extracted in 50% glycerin or the glycerin is added after extraction. Fifty percent glycerin inhibits microbial growth and is more stable than aqueous allergen extract products. 0.4% phenol is added to prevent microbial growth.

Alum Precipitated—Allergenic proteins are precipitated with a solution of aluminum hydroxide (alum), forming a complex that results in slower release of the allergen. Used for treatment (but, not skin test diagnosis) due to their slow release nature. Larger doses of extract can be given at less frequent intervals, and they are reported to have a lower incidence of systemic reactions.

Lyophilized—Freeze-dried preparations existing in powder or dry cake form that need to be reconstituted. It is recommended that human serum albumin (HSA) diluent be used. Venom products are lyophilized.

Acetone Precipitated (AP)—The AP extraction process involves a specially developed technique designed to concentrate the proteins and remove lower-

molecular-weight nonspecific irritant materials. This process requires up to 50 times as much raw material as other extracts.

Stability of Allergen Extracts

Temperature—Loss of potency occurs when stored at higher temperatures for extended periods of time. To maintain potency, allergen extract products should be stored at 2–8°C (or 36–46°F).

Loss of potency when exposed to high temperatures may be due to heat-labile proteins and loss of potency due to extended periods of time at room temperature or may be due to protease activity in the extract solution. Fifth percent glycerin may protect against the effects of extended exposure to room temperature. Less is known about the effect of freezing on allergen extract solutions; however, numerous freeze-thaw cycles may contribute to loss of potency.

Concentration—Dilute extracts have lower protein content and lose their potency more rapidly than concentrated extract forms.

Vial Volume—Loss of potency is related to protein content. Thus, lower volume in a storage vial may increase loss of potency due to greater surface volume relative to volume of solution, as well as enhanced adsorption of proteins to the container wall.

Proteolytic Enzymes—Allergens with protease enzymes include cockroach, molds, and dust mite. Though dust mite products in the US do not seem to have adverse effects on pollen extracts. Consideration should be given to keeping extracts that have high proteolytic activity, such as fungi and cockroach, separate from pollen extracts. Grass pollen extracts are particularly susceptible to proteolytic enzymes.

Flash Card Q16

Which of the following describes a nonstandardized extract: AU/mL, BAU/mL, PNU/mL?

Presence of Stabilizers and Preservatives (Diluents)

Glycerin—At 50%, glycerin is the diluent best known to maximize the stability of an allergen extract product; it also inhibits bacterial growth. It appears to inhibit proteolytic enzymes, and its effectiveness decreases with a decreasing percentage of glycerin (e.g., 50% > 25% > 10%).

Flash Card Q17

Which extracts most consistently demonstrate proteolytic activity?

Human Serum Albumin (HSA)—This diluent has a preservative effect due to its reducing adsorption of allergenic proteins to vial surface. It is more effective than glycerin in protecting products from phenol denaturation.

Flash Card Q18

What diluent reduces allergen adsorption to vial surfaces?

Phenol—A 0.4% solution of phenol is the preservative added to the allergen extract solution to prevent microbial growth; but, it can break down allergenic proteins in allergen extract products containing 50% glycerin.

AUTOANTIGENS

The major factors that contribute to the development of autoimmunity are genetic susceptibility and environmental triggers.

Infections and tissue injury may alter the way in which self-antigens are displayed to the immune system, leading to a failure of self-tolerance.

- **Molecular mimicry**: Antigens associated with a microbe may cross-react with autologous antigens that involve normally dormant cells, which have escaped thymic deletion and can react with self-antigens.

In a phenomenon known as **epitope spreading**, autoimmune reactions initiated against one antigen that injures tissues may result in the release and alterations of other tissue antigens, activation of lymphocytes specific for these other antigens, and exacerbation of the disease.

In systemic autoimmunity, most of the autoantibodies do not appear to be directly pathogenic, yet they are often highly specific for a particular disease. There are also a number of models that indicate roles of various autoantigens in disease pathogenesis.

Flash Card A16

PNU/mL (protein nitrogen units/mL)

Although thousands of molecules are potential target autoantigens, the number of frequently targeted molecules is extremely restricted. This restriction has led to the proposal that the targeted autoantigens may have properties that make them immunogenic.

Disease-Associated Self-Antigens

Flash Card A17

Mold, cockroach

Self-antigens include autoantigens that are associated with autoimmune disease (organ-specific or systemic) and tumor antigens. A common feature of these disease-linked self-antigens is the appearance of antibodies against them as the disease progresses. A list of human diseases associated with specific autoantigens can be seen in Tables 11-7, 11-8, and 11-9.

Flash Card A18

Human serum albumin (HSA)

Table 11-7. Examples of Autoantigens Associated with Cell- or Organ-Specific Autoimmunity

Autoantigen	Clinical Association
Acetylcholine receptor	Myasthenia gravis
Actin	Chronic active hepatitis and primary biliary cirrhosis
Adenine nucleotide translocator (ANT)	Dilated cardiomyopathy and myocarditis
Aromatic L-amino acid decarboxylase	Autoimmune polyendocrine syndrome type I (APS-I)
Asialoglycoprotein receptor	Autoimmune hepatitis
Bactericidal/permeability-increasing protein (Bpi)	Cystic fibrosis vasculitides
Calcium-sensing receptor	Acquired hypoparathyroidism
Cholesterol side-chain-cleavage enzyme CYPlla)	APS-I
Collagen-type IV α3-chain	Goodpasture's syndrome
Cytochrome P450 2D6 (CYP2D6)	Autoimmune hepatitis
Desmin	Crohn's disease and coronary artery disease
Desmoglein 1	Pemphigus foliaceus
Desmoglein 3	Pemphigus vulgaris
F-actin	Autoimmune hepatitis
GM gangliosides	Guillain-Barré syndrome (GBS)
Glutamate decarboxylase (GAD65)	Type 1 diabetes and stiff person syndrome (SPS)
Glutamate receptor (GLUR)	Rasmussen's encephalitis
H/K ATPase	Autoimmune gastritis
17-α-Hydroxylase (CYP17)	APS-I
21-Hydroxylase (CYP21)	Addison's disease
IA-2 (ICA512)	Type 1 diabetes
Insulin	Insulin Type 1 diabetes and insulin hypoglycemic syndrome (Hirata disease)
Insulin receptor	Type B insulin resistance, acanthosis, and systemic lupus erythematosus (SLE)
Interphotoreceptor retinoid-binding protein (IRBP)	Uveitis
Intrinsic factor type 1	Pernicious anemia
Leukocyte function-associated antigen (LFA-1)	Treatment-resistant Lyme arthritis
Myelin-associated glycoprotein (MAG)	Polyneuropathy
Myelin basic protein	Multiple sclerosis and demyelinating diseases
Myelin oligodendrocyte glycoprotein (MOG)	Multiple sclerosis
Myosin	Rheumatic fever
p-80-Coilin	Atopic dermatitis
Pyruvate dehydrogenase complex-E2 (PDC-E2)	Primary biliary cirrhosis
Sodium iodide symporter (NIS)	Graves' disease and autoimmune hypothyroidism

Table 11-7. Examples of Autoantigens Associated with Cell- or Organ-Specific Autoimmunity, cont.

Autoantigen	Clinical Association
SOX-10	Vitiligo
Thyroid and eye muscle-shared protein	Thyroid associated ophthalmopathy
Thyroglobulin	Autoimmune thyroiditis
Thyroid peroxidase	Autoimmune Hashimoto's thyroiditis
Thyrotropin receptor	Graves' disease
Tissue transglutaminase	Celiac disease (CD)
Transcription coactivator p75	Atopic dermatitis
Tryptophan hydroxylase	APS-I
Tyrosinase	Vitiligo and metastatic melanoma
Tyrosine hydroxylase	APS-I

Table 11-8. Examples of Autoantigens Associated with Systemic Autoimmunity

Autoantigen	Clinical Association
Adrenocorticotropic hormone (ACTH)	ACTH deficiency
Aminoacyl-tRNA histidyl synthetase	Myositis and dermatomyositis
Aminoacyl-tRNA synthetase (several)	Polymyositis and dermatomyositis
C1 inhibitor	Autoimmune C1 deficiency
C1q	Systemic lupus erythematosus (SLE) and membrane proliferative glomerulonephritis (MPGN)
Cardiolipin	SLE
Carbonic anhydrase II	SLE, Sjögren's syndrome, and systemic sclerosis
Collagen (multiple types)	Rheumatoid arthritis (RA), SLE, and progressive systemic sclerosis
Centromere-associated proteins	Systemic sclerosis
Cytokines (IL-1α, IL-1β, IL-6, IL-10, LIF)	RA, systemic sclerosis, and normal subjects
DNA-dependent nucleosome-stimulated ATPase	Dermatomyositis
Fibrillarin	Scleroderma
Fibronectin	SLE, RA, and morphea
Glucose-6-phosphate isomerase	RA
β2-Glycoprotein I (β2-GPI)	Primary antiphospholipid syndrome
Glycoprotein IIb/IIIg and Ib/IX	Autoimmune thrombocytopenia purpura
Golgin (95, 97, 160, 180)	Sjögren's syndrome, SLE, and RA
Heat shock protein	Various immune-related disorders
Hemidesmosomal protein 180	Bullous pemphigoid, herpes gestationis, and cicatricial pemphigoid
Histone H2A-H2B-DNA	SLE

Table 11-8. Examples of Autoantigens Associated with Systemic Autoimmunity, cont.

Autoantigen	Clinical Association
IgA	Immunodeficiency
IgE receptor	Chronic idiopathic urticaria
Keratin	RA
Ku-DNA-protein kinase	SLE
Ku-nucleoprotein	Connective tissue syndromes
La phosphoprotein (La 55-B)	Sjögren's syndrome
Myeloperoxidase	Necrotizing and crescentic glomerulonephritis (NCGN) and systemic vasculitis
Oxidized LDL (OxLDL)	Atherosclerosis
Proteinase 3 (PR3)	Wegener granulomatosis and Churg-Strauss syndrome
Ro52	Sjögren's syndrome, rheumatoid arthritis, and systemic lupus erythematosus
RNA polymerase I-III (RNP)	Systemic sclerosis and SLE
Signal recognition protein (SRP54)	Polymyositis
Topoisomerase-I (Scl-70)	Scleroderma, Raynaud's syndrome or phenomenon (RP)
Tubulin	Chronic liver disease and visceral leishmaniasis
U1 ribonucleoprotein complex	Mixed connective tissue disease
Vimentin	Systemic autoimmune disease

Table 11-9. Examples of Autoantigens Associated with Cancer and Paraneoplastic Autoimmunity

Autoantigen	Clinical association
Amphiphysin neuronopathy	Small lung cell cancer
Cyclin B1	Hepatocellular carcinoma
DNA topoisomerase II	Liver cancer
Desmoplakin	Paraneoplastic pemphigus
Gephyrin	Paraneoplastic stiff man syndrome
Hu proteins	Paraneoplastic encephalomyelitis
Neuronal nicotinic acetylcholine receptor	Subacute autonomic neuropathy and cancer
p53	Cancer and systemic lupus erythematosus (SLE)
p62 (IGF-II mRNA-binding protein)	Hepatocellular carcinoma
Recoverin	Cancer-associated retinopathy
Ri protein	Paraneoplastic opsoclonus myoclonus ataxia
β IV spectrin	Lower motor neuron syndrome
Synaptotagmin	Lambert-Eaton myasthenic syndrome (LEMS)
Voltage-gated calcium channels	LEMS
Yo protein	Paraneoplastic cerebellar degeneration

Conclusion

A number of autoantigens have been either cloned and sequenced, or purified. Many of these are commercially available as recombinant proteins and can be used in immunoassays to detect autoantibodies in patients' sera.

INFECTIOUS AGENTS

Human Immunodeficiency Virus (HIV)

HIV is a member of the lentivirus group of retroviruses. It is an enveloped virus with a diploid single-stranded ribonucleic acid (ssRNA) genome. Notable proteins are discussed in Table 11-10.

Table 11-10. Notable Proteins

Gene	Proteins Encoded	Function
env	gp41	Hydrophobic portion mediates viral fusion with cell membrane. Target of fusion inhibitor drugs (e.g., enfuvirtide).
	gp120	Mediates virion binding to CD4 receptor. Rapid mutation rates in this gene have hampered the development of effective vaccines.
gag	p24	Main component of the viral capsid. Because its structure is reasonably well-conserved, it is one of three proteins tested for in a diagnostic Western blot.
	p6, p7	Nucleocapsid
	p17	Matrix
pol	Reverse transcriptase	RNA-dependent DNA polymerase. Also possesses ribonuclease and DNA-dependent DNA polymerase activity. Low-fidelity polymerase with high mutation rate. Target of reverse transcriptase inhibitors (e.g., nucleoside or nucleotide analog, and non-nucleoside inhibitors).
	Integrase	Responsible for viral integration into host genome. Target of raltegravir (integrase inhibitor).
	Protease	Aspartic protease necessary for viral protein production. Target of protease inhibitors
tat	Tat	Regulation of viral transcription. Diffusible action may be responsible for cell death of uninfected T-cells.
rev	Rev	Facilitates nuclear export of viral messenger ribonucleic acids (mRNAs). Plays role in late-phase gene expression.
nef	Nef	Reduces expression of major histocompatibility complex (MHC) class I molecules, inhibiting destruction of infected cells by CD8+ cytotoxic T-cells.
vif	Vif	Causes ubiquitinization and destruction of APOBEC3G, an antiviral protein that would otherwise cause hypermutation and destruction of viral genome.
vpr	Vpr	Facilitates nuclear import of HIV preintegration complex.
vpu	Vpu	Facilitates viral budding from infected cells.

Staphylococcus aureus

Staphylococcus aureus is clinically relevant bacterium as it produces a number of unique virulence factors. In addition, *S. aureus* may acquire resistance to methicillin by acquisition of *mecA* gene location on the staphylococcal cassette chromosome mec (SCCmec). Table 11-11 shows the virulence factors and functions of *S. aureus*.

Molecular Mimicry

Molecular mimicry describes when an environmental agent triggers the development of an immune response against an antigen that is similar enough to a self-antigen to cause autoimmunity. Several examples of molecular mimicry have been observed in disease processes:

- Reiter's syndrome (RS), following infections with *Chlamydia* or *Shigella*
- Guillain-Barré syndrome (GBS) following *Campylobacter* infections
- Rheumatic fever caused by the resemblance of the *Streptococcus pyogenes* M protein with cardiac myosin

Table 11-11. Virulence Factors and Functions of *Staphylococcus aureus*

Virulence Factor	Function
Protein A	Binds Fc region of IgG1, IgG2, and IgG4, inhibiting antibody-mediated clearance.
Clumping factor	Activates fibrinogen to insoluble fibrin, causing aggregation of bacteria.
Capsule	Acts to inhibit phagocytosis of bacteria by covering bound opsonins.
Cytotoxins	A group of five cytolytic toxins (α, β, γ, and δ toxins and P-V leukocidin).
Exfoliative toxins (ETA, ETB)	Two serine proteases that cleave desmoglein 1 in the stratum granulosum of the epidermis, resulting in staphylococcal scalded skin syndrome (SSSS), which is also known as Ritter von Ritterschein disease (in newborns), Ritter disease, and staphylococcal epidermal necrolysis.
Toxic shock syndrome toxin-1 (TSST-1)	Superantigen that elicits its response by binding to major histocompatibility complex (MHC) class II molecules outside of the peptide-binding groove and to the variable region of the TCR β chain. This leads to a massive release of cytokines (most notably TNFa, IL-1, and IL-6) and the ensuing capillary leakage syndrome. Of note, a disproportionately large number of superantigen-activated T cells express the skin-homing cutaneous lymphocyte antigen (CLA), which explains the propensity of bullous manifestations in toxic shock and also likely contributes to *S. aureus* colonization in atopic dermatitis.
Enterotoxins C and D	Enterotoxins C and D are found in contaminated food as preformed toxins. These both function as superantigens.

Abbreviations: Il, interleukin; TCR, T-cell receptor; TNF, tumor necrosis factor.

FOODS

Food allergens belong to a limited number of protein families with different molecular properties, which may mean routes of sensitization differ for different allergen families. For example, food allergens that sensitize through the gastrointestinal tract have features that enhance their stability to thermal and proteolytic denaturation. Table 11-12 gives a few examples of these protein families.

Table 11-12. Examples of Food Allergen Protein Families

Allergen Family	Function/Clinical Implication	Source (Allergen)
Food Allergens of Animal Origin		
Tropomyosins	Bind to actin in muscle, increasing thin filament stability and rigidity. Invertebrate tropomyosins are highly homologous and tend to be allergenic—those from crustaceans (e.g., shrimp, crab, crawfish, and lobster), arachnids (house dust mites), insects (cockroaches), and mollusks (squid, snails)—whereas vertebrate tropomyosin tends to be nonallergenic.	Brown shrimp (Pen a 1), Crab (Cha f 1), Oyster (Cra g 1, Cra g)
Parvalbumin	Control the flow of calcium from troponin C back to membrane-bound pumps after a muscle contraction	Salmon (Sal s 1.01), Tuna (Thu o 1.01), Carp (Cyp c 1.01), Frog (Ran e 1)
Caseins	Form stable micellar calcium phosphate protein complexes in mammalian milk. Explains some of the cross-reactivities amongst mammalian milks.	Cow (Bos d 8)
Food Allergens of Plant Origin		
11S (legumin like) globulins	Seed storage protein	Peanut (Ara h3, Ara h4), Soy (Glycinin), Buckwheat (Fag e 1), Cashew (Ana o 2)
Bet v 1 superfamily	Pathogen-related protein 10, comprises many of the class 2 proteins that cause OAS/PFAS	Apple (Mal d 1), Cherry (Pru av 1), Pear (Pyr c 1), Carrot (Dau c 1)
Nonspecific lipid transfer proteins	Function unknown, maybe involved in transport of suberin monomers. Highly resistant to heat and proteolytic digestion. While a class I allergen, it is concentrated in the peel of fruits and explains why some patients with true allergy to fruits can tolerated the peeled flesh.	Apricot (Pru ar 3), Cherry (Pru av 3), Peach (Pru p 3), Strawberry (Fra a 3), Grape (Vit v 1), Walnut (Jug r 3), Lettuce (Lac s 1), Corn (Zea m 14),
Chitinases	Enzymes catalyze the hydrolysis of chitin polymers. Class I chitinases contain *hevein* domain which shares high sequence identity with *Hevea brasiliensis* (latex)	Avocado (Pers a 1), Banana (Mus xp Chitinase)
Profilins	Regulates actin polymerization and depolymerization during cell movement and signaling. Present in some form in all eukaryotic cells. Heat labile. Risk factor for allergic reactions to multiple pollens and for oral allergy syndrome/pollen-food allergy syndrome (OAS/PFAS).	Pear (Pyr c 4), Cherry (Pru av 4), Celery (Api g 4), Latex (Hev b 8),

Key Fact

Multiple families of food allergy proteins may present similarly, but may infer different prognosis on the food allergy. Bet v 1 superfamily pathogen-related protein (PRP), nonspecific lipid transfer proteins and profilins all involve similar families (apple, pitted fruits etc). Profilins and Bet v 1 superfamily PRP both may present as OFAS and are class 2 allergens. Lipid transfer proteins, however, are class 1 allergens that cover a similar spectrum of foods.

Food allergens can be categorized as follows:

- Class 1: Traditional food allergens (Table 11-13)
- Class : Consequence of an allergic sensitization to inhalant allergens (i.e. associated with oral allergy syndrome [OAS]) (Table 11-14)

Having a basic understanding of allergens families helps to explain cross-reactivity amongst allergens and phenomenon such as oral allergy syndrome/pollen-food allergy syndrome (OAS-PFAS)

Most Common Food Allergens

Cow's Milk

- Prevalence: **Most common** food allergy in children
- Allergens: Casein and whey. Pasteurization does not denature these proteins.
- Cross-reactivity: Among various types of milk (i.e., cow, goat, and sheep). Cow's milk has a cross-reactivity of 10% with beef, 92% with goat's milk, and 4% with mare's (or horse's) milk.

Egg

- Prevalence: Allergy is typically to the egg white; the yolk is less allergenic.
- Allergens:
    - Egg white has 23 glycoproteins. The major ones are ovomucoid (heat-stable), ovalbumin (heat-labile), and ovotransferrin.
    - The common egg yolk proteins are apovitellin livetin, and vosvetin.

Wheat

- Prevalence: Allergy has a prevalence of 0.4% in children.
- Allergens: Globulin and glutenin are the major allergenic fractions in IgE-mediated reactions, gliadin in celiac disease, and albumins in baker's asthma.
- Cross-reactivity: The grains have a 20% cross-reactivity with each other.

Peanuts—In the legume family.

- Prevalence: The major food allergen beyond 4 years of age.
- Allergens: The major allergens that cause severe clinical reactions are Ara h1, Ara h2, and Ara h3.
    - Ara h8 is associated more with oral allergy syndrome (OAS).
    - Ara h 2 is the dominant peanut allergen detected in 90–100% of patients with peanut allergy.
    - Ara h 2 plasma sIgE test levels provide higher diagnostic accuracy than whole-peanut plasma sIgE levels.
- Cross reactivity: 5% cross-reactivity with other legumes (e.g., as peas).

Soybeans—A second legume also an important player in food allergens.

- Prevalence: 0.4% in children.

Flash Card Q19

What is the dominant peanut allergen detected in 90–100% of patients with peanut anaphylaxis?

Flash Card Q20

Allergy to which peanut allergen confers the best prognosis***?***

- Cross-reactivity: 5% cross-reactivity with peanuts. The soy proteins are Gly m3.

Tree Nuts

- Prevalence: 0.6% of Americans.
- Cross-reactivity: Major cross-reactivity among the tree nuts. About 35–50% of peanut-allergic patients can also be allergic to at least one tree nut.

Fish

- Allergens: Very susceptible to heat processing. The major allergen is Gad c 1 in codfish and Sal s1 in salmon**. In finned fish, parvalbumin is the dominant allergen.**

Shellfish—Divided into two families: mollusks (e.g., clams, oysters, and mussels) and crustaceans (shrimp, crab, and lobster).

- Allergen: The major allergen is tropomyosin, which is also found in other invertebrates (e.g., cockroach and dust mite).
- Cross-reactivity: 75% with other shellfish. Cross-reactivity between shellfish and mollusks is not well defined.

Table 11-13 provides an overview of the most common food allergens.

Key Fact

Within the tree nut family, the strongest probability of cross-reactivity is between pistachio and cashew, and also walnut and pecan.

Mnemonic

To recall common food allergens, remember:

- **Milk** proteins are the **bos**s, especially in kids under 1 year of age.
- The chicken **gal**lantly defends his **eggs**.
- The **ar**rog**a**nt **peanut** scares parents.
- **Fish gad**da swim upstream.
- **Soy gly**des into many recipes.
- A bad **Apple** is **mal.**
- **Jug**head hits his head on **walnuts.**
- Most people are **pru**dent about their fruit, only picking the freshest **plums**, **peaches**, and **cherri**es.
- Ella eats **corn** with **zea**l.

Flash Card A19

Ara h 2

Flash Card A20

Ara h 8

Table 11-13. Major Class I Food Allergens

Foods	Proteins	Food Allergen
Cow's milk	Caseins αs1-Casein αs2-Casein β-Casein κ-Casein	Bos d 8
	Whey β-Lactoglobulin α-Lactalbumin Serum albumin	Bos d 5 Bos d 4 Bos d 6
Chicken egg white	Ovalbumin Ovomucoid Ovotransferrin	Gal d 1 Gal d 2 Gal d 3
Wheat	Globulin Glutenin	
Peanut	Vicilin Conglutin Glycinin	Ara h 1 Ara h 2 Ara h 3
Soybean	Glycinin G1 acidic chain Profilin	Gly m 3
Fish	Codfish—Parvalbumin	Gad c 1
	Salmon	Sal s 1
Shrimp	Tropomyosin	Pen a 1

Homology of Food Proteins

Many foods are related botanically or by a high degree of homologous proteins (Table 11-14).

Table 11-14. Major Class II Food Allergens: Major Plant-Derived Allergens

Plant Allergen	Protein	Food	Food Allergen
Latex-fruit cross reactivity	Class I chitinases	Avocado, chestnut, banana	
	Thaumatin-like	Cherry	Pru A2
		Apple	Mal d2
Birch	Bet v1 homologues = pathogen-related proteins 10	Apple	Mal d 1
		Cherry	Pru a1
		Pear	Pyr a1
		Celery	Api g1
		Carrot	Dau c1
		Potato	
Celery-mugwort spice syndrome	Bet v2 homologues = Profilin	Latex	Hev b 8
		Celery	Api g 4
		Potato	
		Cherry	Pru av 4
		Pear	Pyr c 4
		Peanut	Ara h 5
		Soybean	Gly m 3
		Apple, tomato, and carrot	
	Lipid-transfer proteins	Peach	Pru p
		Apple	Mal d3
		Soy	
Seed Storage Proteins	2S albumin	Mustard	Sin a1
		English walnut	Jug r1
		Rapeseed and Brazil nut	
	Vicilin	Peanut	Ara h1
		Walnut	Jug r1
	Conglutin	Peanut	Ara h2
	Glycinin	Peanut	Ara h3
		Soy	
	β-Glycinin	Soy	

Table 11-15 highlights the interactions between pollens and foods in pollen-food allergy syndrome.

Table 11-15. Cross-Reacting Pollens and Foods

Pollen	Foods
Birch = Bet v1	Apple, apricot, carrot, cherries, plums, potato, kiwi, celery, hazelnuts, almonds, and walnuts
Ragweed = Amb a1	Banana, cucumber, melon (i.e., watermelon, cantaloupe, honeydew), zucchini, dandelions, and chamomile tea
Grass	Peaches, celery, melons, tomatoes, and oranges
Mugwort	Celery, apple, kiwi, peanut, carrots, and sunflower
Alder	Celery, pears, apples, almonds, cherries, hazelnuts, peaches, and parsley
Latex	Bananas, avocado, kiwi, chestnut, papaya, tomato, and potato

VENOM ALLERGEN AND ANTIGENS

Venom Composition

Key Fact

Alkaloids in fire ant venom cause the sterile pustule, but do not contribute to its allergic potential.

The major allergen of honeybee venom is **phospholipase A2**. **Melittin** is a unique honeybee allergen and makes up 50% of the venom protein. The major allergen of vespid venoms is **antigen 5**, unique to this group. Vespid **phospholipase A1** is also a major allergen. Both apids and vespids contain **hyaluronidase**. Imported fire ant (IFA) venom is primarily composed of **alkaloids**, with a small amount of aqueous proteins that are similar to other venoms and are responsible for allergic reactions (Table 11-16).

Cross-Reactivity of Venoms

Key Fact

Imported fire ant (IFA) venoms are not commercially available; whole-body extract (WBE) is used for diagnosis and treatment of fire ant allergy.

Phospholipase A2, hyaluronidase, and acid phosphatase are similar in patients who are allergic to either honeybee and bumblebee stings; and sera from many honeybee-allergic patients shows cross-reactivity with bumblebee venoms.

Venoms among the vespids and *Polistes* wasp demonstrate extensive cross-reactivity. Hyaluronidases show some cross-reactivity among families, but phospholipases do not cross-react between families. The venom of *Solenopsis invicta* (*S. invicta*, also known as the red imported fire ant) and *Solenopsis richteri* (*S. richteri*, also known as the black imported fire ant and as its own species) have extensive cross-reactivity.

Table 11-16. Stinging Insect Allergens

Family	Subfamily	Venom Allergen	Name/ Function	Other
Apidae	Honeybee	Api m 1	**Phospholipase A2**	The major allergen; cross-reactive with Bom p1 •Used to standardize extracts
		Api m 2	Hyaluronidase	A major allergen
		Api m 3	Acid phosphatase	
		Api m 4	Melittin	Unique antigen to this group
	Bumblebee	Bom p 1	Phospholipase A2	Cross-reactive with Api m1
		Bom p 4	Protease	
Vespidae	Vespinae and Polistinae	Grp 1	Phospholipase A1	A major allergen; not cross-reactive with phospholipase A2 in Apidae
		Grp 2	**Hyaluronidase**	A major allergen; similar to hyaluronidase of Apidae •Used to standardize extracts
		Grp 3	Acid phosphatase	
		Grp 4	Protease	
		Grp 5	**Antigen 5**	The major allergen; unique antigen to this group
Formicidae	Fire ants	Sol i 1	Phospholipase A1	Cross-reacts with sera from vespid-allergic patients
		Sol i 2	Unknown protein	
		Sol i 3	Antigen 5 protein family	Has homology with vespid antigen 5, but no cross-reactivity
		Sol i 4	Unknown protein	Has homology with Sol i 2 but no cross-reactivity

Standardization

Honeybee venoms are standardized for their content of **phospholipase A** and *Vespula* venoms are standardized for their content of **hyaluronidase**. The most common source for venom proteins in stinging insects are from the venom sacs of bees, wasps, and hornets. Fire ant venom proteins have been found to be present in sufficient concentration in nonstandardized WBE to be suitable for use as diagnostic allergens and therapeutic vaccine

Flash Card Q21

What is the major allergen for honeybee? What is the major allergen for yellow jacket?

Flash Card Q22

What venom protein is most cross-reactive between honeybee and yellow jacket?

Flash Card Q23

What venom protein is used to standardize honeybee extract? What is used to standardize yellow jacket and wasp extracts?

Stability

Proteins in concentrated venom solutions are relatively stable and will remain constant through the manufacturer's expiration date, whereas diluted reconstituted vaccines are less stable. Smaller proteins in venoms are not as stable; so, processed venoms are not as potent as venom from a natural sting. Extracts should be stored at 4°C (or 36°F) to decrease rate of potency loss. Proteases in venoms can decrease stability, which can be attenuated by mixing with solutions of glycerin or human serum albumin. Human serum albumin also helps decrease adsorption of allergenic proteins to storage vials.

Flash Card A21

Phospholipase A2 for honeybee. Antigen 5 for yellow jacket

Flash Card Q22

Hyaluronidase

Flash Card Q23

Phospholipase A2 for honeybee. Hyaluronidase for yellow jacket and wasp

ABOUT THE EDITORS

Tao Le, MD, MHS

Dr. Le developed a passion for medical education as a medical student. He currently edits more than 15 titles in the *First Aid* series. In addition, he is the founder of the USMLE-Rx online video and test bank series as well as a cofounder of the *Underground Clinical Vignettes* series. As a medical student, he was editor-in-chief of the University of California, San Francisco (UCSF) Synapse, a university newspaper with a weekly circulation of 9000. Dr. Le earned his medical degree from UCSF in 1996 and completed his residency training in internal medicine at Yale University and fellowship training at Johns Hopkins University. At Yale, he was a regular guest lecturer on the USMLE review courses and an adviser to the Yale University School of Medicine curriculum committee. Dr. Le subsequently went on to cofound Medsn, a medical education technology venture, and served as its chief medical officer. He currently has an interest in medical education and education research at the University of Louisville.

Bret Haymore, MD

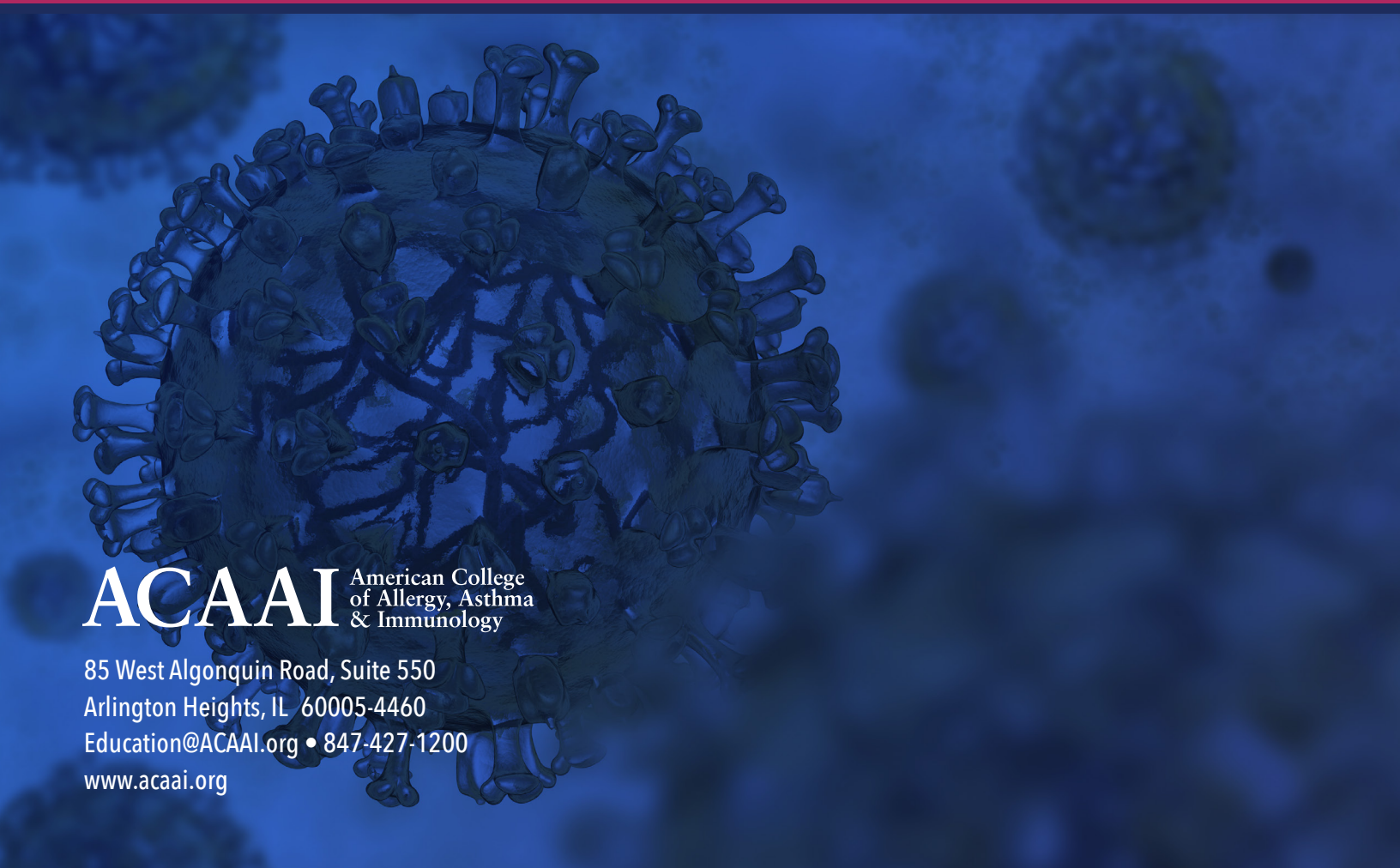
Dr. Haymore received his medical degree from the Penn State College of Medicine in 2002 where he was also elected to the AOA. He completed residency training in internal medicine at William Beaumont Army Medical Center where he remained as chief medical resident. He completed his allergy-immunology fellowship at Walter Reed Army Medical Center and remained as the clinical service chief until July 2011. Currently he is medical director at *Breathe*America Tulsa and assistant professor in the Department of Medicine at the University of Oklahoma College of Medicine-Tulsa. He has received numerous teaching and research awards along with having served as chair of the FIT section on the ACAAI Board of Regents from 2007-2008. He enjoys many activities with his wife and five children including sports and outdoor activities.

Vivian Hernandez-Trujillo, MD

Dr. Hernandez-Trujillo has had an interest in medical education since 1999. She earned her medical degree from Albany Medical College in 1999 and completed her residency training in pediatrics at Miami Children's Hospital and allergy and immunology fellowship training at the University of Tennessee-Memphis. Dr. Hernandez-Trujillo has received teaching awards for her work with pediatric residents. She has written textbook chapters and peer-reviewed articles and lectures regularly, both nationally and internationally, on allergy and immunology topics. She is involved in national and international education on the topics of anaphylaxis, food allergy, allergic rhinitis, and primary immunodeficiency diseases. She is the director of the Division of Allergy and Immunology at Miami Children's Hospital, and Clinical Assistant Professor at the Herbert Wertheim College of Medicine in Miami, Florida. She enjoys dancing, music, spending time with her family, and traveling.

Gerald Lee, MD

Dr. Lee received his medical degree from Case Western Reserve University in 2003. He completed residency training in a combined Internal Medicine/Pediatrics residency at St. Vincent’s Hospital in New York, NY where he also was the pediatric chief resident. He completed his fellowship in allergy/immunology at Cincinnati Children’s Hospital Medical Center in 2011 and is currently an assistant professor at the University of Louisville. He is currently pursing a Master of Education for medical educators and sits on the Educational Policy Committee for the medical school. He is also vice-chair of the ACAAI continuing medical education committee. In his spare time he enjoys marathon running and fiddling with technology.



ACAAI American College of Allergy, Asthma & Immunology

85 West Algonquin Road, Suite 550
Arlington Heights, IL 60005-4460
Education@ACAAI.org • 847-427-1200
www.acaai.org