1. Defects in ______ causes a combined immunodeficiency of B & T-cells associated with increased radiosensitivity in humans.
   A. RAG 1 and RAG 2
   B. DNA-PK
   C. Artemis
   D. Ku70:Ku80

2. The majority of T-cell receptor diversity is concentrated into which regions?
   A. CDR2
   B. TCRa
   C. CDR1
   D. TCR g:d

3. Fc region of antibodies participate in which of the following actions?
   A. Bind Fcg receptors on neutrophils & macrophage to facilitate phagocytosis
   B. Bind tightly to toxin or virus and prevent recognition by host cell
   C. Bind C1q complement protein to initiate alternative pathway of complement activation
   D. Deliver antibodies into secretions (tears, maternal milk), across mucosal membranes and into fetal circulation by passive diffusion.

4. Polymerization of IgM & IgA is made possible by covalently linking cysteine residues present in the C region to residues on which of the following?
   A. Polyadenylation sites
   B. J segment
   C. FcRN
   D. J chain

5. The “12/23 Rule” is defined as?
   A. Nonamer and Heptamer conserved blocks of nucleotides present within the (RSS) Recombination Signal Sequence.
   B. A gene segment flanked by RSS with a 12-bp spacer can only be joined to one flanked by 23-bp spacer.
   C. The number of antigen binding sites present on transmembrane and soluble pentameric IgM.
   D. The number P-nucleotides and N-nucleotides added by Tdt, respectively.

6. In RAG-dependent V(D)J rearrangement, once the RAG complex has synapsed with the second RSS, endonuclease activity of which of the following parts of the V(D)J recombinase makes single-stranded cuts in the DNA backbone?
A. DNA-PK  
B. TdT  
C. DNA ligase IV  
D. RAG

7. A baby presents with generalized erythroderma, desquamating skin, diarrhea, and recurrent bacterial infections. Lab results are notable for absence of circulating B cells and autoreactive T cells. Further studies reveal a mutation in which of the following?  
A. DNA-PK  
B. RAG-1 or RAG-2  
C. DNA ligase IV  
D. TdT

8. Which part of the TCR maintains direct contact with the antigenic peptide component of the peptide: MHC complex?  
A. CDR1  
B. CDR2  
C. CDR3  
D. TCR-alpha C region

9. Which of the following immunoglobulin subclasses can activate the classical pathway of complement via interaction with C1q?  
A. IgE only  
B. IgG only  
C. IgA only

10. Transmembrane forms of immunoglobulins are derived when the heavy-chain RNA sequence is cleaved and polyadenylated at which site?  
A. pAₚ site  
B. pAₘ site  
C. Constant region  
D. DJ region

Answers  
1. C, page 166  
Defects in Artemis produce combined immunodeficiency of B & T-cells associated with increased radiosensitivity. SCID due to mutations in DNA-repair genes is called irradiation-sensitive SCID.

2. B, pages 172–173  
Structural diversity of TCR is attributable mainly to combinatorial and junctional diversity generated during gene rearrangement. Most variability in the TCR is within junctional regions, encoded by V, D, J regions. TCRα locus contains more J gene segments, thus the variability generated in this region is even greater for T-cells than immunoglobulins. Thus, most of the diversity resides in CDR3 loops that contain junctional region and form center of antigen binding site.
3. A, page 175
Fc regions of antibodies have 3 main functions: 1) bind specialized Fc receptors on immune effector cells including Fcg on phagocytes to induce phagocytosis, 2) bind C1q to initiate classical complement activation (not alternative) and 3) engage specific receptors like FcRN, to induce active transport of immunoglobulin into different body compartments. Fab region of antibodies bind toxins and viral components in attempts for neutralization and prevent host recognition.

4. B, page 178
IgA & IgM can form multimers. Tailpiece of C regions contain cysteine residue essential for polymerization. A separate chain, called the J chain, promotes polymerization by linking to the cysteine of tailpiece.

5. B, page 161
A gene segment flanked by RSS with a 12-bp spacer typically can be joined only to one flanked by a 23-bp spacer RSS. Therefore, a D gene segment can be joined to a J gene segment and a V gene segment to a D gene segment but V gene segments cannot join V gene segments directly, as both V & J are flanked by 23bp spacers and D gene segments have 12-bp spacers on both sides.

6. D, pages 164-165
In the cleavage step, the endonuclease activity of RAG makes single-stranded cuts in the DNA backbone between each coding segment and its RSS. This leads to formation of covalently closed DNA hairpin ends. The endonuclease activity is thought to reside in RAG-1.

7. B, page 166
This clinical presentation is consistent with Omenn syndrome which is due to partial V(D)J recombinase activity stemming from mutated RAG-1 or RAG-2.

8. C, page 171
The less variable CDR1 and CDR2 loops of a T-cell receptor will mainly contact the relatively less variable MHC component of the ligand, whereas the highly variable CDR3 regions will mainly contact the unique peptide component.

9. D, pages 174–175
Only IgG and IgM can bind C1q to trigger complement.

Transmembrane and secreted forms of immunoglobulins are derived from the same heavy-chain sequence by alternative RNA processing. Each heavy-chain C gene has two potential polyadenylation sites (pAs and pAm) and depending on where gene splicing occurs, the SC sequence may or may not be removed. When the transcript is polyadenylated and cleaved at the pAm site, the SC sequence is removed and a transmembrane form of Ig results.
Chapter 5, Part II (pages 179- end of chapter): The Generation of Lymphocyte Antigen Receptors

Prepared by Tara Shankar, MD, Children’s Hospital of Pittsburgh of UPMC, and Andrew Nickels, MD, Mayo School of Graduate Medical Education

1. Secondary diversification occurs by what three methods?
   A. Gene rearrangement, VDJ recombination, somatic hypermutation
   B. Somatic hypermutation, class switching, gene conversion
   C. Gene conversion, VDJ recombination, affinity maturation
   D. Affinity maturation, class switching, VDJ recombination

2. What enzyme initiates secondary diversification of antibody?
   A. AID
   B. RAG
   C. UNG
   D. APE 1

3. Which of the following is true about AID?
   A. It is expressed on both T and B cells.
   B. It can bind to and deaminate only single strand DNA.
   C. It deaminates adenosine residues.
   D. It excises an abasic residue to create a single-strand DNA nick.

4. What is a consequence of somatic hypermutation?
   A. IgM can switch to the other immunoglobulin classes.
   B. Provides antibodies with the same antigen specificity but distinct effector capacities.
   C. B cells expressing mutant immunoglobulins that bind antigen better are selected against.
   D. Creation of high-affinity secreted antibodies.

5. A 4-year-old male presents with recurrent ear and sinus infections. Laboratory studies obtained demonstrate low IgG, low IgA, and elevated IgM. Lymphocyte subsets are normal but flow cytometry demonstrates low switched memory B cells. Gene sequencing confirms AID deficiency and the patient is started on IVIG. Why is the patient not placed on PCP prophylaxis?
   A. HSCT should be considered first given low survival rate without transplantation
   B. The patient had appropriate T cell numbers on lymphocyte subsets
   C. AID is expressed specifically in activated B cells, not T cells
   D. IVIG will provide appropriate PCP coverage

6. Apurinic/apyrimidinic endonuclease 1 (APE 1) has the following function in secondary antibody diversification?
   A. Removes the uracil base from the uridine to create an abasic site in the DNA.
   B. Excises the abasic residue to create a single strand discontinuity (known as a single-strand nick) in the DNA at the site of the original cytidine.
   C. Bind to and deaminate single stranded DNA only (not double stranded DNA).
   D. Deaminate cystidine residues in the immunoglobulin V regions.
7. The $V_H$-region exons expressed by any given B Cell are determined while the B Cell is in what anatomic location?
A. Lymph node
B. Peripheral blood
C. Bone marrow
D. Spleen

8. A 7-year-old boy presents to clinic with a history of two chest x-ray-documented pneumonias and a wobbly gait. He began walking at a normal age, but never developed appropriate gait. His parents report that about 4 years of age they noticed his right eye had extra blood vessels and recently they have noticed he has developed difficulty holding a smooth gaze. You make the appropriate diagnosis. Patients with this condition sometimes have impaired class switching due to mutations in what DNA repair protein?
A. Apurinic/apyrimidinic endonuclease 1 (APE 1)
B. Activation-induced cytidine deaminase (AID)
C. Uracil-DNA glycosylase (UNG)
D. DNA-PKcs-family kinase ATM

9. Which of the following statements is accurate about the similarities/differences between class switch recombination and V(D)J recombination?
A. V(D)J recombination occurs only after antigen stimulation and class switch recombination occurs without antigen stimulation.
B. Class switch recombination is not random and is directed external signals; V(D)J recombination is a random process.
C. Class switch recombination requires RAG enzymes and V(D)J does not.
D. All class switch and V(D)J recombination results are productive.

10. Which of the following events is an irreversible event occurring only in the B Cell (i.e. not T Cell) that is due to DNA point mutation?
A. Switch recombination
B. IgM, IgD expression on surface
C. Somatic hypermutation
D. Junctional diversity

Answers
1. B, page 179
Diversification is achieved through three mechanisms: somatic hypermutation, class switching, and gene conversion—which alter the sequence of the secreted immunoglobulin in distinct ways.

2. A, page 180
Like RAG-mediated V(D)J recombination, all these processes result in irreversible somatic mutation of the immunoglobulin genes, but unlike V(D)J recombination they are initiated by an enzyme called activation-induced cytidine deaminase (AID).

3. B, page 180
AID can bind to and deaminate single-stranded DNA but not double-stranded DNA.

4. D, page 183
Hypermutation is simply an adaptive specialization for B cells to make very high-affinity secreted antibodies.

5. C, page 180
AID is expressed specifically in activated B cells. These processes do not occur in T-cell receptor genes.

6. B, page 182
APE1 Excises the abasic residue to create a single strand discontinuity (known as a single-strand nick) in the DNA at the site of the original cytidine. Uracil-DNA glycosylase (UNG) removes the uracil base from the uridine to create an abasic site in the DNA. Activation-induced cytidine deaminase (AID) bind to and deaminate single stranded DNA only (not double stranded DNA) and act to deaminate cystidine residues in the immunoglobulin V regions.

7. C, page 184
The VH- region exons expressed by any give B Cell are determined during its early differentiation in the bone marrow.

8. D, page 186
The diagnosis is Ataxia telangiectasia. Class switching is sometimes impaired in the disease ataxia telangiectasia due to mutations in the DNA-PKcs-family kinase ATM.

9. B, page 186
Class switch recombination is not random but is directed by external signals such as those provided by T-cells. A is false: class switch recombination occurs after antigen stimulation and not during the B-Cell development in the bone marrow (like V(D)J recombination does). C is false: Class switch recombination does not require RAG enzymes and V(D)J recombination does. D is false: some V(D)J recombination does not lead to a product.

10. C, page 187 Fig. 5.26
Somatic hypermutation is an irreversible, B Cell only event that is due to DNA point mutation. Switch recombination is a B Cell only process and is irreversible but is due to somatic recombination of DNA. IgM, IgD expression on surface is a B cell only event but is reversible and due to differential splicing of RNA. Junctional diversity occurs in both B and T cells, is irreversible, and is due to imprecise joining, N-sequence insertion in DNA.