Allergy and Immunology Board Review Corner

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Question 1: Which of the following is best described as a large group of secreted proteins with diverse structures and functions, which regulate cell migration and movement of the cells of the innate and adaptive immune system?

a. Antibody
b. Chemokines
c. Antigens
d. Immunogens

Answer: B. Page 2. Chemokines are structurally related class of cytokines that regulate cell migration and movement in the immune system.

Question 2: Which of the following reactions would you expect to occur within 0-6 hours of an active infection?

a. Naïve B-Cell activated by antigen presenting cell with T Cell help.
b. Class switch recombination of activated B Cells within the lymph nodes
c. Alternate complement system activation
d. Increase T-Cell secretion of IL-2 to promote clonal proliferation of antigen specific T-Cells

Answer: C. Page 2-3. Of the choices, the activation of the complement system is the only innate immune system mechanism in the list of choices. The innate immune system is activated in the first 6 hours to 12 hours of an infection. The remainder of the choices are involved in the adaptive immune response, which typically takes >12 hours to fully begin responding.

Question 3: Which of the following is a characteristic of the innate immune system?

a. Immunologic memory derived specifically from past antigen exposures
b. Limited diversity that is dependent on germline encoded receptors
c. Lymphocytes are the major cell type
d. Receptors that are produced via DNA recombination

Answer: B. Page 3 Table 1-2. The innate immune system has limited diversity that is derived from germline encoded mechanisms. These do not the change in the face of infection or a specific antigen.

Question 4: A 28 year old male is brought into the emergency room after a snake bite. In the course of treating the patient, he is given snake “anti-venom”. This is an example of which of the following concepts?
a. Adaptive immunity
b. Active Immunity
c. Cellular Immunity
d. Passive Immunity
e. Autoimmunity

Answer: D. Page 4. Administration of anti-venom is a form of passive immunization. Antibodies against the snake toxin are given. This allows the conferring of resistance to the toxin rapidly without having to wait for the adaptive immune system to develop a full response.

Question 5: Which feature of the adaptive immune system is best described by the following statement: ensures that the immune response to a microbe (or non-microbial antigen) is targeted to that microbe (or antigen)?

a. Specificity
b. Diversity
c. Memory
d. Specialization
e. Clonal expansion

Answer: A. Page 13. Specificity ensures that the immune response to a microbe (or non-microbial antigen) is targeted to that microbe (or antigen)?

Question 6: Which feature of the adaptive immune system is best described by the following statement: Increases the ability to combat repeat infections by the same microbe?

a. Specificity
b. Diversity
c. Memory
d. Specialization
e. Clonal expansion

Answer: A. Page 13. Memory increases the ability to combat repeat infections by the same microbe/antigen.

Question 7: In the context of the adaptive immune system, which of the follow cells is thought to help macrophages to eliminate ingested microbes and also help B cells to produce antibodies?

a. CD8+ Lymphocytes
b. CD4+ Lymphocytes
c. NK Cells
d. Dendritic Cells
Answer: B. Page 10-12. CD4+ helper T Lymphocytes have a number of helper functions including assisting macrophages to produce antimicrobial substances to kill phagocytized material and also helps B cells activate and produce antibodies.

Question 8: What cytokine is secreted by activated naïve CD4+ T cells and acts as a growth factor to stimulate the clonal expansion of the antigen specific T Cells?

a. IL-25
b. IL-16
c. IL-12
d. IL-8
e. IL-2

Answer: E. Page 11. IL-2 is secreted by activated CD4+ T Cells and acts as a growth factor to stimulate clonal expansion of the antigen specific T cell.

Question 9: Which of the following component of the immune system is thought to play a crucial role in the initiation and development of the adaptive immune system by capturing and displaying the specific antigens?

a. T Lymphocytes
b. Antibodies
c. Complement
d. Dendritic Cells

Answer: D. Page 8. Antigen presenting cells play a crucial role in the initiation and development of the adaptive immune response. Dendritic cells are the most specialized of the APCs and capture microbial antigens to present to other components of the immune system.

Question 10: What effect do microbes typically have on antigen presenting cells?

a. Up-regulation of costimulatory molecules
b. Up-regulation of molecular mechanisms for class switch recombination
c. Down-regulation of the DNA repair gene TdT
d. Down-regulation of MHC class I and II on the surface of the APC

Answer: A Page 10. T-Cells need more than just the antigen to become activated. Antigen presenting cells have “costimulators” that allow the T-Cells to become active. When APCs encounter an antigen, their costimulatory molecules are often upregulated. APCs do not produce antibodies, which would require class switch recombination. APC’s do not undergo changes to their somatic DNA that require TdT for their immune function.
Question 1. Kupffer cells, microglial cells and osteoclasts are all examples of what type of immune system cell?

   a. Dendritic cells  
   b. Macrophages  
   c. Monocytes  
   d. Neutrophils

Answer: B. Pages 14-15. These cells are all examples of macrophages. Upon tissue damage, or infection, monocytes are rapidly recruited to the tissue where they differentiate into tissue macrophages. Additionally, many tissues are populated with long-lived resident macrophages derived from yolk sac or fetal liver precursors during fetal development. These resident macrophages assume specialized phenotypes depending on the organ where they reside. Examples include: microglial cells in the brain, alveolar macrophages in the lungs, Kupffer cells in the liver, sinusoidal macrophages in the spleen and osteoclasts in the bone.

Neutrophils are produced in the bone marrow and arise from precursors that also give rise to mononuclear phagocytes (monocyte, macrophage), however, these cells are polymorphonuclear and mediate the earliest phases of inflammatory reactions.

Dendritic cells are antigen presenting cells. They capture and display antigens to T-lymphocytes in cell-mediated and humoral immune responses. Unlike macrophages, dendritic cells do not ingest and kill microbes or dead host cells. Subsets of dendritic cells include: classical, plasmacytoid, inflammatory and Langerhans cells.

Question 2. Which of the following immune system cells contain cytoplasmic granules filled with defensins and cathelicidins?

   a. Basophils  
   b. Eosinophils  
   c. Mast Cells  
   d. Neutrophils

Answer: D. Pages 14-16. Neutrophils contain granules of two types. The majority, called specific granules, are filled with enzymes such as lysozyme, collagenase and elastase. These granules do not stain strongly with basic or acidic dyes which distinguishes neutrophil granules from those of basophils and eosinophils. The remainder of the granules of neutrophils, called
azurophilic granules are lysosomes that contain enzymes and other microbiocidal substances including defensins and cathelicidins.

Basophils contain granules that bind basic dyes and are capable of synthesizing many of the same mediators as mast cells, such as heparin, histamine and leukotrienes. Eosinophilic granules contain enzymes, such as eosin, that are harmful to the cell walls of parasites but can also damage host tissues. Mast cells contain abundant cytoplasmic granules filled with histamine and other mediators.

Question 3. What cytokine is essential for mast cell development?

   a. c-KIT ligand  
   b. CD40 ligand  
   c. Fas ligand  
   d. Flt3 ligand

Answer: A. page 16 Stem cell factor, or c-KIT ligand, is essential for mast cell development.

CD40 ligand binds to CD40 which delivers signals leading to activation of B cells, macrophages and endothelial cells. Flt3 ligand binds to Flt3 tyrosine kinase on precursor cells and is responsible for dendritic cell maturation. Fas ligand binds to Fas which delivers signals leading to apoptotic death.

Question 4. Which of the following promotes eosinophil maturation from myeloid precursors?

   a. IL-2  
   b. IL-4  
   c. GM-CSF  
   d. M-CSF

Answer: C. page 17. The cytokines GM-CSF, IL-3 and IL-5 promote eosinophil maturation from myeloid precursors. IL-2 promotes proliferation and differentiation of naïve T-lymphocytes into effector and memory T cells. It also promotes regulatory T cell development, NK cell proliferation and activation and B cell proliferation.

   IL-4 promotes isotype switching to IgE, proliferation of mast cells, TH2 proliferation and differentiation and plays a role in macrophage activation.

Cells of the macrophage lineage arise from committed precursor cells in the bone marrow, driven by monocyte (or macrophage) colony-stimulating factor (M-CSF).
Question 5. What cytokine is responsible for dendritic cell maturation?

a. Flt3
b. Flt3 ligand
c. IL-2
d. IFN-gamma

Answer: B. Page 17. Maturation of dendritic cells is dependent on a cytokine called Flt3 ligand which binds to the Flt3 tyrosine kinase receptor on the precursor cells.

IL-2 promotes proliferation and differentiation of naïve T-lymphocytes into effector and memory T cells. It also promotes regulatory T cell development, NK cell proliferation and activation and B cell proliferation.

IFN-gamma promotes classical activation of macrophages, isotype switching to opsonizing and complement fixing IgG subclasses and TH1 differentiation. It also promotes increased expression of class I and class II MHC molecules and increased antigen processing and presentation to T cells.

Question 6. What subpopulation of dendritic cells are early cellular responders to viral infection?

a. classical dendritic cells
b. follicular dendritic cells
c. inflammatory dendritic cells
d. plasmacytoid dendritic cells

Answer: D. Pages 17-18. Plasmacytoid dendritic cells recognize nucleic acids of intracellular viruses and produce soluble proteins called type I interferons which have potent antiviral activities. This subpopulation of dendritic cells are early responders to viral infection.

The majority of dendritic cells in skin, mucosa, and organ parenchyma are called classical or conventional dendritic cells. They respond to microbes by migrating to lymph nodes where they display microbial antigens to T-lymphocytes.

Follicular dendritic cells have membranous projections that are found intermingled in collections of activated B cells in the lymphoid follicles of lymph nodes, spleen and mucosal lymphoid tissues. Follicular dendritic cells are not derived from precursors in the bone marrow and are unrelated to the dendritic cells that present antigen to T-lymphocytes. These cells display protein antigens on their surface for recognition by B-lymphocytes.
Inflammatory dendritic cells may arise from monocytes in inflamed tissues.

Question 7. Of the following anatomic sites, the largest proportion of lymphocytes can be found where?

a. blood
b. bone marrow
c. respiratory tract
d. spleen

Answer: D. Page 18

The total number of lymphocytes in a healthy adult is approximately $5 \times 10^{11}$. Of these, ~2% are in the blood, ~4% in the skin, ~10% in the bone marrow, ~15% in the mucosal lymphoid tissues of the gastrointestinal and respiratory tracts and ~65% in the lymphoid organs (lymph nodes and spleen).

Question 8. Your patient has a known diagnosis of X-linked severe combined immunodeficiency (SCID). Which of the following phenotype markers would you expect to be present on flow cytometry?

a. CD19
b. CD25
c. CD45
d. CD56

Answer: A. Page 19, 444 X-linked SCID is characterized by impaired maturation of T cells and NK cells and greatly reduced numbers of mature T cells and NK cells but the number of B cells is usually normal or increased (T-B+NK-). Therefore, you would expect to see normal or increased numbers of B cell markers such as CD19 and CD20.

Question 9. Naïve B cells express this chemokine receptor, which recognizes a chemokine, CXCL13, produced by the lymph node follicle by follicular dendritic cells.

a. CCR5
b. CCR7
c. CXCR4
d. CXCR5
Chemokine receptors are located on the surface of various cells. These receptors transduce signals stimulating the migration of leukocytes. All chemokine receptors are members of the seven-transmembrane alpha-helical, G protein-coupled receptor family. Each of these receptors binds a different set of chemokines.

- **CXCR5** binds **CXCL13**.
- **CCR5** binds **CCL3, CCL4 and CCL5**.
- **CR7** binds **CCL19 and CCL21**.
- **CXCR4** binds **CXCL12**.

**Question 10.** Susceptibility to recurrent infections with intracellular fungi and catalase positive bacteria is an example of what type of immune disorder?

a. defect in cell-mediated immunity  
b. defect in complement  
c. defect in humoral immunity  
d. defect in phagocytic cells

Answer: D. Page 13-15, 439. Chronic Granulomatous Disease is an example of a phagocytic disorder. CGD is characterized by recurrent infections with intracellular fungi and bacteria (think SBANS – Serratia, Burkholderia, Aspergillus, Nocardia and Staphylococcus). Many of the organisms that are particularly troublesome in CGD patients produce catalase, which destroys the microbiocidal hydrogen peroxide that may be produced by host cells from the residual reactive oxygen radical superoxide. Because the infections are not controlled by phagocytes, they stimulate chronic cell-mediated immune responses, resulting in T cell-mediated macrophage activation and the formation of granulomas composed of activated macrophages.

Deficiencies of complement proteins are the causes of various disease. Deficiencies in alternative pathway components result in increased susceptibility to pyogenic bacteria. Patients with deficiencies in the terminal complement components have a propensity for disseminated infections by Neisseria bacteria.

B cell deficiency is characterized by increased susceptibility to pyogenic bacteria, enteric bacteria and viruses and some parasites.  
T cell deficiencies are characterized by increased susceptibility to opportunistic organisms such as Pneumocystis jiroveci, many viruses, atypical mycobacteria, and fungi.
Allergy and Immunology Review Corner: *Cellular and Molecular Immunology, 8th Edition*
By Abul K. Abbas, MBBS; Andrew H. H. Lichtman, MD, PhD; and Shiv Pillai, MBBS, PhD.

Chapter 2 (pages 22–33): Cells and Tissues of the Immune System

*Prepared by Kristen Dazy, MD, Scripps Clinic Medical Group*

1. Which of the following surface proteins is highly expressed on effector T cells and indicative of recent activation?
   A. IL-2R (CD25)
   B. L-selectin (CD26L)
   C. IL-7R (CD127)
   D. CCR7

2. The epithelial component of the thymus is derived from invaginations of what germ cell layer?
   A. Endoderm
   B. Mesoderm
   C. Ectoderm
   D. Neural crest

3. The thoracic duct and right lymphatic duct drain return fluid to the blood stream via which major vessel?
   A. Aortic arch
   B. Superior vena cava
   C. Inferior vena cava
   D. Subclavian vein

4. Where does B-cell proliferation and generation of memory B cells take place within the lymph nodes?
   A. Germinal center
   B. Paracortex
   C. Subcapsular sinus
   D. Medulla

5. Chemokines promote naïve T cells to enter the T cell zones of the lymph node via which of the following?
   A. Afferent lymphatic vessels
   B. Medullary sinus lymphatic vessels
   C. High endothelial venules
   D. Marginal sinus

6. The chemokines CCL19 and CCL21 promote the migration of naïve T cells into the T cell zones of the lymph node by binding to which receptor located on the surface of naïve T cells?
A. CCR7  
B. CXCR5  
C. IL-7R  
D. IL-2R 

7. Circulating naïve B cells enter lymph nodes and are attracted into the follicles by which chemokine?  
   A. CCL19  
   B. IL-3  
   C. IL-7  
   D. CXCL13 

8. During fetal life, which cell types are responsible for the development of lymph nodes as well as other secondary lymphoid organs?  
   A. Fibroblastic reticular cells (FRCs)  
   B. Lymphoid tissue-inducer cells  
   C. Follicular dendritic cells (FDCs)  
   D. Naïve T cells 

9. Which of the following is not a true statement regarding the spleen?  
   A. The spleen is responsible for removing aging and/or damaged blood cells from the circulation.  
   B. Asplenic patients are susceptible to disseminated infections with encapsulated bacteria, including pneumococci and meningococci.  
   C. Blood is carried out of the spleen through the splenic vein, which drains into the portal circulation.  
   D. The spleen recognizes and removes un-opsonized microbes from the circulation. 

10. The anatomic arrangement of antigen presenting cells, B cells, and T cells within the spleen helps to promote the interactions required for the efficient development of humoral immune responses. In which part of the spleen do these actions take place?  
    A. Red pulp  
    B. White pulp  
    C. Hilum  
    D. Vascular sinusoids  
    E. 

Answers  
1. A, pages 22–23, Table 2-3.  
Both CD4 and CD8 effector T cells usually express surface proteins indicative of recent activation, including CD25 (a component of the receptor for T cell growth factor IL-2), and altered patterns of adhesion molecules (selectins and integrins). Choices B, C, and D are all highly expressed on naïve T cells rather than effector T cells. 

2. C, page 27.  
The epithelial component of the thymus is derived from invaginations of the ectoderm in the developing neck and chest of the embryo, forming structures called branchial pouches.
The efferent lymph vessel at the end of a lymph node chain joins other lymph vessels, eventually culminating in a large lymphatic vessel called the thoracic duct. Lymphatics from the right upper trunk, right arm, and right side of the head drains into the right lymphatic duct. The thoracic and right lymphatic ducts both empty into the superior vena cava, thus returning fluid to the blood stream.

Germinal centers develop in response to antigenic stimulation. They are sites of remarkable B cell proliferation, selection of B cells producing high-affinity antibodies, and generation of memory B cells and long-lived plasma cells. The T lymphocytes are located mainly beneath and more central to the follicles, in the paracortical cords, often called the paracortex.

T cell rich zones, called the paracortex, contains a network of fibroblastic reticular cells (FRCs), many of which form the outer layer of tube-like structures called FRC conduits. These conduits begin at the subcapsular sinus and extend to both medullary sinus lymphatic vessels and cortical blood vessels, call high endothelial venules (HEVs). Naïve T cells enter the T cell zones through the HEVs, specifically promoted by chemokines produced by stromal cells in the T cell zones of the lymph node.

Naïve T cells express a receptor called CCR7 that binds the chemokines CCL19 and CCL21 produced by stromal cells in the T cell zones of the lymph node. These chemokines promote naïve T cell movement from the blood, through the walls of the high endothelial venules (HEVs), and into the T cell zone. The chemokine CXCL13 and its receptor CXCR5 are responsible for B cell migration into the follicles.

Naïve B cells express high levels of the receptor CXCR5, which recognizes a chemokine, CXCL13, produced only in follicles by follicular dendritic cells (FDCs). Studies of CXCR5 knockout mice lack B cell-containing follicles in lymph nodes and spleen.

The development of lymph nodes, as well as peripheral lymphoid organs, depends on lymphoid tissue-inducer cells and the coordinated actions of several cytokines (e.g., lymphotoxin-α, lymphotoxin-β), chemokines, and transcription factors. These cells are a subset of innate lymphoid cells. Cytokines produced by these cells go on to activate stromal cells, such as FRCs and FDCs, to secrete additional cytokines, which help organize the structure of lymphoid organs.

The spleen is a highly vascularized organ whose major functions are to remove aging and damaged blood cells and particles (such as immune complexes and opsonized microbes) from the circulation and to initiate adaptive immune responses to blood-borne antigens. Macrophages within the red pulp of the spleen serve as an important filter for the blood and remove antibody-
coated (opsonized) cells and microbes. Individuals lacking a spleen are therefore susceptible to disseminated infections with encapsulated bacteria, such as pneumococci. Blood enters the spleen via the splenic artery and leaves via the splenic vein, which drains into the portal circulation.

The splenic parenchyma is anatomically and functionally divided into red pulp, which is composed mainly of blood-filled vascular sinusoids, and lymphocyte-rich white pulp. The white pulp is arranged into T cell (periarteriolar lymphoid sheath) and B cell (follicles) zones. This segregation is a highly regulated process and is dependent upon the production of different cytokines and chemokines in the stromal cells in these different areas, analogous to the case for lymph nodes. This arrangement is crucial to initiate the development of the adaptive humoral immune response to blood-borne antigens.

**Review Questions**

**Allergy and Immunology Review Corner: Cellular and Molecular Immunology, 8th Edition**

By Abul K. Abbas, MBBS, Andrew H. H. Lichtman, MD, PhD and Shiv Pillai, MBBS, PhD.

**Chapter 3 (pages 38–45): Leukocyte Circulation and Migration into Tissues**

*Prepared by Kara Wada, MD, Ohio State University/Nationwide Children's Hospital*

1. Which molecule found on leukocytes promotes their adhesion to endothelial cells?
   A. Sialomucin
   B. P-selectin
   C. L-selectin
   D. E-selectin

2. Which best describes the properties of integrins?
   A. They mediate adhesion of cells to other cells or to the extracellular matrix through non-specific binding interactions with various ligands.
   B. They normally are present in a high-affinity state on the endothelial surface.
   C. They have the ability to respond to intracellular signals by decreasing their affinity for ligands.
   D. Chemokines induce membrane clustering of integrins.

3. The HIV virus uses which chemokine receptors as co-receptors?
   A. CCR5 and CXCR4
   B. CCR5 and Mac-1
   C. LFA-1 and CXCR4
   D. Mac-1 and LFA-1

4. Chemokines are a large family of cytokines with diverse functions. Which best describes a property of chemokines?
A. Chemokines are large molecules with three or more external disulfide bonds.
B. Chemokines are only produced by leukocytes.
C. Chemokines are essential for the recruitment of leukocytes from blood vessels to extravascular sites.
D. Chemokines are not involved in lymphoid organ development.

5. Selectins, integrins and chemokines work together to regulate migration of leukocytes into the tissues. What best describes this process?
   A. Selectin-mediated rolling on the endothelium, chemokine-mediated increased in integrin affinity, stable integrin-mediated adhesion of leukocytes to the endothelium and transmigration between endothelial cells.
   B. Selectin-mediated adhesion on the endothelium, chemokine-mediated increased in integrin affinity, stable integrin-mediated rolling of leukocytes to the endothelium and transmigration between endothelial cells.
   C. Selectin-mediated rolling on the endothelium, chemokine-mediated decreased in integrin affinity, stable integrin-mediated adhesion of leukocytes to the endothelium and transmigration between endothelial cells.
   D. Selectin-mediated rolling on the endothelium, chemokine-mediated increased in integrin affinity, stable integrin-mediated adhesion of leukocytes to the endothelium and transmigration through endothelial cells.

6. The interplay of selectins, integrins and chemokines in leukocyte migration is essential in normal host defense. What defect is responsible for leukocyte adhesion deficiency type 1 (LAD-1)?
   A. A deficiency in the α subunit of LFA-1 and Mac-1
   B. A lack in the golgi fucose transporter needed to express the carbohydrate ligands for E-selectin and P-selectin.
   C. A mutation in the signaling pathways linking chemokine receptors to integrin activation.
   D. A deficiency in the β subunit of LFA-1 and Mac-1.

7. Migration of the inflammatory infiltrate generally follows the same pattern and is directed by chemokines and their receptors. Which pairing of cell types and chemokine receptor is correct?
   A. Monocytes & CCR7
   B. Neutrophils & CXCR1
   C. Monocytes & CCL2
   D. Neutrophils & CXCL2

8. Migration of naïve T cells depends on efficient homing mechanisms.
   A. Naïve T cells are delivered through venous blood flow into the high endothelial venules (HEVs).
   B. Rolling of naïve T cells on the HEVs is mediated by P-selectin.
   C. Firm adhesion of naïve T cells is mediated by LFA-1.
   D. Chemokines that deactivate the naïve T cell integrins to a low-affinity state are CCL19 and CCL21.
9. How is S1P responsible for the exit of naïve T cells from the lymph nodes?
   A. S1P is in higher concentrations in the tissues attracting naïve T cells away from the lymph node.
   B. S1P binds to S1PR1 on T cells directing movement toward higher concentrations of S1P.
   C. S1P lyase degrades S1P and is responsible for the higher levels in the tissues.
   D. Naïve T cells are coated in S1PR1 receptors allowing for immediate interaction with the S1P.

10. Central memory T cells are characterized by what phenotype?
   A. CD45RO+ with high expression of CCR7 & L-selectin
   B. CD45RO+ with low expression of CCR7 & L-selectin
   C. CD45RO+ with high expression of CXCR5
   D. CD45RO+ with low expression of CXCR5

Answers
   L-selectin is expressed on leukocytes. Their ligands are sialomucins on endothelial cells, whose expression is increased by cytokine activation of the cells. E-selectin and P-selectin are also found on endothelial cells.

   Integrins mediate adhesion through specific binding interactions. They are normally present in a low-affinity state. They will respond to intracellular signaling by increasing their affinity for ligands and chemokines will induce clustering on the membrane surface.

   CCR5 and CXCR4 act as co-receptors for the HIV. Mac-1 and LFA-1 are integrins and not chemokine receptors.

   Chemokines are 8-10-kD polypeptides that contain two internal disulfide bonds, which are produced by leukocytes, endothelial cells, epithelial cells and fibroblasts. They are essential for the recruitment of leukocytes from blood vessels to extravascular sites and are involved in the development of lymphoid organs.

5. A, pages 41–42.
   Selectin-mediated interactions slow down the leukocyte, release of chemokines increase integrin affinity allowing the stronger integrin-mediated adhesion to occur. This allows the leukocyte to then migrate between the endothelial cells by transiently disrupting the adherens junction proteins.

   Patients with LAD-2 lack the golgi fucose transporter thus are unable to express the carbohydrate ligands for E-selectin and P-selectin. LAD-3 is characterized by a mutation in the signaling
pathways. LAD-1 is characterized by an autosomal recessive deficiency in the $b$ subunit of LFA-1 and Mac-1.

7. B, page 42, Table 3.2 on page 40.
In the nomenclature, all chemokine receptors contain the letter R whereas chemokines contain the letter L. CCR7 is responsible for T and dendritic cell migration in the lymph nodes. CXCR1 and CXCR2 are responsible for neutrophil migration.

8. C, page 44.
Naïve T cells are delivered through arterial blood flow to the HEVs where rolling is mediated by L-selectin and firm adhesion is mediated by LFA-1. CCL19 and CCL21 are chemokines that activate the naïve T cell integrins to a high affinity state.

S1P is a lipid chemoattractant which binds to S1PR1 on T cells. S1P is found in higher concentrations in the blood and lymph. It is degraded by S1P lyase in the tissues. The binding of S1P to S1PR1 results in directed movement of T cells along the S1P concentration gradient and out of the lymph node parenchyma. Circulating T cells have very little S1PR1 due to high blood levels causing internalization of this receptor. It takes several hours for S1PR1 to be re-expressed thus allowing naïve T cells to interact with antigen presenting cells in the lymph node before exiting back into the circulation via the efferent lymphatic.

10. A.
Central memory T cells are CD45RO+ with high expression of CCR7 & L-selectin. Effector memory T cells are defined as CD45RO+ with low expression of CCR7 & L-selectin. CXCR5 is a chemokine receptor found on B cells, which is found on mature naïve B cells.
Chapter 4 (pages 62-74): Innate Immunity

Prepared by Reenal Patel, MD, UMDNJ New Jersey Medical School

1. The scavenger receptor, CD36, functions as a coreceptor for which TLR?
   a. TLR ½
   b. TLR 3
   c. TLR 4
   d. TLR 2/6

2. Which of the following is false for innate lymphoid cells (ILCs)?
   a. They depend on Il-7 for development
   b. They express Id2 transcription factor
   c. They are thymus derived cells with lymphocyte morphology that serve diverse antimicrobial functions.
   d. They emerge fully capable of performing effector functions without the need for clonal expansion and differentiation.

3. Which of the following mast cell mediators are not preformed?
   a. Tryptase
   b. Platelet-activating factor
   c. Heparin
   d. Chymase

4. Which cell is a major source of IL-1?
   a. Lymphocytes
   b. Neutrophils
   c. Mononuclear phagocytes
   d. Dendritic cells

5. Which of the following cytokines are NOT associated with the acute inflammatory response?
   a. IL-1
   b. TNF
   c. IL-6
   d. IL-12

6. Which of the following complement members are responsible for Neisseria bacterial infections?
   a. C1r
   b. C2
   c. C5-9
7. Plasmacytoid dendritic cells are a subset of dendritic cells. Plasmacytoid dendritic cells express an abundant amount of TLRs, which of the following TLRs do they express?
   a. TLR 2
   b. TLR 4
   c. TLR 6
   d. TLR 7

8. Which of the following carbohydrate receptors play a role in promoting HIV-1 infection in T-cells?
   a. CD207
   b. CD209 (DC-SIGN)
   c. Dentin-1
   d. CD 206

9. Which receptor on NK cells is a low-affinity receptor IgG antibodies?
   a. CD16
   b. NKG2D
   c. DAP10
   d. MHC Class I

10. Which of the following cytokines stimulate NK function?
    a. IL-1
    b. IL-6
    c. IL-12
    d. IL-20

Answers
   CD36, a scavenger receptor, is expressed on macrophages, and mediates the phagocytosis of microorganisms. It also functions as a corrector in TLR2/6 recognition and response to bacterially derived lipoteichoic acid and dilated lipopeptides.

   Innate lymphoid cells are bone marrow derived cells with lymphocyte morphology. These cells arise from a common bone marrow precursor identifiable by expression of the Id2 transcription factor, they depend on Il-7 for development, and unlike lymphocytes of the adaptive immune system, they emerge fully capable of performing effector functions without the need for clonal expansion and differentiation.

   Mast cells contain abundant cytoplasmic granules and are filled with inflammatory mediators that are released when the cells are active. The preformed mediators are histamine, tryptase, carboxypeptidase, chymase and heparin. Mast cells also synthesize and secrete lipid mediators, like prostaglandin, leukotrienes, and platelet activating factor.
The major cellular source of IL-1 is activated mononuclear phagocytes. IL-1 is also produced by many cell types other than macrophages, such as neutrophils, epithelial cells, and endothelial cells.

5. D, page 73
The earliest response of the innate immune system to infection and tissue damage is the secretion of cytokines by tissue cells, which is critical for the acute anti-inflammatory response. The three most important pro-inflammatory cytokines of the innate immune system are TNF, IL-1, and IL-6.

Genetic deficiencies in MAC formation increase the susceptibility to *Neisseria* bacteria. *Neisseria* bacteria have thin cell walls that make them susceptible to the lytic action of the MAC (C5-9).

7. D, page 64.
Plasmacytoid dendritic cells express abundant amounts of endosomal TLRs-TLR 3, 7, 8 and 9.

DC-SIGN (CD 209) play a pathogenic role in promoting HIV-1 infection of T cells. The HIV-1 gp 120 envelope glycoprotein binds to DC-SIGN on dendritic cells in mucosal tissues, the dendritic cells carry the virus through lymphatics to draining lymph nodes, and the virus is the transferred to and infects CD4+ T cells.

CD16 is a low affinity receptor IgG antibodies. CD16 binds to the Fc regions of certain types of antibodies called IgG1 and IgG3.

IL-12, IL-15, IL-18, and type I interferons are the major cytokines of the innate immune system that stimulate the NK function.

**Review Questions**

**Allergy and Immunology Review Corner:** Cellular and Molecular Immunology, 8th Edition
By Abul K. Abbas, MBBS, Andrew H. H. Lichtman, MD, PhD and Shiv Pillai, MBBS, PhD.

**Chapter 4 (pages 62-85):** Innate Immunity

1. Which of the following is a commonly used adjuvant?
   a. ALUM
   b. KOH
   c. TLR3
   d. IL-15
2. TNF has which of the following important functions?
   a. Promotes myocardial contractility
   b. Increase adipose tissue formation
   c. Causes intravascular hemolysis
   d. Increases leukocyte production in the bone marrow

3. Which TLR can use the adapter protein TRIF and activate IRF3 and IRF7 transcription factors?
   a. TLR 3 only
   b. TLR 4 and 5
   c. TLR 3 and 4
   d. TLR 4 only

4. Which of the following is the critical step in complement activation leading directly or indirectly to all of the initial effector activities of the complement system?
   a. C1q interaction with pathogen surface.
   b. Cleavage of C5 resulting in generation of C5b important for opsonization.
   c. Cleavage of C5 resulting in generation of C5b important for opsonization.
   d. Hydrolysis of C3 to C3 (H2O) to initiate deposition of C3 convertase on microbial surfaces.

5. Which of the following is accurate regarding the stimulation of the adaptive immune system in terms of the two signal hypothesis?
   a. Signal 1 includes various cytokines and costimulatory molecules.
   b. Signal 2 is described as the presence of antigen.
   c. Signal 1 + 2 leads to induction of the adaptive immune response when a dangerous infection is present.
   d. Signal 1+2 leads to immediate destruction of self-antigens, thus resulting in autoimmune disease.

6. What is the correct effector function of Type 1 interferon?
   a. Type 1 Interferon decreases the cytotoxicity of NK cells and CD8+ CTLs
   b. Type 1 Interferon promotes the differentiation of Th17 subpopulation of cells
   c. Type 1 Interferon upregulates the expression of Class 1 MHC molecules
   d. Type 1 Interferon increase release of lymphocytes out of the lymph node

7. Which of the following is an inhibitory receptor of NK cells?
   a. NKG2A
   b. NKG2D
   c. NKG2C
   d. NKp46
8. Which of the following is considered a property of the cathelicidin family?
   a. Naïve They are made of dendritic cells in the skin in response to infection.
   b. They are made as active propeptides composed of two linked domains and are processed after secretion.
   c. They are stored in primary granules and are activated by fusion of these primary granules into secondary granules.
   d. They lack disulfide bonds that stabilize molecules such as defensins.

9. Gout is a painful inflammatory condition of the joints that has been known to cause deposition of monosodium urate crystals in joints, and that these crystals can activate the inflammasome. As a result of understanding the function and pathway of the inflammasome, which could be a possible therapeutic target for treating gout?
   a. IL-1 antagonists
   b. IL-2 antagonists
   c. Pro-IL1 beta secretagogue
   d. TLR 3 inhibitor

10. An important function of an adjuvant is:
    a. To activate T cells directly.
    b. Decrease the expression of co-stimulatory molecules.
    c. Prevent migration of dendritic cells into the lymph nodes.
    d. To activate dendritic cells to express more major histocompatibility molecules.

Answers
   Adjuvants are substances that need to be administered together with purified protein antigens to elicit maximal T cell dependent immune response and work by stimulating innate immune responses at the site of antigen exposure. Many adjuvants are microbial products such as killed mycobacteria, LPS (i.e. that engage TLRs). Alum is a routinely used adjuvant in human vaccines (composed of aluminum hydroxide or aluminum phosphate).

2. D, pages 82.
   Functions of TNF shown in Figure 4-4 include increasing leukocyte production in bone marrow, inhibiting myocardial contractility and vascular smooth muscle tone, causing intravascular thrombosis, induction of muscle wasting and wasting of fat cells leading to cachexia.

   TLR 3 signals through TRIF and therefore activates IRF 3 and induces expression of type 1 interferons. TLR4 signals through both MyD88 and TRIF and is able to induce both types of responses.

4. C, pages 73.
Cleavage of C3 is the critical step in complement activation leading directly or indirectly to all of the effector activities of the complement system. C5 cleavage is involved in “late events” including formation of MAC.

The activation of lymphocytes requires two distinct signals, the first being antigen and the second being molecules that are produced during innate immune responses to microbes or injured cells. This idea is called the two-signal hypothesis for lymphocyte activation. The requirement for antigen (signal 1) ensures that the ensuing immune response is specific. The requirement for additional stimuli triggered by innate immune reactions to microbes (signal 2) ensures that the adaptive immune responses are induced when there is a dangerous infection and not when lymphocytes recognize harmless antigens, including self-antigens. The molecules produced during innate immune responses function as second signals for lymphocyte activation including co-stimulators (for T cells), cytokines (both T and B cells), and complement breakdown products (for B cells).

Type 1 interferons signal through the type 1 interferon receptor, activate transcription of several genes that confer on the cells a resistance to viral infection. They also cause sequestration of lymphocytes in lymph nodes thus maximizing the opportunity for encounter with microbial antigens. Type 1 interferons increase the cytotoxicity of NK cells and CD8+ CTLs and promote differentiation of naïve T cells to the Th1 subset of helper T cells. Finally, Type 1 interferons upregulate expression of class 1 MHC molecules and thereby increase the probability of viral infected cells will be recognized and killed by CD8+ CTLs.

7. A, page 70.
NKG21 is inhibitory receptor, all other listed above are activating receptors as demonstrated by figure 4-7.

Cathelicidins are made constitutively by neutrophils, macrophages, and keratinocytes in the skin in response to infection. They are made as inactive propeptides composed of two linked domains and are processed before secretion. Cathelicidins are stored in secondary granules and are activated by proteolytic cleavage when these granules fuse with the phagosome. This family of antimicrobial peptides lack disulfide bonds that stabilize molecules such as defensins.

IL-1 antagonist or blockage of inflammasome could be useful therapeutic target in treatment of gout.

Adjuvants activate dendritic cells to express more major histocompatibility molecules that are part of the antigen (signal 1) that T cells recognize, increase the expression of costimulators (signal 2) and cytokines needed for T cell activation, and stimulate migration of the dendritic cells to lymph nodes where T cells are located.
FIT Board Review Corner – June 2015
Welcome to the FIT Board Review Corner, prepared by Andrew Nickels, MD, and Sarah Spriet, DO, senior and junior representatives of ACAAI’s Fellows-In-Training (FITs) to the Board of Regents. The FIT Board Review Corner is an opportunity to help hone your Board preparedness.

Review Questions

Allergy and Immunology Review Corner: Cellular and Molecular Immunology, 8th Edition
By Abul K. Abbas, MBBS, Andrew H. H. Lichtman, MD, PhD and Shiv Pillai, MBBS, PhD.

Chapter 5 (pages 90-104): Antibodies and Antigens

Prepared by Sahila Smith, DO, Larkin Comm Hosp/Nova Southeastern Univ

1. Effector functions are carried out by which region of the immunoglobulin structure?
   a. V regions of heavy chains
   b. C regions of heavy chains
   c. C region of light chains
   d. V region of light chains

2. A 21-year-old female presents with morning stiffness lasting for sixty minutes, joint swelling of bilateral hands, wrists and ankles. Her exam reveals MCP joint synovial edema, erythema and warmth. Laboratory data includes positive rheumatoid factor, elevated CRP and ESR. Anti-citrullinated peptides were also positive. She has been started on adalimumab (Humira) which is a monoclonal antibody directed against TNF alpha.
   Which of the following describes the process by which monoclonal antibodies are made?
   a. Fusing T cells from an immunized animal (mouse) with an immortal sarcoma cell line and growing cells under appropriate conditions and growth medium.
   b. Fusing B cells from an immunized animal (mouse) with an immortal myeloma cell line and growing cells under conditions in which non infused cells cannot survive.
   c. Fusing dendritic cells from an immunized animal (mouse) with an immortal adenocarcinoma cell line and growing cells under appropriate conditions and growth medium.
   d. Fusing B cells from an immunized animal (mouse) with an immortal stromal cell line and growing cells under conditions in which non infused cells cannot survive.

3. Which of the following best represents structural feature of antibody variable regions?
   a. Hypervariable regions consists of three loops of β pleated sheets of V region of both the heavy chain and light chain which are brought together to bind antigen.
   b. Hypervariable regions consists of three loops of β pleated sheets of C region of both the heavy chain and light chain which are brought together to bind antigen.
   c. The constant region of the light chain has more variability in binding antigen than variable region of the light chain.
   d. The carboxyl-terminal end determines the hypervariable region to bind a multitude of antigens.
4. The ability for antibodies to distinguish between small differences in chemical structure is also known as:
   a. Affinity maturation
   b. Diversity
   c. Specificity
   d. Repertoire

5. A protein antigenic determinant that then becomes phosphorylated is called a:
   a. Conformational determinant
   b. Linear determinant
   c. Neoantigenic determinant
   d. Epitope

6. The strength of the binding between a single combining site of the antibody and an epitope of an antigen is called:
   a. Affinity Maturation
   b. Affinity
   c. Diversity
   d. Avidity

7. A 23-year-old female presents with abdominal pain and diarrhea which is foul smelling. She reports eating raw meat. A complete blood count reveals eosinophilia. Which of the following can explain the increase in eosinophils?
   a. Naïve +IgD and +IgM B cells recognize parasite antigen and thus switch to +IgE isotype to carry out the process of antigen elimination.
   b. B cells switching to the IgG isotype also prolongs the effectiveness of humoral responses.
   c. +IgM B cells recognize linear determinants of parasites and IgM progeny rid the body of parasites.

8. A 35-year-old male presents to your office with fever, chills, headache and photophobia. On exam he has positive Kerning’s sign. Cerebral spinal fluid from lumbar puncture reveals gram’s positive diplococci. Broad spectrum antibiotics are started. Patient’s symptoms improve after three days. Which of the following describes how effector functions are initiated?
   a. Ig molecules without antigen can trigger effector functions.
   b. Antibodies bound with antigen care able to trigger effector functions via a process called diversity.
   c. Antibodies with bound antigens activate effector mechanisms with two or more adjacent antibody Fc portions which are needed to bind and trigger complement pathway.

9. The hinge region of antibodies allows for flexibility in the molecule. A single antibody mat attach to a single multivalent antigen by more than one binding site. Which of the following describes avidity as is relates to antibody binding antigen?
   a. Monovalent epitopes spaced apart has low avidity interaction
   b. Bivalent epitopes bound by one molecule of IgG has high avidity and high affinity.
   c. If the affinity is low then the avidity must also be low.
   d. IgM molecules have ten identical sites to bind polyvalently to multiple epitopes at once leading to very high avidity.
10. An immunology student is running an experiment with rabbit IgG in the laboratory. The student adds pepsin to one dish and finds the Ig molecules breaks into distinct pieces. She then adds papain into another dish and a similar reaction occurs. Which of the following accurately demonstrates the immunology student’s observations?

a. Papain cleaves rabbit IgG into three distinct pieces; two identical V\textsubscript{L} and C\textsubscript{L} called Fab (fragment, antigen binding). The third piece is CH\textsubscript{2} and CH\textsubscript{3} domains which is crystalized and called (fragment, crystalizable). Pepsin results in proteolysis distal to hinge region equaling both V\textsubscript{L} and C\textsubscript{L}. Hinge and disulfide bonds are intact and this molecule is also known as F(ab’)\textsubscript{2}. The remaining C region is broken down into various peptides.

b. Pepsin cleaves rabbit IgG into three distinct pieces; two identical V\textsubscript{L} and C\textsubscript{L} called Fab (fragment, antigen binding). The third piece is CH\textsubscript{2} and CH\textsubscript{3} domains which is crystalized and called (fragment, crystalizable). Papain results in proteolysis distal to hinge region equaling both V\textsubscript{L} and C\textsubscript{L}. Hinge and disulfide bonds are intact and this molecule is also known as F(ab’)\textsubscript{2}. The remaining C region is broken down into various peptides.

c. Papain cleaves rabbit IgG into two distinct pieces; one V\textsubscript{L} fragment called Fab (fragment, antigen binding). The second piece is two identical CH\textsubscript{2} and CH\textsubscript{3} domains which is crystalized and called (fragment, crystalizable). Pepsin results in proteolysis of the Ig molecule leaving various peptide fragments.

d. Papain does not cause any proteolysis and thus results in intact Rabbit IgG. Pepsin results in proteolysis distal to hinge region equaling both V\textsubscript{L} and C\textsubscript{L}. Hinge and disulfide bonds are intact and this molecule is also known as F(ab’)\textsubscript{2}. The remaining C region is broken down into various peptides.

Answers
Effector functions are mediated through C regions of heavy chains, but these functions are triggered by binding of antigens to the combining site in the V region.

2. B, page 95.
Georges Kohler and Cesar Milstein in 1975 infused an animal (mouse) with an immortal myeloma cell line and grew cells under conditions in which non infused cells cannot survive. The resultant fused cells are called hybridomas.

Hypervariable regions consists of three loops of β pleated sheets of V region of both the heavy chain and light chain which are brought together to bind antigen.

4. C, pages 102-103
Experiments performed in the 20\textsuperscript{th} century demonstrated that antibodies made in response to an amino-benzene hapten with a meta-substituted sulfonate. Groups would bind strongly bind to this hapten but weakly or not at all to ortho- or para-submitted isomers. These antigens are structurally similar and differ only in the location of the sulfonate group on the benzene ring.

Proteins may be subjected to modifications such as glycosylation, phosphorylation, ubiquitination, acetylation, and proteolysis. These modifications, by altering the structure of the protein, can produce new epitopes. They too can be recognized by specific antibodies.

The affinity is commonly represented by a dissociation constant (Kd), which indicates a stronger or higher affinity interaction because a lower concentration of antigen and of antibody is required for complex formation.

After stimulation by an antigen, a single clone of B cells may produce antibodies with different isotypes that nevertheless possess identical V domains and therefore identical antigen specificity. Naive B cells simultaneously express IgM and IgD that function as membrane receptors for antigens. When these B cells are activated by foreign antigens, typically of microbial origin, they may undergo a process called isotype switching.

The reason that only antibodies with bound antigens activate effector mechanism is that two or more adjacent antibody Fc portions are needed to bind to and trigger various effector systems, such as complement proteins and FcRs of phagocytes.

The hinge region of antibodies allows for flexibility in the molecule. A single antibody may attach to a single multivalent antigen by more than one binding site. IgM molecules have ten identical sites to bind polyvalently to multiple epitopes at once leading to very high avidity.

10. A, pages 90-91, figure 5-3 A.
Papain cleaves rabbit IgG into three distinct pieces; two identical VL and CL called Fab (fragment, antigen binding). The third piece is CH2 and CH3 domains which is crystalized and called (fragment, crystalizable). Pepsin results in proteolysis distal to hinge region equaling both VL and CL. Hinge and disulfide bonds are intact and this molecule is also known as F(ab')2. The remaining C region is broken down into various peptides.

Review Questions

Allergy and Immunology Review Corner: Cellular and Molecular Immunology, 8th Edition
By Abul K. Abbas, MBBS, Andrew H. H. Lichtman, MD, PhD and Shiv Pillai, MBBS, PhD.

Chapter 5 (pages 97-105): Antibodies and Antigens

Prepared by Priscilla Wong, MD, Wilford Hall Ambulatory Surgical Center
All/Immun 59 MDOS/SG05A

1. Which antibody isotype has the shortest half-life in circulation?
   a. IgA
   b. IgG
   c. IgE
   d. IgM
2. Which antibody is a pentamer?
   a. IgA
   b. IgG
   c. IgE
   d. IgM

3. Therapeutic agents such as abatacept (CTLA4-Ig) and etanercept (TNFR-Ig) are fusion proteins where the biologically active parts of the target proteins are fused to the Fc region of human IgG. To what receptor does IgG bind enabling its long half-life?
   a. FcγRI
   b. FcRn
   c. FcαRI
   d. FcγRIII

4. A mature, naive B cell expresses which of the following?
   a. IgG
   b. Membrane IgM
   c. Membrane IgM and IgD
   d. Cytoplasmic μ heavy chain and pre-B cell receptor

5. In the tissues of patients with an immune complex disease like systemic lupus erythematosus, which of the following is present when polyvalent antigens are mixed with antibody in a test tube during a clinical flare?
   a. Zone of antigen excess
   b. Zone of equivalence
   c. Zone of antibody excess
   d. Zone of neutrality

6. The affinity of the antibody and the avidity contribute to the strength of antigen-antibody interactions. Which of the following antigen-antibody combinations represents the strongest interaction?
   a. Monovalent antigen – IgE
   b. Monovalent antigen – IgM
   c. Polyvalent antigen – IgG
   d. Polyvalent antigen – IgM

7. In affinity maturation, the average binding affinity of an antibody for antigen changes in which progression?
   a. $K_d \ 10^{-1}$ to $K_d \ 10^1$
   b. $K_d \ 10^{-9}$ to $K_d \ 10^{-11}$
   c. $K_d \ 10^1$ to $K_d \ 10^3$
   d. $K_d \ 10^4$ to $K_d \ 10^6$
8. The effector functions of immunoglobulins are mediated by which portion of the molecule?
   a. Antigen binding site
   b. Fab
   c. Fc region
   d. V region

9. Which antibody is efficiently secreted through mucosal epithelia and is the major class of antibody in mucosal secretions and human milk?
   a. IgA
   b. IgE
   c. IgM
   d. IgG

10. During affinity maturation, which of the following occurs?
    a. Somatic mutations in the CH region
    b. Germline mutations in the V region
    c. Germline mutations in the V region
    d. Somatic mutations in the V region

Answers
IgE has a very short half-life of 2 days in the circulation, circulating IgA has a half-life of about 3 days, and circulating IgM has a half-life of about 4 days. In contrast, circulating IgG molecules have a half-life of about 21-28 days.

For pentameric IgM, a single antibody may bind at up to 10 different sites" whereas "IgG or IgE can involve at most 2 binding sites."

"The long half-life of IgG has been used to provide a therapeutic advantage for certain injected proteins by producing fusion proteins containing the biologically active part of the protein and the Fc portion of the IgG. The Fc portion enables the proteins to bind to the FcRn and thus extends the half-lives of the injected proteins. One therapeutically useful fusion protein is TNFR-Ig"…"Another therapeutically useful fusion protein is CTLA4-Ig."

Mature B cells express membrane forms of IgM and IgD. These membrane Ig receptors serve as cell surface receptors that recognize antigens and initiate the process of B cell activation.

5. B, page 102, Figure 5-14.
"If a polyvalent antigen is mixed with a specific antibody in a test tube, the two interact to form immune complexes. At the correct concentration, called a zone of equivalence, antibody and antigen form an extensively cross-linked network of attached molecules such that most or all of the antigen and antibody molecules are complexed into large masses."…"If a zone of equivalence is reached in
vivo, large immune complexes can form in circulation. Immune complexes that are trapped or formed in tissues can initiate an inflammatory reaction, resulting in immune complex diseases.”

“Polyvalent antigens will have more than one copy of a particular determinant. Although the affinity of any one antigen-binding site will be the same for each epitope of a polyvalent antigen, the strength of the attachment is called the avidity and is much greater than the affinity of any one antigen-binding site. Thus, a low-affinity IgM molecule can still bind tightly to a polyvalent antigen because many low-affinity interactions (up to 10 per IgM molecule) can produce a high avidity interaction.”

“The affinity is commonly represented as a dissociation constant (Kd) which indicates how easy it is to separate an antigen-antibody complex into its constituents. A smaller Kd indicates a stronger or higher affinity interaction because a lower concentration of antigen and antibody is required for complex formation. The Kd of antibodies produced in typical humoral immune responses usually varies from $K_d \ 10^{-7}$ to $K_d \ 10^{-9}$ M….” Affinity maturation results in an increase in the average binding affinity of antibodies for antigen as a humoral immune response evolves. Thus, an antibody produced during a primary immune response to a protein antigen often has a Kd in the range of $K_d \ 10^{-9}$ to $K_d \ 10^{-11}$; in secondary responses, the affinity increases, with a $K_{d}$ of $10^{-11}$”

“Many of the effector functions of immunoglobulins are mediated by the Fc portions of the molecules, and antibody isotypes that differ in these Fc regions perform distinct functions.”

“IgA can be secreted efficiently across mucosal epithelia and is the major class of antibody in mucosal secretions and milk.”

10. D, page 103, Figure 5-15.
“A mechanism for the generation of high-affinity antibodies involves subtle changes in the structure of the V regions of antibodies during T cell-dependent humoral immune responses to protein antigens. These changes come about by a process of somatic mutation in antigen-stimulated B lymphocytes that generates new V domain structures, some of which bind the antigen with greater affinity than did the original V domains (Fig 5-15)”
FIT Board Review Corner – July 2015

Welcome to the FIT Board Review Corner, prepared by Andrew Nickels, MD, and Sarah Spriet, DO, senior and junior representatives of ACAAI’s Fellows-In-Training (FITs) to the Board of Regents. The FIT Board Review Corner is an opportunity to help hone your Board preparedness.

Review Questions

Allergy and Immunology Review Corner: Cellular and Molecular Immunology, 8th Edition
By Abul K. Abbas, MBBS, Andrew H. H. Lichtman, MD, PhD and Shiv Pillai, MBBS, PhD.

Chapter 6 (pages 119-133): Major Histocompatibility Complex Molecules and Antigen Presentation to T Lymphocytes

Prepared by Tara Shankar, MD, Children’s Hospital of Pittsburgh of UPMC

1. What is the principal cytokine involved in stimulating expression of class II molecules in APCs?
   a. IFN-alpha
   b. IFN-beta
   c. IFN-gamma
   d. IL-1

2. What is the binding site for CD8 on the Class I MHC molecule?
   a. a1
   b. a2
   c. a3
   d. b2

3. MHC Class I molecules accommodate peptides of what length?
   a. 4-9
   b. 8-11
   c. 10-20
   d. 12-30

4. What type of interaction does a peptide and MHC molecule have?
   a. Saturable, slow off
   b. Saturable, fast off
   c. Non saturable, slow off
   d. Non saturable, fast off
5. Listeria monocytogenes can resist microbiocidal mechanisms by producing listeriolysin which allows the bacteria to escape from vesicles into the cytosol. What type of molecule would then present the listeria microbial protein?
   a. Class I MHC
   b. Class II MHC
   c. HLA DM
   d. CIITA

6. HSV has evolved to block the TAP transporter. How does this evolutionary change allow HSV to evade the host immune response?
   a. The invariant chain peptide cannot be removed to make the peptide binding cleft of class II molecules available.
   b. Cytosolic peptides cannot be transported into the ER where they can associate with a class II MHC molecule.
   c. The invariant chain peptide cannot be removed to make the peptide binding cleft of class I molecules available.
   d. Cytosolic peptides cannot be transported into the ER where they can associate with a class I MHC molecule.

7. Where do processed peptides associate with class II MHC molecules?
   a. Endoplasmic reticulum
   b. Endosomal vesicle
   c. Surface of the APC
   d. Endocytic vesicle

8. What is the function of HLA-DM?
   a. Transports cytosolic peptides to the ER.
   b. Brings the TAP transporter into a complex with the class I MHC molecules awaiting arrival of peptides.
   c. Chaperones the folding and assembly of class II MHC dimers.
   d. Removes CLIP to make the peptide cleft available.

9. Though most ingested proteins do not enter the class I pathway, in cross-presentation, ingested antigens are transported from vesicles to the cytosol where peptides enter the class I pathway. This process is unique to which cell?
   a. Macrophages
   b. NK cells
   c. Dendritic cells
   d. Gamma delta T cells

10. What molecule is structurally homologous to the class I MHC molecule and displays lipid antigens for recognition by NKT cells?
    a. CD1
    b. CD2
    c. CD3
    d. CD4
Answers
“IFN-gamma is the principal cytokine involved in stimulating expression of class II molecules.”

2. C, page 121.
“The alpha3 segment of the alpha chain…contains most of the binding site for CD8.”

“Class I molecules can accommodate peptides that are 8-11 residues long.”

“The association of peptides and MHC molecules is a saturable interaction with a very slow off rate…This extraordinarily slow off rate of peptide dissociation from MHC molecules ensures that after an MHC molecule has acquired a peptide, it will display the peptide long enough to maximize the chance that a particular T cell will find the peptide it can recognize and initiate a response.”

5. A, page 125.
“Protein antigens that are present in the cytosol…generate class I associated peptides that are recognized by CD8+ T cells.”

“Because antigenic peptides for the class I pathway are generated by proteases in the cytosol…but class I MHC molecules are synthesized in the ER, a mechanism is needed to deliver cytosolic peptides into the ER. This delivery is mediated by a dimeric protein called transporter associated with antigen processing (TAP).”

7. B, page 130
“Within the endosomal vesicle, the invariant chain dissociates from class II MHC molecules…and antigenic peptides are then able to bind to the available peptide binding clefts of the class II molecules.”

“CLIP must be removed so that the cleft becomes accessible to antigenic peptides produced from extracellular proteins. This removal is accomplished by the action of a molecule called HLA-DM.”

“Ingested antigens are transported from vesicles to the cytosol for where peptides enter the class I pathway. This permissiveness for protein traffic from endosomal vesicles to the cytosol is unique to dendritic cells.”

10. A, page 133.
“NKT cells recognize lipids and glycolipids displayed by the class I-like non-classical MHC molecule called CD1.”
Review Questions

Allergy and Immunology Review Corner: Cellular and Molecular Immunology, 8th Edition
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Chapter 7 (pages 137-150): Immune Receptors and Signal Transduction

Prepared by Erin Kemp, DO, Ohio State University Hospital

1. Btk is a tyrosine kinase of the Tec family that recognizes which of the following molecules:
   a. Leucine
   b. PIPI3
   c. Proline
   d. Ubiquitin

2. Which of the following receptors contains an immunoreceptor tyrosine-based inhibitory motif (ITIM) in its cytoplasmic tail?
   a. CD3ζ
   b. FcεRI
   c. FcγRIIB
   d. Igα

3. Which of the following components of the T cell receptor complex serve as the binding site for a peptide-MHC complex?
   a. αβ
   b. εδ
   c. εγ
   d. ζζ

4. Signal transduction for the T cell receptor complex is mediated by which component?
   a. α
   b. β
   c. β2m
   d. ζ

5. Which portion of the TCR complex serves as a binding site for microbial superantigens?
   a. α
   b. β
   c. δ
   d. ζ

6. After recognition of MHC-peptide complexes by the TCR, phosphorylation of ITAMs on the CD3 and ζ chains is initiated by which kinase on the CD4/CD8 coreceptor?
a. Btk  
b. LAT  
c. Lck  
d. ZAP-70

7. Which of the following MAP kinases is activated through the Ras G protein pathway?  
a. ERK  
b. JAK  
c. JNK  
d. STAT

8. Which of the following G protein pathways leads to activation of the AP-1 transcription factor through the MAP kinase JNK?  
a. DAG  
b. PLCγ1  
c. Rac  
d. Ras

9. Which of the following is the initial step leading to an increase in cytosolic free calcium as a result of TCR activation?  
a. DAG activates PKC  
b. Grb-2 docks to phosphorylated LAT  
c. PLCγ is phosphorylated and hydrolyzes PIP2 into IP3  
d. STIM1 activates opening of the CRAC channel

10. Which of the following transcription factors is activated by calcineurin and results in expression of genes for IL-2, IL-4, and TNF?  
a. AP-1  
b. c-Fos  
c. NFAT  
d. NF-κB

Answers  
1. B, page 140, figure 7.3.  
Btk is a member of the TEC family of tyrosine kinases, which recognizes PIP3 on the inner leaflet of the plasma membrane via a pleckstrin-homology (PH) domain. Proline is recognized by SH3 homology domains.

2. C, page 142, figure 7.5.  
FcγRIIB is an inhibitory B cell receptor that contains an ITIM. CD3ζ exists as a homodimer in the T cell receptor complex, and each of the z chains contain 3 ITAMs each. FcεRI is an ITAM-
containing B cell receptor, and Igα is associated with membrane-bound Ig molecules which also contains an ITAM.

The T cell receptor complex consists of a collection of homodimers and heterodimers. The αβ heterodimer serves as the binding site for a peptide-MHC complex. Each TCR complex also contains one CD3 γε heterodimer and one δε heterodimer, as well as a ζζ homodimer, all of which contain ITAMs on their cytoplasmic tails.

The TCR complex is made up of multiple polypeptide chains. The TCR α and β chains bind antigen, but have short cytoplasmic tails without signal transduction capability. Signal transduction is mediated through the cytoplasmic tails of the ζζ homodimer, or through one of the CD3 heterodimers (γε or δε).

The α and β chain each contain 3 complementarity-determining regions (CDRs) that contribute to the variability of the TCR. The β chain contains a fourth CDR that serves as the binding site for superantigens.

6. C, page 147, figure 7.11.
The cytoplasmic tails of CD4/CD8 contain the Src family kinase Lck. After the TCR binds an MHC-peptide complex, the CD4/CD8 coreceptor brings Lck close to the ITAMs on the cytoplasmic tails of the CD3 and ζ chains, allowing the ITAMs on these chains to be phosphorylated. Once phosphorylated, the ITAMs on the ζ chain serves as a docking site for ZAP-70. LAT is an adaptor protein phosphorylated by ZAP-70. Btk is a kinase involved in B cell signaling.

During TCR activation, ZAP-70 phosphorylates LAT, allowing Grb-2 to dock. SOS is then recruited to a site on Grb-2, which exchanges GTP for GDP on the Rac molecule, resulting in Rac-GTP. Rac-GTP activates Raf, which activates MEK-1, which activates the MAP kinase ERK.

8. C, pages 151-52, figure 7.14
Ras and Rac are both G proteins. The Ras pathway activates AP-1 through the ERK MAP kinase, while the Rac pathway activates AP-1 via JNK. DAG and PLCγ1 are not G proteins, and are involved in activation of PKC.

LAT recruits PLCγ1 to the plasma membrane where it is phosphorylated and hydrolyzes PIP2 into IP3 and DAG. IP3 stimulates the release of membrane-sequestered calcium stores, which is sensed by STIM1 and leads to opening of the CRAC channel. DAG activates PKC.

10. C, pages 152-54, figure 7.16.
NFAT is involved in expression of IL-2, IL-4, and TNF. c-Fos is a component of the AP-1 transcription factor, and NF-κB is a transcription factor important in innate immune cell signaling.
Welcome to the FIT Board Review Corner, prepared by Andrew Nickels, MD, and Sarah Spriet, DO, senior and junior representatives of ACAAI’s Fellows-In-Training (FITs) to the Board of Regents. The FIT Board Review Corner is an opportunity to help hone your Board preparedness.

Review Questions

Allergy and Immunology Review Corner: Cellular and Molecular Immunology, 8th Edition
By Abul K. Abbas, MBBS, Andrew H. H. Lichtman, MD, PhD and Shiv Pillai, MBBS, PhD.

Chapter 8 (pages 172-196): Lymphocyte Development and Antigen Receptor Gene Rearrangement
Prepared by Lorraine Anderson, MD, VA Greater Los Angeles Healthcare System

1. The maturation of B cells from progenitors occurs mostly in __________ before birth and __________ after birth.
   a. Fetal bone marrow; bone marrow
   b. Fetal spleen; liver
   c. Fetal liver; spleen
   d. Fetal liver; bone marrow

2. Precursors of T lymphocytes leave the fetal liver before birth and bone marrow later in life and complete their maturation in the
   a. Spleen.
   b. Liver.
   c. Bone marrow.
   d. Generative lymphoid organs.

3. The following transcription factors drive the development of B cell lineages (Follicular B cells, marginal zone B cells, B-1 B cell) from uncommitted progenitors.
   a. EBF, Notch 1, E2A
   b. Notch 1, GATA 3
   c. EBF, E2A, Pax-5
   d. Notch 1, Pax-5

4. The following transcription factors drive the development of T cells lineages (αβ T cells, γδ T cells) from uncommitted progenitors.
   a. Notch 1, GATA 6
   b. Notch 1, GATA3
   c. EBF, E2A, Pax-5
   d. Notch 1, Pax-5
5. In T cells IL7 is required for proliferation. Mutations in the common γ chain of the IL-7 receptor causes an immunodeficiency disorder called X-Linked Severe combined immunodeficiency disease (X-SCID). This disease is characterized by
   a. Block in T and B cell development.
   b. Block in T, NK cell and B cell development.
   c. Block in T and NK cell development but normal B cell development.
   d. Block in T, NK cell, B cell and dendritic cell development.

6. Lymphocyte development is a stepwise process guided by 2 check points before the lymphocyte can be selected to “mature.” What is the first check point during lymphocyte development?
   a. Expression of the pre-B/T antigen receptor.
   b. Positive selection.
   c. Negative selection.
   d. Clonal deletion and receptor editing.

7. Negative selection is a process important in maintaining central tolerance. Which statement accurately describes Negative selection?
   a. A process that selects lymphocytes that weakly recognize self-antigens for apoptosis.
   b. A process that eliminates or alters developing lymphocytes whose antigen receptors bind strongly to self-antigens.
   c. A process that selects lymphocytes that weakly recognize self-antigen for maturation.
   d. A process that eliminates or alters developing lymphocytes whose antigen receptors bind weakly to self-antigens.

8. Genes that encode antigen receptors of B and T cells are generated by the rearrangement of the variable (V) region gene segments with diversity (D) and joining (J) gene segments. Which statement accurately identifies the correct gene segment rearrangement?
   a. The Ig light chain protein (κ or λ) V domain is encoded by the V, D, and J gene segments while the Ig heavy chain protein V domain is encoded by the V and J gene segments.
   b. The Ig light chain protein (κ or λ) V domain is encoded by the V and D gene segments; while the Ig heavy chain protein V domain is encoded by the V,D, and J gene segments.
   c. The Ig light chain protein (κ or λ) V domain is encoded by the V and J gene segments; while the Ig heavy chain protein V domain is encoded by the V and J gene segments.
   d. The Ig light chain protein (κ or λ) V domain is encoded by the V and J gene segments; while the Ig heavy chain protein V domain is encoded by the V,D, and J gene segments.

9. Which TCR gene loci has similarities to the Ig heavy chain locus?
   a. TCR α and TCR β.
   b. TCR α and TCR γ.
   c. TCR β and TCR δ.
   d. TCR β and TCR λ.

10. The sequential events during V (D) J recombination
    a. Double stranded break (Rag-1, Rag-3), hairpin opening (TdT), end-processing (Artemis), non homologous end joining (Ku70, Ku80, DNA-PK, XRCC4, DNA ligase IV).
b. Double stranded break (Rag-1, Rag-2), hairpin opening (Artemis), end-processing (TdT), non homologous end joining (Ku70, Ku80, DNA-PK, XRCC4, DNA ligase IV).

c. hairpin opening (Artemis), Double stranded break (Rag-1, Rag-2) end-processing (TdT), non homologous end joining (Ku70, Ku80, DNA-PK, XRCC4, DNA ligase IV).

d. non homologous end joining (Ku70, Ku80, DNA-PK, XRCC4, DNA ligase IV), Double stranded break (Rag-1, Rag-2), hairpin opening (Artemis), end-processing (TdT).

11. Which enzyme(s) is (are) responsible for junctional diversity by adding P and N nucleotides?
   a. Artemis.
   b. DNA ligase IV.
   c. Rag 1, Rag 2.
   d. TdT.
   e. Ku70, Ku80, DNA-PK.

12. The most immature cortical thymocytes are_____________ and considered to be a the pro-T cell stage and express the following protein(s)z:
   a. Double positive T cells; Artemis.
   b. Double negative T cells, Rag1 and Rag 2.
   c. Double positive T cells, Rag 1 and Rag 2.
   d. Double negative T cells, Artemis.

13. Negative selection of T cells occurs in the thymic cortex at the double positive T cell stage and in the thymic medulla at the single positive T cell stage. What mutation results in autoimmune polyendocrine syndrome?
   a. Btk.
   b. Artemis.
   c. AIRE.
   d. Rag-1 and Rag2.

Answers
The maturation of B cells from progenitors committed to this lineage occurs mostly in the bone marrow and before birth in the fetal liver.

Precursors of T lymphocytes leave the fetal liver before birth and bone marrow later in life and complete their maturation in the thymus.

3. C, page 173 Figure 8-2.

4. B, page 173 Figure 8-2.

X linked SCID is characterized by a block in T cell and NK cell development with normal B cell development, i.e. T(-)B(+)NK(-).
The assembled pre-BCR and pre-TCR complexes provide signals for survival, for proliferation, for the phenomenon of allelic exclusion and for the further development of early B and T lineage cells. Thus expression of the pre antigen receptor is the first checkpoint during lymphocyte development.

Negative selection is a process that eliminates or alters developing lymphocytes whose antigen receptors bind strongly to self-antigens present in generative lymphoid organs. T cells with high affinity for self-antigens are eliminated by apoptosis a phenomenon known as clonal deletion. Strongly self-reactive immature B cells may be induced to make further Ig gene rearrangements in phenomenon called receptor editing. If editing fails, the self-reactive B cells die, also called clonal deletion. Negative selection of immature lymphocytes is an important mechanism for maintaining tolerance to many self-antigens; this is also called central tolerance because it develops in the central (generative) lymphoid organs.

8. D, page 178 Figure 8-5.
The Ig light chain protein (κ or λ) V domain is encoded by the V and J gene segments; while the Ig heavy chain protein V domain is encoded by the V,D, and J gene segments

In the TCR β and δ proteins, the V domain is encoded by the V, D and J gene segments, and in the TCR α or γ proteins the V domain is encoded by the V and J gene segments. Thus the TCR β and δ loci also have D segments like the Ig heavy chain locus.

10. B, page 182 Figure 8-10.

The addition of new nucleotides is mediated by the enzyme terminal deoxynucleotidyl transferase (TdT)

The most immature cortical thymocytes are called double negative thymocytes. Rag-1 and Rag-2 proteins are first expressed at the double negative stage of T cell development and are required for the rearrangement of TCR genes.

In the medulla, medullary thymic epithelia cells express a nuclear protein called AIRE (autoimmune regulator) that induced the expression of a number of tissue-specific genes in the thymus. The AIRE dependent expression in the thymus makes many tissue specific peptides available for presentation to developing T cells, facilitating the deletion (negative selection) of these cells. A mutation in the gene that encodes AIRE results in an autoimmune polyendocrine syndrome.
Review Questions

**Allergy and Immunology Review Corner**: Cellular and Molecular Immunology, 8th Edition
By Abul K. Abbas, MBBS, Andrew H. H. Lichtman, MD, PhD and Shiv Pillai, MBBS, PhD.

**Chapter 9 (pages 202-211): Activation of T Lymphocytes**

*Prepared by Sarah Spriet, DO, Walter Reed National Military Medical Center*

1. Which receptor and ligand pair results in costimulation of naïve T cells and the generation of regulatory T cells?
   - a. CD28 : B7
   - b. CTLA-4 : B7
   - c. ICOS : ICOSL
   - d. PD1 : PDL2

2. Identify the anti-apoptotic protein which promotes T cell survival.
   - a. Bax
   - b. Bcl-2
   - c. Bim
   - d. Fas

3. Identify the correct statement regarding costimulators below.
   - a. Of all potential APCs, macrophages express the highest levels of costimulators and, thus, are the most potent stimulators of naïve T cells.
   - b. One of the major functions of adjuvants in T cell activation is to stimulate the expression of costimulators on APCs.
   - c. T cells recognize antigen (with or without B7 costimulators), causing expression of CD40 on T cells.
   - d. Ox40 (CD134) is a TNFR family member expressed on naïve T cells that functions to maintain cell survival and sustained responses.

4. Identify the receptor that has a negative regulatory effect on T cells.
   - a. CD28
   - b. ICOS
   - c. CTLA-4
   - d. CD40

5. What surface protein binds to and reduces expression of S1PR1 resulting in the retention of activated T cells in lymphoid organs?
   - a. CD25
   - b. CD40L
   - c. CD62L
   - d. CD69
6. What immunobiologic agent acts on CD80/CD86 to block costimulation and T cell activation?
   a. Abatacept
   b. Adalimumab
   c. Basiliximab
   d. Etanercept

7. Which of the following chains is unique to the IL-2 receptor?
   a. α
   b. β
   c. γ
   d. δ

8. What is the most important cytokine for the maintenance of memory CD4+ and CD8+ T cells?
   a. IL-2
   b. IL-3
   c. IL-4
   d. IL-7

9. What protein chain of the IL-2R is non-functional in X-linked SCID?
   a. CD25
   b. CD122
   c. CD127
   d. CD132

10. Identify the correct statement regarding memory T cells.
    a. Central memory T cells express CC47 and L-selectin and home mainly to lymph nodes.
    b. Central memory T cells home to mucosal tissues and produce IFN-γ in response to antigenic stimulation.
    c. The most reliable phenotype markers for memory T cells appear to be the surface expression of the IL-7 receptor and CD28.
    d. The maintenance of memory T cells requires cytokines and antigen recognition.

Answers
1. A, pages 202-204.
The proliferation and differentiation of naïve T cells require signals provided by molecules on antigen presenting cells, called costimulators, in addition to antigen-induced signals. The best characterized costimulatory pathway in T cell activation involves the T cell surface receptor CD28, which binds the co-stimulatory molecules B7-1 (CD80) and B7-2 (CD86) expressed on activated APCs. Numerous receptors homologous to CD28 and their ligands homologous to B7 have been identified, and these proteins regulate T cell responses both positively and negatively. Binding of ICOS:ICOSL leads to co-stimulation of effector and regulatory T cells and generation of follicular helper T cells. Whereas, CTLA-4 and PD-1 are inhibitory receptors of the CD28 family. Of the possible answers, only CD28 binding to B7 results in co-stimulation of naïve T cells.
Costimulatory signaling via CD28 amplifies signaling pathways that are also induced downstream of the T cell receptor and may trigger additional signals that cooperate with TCR-induced signals. PI3-kinase is recruited to the cytoplasmic tail of CD28, and this in turn activates the downstream pro-survival kinase Akt as well as Itk and PLCγ, which can trigger calcium signaling. CD28 can also contribute to the activation of the JNK MAP kinase via the Rac small G protein and can amplify the activation of the NF-κB pathway. The net result of these signaling pathways is the increased expression of anti-apoptotic proteins such as Bcl-2 and Bcl-XL, which promote survival of T cells; increased metabolic activity of T cells; enhanced proliferation of the T cells; production of cytokines such as IL-2; and differentiation of the naïve T cells into effector and memory cells.

BH3-only proteins are sensors of cell stress that bind to and influence death effectors and regulators. In lymphocytes, the most important of these sensors is a protein called Bim. Activated Bim binds to two pro-apoptotic effector proteins of the Bcl-2 family called Bax and Bak, which oligomerize and insert into the outer mitochondrial membrane, leading to increased permeability.

In CD4+ T cells, the most important death receptor is Fas and its ligand is FasL. Their binding activates a cascade of caspases, which ultimately cause the apoptotic death of the cells. The same pathway of apoptosis may be involved in the elimination of self-reactive B lymphocytes also in the periphery.

Many adjuvants are products of microbes, or mimic microbes, and one of their major functions in T cell activation is to stimulate the expression of costimulators on APCs. Of all potential APCs, mature dendritic cells express the highest levels of costimulators and, as a result, are the most potent stimulators of naïve T cells. T cells recognize antigen (with or without B7 costimulators), causing expression of CD40L on T cells. Ox40 is a TNFR family member expressed on activated CD4+ and CD8+ T cells that functions to maintain cell survival and sustained responses.

CTLA-4 and PD-1 are critical inhibitors of T cells and are sometimes called coinhibitors. Binding of B7 to CTLA-4 inhibits the initial activation of T lymphocytes in secondary lymphoid organs. Binding of PD-L2 to PD-1 inhibits the activation of effector cells, especially in peripheral tissues. CD28 and ICOS are costimulators. CD40 is a member of the TNFR superfamily expressed on B cells, macrophages and dendritic cells. CD40 has functions in the activation of macrophages in cell-mediated immunity and activation of B cells in humoral immune responses. Activated helper T cells express CD40L, which engages CD40 on the APCs and activates the APCs to make them more potent by enhancing their expression of B7 molecules and secretion of cytokines that promote T cell differentiation. Thus, the CD40 pathway indirectly amplifies T cell responses by inducing costimulators on APCs, but CD40L does not itself function as a costimulator for T cells.

After the initiation of activation by antigen recognition and costimulator binding, there are characteristic changes in the expression of various surface molecules in T cells. Within a few hours of activation, T cells increase their expression of CD69, a plasma membrane protein. This protein binds to and reduces surface expression of the sphingosine 1-phosphate receptor S1PR1, a receptor that mediates the egress of T cells from lymphoid organs.
The consequence of decreased S1PR1 expression is that activated T cells are retained in lymphoid organs long enough to receive the signals that initiate their proliferation and differentiation into effector and memory cells. After cell division, CD69 expression decreases, the activated T cells re-express high levels of S1PR1, and therefore effector and memory cells can exit the lymphoid organs.

CD25 is a cytokine receptor that enables activated T cells to respond to the growth-promoting cytokine IL-2. The expression of CD40L enables activated T cells to mediate their key effector functions, which are to help macrophages and B cells. In addition, CD40L on the T cells activates dendritic cells to become better APCs. During activation, T cells reduce expression of molecules that bring them to the lymphoid organs such as L-selectin (CD62L) and the chemokine receptor CCR7 and increase the expression of the molecules that are involved in their migration to peripheral sites of infection and tissue injury.

Abatacept (Orencia) is a selective costimulation modulator. It is a fusion protein composed of the Fc region of human IgG fused to the extracellular domain of CTLA-4. Abatacept binds to the CD80 (B7-1) and CD86 (B7-2) molecules and prevents the second signal, thereby preventing T cell activation. This medication is currently being used for the treatment of Rheumatoid Arthritis refractive to anti-TNF therapy.

Adalimumab (Humira) is a fully human anti-TNF-alpha monoclonal antibody. It binds to TNF-alpha and decreases the inflammatory response associated with autoimmune diseases. This medication is approved for treatment of several conditions including RA, Crohn’s Disease and Ankylosing Spondylitis.

Basiliximab (Simulect) is a chimeric mouse-human monoclonal antibody antibody to the α chain (CD25) of the IL-2 receptor of T cells. It is used to prevent rejection in organ transplantation, especially in kidney transplants.

Etanercept (Enbrel) is another TNF antagonist. Its uses include symptoms reduction in patients with RA, JIA, Psoriatic Arthritis, Plaque Psoriasis and Ankylosing Spondylitis.

Of the three chains, only IL-2α is unique to the IL-2R. IL-2 binds to the alpha chain alone with low affinity, and this does not lead to any detectable cytoplasmic signaling or biologic response. The beta chain is also part of the IL-15 receptor. The gamma chain is shared by multiple cytokine receptors involving those for IL-4, IL-7, IL-9, IL-15, and IL-21. The IL-2 receptor has no delta chain.

The most important cytokine for the maintenance of memory T cells is IL-7, which also plays a key role in early lymphocyte development and in the survival of naïve T cells. Memory CD8+ cells also depend on the related cytokine IL-15 for their survival. IL-7 and IL-15 induce the expression of anti-apoptotic proteins and stimulate low level proliferation, both of which maintain populations of memory T cells for long periods.
IL-2 is involved in the proliferation and differentiation of T cells into effector and memory cells. It also promotes regulatory T cell development, survival and function. IL-2 is also involved with the proliferation and activation of NK cells as well as B cell proliferation.

IL-3 induces maturation of all hematopoietic lineages.

IL-4 acts on B cells, T cells, Macrophages and Mast cells. In regards to T cells, IL-4 is involved in TH2 differentiation and proliferation.

X-linked SCID is caused by mutations in the gene encoding the common gamma chain (CD132) which is shared by the receptors for the interleukins IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21. CD25 refers to IL-2R alpha chain, CD122 refers to IL2R beta chain and CD127 refers to the IL7R alpha chain.

10. A, page 211.
Central memory T cells express CC47 and L-selectin and home mainly to lymph nodes. Effector memory T cells do not express CCR7 or L-selectin and home to peripheral sites, especially mucosal tissues. On antigenic stimulation, effector memory T cells produce effector cytokines such as IFN-γ or rapidly become cytotoxic, but they do not proliferate much. The most reliable markers for memory T cells are IL7R and CD27. Maintenance of memory T cells is dependent on cytokines but does not require antigen recognition.
FIT Board Review Corner – September 2015

Welcome to the FIT Board Review Corner, prepared by Andrew Nickels, MD, and Sarah Spriet, DO, senior and junior representatives of ACAAI’s Fellows-In-Training (FITs) to the Board of Regents. The FIT Board Review Corner is an opportunity to help hone your Board preparedness.

Review Questions

Allergy and Immunology Review Corner: Cellular and Molecular Immunology, 8th Edition
By Abul K. Abbas, MBBS, Andrew H. H. Lichtman, MD, PhD and Shiv Pillai, MBBS, PhD.

Chapter 10 (pages 213-230): Differentiation and Functions of CD4+ Effector T Cells
Prepared by Kristen Dazy, MD, Scripps Clinic Medical Group

1. Inflammation, consisting of leukocyte recruitment and activation, accompanies many of the reactions of CD4+ T lymphocytes and may damage normal tissue. What is another name for this T-cell dependent injurious reaction?
   a. Immediate hypersensitivity
   b. Cytotoxic hypersensitivity
   c. Immune complex mediated hypersensitivity
   d. Delayed-type hypersensitivity

2. What is the signature cytokine produced by the T\(_H\)1 subset of CD4+ effector T cells?
   a. INF-\(\gamma\)
   b. TNF-\(\alpha\)
   c. IL-4
   d. IL-22

3. INF-\(\gamma\) helps stimulate T\(_H\)1 differentiation by directly activating which transcription factor?
   a. T-bet
   b. STAT1
   c. STAT4
   d. STAT6

4. T\(_H\)1 cells activate macrophages by contact-mediated signals including INF-\(\gamma\) and what other cell surface marker?
   a. CXCR3
   b. CCR5
   c. CD40L
   d. CCR8

5. A 14-month old boy presents to the hospital with a two day history of non-productive cough, shortness of breath, and fever. On arrival, he is febrile to 103.2 degrees and O2 saturation is 89%. Chest x-ray reveals bilateral infiltrates. He is empirically started on broad-spectrum antibiotics, however, his respiratory status declines over the next 24 hours and he is intubated
for respiratory failure. Bronchoalveolar lavage is performed and is positive for *Pneumocystis jiroveci*. On detailed history, you determine that he has been hospitalized several times since birth due to severe, recurrent respiratory infections. Immune deficiency work-up has previously revealed low IgG and normal IgM levels. Which of the following is most likely responsible for this child’s presentation?

a. ADA deficiency  
b. CD40L mutation  
c. JAK3 mutation  
d. STAT3 mutation

6. Which of the following transcription factors acts as the master regulator of Th2 differentiation by enhancing expression of the Th2 cytokine genes IL-4, IL-5, and IL-13?

a. STAT1  
b. STAT4  
c. STAT6  
d. GATA-3

7. Which of the following cytokines is responsible for B cell Ig heavy chain class switching to both the IgE and IgG4 isotypes?

a. IL-4  
b. IL-5  
c. IL-12  
d. INF-γ

8. What is the major cell type that is recruited by Th17 cells to combat extracellular microbes?

a. Neutrophils  
b. Monocytes  
c. Eosinophils  
d. Basophils

9. Which cytokine is produced by activated Th17 T cells and serves to maintain barrier function of epithelial tissue, particularly of the skin and GI track, by stimulating repair mechanisms and producing anti-microbial peptides?

a. IL-17  
b. IL-18  
c. IL-21  
d. IL-22

10. Which of the following cell types are considered a subset of T cells and are able to recognize a wide variety of antigens without a requirement for MHC-associated presentation?

a. Th2 T cells  
b. αβ T cells  
c. γδ T cells  
d. Th17 T cells

Answers
Delayed-type hypersensitivity (DTH) is a term that refers to tissue damage caused by a T cell mediated immune response. DTH frequently occurs together with protective cell-mediated immunity against microbes and may be the cause of much of the pathology associated with certain types of infections.

The signature cytokines produced by T cell subsets determine their effector functions and roles in disease. The signature cytokine produced by T\(_{H1}\) CD4+ T cells is INF-\(\gamma\) which occurs in response to intracellular microbes that activate dendritic cells, macrophages, and NK cells.

INF-\(\gamma\) directly activates transcription factor STAT1 which in turn stimulates expression of another transcription factor, T-bet. T-bet then promotes INF-\(\gamma\) production through a combination of direct transcriptional activation of the INF-\(\gamma\) gene and by inducing chromatin remodeling of the INF-\(\gamma\) promoter region. IL-12 contributes to T\(_{H1}\) differentiation by binding to receptors on antigen-stimulated CD4+ T cells and activating transcription factor STAT4, which also enhances INF-\(\gamma\) production. STAT6 is a transcription factor involved in production of the T\(_{H2}\) subset of CD4+ T cells.

4. C, pages 221-222.
When T\(_{H1}\) cells are stimulated by antigen, the cells express CD40L on their surface and secrete INF-\(\gamma\). The actions of INF-\(\gamma\) on macrophages synergize with the actions of CD40 ligand and together they are potent stimuli for macrophage activation. T\(_{H1}\) cells also express high levels of the chemokine receptors CXCR3 and CCR5 which bind to other chemokines elaborated in tissues during innate immune responses.

5. B, page 222.
Inherited immunodeficiencies, as well as gene knockout mice, have established the critical importance of CD40-CD40L interactions. X-linked hyper-IgM syndrome results from inherited mutations in CD40L which makes patients susceptible to infections with otherwise harmless intracellular microbes, including the intracellular fungus \textit{Pneumocystis jiroveci}, which require T cell-dependent macrophage activation in order to be eradicated. As expected, these patients also have defects in helper T cell-dependent antibody production.

L-4 stimulates T\(_{H2}\) development by activating the transcription factor STAT6, which, together with TCR signals, induces expression of GATA-3. This transcription factor acts as a master regulator of T\(_{H2}\) differentiation by enhancing T\(_{H2}\) cytokine genes (IL-4, IL-13) which are located in the same genetic locus. GATA-3 works by directly interacting with the promoters of these genes and also by causing chromatin remodeling.

IL-4 stimulates B cell Ig heavy chain class switching to the IgE isotype and also enhances switching to IgG4 (in humans) and inhibits switching to the IgG2a and IgG2c isotypes (in mice), both of which are stimulated by INF-\(\gamma\). IL-13 can also contribute to switching to the IgE isotype.

**TH**17 cells combat microbes by recruiting leukocytes, mainly neutrophils, to sites of infection and serve as a major defense mechanism against extracellular bacteria and fungi. IL-17 is produced by TH17 cells and stimulates the production of chemokines and other cytokines that recruit neutrophils and, to a lesser extent, monocytes to the site of T cell activation. It also enhances neutrophil generation by increasing the production of G-CSF and the expression of its receptors.

9. DC, page 227.
IL-22 is a member of the type II cytokine family. It is produced by activated T cells, particularly TH17 cells, and by some NK cells and group 3 innate lymphoid cells. IL-22 is produced in epithelial tissues, especially of the skin and gastrointestinal track, and serves to maintain epithelial integrity, mainly by promoted the barrier function of epithelia, by stimulating repair reactions, and by induction production of anti-microbial peptides.

In addition to CD4+ and CD8+ T cells, there are smaller populations of T cells that have distinct features and are thought to serve specialized functions in host defense. The best defined of these subsets are γδ T cells and NKT cells. Both of these T cell subsets express receptors of limited diversity (suggesting that they have evolved to recognize a small group of microbial antigens) and are able to recognize various antigens without a requirement for MHC-associated presentation. They are both abundant in epithelial tissues, such as the gastrointestinal track.
Review Questions

**Allergy and Immunology Review Corner:** Cellular and Molecular Immunology, 8th Edition
By Abul K. Abbas, MBBS, Andrew H. H. Lichtman, MD, PhD and Shiv Pillai, MBBS, PhD.

**Chapter 11 (pages 231-239):** Differentiation and Functions of CD8+ Effector T Cells

Prepared by Kara Wada, MD, Ohio State Univ/Nationwide Children's Hospital

1. Which of the following are mechanisms are employed by viruses in an attempt to evade host defenses?
   a. Viruses reside in the nucleus where they are inaccessible to killing mechanisms.
   b. Viruses do not use the host cell’s machinery to replicate.
   c. Viruses use cell surface molecules to gain entry into the host cell.
   d. Viruses do not alter cell surface molecules of the host cell.

2. CD8+ T lymphocytes are effector T cells responsible for the following functions:
   a. Elimination of intracellular microbes, tumor cells and acute organ allograft rejection.
   b. Elimination of extracellular microbes and tumor cells.
   c. Elimination of intracellular microbes, tumor cells and chronic organ allograft rejection.
   d. Elimination of extracellular microbes, tumor cells and chronic organ allograft rejection.

3. Cross presentation is a critical step in the antigen presentation to CD8+ T cells. Which of the following best explains this process?
   a. Specialized dendritic cells ingest infected cells, transfer the protein to the cytosol and process the antigen to enter the class I MHC presentation pathway for recognition by CD8+ T cells.
   b. Specialized B cells ingest infected cells, transfer the protein to the cytosol and process the antigen to enter the class I MHC presentation pathway for recognition by CD8+ T cells.
   c. Specialized dendritic cells ingest infected cells, transfer the protein to the cytosol and process the antigen to enter the class II MHC presentation pathway for recognition by CD8+ T cells.
   d. Specialized dendritic cells ingest infected cells, transfer the protein to the cytosol and process the antigen to enter the class I MHC presentation pathway for recognition by CD4+ T cells.

4. CD4+ helper T cells are not necessary for CD8+ T cell responses in which of the following circumstances?
   a. Latent viral infections
   b. Infection of an antigen presenting cell
   c. Organ transplant rejection
   d. Tumor recognition

5. Helper T cells are thought to promote CD8+ T cell activation by which of the following mechanisms?
a. Expression of CD40
b. Production of IL-15
c. Production of IL-21
d. Production of TNF-α

6. CD8+ T cells may be initiated in some chronic viral infections but then gradually diminishes over time. This phenomenon is called:
   a. Tolerance
   b. Cross presentation
   c. Exhaustion
   d. Consumption

7. What functional and phenotypic changes are found in exhausted CD8+ T cells?
   a. Decreased production of INFγ
   b. Decreased expression of PD-1
   c. Increased production of IL-21
   d. Presence of natural antibodies that block PD-1

8. CTL-mediated killing involves a specific series of events initiating with recognition. Which is the correct pairing found in the immunologic synapse of CTL-mediated killing?
   a. Target cell with class I MHC molecule with peptide: LFA-1
   b. ICAM-1:LFA-1
   c. ICAM:CD8 coreceptor
   d. ICAM:TCR

9. CTLs and NK cells mediate killing through the use of cytotoxic proteins. Which cytotoxic protein is correctly described?
   a. Granzymes function analogous to C9 complement
   b. Perforin functions to facilitate delivery of the other cytotoxic granules into the cytosol of the target cell.
   c. Serglycin is a serine protease that activates caspases.
   d. Perforin serves to assemble a complex of many different types of cytotoxic proteins

10. A patient presents with a history of recurrent Mycobacterial infections, difficult to clear malaria and reactivation of EBV. Which type of immunodeficiency would you be most concerned about?
    a. TLR defect
    b. HIV/AIDS
    c. CD8+ T cell defect
    d. Macrophage defect

Answers
Viruses have evolved to use various cell surface molecules to gain entry into host cells and then use the host cell’s machinery to replicate and disseminate from one host cell to the next. They tend to reside in the cytosol thus further avoiding intrinsic microbicidal mechanisms.

The function of CD8+ cytotoxic T lymphocytes include elimination of a variety of intracellular microbes, eradication of tumor cells and play critical roles in the acute rejection of organ allografts.

Specialized dendritic cells ingest infected cells, transfer the protein to the cytosol and process the antigen to enter the class I MHC presentation pathway for recognition by CD8+ T cells. See Fig. 6.20.

The requirement for helper cells varies according to type of antigen exposure. Strong innate response and direct infection of APCs do not require CD4+ help whereas latent viral infections, tumors and organ transplant responses do required CD4+ interactions.

Helper T cells may promote CD8+ T cell activation by secretion of cytokines that stimulate the differentiation of CD8+ T cells and through expression of CD40L which can bind to CD40 on the APC. IL15 is important for memory CD8 survival but IL21 is known to be produced by CD4 T cells and plays a role in the induction of CD8 T cell memory and prevention of exhaustion.

Exhaustion is a term that is used to imply that the effector response does develop but then is actively shut down. This occurs in chronic viral infections.

Exhausted CD8+ T cells demonstrate decreased INFγ and increased expression of inhibitory receptors such as PD-1. Presence of IL-21 helps prevent exhaustion from occurring and antibodies directed against PD-1 are being considered therapeutically in the treatment of tumors ad chronic viral infections.

Target cell with class I MHC molecule with peptide is a ligand for the CR and CD8 coreceptors. ICAM-1 binds to LFA-1.

Granzymes A, B, and C are serine proteases at cleave proteins and activate caspases, perforin functions analogous to C9 complement and also facilitates delivery of other granzymes. Serglycin serves to assemble a complex containing granzymes and perforin.

CTLs are crucial for host defense against intracellular bacteria, malaria, and latent viral infections such as EBV.
Welcome to the FIT Board Review Corner, prepared by Andrew Nickels, MD, and Sarah Spriet, DO, senior and junior representatives of ACAAI's Fellows-In-Training (FITs) to the Board of Regents. The FIT Board Review Corner is an opportunity to help hone your Board preparedness.

Review Questions

Allergy and Immunology Review Corner: Cellular and Molecular Immunology, 8th Edition
By Abul K. Abbas, MBBS, Andrew H. H. Lichtman, MD, PhD and Shiv Pillai, MBBS, PhD.

Chapter 12 (pages 239-250): B Cell Activation and Antibody Production
Prepared by Kathryn R. Wessell, DO, University Hospitals Richmond

1. Antibody responses to protein antigens that require the antigen to be internalized by specific B cells, processed, and then the peptides to be presented to CD4+ helper T lymphocytes which then activate the B cells are called:
   a. T-independent antigens
   b. T-dependent antigens
   c. Non-protein antigen response
   d. Non-polysaccharide antigen response

2. Antigen and other stimuli stimulate the proliferation and differentiation of the specific B cell clone. B cell proliferation results in a B cell progeny of the clone that undergoes 4 primary endpoints: antibody secretion, Isotype switching, affinity maturation and ________________.
   a. Recruiter B cells
   b. Mature B cells
   c. Memory B cells
   d. Secondary B cells

3. The primary antibody immune response is different from the secondary antibody in many ways. The primary response peak is smaller, the antibody affinity is lower, and the antibody isotype is usually IgM > than IgG, compared to the antibody isotype of the secondary response being typically a relative increase in IgG and ______________ in certain situations.
   a. IgA or IgE
   b. IgM or IgA
   c. IgM or IgD
   d. IgA or IgD

4. One of the major distinctions between the forms of antigens recognized by B lymphocytes compared to T lymphocytes is that the antigen that is presented to B cells is generally:
   a. Processed by antigen presenting cells
   b. Presented in its intact, native conformation
   c. Small antigens are taken up by macrophage in the subcapsular sinus
   d. Large antigens are delivered to follicles via conduits
5. B cell activation is facilitated by the ___________ co-receptor on B cells, which recognize complement fragments covalently attached to the antigen or that are part of the immune complexes containing the antigen.
   a. CR4/CD 19
   b. CR4/CD 21
   c. CR2/CD 21
   d. CR2/CD 19

6. There are 4 major functional responses induced by antigen-mediated cross-linking of the BCR (B cell receptor) complex. These include expression of proteins that promote survival and cell cycling, antigen presentation increased B7 expression that aides in the interaction with helper T cells, increased expression of cytokine receptors that results in responsiveness to cytokines, and increased expression of __________ that leads to migration of the B cell from the follicle to T cell areas.
   a. CCR7
   b. CCR8
   c. CCR9
   d. CCR10

7. Antigen activated helper T cells and B cells move toward one another in response to chemokine signals and make contact with one another adjacent to the edge of:
   a. Germinal centers
   b. T cell zone
   c. Primary follicles (B cell zone)
   d. Capsule of the lymph node

8. The interaction of ____________ in T-Dependent B cell activation stimulates B cell proliferation and differentiation.
   a. CD40 on T cells and CD40 Ligand on B cells
   b. CD 40 Ligand on T cells and CD40 on B cells
   c. CD 40 on Both T cells and B cells
   d. CD40 Ligand on Both T cells and B cells

9. The germinal center consists of a light zone and dark zone. Proliferating B cells accumulate in the dark zone of the germinal center. The small non-dividing progeny of the B cells migrate to the adjacent light zone where they contact __________________________.
   a. Reticular dendritic cells
   b. Follicular dendritic cells
   c. Mantle zone dendritic cells
   d. Antigen presenting dendritic cells

10. Differentiation of T_{FH} cells from naïve CD4+ T cells requires two steps:
    a. Initial activation by B Cells and subsequent activation by antigen-presenting dendritic cells
    b. Initial activation by the follicular dendritic cell and subsequent activation by the antigen-presenting dendritic cell
c. Initial activation by the antigen-presenting dendritic cells and subsequent activation by B cells
d. Initial activation by B cells and subsequent activation by follicular dendritic cells.

Answers
Antibody responses to protein antigens that require that the antigen be internalized by specific B cells, processed, and then that the peptides be presented to CD4+ helper T lymphocytes, which then activate the B cells are called T-dependent antigens. T-independent antigens do not require antigen-specific helper T lymphocytes. Examples are non-protein antigens with repeating determinants such as polysaccharides, some lipids, and nucleic acids.

2. C, page 240, Figure 12-1.
Memory B cells survive in a resting state without secreting antibodies for many years, but they mount rapid responses on subsequent encounters with the antigen.

3. A, page 241, Figure 12-2.
The secondary response has an antibody isotype with a relative increase in IgG and under certain situations an increase in IgA or IgE, compared to IgM, the antibody isotype that predominates in the primary antibody response.

Antigens that are presented to B cells are generally presented in their intact, native conformation and not processed by antigen presenting cells. Small antigens are delivered to naïve B cells in follicles via conduits and larger antigens are delivered to naïve B cells by macrophages in the subcapsular sinus and by dendritic cells in the medulla.

5. C, page 244, Figure 12-5.
The CR2/CD21 coreceptor on B cells facilitates B cell activation. CR2/CD21 enhances BCR (Bell Cell Receptor) signaling.

6. A, page 245, Figure 12-6.
The functional response of migration of B cells toward T cells is a result of the expression of CCR7.

7. C, page 246, Figure 12-8
T cells and B cells make contact adjacent to the edge of primary follicles.

On activation, helper T cells express CD40 ligand (CD40L) which engages its receptor, CD40, on antigen-stimulated B cells and induces B cell proliferation and differentiation. Cytokines produced by the helper T cells also contribute to B cell responses.

Follicular dendritic cells (FDCs) are found only in lymphoid follicles. Proliferating B cells accumulate in the dark zone of the germinal center, which contains neither FDCs nor T cells. The small non-dividing progeny of the B cells migrate to the adjacent light zone, where they come into close contact with the processes of FDCs and \( \text{T}_{\text{FH}} \) cells, and this is where subsequent selection events occur.

10. C, pages 250 and 251, Figure 12-3. Differentiation of \( \text{T}_{\text{FH}} \) cells from naïve CD4+ T cells requires initial activation by antigen presenting dendritic cells, then activated T cells express high levels of CXCR5, subsequently the interaction of ICOS with ICOS ligand on activated B cells promotes the differentiation of T cells into \( \text{T}_{\text{FH}} \) cells.
Review Questions

**Allergy and Immunology Review Corner:** Cellular and Molecular Immunology, 8th Edition
By Abul K. Abbas, MBBS, Andrew H. H. Lichtman, MD, PhD and Shiv Pillai, MBBS, PhD.

**Chapter 13 (pages 276-287):** Effector Mechanisms of Humoral Immunity

*Prepared by Shalia E. Smith, DO, Larkin Comm Hosp/Nova Southeastern Univ*

1. Which of the below explanations accurately describe why free IgM cannot bind C1q?
   a. Free IgM is a pentameric and are in a configuration that is inaccessible to C1q
   b. IgG molecules bind the Fc portion of IgM making C1q inaccessible to bind
   c. IgM is most efficient in complement binding
   d. IgG is most efficient in complement activation

2. Classical complement activation is essential for ridding the host of various microbes. Which statement best describes the components in C3 convertase?
   a. Binding of C3b to antigen surface thus cleaving C5
   b. Cleavage of C4 to C4b, then binding of C2, then cleavage of C2 leaving C4bC2a
   c. Cleavage of C4 to Ig-associated C1q allowing formation of C3 convertase
   d. C3 binds to C1q and cleaves C5 making C3 convertase

3. How is the lectin pathway activated?
   a. Microbial polysaccharide antigen binds to IgG and this complex binds to free lectins in serum, thus activating lectin pathway
   b. C1q binds antigen (polysaccharide) bound to mannose and other lectins
   c. C3b binds to circulating lectin molecules and then bind to microbial polysaccharides
   d. Activation is triggered in the absence of antibody. Instead microbial activation is triggered in the absence of antibody. Instead microbial

4. Which of the following describes how the host is safe from enzymatic activity in the alternative pathway?
   a. Microbial surfaces degrade C3 convertase thus allowing rapid expansion of microbes
   b. Mammalian cells contain markers which inhibit C3bBb from binding
   c. If the C3bBb complex if bound to mammalian cells, it’s rapidly degraded and the activation is inhibited by regulatory proteins
   d. C3 convertase does not bind mammalian cells due to configuration of active sites

5. Which of the below complement components is the most integral to formation of the membrane attack complex?
   a. C5 convertase
   b. C6
   c. C9 convertase
   d. C8
   e. C7 inhibitors

6. Complement receptor 2 (CR2 or CD21) functions to....
a. Stimulate humoral immune responses by enhancing B cell activation by antigen and by promoting the trapping of antigen-antibody complexes in germinal centers
b. Promotes phagocytosis of C3b- and C4b- coated particles and clearance of immune complexes from circulation
c. Function as a receptor for the iC3b fragment generated by proteolysis of C3b
d. Be expressed on Kupffer cells in the liver

7. A family is seen by you for recurrent swelling in arms, abdomen, and face. These episodes are not ameliorated by histamine blockade and last for 1-2 days. One family member had to be intubated due to laryngeal edema and respiratory failure.

Laboratory values:
- C1 INH <20% of normal
- C4 markedly decreased

You start the family on C1 INH replacement therapy. They ask you about the pathophysiology of this regulatory protein. You tell them:

a. This autosomal dominant disease causes deficient C1 INH which causes excessive degradation and consumption of C4 and C2 leading to increased fluid in tissues causing pain and swelling
b. Sun exposure causes mutations in C1 INH and increased degradation of C4 thus making bradykinin induced swelling
c. Increases in C4 causes C1 INH malfunction thus leading to swelling
d. This autosomal recessive disease causes deficiency in C1 INH, C4 and C2 which leads to clinical manifestations

8. You are asked to give a lecture to medical students on the functions of complement in humoral immunity and innate immunity. They inform you that they know complement is responsible for opsonization and phagocytosis. You then tell them how complement also causes inflammation. Which statement best describes this?

a. Complement mediated lysis of foreign organisms is mediated by membrane attack complex
b. The proteolytic fragments C5a, C4a, and C3a induce inflammation by activating mast cells, neutrophils, and endothelial cells
c. Immune complexes are NOT made more soluble by complement and does not aid in clearance
d. C3b binds to CR4 on B cells and facilitates B cell activation and the initiation of humoral responses

9. A 20 year old male is admitted to the ICU with sepsis due to meningitis. This is his fourth episode of meningitis in one year. He is sexually active and his CD4+ counts are normal. Infectious Disease staff ask you if there is any immune deficiency that could explain these recurrent severe infections. You tell them:

a. No. This patient is just unlucky or you all have not given him the right antibiotics
b. Yes, deficiencies in the terminal complement components including C5, C6, C7, C8 and C9 predispose patients to disseminated infections with Neisseria meningitidis and Neisseria gonorrhoeae
c. Yes, deficiency in factor H will cause this
d. Yes, deficiency in the terminal complement components can cause lupus
10. A 27 year old female plans to breastfeed her newborn. She says she read on the internet that babies have a weak immune system and that breast feeding protects them. She wants to know how this works. Which of the following describes how this works?
   a. A 27 year old female plans to breastfeed her newborn. She says she read on the internet that babies have a weak immune system and that breast feeding protects them. She wants to know how this works. Which of the following describes how this works?
   b. Maternal IgE in breast milk provided passive immunity to bacterial microbes
   c. Ingested maternal IgA and IgM colonize the gut and stop opportunistic infections
   d. Maternal IgA is the only type of antibody a mother can provide her infant after birth

Answers
   Free IgM is not accessible to C1q, but once IgM binds to antigen, the conformational shape is altered and exposes the sites of Fc portions so that C1q can bind.

2. B, pages 276-278, Figure 13-9.
   C4 first binds to Ig-associated C1q and then is cleaved by C1r2S2 enzyme; covalent attachment of C4b to antigenic surface and antibodies. Then C2 binds to C4 and is cleaved into C2a and C2b. C4b and C2a make C3 convertase or C4b2a.

   Lectin pathway is antibody independent process. Microbial polysaccharides are bound by circulating lectins. MASP act at enzymatic proteins cleaving C4 and the rest of the pathway is akin to classic pathway.

   Alternative pathway activation readily occurs on microbial cell surfaces and not on mammalian cells page.

5. A, page 279.
   C5 convertase from any pathway initiates the last stages of complement pathway which leads to formation of membrane attack complex.


10. A, page 287.
Welcome to the FIT Board Review Corner, prepared by Sarah Spriet, DO, and Tammy Peng, MD, senior and junior representatives of ACAAI's Fellows-In-Training (FITs) to the Board of Regents. The FIT Board Review Corner is an opportunity to help hone your Board preparedness.

**Review Questions**

**FIT Board Review Corner – December 2015**

Allergy and Immunology Review Corner: Cellular and Molecular Immunology, 8th Edition
By Abul K. Abbas, MBBS, Andrew H. H. Lichtman, MD, PhD and Shiv Pillai, MBBS, PhD.

Chapter 14 (pages 294-316): Regional Immunity: Specialized Immune Responses in Epithelial and Immune Privileged Tissues
*Prepared by Niti Agarwal, MD, New York and Presbyterian Hospital*

1. Which of the following pairs are correct regarding immunity in the gastrointestinal tract?
   a. M cells: luminal antigen processing
   b. Paneth cells: neutralization of microbes in the lumen
   c. Secretory IgA/IgM: defensin production
   d. Intestinal epithelial cells: luminal antigen sampling

2. DC subsets in the GI tract have which of the following important functions?
   a. Mesenteric antigen sampling
   b. T cell tolerance induction and effector T cell activation
   c. Induction of B cell IgG class switching
   d. Imprinting gut homing phenotypes of strictly B cells

3. Which of the following tissues encompasses the greatest number of lymphocytes?
   a. Bone marrow
   b. Spleen
   c. Skin
   d. GI tract

4. Which of the following statements is accurate in regards to IGA class switching in the gut?
   a. IgA class switching can occur by only T cell dependent mechanisms
   b. DCs in the subepithelial dome of Peyers patches capture bacterial antigens delivered by M cells and migrate to interfollicular zone where they present antigen to CD8+ T cells
   c. TLR ligand activated DCs induce IgA class switch through factors such as BAFF, APRIL, and TGF-B
   d. B cell class switching to IgA is stimulated primarily through TGF-beta

5. IBD is thought to be related to which of the following immune mechanisms?
   a. Defects in innate immunity to gut commensals such as defensins and NOD2 cytoplasmic innate immune sensors
   b. Abnormalities primarily in the TH2 and TH17 immune response
c. Adequate T reg mediated suppression of immune responses to commensal organisms
d. Mutations in select genes relating to cell apoptosis

6. Which of the following immunodeficiency is a result of FOXP3 mutations resulting in failure to
develop proper T regulatory response leading to immune dysregulation, polyendocrinopathy,
enteropathy, and autoimmunity?
   a. Complete DiGeorge
   b. Nethertons Disease
   c. Deficiency of Interleukin 1 receptor antagonist
   d. IPEX

7. Which of the following is considered an immune privileged site?
   a. Brain
   b. Conjunctiva
   c. Scrotum
   d. Skin

8. Which of the following pairs is correct regarding the cutaneous immune system?
   a. Epidermis: innate immune defense function/physical barrier protection to microbial
      invasion
   b. Keratinocytes: mixed population of mast cells, macrophages, and DCs mediating
      inflammatory response
   c. Dermis: secrete defensins as well as inflammatory cytokines to various PAMPS and
      DAMPS
   d. Keratinocytes: IL-18 and IL-22 induce expression of defensins in keratinocytes

9. Which describes the function of alveolar macrophages best?
   a. Maintaining an anti-inflammatory phenotype
   b. Activate T cell responses as well as antigen presentation of airway Dendritic cells
   c. Expression of IL-5, nitric oxide, and TGF-beta
   d. Highly phagocytic compared with resident macrophages

10. Skin homing molecules include which of the following?
    a. CCR3
    b. CC10
    c. CLA
    d. CCL26

Answers
M cells involved with luminal antigen processing is correct. Paneth cells are involved with defensing
production, Secretory IgA/IgM involved with neutralization of microbes in the lumen. Intestinal
epithelial cells are involved with mucus secretion, and finally, DC subsets are involved with luminal
antigen sampling amongst other functions.

DC subsets in the GI tract are involved with lamina propria antigen sampling, T cell tolerance induction and effector T cell activation, induction of B cell IgA class switching, and imprinting gut homing phenotypes of both B and T cells.

3. B, page 294, Figure 13-2.
The spleen has greatest numbers of lymphocytes, followed by bone marrow and GI tract which have similar numbers, followed by skin.

4. C, page 303, Figure 13-7.
Antigens that are presented to B cells are generally presented in their intact, native conformation and IgA class switching occurs through both T cell dependent and independent mechanisms. DCs in the subepithelial dome of Peyers patches capture bacterial antigens delivered by M cells and migrate to interfollicular zone where they present antigen to CD4+ T cells. TLR ligand activated DCs induce IgA class switch through factors such as BAFF, APRIL, and TGF-B. B cell class switching to IgA is stimulated through action of both TGF-beta and through T cell CD40L binding to B cell CD40.

IBD is thought to be due to many different immune mechanisms including the following: Defects in innate immunity to gut commensals such as defensins and NOD2 cytoplasmic innate immune sensors, abnormalities primarily in the TH1 and TH17 immune response, inadequate T reg mediated suppression of immune responses to commensal organisms, and mutations in select genes relating to cell autophagy.

IPEX also known as immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX), including severe gut inflammation as well as autoimmunity, is due to FOXP3 mutations leading in failure of Tregs to develop.

Immune privileged sites are tissues where immune responses are not readily initiated, including the brain, anterior chamber of the eye, and testis. Mechanisms include tight junctions of endothelial cells in blood vessels at these sites, local production of immunosuppressive cytokines, and expression of cell surface molecules that inactivate or kill lymphocytes.

Epidermis is involved with innate immune defense function/physical barrier protection to microbial invasion. Keratinocytes secrete defensins as well as inflammatory cytokines to various PAMPs and DAMPs, and the dermis has a mixed population of mast cells, macrophages, and DCs mediating inflammatory response. Additionally, IL-17 and IL-22 induce expression of defensins in keratinocytes.

Alveolar macrophages represent the majority of free cells within the alveolar spaces. These cells are functionally distinct from macrophages in most other tissues in that they maintain an anti-inflammatory phenotype. They express IL-10, NO, and TGF-beta, and are poorly phagocytic compared with resident macrophages. Aloveolar macrophages inhibit T cell responses as well as the antigen presentation function of CD103+ airway DCs.
CLA, CCR4, and CCR 10 are all examples of skin-homing molecules. In addition, T cell expression of CCR4 and CCR10 which bind to chemokines CCL17 and CCL27 are also required for T cell trafficking to the skin.