This exam consists of 21 questions on 7 pages for a total of 100 points. On questions with choices, all of your answers will be graded and you will receive the highest scoring answer. Please use the space provided, or the back of the previous page.

1. (5 pts) Select one difference between the innate and acquired immune system. Summarize that difference and use one example from each branch of the immune system (innate or acquired) to illustrate your answer.

<table>
<thead>
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<th>Innate</th>
<th>Acquired</th>
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<tbody>
<tr>
<td>Ever present (physical barriers such as skin are always there)</td>
<td>Induced (takes ~7 days for antibody production)</td>
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<tr>
<td>Low specificity (TLR have some specificity)</td>
<td>High specificity (e.g. Ab)</td>
</tr>
<tr>
<td>No memory (same response each time)</td>
<td>Memory - Bmem cells are created, giving a more intense secondary response.</td>
</tr>
<tr>
<td>Fast - mast cells release histamine in a few min</td>
<td>Slow (takes ~7 days for antibody production)</td>
</tr>
<tr>
<td>Not adaptive (same response each time)</td>
<td>Adaptive - class switching, affinity maturation</td>
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2. (3 pts) Please select one of the following items and briefly describe why the item represents both a physical barrier and a mechanical (or physiological) barrier to pathogens.

Choice A: Mucosal membrane.
Choice B: Skin

Choice A: Mucosal membrane is a physical barrier because it blocks pathogen entry and pathogens are trapped in the mucus. It is a mechanical/physiological barrier because the mucus (along with pathogens) by cilia.

Choice B: Skin is a physical barrier because it blocks pathogen entry. Physiological barriers include (only one needed for full points). Low pH, fatty acids (detergent activity), antibacterial peptides.

3. (6 pts) In what way are the three complement pathways similar? In what way do they differ? Your answer should comment on the activation processes and the list convergent down-stream events that occur after activation.

Similarities: All converge on C3 convertase, and then follow a common path to C5 convertase and then the formation of the Membrane attach complex (MAC). (2 pts)

Differences:
i) Activation (3 pts)
alternative - spontaneous, with the conversion of C3 to iC3
lectin: by binding to mannose via mannose binding lectin, followed by activation of MASP1 and MASP2
classical: by either antibody or c-reactive protein interacting with C1q, followed by activation of C1r and C1s.

Convertases are different between alternative and lectin/classical pathway: (1 pt)
Alternative - C3bBb and C3b(Bb)2
Lectin and classical - C4bC2a and C4bC2aBb

4. (5 pts) How is C3b formed and what is one of its roles in either the innate or acquired immune system (you can also comment on the role of its degradation products, e.g. C3dg)

It is formed from C3 by C3 convertase (2 pts)
Functions:
Opsin (3 pts, only one of the following required for full points.)
- Enhances receptor mediated endocytosis of pathogens by macrophages,
- Enhances clearance of pathogens by RBCs in spleen via the CR on the RBC
- Enhances clearance of immune complexes by macrophages.
- Enhances activation of B-cells via their complement receptor.
5. (3 pts) Why is it important to regulate complement pathways? Provide one example of how this pathway is regulated.

So that we don’t form membrane attack complexes on our own membranes that would lead to cell lysis (2 1/2 pts)

Regulation (1/2 pt)
- Factor H, MCP – prevent binding of Bb to C3b
- Factor I – degrades C3b
- DAF – causes loss of Bb from C3b
- CD50 (protectin) – interferes with MAC complex formation.

6. (1 pt) Regarding the major effector functions of macrophages, which of the following specifically triggers inflammation and serves to enhance adaptive immunity?
   a) Secretion of IL-1, IL-6, IL-12 and TNF
   b) Phagocyte oxidase and iNOS producing ROS and NO, respectively
   c) Secretion of fibroblast growth factors such as FGF, and angiogenic factors such as VEGF
   d) Downregulation of inhibitory MHC I expression at the cell surface during viral infections

7. (1 pt) Upon phagocytizing a bacterium, an activated macrophage releases cytokines and chemokines to attract and activate neighboring neutrophils. This action can be described as ______ signaling.
   a) autocrine
   b) paracrine
   c) endocrine
   d) neurocrine

8. (6 pts) Please do one of the following choices:
   Choice A: Briefly describe the molecular signaling cascade of a chemokine-activated 7-transmembrane G-protein coupled chemokine receptor (GPCR).
   Choice B: Briefly trace the intracellular molecular signaling events that result from cytokine IL-2 binding the IL-2R, including what happens to the IL-2R and subsequent downstream events, ending with gene expression.

   Choice A: In the resting state, the intracellular domain of the GPCR in bound to Gaβγ. Ga is GDP bound and thus inactive. Upon binding a chemokine like IL-8, GDP is exchanged for GTP, thus creating an active Ga, which releases from the GPCR. Ga-GTP activates an effector molecule in the cell cytoplasm, triggering a signal cascade into the nucleus, resulting in gene expression. Once the chemokine releases, GTP is hydrolyzed, restoring Ga-GTP to Ga-GDP = resting state ready for reactivation.

   Choice B: IL-2 binds to the IL-2R, causing a conformational change in the IL-2R. This change in structure recruits the JAK kinases and brings their respective phosphorylation targets (TYR residues) into proximity. JAK phosphorylation of IL-2R TYR residues recruits STAT, which binds to TYR-P residues. JAK then phosphorylates STAT, forming a STAT homodimer, which travels to the nucleus, where it binds to response elements for target genes, such as IL-2 ad other pro-inflamm cytokines.
9. (3 pts) Briefly describe or list three specific type I IFN-induced anti-viral effects, at the molecular level, that can occur in virus-infected cells.

Any three of the following.

1. inhibition of viral protein translation
2. degradation of viral dsRNA
3. inhibition of viral gene expression and virion assembly
4. expression of proteasome for viral protein degradation and MHC I loading/presentation

10. (5 pts) NK cells, like CD8+ cytotoxic T-lymphocytes, can recognize and kill host cells infected by an intracellular pathogen using a unique granule-based mechanism. Describe how NK cells function in the presence of healthy vs. virally-infected host cells and how this granule-based system leads to host cell killing.

NK cells recognize and kill without clonal expansion. In normal cells, the NK activating receptor gets phosphorylated but MHC I-self Ag complex activates KIR (killer inhibitory receptor), which inhibits NK cell function via dephosphorylation of the activating signal. Some viral infections can decrease MHC presentation at the PM surface in an effort to escape TCTL recognition and killing; however, this effectively removes inhibitory signal, thus affording NK cell activation.

Activated NK cells fuse perforin and granzyme containing granules with their PM to exocytose these proteins. Perforin forms pores in the host cell membrane, allowing entry of granzyme, which enters virus-infected cell and triggers apoptosis.

NK cells also activated via ADCC, binding to Fc regions of Abs bound to foreign surface antigens of virally infected cells.

11. (6 pts) In response to a minor injury to the skin and exposure to an extracellular pathogen, briefly describe the innate inflammatory immune response. Include in your description a) the key responding immune cells and soluble factors, b) any cytokines/inflammatory molecules that are secreted, and c) how the pathogens are killed.

Upon exposure to bacteria, resident macrophages begin to phagocytose pathogens and release pro-inflamm cytokines (IL-1, IL-6, TNF-a) and chemokines (IL-8).

Mast cells that bind C3a and C5a and tissue cells immediately release histamine and prostaglandins, which cause vasodilation of the nearby capillaries → increased vascular permeability → brings more blood and immune cells to injury site (inflammation and swelling). Circulating monocytess and neutrophils are recruited to the injury site via chemokines and interacting with adhesion molecules on endothelial cells. Upon entering the injury sites, these cells become activated, engulfing pathogens and releasing more cytokines.

During this response, the clotting cascade begins to create a clot to wall off the damage and begin tissue repair.
12. (3 pts) Briefly describe antigen (peptide) presentation on either class I or class II MHC. Your answer should indicate:
   i) source of the peptide,
   ii) type of cells that present on the class of MHC that you selected.
   iii) The type of T-cell that would recognize the MHC that you selected.

   Class I: Peptides are synthesized inside the cell (all cells) or obtained externally (Dendritic cells only). The MHC-peptide complex is recognized by T_C cells.

   Class II: Peptides are obtained externally and only presented on professional antigen presenting cells, B-cell, macrophage, or dendritic cell. The MHC-peptide complex is recognized by T_H cells.

13. (5 pts) Briefly discuss either of the two mechanism by which T_C cells are activated to T_CTL.

   1. Interaction with dendritic cell that has been activated by a previous encounter with a T_H cell. The two signals that are required are:
      a) Class I MHC-foreign peptide: TCR interaction.
      b) b7 – CD28 interaction

   2. Interaction with any cell presenting a foreign peptide on class I. This requires co-stimulation by IL2 from a nearby activated T_H cell.

14. (5 pts) Please do one of the following two choices:

   Choice A: Briefly describe the circulation of lymph and briefly discuss why the circulation of lymph is an important component of the acquired immune system.

   Choice B: What properties of the lymph nodes (and the spleen) important in the activation of cells in the acquired system?

   Choice C: How is mucosal tissue protected by the acquired system – what are the important cell types and what specific antibody is produced?

   Choice A: Lymph fluid (and lymphocytes) leave the blood (at capillary beds) and enter the lymphatic vessels. The fluid and cells pass through lymphnodes and eventually return to the blood. This allows B and T cells to sample different parts of the body in responding to pathogens. This is important because the high specificity of the BCR and the TCR implies that only a small number of B and T cells can respond to a particular pathogen.

   Choice B: Both of these organs have a high concentration of B and T cells, increasing the likelihood that a productive interaction can occur between a B cell and a T cell – allowing for activation of the B cell. Because of the specificity of the TCR, only a small subset of Th cells can activate a B-cell.

   Choice C: Specialized M-cells in the mucosal membrane bring antigen across where it is processed by proAPCs. B-cell response results in the production of IgA, which can cross the mucosal membrane, providing protective immunity outside the body.
15. (4 pts) Sketch the immunoglobulin component of a B-cell receptor. Indicate on your diagram the location of antigen binding, F\textsubscript{ab} fragment, and F\textsubscript{v} fragment.

- Diagram should be Y-shaped, two light and two heavy chains
- Two antigen binding sites should be indicated.
- Fab should be entire light and first two Ig domains of heavy (Vh and CH1)
- Fv should just be the two variable domains (Vh VL)

(If they indicated the F\textsubscript{c} region instead of F\textsubscript{v} – give full credit, since there was some confusion during the exam).

16. (5 pts) Please do one of the following choices:

**Choice A:** Why are IgG molecules good at physical blocking of pathogens, but IgM is more proficient at agglutination of pathogens.

**Choice B:** Why is IgM particularly good at activating complement while most forms of IgG are not.

**Choice C:** How do Fc receptors enhance pathogen destruction by either macrophages or NK cells?

**Choice D:** What immunoglobulin is responsible for allergies? What cell does it bind to?

**Choice E:** How do babies benefit from the immune system of their mothers? What antibodies are involved and how are they transferred to the baby?

- **Choice A:** The smaller IgG (monomer versus IgM pentamer) can reach a higher density on the surface of the pathogen.
- **Choice B:** Activation of complement (classical pathway) requires two Fc regions within 40-50 Å of each other. Since IgM is a pentamer with 5 Fc regions, it is easy to accomplish this.
- **Choice C:** Antibodies bound to the pathogen will target the pathogen-antibody complex to the Fc receptor on the macrophage, leading to efficient internalization of the pathogen by receptor mediated endocytosis. In the case of NK cells, the interaction between the Fc and the Fc receptor will activate the NK cell and cause the antibody bearing cell to be killed (only necessary to discuss macrophage or NK cells).
- **Choice D:** IgE binds to Fc receptors on mast cell. The antibodies are present in a sensitized individual and the only thing that is required for degranulation of mast cells (histamine release) is the crosslinking of the Fc receptors by the antigen.
- **Choice E:** The mother’s IgG crosses the placenta, providing immunity in utero. Breast milk contains IgA that provides protection of the mucosal membranes in the gut of the feeding infant.

17. (3 pts) The following is a segment of DNA:

i) Correct the mistakes in the diagram. Briefly justify your answer.

<table>
<thead>
<tr>
<th>V1</th>
<th>V2</th>
<th>V3</th>
<th>D1</th>
<th>D2</th>
<th>D3</th>
<th>J1</th>
<th>J2</th>
<th>J3</th>
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<tr>
<td>1</td>
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ii) Does this DNA represent the light or heavy chain? Why?

- i) The joining of segments requires that a one-turn be paired with a two turn. In this diagram V cannot join to D. The "1" turn signals at the end of each V-segment should be "2" (2 pts)
- ii) Heavy, since there are D segments. (1 pt)
18. (6 pts) Select one of the following and briefly describe how it occurs and its contribution to the diversity of antibodies.

**Choice A:** p-bases
**Choice B:** n-bases
**Choice C:** junctional diversity (imprecise joining)

**Choice A:** p-bases are added during repair of the hairpin during joining. They increase diversity because they represent new sequences.

**Choice B:** n-bases are added by terminal transferase during the joining of segments in the heavy chain. They increase diversity because the bases added are random.

**Choice C:** When the 1 and 2 turn joining occurs the actual position of the joint is not precise. This can lead to loss of bases, changing the amino acid sequence.

19. (10 pts) Complete the following image of B-cell development and activation by labeling a) the correct stages of B-cell development; b) what type of V(D)J recombination happens at which stage, c) what surface Ig molecules are expressed at which stage, and d) where the key checkpoints occur. Also, below, please describe at least one attribute/function of plasma and memory B-cells.

<table>
<thead>
<tr>
<th>Bone marrow</th>
<th>Lymph node</th>
<th>blood</th>
<th>Secondary organ</th>
</tr>
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<tbody>
<tr>
<td>(1pt)</td>
<td>(2 pts)</td>
<td>(2 pts)</td>
<td>(1 pt)</td>
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20. (5 pts) Please do one of the following choices:

Choice A. Briefly describe the process of allelic exclusion in relation to the Ig heavy chain during B-cell development.

Choice B: In B-cell development there are two self-tolerance checkpoints: one in the bone marrow and one in the lymph node. Briefly describe what happens at each checkpoint during b-cell development and why.

Choice A: One allele is randomly selected from the two germ line genes, V(D)J recombination occurs, creating a HC IG molecule. Paired with a surrogate LC, the Ig molecule is expressed on the cell surface and tested with self-Ag. If the reaction is too strong, the HC is removed and the other allele is rearranged, placed on the surface with a surrogate LC and tested. If it does not pass the HC checkpoint, the cell undergoes apoptosis. If it does pass, the LC is rearranged to create a functional IgM.

Choice B: At each checkpoint, self-Ag is presented to the developing B-cell.

Membrane self-antigens: One of the following can happen:
- B-cell can be eliminated via induction of apoptosis.
- A new light chain can be tried.

Soluble self-antigens:
- B-cell becomes anergic - non-responsive, can't be activated.

This helps to prevent autoimmune reactions.

21. (10pts) Describe or draw+annotate the key steps in a T\textsubscript{H} cell dependent activation of a B cell, including the key molecules that must be expressed to generate both activating signals #1 and #2.

See Lecture 10 notes for drawing.

Must include BCR + CR2;

MHC I + Ag/TCR&CD4;

CD40/CD40L;

B7/CD28; ICAM/LFA;

cytokines released from TH cell; and mention that B-cell undergoes clonal selection and proliferation, class switching, affinity maturation → Plasma cells & B memory cells, and T\textsubscript{H} cell undergoes proliferation (give credit if these have already been mentioned in question 19)