1. Which of the following require the thymus?
   A. B Cell Differentiation
   B. Antigen Processing and Presentation of Materials from a Peripheral Infection
   C. Rearrangement of Germline Gene Segments to Code for Variable Portions of Antibody Molecules
   D. Rearrangement of Genes for the T Cell Antigen Receptor and T Cell Maturation
   E. Differentiation of Granulocytes

2. All of the following are true about CD8 T cells
   EXCEPT that they
   A. major mediators of ADCC.
   B. use perforin to mediate lysis of virus-infected cells.
   C. respond to antigenic peptides presented by MHC class I molecules.
   D. are part of adaptive immunity.
   E. make IFN-γ.

3. All of the following are important functions of IFN-α/β EXCEPT that they
   A. induce resistance to viral infections.
   B. promote MHC class I expression.
   C. are elicited in response to a viral infection.
   D. are important growth and proliferation factors for T cells.
   E. are part of innate defense mechanisms.

4. IL-12 is important for which of the following?
   A. Eosinophil Differentiation
   B. CD28 Expression
   C. TNF Production
   D. IFN-γ Production
   E. CD56 Expression
5. You are a physician in the emergency room at Rhode Island Hospital. A young girl, of about 6 years of age, has stepped on a rusty nail. She is not certain that she has ever been vaccinated for protection against tetanus toxin. Based upon what you have learned, which of the following would you do?
   a. Immunize her with the toxoid and give her passive antibody against tetanus.
   b. Immunize her with the toxoid.
   c. Give her passive antibody against tetanus toxin only.
   d. Give her IL-4.
   e. Send her home without treatment.

6. Upon her return from South America, an otherwise normal 12 year old girl is found to be infected with a helminth parasite. She is not doing well because of an endogenous Th1 rather than a Th2 type cytokine response. Which of the following would you expect to see?
   A. IgE
   B. IFN-α/β Production
   C. IL-5
   D. Eosinophilia
   E. IL-2 and CD4 T cell IFN-γ Production

7. An individual is genetically deficient for CD3e. Which of the following would you expect to be blocked?
   A. Immunoglobulin Gene Rearrangement
   B. Cell Surface Expression of A Functional T Cell Receptor for Antigen on Mature T Cells
   C. Rearrangement of the Genes for the T Cell Receptors for Antigen, α, β, γ and δ
   D. Granulocyte Development
   E. NK Cell Development

8. All of the following components can be part of innate immune responses EXCEPT
   A. natural killer cells.
   B. alternate pathway of complement activation.
   C. MAC.
   D. B cells.
   E. macrophages.

9. Complement can do all of the following EXCEPT
   A. lyse bacteria.
   B. promote phagocytosis though opsinization.
   C. promote antigen processing and presentation.
   D. mediate lysis through a Fas ligand-dependent pathway.
   E. increase vascular permeability.
QUESTIONS 10 - 11
ARE IN A FORMAT ALLOWING ONE OR MORE CORRECT ANSWERS
Each question in this section is worth 5 points. (Clearly mark each possibility, i.e. 1, 2, 3, and 4, as true or false for partial credit.) The section is worth a total of 10 points.
One or more of the numbered options in this section is/are correct.
a = 1, 2, 3 are correct  b = 1, 3 are correct  c = 2, 4 are correct  d = 4 only is correct  e = 1, 2, 3, 4 are all correct

10. Which of the following are important mediators for promoting inflammation?  
   ✗ 1. C5a  
   ✔ 2. Antibodies  
   ✔ 3. MIP-1α  
   ✗ 4. IL-5

11. Which of the following statements about endotoxic shock are true?  
   ✔ 1. It is induced by high concentrations of the lipopolysaccharide (LPS) endotoxin from gram negative bacteria.  
   ✗ 2. MHC class I molecules aggravate the condition.  
   ✔ 3. It can result in vascular collapse and conditions incompatible with life.  
   ✗ 4. The cytokines TNF, IL-1 and IL-6 are induced independently of each other.
12. In a major heavy weight fight, one boxer (boxer A) bites the ear of his opponent (boxer B) and a lot of blood gets spread all over both players. How would you determine if either boxer was infected with HIV as a result of the incident?

The best way to screen for HIV infection is to look for HIV-Ab using an ELISA immediately after the incident. In 6-8 months later, in using an ELISA, HIV-Ab is allowed to wells of microtiter plates. A sample of blood from each boxer is placed in separate wells. If the blood contains HIV-Ab, it will bind the fixed Ag and remain behind after the wells are washed. A second Ab, which is anti-human Ig from mice, is added to the wells. If a well contains an Ab-Ag complex, the anti-human Ig will bind to the anti-HIV Ab, forming a 'sandwich'. The second Ab is linked to an enzyme capable of catalyzing a color change reaction. The wells are washed to remove unbound Ab, substrate is added. Formation of the colored product indicates that anti-HIV Ab was present in the initial sample. Ab concentration can be estimated by comparison to a standard curve. Screening is required at this 6-8 months because initial Ab concentrations may be too low to detect, or not present at all.

13. Explain chronic granulomatus disease (CGD). How might you expect it to present? How would you diagnose it? How might you treat an individual with this condition?

CGD is a genetic deficiency in the respiratory burst pathway. This is characterized by a deficient NADPH oxidase that is used by phagocytes to produce oxygen radicals (O2) to be used for oxidative killing of microorganisms (usually bacteria). It presents as an increased susceptibility to bacterial infection with a chronic immune response as the acute inflammation is unable to clear it. Histologically, there is formation of granulomas in an attempt to wall off or contain the infection. You would diagnose it by histology: identification of bacteria which do not normally invade granulomas in the granulomas. You would also assess the rate of production of H2O2 in blood, which requires an intact respiratory burst pathway. Low H2O2 & granulomas of non-gram-negative bacteria are diagnostic of the condition. You would treat an infection with high dose, broad spectrum antibiotics to prevent initial infection.
14. What are NK cells? Be certain to give two characteristic markers. Give three major functions for the cell type.

NK, or natural killer cells, are non-B, non-T cells derived from the lymphoid lineage. They are CD4^+CD8^+ but do contain characteristic FcγR and CR3 on their cell surfaces. NK cells function in innate immunity by lysing virally infected cells which down-regulate MHC-I display. This occurs because NK cells have a KIR (killer inhibitory receptor) capable of recognizing MHC-I, & presentation of MHC-I prevents NK cell lysis.

NK cells can be recruited into specific immune via their FcγR. This allows NK cells to use IgG to target, or direct them, to infected cells or infectious molecules which the IgG is capable of binding. After targeting, the NK cells lyse the cell or organism, unless inhibited.

Finally, NK cells can be induced by cytokines to produce IFN-γ, which is a key step in fighting off bacteria. This induction is part of the cytokine activation of NK by TNF-α & IL-1.

NK cells lyse by way of perforin/granzyme B pathways, or presentation of FasL to Fas receptors.

15. How do T cells help B cells? Please give at least three different molecules involved in the process and at least two different consequences for the B cell response.

T helper cells produce cytokines which are required for B cell proliferation, differentiation into plasma, & memory cells, & heavy-chain isotype switching.

Th production of IL-2 (T-cell autocrine growth factor) stimulates B cells to proliferate, as do IL-4 & IL-5 from Th2. IL-2, 4, 5 are also responsible for inducing secretion of Ig by B cells. These cytokines, along with monocyte/macrophage derived cytokines, induce differentiation into memory cells.

Th cytokines are also capable of inducing isotype switching to Ig types appropriate for the infection. IFN-γ is capable of switching bovine IgG isotypes (x nucleo) IL-4 induces IgG1 production & IL-5 induces IgA production.

T cell help requires there be peptide antigen for the T cells to recognize. B cells are capable of presenting the T cells, & in doing so allow T cells to present co-stimulatory signal via B cells. This prevents energy or deletion in the face of a "weak" induction.

Without T cell help, B cells might not respond to antigen, do not form memory cells & do not isotype switch.
16. What is fluorescence-activated flow cytometry (FACS)? Explain how it might be used for diagnosing a specific T-cell deficiency. Be certain to give details of the test, identify the molecule(s) being measured, and discuss the limitations to be considered in interpreting the results.

FACS is used to sort cells based on surface markers for which specific monoclonal Abs exist. The Abs to be used (for the surface markers chosen to sort by) are labeled with different fluoresceins. The sample (cells labeled with Abs) are mixed, allowing the cells to be "painted" based on their surface markers. The sample is then pumped at a rate past a laser/flow detector, generally. This is attached to a computer which analyzes the fluorescence wavelengths at the surface of the cell which was just flowing through the detector. The computer is also attached to magnets which direct the flow of the sample into a well or tube which collects cells of the same type. The computer also keeps count so that the relative percent composition of the sample can be calculated. This could be used to diagnose a T-cell deficiency by sorting a sample of T-cytophages using anti-CD3 & anti-CD8 Abs. Absence of a peak could indicate a deficiency in the T-helper or CTL line.

17. Which immune system components are most important for protection during secondary exposure to influenza virus? How do they work? Why are they important during challenges with this agent? Why are they less effective during primary infections?

B-memory cells & Ig against the flu virus are the most important for 2nd response. Memory cells formed during the initial exposure allow a rapid release of specific IgG in large amounts. The IgG "maps up" viral particles & prevents them from infecting cells. IgG also activates complement & together they opsonize the Ag-Abs complexes for clearance by the RES system. IgG is capable of targeting the cells to kill virally infected cells, preventing viral replication. These are much less effective in primary infections because there is no memory B-cells, so clonal selection & affinity maturation need to take place. Finally, more processing & presentation of viral antigens need to take place to induce T-cell help for isotype switching & memory cell formation.
18. Describe the major antigen processing pathway for presentation during a viral infection. Give at least two major characteristics required to be a good antigen presenting cell (APC) under these conditions. Can cells be induced to be better APCs for these agents? If so, give a mechanism.

Presentation of the viral antigens takes place on MHC-I. This pathway is as follows:
1. Viruses use host machinery to synthesize proteins.
2. This, along with all proteins, is ubiquitinated & sent to the ER proteosome.
3. Small peptides of viral peptide is brought into the ER by TAP, using ATP.
4. Meanwhile, MHC-I & beta-2m subunits are transcribed & translated in ER.
5. Calnexin helps the chains fold & they form a loose heterodimer.
6. The oligomer associates with TAP.

Then:
1. Viral peptides bind in the alpha chain binding site, stabilizing the heterodimer.
2. Complete peptides/MHC-I leaves the ER & is transported by way of the Golgi to the cell surface.

IFN-αβ can upregulate transcription/translation of MHC-I genes, which results in more presentation of viral antigen.

19. Describe major pathways to Th1 type T cell responses during an infection. Be certain to include the initial/innate response components required, and to specifically define the characteristics of Th1 T cells. When would these responses be important for host defense?

Th1 responses are important in defense against bacterial infections. The macrophages phagocytose bacteria & produce IL-12. This is the key step in initiating a Th1 response. They process & present bacterial antigen on MHC-II & display to CD4+ T cells. Antigen presentation & IL-12 direct the T-cells to become Th1. (The macrophages must have IFN-γ to continue. T-cells.) The Th1 cells start making IL-2, IFN-γ & TNF-α. The IFN-γ activates the macrophages & allows them to clear the bacterial infection by oxidative killing.
20. Describe the steps for immunoglobulin heavy chain class switching from IgM to IgG. Are the events antigen dependent? Do they always result in functional products? What are the functional consequences of successful completion to the host?

1. Thy is bound by B-cell membrane IgM, and phosphorylated & processed for presentation to T-cells.
2. T-helper cytokines or non-T-cytokines (IL-6) activate isotype switching.
3. Receptors rem bargain the Ig heavy chain DNA by joining the switch region 3' to the Vβ region & 5' to the γ region.
4. mRNA transcripts are made which contain the Vβ & γ regions.
5. Splicing of mRNA results in an IgG message which has the same binding specificity as the IgM.

Isotype switching always results in functional product as the Vβ region has already undergone successful recombination & the enzyme for the γ is left alone. Non-functional products would only result from mutant recombination or mutation of switch regions.

Isotype switching also deletes (permanently)inactive constant regions.

In this example, the B-cell could never go from IgG back to IgM or IgD. Theoretically it could be driven to IgE or IgA, as those sequences still exist.

The class switching is not antigen dependent. (Although initial B-cell activation is T-cell dependent.)

21. What are chemokines? Remember to address structure and function in the definition. Can they mediate a direct anti-microbial effect? If so, how and under what conditions?

Chemokines are "chemotactic cytokines" which are small peptides capable of causing chemotaxis of various immune cells. They are produced to attract immune cells to the sites of inflammation. They have a "loop formation" and are subdivided into families by their internal domain. There are the CXC family (chemotactic for neutrophils), the CC family (chemotactic for monocytes, amongst others) & the C family (chemotactic for lymphocytes).

They are capable of anti-microbial effects. One example is by blocking entry of HIV into cells by blocking viral receptors. In the case of non-Tropic strains, they can block the CCR5 receptor, & in the case of T-Tropic strains, they can block the CXCR4 receptor. These are normal cytoplasmic receptors which the virus has co-opted to host cell entry.
22. Describe an aspect of the immune system or the immune response to challenge which YOU find particularly interesting but was not addressed in the earlier parts of the exam. (This is your chance to let me know about something you studied very thoroughly.) Explain why you find it interesting. Be certain to provide at least 5 different, specific and precise, immunological details about this aspect.

The generation of Ig molecules from germline DNA in B-cells is an interesting subject. The regulatory steps involved make it hard to believe that it just evolved. The B-cells also get a number of chances to get it right before they're scrapped, demonstrating that it's not something that just happens.

The first step involves a D-J recombination on one heavy chain allele. This is followed by a V-D-J recombination. Multiple V, D, J segments allow for diversity, along with immunoglobulin's N-terminal addition of bases. Both steps use RNA/DNA primer products. This possible heavy chain is transcribed as IgM and most of it is kept for reaching the membrane by B-cells. Some molecules associate with surrogate light chains and get expressed on the cell surface. This equals an unsuccessful recombination and inactive recombination of the second heavy chain allele. (Tone: Exemplify) It also signals initiation of light chain recombination. If the light heavy chain is not successfully recombination, the B-cell dies.

Light chain recombination is similar, but it only involves a V-J recombination. The B-cell also has five choices at a light chain, and each allele contains a.

K and J site. Successful light chain recombination leads to assembly of the Ig in the ER and bridge of the chains. Surface expression of complete inhibits recombination of other light chain loci (light-chain exclusion).