1. (4 pts) DNA rearrangements occur in the generation of heavy and light chains in B-cells and in class switching. Compare and contrast the two events in terms of:
   i) When and where these events occur in B-cell development (1 pt).
   ii) The immunological benefit of each event (3 pts).

2. (6 pts) Two patients present themselves to an emergency room. Both patients are infected with Pseudomonas. The first patient is having difficulty fighting the infection and has shown very high levels of bacteria in their blood throughout the entire course of infection. The second patient has had relatively low levels of bacteria during the infection. You collect serum samples from the individuals and find:
   - The first patient has IgG antibodies that recognize the bacteria, but they are low affinity.
   - The second patient has high affinity IgG antibodies that are proficient at eliminating the bacteria.

Explain the different response of each patient.
3. (4 pts) In the production of hybridomas, immunized B-cells are fused with the myeloma cells.
   i) What is the end goal in this process? Why are hybridomas useful?
   ii) Please answer one of the following questions:
       Choice A: Why are myeloma cells used as the fusion partner?
       Choice B: What is the selection method to ensure that only fused cells are obtained?

4. (4 pts) What are BiTE antibodies and how are they used to eliminate cancer cells in a patient? Briefly describe how the cancer cells are recognized and how they are killed.

5. (2 pts) What is the difference between a primary and a secondary antibody in an immunoassay?

6. (2 pts) Why are ELISA assays more sensitive than other immunoassays, such as precipitation?
7. (10 pts) Please do one of the following two choices:

**Choice A:** Briefly describe how a FACS instrument works and then briefly discuss how you could quantify the number of cells in one of the following two scenarios (a or b):

a) the amounts of $T_h$, $T_c$, and double positive $CD4^+$ and $CD8^+$ cells during development of T-cells in the thymus.

b) Differentiation between B-cells just prior to leaving the bone marrow, naïve B, and activated B cells in the lymph node.

**Choice B:** The following graph shows the response unit from an SPR experiment to measure antigen binding to two different antibodies, A or B. In both cases the antibody was immobilized on the gold surface and an equal amount of antigen was added. Please answer all of the following questions:

i) Briefly explain why there is a change in the response unit when antigen binds.

ii) Do both antibodies have the same affinity for their antigen? Why?

iii) Which antibody has the faster on-rate, A or B? Why?

8. (8 pts) Please do one of the following.

**Choice A:** Describe an immune assay to measure the concentration of a peptide hormone in human serum.

**Choice B:** Describe an immune assay to measure the concentration of anabolic steroids in blood samples.

**Choice C:** After performing a western blot with a monoclonal antibody you detect 2 bands. Briefly describe how a Western blot works and why you might have detected two bands.

**Choice D:** What type of immune assay could you use to distinguish $T_h1$ cells from $T_h2$ cells? Briefly describe how it would work.

**Choice E:** Design an immunoassay to determine whether a pregnant woman would require Rhogam injections prior to delivery of her children (assume genotype of the father is unknown).
9. (6 pts) Compare and contrast the peptide binding properties of class I versus class II MHC. In what way are the binding similar, in what way is it different?

10. (8 pts) The diagram on the right shows the gene structure of the MHC region of an inbred (homozygous) laboratory strain of an organism. The numbers below each gene represent the allele number. There are 100 alleles for each gene within the population. You catch a wild-type member of this species (outbred) and obtain a DNA sample and sequence its MHC locus.
   i) What word, or term, could you use to distinguish the different alleles in MHC locus of these creatures (1 pt)?
   ii) How many different class I MHC molecules would be found on the outbred individual? Briefly justify your answer (2 pt).
   iii) How many different class II MHC molecules would be found on an antigen presenting cell from the inbred animal? Briefly justify your answer (2 pt).

   iv) An inbred and the outbred animal were infected with a virus. The inbred animal survived the infection with a vigorous antibody response and the outbred animal survived the infection with a vigorous $T_{CTL}$ response. Briefly explain this outcome (3 pts).
11. (6 pts) A large number (~$10^8$) of different BCR and TCR can be generated by an organism. Briefly discuss what mechanisms give rise to this high level of diversity. Elaborate on the differences between the generation of diversity in the BCR versus the TCR.

12. (6 pts) You have isolated an antibody that is specific for the disaccharide (Glucose)-(Glucosamine) and you would like to determine which chains of the antibody are responsible for binding to which part of the antigen. You obtain B-cells that produce the antibody against the disaccharide. You clone the rearranged genes for the heavy and light chain genes. You produce three separate transgenic mouse lines by integrating either the rearranged heavy chain (line H) or the rearranged light chain (line L) or both (line H+L) in the mouse genome.

i) Approximately how many different antibodies would you find in the H+L line? Briefly justify your answer (1 pt).

ii) You inject your antigen into all three lines and obtain antibodies against your antigen, as expected. You now inject a modified antigen: (Ribose)-(Glucosamine) and find that you obtain antibodies only from the transgenic mouse line that received the heavy-chain gene. Which chain on the antibody is recognizing the glucose portion of the antigen? Briefly justify your answer. (3 pts)

iii) Is injection of the pure disaccharide sufficient to generate antibodies, or would you have to inject something else? Briefly explain your answer. (1 pt)

13. (1 pt) The following diagram represents the TCR-MHC complex. Which hypervariable loops on the β and α chains are principally involved with recognizing allelic differences on the MHC (circle answer).

1) 2) 3)
14. (1pt) The generation of secreted Ig rather than membrane-bound Ig results from...
   A. V(D)J recombination
   B. VJ recombination
   C. alternative splicing of Ig mRNA transcript
   D. post-translational cleavage and removal of transmembrane domain

15. (4pts) Briefly describe the activation of a B-cell in a T<sub>H</sub> cell–independent manner. In what way(s) do the activated B-cells differ after T-cell independent versus T-cell dependent activation? Which mechanism results in a more robust antibody-mediated immune response? *Illustrations are welcome.*

16. (5pts) Describe at least four characteristics of a secondary B-cell response to a given pathogen, and how these differ from the primary response to the same pathogen. *Feel free to provide a graphical illustration to supplement your discussion.*

17. (2pts) Nearing the end of a controlled immune response, briefly describe at least one mechanism for shutting down activated B-cells and one mechanism for T-cells.
18. (8pts) Using this rearranged DNA sequence as the starting point, 1) draw the linear configuration for the intermediate RNA structure for IgM and IgD. 2) Following alternative RNA splicing, draw the mature mRNA, with all of the correct segments and exons labeled, for membrane-bound IgM vs. IgD immunoglobulins.

Rearranged DNA:

![DNA Structure Diagram]

19. (1pt) A young patient presents with a severe, multifactorial autoimmune disease that attacks several different types of organs. Upon analyzing tissue samples from the patient’s thymus, you notice that there are various clones of self-reactive T-cells. A mutation in which of the following most likely explains this autoimmune disorder?

A. An inactivating mutation in AIRE (autoimmune regulator) transcription factor
B. An inactivating mutation in both alleles for the TCR Beta chain
C. An inactivating mutation in both alleles for the BCR Heavy chain
D. An inactivating mutation in RAG1 and RAG 2

20. (1pt) I hand you a blood sample from a patient with persistent allergic reactions to perfumes and other fragrances. You analyze the blood and observe elevated levels of IL-4 and IL-5, as well as IgG1. Which Th cell response is most likely responsible?

A. Th1
B. Th2
C. Th17
D. ThReg

21. (1pt) Immunity to extracellular bacteria is best accomplished by...

A. CD4+ Th1 activation of macrophages via CD40L and secretion of IFN-γ
B. CD4+ Th2 activation of IgE isotype switching of B-cells
C. CD4+ Th17 activation of neutrophils and monocytes via IL-17 secretion
D. CD4+ T-reg activation of CD8+ cytotoxic T-cells

Points on Page:________
22. (4pts) Briefly discuss what happens at each of the four key checkpoints associated with T-helper cell development. *Illustrations are welcome.*

23. (2pts) Briefly describe how superantigens, such as the Staphylococcal enterotoxin, induce a life-threatening immune response? Include mention of roughly what percent of T-cells are activated due to superantigen activation in comparison to typical T-cell activation and why this is dangerous to the host.

24. (4pts) Briefly describe the two required signals for APC activation of naïve CD4+ T-cells. Also, discuss the unique properties of IL-2R expression and activation on T-cells and what role it plays in T-cell activation.