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).

   i) 
   - Generation of the H + L chains occurs in the bone marrow at the very beginning of B-cell development - the first step in the generation of a B-cell.
   - Class switching occurs after activation, usually in the lymph nodes (or other secondary lymphatic tissues).

   ii) 
   - The generation of H + L chains by DNA recombination allows the production of many different chains increasing the diversity of antibodies in an organism.
   - Class switching changes the heavy chain of the antibody, altering biological function which makes the antibody more effective against pathogens.

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.

   - The first patient never underwent affinity maturation. They must have AID (since they class switched), but the high levels of pathogen reduced the selective pressure during affinity maturation. All antibodies were saturated due to the high level of antigen, regardless of their affinity.

   - The second patient shows affinity matured antibodies, the lower level of pathogen generated positive selective pressure of higher affinity antibodies - those B-cells producing higher affinity antibodies proliferated to a greater extent.
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?

   i) Hybridomas produce monoclonal antibodies - a single defined antibody which is a well defined reagent compared to polyclonal antibodies. They are also immortal and can produce antibodies forever.

   ii) **Choice A:** Myeloma cells are immortal - and will confer immortality on B-cells, which would otherwise die in culture.
       **Choice B:** HAT media suppresses de nova synthesis of purines and pyrimidines. The B cells (and hybridomas) are HGPRT¹ and can utilize the hypoxanthine (H) to make purines for DNA synthesis.

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.

   • BiTE antibodies consist of the Fv fragment of an antibody that recognizes CD3 - the signaling chains of the TCR and the Fv fragment that recognizes a tumor antigen.
   • They bring T̄C cells to the tumor cell and activate them, and they become cytotoxic T̄CTL cells. The T̄CTL cells kill the tumor cell by perforin and granzyme induced apoptosis.

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

   Primary antibody - recognizes the antigen
   Secondary antibody - recognizes the Fc region of the primary

6. (2 pts) Why are ELISA assays more sensitive than other immunoassays, such as precipitation?

   The assay utilizes an enzyme. Consequently a single antibody-Enzyme can produce many products, **amplifying** the signal.
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 $T_{H1}$, $T_{C2}$, 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?

**Choice A:**

FACS:
- Bind fluorescent Ab to surface of cell.
- Generate droplets that have one cell and are charged.
- Measure the fluorescence of the droplet.
- Sort the cells, by electrostatic deflection, according to the nature of its fluorescence.

a) Use different fluorophores on the anti-CD4 and anti-CD8 antibodies (e.g. anti-CD4-red/anti-CD8-green).
- $T_{H1}$ cells will have high levels of red fluorescence and low levels of green fluorescence.
- $T_{C2}$ cells will have low levels of red fluorescence and high levels of green fluorescence.
- Double positive cells will have high levels of both red and green fluorescence.

b) Use labeled anti IgM, IgD, and b7 antibodies. (three different colors)
- preB - IgM only (or no antibodies)
- naïve B - IgM and IgD
- activated - high levels of b7 (and potentially loss of IgM if class switched)

**Choice B:**

i) The response unit is a measure of the angle of reflection from the glass/gold surface. The angle changes with refractive index, which is affected by the mass loaded on the surface, as the antigen bond the mass increases, so the refractive index changes and the RU changes.

ii) Yes, because the equilibrium value for the response using is the same.

iii) A has the faster on-rate since the rate of change in the RU is faster.

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:** Determine whether a pregnant woman may require Rhogam injections prior to delivery of her children (assume genotype of the father is unknown).

**Choice A:** Could do indirect ELISA (use serum to place antigen in well), sandwich ELISA using two different antibodies, Western, RIA using labeled peptide. Need to provide some details regarding the method.

**Choice B:** RIA only. Need to provide some details regarding the method.

**Choice C:** SDS-PAGE, transfer to membrane, binding of Enz-linked antibody, add substrate. Two bands are due to i) cleavage, ii) modification. Neither of which affect the epitope.

**Choice D:** These differ by the type of cytokines produced. $T_{H1}$ - INFγ, IL-2, TNF, $T_{H2}$ - IL4, IL5. Could do an ELISPOT or a western blot using antibodies specific for different cytokines. Need to briefly describe how either works.

**Choice E:** Use an agglutination assay with anti-Rh factor antibodies to detect the presence of Rh factor on mothers RBC. If absent she should have a Rhogam shot. Need to mention the requirement for primary and secondary antibodies.
9. (6 pts) Compare and contrast the peptide binding properties of class I versus class II MHC. In what way are they binding similar, in what way is it different?

- Both recognize the mainchain atoms of the peptide (2 pts)
- Peptide is in an extended conformation (1 pt)
- One or two residues are recognized via their sidechains (anchor residues). (2 pts)
- Class I has a restricted length 8-9 residues, no length restriction for class II. (1 pt)

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.

The alpha and beta labels were accidentally omitted due to a problem during copying.

i) What word, or term, could you use to distinguish the different alleles in MHC locus of these creatures (1 pt)? Haplotype

ii) How many different class I MHC molecules would be found on the outbred individual? Briefly justify your answer (2 pt).

Two, although there is one class I gene (A) it is likely that the alleles are different, so the individual would be heterozygous. Each allele would produce a different protein

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).

- The inbred is homozygous, so it is only necessary to consider gene products from one chromosome, exactly the same gene products would be generated from the other chromosome.
- The will be one type C, and two type E MHC II. C and E cannot pair, and we get two Es because either Ea1 or Ea2 can pair with Eβ

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<sub>CTL</sub> response. Briefly explain this outcome (3 pts).

- The inbred and outbred animals likely have different alleles for both class I and class II.
- The inbred has alleles on its class II genes that can present peptides from the virus on B-cells.
- The outbred has alleles on its class I gene that can present peptides from the virus on dendritic cells, allowing activation of T<sub>C</sub> cells. It must also have some alleles that can present on class II, so that T<sub>H</sub> cells could be activated by APCs to aid in activation of the T<sub>C</sub> cells.
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.

The diversity for both types of receptors is due to
1. Combinatorial joining of VDJ and VJ segments
2. Imprecise joining
3. P-base addition
5. Pairing of chains (H&L, α&β)

The two major differences between the BCR and the TCR are
1. N-base addition only happens on the heavy chain, not the light chain. It occurs on both the β and α chain for the TCR.
2. In the case of the β chain, both VDJ and VJ joining can occur, increasing the diversity of the HV loop 3.

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).

- One - the rearranged genes would suppress rearrangement of the endogenous H & L genes due to allelic exclusion.

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)

- The light chain recognizes the first sugar (glucose) while the heavy chain recognizes the second sugar (glucosamine).
- In the heavy chain transgenic mouse line the antibodies that are produced would all have the same heavy chain, which can only recognize glucosamine. The light chain can be any of a thousand or so, one of which can recognize the ribose on the modified antigen, consequently the Ab that recognizes the ribose-glucosamine would have a different light chain.

iii) Is injection of the pure disaccharide sufficient to generate antibodies, or would you have to inject something else? Briefly explain your answer. (2 pt)

- The carbohydrate would have to conjugated to a carrier protein that is foreign to the organism, so that a foreign peptide can be presented on class II MHC by the B-cell.

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)

(Box is the TCR, oval shapes are the α and β chains, zig-zags are the peptide binding helices of the MHC. HV loops 1 and 3 recognize both the peptide and the MHC, HV loop 2 only contacts just the MHC)
This portion of the exam consists of a total of 33 points. Please use the space provided, or the back of the previous page.

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.*

   T<sub>H</sub>-indep activation:
   - Involves B-cell BCR interacting with antigen with repeating epitope, like a carbohydrate or nucleic acid, which triggers moderate BCR signaling. Lacking B7-CD28 makes this not as robust.
   - Results in: low affinity IgM, no isotype switching, no affinity maturation, and no memory cell creation

   T<sub>H</sub>-dependent activation:
   - Results in: high affinity IgM, Isotype switching, affinity maturation, and memory cell creation
   - Signal 1 (B-cell MHC+Ag → T<sub>H</sub> cell TCR) + Signal 2 (B-cell B7 → T<sub>H</sub> cell CD28) are required for full B-cell activation and produce a more robust immune response.
   - T<sub>H</sub> cell secretes activating cytokines that augments B-cell activation.

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.*

   Upon 2<sup>nd</sup> exposure:
   - There is a more rapid AND robust immune response than the primary response
   - Clonal expansion of activated B-cells occurs during both
   - Activated B-cells undergo additional affinity maturation and isotype switching, producing Abs with greater affinity
   - The resulting memory B-cell population is larger than before and will respond even more quickly upon tertiary infections.

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.
   - B-cells: B-cell express Fc receptors, and when the B-cell FcR binds to the Fc region of an antibody that is bound to a pathogen, the intracellular domain of the FcR activates a protein phosphatase, which removes activating phosphates from the nearby phosphorylated BCR-associated signaling Iga/β proteins.
   - T-cells: T-cells express CD28, the requisite co-receptor for full activation of target effector cells, such as B-cells. T-cells express CTLA-4, which counters CD28 by binding to B7 with greater affinity than CD28, and shuts down the TCR signaling.
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:

Solution (4 pts each drawing):

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 T cell response is most likely responsible?
   A. T\textsubscript{H}1
   B. T\textsubscript{H}2
   C. T\textsubscript{H}17
   D. T\textsubscript{Reg}

21. (1pt) Immunity to extracellular bacteria is best accomplished by...
   A. CD4+ T\textsubscript{H}1 activation of macrophages via CD40L and secretion of IFN-γ
   B. CD4+ T\textsubscript{H}2 activation of IgE isotype switching of B-cells
   C. CD4+ T\textsubscript{H}17 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.

Hemopoietic stem cell (HSC) travels from bone marrow to thymus = thymocyte

1st: V(D)J rearrangement of Beta chain. Paired with surrogate alpha chain, the beta chain is tested to see if the chain is functional. If OK → clone survives and expands in #.


3rd: Positive selection: various T-cells (same beta but different alpha chains) are tested to see if TCR can recognize self-MHC. If moderate interaction, that clone survives. If weak, then death via apoptosis.

4th: Negative selection: Same T-cells are tested to see if TCR has too strong a reaction to self MHC + self peptide Ag expressed due to AIRE. IF OK → survival and sent out to fight! IF too strong, then death via apoptosis.

(Both steps 3 and 4 occur simultaneously, so the exact order is not critical)

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.

Superantigens induce massive polyclonal T-cell activation via binding non-specifically to both the MHC II and TCR when APCs engage naïve T-cells. Compared to the typical 0.0001 to 0.001% T-cell activation, superantigens can activate over 10% of T-cells, resulting in massive IFN-γ secretion by effector T_{H} cells, which induces secretion of pro-inflammatory cytokines IL1, IL6, TNF-alpha from activated macrophages.

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.

(1pt) 1st: MHC II + foreign peptide on APC → TCR + CD4 + CD3 co-signaling molecules

(1pt) 2nd: B7 on APC → CD28 on CD4+ T-cell (this interaction is augmented by CD40-CD40L interaction)

(2pts) IL-2R: exists as a dimer of gamma & beta on all resting t-cells. Upon activation, IL-2 autocrine secretion by the t-cell triggers the production of IL-2R alpha subunit, which when combined with the gamma and beta chains, forms the fully active IL-2R trimer, which signals survival, proliferation and differentiation of that activated T-cell.