Instructions: This exam contains 15 questions on 6 pages for a total of 100 points. On questions with choices/options all of your attempts will be graded and you will be awarded the highest grade.

1. (4 pts) Please do one of the following choices.
   - Choice A: What are hybridomas? Why is it advantageous to generate them?
   - Choice B: Hybridomas are selected using “HAT” media. Pick H, A, or T and discuss its role in the selection process.

   **Choice A:** Hybridomas are fusions between B-cells and myeloma cells. They are immortal and will produce a single antibody indefinitely. They will produce a monoclonal antibody that will recognize only one epitope, potentially reducing cross-reactivity seen with polyclonal antibodies.

   **Choice B:**
   - A – aminopterin – inhibits de-novo synthesis of nucleotides, forcing the cell to use the salvage pathway.
   - H & T are hypoxanthine and thymine, both precursors for DNA synthesis using the salvage pathway. The enzyme HGPRT, allows the use of hypoxanthine. This enzyme is provided by the B-cell. Thus hybridomas will survive in HAT media: Myeloma cells lack this enzyme and will die.

2. (4 pts) Discuss one method by which antibodies can be used to treat cancers, you can discuss BiTE antibodies if you wish.

   1) Bind to and inhibit a growth factor receptor that is overexpressed on the surface of breast cancer cells.
   2) Antibody that is specific for a tumor cell antigen, labeled with toxin, can carry the toxin to the tumor cell, increasing the local concentration of the toxin.
   3) Antibody binding to tumor cells can cause NK cells to kill the tumor cell via the Fc receptor.
   4) BiTE antibodies are bifunctional and contain an Fv for CD3 (TCR signaling chains) and a Fv for a tumor antigen on the other end. This BiTE antibody brings the Tc cell and the tumor cell together, the Tc cell is activated and kills the tumor cell.

3. (7 pts) Please do one of the following two choices:
   - Choice A: Briefly describe how a FACS instrument can be used to quantify the amounts of different cell surface antigens on one cell.
   - Choice B: Briefly describe SPR (surface plasmon resonance) and describe how it is used to measure the binding affinity of antigens to antibodies.

   **Choice A:** The FACS instrument can measure the fluorescence emitted from one cell with a drop by excitation with a laser. The amount of fluorescence is proportional to the amount of bound fluorescent antibody, which is proportional to the amount of antigen (note: there is no need to sort the cell).

   **Choice B:** In SPR light is reflected off of a gold covered glass plate. At a critical angle the light is absorbed by the gold. The critical angle depends on the amount of protein attached to the gold surface. Antibodies are placed on the gold and as antigen binds the critical angle changes. The change in the critical angle (response unit) is proportional to the amount bound. By measuring this at different ligand concentrations the Kd can be measured.
4. (10 pts) Please do one of the following choices:

**Choice A:** Describe an immuno assay, besides FACS, that you could perform to detect the amount of b7 on the surface of antigen presenting cells.

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

**Choice C:** Why is it usually better to use denatured proteins when raising antibodies for a Western blot (your answer should include a description of the relative binding energies for antibody-antigen interactions and why they would differ between native and denatured proteins.)

**Choice A:**
Antibodies against b7 would be required. Possible answers include:

- **Indirect:** Adhere cells to surface, add anti-b7 antibodies, wash, add enzyme linked secondary antibody.
- **Sandwich:** Ab on the surface, add cells, wash, add antibody (different source), followed by E-linked secondary.

**What won’t work:** ELISPOT – measures secreted proteins, Western – measures total protein, RIA – difficult to get radioactive b7.

**Choice B:**

- **Indirect:** Use serum to coat well, add specific antibody, followed by E-linked secondary.
- **Sandwich:** Coat well with Ab, add serum, second E-linked antibody.
- **RIA:** Use radioactive peptide and measure binding competition to Ab immobilized on plate.
- **Western:** Difficult to separate small peptides on gel. ELISPOT – measures secreted proteins, therefore not useful.

**Choice C:**
Westerns detect proteins after SDS-page, i.e. usually in their denatured state. Therefore antibodies to the denatured protein are likely to be better. Antibodies against the native protein will only recognize one conformation, and the epitope could be discontinuous. The peptide may assume more than one conformation and will be a continuous epitope, both of which may be more favorably recognized by antibodies against the denatured protein.

5. (9 pts) The following shows the gene structure of the MHC region of an organism. Briefly justify your answers for all parts.

i) How many different class I MHC molecules would be found on an inbred individual (1 pt)?

One – since there is only one gene for class one, and in an inbred animal, the alleles will be the same, as will the protein product.

ii) How many different class I MHC molecules would be found on an outbred individual (1 pts)?

Two – it is likely the alleles are different, therefore different proteins.

iii) How many different class II MHC molecules would be found on an inbred APC (2 pts)?

Total of three. One of type C, and two of type E. The protein products from the other chromosome will be identical.

iv) Two different inbred strains (A^k and A^b) are infected with a virus. The A^k strain generally survives the infection, while the A^b strain does not. Give one possible explanation for this observation (5 pts).

These two strains differ in their MHC alleles. The surviving strain must be able to present peptides and have the peptide recognized bound to A^k. The A^b strain: i) cannot bind viral peptides to its class I MHC, or does not have a T-cell receptor that can recognize viral peptides presented on A^b.
6. (6 pts) Assume that the recombination signal sequence (RSS) is a one (1) turn sequence on the 5' side of the J segment for both the β-chain and heavy chain of the TCR and BCR, respectively. The RSS sequences that surround the D-segments are __1-D-2____ for the β-chain, while the RSS sequences that surround the D-segments on the heavy chain are __2-D-2_____. Briefly justify your answer.

This signal on the right of the D-segment must be 2 to allow joining to H (2+1 rule). Since VJ joining is possible in the β-chain, the signal downstream from the V must be 2 as well. Since the V-segment has to also join a D segment, there must be a 1 turn signal sequence on the 5' end of the D. Since VJ joining is forbidden in the heavy chain, the V segment must have a 1, and to allow D joining, then there must be a 2 turn signal on the 5' end of the D segment. (4pts)

7. (10 pts) Please do one of the following two choices (a labeled diagram is a suitable answer).

**Choice A:** Compare and contrast the structure of TCR to the BCR. How are they similar? How do they differ?

**Choice B:** Compare and contrast the structure of a class I MHC and a class II MHC. How are they similar? How do they differ?

**Choice A:**
Both are membrane bound, with signaling chains.
A TCR is essentially equivalent to a Fab fragment, the Ab will have a constant region.
Both have Ig folds
Both have variable domains (V_l, V_H, V_α, V_β)
Both have 3 hypervariable loops that recognize antigen
TCR has an additional hypervariable loop.

**Choice B:**
Both are attached to the membrane.
Section closest to membrane consists of two Ig folds. In class I - the α3 domain + β2 microglobin. In class II - the two carboxy terminal domains of two chains.
Both have a peptide binding domain furthest from the membrane, consisting of two helices on each side of a b-sheet platform.
Ends of the peptide binding groove are closed in class I, limiting the length of the peptide to 8-9 residues

8. (2 pts) What is the role of the anchor residue in MHC-peptide interactions?
Indicates an interaction between the sidechain of the peptide and the MHC.
Increases the specificity of the peptide recognition, so that only a limited (but still large) number of peptides can be displayed on an MHC.
9. (7 pts) How does the introduction of a pre-rearranged TCR α or β chain (or both) affect the diversity of TCR in the transgenic animal (5 pts)? Briefly discuss/describe how transgenic animals were used to investigate the structure of the TCR-MHC-Peptide complex or T-cell maturation (2 pts).

The rearranged gene inhibits the rearrangement of the endogenous genes, due to allelic exclusion. All of the T-cells will express the same transgene, reducing the diversity to zero.

a) Peptide - MHC interactions. Can test whether peptide variants (different sequences) can activate T-cells because only a single TCR is available. Otherwise, activation would likely occur because of the large number of possible TCRs found in a normal (non-transgenic) animal.

b) T-cell maturation. Generate a TCR that recognizes a male specific peptide. Male transgenic mice will have no T-cells because all of their T-cells have the same receptor - proving that there is negative selection for self peptides.

10. (6 pts) What is the most significant difference between the checkpoints in B-cell maturation and T-cell maturation? Be sure to discuss the molecular basis for this difference (i.e. what is the nature of the protein-protein interaction?).

The most significant difference is that T-cells must also recognize self-MHC (4 pts). This is accomplished by the TCR recognizing both conserved and polymorphic residues on the MHC during maturation in the thymus (2 pts).

11. (6 pts) T_{Ho} cells emerge from the thymus and differentiate into T_{H1} and T_{H2} cells. Please answer the following questions:

i) What is responsible for the differentiation of these T-cell subsets (1 pt)?
   Cytokine environment (1 pt). IL12 for TH1 and IL4 for TH2

ii) Name one cytokine that is produced by either subset and describe the role of that cytokine in the acquired immune response (2 pts)?

TH1:
   IL-2 – activates Tc cells, TNFβ – inflammation, INFγ- activates macrophages, MCP – attracts monocytes to tissue, MIP – inhibits macrophages from migrating away from tissues.

TH2: IL4 and IL5, general B-cell activation, class switching to IgE.

iii) Which T-cell subset is more proficient at fighting intra-cellular pathogens and multi-cellular parasitic infections? Briefly justify your answer (3 pts).

TH1: Activated Tc cells and macrophages are more able to destroy a cell infected with an intracellular pathogen, or a multi-cellular parasite.

12. (4 pts) Please do one of the following choices:
   Choice A: What is toxic shock syndrome – what is the immunological basis of this condition?
   Choice B: Under what circumstances will a person develop dengue fever? Briefly justify your answer.

Choice A: Toxic shock syndrome is due to release of superantigens from strep or staph bacteria that crosslink MHC II and Th cells non-specifically. This leads to the activation of a large number of Th cells, causing systemic inflammation.

Choice B: On the second infection with a different serotype, the antibodies against the first serotype do not activate the 2nd serotype, but enhance the transport of the virus into the macrophage, infecting it. Macrophage also becomes activated and recruits Th cells, which recruit more macrophages, leading to severe inflammation.
13. (10 pts).

i) Compare and contrast antigenic drift, antigenic shift, and multiple serotypes (6 pts).

ii) Give one example of a pathogen that utilizes any one of the above three (1 pt).

iii) What do all of these have in common with regard to evasion of the immune system by pathogens? (2 pts).

iv) Give one method of immune evasion by pathogen that does not involve the above three (1 pt).

i) Antigenic drift – small changes in epitopes due to single mutations: HIV, seasonal flu.

Antigenic shift – large changes in epitopes due to gene conversion: pandemic flu, sleeping sickness, N. gonorrhea – pili

Multiple serotypes – large number of different antigen sequences exist in the population. The antigens do not change on a single organism. Strep, dengue virus.

iii)

Present different antigens – so that the response to one may not be effective against the different antigen.

iv) Inactivation of IgA (N. gonorrhea), attachment of pili to mucosal membranes (N. gon), inhibition of MAC complex (N. gon), cell surface capsule that makes it difficult to phagocytosis (Strep), intracellular infection – hidden antigens (TB, leprosy)

14. (5 pts) Briefly discuss the roles of basophils and mast cells in the response to multi-cellular parasites, such as schistosomes.

Basophils produce IL-4 which enhances TH2 cells, TH2 cells secrete IL4, IL5 which enhances IgE production. (1 pt)

Mast cells have anti-parasite IgE on their surface and degranulate when the IgE binds to the parasite. (2 pts)

Mast cell secretes (2 pts)

TNFα: increases vascular permeability – fluid and cells enter region.

Histamine: increases vascular permeability – fluid and cells enter region, smooth muscle contraction.

Fluid and smooth muscle contractions serve to eliminate parasite.
15. (10 pts) Please do one of the following choices. You answer should include a description of the molecules and cells involved.

**Choice A:** After treatment with penicillin for 10 days a patient become anemic and trace amounts of hemoglobin are found in their urine. What is the cause of these symptoms?

**Choice B:** A snake trainer is bitten by his pet cobra and goes into anaphylactic shock and dies. Why does this occur?

**Choice C:** A patient is treated with an antibody for chemotherapy. After 10 days a patient develops a rash over most of their body. What is the cause of this rash?

**Choice D:** Several days after a hike in the Canadian woods, the hiker develops blisters on their ankles. What is the cause of these blisters?

**Choice A (Type II HS):**
The penicillin has modified a cell surface protein, likely on red blood cells.
The modified cell surface protein is regarded as foreign, thus antibodies have been formed.
The antibodies bind to the surface of red blood cells, causing lysis of the RBC by the membrane attack complex from complement. NK cells, via their Fc receptor, will damage RBC. Loss of RBCs lead to anemia, released hemoglobin is in the urine.

**Choice B (Type I HS):** The trainer has been bitten before and has pre-existing Ab to venom on mast cells.
Current bit has injected venom enters the blood and travels to the lungs.
Mast cells adjacent to the bronchial tubes degranulate, release histamine, causing contraction of smooth muscle cells, closing down airway.

**Choice C (Type III HS):** The antibody is regarded as foreign by the patient and antibodies have been generated. These form immune complexes with the chemotherapeutic antibodies which get lodged in the skin. Complement is activated, mast cells degranulate due to C3a and C5a, releasing histamine which helps recruit neutrophils to the areas. Neutrophils become activated and secrete lytic enzymes, oxidative oxygen species that cause inflammation (rash).

**Choice D (Type IV HS):** The hiker has touched poison ivy and the oil from the plant has modified some skin proteins. The modified peptides are presented to, and activate, TH1 cells. In this case it is a second exposure, so the memory T-cells must be activated. The T-cells produce TNFβ that causes inflammation. Other cytokines released by T-cells recruit macrophages which become activated causing inflammation and fluid flow into the tissue - hence the blisters.

**Bonus Questions (2 pts each)**

1. How do allergy shots work to reduce allergic reactions?

   *They cause an increase in the production of IgG against the antigen. The IgG binds instead of IgE.*

2. Why is there a higher incidence of allergies in developed countries, such as the United States?

   *The allergic reaction (HS I) is essentially the same as that to multi-cellular parasites. In developed countries, the incidence of infection with parasites is small and thus there is capacity to mount a response to harmless antigens, causing allergies.*