1. (10 pts) Imagine that you are a stem cell that is destined to become a $T_{H1}$ cell.
   a) (8 pts) Describe the steps involved in your genesis, beginning as a stem cell. Your answer should provide information on the following:
      i) Physical location of events.
      ii) Any changes in the DNA that occur during your development.
      iii) Checkpoints in your development and the immunological importance of those checkpoints.
   b) (1 pt) Why did you become a $T_{H1}$ cell instead of a $T_{H2}$ cell?
   c) (1 pt) What type of pathogen are you best to combat, intra-cellular or extracellular? Why?

a) Begin in bone marrow as a stem cell.
Migrate to thymus
Express $\beta$ chain by VDJ joining
Checkpoint for the production of a functional $\beta$ chain using a surrogate $\alpha$ chain, so that a functional TCR will be produced.
Expression of CD4 and CD8
Expression of a chain, by VJ rearrangement.
Checkpoint for functional a chain, so that a functional TCR will be produced.
Checkpoint for positive selection for binding to self-MHC, so that T-cells can interact with APCs
Negative selection for binding to self peptides, to avoid self-reactivity.
(moderate affinity TCRs are allowed to progress)
Loss of CD4 or CD8
Release to blood
Home to a lymph node
Activation.
Development to $T_{H2}$
(4 pts for four checkpoints, 2 points for correct order of events, 2 pts for correct location.)

b) My environment was rich in IL 12, a cytokine that promotes $T_{H1}$ development

c) Intracellular, because the cytokines that I secrete will activate $T_C$ cells and macrophages, which are effective at killing other infected cells.

2. (2 pts) The following is a list of events that could be associated with B and/or T-cell development. Please indicate next to each event the following: “B” if it relates to B-cells only, “T” if it relates to T-cells only, and “B+T” if it applies to both.

   ___ T only____ Joining of V and J segments in the generation of heavy or $\beta$ chain. (HC is VDJ only)

   ___ B and T____ Pairing of 1 turn and 2 turn RSS sequences by Rag1/Rag2

   ___ T only_____ Nbase addition during the generation of the Light chain or $\alpha$ chain. (N base only on $\alpha$)

   ___ B and T____ Pbase addition during the generation of the chains.
3. (2 pts) What common structural feature of both antibodies and TCR is important for a large increase in the diversity of these receptors [Hint: the same structural feature stabilizes MHCs]?

Pairing of the variable domains is facilitated by strong protein-protein interactions between the constant domains which are adjacent to the variable domains. This pairing between the Ig folds also stabilized MHCs.

4. (1 pt) What enzyme is required for both class switching and affinity maturation? What does this enzyme do?

Activation induced cytosine deaminase. It converts cytosine to uracil.

5. (2 pts) Why is it useful for class switching to occur? What advantage does it provide to the organism?

It allows the organism to tailor the biological response. For example, class switching to IgE is good for mucosal infections, IgA for secretion of the antibody across mucosal membranes.

6. (2 pts) What is the outcome of affinity maturation and why is it beneficial for affinity maturation to occur, especially after class switching?

Affinity maturation increases the affinity of antibodies to the antigen. It is important because the avidity drops after class switching due to a reduction in the number of binding sites. Increasing the affinity can restore the amount of binding.

7. (9 pts) Mice that are exposed to low levels of pathogen can show an increase in affinity by several hundredfold. However if the same mice of the same type are exposed to high levels of antigen, there is no increase in affinity.

i) Explain this result (6 pts).

ii) How would you measure the affinity of antibodies to antigen? Briefly describe the experimental technique and how you would analyze the data (3 pts).

i) Affinity maturation occurs due to random mutations in the V regions of the light and heavy chain. The mutations can increase or decrease the affinity (or leave it the same). B-cells with higher affinity receptors are selected due to the fact that they can capture more antigen, present more peptides to T-cells. This selective advantage is only possible at low antigen concentration. At high antigen concentration, all B-cells will be saturated with antigen, so there is no selective advantage and the average affinity will remain the same.

ii) Surface Plasmon Resonance (1 pt).
Bind the antibody to the surface, flow antigen in, measure the response (change in the critical angle) (1 pt)
Plot the response versus antigen concentration to get a binding curve, $K_D$ is the antigen concentration that gives $1/2$ saturation. Alternatively, plot a Hill plot, x-intercept is $\log K_D$. (1 pt)
8. (4 pts) Please do one of the following choices:

**Choice A:** B-cells normally have Fc receptors on their surface. What are the consequences of a mutation that would prevent expression of the Fc receptor? [Hint: What is the role of the Fc receptor?]

**Choice B:** Assume that B-cells lost the ability to express MHC II. Under what conditions could they still be activated?

**Choice C:** When making antibodies against small organic molecules, it is necessary to couple the organic molecule to a carrier protein. Why is this necessary?

**Choice A**
The B-cell would lose the ability to terminate the activation process when there is excess antibody present (signaling that the pathogen has been cleared). This would be a form of B-cell cancer - uncontrolled proliferation of B-cells.

**Choice B**
The can still be activated by multi-valent antigens, i.e. repeating units on the surface of a pathogen. This does not require MHC-TCR interaction. Only IgMs are produced, these are of low affinity, and no memory cells are made.

**Choice C**
In order to produce antibodies it is necessary that the B-cell be activated by Th cells. This can only occur if a peptide is presented on class II MHC. Thus the antigen must always have a protein component. In this case, the B cell receptor recognizes the hapten, but the peptide from the carrier protein is presented on MHC II. The carrier protein would be from a different species, so would be perceived as being foreign.

9. (4 pts) Please do one of the following choices.

**Choice A:**

i) What are the difference between polyclonal and monoclonal antibodies? Why is it generally better to use monoclonal antibodies for most applications that involve antibodies?

ii) After fusing B-cells to myeloma cells you are selecting for hybridomas using HAT media. Why are you growing the cells in HAT media?

**Choice B:** Briefly discuss how antibodies (or antibody fragments) can be used to treat disease. The DNA coding for these antibodies is usually generated by recombinant DNA technology as opposed to using the entire mouse coding region. Why are recombinant methods used to generate these antibodies?

**Choice A:**

i) Monoclonal antibodies are homogeneous and have a well defined epitope. Polyclonal antibodies consist of a collect of different antibodies that recognize different epitopes. Consequently, it would be more likely to have cross reactivity with polyclonal antibodies.

ii) HAT = hypoxanthine, aminopterin, thymine. Aminopterin suppresses de novo synthesis of nucleotides. Hypoxanthine and thymine are required for the salvage pathway, but can only be used if the cell has HGPRT. This gene is provided by B-cells but lacking in the myeloma cells.

**Choice B:**

Antibodies can be used to target tumor antigens, bringing toxins to the tumor location. They can also block growth factor receptors that are up-regulated on a number of tumors. When combined with recognition of CD3 (Bite antibodies) they can target T_{CTL} to tumor cells. The mouse part of the antibodies are replaced by human sequences, to avoid an immune response to the antibodies.
10. (10 pts) Please do one of the following choices. How could you determine

**Choice A:** the relative number of immature B and naïve B cells in a lymph node?

**Choice B:** the relative number of naïve versus activated B cells in a lymph node?

**Choice C:** the relative number of T<sub>C</sub> versus T<sub>H</sub> cells in a lymph node?

**Choice D:** how many T<sub>H1</sub> versus T<sub>H2</sub> cells might be present in a population of T<sub>H</sub> cells.

**Choice E:** if an individual had been vaccinated against measles virus.

**Choices A-C:**

These all involve detecting the appropriate cell surface markers by FACS. In general two antibodies would be required, one that is specific for each cell type. The antibodies would be labeled with different fluorophores and the relative intensity of each fluorescent signal would be used to distinguish between the cell types.

**A:** Immature B cells do not express IgD as part of their B-cell receptor. Naïve B-cells express both IgM and IgD, thus anti-IgD and anti-IgM fluorescently labeled antibodies could be used.

**B:** During activation, B-cells will up-regulate b7 or cytokine receptors. So using a fluorescent antibody that bound to b7 could be used. To be sure that you have B-cells, an antibody that recognizes IgM would be required.

**C:** These two cells are distinguished by high levels of CD4 (T<sub>H</sub>) or CD8 (T<sub>C</sub>).

**Choice D:** Since you are detecting secreted proteins, and ELISPOT assay is appropriate. The two cell types can be distinguished by secretion of INFγ, IL-2, or TNFβ (T<sub>H1</sub>) or IL-4 and IL5 (T<sub>H2</sub>). The well could be coated with anti-IL2 and anti-IL4 antibodies, each with a different enzyme linked to them so that the products could be distinguished.

**Choice E:** In this case you need to detect the presence of antibodies against the measles virus. This could be done with an indirect ELISA. Antigens from the virus (or intact virus) would be immobilized on the plate, and serum from the patient would be added. Detection would require the use of an enzyme linked secondary antibody.

11. (6 pts) Compare and contrast the structures of class I versus class II MHC molecules. How are they similar, how do they differ.

**Both are membrane attached.**

**Both have a membrane proximal Ig fold domain.** In the case of class I one is from the MHC the other is β2-microglobulin, in the case of class II, both are found in the MHC chains (α and β chains).

**Both have a peptide binding trough that has a β-sheet base, walled by two alpha helices (2 pts).** In the case of class I the ends of the trough are blocked off, they are open in a class II
12. (6 pts) A single MHC molecule can display a large number of peptides, but not an infinite number.
   i) What features of the MHC peptide interaction are responsible for this degree of specificity?
   ii) Why is this degree of specificity beneficial for generating an immune response?

   i) With the exception of one or two anchor residues (conserved in the peptide) The MHC recognizes the mainchain atoms of the peptide, so that it is not very sequence specific. All peptides that contain the anchor residues could bind to the MHC.

   ii) It increases the likelihood that an APC will be able to activate a T-cell because the recognition of the MHC-peptide by the TCR is very specific and only a small number of peptides may be able to activate the T-cell.

13. (6 pts) Describe the interaction of the TCR with MHC/peptide and comment on the specificity of recognition, both with respect to the peptide and the MHC.

   The TCR receptor binds to both the MHC and the peptide (2 pts).
   It interacts with both conserved and polymorphic residues of the MHC, thus it will only recognize self-MHC (2 pts).
   The α and β chains recognize different parts of the complex, with the α-chain recognizing $\frac{1}{2}$ of the MHC-peptide surface and the β-chain recognizing the adjacent $\frac{1}{2}$. (2 pts)
   Hypervariable loops 1 &3 mostly recognize the peptide and 2 recognizes the MHC (+1/2 bonus).
14. (8 pts) Assume that an animal has the following organization of its MHC genes:

-----A-------B--------E_α-------E_β-------F_α-------F_β1-------F_β2-------

i) Which genes code for class I MHC and which code for class II MHC? Briefly justify your answer (2 pts).
i) A and B are class I because they consist of only a single chain. E and F will code for class II, because there is an α and a β chain.

ii) How many different class I proteins would you find on the cell surface of a typical outbred animal? Briefly justify your answer (2 pts).
ii) If it is an outbred animal, then it is likely to have different alleles of each gene and therefore will produce different proteins from the same gene. There will be two type A and two type B, a total of four different class I.

iii) Outbred animals are generally more effective at fighting viral infections as opposed to inbred animals. Why is this so? (4 pts).
iii) Outbred animals typically will have different alleles at each of the MHC genes, thus producing more variants of MHC on their cells. Each variant has the ability to present slightly different peptides (i.e. they recognize different anchor residues), thus increasing the number of different peptides that can be presented from a pathogen, increasing the chance that T-cell activation will occur.

15. (8 pts) You are studying the activation of T-cells by peptides derived from the HIV virus with the intent of generating killer T_c for the treatment of HIV infected individuals. You isolate a T-cell that can be activated by the HIV virus and clone the genes for its α and β genes.

i) The HIV virus is known to undergo a considerable number of mutations. How would you test whether your particular TCR would be able to identify variations in the sequence of the HIV peptide so that your killer T_c could be used to treat many different viral mutations (5 pts).

i) You would first make a transgenic mouse that expressed the cloned α and β genes. The presence of the cloned genes would suppress the rearrangement of the endogenous VDJ and VJ segments, so that most of the T-cells produced in the transgenic mouse would be homogeneous.

In vivo you could infect the mouse with the different HIV mutants and look for activation of T-cells.
In vitro you could expose the T-cells to APCs that were loaded with the different HIV peptides and look for activation.
Note: you could not activate with just MHC-peptide because of the need for co-stimulatory signals.

ii) Although you may be able to produce a killer T_c cell that recognizes many different forms of HIV, why is this unlikely to be an effective treatment for the majority of HIV patients? (Hint: See Question 13) (3 pts).

ii) The TCR would only recognize these peptides in the context (bound to) the MHC allele of the source of the TCR genes. Because of the large number of alleles this TCR will not be activated by MHC in most of the patients. (Antigenic drift in HIV was also accepted as an answer).