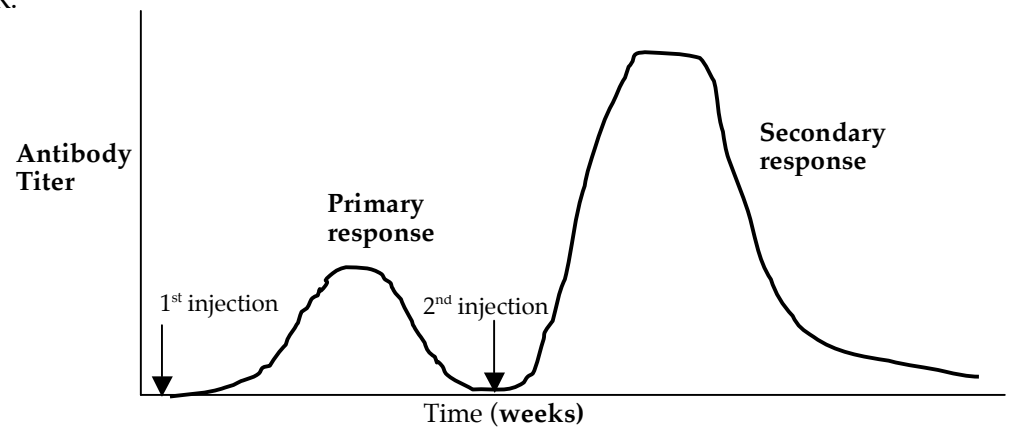
Solution Key - 2010 7.012 Problem Set 8

Question 1

You have purified a novel protein, which you call **Protein R** as a part of your summer project. You would like to further characterize this protein. Accordingly, you want to develop antibodies against this protein. You inject Protein R into a rabbit and after one month you draw some blood from this rabbit and determine that the rabbit's immune system has developed antibodies against Protein R. You wait for one month and then inject Protein R again into the same rabbit. You observe a stronger secondary immune response developing within a few days against Protein R.

a) On the graph below, draw the primary and secondary immune responses of the rabbit against Protein R.



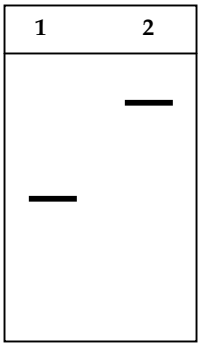
b) Why is the secondary immune response **faster** and **stronger** compared to the primary immune response?

During the primary immune response, the memory B cells, against the specific antigen, are generated. These B cells express surface IgM molecules against the specific antigen. Furthermore, they can also proliferate to form more memory B cells and plasma cells that produce the IgG antibody to counteract the antigen infection. During the secondary immune response, the memory B cells generated during the primary immune response immediately start proliferating to generate more of their own kind and also the IgG producing plasma cells to counteract the viral infection thus making the response faster and stronger compared to primary response.

c) Which **two classes** of B cells in the immune system **produce antibodies** during the primary and secondary immune responses?

B cells (both memory B cells and plasma B cells) produce antibodies against the foreign antigen.

d) The immune system's first encounter with an antigen is via the IgM class of antibodies. Once the response is initiated, the immune cells produce the IgG class of antibodies that circulate in the blood stream. You isolate the antibodies produced against Protein R, resolve them on a protein gel **based on their molecular weight** and obtain a profile as shown in the schematic below.



Protein gel

Higher molecular weight

Lower molecular weight

i. Identify the two classes of antibodies in lane 1 and lane 2 of the protein gel.
   - Lane 1 of the protein gel. *IgG*
   - Lane 2 of the protein gel. *IgM*

ii. Do the two classes of antibodies have …
   - identical or different antigen binding sites?
   - same number of antigen binding sites (Yes/No)? **Explain**.

It has different number of antigen binding sites. Since IgG is monomer it has 2-antigen binding sites/IgG molecule in comparison to 10 binding sites/ IgM molecules, which is a pentamer.

Question 1, continued

e) Towards the end of the primary and / or secondary immune response, you incubate the two cell-types that you have identified in part d(ii) with a fluorescent dye (Annexin V). This dye specifically binds to terminally differentiated cells that are undergoing apoptosis. You then sort the cells using a flow cytometer and observe two distinct populations of the cells: population 1 being negative for annexin staining and population 2 being positive for Annexin staining.

i. Identify the cell-types that **most likely** make up
   - Population 1: *Memory B cells*
   - Population 2: *Plasma B cells*

ii. Which of the two cell-types shows a higher potency (number of fates a cell can acquire)? **Explain** why you selected this option.

Memory B cells are more potent since they can divide to make more of their own kind and also generate plasma B cells. In comparison, plasma B cells produce and secrete the IgG antibodies and then die.

f) Besides the cells that you have identified in different parts of this question, what roles do the **T – helper cells** and **antigen presenting cells (APC)** play in the production of antibodies? *Note: in your answer please specify the cell surface proteins that are involved.*

The helper T cells are involved in the antigen recognition and clonal expansion of the B cells. When a B cell ingests antigen, it attaches epitopes of the pathogen's proteins to a MHC-II protein. This complex is moved to the cell membrane, where it can be recognized by a T helper cell (T_H) that are activated by the antigen presenting cells (APC cells) displaying the epitope through the MHC-II. When a B cell processes and presents the same antigen to the primed T_H cell, the T_H cell secretes cytokines that activate the B cell. These cytokines trigger B cell proliferation and differentiation into plasma cells that produce the IgG antibodies, which act against the antigen.

g) Briefly describe the three major mechanisms by which an antigen reacting with an antibody may be either destroyed or neutralized.

The following are the three major mechanisms for antigen disposal:
- *Neutralization: The antibodies bind to the antigens located on the surface of the virus and neutralize it by blocking its ability to bind to a host cell.*
- *Opsonization: The antibodies bind to the antigens on the bacterial surface to promote the phagocytosis of the bacteria by macrophages.*
- *Activation of complement system: The antibodies bind to an antigen located on the surface of a foreign cell to activate the complement system which then forms a membrane attack unit. This unit forms pores in the membrane of the foreign cells allowing water and ions to rush in. The foreign cell therefore swells and ultimately dies.*

h) Herceptin is a monoclonal antibody that can specifically bind to the HER receptors that are over-expressed in 25-30% of breast cancer patients.

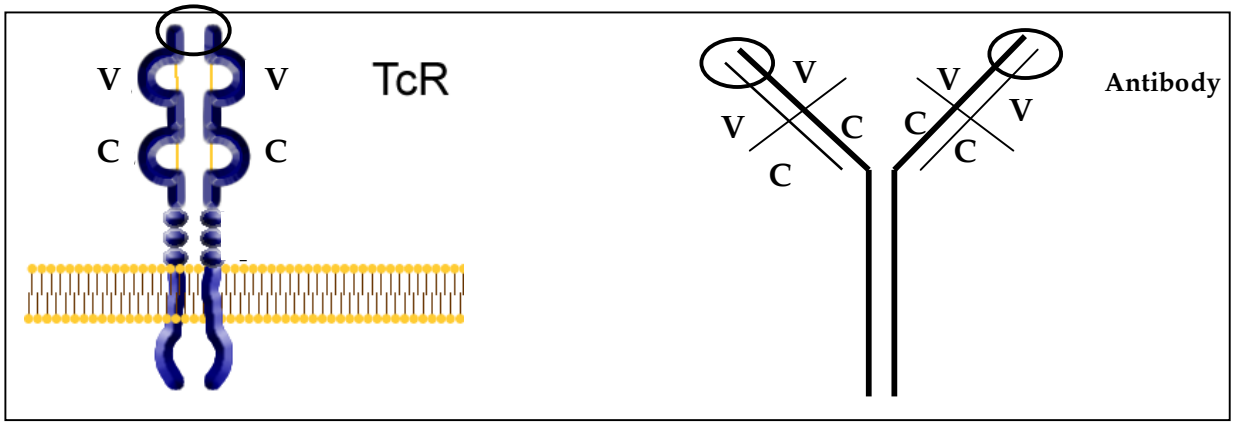
i. In terms of the epitopes being recognized, how do monoclonal antibodies differ from the polyclonal antibodies?

Each monoclonal antibody recognizes a single epitope. In comparison, polyclonal antibodies represent a mixture of different antibodies that can recognize different epitopes on the surface of the same antigen.

ii. How many HER-2 binding sites are there on each Herceptin molecule?

2 HER-2/herceptin molecule

Question 2
The following is a schematic of the structure of T cell receptor (TcR) and an antibody molecule.



a) On the diagram, for both molecules
- **Circle all** the antigen binding sites
- Label the variable regions
- Label the constant regions.

b) Both TcR and antibody molecules exhibit a quaternary structure. Name **the covalent bond(s)** that stabilize the quaternary structure of these two protein macromolecules.
You can include all the non-covalent bonds but you must have the disulfide bond (S-S) as a part of your answer.

c) Besides binding to a specific antigen,
i. What are the other surface proteins with which the TcRs interact during the **cell mediated immune response**? *MHC-I (and CD8)*

ii. What are the other surface proteins with which the TcRs interact during the **humoral immune response**? *MHC-II (and CD4)*

d) There are 20,000 genes in the human genome. Yet there is a specific TcR and / or specific antibody against each antigen. Briefly describe the **major mechanism required** for generating the TcR and antibody diversity given the number of genes that we have.
The diversity is generated through gene rearrangement i.e. V, D, J joining. Further diversity can be generated by processes such as somatic hyper mutations and imprecise joining of nucleotides at the joining points.

e) Sometimes, the immune system turns against particular molecules of the body resulting in autoimmune diseases. The examples include lupus, rheumatoid arthritis, diabetic mellitus and multiple sclerosis. Briefly describe how the self-reacting T cells are eliminated during the development of immune system.
*This can be explained by **Clonal Deletion/ negative selection theory** according to which self-reactive lymphoid cells are destroyed during the development of the immune system in an individual.*

f) Organ transplant is required in various clinical conditions. One has to consider various criteria in order to reduce the chances of transplant rejection.
i. Which surface molecule(s) is critical for the success of organ transplant? *MHC-I*

ii. Which cell types express this molecule on their cell surface?
MHC-I surface molecules are located on the membrane of all the cells except for mature red blood cells.

iii. Why do we see a higher success rate of organ transplant if the donor is a monozygotic twin compared to a sibling?
The monozygotic twins are genetically identical to each other and should express identical MHC I molecules. Therefore the graft cells will be recognized as normal.

Question 3

As a clinician you come across a couple that were diagnosed as having a mild form of familial hypercholesterolemia (FH). Their son however has a more severe form of FH. Both the parents are able to take a medication that increases the rate of uptake of low-density lipoproteins (LDL) by the liver. The medication works by increasing LDL- receptors. You find that although this medication works for both parents it does not work for their son.

a) What is the genetic explanation for the son having a more severe form of FH than either of his parents?

Both the parents are heterozygous for FH (+/fh) and therefore have one wild type and one mutant copy of LDL-R gene. Their son is however homozygous (fh/fh) and thus has two copies of mutant LDL-R gene which causes a severe form of FH.

b) Propose a reason to explain why does the medication taken by the parents not work for the son?

The drug taken by the parents upregulates transcription of wild type LDL-R gene, thus increasing the total amount of functional LDL-receptor in the liver cells. The medication does not work for the son since his liver is not capable of making any functional LDL-receptor because he has no wild type copy of LDL-R gene.

c) Suppose you were able to surgically remove some tissue from the son's liver, grow the liver tissue in the culture and expose the cells to radio- labeled LDL. If you observe that the radio labeled LDL was found on the surface of the liver cells, what explanation could you provide for the molecular basis of the son's form of FH?

The son most likely has a mutation in the LDL-receptor gene such that it can bind LDL but it cannot internalize the LDL bound LDL-receptor complex.

d) You decide to cure this disease using gene therapy. Explain how gene therapy may be useful in treating this patient. What disadvantage may the somatic cell gene therapy have over reproductive gene therapy?

Gene therapy *is the insertion of genes into an individual's cells and tissues to treat a disease such as FH in which a deleterious mutant LDLR allele is replaced with a functional one. Gene therapy may be classified into the following types.* ***Germ line gene therapy;*** *In the case of germ line gene therapy, germ cells, i.e., sperm or eggs, are modified by the introduction of functional genes, which are ordinarily integrated into their genomes. Therefore, the change due to therapy would be heritable and would be passed on to later generations. This new approach, theoretically, should be highly effective in counteracting genetic disorders and hereditary diseases. However, many jurisdictions prohibit this for application in human beings, at least for the present, for a variety of technical and ethical reasons.* ***Somatic gene therapy;*** *In the case of somatic gene therapy, the therapeutic genes are transferred into the somatic cells of a patient. Even if 10-15% of cells receive the wild type copy of the gene, the amount of protein made may be sufficient enough for the affected individual to lead a normal life. With such a therapy any modifications and their corresponding effects will be restricted to the individual patient only, and will not be inherited by the patient's offspring.*

Question 4

Human immunodeficiency virus (HIV) is a retrovirus. Its genome is a single (+) stranded RNA that is packaged with the reverse transcriptase enzyme within a protein capsid. This is further packaged into an envelope that is derived from the plasma membrane of the host cell in which the virus had replicated. The surface of the envelope is covered with the envelope glycoprotein, called gp120.

a) HIV specifically infects the T- helper (T_H) cells of the human immune system. If HIV enters the host cell by means of host receptor recognizing a viral protein, what would be the most likely **ligand** and its **corresponding receptor** during HIV infection?

The gp120 protein on the surface of HIV envelop binds to the CD4 receptor on the surface of T helper cells and this ligand-receptor binding event is the first step of infection.

Question 4, continued…

b) Some individuals are resistant to HIV infection even after repeated exposure. Assuming that these individuals express a normal level of the functional receptor that you have recognized above, how can you explain their resistance to HIV?

The gp41 protein on the surface of virus binds to a chemokine receptor (CCR) on the surface of T helper cells. If a Person shows a homozygous mutation for the CCR gene (CCR-/ CCR-) he/she will not have the chemokine receptor and will not contract AIDS even after repeated exposure to HIV.

c) Once an infection has taken place the RNA genome has to be made into the double stranded DNA that integrates into the host cell genome. Given that these steps are all performed by the reverse transcriptase enzyme, provide two-enzymatic activities of this enzyme.

The following are the three steps required to produce the double stranded DNA from the single (+) stranded RNA genome.step1: Synthesis of the complementary strand of DNA using the (+) stranded RNA genome as the template, step 2: Degradation of the (+) strand RNA template, step3: Synthesis of the double stranded DNA using the complementary strand of DNA as the template. Given these three steps are all performed by the reverse transcriptase enzyme, the activities of this enzyme are: RNA directed DNA polymerase activity, RNAse activity and DNA directed DNA polymerase activity. It is to be noted that this enzyme also works as an integrase to integrate the cDNA copy of the virus genome into the genome of the host cell.

d) In recent years, therapies have been developed to fight AIDS using nucleotide analogs. The drug used to combat AIDS is Azidothymine (AZT). The structure of AZT is very similar to thymidine except that in AZT, the 3'-OH group on the deoxyribose sugar is replaced by an azido (N_3) group. Which process of the life cycle of HIV do you think is inhibited by AZT?

AZT is a thymidine analogue (a nucleotide used in the synthesis of DNA. Therefore AZT interferes with the synthesis of DNA from RNA by reverse transcriptase. This enzyme incorporates AZT more effectively into the growing DNA chain and this blocks the further elongation of the chain because the growing end has no 3'-OH group on the deoxyribose sugar. So the viral concentration decreases over time with response to the treatment.