

Bio257 Immunology Problem Set #2

1. What is the advantage of a B cell undergoing somatic hypermutation after it binds to an antigen? Would the change involve a change to another isotype, allotype or idiotype? Explain your choice.

2. Although we predominantly think of viruses as intracellular pathogens and bacteria as extracellular pathogens, there are many types of bacteria that live and multiply within cells. While escaping the dangerous world outside host cells can be advantageous for intracellular bacteria, there are also immune challenges once a bacterium has taken up residence in a host cell.

a) Early in infection, intracellular and extracellular pathogens face similar challenges from the innate immune system.

Describe two (2) innate immune defenses (other than phagocytosis) that bacteria face upon entering a host. Make sure to include what makes these defenses effective against bacteria. You can include innate immune defenses that cooperate with the adaptive immune system.

b) *Shigella flexneri* is an intracellular bacterium that causes dysentery and it enters host cells through phagocytosis by the host cell. At low pH, however, *Shigella* has the ability to disrupt membranes and escape into the cytoplasm.

Why is it advantageous for *Shigella* to escape from phagocytic vesicles (i.e. what would happen if the bacterium remained in the vesicle)? How would an inability to escape the vesicle affect *Shigella* bacteria that have not yet entered host cells? Describe the cells and molecules involved.

c) You discover a bacterium, *S. bodoynia*, that can enter directly into the cytoplasm of host cells. Interestingly, you find that *S. bodoynia* secretes a protein called TBP that binds tightly to the TAP protein and blocks its function.

How would TBP help protect *S. bodoynia* from the host immune system? Describe the cells and molecules involved.

d) You delete the TBP gene from *S. bodoynia* in the hopes that this mutant bacterium will have lowered virulence. You test the mutant (TBP-) and wildtype bacteria in two inbred mouse strains A and B and find that while the wildtype bacteria kill both types of mice, the mutant bacteria only kills the A strain.

Based on what you know about immunology, give a hypothesis for why strain B is resistant and strain A is sensitive to the TBP- bacteria. Explain your hypothesis in terms of the cellular and molecular interactions in the infected mouse strains.

e) To try to help strain A mice, you decide to use irradiation to kill the T-cells in strain A and to replace them with T cells from a strain B mouse. You make sure that the donor strain B mouse has been immunized with *S. bodoynia* proteins.

Will the *S. bodoynia*-specific T-cells from strain B help protect strain A from subsequent *S. bodoynia* infection? Why or why not (explain in terms of cells and molecules involved)?

3. The availability of technology to disrupt genes in mice has allowed researchers to test the importance of individual genes for proper immune system function. One possible phenotype for the disruption of genes involved in immunity is severe combined immunodeficiency (a SCID phenotype). Whereas deletion of gene(s) that encode certain protein enzyme(s) of the V(D)J recombinase leads to a SCID phenotype, deletion of other V(D)J recombinase gene(s) does not result in such a severe phenotype.

- a) What are the four key enzymatic proteins involved in V(D)J recombination?
- b) What cell types will be affected by deletion of the genes for these proteins and why?
- c) For each protein, predict whether the deletion of the gene encoding that protein will lead to a SCID phenotype and explain your prediction.
- d) For each gene deletion that you believe will NOT have a SCID phenotype, predict what the effects of the gene deletion will be and explain your prediction.

4. Immunologists have constructed a strain of mice in which both alleles of the gene for β_2 -microglobulin have been deleted. If one of these mice is infected with Theiler's virus (a mouse relative of poliovirus), its immune system has a hard time fighting the infection.

- a) Explain why lacking β_2 -microglobulin causes these mice to be susceptible to viral infection. What cells would be normally be involved in fighting infection and how are they disabled in these mice?
- b) You believe that the immune system of these mice would be able to defend them from infection with the bacteria *E. coli* or *Staphylococcus aureus*. Explain this hypothesis. What cells and molecules are involved in fighting such extracellular bacteria?

5. Consider the antibody-based assays that we have discussed: ELISA, immunofluorescence microscopy, immunoelectron microscopy, immunoblotting, flow cytometry, Ouchterlony/double immunodiffusion.

- a) For each of these assays, would you describe the assay as qualitative or quantitative? Why?
- b) For each assay, think of an experiment you could perform using the assay--what question would the assay allow you to address, what results would you expect and what controls would you need to perform to interpret your results (or to make the results quantitative rather than qualitative).

Additional questions from the textbook

These questions will be useful to make sure you have mastered the material we have covered although they are not too similar in format to exam questions. Some important material may not be covered by these questions, so make sure to go over your notes thoroughly and ask me questions about topics you find confusing!

Chapter 5: 5, 7, 14

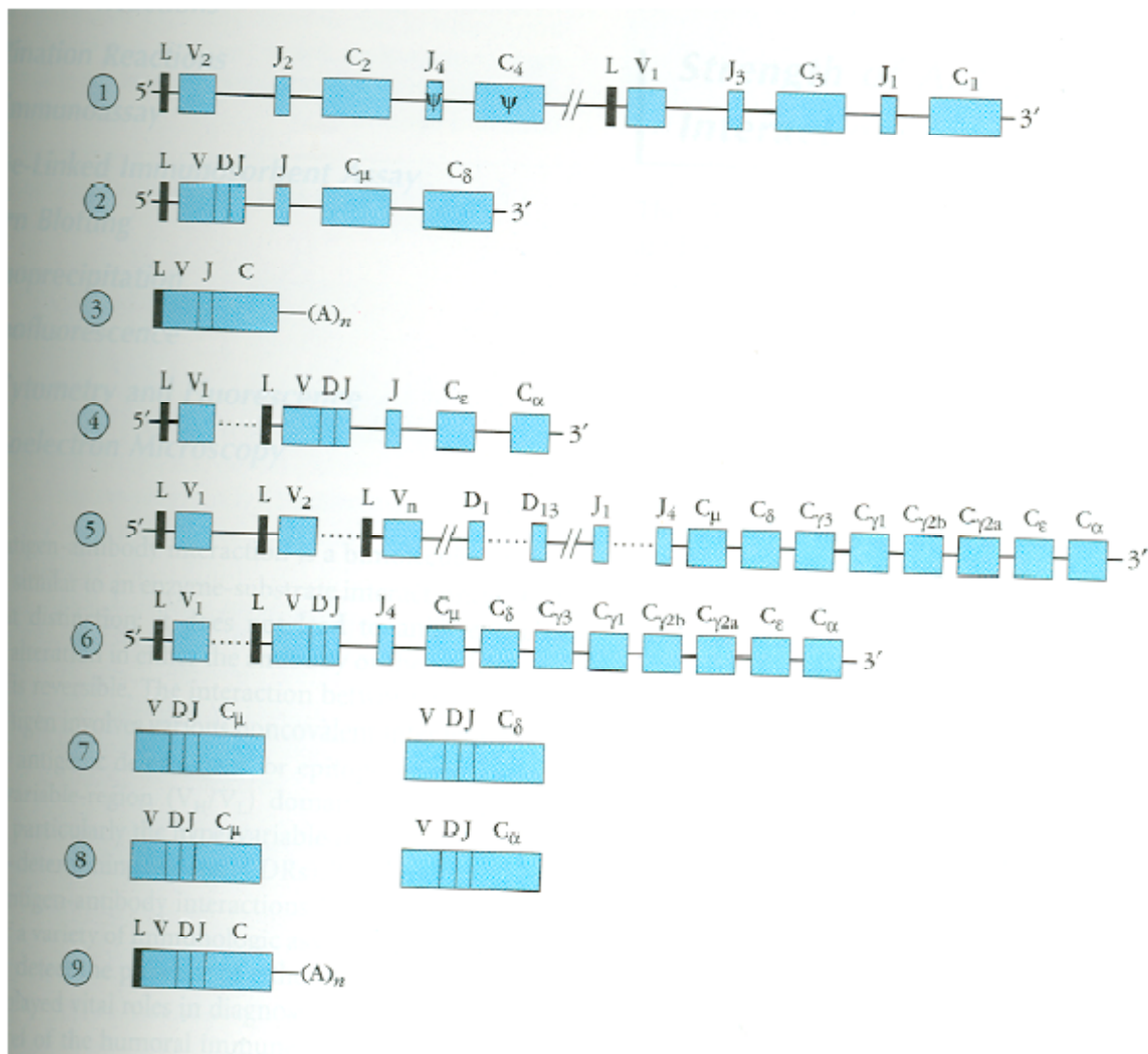
Chapter 8: 5, 16, clinical focus question

Chapter 9: 3, 4a&b, 5, 8

This question from the previous edition of the textbook is a little silly (notice it was removed in our edition), since drawing DNA, RNA and proteins using the same symbols misrepresents what is happening in cells. In spite of that issue, I think the exercise is valuable in having one think through the process of gene rearrangement and expression.

“For each immunoglobulin heavy-chain or light-chain DNA, mRNA or polypeptide (a-g) described below, select the corresponding schematic diagram (1-9) in the figure below.

- _____ Heavy-chain DNA in a liver cell
- _____ λ -chain DNA in a liver cell
- _____ Heavy-chain DNA in a mature B cell
- _____ Primary RNA transcript encoding heavy chain in a mature B cell
- _____ mRNA encoding light chain in a mature B cell
- _____ Heavy-chain proteins observed on the membrane of a mature B cell before any class switching
- _____ Heavy-chain DNA in a plasma cell secreting IgE” (Goldsby *et al.*, 2000)



Reference: Goldsby, R.A., Kindt, T.J. and Osborne, B.A. (2000) Kuby Immunology, 4th ed. (New York: Freeman) p. 147, 171.