CHAPTER 3. Antigens.
1. What are the definitions for: antigens, epitopes, immunogens and antibodies.
2. What are the differences between immunogenicity and antigenicity? How can you explain that happens in order to induce an immune response need to be conjugated to a carrier protein?
3. Be prepared to explain for example, why one would expect that the humoral immune response to the GP120 HIV virus receptor would be expected to elicit antibodies with many different recognitions (for different antigenic determinants). In this respect, know what is meant by a polyclonal antibody response.
4. Why antigens need to be greater than 5,000 molecular weight in order to efficiently stimulate an immune response. Think about why that might be so. Overall, you need to know the properties that contribute to the immunogenicity of an antigen.
5. What are adjuvants? What are the most commonly used adjuvants? How do they work?
6. For what sorts of antigens do we mount the strongest and the weakest immune responses?
7. You need to be familiar with how antigens are seen by T and B cells? (Table 4.2)
8. What do we mean by sequential or conformational epitopes?

Immunoglobulins: Structure and Function.
1. Be able to describe and draw the basic structure of an antibody molecule indicating: antigen binding sites, biological activity site, constant domains, variable domains, hinge regions, intra- and extra-chain disulfide bonds, carbohydrate-binding sites, carboxy and amino ends, etc.
2. Be familiar with the roles of the different parts of the antibody molecule (ex. antigen binding)
3. Be able to describe experiments used to demonstrate antibody structure: pepsin, papain, and mercaptoethanol treatments. What are the end products?
4. Be able to know the types of light and heavy chains? Types of light and heavy chains.
5. You need to know what is an immunoglobulin fold. How many domains are present in each class of antibody? What many domains are present in IgG compared to IgE?
6. Be able to know what makes a molecule part of the immunoglobulin superfamily. Provide examples.
7. What are complementary-determining regions (CDRs)? Be able to identify within the variable regions the CDRs. How many? Why are they important?
8. Be able to describe the antibody-mediated effector functions (opsonization, etc).
9. Know 2-3 properties for each antibody type (fixes complement, crosses placenta, etc.)
10. How IgA (and to a lesser degree IgM) acquires its secretory component?
11. Know the difference between isotype and allotype.
12. Be able to describe the structure and properties of the “B cell receptor”.
13. Be familiar with at least ONE member of the Ig superfamily that recognizes antigens, receptors, and signaling.
14. Be able to discuss in favor or against the benefit of a monoclonal vs a polyclonal antibody.

CHAPTER 5. Organization and Expression of immunoglobulin genes.
1. Be able to associate immunoglobulin expression during B cell development with each developmental stage (lymphoid cell --------------> mature B cell).
2. Be able to outline the gene organization of immunoglobulin heavy chains (mouse only).
3. Be able to understand figure in your book that led to understanding of Ig gene rearrangement. Why when a light chain probe was used against myeloma and embryonic DNA we obtained one and two bands respectively? What was shown by this experiment?
4. Be able to outline the steps in the generation of a light chain (kappa only!) or a heavy chain arrangement. I will provide in the exam the antibody gene segments and you should be able
to do the arrangement. Remember, we will use six steps similar to those used in class (and in the lab).

5. Be able to describe the steps in the recombination event (from question 4).

6. Understand that in the reciprocal recombination events producing the final variable gene sequence, that there are signal sequences at which the recombination events occur. These signal sequences are targets for recombination enzymes called RAG1 and RAG2 in a synapse at the recombination site.

7. Why is junctional flexibility important to the overall diversity of the antibody molecule?
Understand in general terms that additional diversity is produced by “controlled slop” in joining the two variable regions. This additional diversity is partially produced by extra nucleotides at the splice junction (P nucleotides) arising from the peculiar way that the initial cuts are made and processed in the initial steps of the recombination. Further diversity arises from additional nucleotides added in a random sequence to the ends of the joining sequences by deoxynucleotidyl terminal transferase (Tdt) before ligation between the ends occurs (N nucleotides).

8. The recombination signal sequences (RSS) are such that, in a single-stranded form, they can hybridize (make base pairs) with each other because of their palindromic nature and because the two ends of the signal sequences are the same for RSS 1 and RSS 2. One of these signal sequences is referred to as a two turn (23 aa) sequence and the other one as a one turn sequence (12 aa). Be able to rearrange several V-J and V-DJ regions containing one turn or two turn signal sequences. Remember, recombination can only occur between a two turn sequence and a one turn sequence.

9. Be able to understand the mechanisms by which antibody diversity is generated: multiple germ-line gene segments, combinatorial V-(D)-J joining, junctional flexibility, P-nucleotide addition, N-nucleotide addition, and somatic hyper-mutation.

10. Be able to describe the process, which (allelic exclusion, light chain exclusion) which restricts the number of heavy and light gene rearrangements per cell so that any cell never has two rearranged heavy or light variable genes. How is this process related to the use of kappa versus lambda chain gene sets.

11. Finally, additional diversity arises throughout the variable region but predominantly at the hypervariable regions (CDR regions) from hypermutation rates exclusively in the variable region (not spilling over into the constant region). The diversity, which arises from the hypermutation, is selected for in a classic evolutionary process according to which mutations allow the antibody to more strongly bind the antigenic epitope against which the immune response is being made. You must be able to describe all of these processes along with appropriate drawings.

12. Class switching occurs by similar recombination events in the spacers between the constant genes on the locus coding for the heavy chain. This splicing occurs at “signal sequences” (switch regions) which are very different from the RSS sequences described above. These signal sequences consist of short sequences, which are repeated many times. Be familiar with the role played by certain cytokines to promote preferential class switching to certain constant genes. If you are familiar with question 2 then you will get this question right!

13. Know why it is that any particular B lymphocyte may only synthesize and secrete one class of antibody at one time. Also that having switched from one isotype of antibody to another, that B lymphocyte may never again synthesize the isotype previously made.

14. What is the evidence that high mutation rates occur predominantly in the three hyper variable regions during the process of an immune response. Do these high mutation rates only occur in the primary immune response or do they pick up again during a secondary immune response.

15. What is the evidence that the high mutation rates actually steadily improve the specificity and binding strength of the antibody produced as the immune response progresses (higher affinity). Be able to explain why this process is actually evolution in action (mutation and
natural selection) although tremendously speeded up. Why is the high mutation rate not harmful to the B cells in which it is occurring? Be able to defend the premise that this process of evolution is critically important to the quality of the antibodies produced. (Remember: less antigen vs higher antibody affinity).

16. Explain why it is that the probability of a successful rearrangement of a heavy or light variable is so low (about 8%).

17. Why is it that the junctional diversity produced by addition of “P” and “N” nucleotides mostly the CDR3 region and not the other two regions? Do you think that it is a coincidence that CDR3 region is the most important in determining antibody specificity?

18. Be able to describe the structural differences between the membrane bound antibody versus the secreted antibody at the carboxy terminal end of the polypeptide chain. Why is the carboxy terminal end of the membrane bound antibody made the way that it is? What is the mechanism that determines whether an antibody will be secreted or membrane-bound?

19. Similar to the question above be able to describe the mechanism by which the choice is made to express the IgM constant gene versus the IgD constant gene. Alternatively, how can we explain the presence of IgD and IgM on the surface of a mature B cell?

CHAPTER 6. Antigen- Antibody Interactions.

1. Be able to describe the nature of the bonds made between the antigen and antibody. Given the nature of these bonds, be able to describe the reasons that the antigen and antibody must interact in a way so that the antibody reaction site is a mirror image of the topology of the binding surface of the antigen. Why can cross reactivity occur between two antigens with a single antibody?

2. Be able to interpret the concepts of affinity and avidity as they relate to monoclonal and polyclonal antibodies. Do not forget the concept of cross-reactivity!!! (last exam question!)

3. Be able to outline the basic steps of techniques describing the usefulness of antibodies: double diffusion, radial immunodiffusion, SDS-PAGE, ELISA (seen in the lab; for Ag or Ab quantification), ELISPOT, Western blotting, immunofluorescence, and FACS. Which one are most sensitive?

4. Be familiar with the different versions of the ELISA technique.

5. How would you purify T cells from a lymph node or spleen? Check magnetic isolation and FACS. What are the common fluorochromes used in immunofluorescence/FACS? Remember, in FCAS isolation you start with 10,000 cells (100%) and you have to provide % of cells in each of your quadrants.