Problem Set #4

1) What is responsible for the initial lag in the production of antigen-specific antibody observed after the initial injection of antigen? What happens to this lag period upon subsequent exposure to the same antigen and why?

The initial lag is due to the time required for the appropriate rare antigen-specific B cells to co-localize and interact with antigen and with rare antigen specific T cells if required. Then, there are several rounds of proliferation, followed by differentiation into plasma cells for IgM secretion. This takes several days. Upon re-exposure to the same antigen, pre-existing memory B cells specific for the antigen are present in higher concentration and have already received their initial activating signals from T helper cells so they start dividing and differentiating right away-- hence a much shorter lag.

2) How can an antigen activate B cell directly, without T cell help?

If it's a polyvalent antigen, it can directly crosslink individual IgM molecules on the surface of the B cell resulting in cellular activation.

3) Do T and B cells have to recognize the same epitope on a complex antigen in order to cooperate with one another? Why or why not?

No. Most often, B and T cells recognize different epitopes of the same complex antigen. This is because B cells recognize intact antigen, but T cells recognize antigen which has been ingested by an antigen presenting cell (APC; many phagocytes), chopped up into pieces, and displayed on the cell surface along with an MHC molecule. Hence, the B and T cell epitopes need only be part of the same macromolecular complex.

4) Which of the following require alterations at the (a) DNA level, (b) RNA level, (c) protein level:

a) antigen receptor gene assembly **DNA**
b) class switching **DNA**
c) membrane vs. secreted form of Ig **RNA**
d) biosynthesis of IgA dimer **protein**
e) somatic hypermutation **DNA**

5) Many resting B cells express both IgM and IgD. How is this possible? Some splenic B cells in immunized mice have both IgG and very low levels of IgM on the cell surface. How is this possible?

IgM and IgD can be co-expressed on a mature B cell since class switch recombination is NOT required for IgD expression. It's expression is due to regulated readthrough of

transcription from the Cμ exons down to the Cδ exons, followed by RNA splicing of the VDJ exon to the Cδ exons. Both μ and δ mRNA are made in the same cell. Transient IgM and IgG co-expression might be due to pre-existing IgM in a cell which has recently undergone class-switch recombination to IgG. You can detect the IgM until it gets turned over.

6) Which of the following factors are required for both V(D)J recombination and class switching: RAG1, Ku70, RNA polymerase II, AID (activation-induced cytidine deaminase)?

V(D)J, both, neither (not sure), class switching

7) Is somatic hypermutation limited to the expressed Ig alleles in a B cell? Design an experiment to find out (extra credit).

Many possible answers. 1) PCR amplify VDJ junctions from immunized splenocytes. Sequence a group of them. Look at those which are out of frame rearrangements (they are not expressed as HC protein). Look for mutations compared to germline sequences. 2) You may want to test whether non-Ig loci undergo mutation. Use PCR to amplify exons of other non-Ig genes known to be expressed in germinal center B cells. Clone and determine sequence. Scan for mutations.

8) What cellular and molecular interactions are required to activate switch recombination? How is switch recombination regulated?

Requires B cell- T cell interaction. Both cells must be specific for the same antigen (although it might be that they are specific for distinct epitopes on that antigen). B cell uses its B7 protein to bind to CD28 on the T cell surface and its MHC class II molecule to present a fragment of antigen to the T cell, and the T cell uses its gp39 (also known as CD40 ligand) to engage CD40 on the B cell surface. The T cell also secretes cytokines at the B cell. These cytokines activate specific "switch transcripts" from promoters upstream of the various switch sequences, targeting the switching reaction. The newly discovered cytidine deaminase AID is involved, but it is uncertain how.

9)Explain how somatic mutation results in increases in antibody affinity during an immune response?

Mutations are generated across variable exons of heavy and light chain genes leading to the synthesis of altered surface Ig on the B cell. The B cell is in the germinal center and must compete for antigen binding with other B cells. Mutations which increase affinity

for antigen result in more rapid cell division and the prevention of apoptosis. Mutations which decrease binding end up resulting in apoptosis of that B cell. Hence, selection.

10) List the genetic events which must occur properly in order to produce a high-affinity IgG specific for a novel antigen. Name three genetic diseases which interfere with one or several of these events.

V(D)J recombinaton	*scid due to RAG-deficiency, DNA-PK mutation, ADA deficiency, Il7Rγ defect, etc.*
Class-switch recombination	
Somatic hypermutation	