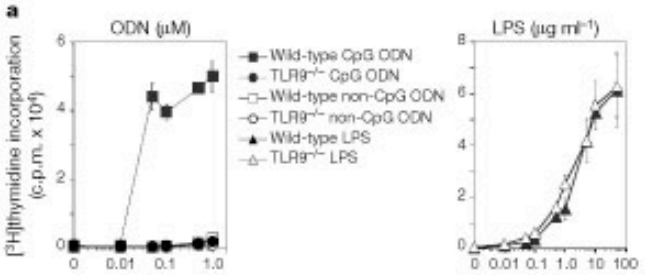
Problem Set MCB150 Spring 07 (Midterm questions from Fall 06)

For Discussion Sections 1 and 2:

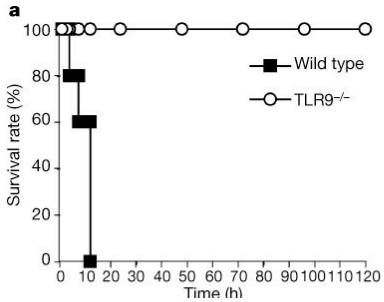
1a. (5 pts) What are PRRs and PAMPs? Give two examples of each.

1b. (4 pts) What does TLR9 recognize? Explain why its ligand is unique to microbes versus mammalian cells?

1c. (4 pts) Below is a figure from our discussion section paper showing the proliferation of splenocytes from wild and knock-out mice treated with the indicated ligands. Explain why LPS treated splenocytes were included in this experiment.



1d. (4 pts) Below is another figure from our discussion section paper showing the survival of mice following injection with CpG oligodinucleotides (ODN). What are the wild type mice dying from? Why are the knockout mice surviving?



2. (6 pts) For each technique listed below, indicate which of the statements below apply.

Western blot

Flow cytometry

FACS

Sandwich ELISA

RIA

Immunofluorescence microscopy

a) Can be used to determine the number of cells in a cell suspension that express a particular antigen.
b) Sensitive method used to determine the quantity of antigen in a complex sample.
c) Can be used to determine the molecular weight of an antigen.
d) Requires 2 different antibodies to the same antigen.
e) Provides anatomical/spatial information about location of antigen.
f) Can be used to detect multiple different antigens simultaneously in the same sample.
g) Can be used to physically isolate cells based on marker expression

3. (5 points) Below is a ribbon diagram of a light chain. Indicate on the diagram the regions that correspond to the :

V domain
C domain
Intrachain disulfide bond
HV loops
part of the light chain that corresponds to the V-J DNA rearrangement junction

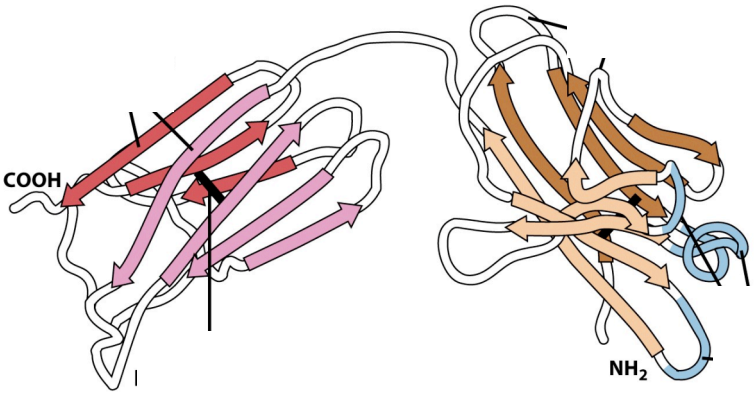


Figure 4-11a
Kuby IMMUNOLOGY, Sixth E

4. (4 pts) In order to generate antibodies in a rabbit against the hapten DNP, you need both a carrier and an adjuvant. Briefly explain both terms and explain how each one contributes to antibody production.

5. (8 pts) For each antibody isotype listed below, indicate which of the statements below apply.

IgG

IgM

IgD

IgE

IgA

a) Dimeric (2 antigen binding sites/molecule).
b) Tetrameric (4 antigen binding sites/molecule).
c) Decameric (10 antigen binding sites/molecule).
d) First antibody secreted in an immune response.
e) Production requires class switch recombination.
f) Exists in only a transmembrane form.
g) Binds to Fc receptors on mast cells.

(10 points total, 2 points each) What is (are) each of the following and why are they important. (Two sentence limit for each):

myD88

defensins

For Discussion Sections 3 and 4
What is (are) each of the following and why are they important. (Two sentence limit for each):

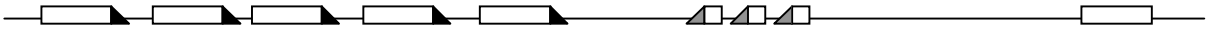
Allelic exclusion

follicular dendritic cell

λ5

germinal center

7a. (5 pts) Below is a simplified diagram of an unrearranged light chain locus with the 1-turn RSS shown in black and the 2-turn RSS in grey. Label the gene segments and indicate on the diagram where a rearrangement might take place. Sketch the rearranged DNA locus below.



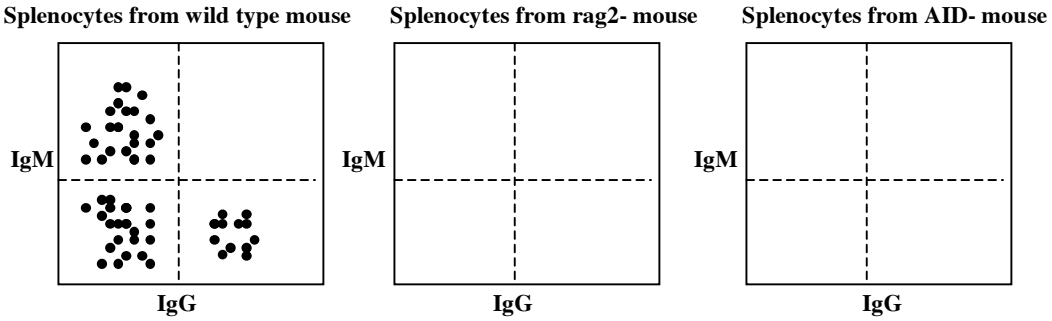
7b. (6 pts) Sketch a *simplified* diagram (similar to the light chain locus diagram show above) of an unrearranged heavy chain locus, labeling the location of the 1-turn and 2 turn RSS and the gene segments. Indicate where rearrangements might take place and sketch a rearranged DNA locus version of the heavy chain locus below the un-rearranged DNA locus.

7c. (5 pts) Briefly explain why receptor editing can occur efficiently with the light chain locus, but not the heavy chain.

8. (8 pts) Below are listed several events that occur during B cell development. Fill in the table below to indicate which statements apply to which events.

statement / event	Directly involves rag proteins	Directly effected by AID mutation	Alters the DNA encoding antibody proteins	Occurs after antigen encounter	Involves alternative mRNA processing
Class switch to IgG, IgA, IgE					
Production of secreted antibody					
Kappa V-J joining					
Somatic hypermutation					
IgD expression					

9a. (5 pts) The left panel below shows an idealized flow cytometry plot of spleen cell from wild type mice. In the plots to the right, fill in what results you would expect for spleen cells from a rag2 gene knockout mouse and an AID gene knockout mouse.



9b. (3 pts) For the AID knockout mouse, you decide to further investigate the defect by examining the average affinity of antibodies (determining the equilibrium binding constants) produced in mutant mice after repeated immunizations with chicken ovalbumin (ova). Your first step is to generate a panel of monoclonal antibodies from immunized mutant and wild type mice. Explain why you need to include this step.

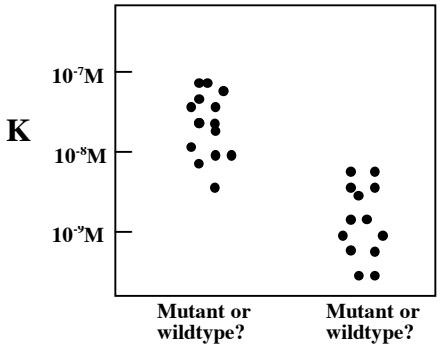
9c. (2 pt) You notice that all of the monoclonals generated from the knockout mice are secreting IgM, whereas those from the mutant are producing predominatly IgG. Explain this observation.

9d. (6 pts) You decide to initially evaluate your monoclonal antibodies using assays that rely on lattice formation. Under which conditions would you expect antibody-antigen lattices to form:

a) individual monoclonal antibodies with soluble, monomeric ovalbumin (ova).
b) mixture of several different monoclonals with soluble, monomeric ova.
c) individual monoclonal antibodies with cells displaying ovalbumin on their surface as a transmembrane protein.

9e. (4 pts) In order to determine the affinities of your monoclonal antibodies for ovalbumin, you decide to use the technique of Surface Plasmon Resonance using a biosensor that detects changes in resonance angle as a read out of the degree of protein binding to another protein immobized on a chip. You consider 2 different setups: (a) antibody immobilized on chip with soluble antigen or (b) antigen immobilized on chip with soluble antibody. Say which setup would be better for determining equilibrium binding constants and briefly explain why.

9f. (3 pts) After completing your affinity measurements, you generate the graph of equilibrium dissociation constants (K_d) shown below. Indicate on the graph which samples are from mutant or wild type mice. Briefly explain your answer.



9g. (3 pts) You find that the antibodies from the mutant mice bind at least as tightly (avidly) to cells displaying ovalbumin as those from wild type mice, in spite of the affinity differences seen in the graph above. Explain this observation.