

Biology 257 Immunology Fall 2005

Midterm #1

Instructions

This exam is designed to take ~2 h. The relative number of points should give you a rough idea of how much time to spend per question. The **space** that has been left between questions **roughly approximates** how long your answers will be, depending on the size of your handwriting. **Aim for CLEAR, CONCISE, COMPLETE answers--most questions on this exam only require 1-3 sentences maximum per section. Diagrams are great** if they help you explain your answer, but they are not required.

Hand in all sheets, including scrap paper, before you leave and **make sure your name is on this front page.**

Question 1 (25 pts. total) The instructors for an immunology course are preparing a lab for next month. They take two highly inbred strains of mice, CBA (H-2^{k/k}) and C57L (H-2^{b/b}), and inject one mouse of each type with the soluble protein ovalbumin from chicken eggs. They also infect a different mouse of each type with influenza virus.

a) (2 pts) What types of molecules are definitely different in H-2^{k/k} and H-2^{b/b} mice?

b) (2 pts) Which lymphocyte cell types does one expect to be activated in spleens from mice injected with ovalbumin?

c) (7 pts) Which lymphocyte cell types does one expect to be activated in spleens from mice infected with influenza? Are these types the same as or different from those in b? Explain why these types are the same or different.

After a week, the instructors sacrifice the mice and remove their spleens. Unfortunately soon thereafter, ethanol spills on the tubes containing the spleens, smearing the labels. Luckily, a student walks into the room at their moment of despair and designs two tests to figure out which spleen is from which mouse.

Assay #1:

1. H-2^{b/b} and H-2^{k/k} cells are preincubated with _____.
2. The cells are exposed to ⁵¹Cr and then overlaid with splenocytes from each mouse.
3. After 16 h, the amount of ⁵¹Cr in the culture medium and in the cells is determined.

Assay #2:

1. Antigen-presenting cells (H-2^{b/b} and H-2^{k/k}) are preincubated with _____.
2. The cells are then overlaid with splenocytes from each mouse.
3. Cytokine production is measured.

d) (5 pts) Briefly explain what is happening in Assay #1. Make sure to include which cell types from the spleen will be detected (indicate by underlining) and whether one preincubates the cells in step 1 with ovalbumin or influenza virus.

1e) (5 pts) Briefly explain what is happening in Assay #2. Make sure to include which cell types from the spleen will be detected (indicate by underlining) and whether one preincubates the cells in step 1 with ovalbumin or influenza virus.

The results of the tests are shown in **Table 1**.

f) (4 pts) Which spleen came from which mouse?	Spleen#
CBA (H-2^{k/k}) + ovalbumin	_____
C57L (H-2^{b/b}) + ovalbumin	_____
CBA (H-2^{k/k}) + influenza virus	_____
C57L (H-2^{b/b}) + influenza virus	_____

Question 2 (29 pts total) You have just started a research project on a certain protein and are thrilled to find out that someone in the lab next door has two hybridoma cell lines (A and B) that produce monoclonal antibodies that recognize the protein. You get a tube of each purified antibody from your neighbor, but before you can label them, they slip out of your hands and one lands under a nearby table and the other lands under the fridge. You manage to fish them out and then label them T (table) and F (fridge). Rather than asking for another tube of each antibody, you decide to use your understanding of immunology to figure out which antibody (T or F) was produced by which hybridoma (A or B).

The first step you take is to dilute some of each antibody sample with SDS buffer containing dithiothreitol (DTT, a reducing agent) and analyze the samples by SDS-PAGE. You run three gels. You treat one gel with Coomassie blue stain. For the other two gels, you transfer the protein(s) to a membrane and perform Western blot analysis: one blot you probe with an anti- λ -chain antibody and the other blot you probe with an anti- κ -chain antibody. Your results are shown in **Fig. 2A**.

a) (3 pts) Why do you see two bands in each lane in the Coomassie-stained gel?

b) (2 pts) In contrast, why do you only see a maximum of one band per lane in the western blots?

2c) (2 pts) What do the western blots reveal about the two antibodies?

Luckily, when you received the tubes of antibody, your neighbor also gave you a rough diagram (**Fig. 2B**) of the λ -chain and κ -chain loci in each hybridoma as well as of the loci in liver cells (she determined the DNA sequence for part of the λ -chain locus and for the whole κ -chain locus).

d) (2 pts) Why do the κ loci look different in the liver cells than in the hybridoma cells?

e) (5 pts) Name all the antibody chains that you expect to be produced by hybridoma A. Briefly explain your prediction.

f) (5 pts) Name all the antibody chains that you expect to be produced by hybridoma B. Briefly explain your prediction.

g) (2 pts) Which antibody (T or F) do you expect to have been produced by which hybridoma (A or B)?

Although you are happy to have solved your puzzle, you are still curious about the diagram. In **Fig. 2B**, the κ allele 1 in hybridoma A looks the same as the κ allele 1 in hybridoma B, yet you think that they must be different from one another. You develop a hypothesis about how κ allele 1 differs between the two hybridomas and decide to test it by asking your neighbor for the actual DNA sequence of the two loci.

h) (2 pts) What evidence in Fig. 2B leads you to believe that the sequence of κ allele 1 is in fact different in the two hybridomas?

2i) (6 pts) What difference do you expect to find in the DNA sequence of the k allele 1 between hybridomas A and B? Give one possible explanation for how this difference might have arisen.

Question 3 (12 pts total) In a hypothetical experiment, a disease is induced in three highly inbred dogs that causes their adaptive immunity to be majorly hindered. Following disease induction, the effects of two different yeasts (which differ in one of their cell wall glycoproteins) on these immunocompromised dogs are observed (see table below). One of the dogs is simultaneously injected with serum from a healthy dog.

	<u>Yeast</u>	<u>Serum from a healthy dog</u>
Dog 1	A	no
Dog 2	B	no
Dog 3	B	yes

Days after being injected with the yeast, the dogs 1 and 3 are still alive and well, but dog 2 is extremely sick. Use your knowledge of immunology to answer the following questions:

a) (5 pts) Why might dog 1's body have been able to defend against yeast A whereas dog 2's body was not able to fight off yeast B?

b) (5 pts) How did dog 3 escape the effects of yeast B suffered by dog 2?

c) (2 pts) What can we guess about the history of the healthy dog from the results of this experiment?

Question 4 (34 pts total) Your immunology class is producing monoclonal antibodies that recognize lymphocyte cell surface molecules. One of the antibodies recognizes a novel molecule that you name CD555. You want to use a fluorescence microscope to test whether there are certain areas of the spleen that contain cells that express CD555.

a) (4 pts) Why do you think there may be CD555-expressing cells in the spleen?

Although you have purified lots of the anti-CD555 antibody, you and your classmates decide that, to save time on your experiments, you will make a single-chain antibody that is fused to the green fluorescent protein (GFP). Like all proteins, GFP is composed of amino acids, but it has the cool property of fluorescing on its own, without an extra fluorescent molecule being attached to it. Diagrams of the expression plasmids you have made (A, B, C and D) are shown in **Fig. 4A**.

b) (5 pts) What do VH and VL stand for? Explain why you have inserted these regions in your plasmids.

c) (5 pts) Why did you make sure that the number of nucleotides in the linkers between the different DNA segments were in multiples of 3 (i.e. $12=3 \times 4$, $51=3 \times 17$)?

You put each plasmid into bacteria, allow the bacteria to synthesize the protein, lyse the bacteria and purify the proteins. Now you are ready to use the fluorescence microscope to test whether there are certain areas of the spleen that contain cells that express CD555.

d) (7 pts) Why would you prefer to use one of these GFP fusion proteins rather than the original monoclonal anti-CD555 antibody in your experiments? Which step can you avoid and why can you skip it?

Once you put your slides under the microscope, you find that only one of the four single-chain antibodies works. You name this winning protein scAb-GFP.

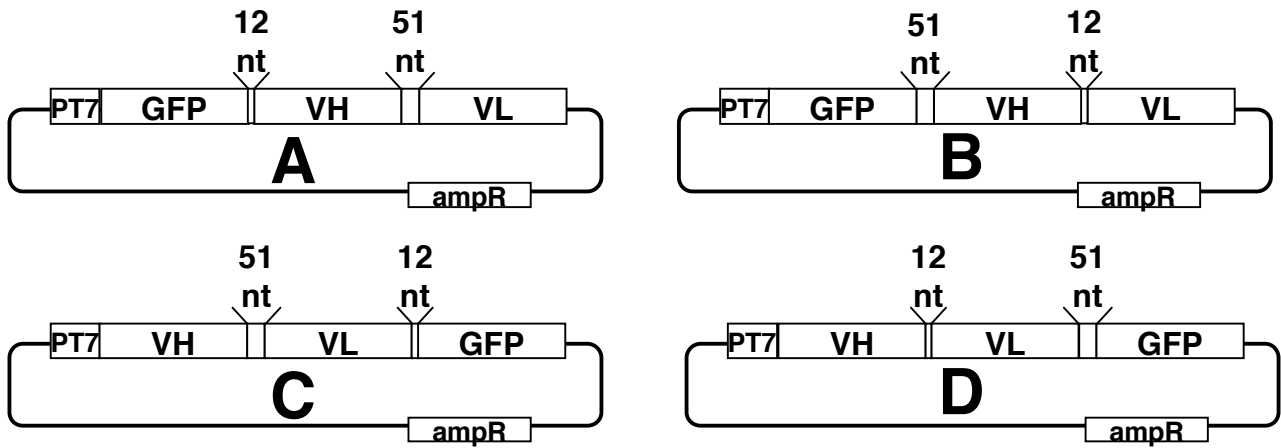
e) (7 pts) Which plasmid (A, B, C or D) do you think encodes scAb-GFP and why?

Excited that you have one protein that works for microscopy, you decide to use flow cytometry to figure out which types of cells have CD555 on their surface. You fix two samples of spleen cells and incubate them with scAb-GFP. One sample you also incubate with a rhodamine-conjugated anti-CD4 antibody and the other you also incubate with a rhodamine-conjugated anti-CD8 antibody (rhodamine is a red fluorescent molecule). You then run each sample through a flow cytometer and collect the data in **Fig. 4b**.

f) (6 pts) What type of cells do you think are most likely to have CD555 on its surface? Briefly explain your reasoning.

Extra credit (2 pts): Which of the types of molecules that we have discussed this semester were featured in the New York Times yesterday as potential targets for new drugs to fight diseases from cancer to hepatitis?

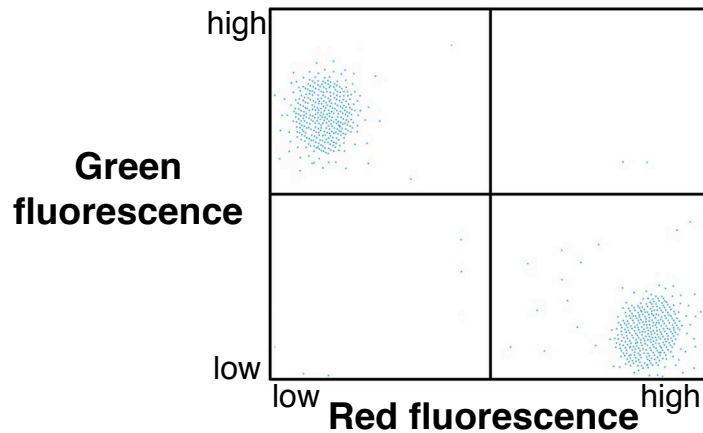
Fig. 4A: Expression plasmids for GFP fusion proteins



nt=nucleotide

Fig. 4B: Flow cytometry analysis of splenocytes

scAb-GFP + rhodamine-anti-CD4



scAb-GFP + rhodamine-anti-CD8

