

Biology 257 Immunology Fall 2005: Midterm #1 ANSWER KEY

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?
class I and class II MHC (major histocompatibility complex) proteins (TCR)

b) (2 pts) Which lymphocyte cell types does one expect to be activated in spleens from mice injected with ovalbumin? **helper T cells (T_H cells), B cells**

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. **T_H cells, B cells, cytotoxic T (T_C) cells**

Since ovalbumin is a soluble protein, it can be recognized directly by B-cell receptors, but, as an exogenous antigen, it also is taken up (endocytosed) by antigen-presenting cells (APCs) for presentation to helper T cells. Since influenza virus starts outside cells, T_H and B cells are similarly activated, but since viral proteins are also present within cells after infection, viral peptides can be presented as endogenous antigens on class I MHC molecules to activate cytotoxic T cells.

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.

In this assay, preincubation of cells with *influenza virus* allows the virus to enter the cells, where viral proteins can be digested by the proteasome, allowing viral peptides to enter the endoplasmic reticulum and bind to class I MHC molecules. The cells take up the radioactive chromium. Release of ⁵¹Cr after incubation with splenocytes indicates the presence of cytotoxic T cells with T-cell receptors (TCR) that lyse cells that display a matching antigenic influenza peptide in the context of a matching MHC class I molecule.

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.

In this assay, APCs will endocytose/phagocytose *ovalbumin*, digest it into peptides and present it on MHC class II molecules. Any ovalbumin-specific helper T cells in the spleen will recognize presented ovalbumin peptides and signal the T_H cell to secrete cytokines.

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 | <u>4</u> |
| C57L (H-2 ^{b/b}) + ovalbumin | <u>1</u> |
| CBA (H-2 ^{k/k}) + influenza virus | <u>3</u> |
| C57L (H-2 ^{b/b}) + influenza virus | <u>2</u> |

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?

The disulfide bonds that link the heavy and light chains of the antibodies were broken by the DTT, so the chains migrate separately on the denaturing gel, with light chains migrating farther down the gel.

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

Since the anti- λ and anti- κ antibodies only bind to light chains, only the lower band is visualized by western blot.

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

The F antibody has a λ light chain and the T antibody has a κ light chain.

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?

The rearrangement of immunoglobulin gene segments, as indicated by the removal of internal V and J segments, is activated only in B cells, not in liver cells or any other cell types. Hybridoma cells are created by the fusion of B cells and myeloma cells.

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

Heavy chain and λ light chain encoded by allele 1. Since both κ alleles and one λ allele have been rearranged, both κ gene rearrangements must have been unproductive, allowing λ gene rearrangement to occur.

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

Heavy chain and κ light chain encoded by allele 1. Since only one κ allele was rearranged, it must have been a productive rearrangement, inhibiting rearrangement of the other κ and λ light chain alleles (allelic exclusion).

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

Hybridoma A=antibody F; Hybridoma B=antibody T

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?

As stated in d and e above, since a λ allele was rearranged in hybridoma A, both κ gene rearrangements must have been unproductive, whereas the rearranged κ allele 1 in hybridoma B produces full-length light chains, since further rearrangements were inhibited.

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

The V, J, and C regions of the κ allele 1 in hybridoma B should all be joined to form a continuous reading frame. The κ allele 1 in hybridoma A is likely to contain an in-frame stop codon or an altered reading frame (frameshift) where the V and J segments have been joined, resulting in either no translation or improper translation of the downstream constant region. An in-frame stop codon or a frameshift could have been the result of either nucleotide addition after RAG cutting or of "junctional flexibility" in the joining of coding segments.

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?

Since the yeasts differ in a cell wall glycoprotein, this protein in yeast A may bind to a pattern recognition receptor (e.g. a Toll-like receptor) on phagocytes, directing the phagocyte to engulf and digest the yeast. If pattern recognition receptors on macrophages recognize no surface proteins of yeast B, in the absence of an adaptive immune response, this breed of dog appears to have no line of defense against yeast B.

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

Antibodies made by the healthy dog could bind to epitopes of antigens on the surface of yeast B and "opsinize" the yeast, directing it to bind to Fc receptors on macrophages or other phagocytes, which can then eliminate the yeast.

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

The healthy dog must have been exposed to yeast B (or a yeast with closely related surface antigens) sometime in the past.

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?

As an organ of the immune system, the spleen has many B and T lymphocytes and the monoclonal anti-CD55 antibody recognizes a lymphocyte surface protein.

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.

VH=heavy chain variable region, VL=light chain variable region. Since the variable regions of the heavy and light chains together form the part of an antibody that binds specifically to antigen, these regions are required to allow binding to CD555.

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. $12=3 \times 4$, $51=3 \times 17$)?

The 5' gene segments must be in the same reading frame as the 3' gene segments so that the proper amino acid sequences are maintained in the protein. Since there are 3 nucleotides per codon, multiples of three allow the fusion gene to stay in the right reading frame.

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?

If one used the original monoclonal anti-CD555 antibody, one would have to use an indirect immunofluorescence technique: first the untagged primary antibody binds to its specific antigen on spleen cells on a slide, then the antigen location is detected by using a secondary antibody linked to a fluorescent molecule. Since the scAb-GFP has both antigen specificity and fluorescence, you don't need the secondary antibody.

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?

C is the best looking plasmid. It has two advantages:

- 1. A long linker between VH and VL, which encodes a flexible region that is more likely to allow the two variable regions to fold properly with their N-termini juxtaposed.**
- 2. GFP on the C-terminus of the variable regions. If GFP were on the N-terminus of the VH domain, it might interfere with the ability of the N-termini of the two variable regions to bind to antigen.**

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.

Since cells that have high levels of green fluorescence have low levels of anti-CD4 red fluorescence but high levels of anti-CD8 red fluorescence, cells that have CD555 are predominantly CD4-CD8+. The most common cell type with this combination is cytotoxic T cells.

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?

Toll-like receptors!