

Student-written practice questions: Midterm 1

One of these questions will appear on the midterm. For the most part I have tried to stick with minimal editing for clarity, but note that your question may have an adjustment or addition that could affect the interpretation of the question.

If you need to make assumptions to answer any question, make sure that you describe your assumptions!

1. Your friend has been getting sick very frequently lately, and you think she may have something wrong with her immune system. She goes to see a doctor, and he runs some tests. He informs your friend that she has a defect in her Class I MHC molecules, in that two of the amino acids in the loop connecting the alpha-1 and alpha-2 regions are not the amino acids that are normally found in this loop. Both of the two mutations change Cys (side chain -SH) to Ser (side chain -OH). Your friend is very worried and very confused.

a. Explain to your friend how such these mutations would affect her immune system, using the appropriate molecules and processes in your explanation.

You also know, as an immunology student, that your friend's immune system is not completely compromised by this disorder.

b. Explain to your friend why her immune system is not completely compromised in spite of the disorder.

Your friend asks if she could get injections of normal Class I MHC molecules to help her immune system.

c. Explain to her why this treatment would not be beneficial.

2. Your research lab has decided to begin studying molecule X, a very small, newly discovered molecule that has recently been implicated to play a crucial role in some horrible disease. You want to perform western blots in your future experiments, but anti-X antibodies are not readily available yet, so you decide to produce your own. You inject rats with molecule X and perform an indirect ELISA on the serum you collect from these rats. After the addition of the secondary antibody, you see little to no color in the test well.

a. What does this result indicate about the serum collected from the experiment?

b. Describe two strategies you could try in your next attempts to inject the rats with X to be more successful at obtaining anti-X antibodies and why you believe these strategies may help you achieve your goal.

3. Unfortunately one of your friends has the symptoms of diphtheria and because of your new- found immunology skills you think you are able to help him out to discover what he actually has. You decide that you are going to inject diphtheria toxin into a mouse to see if the mouse has similar symptoms to your friend. After twenty-four hours the mouse does not show any signs of infection.

a. Explain why your friend has symptoms of diphtheria while the mouse may not show any signs of the illness.

You then remove serum from the mouse and decide to inject the serum into a guinea pig in hopes that the guinea pig will have symptoms similar to your friend. After four hours you decide that you are going to run an indirect ELISA, so you coat the wells with diphtheria toxin. You are a pro with running ELISAs so you wash after you coat the wells (and after each of the following steps). You then remove serum from the infected guinea pig and place it into the well. You place enzyme-linked secondary antibodies that you know will detect guinea pig antibodies, as you have run this test before, but when you place the substrate into the well, there is no color change.

b. What does this result mean and why do you think there was no color change? (i.e. describe a hypothesis to explain the lack of color change)

c. Describe how you would test your hypothesis in (b).

4. You recently learned researchers have identified a cytoplasmic protein in certain species of monkeys that confers with natural HIV-1 immunity. You have a pet monkey, Reeses, and you want to test her for her natural immunity to HIV-1. You contact the researchers and find out that the protein name is TRIM5 α , and it interferes with viral replication by binding to the HIV-1 capsid as soon as the virus enters the cell. They agree to send you antibody that recognizes TRIM5 α .

a. Given the choice of flow cytometry, immunofluorescence microscopy, and Western blotting, which method would you use to test if your monkey has cells that express the TRIM5 α protein? Why would you choose this method over the other two methods?

b. How would you design and carry out your experiment (including controls)? What would a positive result look like?

The researchers also found that humans have a similar form of TRIM5 α that differs by one amino acid - ours has a charged amino acid residue in the C-terminus, while the monkey protein does not (it is either uncharged or not there). You want to test yourself too to see if you are naturally resistant to HIV-1.

c. Can you use the same antibody used in Reese's study? Why or why not?

5. Chronic Granuloma Disease (CGD) is characterized by the inability of granulocytic cells to produce an O_2^- superoxide anion. This superoxide anion is then converted into oxidizing agents that render phagocytosed antigens harmless. Neutrophils are often considered the most important granulocytes because they are the first to arrive at the site of an infection.

a. Based on what you know about the function of neutrophils, why is this disease particularly acute? Be sure to include what type of immunity neutrophils are classified under.

b. Design an experiment that would allow you to test whether or not a patient has CGD. Be sure to explain what results you would expect if the patient tests positive for CGD as well as the results you would expect if the patient tested negative for CGD. Also include any controls you feel are necessary to interpret your results. You have the following reagents and a modernly equipped diagnostic lab.

-Dihydrorhodamine, a fluorochrome that fluoresces when oxidized

-Purified leukocytes from a chronically ill patient

6. A nurse in a busy hospital is in charge of giving two patients blood transfusions. The nurse realizes she has not labeled the two bags of blood to be transfused and is not sure which bag is for which patient.

a. From an immunological standpoint, what problems might giving the wrong blood transfusion to a patient cause?

The nurse decides to figure out which bag of blood she should give to each patient by performing an Ouchterlony double-diffusion test. The reagents she has available are serum from patient #1, serum from patient #2, and blood from each transfusion blood bag.

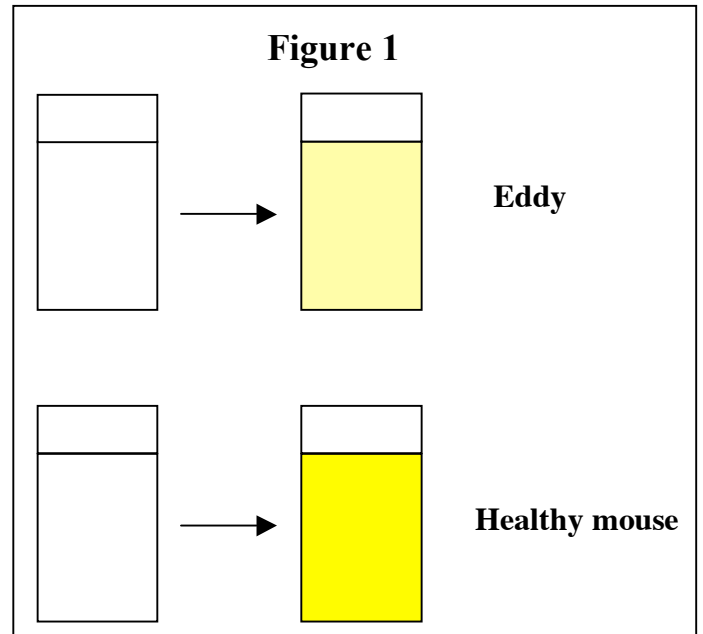
b. How should she set up the test and how should she interpret the results (drawing a picture may be helpful)? Do any of the reagents need to be manipulated before setting up the assay? If so, why?

During the 24 hours it takes the nurse to perform the Ouchterlony assay, one of the patients severely deteriorates and now needs a heart transplant.

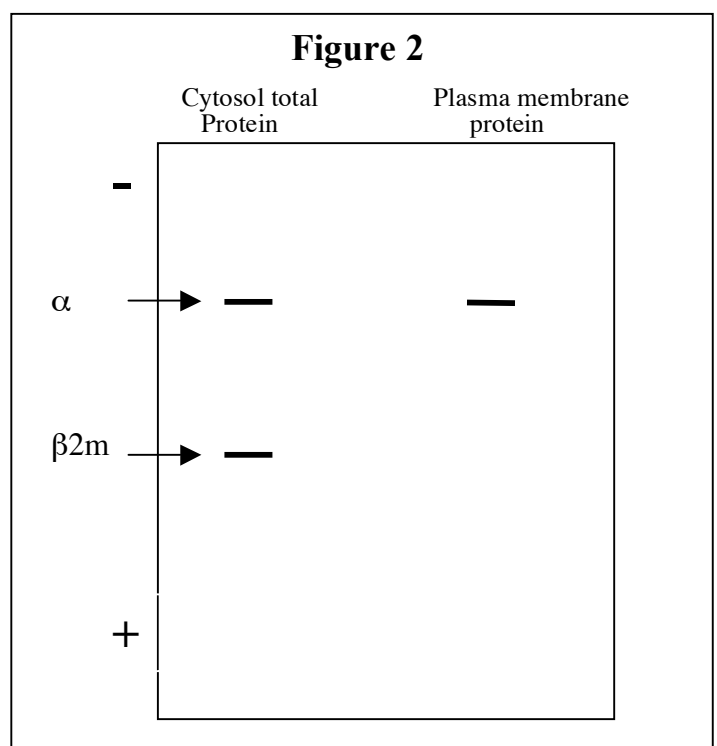
c. Why is it much harder to match a donor and a recipient for a heart transplant than for a blood transfusion?

7. You are studying the immune response of mice to different disease-causing agents. Since you work in a very well-funded lab, ever since their birth your mice have been kept in a completely sterile environment that allows absolutely no virus or bacteria to get to them. One day somehow an airborne strain of the virus gets released in the lab and gets to the mice. Most of your mice get very mild symptoms but are completely healthy in a few days. However, one of the mice, Eddy, demonstrates very bad symptoms and after a day it seems almost dead. You realize that there must be something very wrong with Eddy and you are curious to learn what the problem is. You take blood and tissue samples from Eddy and then inject him with blood serum from the healthy mice. After multiple shots he gets better.

First you perform indirect ELISA with the mouse blood using wells coated with viral coat protein. As a control you perform the same experiment with blood from a mouse that survived the infection and is healthy again. You use a secondary antibody with an enzyme that turns a chemical in the well yellow. Your results are shown in Fig. 1



Now you perform some experiments on the mouse tissue samples that you have. You lyse the cells by vortexing and then perform a series of centrifugations that separate the plasma membrane from the cytosol and all the organelles. You then do a Western on the two samples and probe the membrane with a mixture of two anti-MHC I antibodies (one recognizes the α chain and the other recognizes β 2-microglobulin). These results are shown in Fig. 2.



Explain what may be causing the inability of Eddy to fight the viral attack. Make sure to relate your explanation to all of your experimental results.