

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. **Read the entire question before** starting to **answer** it. The **space** that has been left between questions **roughly approximates** how long your answers will be, depending on the size of your handwriting. If you need it, there is **extra space on the back of the exam**. **CLEAR, CONCISE, COMPLETE** answers will receive full credit.
- To start, find a question with which you feel comfortable.
- If you feel you need to **make an assumption to answer a question, state the assumption explicitly**.

1. (16 pts total) Chronic Granuloma Disease (CGD) is characterized by the inability of granulocytic cells to produce an O₂⁻ 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. (5 pts) 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.

Neutrophils are phagocytic "first-responder" cells of the innate immune system. When an infectious agent enters the body, neutrophils can leave the bloodstream and move toward the site of infection through chemotaxis. The ability of these cells to phagocytose foreign objects (through the use of pattern recognition receptors such as Toll-like receptors or through opsinization by complement proteins) helps them mount an immediate response to infection. Therefore, if these cells (the most numerous white blood cell) are not effective because they cannot digest foreign antigens, the pathogen will be able to multiply and establish a larger infection during the time necessary for the adaptive immune system to activate (which takes much longer than neutrophil action).

Note: Dendritic cells, macrophages and B cells express MHC II, neutrophils do not.

b. (11 pts) 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

I would incubate leukocytes from this patient, from a healthy donor and from a donor known to have CGD with dihydrorhodamine (assumption: DHR can cross membranes by diffusion). I would then use a flow cytometer to count the number of leukocytes fluorescing at the appropriate wavelength for the fluorochrome. The healthy donor should have a large number of fluorescent leukocytes, in which the fluorochrome has been oxidized by superoxide anions in granulocytes. The known CGD donor should have few fluorescent leukocytes. If the patient's numbers are close to that of the known CGD donor, the patient may have CGD.

Note: One could use a fluorescence microscope for detection, or link DHR to another molecule to enhance its uptake by phagocytes (as long as linking it does not change its fluorescent properties).

2. (36 pts total) You are studying public health and immunology at the University of New Mexico and part of your project is to make a monoclonal antibody that recognizes hantavirus, a virus that was responsible for a 1993 Acute Respiratory Distress Syndrome (ARDS) outbreak in the southwest USA. After you complete your screening, you choose to work with a hybridoma that produces a monoclonal anti-hantavirus IgA antibody. Shortly after you select this hybridoma, there are five patients who present with ARDS in the Gallup Medical Center, three of whom die before they can be treated. The doctors allow you to take small lung samples from these ARDS victims to test whether they may have died from hantavirus infection.

a. (8 pts) Describe an experiment using your monoclonal antibody to test whether the deaths might be due to hantavirus (including proper controls and your expected results).

Since I only have a small lung sample from the ARDS victims, I would fix these samples on a glass slide, incubate them with my monoclonal antibody (mAb), wash the slide then incubate with a fluorescently labeled goat anti-mouse IgA antibody. After washing the slide, I would look at the slide with a fluorescence microscope and compare the patients' samples to positive (e.g. hantavirus-infected cells grown in culture) and negative (e.g. uninfected cultured cells) controls treated the same way. Another important negative control would be to incubate a small sample from the victims with only the secondary antibody, to make sure there is not background binding of the secondary antibody. If the patients were infected by hantavirus, their cells should fluoresce, as should the infected cultured cells, whereas uninfected cells and lung samples only incubated with secondary antibody should not fluoresce.

(Note: other techniques might also work, but should take into consideration the small size of the tissue samples.)

Your housemate asks whether your monoclonal antibody could be used therapeutically to help ARDS patients, but you think it will be more useful for diagnosis of hantavirus infection.

b. (8 pts) How does your friend think your antibody might work therapeutically? Describe what your friend thinks would happen in a patient's body after antibody injection.

My housemate thinks that the mAb would be able to bind to hantavirus in the ARDS patients' bodies. This binding might then a) direct binding of the virus-Ab complex to Fc receptors on phagocytic cells (via the mAb constant region), directing the phagocytosis and digestion of the virus (and leading to helper T cell activation after presentation on MHCII), b) block the ability of the virus to bind to cell surface receptors and therefore prevent infection, or c) cause clumping of viruses, which could also inhibit cell entry. All of these actions will prevent viral replication.

(Note: activation of complement may or may not help, depending on the virus)

c. (8 pts) Explain two possible reasons why your antibody might not work very well therapeutically.

Since mAbs are produced in mice, the Fc region of an IgA antibody from a mouse may not be able to bind to the Fc receptor on human phagocytes, preventing the clearing of the virus by phagocytes. In addition, the mouse antibody may be recognized as foreign by the patient's immune system, which could produce anti-mouse IgA antibodies that direct the phagocytosis and destruction of the mouse IgA before it can block viral infection.

(Note that the second two functions described by my housemate should still be effective, even with a mouse rather than a human antibody).

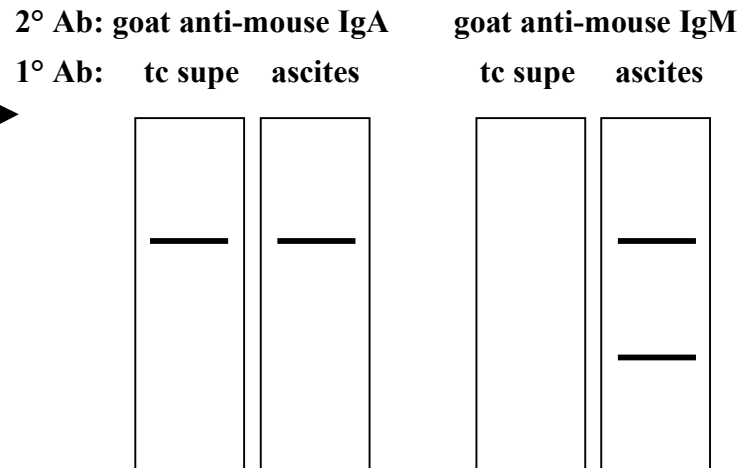
Question 2 cont.

You are growing the hybridoma cells that are producing your antibody in tissue culture and are frustrated by the low concentration of antibody in the tissue culture supernatant. You read about a technique that should allow you to get a higher antibody concentration. In this technique, hybridoma cells are injected into a mouse, where they grow as an "ascites tumor." The "ascites fluid" withdrawn from the mouse usually has a very high concentration of antibody (1000-10,000-fold higher than in a hybridoma tissue culture supernatant).

You create an ascites fluid containing your antibody and decide to test it using an immunoblot assay. You run purified hantavirus samples in two lanes on an SDS-PAGE gel, transfer the resolved proteins to a membrane, cut the gel into two strips, block the strips, and incubate one strip with undiluted tissue culture supernatant (tc supe) and one with a 1/1000 dilution of ascites fluid (ascites). After the washing the strips, you put the strips in secondary antibody solution, and go home for the evening.

When you get home, you realize that your strips are incubating with an enzyme-linked goat anti-mouse IgM antibody rather than an enzyme-linked goat anti-mouse IgA antibody! You return to the lab and repeat the experiment, this time using the proper secondary antibody.

The next morning you decide to finish all four blots. After washing the blots and exposing them to substrate, you find the surprising and disturbing results seen here. → You show your results to your advisor, who suggests that the mouse facility where the ascites fluid was produced should be shut down temporarily until the mouse colony can be cleared of infection.



d. (12 pts) Which results are surprising? Why did these results cause your advisor to suggest that the mouse colony be shut down? Explain what these results suggest had occurred in the mouse colony and why these results lead to that conclusion.

It is surprising that hantavirus proteins are recognized by antibodies in the ascites fluid when enzyme-linked anti-IgM was used as the secondary antibody. Since the mAb produced by the hybridoma is known to be an IgA antibody, the B cell that was fused to the myeloma to create the mAb must have already undergone class switching to go from making IgM to IgA (possibly switching to IgG before to IgA). Since the exons encoding the IgM constant region are excised during class switching, the hybridoma injected into the mouse should not be able to switch back to producing IgM, but should be restricted to making IgA. Therefore, the anti-hantavirus IgM antibodies in the ascites fluid suggest that the mouse used to make the ascites fluid was exposed to hantavirus and therefore had new B cells activated that were making anti-hantavirus IgM (a polyclonal response in which antibodies recognize at least two hantavirus proteins). This result suggests that the mouse colony was infected with hantavirus and therefore the mice in the colony are NOT good candidates for making ascites fluid for a specific monoclonal antibody.

3. (28 pts total) *Mycobacterium bovis bacillus Calmette Guérin* (*M. bovis* BCG) is a bacterium related to *Mycobacterium tuberculosis* that is used as a vaccine for tuberculosis. Both *M. bovis* BCG and *M. tuberculosis* infect macrophages. In experiments to understand what types of immune responses help the body defend against mycobacteria, researchers infected different mouse strains with *M. bovis* BCG.

They looked at two groups of mice: Group X contained mice homozygous for a deletion of a MHC class II β -chain gene and Group Y contained mice heterozygous for this deletion.

While the Group X mice succumbed relatively quickly to infection, the Group Y mice could control infection, although there was still some growth of the mycobacteria in these mice. Spleens were removed from each mouse, mycobacterial antigen was added, and cytokine production was tested. In this assay, the spleens from Group X mice showed no cytokine production, whereas spleens from Group Y mice showed cytokine production.

a. (8 pts) Given these results, which cells of the immune system do you think are important for controlling mycobacterial infection and what leads you to this conclusion? Make sure to explain what is happening in the *in vitro* assay.

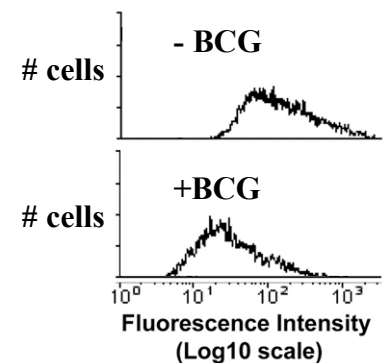
Helper T cells are likely to be critical for fighting mycobacterial infection. Helper T cells recognize antigens presented on class II MHC molecules (MHCII) and respond by producing cytokines to activate other cells of the immune system (i.e. macrophages) to help with infection. Group X mice, which lack MHCII, succumb to infection quickly and their spleens do not have lymphocytes that produce cytokines in response to mycobacterial antigen, which is presented on MHC II by professional antigen-presenting cells (APC) in the spleen, like dendritic cells. The group Y mouse spleens, however, have activated mycobacteria-specific helper T cells, which produce cytokines upon interaction with APCs with MHC II presenting mycobacterial antigens in the assay.

Other researchers were interested in understanding why mycobacteria are successful even in immunocompetent hosts (as suggested by the low level of infection in Group Y mice above). This group of researchers decided to perform *in vitro* experiments with human cells.

Using a monocyte-derived cell line called THP-1 (monocyte=macrophage precursor), in their first experiment, they either infected or did not infect THP-1 cells with *M. bovis* BCG, fixed the cells, stained with an anti-HLA-DR (MHCII) antibody followed by a fluorescein-conjugated secondary antibody and counted fluorescent cells by flow cytometry.

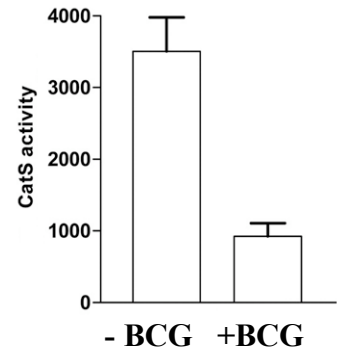
b. (8 pts) What do these results suggest is happening in infected cells? Explain your reasoning.

The flow cytometry results show that infected cells (lower panel) have less MHC II on their surface than uninfected cells (top panel). In this assay, the primary antibody binds to MHC II on the cell surface; therefore the fluorescence intensity indicates levels of surface MHC II. The peak in fluorescence intensity is farther right in the uninfected cells, indicating that more cells have higher fluorescence (and therefore more MHCII) than seen for infected cells.



Question 3 cont.

In their second experiment, the researchers lysed uninfected THP-1 cells and *M. bovis* BCG-infected THP-1 cells and tested the lysates for cathepsin S activity. Cathepsin S (Cat S) is a protease found in some cytoplasmic vesicles that are part of the endocytic pathway. Their results are shown in this figure.



c. (12 pts) Describe a possible mechanism for mycobacteria to evade the immune system suggested by these results and the results in part (b) above. Make sure to describe how the cellular effects of infection shown in these experiments would benefit the mycobacterium.

Mycobacteria may evade the immune system by decreasing cathepsin S activity (either by lowering expression of Cat S or by altering its activity directly) in the endocytic pathway. Cat S may be a protease responsible for digesting either ingested mycobacterial antigen or cleaving the invariant chain that is important for proper loading of peptides onto MHC II molecules. If the invariant chain cannot be cleaved to form the CLIP peptide, it is likely that ingested antigens cannot be exchanged for exogenous antigens and the MHC cannot be transported to the cell's surface. Alternatively, the presence of fewer exogenous peptides may inhibit MHCII presentation on the cell surface. Therefore, by inhibiting this protease, the mycobacterium can lower the recognition of its antigens by helper T cells and therefore the anti-bacterial effects of the cytokines produced by mycobacteria-specific helper T cells.

4. (20 pts total) Frequently children have similar allergies to their parents. You wish to explore this phenomenon, so you design an experiment to test total IgE levels and levels of IgE specific for dust mite allergens within families whose children are at Longfellow School.

a. (8 pts) Describe how you would test people for dust-mite specific IgE.

I would use an indirect ELISA: I would coat plates with dust mite antigens (perhaps ground-up dust mites), incubate dilutions of people's sera in the wells, wash the wells, add an enzyme-linked goat anti-human IgE antibody, wash and then add substrate. The presence of yellow color (for a colorimetric assay) would indicate dust-mite-specific IgE antibody. It would be important to have wells with a dilution series of known human dust-mite-specific IgE for a standard curve to approximate IgE levels in the families (as well as a non-specific IgE primary antibody as a negative control).

The results of your study show that, although there is not a significant correlation between total IgE levels in parents and their own children (some parents with low total IgE have children with high total IgE and vice versa), dust-mite specific IgE are strongly correlated between parents and their children (i.e. if parents have high dust-mite IgE, their children have high dust-mite IgE and if parents have low dust-mite IgE, their children have low dust-mite IgE).

b. (12 pts) Based on what we've discussed so far this semester, explain two reasons why parents and children might have similar allergies as reflected by these specific IgE levels.

Parents and children might have similar allergies because:

a) Children inherit their immunoglobulin genes from their parents. There might be certain V, D and J region allotypes that are more likely (but not certain) to create variable regions in immunoglobulins that can bind to dust mite allergens.

b) Children inherit their MHC genes from their parents. Since MHC molecules vary in their ability to present different peptides, the children and their parents may share MHC haplotypes that either bind strongly or weakly to dust-mite allergen peptides, leading to

strong or weak helper T cell responses (respectively) that would influence B cell activity (or potentially class switching to IgE).

c) If the children and parents live in the same dwelling, they should be exposed to the same allergens, including dust mites. Therefore children and parents living in a house with dust mites may all have activated B cells and T cells that recognize dust mite antigens whereas those living in houses with fewer dust mites may not have had sufficient contact with antigens to activate their adaptive immune responses.