

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. Note that later questions are worth 30 pts each.
- If you feel you need to **make an assumption to answer a question, state the assumption explicitly.**

1. (16 pts total) The cloning of the CCR5 gene in the mid-1990's made a big splash, particularly given its connections to viral infection.

a. (5 pts) What is CCR5 and what is the CCR5 Δ 32 allele? In the study we discussed, what correlations were found between the CCR5 Δ 32 allele and viral infection?

CCR5 is a chemokine receptor found on macrophages and monocytes; the CCR5 Δ 32 allele encodes a form of CCR5 with a C-terminal deletion. Researchers found that this allele was found more frequently in seronegative people than in people whose serum tests positive for HIV infection, suggesting that this shorter form of the protein might protect people from HIV infection.

A group of researchers working on the CCR5 Δ 32 allele performed the following experiment: they isolated CD4⁺ cells from peripheral human blood and introduced a gene to express CCR5 Δ 32, or a gene to express wild-type CCR5, or DNA without a CCR gene (control). They then performed flow cytometry with anti-CCR5, anti-CXCR4 or anti-CD4 antibodies and calculated the mean fluorescence intensity (MFI) for each set of cells. Their results are shown in Fig. 1.

b. (2 pts) What cell type(s) is/are being used in this experiment?

macrophages/monocytes and helper T cells

c. (3 pts) What is the effect of CCR5 Δ 32 expression on these cells?

Expression of the deleted form of CCR5 protein results in lower levels of surface CCR5 and CXCR4 in these CD4⁺ cells.

d. (6 pts) What do these results suggest about the mechanism whereby the CCR5 Δ 32 allele affects viral infection?

These results suggest that expression of this allele decreases the levels of CCR5 (on macrophages/monocytes) and CXCR4 (on helper T cells), which act as coreceptors for HIV. Therefore, CCR5 Δ 32 expression would reduce HIV infection dominantly by actively decreasing cell entry in macrophages/monocytes and potentially in helper T cells (if they express the deleted form of CCR5).

3. (12 pts total) Mouse ear thickness is sometimes used as a measure for immunological response to antigenic challenge. An increase in the thickness of the mouse ear is correlated to a T-cell response to an antigen. Deficiency of T cell response is correlated to little to no change in ear thickness. You have bred two mouse lines, the first with a defect in the gene for CD45RA, and the second is defective for CD45RO. CD45RA is found mainly on naïve T cells while CD45RO is found mainly on memory T cells.

a. (4 pts) What is the role of CD45 in T-cell activation?

CD45 acts as a phosphatase to activate CD4/8-associated Lck by dephosphorylation. The activated Lck will phosphorylate ITAM motifs in CD3 zeta chains. These phosphorylated motifs will serve as a docking site for Zap70, which will activate downstream signaling for T-cell activation (e.g. upregulation of IL-2 and IL-2 receptors).

b. (8 pts) What will be the difference in ear thickness as a measure of T-cell response between the CD45RA and CD45RO knockout mice upon primary exposure to an antigen? Upon secondary exposure?

CD45RA is found on the surface of naïve T cells and activates signaling pathways leading to T cell activation and formation of memory cells (a). Therefore CD45RA knockout (ko) mice will not show any increase in ear thickness, reflecting a lack of T-cell activation on either primary or secondary exposure to antigen. Since CD45RO ko mice still express the RA isoform, naïve T cells will be able to be activated upon primary exposure to antigen, leading to an increase in ear thickness. The lack of the RO isoform on memory T cells, however, will lead to a change in ear thickness upon secondary exposure that looks like that upon primary exposure (i.e. not faster or more extreme as one might expect from a new memory response).

Note: the book mentions effector T cells as having the RO isoform, but it is not clear whether its activity is required for effector function.

4. (30 pts total) Two weeks ago, a team of Japanese researchers published a paper addressing the virulence of Group A streptococci bacteria. In their abstract they note: "Group A streptococcus (GAS) causes [a] variety of diseases ranging from [strep throat] to life-threatening severe invasive diseases, including necrotizing fasciitis."

They started their work by studying a set of GAS strains isolated from patients with invasive and non-invasive infections. They incubated each strain with polymorphonuclear leukocytes (PMNs) in culture for two hours and then determined bacterial survival, as shown in Fig. 3A.

a. (1 pt) What more common name do we use for PMNs? **neutrophils**

b. (4 pts) Briefly describe one mechanism that PMNs could use to kill streptococci in this *in vitro* assay; include important molecules.

Molecules on the surface of the streptococci could bind to a Toll-like receptor or another pattern-recognition receptor on PMNs. The PMNs could then "swallow" the bacterium by endocytosis, then fusing the endocytic vesicle with a phagolysosome, causing digestion of the bacterium (e.g. via reactive oxygen species and proteases)

Note: perforin/granzyme won't work as they don't target prokaryotic cells.

Fig. 3B shows results of another assay in which they incubated PMNs with representative non-invasive (NI) and invasive (I) GAS strains and tested PMN survival by staining with propidium iodide, a membrane-impermeable fluorescent molecule that binds DNA. In addition they tested the invasive strain following deletion of the SLO gene (I-slo Δ). They performed these assays in the presence of an antibody that binds to the bacterial SLO protein (black bars) or in the presence of non-specific control Ig (white bars).

c. (8 pts) What main conclusions about the interaction of PMNs with the different GAS strains would you draw from the data in Fig. 3A&B? Briefly explain your thought process.

While PMNs are capable of killing non-invasive GAS strains (a), the SLO protein produced by bacteria allows the bacteria to kill PMNs (b) and therefore prevent PMNs from digesting them (a). This specificity is shown by the ability of anti-SLO antibody or deletion of SLO from the bacteria to prevent PMN killing.

Your friend thinks that the role of the bacterial SLO gene product involves binding to Fas on the PMN surface, but you disagree.

d. (5 pts) Briefly explain to your friend why you don't think that Fas binding is involved in SLO function.

Fas ligation causes death of target cells via apoptosis in which the contents of the cells are packaged in membrane vesicles prior to digestion by phagocytic cells. Since death of PMNs was detected by the ability of propidium iodide to enter the cell, which requires the membrane to be compromised, the PMNs must be dying by a mechanism other than apoptosis.

In another experiment, the authors incubated representative invasive and non-invasive streptococci with IL-8, and then measured IL-8 after a 1-hour incubation. This time they also tested the invasive strain with a different gene, ScpC, deleted. Their results are shown in Fig. 3C.

e. (12 pts) What do these results suggest about the role of the ScpC gene in the invasive strain? Explain how expression of this gene would influence the severity of streptococcal infection *in vivo*.

If the invasive strain can produce ScpC protein, IL-8 is degraded by the invasive strain. IL-8 is a chemokine that binds to receptors on neutrophils and signals for structural changes in an integrin molecule, allowing the integrin to bind to an ICAM (Ig-like cellular adhesion molecules) on endothelial cells and to cause extravasation of the PMN toward a site of infection. If IL-8 cannot be received by PMNs, they will not be directed toward sites of infection, allowing the GAS to thrive unimpeded, causing a more invasive and severe infection.

5. (30 pts total) You join a lab that has just discovered two new B-lymphotrophic viruses.

Interestingly, both viruses encode proteins with structural similarities to Syk. Your advisor asks you to study the effect of these viruses on B cells and gives you the following reagents: samples of each virus, mice that have been immunized with hen egg-white lysozyme (HEL) and purified HEL.

To begin your experiment, you draw blood from the mice and collect B cells from the blood. Next, you divide the B cells into three tubes and add virus 1 to tube 1, virus 2 to tube 2 and no virus to the third tube. After 24 hours you divide each of the samples into two tubes and add HEL to one tube and not to the other tube. You also add anti-CD40 antibody to all 6 tubes.

After another 24 hours, you count the cells prior to collecting and lysing them. You separate cellular proteins by SDS-PAGE and perform Western blots with antibodies that recognize total Syk (Syk), phosphorylated Syk (P-Syk), total I γ β (I γ β), and phosphorylated I γ β (P-I γ β). Interestingly, although

the western results for the two viruses look identical (Fig. 4A), the cell counts are different between the two viruses (Fig. 4B).

a. (6 pts) Are the western results for uninfected cells what you would expect? Explain your expected results in uninfected cells and indicate any differences from the data in Fig. 4A, if there are any.

Yes. Upon binding of HEL, the B-cell receptors (BCR) will become cross-linked, activating a Src-family kinase, which will phosphorylate $Ig\alpha$ and $Ig\beta$. When Syk binds to phosphorylated $Ig\beta$, it will also become phosphorylated and signal for B-cell activation. The appearance of phosphorylated $Ig\beta$ and Syk upon addition of HEL to uninfected cells is therefore expected. B cells normally express Syk and $Ig\beta$, therefore the presence of these proteins in all blots is expected.

Q3b. (6 pts) Are the cell count results for uninfected cells what you would expect? Explain your expected results in uninfected cells and indicate any differences from the data in Fig. 4B, if there are any. Include why anti-CD40 antibody was added to all tubes.

Similarly to part a, signaling via the BCR and Syk upon HEL addition will lead to B cell proliferation, therefore the increase in B cell number after addition of HEL makes sense. The anti-CD40 antibody is used as an agonist to mimic the interaction between CD40 on the B cell and CD40L on a helper T cell; this signal, in combination BCR cross-linking, increases B cell proliferation.

c. (2 pts) What is the major difference in the western blot between virus-infected and uninfected cells?

Syk is not phosphorylated in infected cells, even in the presence of HEL!

d. (8 pts) How do the counts for cells infected with virus 1 differ from that for uninfected cells? Taking into account experiments in Fig. 4A&B, describe a mechanism involving a viral protein that could account for this difference. How might this effect benefit virus 1?

Infection of B cells with virus 1 results in large amounts of B cell proliferation, regardless of the presence of HEL. Since a protein from virus 1 is similar to Syk, it might mimic a constitutively active form of Syk, causing downstream signaling for B cell proliferation even in the absence of HEL (4B). It's binding to phosphorylated $Ig\beta$ could block the ability of mouse Syk to bind and be phosphorylated (4A). B cell proliferation offers the virus more host cells in which to replicate, increasing the number of viral genomes in the patient.

e. (8 pts) How do the counts for cells infected with virus 2 differ from that for uninfected cells? Taking into account experiments in Fig. 4A&B, describe a mechanism involving a viral protein that could account for this difference. How might this effect benefit virus 2?

Infection of B cells with virus 2 blocks B cell proliferation, even in the presence of HEL. The Syk-like protein in virus 2 may bind to phosphorylated $Ig\beta$ and prevent mouse Syk binding, leading to a block in B-cell activation and proliferation. Blocking B cell activation could help the virus escape from a humoral response that creates virus-2-specific antibodies.