

## Biology 257 Immunology Fall 2005 Midterm #2 Answer Key

**Question 1 (14 pts total)** An extremely rare form of cheese has been discovered in Lappland, but before putting it on the market for cheese-lovers worldwide to enjoy, scientists have decided to test its effects on mice. The results of their tests show that the cheese causes death in all laboratory mice and wild mice except wild mice from Lappland.

Vaccines are then created with an attenuated strain of a microorganism isolated from the cheese and are administered to a small group of American rodents. When vaccinated and unvaccinated mice are offered the irresistible cheese, the vaccinated mice are sick for three days post cheese consumption but ultimately survive, while unvaccinated mice die within 12 hours after cheese consumption.

Spleens of both the vaccinated and unvaccinated mice are removed and high levels of IgG1 are present in the vaccinated mice, while high IgM levels are found in the unvaccinated mice.

**a) (7 pts) Explain why the predominant antibody isotypes differ between the vaccinated and unvaccinated mice. Make sure to include the cellular mechanisms that underlie this difference.**

The vaccinated mice have already had a primary immune response to the microbe, with both microbe-specific B cells and helper T cells being activated. High levels of IgG1 suggest that the B cells have undergone class switching. In contrast, the unvaccinated mice are getting their first exposure to the microbe and the naive B cells when activated will make IgM antibodies since they will not yet have undergone class switching.

**b) (7 pts) Explain two possible molecular/cellular mechanisms whereby vaccination may protect the American mice. Include whether you think the suspect microorganism is an intracellular or extracellular pathogen and why you think it is intra- or extracellular.**

The vaccine has promoted a humoral (antibody) response, suggesting the microbe is extracellular, since antibodies cannot bind to antigens inside the cell. The antibodies could be binding to a toxin produced by the microbe and preventing the toxic effect. Alternatively, the antibodies could be binding to the microbe and targeting it to Fc receptors on a phagocyte, which will then engulf and digest the microbe. In addition, binding of the antibodies to the microbe could activate complement proteins to form a membrane attack complex, leading to lysis of the microbe.

**Question 2 (14 pts total)** Having designed an effective vaccination technique for the cheese micro-organism described in Question 1, you decide first to test the cytokine response in vaccinated mice.

**a) (4 pts) Name 3 cytokines that you might expect to find upon exposure of mice to the cheese micro-organism. Explain why you would expect to find these cytokines.**

Since the vaccine elicits an IgG1 antibody response,  $T_H2$  type cytokines should be present, likely IL-4, IL-5 and IL-10.

**Q2 cont.** Next you decide to test whether vaccination works on mouse strains that lack proteins that you know are important for immune responses. Each of the four mouse strains you test has a different gene deleted (CD4, CD8, CD28 or CD40), but they are otherwise identical. First, you vaccinate mice with the attenuated microbe, then you expose them to the virulent microbe.

**b) (10 pts) For each mouse strain, predict whether it will live or die following vaccination and subsequent challenge. Briefly explain your prediction on a cellular/molecular level, including which cell type expresses the CD protein.**

**CD4-/- DIE.** Binding of **CD4** on the **surface** of **helper T cells** to **MHC class II molecules** on **antigen-presenting cells** is crucial for **activation** of these **T cells** and therefore their **secretion** of **cytokines** for **B-cell activation**. Therefore, without CD4 the mouse won't be able to mount an antibody response.

**CD8-/- LIVE.** **CD8** is found on the **surface** of **cytotoxic T lymphocytes (CTLs)** and is important for their **activation**. Active CTLs are not required for B-cell activation, therefore, without CD8 the mouse will still be able to mount an antibody response.

**CD28-/- DIE.** Binding of **CD28** on the **surface** of **helper T cells** to **B7** on **antigen-presenting cells** is crucial for **activation** of these **T cells** and therefore their **secretion** of **cytokines** for **B-cell activation**. Therefore, without CD28 the mouse won't be able to mount an antibody response.

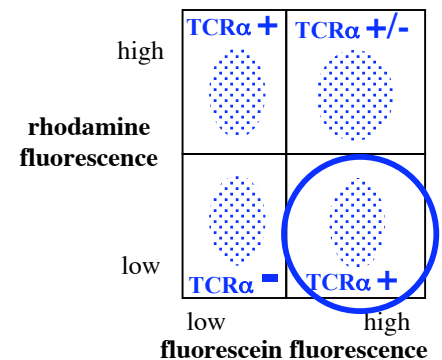
**CD40-/- DIE.** Binding of **CD40** on the **surface** of **B cells** to **CD40 ligand (CD40L)** on **helper T cells** is crucial for **B-cell activation**. Therefore, without CD40 the mouse won't be able to mount an antibody response.

**Question 3 (14 pts total)** You are inspired by the classic experiments from the Zinkernagel lab, so you decide to try to replicate their results in your own lab. You have two inbred mouse strains, AKR (H-2<sup>k/k</sup>) and BALB/c (H-2<sup>d/d</sup>) and LCM virus stocks. First you mate an AKR and a BALB/c mouse and get a litter of mice you call F1 mice. Then you do the following experiment:

1. Remove the thymus from an F1 mouse (#1) and irradiate the bone marrow to kill all cells.
2. Remove the thymus from an AKR mouse and transplant it into mouse #1.
3. Remove bone marrow from another F1 mouse and inject it into mouse #1.
4. Infect mouse #1 with LCM virus.

**a) (2 pts) If you removed the thymus after step 4, stained the thymocytes with rhodamine-conjugated anti-CD4 and fluorescein-conjugated anti-CD8, and analyzed the cells by flow cytometry, draw what you would expect to see on this graph.**

**b) (4 pts) For each quadrant where you have drawn cells, label whether you expect these cells to express TCR $\alpha$ . Use + for all cells expressing TCR $\alpha$ , - for no cells expressing TCR $\alpha$  and +/- for some cells expressing and some cells not expressing TCR $\alpha$ . Assume there are no  $\gamma/\delta$  T cells in the population.**



**Q3, cont.** For your experiment, you decide NOT to remove the thymus, but rather to wait a week and then remove the spleen and to test the splenocytes in a chromium-release assay. After exposing cultured cells to  $^{51}\text{Cr}$ , you either infect them with LCMV or not and then expose them to splenocytes from mouse #1. You find the following results:

Source of cultured cells	Chromium-release into medium	
	<u>LCMV-infected</u>	<u>uninfected</u>
AKR	+	-
BALB/c	-	-

c) (2 pts) On your flow cytometry figure, circle the thymocytes that would be detected in this assay (once they get to the spleen).

d) (6 pts) Briefly explain the results above, including what they reveal about the role of the thymus in immune system development.

The **cytotoxic T lymphocytes** can only kill **infected H-2<sup>k</sup> cells** because of **positive selection** in the thymus whereby only T cells that recognize **MHC** molecules on the surface of cells in the **thymus** can survive. Since the **thymus** was from an **AKR mouse**, only **H-2<sup>k</sup>-recognizing T cells** would **survive positive selection**. **Uninfected cells are not killed** because they do **not present viral antigens**.

**Question 4 (58 pts total)** Viruses have evolved numerous mechanisms to evade the host immune system and this question addresses evasion mechanisms used by three different viruses.

**A. (20 pts)** Cytomegalovirus (CMV) has a 48 kDa glycoprotein that binds to  $\beta 2$ -microglobulin-containing MHC molecules in the endoplasmic reticulum and directs them to the lysosome to be degraded.

i) (4 pts) Which class of MHC contains  $\beta 2$ -microglobulin and why is it advantageous for the virus to send it to the lysosome?

**Class I MHC** molecules contain  **$\beta 2$ -microglobulin**. Since MHC class I molecules present **endogenous antigens** to **cytotoxic T cells**, by preventing MHC class I presentation, CMV can **prevent presentation of peptides** from its own **proteins** and therefore **avoid CTLs recognizing and killing the host cell in which it is replicating**.

ii) (3 pts) What is the main potential disadvantage for the virus in sending this class of MHC molecule to the lysosome?

**Natural killer** cells can **recognize cells without MHC class I molecules** on their surface and can **signal such MHC class I-deficient cells** to undergo **apoptosis**.

**Q4A cont.** Cytomegalovirus makes another protein, UL18, which resembles an MHC molecule but does not present peptides to T cells. People proposed that this protein acts as a "decoy" to get around the disadvantage described in part ii above. A budding immunologist decides to test this hypothesis by infecting mice with either wildtype CMV (WT) or CMV lacking UL18 ( $\Delta$ UL18) and testing for viral yield.

**iii) (8 pts) Assuming that the "decoy" hypothesis is correct, would you expect high or low viral yield after infecting mice with the following viruses. Briefly explain each answer.**

**WT virus: HIGH.** UL18 can bind to a cell surface molecule on NK cells and induce the inhibitory signal to prevent NK cell activation.

**$\Delta$ UL18 virus: LOW.** Since infected cells cannot display UL18 on the surface to bind to the NK cell and inhibit NK cell activation, the binding of the NK cell activation receptor (AR) to a ligand on the infected cell will result in activation of the NK cell, which will then kill the infected cell.

**iv) (5 pts) What would you expect the viral yield to be if you infected lpr/lpr mutant mice (which express little or no Fas) with the  $\Delta$ UL18 virus? Briefly explain why the result is the same as or different from the  $\Delta$ UL18 result in part iii.**

Although NK cells would not be able to signal apoptosis in infected cells by using FasL to bind to Fas on the infected cells, it could still induce apoptosis through the use of perforin and granzyme.

**B. (8 pts)** Kaposi's sarcoma-associated herpesvirus (KSHV) also avoids the immune system by directing cell-surface molecules to be degraded, including not only MHC complexes, but also ICAM-1 and B7.2.

**i) (4 pts) Describe why it would be advantageous to the virus to target ICAM-1 for degradation.**

Binding of ICAM-1 on an infected target cell to LFA-1, an integrin, on CTLs is crucial for forming close contacts that allow the CTL to signal for apoptosis of the infected cell. Alternatively, ICAM-1 might be involved in neutrophil or lymphocyte extravasation and its absence could help limit these cells' access to a site of infection.

**ii) (4 pts) Describe why it would be advantageous to the virus to target B7.2 for degradation.**

The binding of B7 molecules on antigen-presenting cells to CD28 molecules on T cells is a crucial co-stimulatory signal for T-cell activation. By lowering B7 levels, the virus can inhibit T cell activation (potentially either helper T cell or CTL, depending on the specific cell types that express B7.2).

**C. (30 pts)** Poxviruses encode a number of proteins that help with immune system avoidance. Some of these proteins are secreted from poxvirus-infected cells and can bind to cytokines. Alcami and coworkers decided to study a vaccinia virus protein called B18R, which not only binds to interferon  $\alpha$  (IFN $\alpha$ ), but also can bind to the surface of cells after it is secreted. This figure shows the results of the following experiment:

1. Incubate cultured cells in medium lacking (-) or containing (+) purified B18R protein.
2. Remove medium and wash cells.
3. Add fresh medium with (+) or without (-) IFN $\alpha$ .
4. Prepare whole-cell extracts and purify JAK1 protein.
5. Perform a western blot using the purified JAK1 protein and an antibody that binds to phosphotyrosine residues.

i) (3 pts) What is happening during each of the first three steps of the experiment?

1. B18R is binding to the surface of the cells.
2. Any unbound proteins are removed from the cells.
3. IFN $\alpha$  is binding to B18R and/or the cellular IFN $\alpha$ / $\beta$  receptor on the surface of the cells.

ii) (3 pts) Why did they probe the western blot with anti-phosphotyrosine antibody?

When a cytokine binds to its normal cell-surface receptor, the JAK kinase is activated by phosphorylation to initiate intracellular signaling, so this experiment allows them to test for activation of the IFN $\alpha$ / $\beta$  signaling pathway.

iii) (4 pts) Explain why there is a band in lane 2 but not in lane 1.

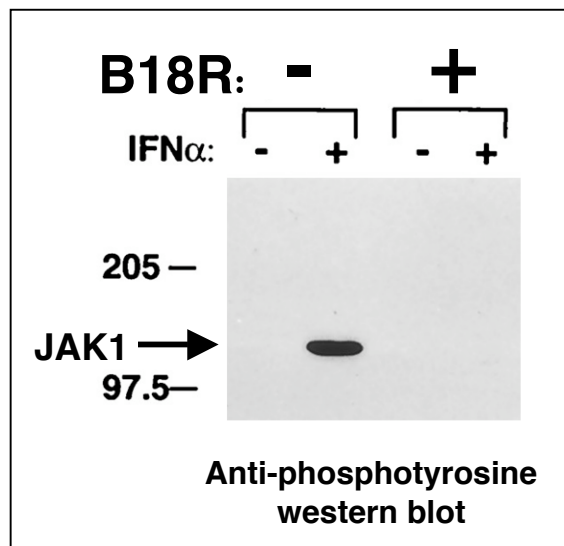
Only in the presence of cytokine should JAK1 and the signaling pathway be activated. Therefore, JAK1 should only be phosphorylated when there is IFN $\alpha$  present.

iv) (4 pts) Explain why there is no band in lane 4 even though there is a band in lane 2.

The B18R protein can bind both to IFN $\alpha$  and to the surface of the cell, but as a soluble protein, it does not have a transmembrane domain to signal into the cell. Therefore, it competes for IFN $\alpha$  with the normal IFN $\alpha$ / $\beta$  receptor and prevents signaling and JAK1 phosphorylation.

v) (5 pts) Based on these results, describe a hypothesis for how B18R might help vaccinia virus evade the immune system.

By binding to IFN $\alpha$ , B18R can prevent this cytokine from binding to its normal receptors and signaling for the shut-off of protein synthesis in the infected cell and nearby cells, allowing the virus to continue to use the host cell machinery to replicate.

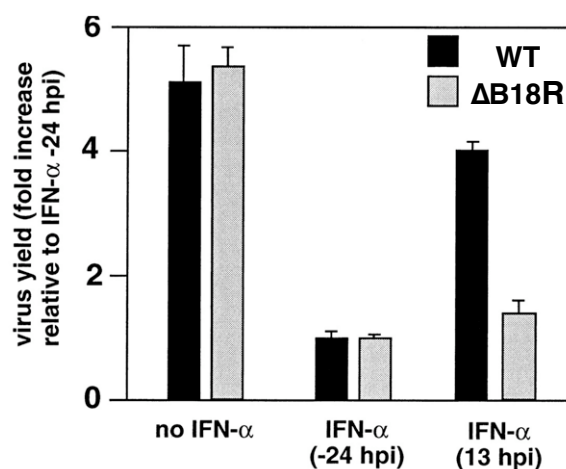


Q4C cont.

vi) (3 pts) What other protein might you test by western blotting with the anti-phosphotyrosine antibody and how would that experiment support your hypothesis?

Either the cellular IFN $\alpha$ / $\beta$  receptor or the STAT transcription factor, both of which are phosphorylated by JAK1 when this signaling pathway is activated.

In another experiment, they tested directly whether B18R affects viral replication by infecting cells with the same amount of WT virus or virus with the B18R gene deleted ( $\Delta$ B18R). They infected cells in the absence of IFN $\alpha$ , or with IFN $\alpha$  present either 24 h before infection (-24 hpi) or 13 hours after infection (13 hpi). They then tested the amount of virus produced by the cells 48 h after the cells had been exposed to virus. Here are their results:



vii) (4 pts) Explain why the two viruses show the same yield when IFN $\alpha$  is added 24 h before infection.

Since the antiviral state was induced before infection with the virus, even if the virus was able to enter the cell, its ability to use the host cell protein synthesis machinery was hindered from the beginning of infection, whether or not the B18R gene was in the viral genome.

viii) (4 pts) Explain the differences in viral yield when IFN $\alpha$  is added 13 h after infection.

The wildtype virus had 13 h to produce B18R, secrete it from the cell and have B18R on the cell surface compete with the normal IFN $\alpha$ / $\beta$  receptor for IFN $\alpha$  binding, facilitating viral replication. Without B18R, the mutant virus could not prevent IFN $\alpha$  from binding to the IFN $\alpha$ / $\beta$  receptors, signaling and shutting down both viral and host protein synthesis.

**Extra credit (1 pt):** Which amino acid has recently been touted as a possible treatment for autoimmune disorders? (feel free to guess, but make sure that your guess is an amino acid!)

**Tryptophan** (proposed activity via a breakdown product)