

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.

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.

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.

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^{-/-}

CD8^{-/-}

CD28^{-/-}

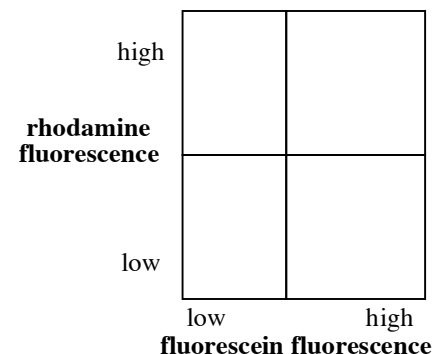
CD40^{-/-}

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^{k/k}) and BALB/c (H-2^{d/d}) 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 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 α . Use + for all cells expressing TCR α , - for no cells expressing TCR α and +/- for some cells expressing and some cells not expressing TCR α . Assume there are no γ/δ 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}Cr , you either infect them with LCMV or not and then expose them to splenocytes from mouse #1. You find the following results:

<u>Source of cultured cells</u>	<u>Chromium-release into medium</u>	
	<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.

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?

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

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 (Δ 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:

Δ UL18 virus:

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 Δ UL18 virus? Briefly explain why the result is the same as or different from the Δ UL18 result in part iii.

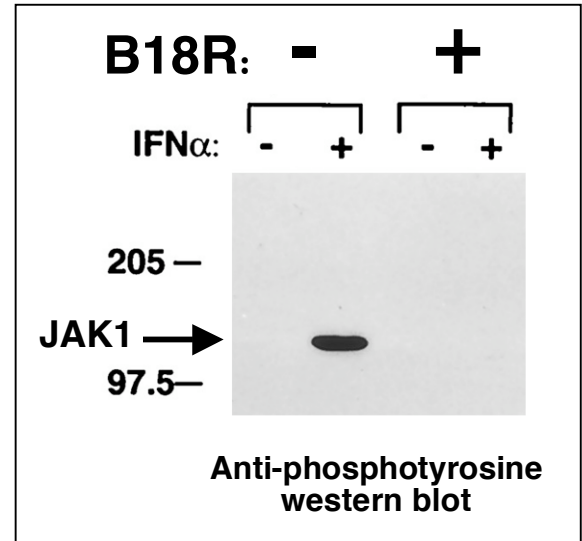
B. (8 pts) Kaposi's sarcoma-associated herpesvirus (KSHV) also avoids the immune system by directing cell-surface molecules to be degraded, including not only MHC complexes, but also ICAM-1 and B7.2.

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

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

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 α (IFN α), 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 α .
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.
- 2.
- 3.

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

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

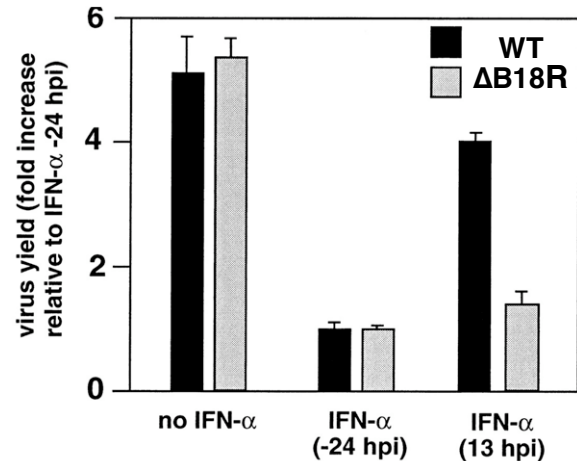
iv) (4 pts) Explain why there is no band in lane 4 even though there is a band in lane 2.

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

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?

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 (Δ B18R). They infected cells in the absence of IFN α , or with IFN α 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 α is added 24 h before infection.

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

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!)