HST175 Midterm Exam Answer Key
10.24.05

Good luck!

Name:_____

Question 1: _____

Question 2: _____

Question 3: _____

Question 4: _____

Question 5: _____

Question 6: _____

Question 7: _____

Question 8: _____

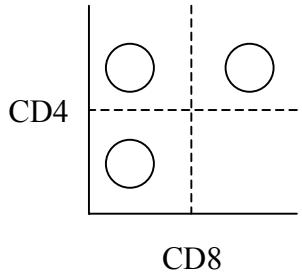
Total Score: _____

Name:_____

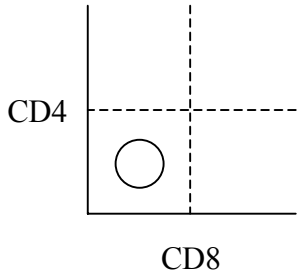
Question 1

Draw the CD4/CD8 FACS profile you would expect to observe for thymocytes from the following mice:

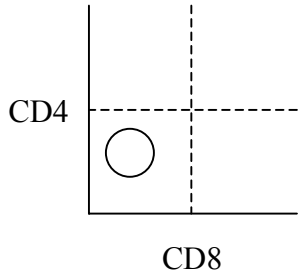
a. $B_2m^{-/-}$



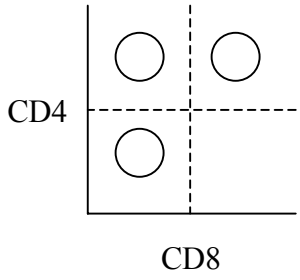
b. RAG $^{-/-}$



c. $pT\alpha^{-/-}$



d. $TAP^{-/-}$



Name:_____

Question 2:

a. Please draw out the steps of deletional VJ recombination in the κ light chain. *Please draw the RSSs and V and J segments in each diagram.*

DELETION

i. The locus before recombination

i. The two intermediates

Figure removed due to copyright reasons.

<needed to show hairpins on V and J>

ii. The final products

b. Please annotate your drawing above by labeling at which stage the following deficiencies would **HALT** VDJ recombination and what part of the structures in your diagrams should have interacted with these molecules, had they been present (please write a **short** explanation for each)

i. Artemis$^{-/-}$ Cannot cleave open hairpin
ii. RAG$^{-/-}$ Cannot make initial single stranded nick
iii. XRCC4$^{-/-}$ and Ligase IV$^{-/-}$ Cannot ligate blunt ends

Name:_____

Question 3:

a. Which molecules in the B cell receptor complex transduce cytosolic signals?
Igα, Igβ

b. What motif fosters this signaling process?

ITAM

c. A weak antigen attempts to bind to the BCR and activate a B cell, but cannot do it alone. With the help of the complement cascade, this antigen is able to activate the B cell using the B cell co-receptors.
    i. What is the molecule from the complement cascade that plays an important role?
    C3d
    ii. What is the molecule on the B cell that acts as a co-receptor?
    CD21

d. Capitalizing on recent media interest, and global concern, a small vaccine company seeks to design a vaccine to elicit an optimal humoral response to avian influenza (H5N1).
    i. They identify a novel glycoprotein on the viral envelope, and the carbohydrate side chain, which has a non-repeating structure is recognized by antibodies in immune individuals. They synthesize the carbohydrate sidechain separately. They propose to initiate phase I trials of a vaccine based entirely on this synthesized carbohydrate with a traditional adjuvant. What type of humoral response will be elicited and what might be the problem with this plan?
    Without a protein antigen, no T cell dependent B cell activation. Without a repeating epitope on the carbohydrate, no effective crosslinking of the BCR and no significant T cell indendent B cell activation.

    ii. What could they do to improve this vaccine (involving the antigen and not the adjuvant)?
    Use the entire glycoprotein or at least mutimerize the carbohydrate sidechain
    iii. Next they focus on a cell mediated response. They hope to elicit optimal APC presentation of viral proteins to CTLs. The avian flu virus (H5N1)is a negative ssRNA virus. What TLR would the viral naturally stimulate in mice and humans (may not be the same)?
    TLR8 in humans and TLR7 in mice

Name:_____

Question 4:

a. You've carefully studied LAD-1 and LAD-2. Unfortunately, your patient has a defect in lymphocyte homing but doesn't seem to have either one ….So, you examine the data:
   - Using a newly invented non-invasive system to monitor intravascular flow, you observe that the patient's cells do not roll on the HEV
   - When the patient's T cells are isolated and injected into a flow chamber (basically, fluid flows over a microscope slide coated with an adhesion molecule, to see how the cells interact with that molecule) coated with ICAM-1, they do not stick
   - A previous physician had diagnosed LAD I and had set up a bone marrow transplant. The patient was unfortunately not cured with a bone marrow transplant from an unaffected, completely HLA-matched twin

Propose a molecular defect in your patient (be specific!) that would explain this data and the cell type that has this defect

HEV defect in PNAd

b. You've probably heard about TYSABRI/Antegrin (Natalizumab), the "selective adhesion molecule" (SAM) that is an $\alpha 4$ integrin blocker that was touted as a therapy for multiple sclerosis by blocking the interaction between VLA4 ($\alpha 4\beta 1$) and its ligand. The promising drug was pulled from the market due to a few patients in the trial developing progressive multifocal leukoencephalopathy (PML).
   PML is a rare and often fatal progressive demyelinating disease due to re-activation of the endemic infection of the JC papovavirus. Reactivation tends to occur in a setting of immunodeficiency (like HIV).

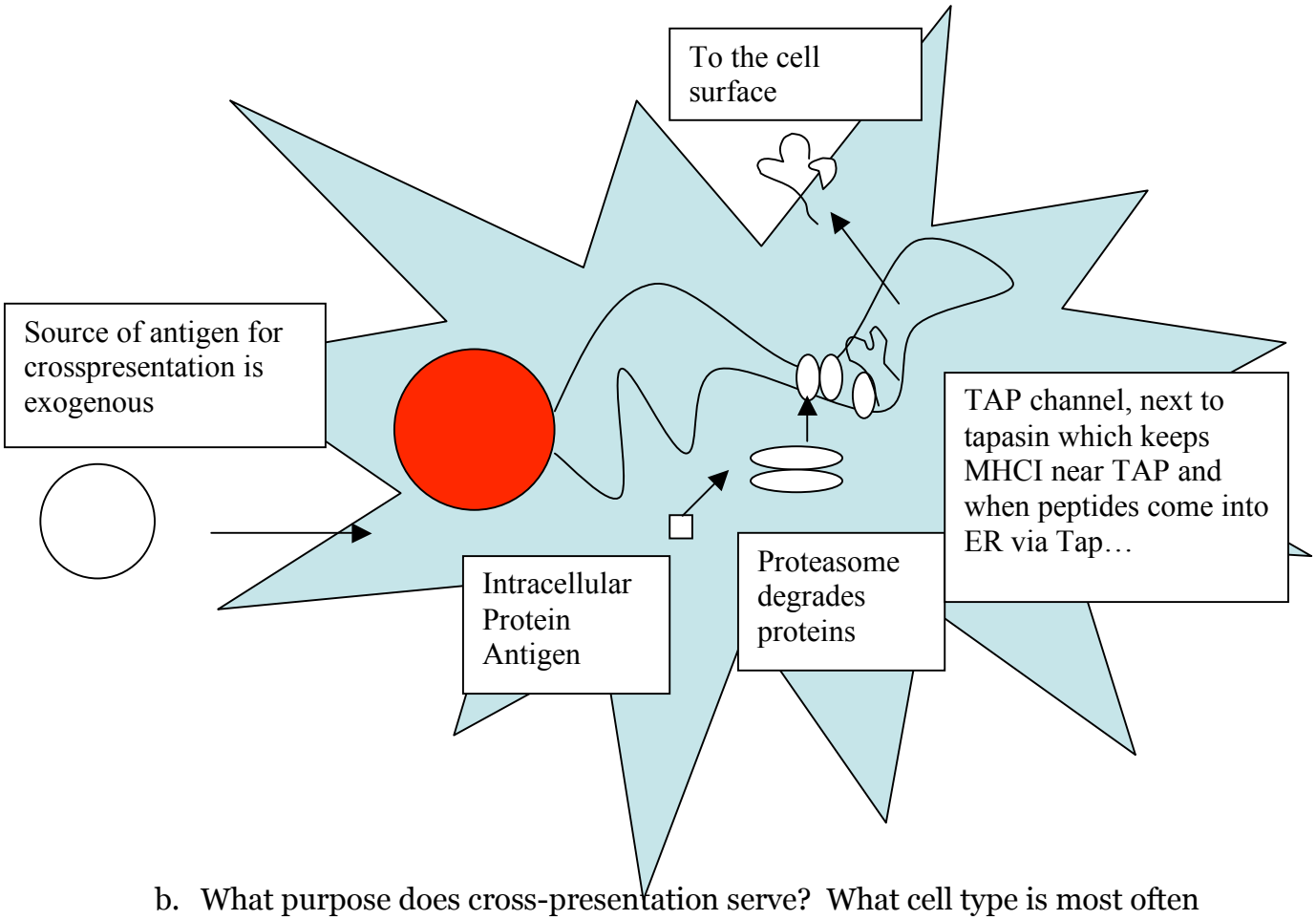
How could this drug have led to this outcome?

Without the ability to stick to the endothelium in peripheral, inflamed tissues (like virally infected CNS) effector and memory T cells can't home to those sites to contain chronic infections and those infections can take over.

Name:_____

Question 5:

a. Please **diagram** the normal process of MHC I antigen processing and presentation, including the source of the antigen, the mechanism by which they are transported, the location they are loaded into MHC I and where they are eventually presented (in the cell below…it's a cell, trust me).



b. What purpose does cross-presentation serve? What cell type is most often discussed as being a cross-presenter?

Some viral infections do not infect APCs—so, you need to convince an APC (usually a DC) to present exogenous antigen on MHC I (when usually it should be presented on MHC II) so that you can activate your CTLs and kill virally infected cells.

c. Please draw the source of peptides for cross-presentation on the figure of the cell above and describe that source below.

Name:_____

Question 6:

a. Different inbred strains have different susceptibilities to some pathogens. For example, while BALB/c mice are extremely susceptible to infection with the intracellular pathogen, Leishmania, C57Bl/6 mice are resistant to Leishmania infections, and are able to easily overcome the infection.

i. What type of T-helper response do you hypothesize the BALB/c mouse is inherently skewed towards?

Th2

ii. If you had a special attachment to a BALB/c mouse, Ralph, who unfortunately became infected with Leishmania, and you had access to recombinant cytokines, what cytokine therapies might you suggest?

IL-12, IFNγ

iii. If your good friend worked for a small biotech start-up, what cytokine inhibitors would you want to have developed to help poor Ralph?

IL-4, IL-5, IL-10

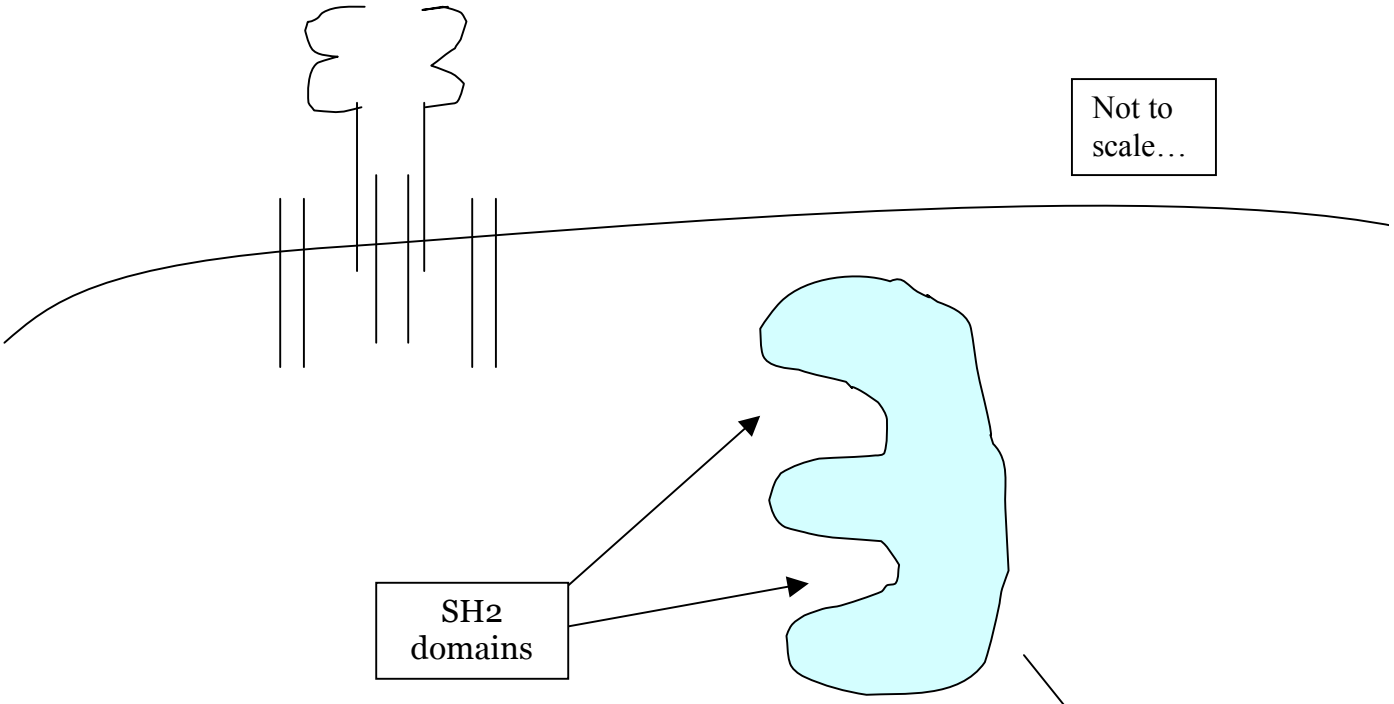
b. Please compare and contrast the major way that the immune system combats intracellular and extracellular **bacteria**, briefly (a bulleted list is ideal).

Intracellular: Th1 activate macrophages

Extracellular: Th2 help B cells to produce antibodies to deal with extracellular bugs

Name:_____

Question 7:



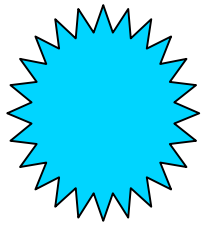
a. A molecule involved in signaling downstream of the TCR has two SH2 domains (depicted above, to the right). What is the name of this molecule? What do the SH2 domains bind to?

Zap70, binds to phosphorylated ITAMs

b. How is this molecule activated? Feel free to use the limited diagram provided above of a TCR.

TCR binds antigen presented on MHC I, src kinases phosphorylate ITAMs, Zap70 gets recruited to the phosphorylated ITAMs and src Kinase activates Zap70

c. What critical downstream molecule initiates a signaling pathway that can be blocked by cyclosporine A and FK506 (on the right)?
PLCγ



Above: this molecules is the downstream target of the molecule with two SH2 domains above and it initiates a pathway that can be inhibited by Cyclosporin A and FK506

Name:_____

Question 8:

a. Wanting to delve deeper into antibody production and optimization, you begin studying downstream signaling cascades relevant to the process. It turns out that PKA is required to activate AID, and the $PKA^{-/-}$ mouse is lethal at day 8.5 of embryogenesis. Since you're curious about the impact of PKA on antibody production, you take a $RAG2^{-/-}$ blastocyst, inject a $PKA^{-/-}$ embryonic stem cell and then implant it into a pseudopregnant female. A mouse pup is born, and you infect it with a virus. *Please describe whether lymphocytes will be seen in this mouse and if so, would isotype switching takes place and comment on the affinity of the resulting antibody response*.

Lymphocytes will be seen. B cells won't be able to class switch or somatic hypermutation (so, low affinity IgM antibodies)

b. Your next project involves making and studying an IP3 $receptor^{-/-}$ mouse. Unfortunately, this deficiency is embryonic lethal. So, you make a conditional knockin—the IP3 receptor is only deleted in T and B cells. These mice survive development, to your great relief. *Would you see T and B cells in the periphery? What stage of development would T and B cells achieve?*

No T or B cells in the periphery—halted because no preTCR or preBCR signaling can take place.

c. A young patient presents with recurrent bacterial otitis media, sinusitis and pneumonias, and a family history of opportunistic infections in siblings and other family members. Considering primary immunodeficiencies, you worry about a defect in B cell function. When you measure antibody levels you find that IgA, IgG and IgE are almost absent, though IgM is normal. *Please list three possible genetic defects that could cause this problem*

1. $CD40^{-/-}$

2. $CD40L^{-/-}$

3. $AID^{-/-}$