

# HST175 Final Exam

**12.13.04** □

**Good luck and thanks for all your hard work this semester!**

**Name:** \_\_\_\_\_

**Question 1:** \_\_\_\_\_

**Question 2:** \_\_\_\_\_

**Question 3:** \_\_\_\_\_

**Question 4:** \_\_\_\_\_

**Question 5:** \_\_\_\_\_

**Question 6:** \_\_\_\_\_

**Question 7:** \_\_\_\_\_

**Question 8:** \_\_\_\_\_

**Question 9:** \_\_\_\_\_

**Question 10:** \_\_\_\_\_

**Question 11:** \_\_\_\_\_

**Total Score:** \_\_\_\_\_

Name:

## Question 1

a. Explain in a stepwise fashion how signals from the T cell receptor induce NFAT activity in the nucleus. Explain briefly how you think FK506 /Tacrolimus (an immunosuppressive drug) works.

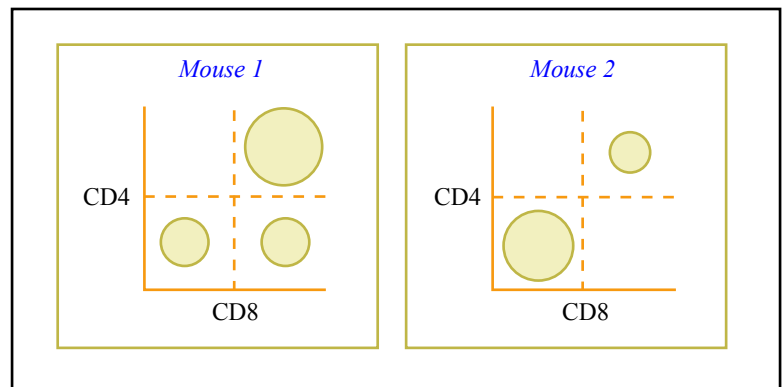
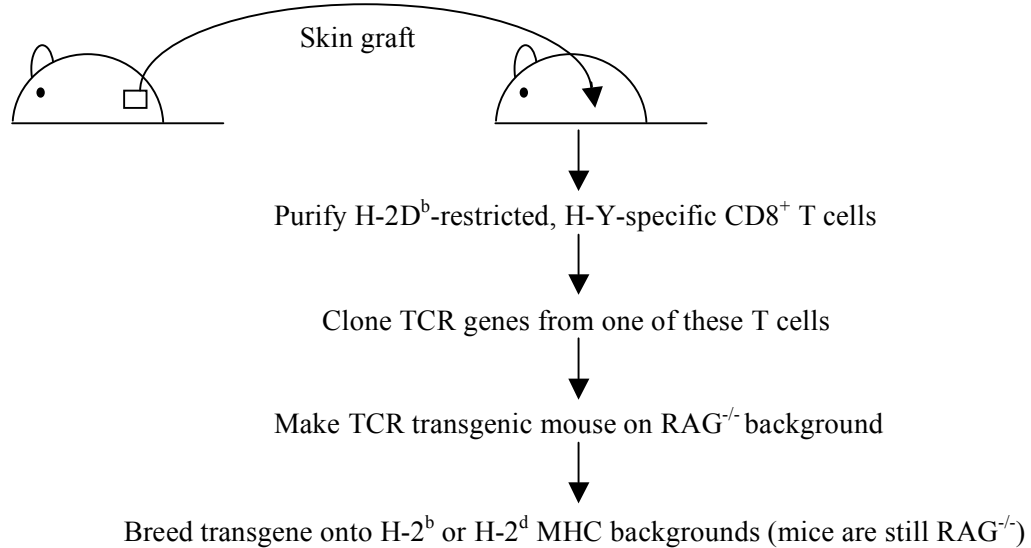
**TCR sees peptide-MHC → lck phos. ITAMS → Zap70  
activated → → PLCg activated → PIP2 to DAG and  
IP3 → Ca<sup>2+</sup> → calcineurin → dephosphorylate NFAT → nucleus**

**FK506 blocks calcineurin from dephosphorylating NFAT.**

## Question 2

A friend of yours at Yale is also taking immunology this semester. Intrigued by the concept of minor histocompatibility antigens, he drops his previous undergraduate thesis and starts working on the H-Y antigen. He first takes skin from a male H-2<sup>b</sup> mouse and grafts it onto a female H-2<sup>b</sup> mouse. The graft is slowly rejected. He waits a week, regrafts skin from the same male H-2<sup>b</sup> mouse onto the same female H-2<sup>b</sup> mouse and finds that the graft is rejected slightly faster this time. He then collects CD8<sup>+</sup> T cells from this female H-2<sup>b</sup> mouse and purifies cells that recognize H-2D<sup>b</sup>:H-Y complexes. He clones the  $\alpha$  and  $\beta$  TCR genes from one of these T cells and makes a transgenic mouse that expresses the H-2D<sup>b</sup>-restricted, H-Y-specific TCR on the RAG<sup>-/-</sup> background. He then breeds this mouse onto two different MHC backgrounds: H-2<sup>b</sup> or H-2<sup>d</sup>.

Unfortunately, he went into lab the day after the Harvard-Yale game, and being a bit hung over, he mixed up all of his cage cards so he doesn't know which cages contain H-2<sup>b</sup> mice and which contain H-2<sup>d</sup> mice. Furthermore, having never worked with mice before, he doesn't know how to determine the sex of a mouse. Determined to avoid embarrassment in lab, he calls you and asks for help. He provides you with thymocyte FACS profiles for three of his mice and asks you to help him determine the MHC background and sex of these three mice. Please help your poor Yale friend and determine the sex and MHC background of these mice, indicating any ambiguities that may be present. Briefly (1-2 sentences) explain to him how you were able to determine the sex and MHC background of each mouse from the FACS plots he provided you.



A) Mouse 1:

Gender: **FEMALE**  
MHC: **H-2Db**

Figures by MIT OCW.

B) Mouse 2 (two pairs of possibilities):

Gender in option 1: **MALE**

MHC in option 1: **H-2Db or H-2Dd**

Gender in option 2: **FEMALE**

MHC in option 2: **H-2Dd**

Name:

### Question 3

A) Please provide a diagram of deletional VDJ signal and coding joint formation (include a diagram of the important parts of the locus before VDJ recombination, the two intermediates, and the end products. Please indicate any regions which are altered in the process).

**See Janeway (or the 2005 midterm answer key)**

B) You have just examined a male infant with recurrent oral candidiasis (thrush) and non-pyogenic lung infections. Another male sibling received a bone marrow transplant four years ago after presenting in a similar manner. Serum immunoglobulins are normal, but the child lacks CD4 T cells, CD8 T cells, and NK cells. The diagnosis of severe combined immunodeficiency is made. What do you think may be the molecular defect in this child?

**IL-2 $\gamma$  chain deficiency**

## Question 4

Viruses have been shown to evade every aspect of innate and adaptive immunity. Viruses which can evade the host's immune system to establish a chronic, persistent infection have a selective advantage over those viruses which are not able to evade the immune system. .

Three examples of viral immune evasion strategies are described below. For each of the three strategies, please state whether virally-infected cells would be killed by i) CTLs and/or ii) NK cells, and explain why target cell killing would or would not occur (1 sentence)

- A)  HIV taken from HLA-A2 positive individuals often harbors mutations in the sole viral epitope presented by HLA-A2, resulting in loss of binding to HLA-A2.
- i) HLA-A2-restricted CTLs –**NO KILLING**
  - ii) NK cells –**NO KILLING**
- B)  Human cytomegalovirus (HCMV) encodes a protein called US3 that binds to MHC Class I molecules and retains them in the ER, so that they never get presented on the cell surface.
- i) CTLs –**NO KILLING**
  - ii) NK cells –**KILLING**
- C)  Poxviruses encode a protein called crmA, which can inhibit caspases, which are proteins in the apoptosis cascade downstream of granzymes and Fas.
- i) CTLs – **NO KILLING**
  - ii) NK cells- **NO KILLING**
- D)  Other viruses have strategies to evade MHC Class II presentation, and therefore CD4<sup>+</sup> T cell activation. Given that eradication of the infected cell requires MHC Class I presentation, give two reasons why would it be advantageous for these viruses to inhibit CD4<sup>+</sup> T cell activation.
- 1) **APC licensing (CD4 Th cell leads to upregulation of CD40 on APC)**
  - 2) **Th help leads to isotype switching and somatic hypermutation (better antibodies)**

### 3) Question 5

Your very tricky colleague Dr. Dark Sircus, has used gene targeting to convert, exclusively in CD8+T cells, a QACRG motif in a cytosolic protein X to a QALRG sequence (strain A). Using the same strategy he has inactivated a protein Y also in CD8+ T cells alone (strain B). Mice A and B were crossed and homozygosed to yield strain A/B in which only CD8+ T cells lack protein Y and exclusively contain the QALRG form of protein X.

He immunized mice from strain A, strain B, and strain A/B with a virus called LCMV and later mixed infected LCMV target cells with activated CTLs from Strain A, Strain B, or Strain A/B. As a control Mice from Strain A were also infected with another virus MHV. The CTL:target ratio was 1:5

At the end of each experiment he used flow cytometry to identify the number of viable target cells and also viable CTLs. He obtained the following results.

Type of CTL	Number of LCMV target cells	Number of CTLs
Mouse A (mutant X, anti MHV)	48,000	9,700
Mouse A (mutant X, anti LCMV)	4,200	9,800
Mouse B (mutant Y, anti LCMV)	4,000	380
Mouse A/B (mutant X, mutant Y, anti LCMV)	4,100	9,700

A) Describe one normal CTL protein that collaborates with perforin to mediate target killing, and state what a potential target of that CTL derived protein might be.

#### Granzyme, caspase 3

B) What kind of protein is protein X?

#### Caspase

C) Describe what you think may be a possible function of protein Y. Explain what you think might be happening, and why, to CTLs from mouse strain B and strain A/B in the above experiment.

#### Blocks caspases from being activated in CTLs

## Question 6

A) You'd previously been working on a vaccine for Hepatitis B and have immunized wild type mice in four different ways:

- 1) purified peptide A derived from a viral coat protein
- 2) peptide A plus a bulky viral protein that binds to peptide A (A-binding protein; ABP)
- 3) peptide A + ABP + DNA with the sequence TGACGTT
- 4) peptide A + ABP + DNA with the sequence TGAGGTT

Will any of these immunizations generate a good humoral immune response? Explain why each immunization may or may not induce a humoral immune response (1 sentence each).

- 1) **no danger signal**
- 2) **no danger signal**
- 3) **has antigen and TLR 9 agonist and induces a good humoral response**
- 4) **no danger signal**

B) Excited about innate immunity, you create TLR 1→10 (all inclusive) knockout mice. Will any of these immunization strategies work for your TLR 1-10 knockout mice? Briefly explain.

**No—w/o a receptor for the adjuvant, you can't get a good response.**

## Question 7

A) Complement subserves many functions. Please list three functions mediated by the complement cascade and its byproducts.

1. **C5a: anaphylotoxin**
2. **C3b: opsonin**
3. **MAC: lysis of target cell**

B) What aspect of the IgM molecule facilitates its ability to initiate the classical complement pathway?  
**pentamer**

C) Fascinated by the germinal center reaction, you find a way to eliminate the follicular dendritic cell. Describe some expected phenotypes of your mice.

**Autoreactivity (if you do get positive selection) or no positive selection at all.**

D) Explain how AID is thought to mediate isotype switching,

**AID → cytidines deaminated to uracils → → two sets of single-strand breaks lead to two double strand breaks in the switch regions → double strand breaks repaired to yield isotype switch.**



## Question 8

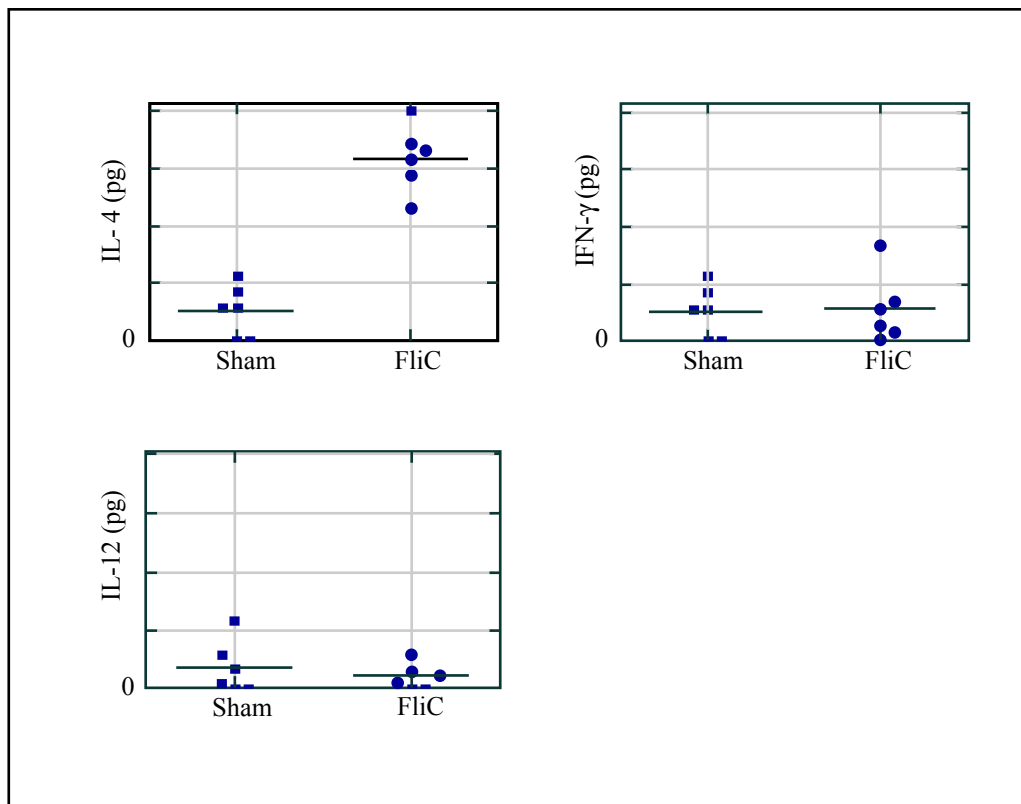
The major immune response against *Salmonella typhimurium* is directed against bacterial flagellae. Each *Salmonella* bacterium can have up to 10 flagellae, which are required for bacterial adhesion and locomotion. These flagella are comprised of the polymerized flagellin protein FliC.

1. Injection of purified, soluble flagellin into mice is known to induce anti-FliC IgG1.

Is this an example of a T-independent or T-dependent activation? Explain.

**This is a T-dependent response → it's a peptide and there has been class switching (which is T cell dependent)**

2. You inject mice with FliC and read out cytokine production in the serum several days later. You get the following results:



Figures by MIT OCW.

A). Are these results indicative of a Th1 or Th2 response? Explain.

### Th2

B) You have found a new strain of mouse (strain X) that is highly susceptible to asthma. You inject one group of 2 week old strain X mice with live *Salmonella* (Group A), and one group of 2 week old strain X mice with FliC (Group B). The mice in Group B all develop asthma by 6 weeks of age, as do your non-i asthma. Provide a hypothesis to explain this phenomenon. Briefly describe a way to test this hypothesis.

### Th1 skew, measure relevant cytokines

**Name:**

C) Different inbred strains have different susceptibilities to some pathogens. For example, 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.

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

**Th2**

- What cytokine modulatory therapy could you imagine giving the BALB/c mouse to improve its ability to combat Leishmania (i.e what cytokines would you want to change the levels of? How might you do that)?

**Block IL-4 or give IFN- $\gamma$**

## Question 9

A large kindred in Utah is being studied to examine susceptibility to a multi-gene disease. In this syndrome, skin rashes are frequently observed in affected individuals, some of whom presented with inflammation of the pleura and the pericardium, while others developed kidney disease. The following results from an affected patient were made available to you.

(Rheumatoid factor: Negative, Anti-double stranded DNA antibodies: Present at high titers, Circulating immune complexes: Positive)

A) What is your first diagnostic choice?

### SLE (lupus)

Affected individuals were found to carry deletions or loss of function mutations in a gene called Mer. This gene was expressed in macrophages and dendritic cells and its sequence predicts an N-terminal signal peptide, a putative extracellular domain, a transmembrane anchor sequence and a tyrosine kinase domain. In order to better understand how this gene contributes to disease, a Mer knockout mouse was created. Thymocytes from wild type and mutant mice were treated with dexamethasone in vitro (dexamethasone induces thymocyte apoptosis) and mixed with macrophages from a normal littermate. Please examine the accompanying figure and legend.

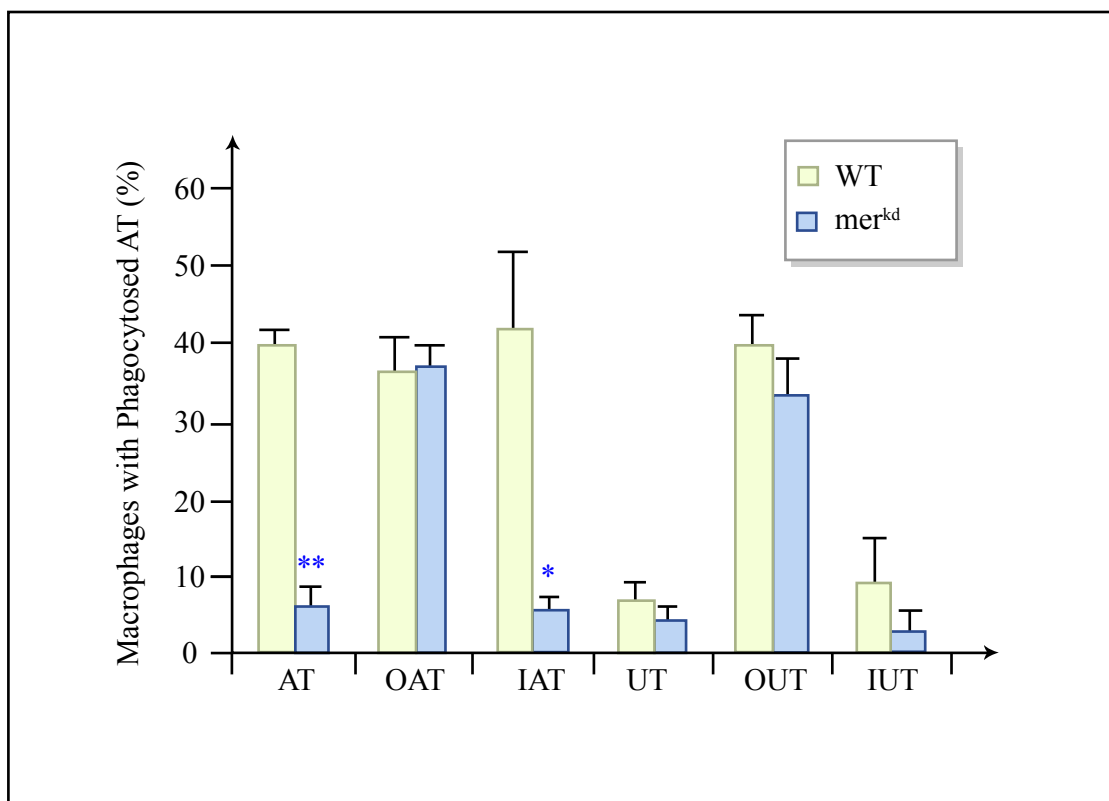


Figure by MIT OCW.

**Figure 1.** Phagocytosis of opsonized thymocytes. Apoptosis was induced in vitro using Dexamethasone. Apoptotic (AT) and untreated /normal thymocytes (UT) were opsonized with a non-activating anti- T cell antibody (an IgG antibody) that recognizes the T cell surface, or isotype-matched non-specific antibody. OAT, AT opsonized with anti-T cell antibodies; IAT, AT incubated with isotype control non-specific antibody; OUT, UT with anti-T cell antibodies; IUT, UT with isotype-matched non-specific antibody. The average and standard error from three experiments are plotted. Black bars, phagocytosis by macrophages from wild-type mice; light grey bars, phagocytosis by macrophages from *mer kd* mice. Asterisk,  $P < 0.05$ ; double asterisk,  $P < 0.001$ .

B) What appears to be the function of Mer? Explain in no more than 3 sentences.

**Mer serves to increase phagocytosis of apoptotic cells**

Name:

C) In a sentence, provide a hypothesis linking loss of Mer function to the human disease diagnosed above in "A".

**When apoptotic cells are not taken up, they remain as antigens. These cells include DNA and can theoretically lead to the development of anti-DNA antibodies (a hallmark of SLE).**

## Question 10

A young girl with a skin disorder was also found to have high levels of IgM, and barely detectable IgG, IgA, or IgE. B cells were purified from her peripheral blood and stained with anti CD40. Flow cytometry revealed normal levels of CD40 on B cells. Total RNA was reverse transcribed and PCR sequencing of CD40 was performed. The CD40 sequence was found to be normal.

Proliferation Assays using  $^3\text{H}$ -thymidine.

Stimulus	Control B cells	Patient's B cells
IL-4	33,000 cpm	35,000 cpm
CD40L	97,000 cpm	870 cpm

A) Make an educated guess as to the nature of the gene defect in this girl. Explain very briefly.

**NEMO (NF $\kappa$ B below CD40)**

B). Do you think that immunization with a pneumococcal polysaccharide vaccine will or will not result in specific antibodies being generated in this girl. Briefly explain your answer.

**Yes → IgM**

C) Will immunization with tetanus toxoid result in similar or different antibodies being generated in this girl and a normal unaffected cousin? Provide a brief explanation for your answer.

**They will have different responses—the patient will only make IgM.**

## Question 11

A young woman enters your office, obviously terrified. She describes sensations of “pins and needles” in her extremities, a broken arm from a recent stretch of “clumsiness” and difficulty with coordination. What really brought her in today was a sudden monocular loss of vision this morning that lasted for 5 minutes and then passed. She’s convinced she has a brain tumor, but you aren’t so sure. She mentions a recent upper respiratory tract infection and asks if that could be related.

A) What disease are you most concerned about?

**MS**

Molecular mimicry is one theory for the initiation of autoimmune disease. Basically, the theory is that an adaptive response to a pathogen leads to a cross-reactive population of cells that then attacks self, usually in a Th1 dependent manner.

B) How could you test the molecular mimicry hypothesis?

**This is a very hard hypothesis to test. You could try to test pathogen levels if you had a suspected pathogen, but the hypothesis does not require persistent antigen (any good hypothesis was given credit).**

C) If molecular mimicry is the mechanism of autoreactive cell generation, describe a cell population that could control these autoreactive cells (include potential effector mechanisms for this cell population).

**Tregs**

D). Another way to treat disease is to alter the ability of effector cells to reach their target. List two ways of preventing these cells from reaching their target tissues.

**Block VLA-4, block E and P selectin**

E) It might be possible to alter the function of the effector cells. Describe a way of achieving this goal therapeutically (i.e. what would you administer)?

**IL-4**

F) Are central and peripheral tolerance redundant? Why or why not?

**No! You can only present a certain set of self-antigens in the thymus (i.e. by AIRE), so there is always the possibility of autoreactive cells escaping central tolerance. In addition, there are non-self antigens that you have to develop tolerance to (like commensal bacteria) so peripheral tolerance is very important.**

Name:

**Bonus:** □

- 1) List three salient differences between Ulcerative Colitis and Crohn's Disease
  - a. **Transmural (CD)**
  - b. **Granulomas (CD)**
  - c. **Whole bowel can be involved (CD)**
- 2) Please explain (in 1-2 sentences) the pathogenesis of anaphylactic shock.

**Anaphylactic shock is an IgE mediated response. After your first (or hundredth) exposure to an antigen, you develop IgE antibodies, which coat Fc receptors on mast cells. Upon the next exposure, you crosslink the IgE on mast cells with the antigen and yield massive mast cell degranulation and vasodilation, airway constriction and systemic organ shutdown.**

- 3) □ Compose a short rhyme that is of deep (the word is used in only a relative sense) immunological significance . The rhyme of about 4 lines must contain "T regs" either at the beginning or the end of one of the lines. If words fail you now you may send an e-mail for extra credit.

Good luck on your last two exams, have a great vacation, and get some rest!