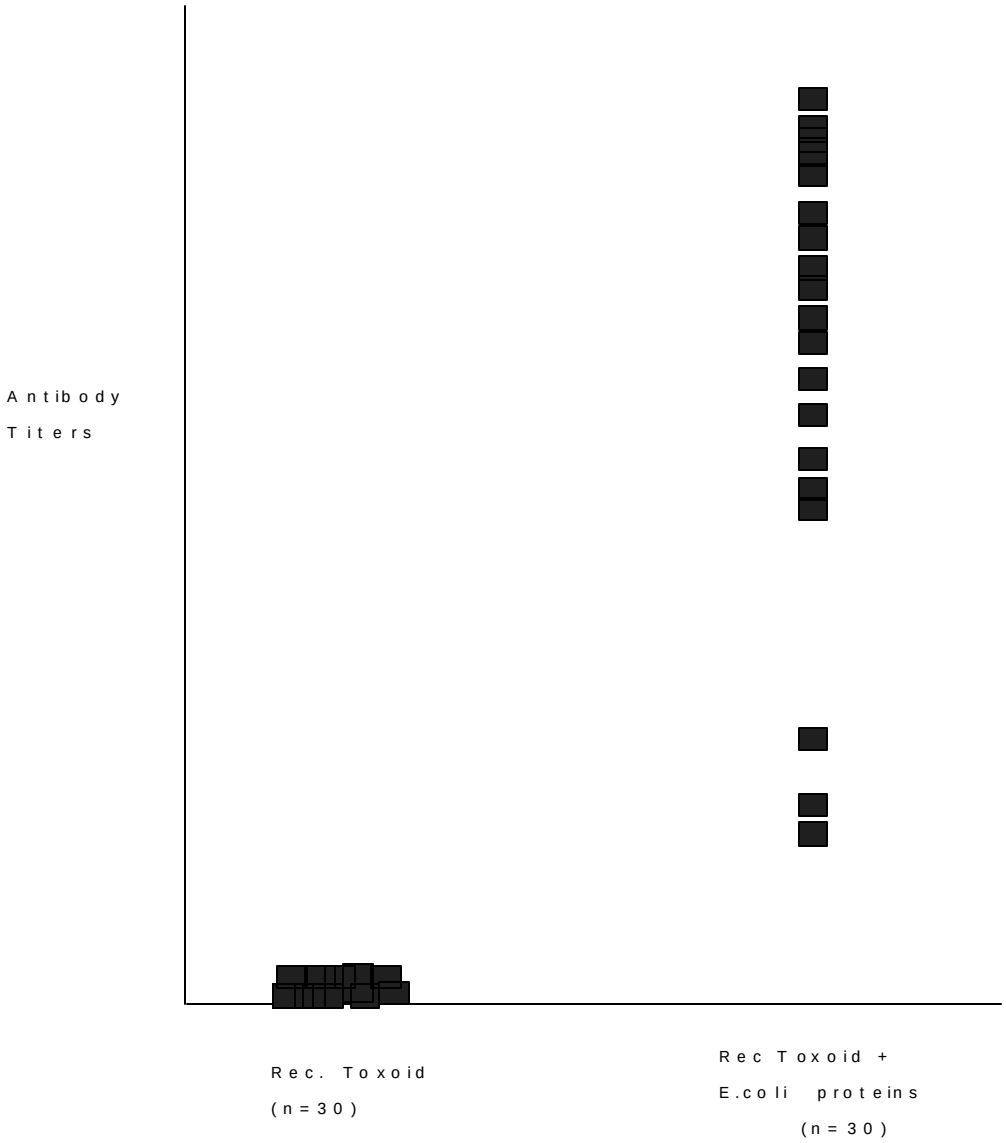
HST 175. Final Examination December 18th 2001. Please be brief!

1. Scientists at your medical school have mutated specific residues in the "protective antigen" (a subunit of anthrax toxin) that renders it non-toxic. Soluble, secreted, recombinant "innocuous" protective antigen (Rec Toxoid) was generated in *E.coli*, and shown to be endotoxin free.

   60 First year HMS students volunteered for a serologic trial. They were divided into two groups that were equivalent in terms of males, females, New Pathway students, and HST students.

   30 students received Rec Toxoid in a pure form. (Group A, on left). The remaining students received the same amount of pure Rec Toxoid mixed with 400 micrograms of a mixture of random *E.coli* derived bacterial proteins (Group B, on right).



a. Serum antibody titers (plotted as the log of the reciprocal of the lowest dilution at which enzyme activity was seen) against the protective antigen (PA) were determined by ELISA after two weeks. In Group A anti-protective antigen titers were uniformly very low. Titers for most students in Group B were on average many fold higher than those in Group A. Explain why the students in group B responded better? (10)

There is no danger signal being innoculated into Group A; only the Rec Toxoid is being innoculated. Thus, antibody titers against this antigen are low since there is no Signal 2 (i.e., no upregulation of B7 on APCs) to activate T cells. Rec toxoid is a protein and does not have identical polymeric units; thus, it cannot activate B cells independently of T cells.

Group B was given a danger signal. What is this danger signal? The fMet present on *E.coli* derived bacterial proteins.

b. While titers in Group B were much higher than those in Group A, they were not uniform. 3 students in Group B actually had titers a little higher than those in Group A (See Figure). Assuming that the actual inoculation procedure was not at fault, what is the most likely explanation for the poor response in these three low-responder students (who are in obvious good health)? Be very brief. (5)

These three individuals have MHC polymorphisms that render them inefficient at presenting foreign peptide derived from the Rec Toxoid.

c. Use a very simple diagram to illustrate how the ELISA could have been used to estimate anti-PA IgG and anti-PA IgM antibodies in serum (5)

Add primary antibody to wells (against PA). Add purified PA, which will bind to the primary antibodies. Add the patient's serum, which will presumably have anti-PA antibodies that bind to the remaining epitopes on the antigen. Add secondary antibody, which binds to the Fc region of the antibodies from the patient's serum. The secondary antibodies are normally conjugated to a fluorescent molecule. You can measure the fluorescence to estimate the antibody titer.

d. Toll like receptors activate the NFκB pathway in antigen presenting cells. This pathway may be important for the transcriptional induction of a cell surface protein that facilitates T cell activation. Briefly outline the NFκB pathway and mention one potential target gene of NFκB in dendritic cells based on the information provided here. (7+3)

The toll-like receptor on APCs activates a member of the TRAF family of proteins, which then activates the IKK complex. This complex phosphorylates IkB, which induces the ubiquitination and proteosomal degradation of IkB. Thus, NF-kB is released from IkB and can migrate to the nucleus, upregulating the transcription of many genes. NFkB upregulates the transcription of B7 in APCs.

2. A mouse model has been created for a rare form of human SCID by knocking out a gene that encodes a novel signaling molecule called PKK. The thymus of this mouse was shrunken in size. Flow cytometry of the thymus was performed as seen in the Figure on the next page.
   a. Where is the major block in development? What checkpoint is likely to have been abrogated? (5).

The block occurs between the transition from Double Negative to Double Positive, so it most likely represents a defect in the pre-TCR or pre-TCR signaling.

A few "breakthrough" CD4 cells are seen in this mouse.
B cell development appears to be normal.

These mice were immunized in two ways. One immunogen was a polysaccharide. Wild type mice that have the same H-2 haplotype as the mutant mice were used as controls. High titer IgM antibodies were seen in all mice (mutant and wild type) in response to this polysaccharide.

A second immunogen was a small molecule – Trinitrophenol, which was covalently conjugated to bovine serum albumin. After immunization, an ELISA was used which specifically detects antibodies against the trinitrophenol moiety.

Graphs removed due to copyright reasons.

Here are the results of this immunization:

	PKK -/-	Wild type
Anti-TNP IgM –	high titers	high titers
Anti-TNP IgG-	not detectable	high titers
Anti TNP IgA	not detectable	high titers

b. Draw a simple diagram demonstrating how TNP-BSA is seen by specific lymphocytes. In this figure or separately, use arrows and your imagination to schematically indicate where relevant epitopes specific for an anti-TNP response may be on this immunogen (12)

The BCR on multiple B cells can recognize all the foreign components of the trinitrophenol or the BSA peptide. The TCR on T cells recognizes only foreign peptide components of the BSA that are presented on MHC. But the T cell cannot recognize the trinitrophenol moiety since it is not a peptide. Therefore, this is an example of a hapten-carrier.

c. Why do you think specific IgG and IgA antibodies were not observed after TNP-BSA immunization in the knockout mice? A very brief answer will suffice (3)

There are no T cells available to induce class switching via CD40L in the knockout mice.

3. Explain how cyclosporin and FK506 function as immunosuppressants (15).

Cyclosporin binds to cyclophilin in the cytosol (FK506 binds to FKBP) and the complex inhibits calcineurin. Therefore, NFAT does not get dephosphorylated, which is required for NFAT activation. Thus, these drugs act as immunosuppresants since NFAT is a required transcription factor for T cell and B cell activation, proliferation, and effector function.

4. A novel asthma gene has been discovered in patients with asthma. Mutations in this gene are linked to high IgE levels. The wild type gene is found to encode a DNA binding protein called NF-Asthma B that binds to a regulatory sequence in the IL-4 gene. The mutant protein cannot bind this regulatory sequence.

a. Do you think NF-Asthma B is a transcriptional activator or a repressor (3)?
b. Are allergens more likely to be polysaccharides or proteins? (3)
c. What is the role of the invariant chain during antigen presentation (9)

a. **Repressor**
b. **Proteins, since they induce class switching to IgE and therefore must be able to activate T cells (CD40L)**
c. **The invariant chain has three functions: First, to bind to the peptide groove on MHC II molecules in order to prevent self-peptides in the ER from binding. Second, to stabilize the MHC II molecule so that it can be transported out of the ER. And, third, the invariant chain contains a dileucine motif that acts as a "zip code" to send the MHC II molecule into vesicles traveling through the lysosomal pathway.**

5. What are caspases? (5)
A viral protein known as v-FLIP structurally resembles Caspase-8 but has no catalytic activity. Would it activate or interfere with apoptosis? (2). Describe the apoptotic pathway which it would most likely influence (7). Outline the role of this pathway in T cell homeostasis. (6).

Caspases are cysteine-requiring aspartate proteases, meaning that they use a cysteine residue in their active site to cleave peptides after aspartate residues. v-FLIP interferes with apoptosis.

The pathway it would influence is the Fas-FasL pathway, where trimeric FasL binds to trimeric Fas. This allows FADD (Fas-Associated Death Domain) to bind to the cytoplasmic side of Fas. FADD then binds to caspase 8, thus activating the autocleavage of caspase 8. Activated caspase 8 then activates caspase 3. Caspase 3 eventually leads to DNA fragmentation via two mechanisms: first, it leads to the cleavage of DNA repair enzymes. And second, it cleaves and inactivates ICAD (Inhibitor of Caspase-Activated DNase). Thus, CAD is activated which results in DNA fragmentation.

In Activation Induced Cell Death (AICD), overstimulated T cells will express FasL that will bind Fas on the same cell (or adjacent T cells). thus, upon clearance of an infection, the Fas pathway is essential for getting rid of activated T cells (as well as activated B cells).