HST 175. Midterm examination 10/23/03. Answer all questions.

I. Mice in which the Rag-2 gene is defective have been generated by gene targeting strategies. These Rag-2-/- mice lack peripheral B and T cells.
a) If thymocytes of Rag-2-/- mice were stained with labeled antibodies against CD8 and CD4, what population or populations of T lineage cells would be detected? (2).

Double negative pro-T cells since pre-TCR signaling needed to go to DP stage. No VDJ recombination.

In the experiment depicted below, bone marrow stem cells that lack NFκB (p50 and p65 are two NFκB family proteins that generally form heterodimers) were obtained from a mouse in which both the p50 and p65 genes had been knocked out. These cells were mixed with Rag-2-/- bone marrow stem cells, and this stem cell mixture was transferred into a recipient Rag-2-/- mouse (henceforth called a reconstituted Rag-2-/- mouse). Although a control (non-reconstituted) Rag-2-/- mouse totally lacked peripheral lymphocytes (not shown here), some lymphocytes were clearly seen in the reconstituted mouse.

Graphs removed due to copyright reasons.

In the figure, the left half of the upper panel represents an analysis of normal (wild type) spleen cells, and the right half is an analysis of spleen cells from a "reconstituted Rag-2-/- mouse".

b) What kind of blood cells can be visualized in the upper panels? What is this kind of analysis called? (2)

Since we are using anti-IgM and anti-IgD, we are visualizing B cells. This is FACS analysis.

c) In the upper right panel some cells are clearly seen in the upper two quadrants. What is their genotype (or genetic makeup)? (3).

Rag-2 +/+ and NF-KB -/-, since VDJ recombination has occurred.

d) Cells in the bottom right quadrant of the upper panels were further analyzed using an additional reagent (see lower panel of figure). What is the phenotype (in terms of cell surface proteins) of the cells that are missing in the reconstituted Rag-2-/- mouse? (3)

IgM^{hi} IgD^{lo} $CD21^{hi}$

e. In the figure above, one of the proteins analyzed is CD21. What does this protein recognize during an immune response (2)? What protein mediates a broadly similar function on cytolytic T cells (2)?

Binds to C3d; acts as a coreceptor for the BCR.
CD8

f. The NFκB pathway is considered to be important in many cell types. Name a receptor on dendritic cells that might be triggered by Gram negative bacteria to activate NFκB (2)? Mention a gene that is likely to be transcriptionally induced in dendritic cells by NFκB that might contribute to T cell activation (2). Where in the dendritic cell would NFκB be found prior to this encounter with Gram negative bacteria (2)?

- **Toll-like receptor**
- **B7**
- **In the cytosol (bound to IKB)**

II. A febrile illness that has incapacitated members of the 101st Airborne in the vicinity of Kirkuk turned out to be an arthropod borne viral illness. The illness was mild in soldiers who expressed HLA-A2, but the absence of HLA-A2 (in 55% of the soldiers) correlated with prolonged fever.

A viral capsid protein (VCP) was identified which contains a peptide which is capable of binding HLA-A2. Five HLA-A2 positive servicemen volunteered to provide peripheral blood during convalescence for studies on immune responses to VCP. VCP was delivered to the cytosol of HLA-A2 expressing B lymphoblastoid cells derived from each volunteer and these cells were used to reactivate CTLs from individual donors. A panel of synthetic peptides resembling the VCP peptide (VINGYSPA) was presented by HLA-A2 expressing targets to CTLs in the course of a series of CTL/chromium release

assays. Each peptide was also tested for its ability to bind HLA-A2. One set of results is summarized below.

Peptide	**HLA-A2 binding**	**CTL activity**
1. V I N G Y S P A	++++	++++
2. V T N G Y S P A	+	+
3. V T N G Y S A A	-	-
4. V I N G F S P A	+ +++	-
5. V I R G Y S P A	++++	+
6. A I N A Y T P G	+++ +	++++

a. Why do peptides 2 and 3 activate CTLs poorly? (2)
Poor binding to HLA-A2

b. What can you infer about the function of the asparagine (N) and tyrosine (Y) residues in this VCP peptide (5).

While not important for strong binding to HLA-A2, these residues are important in that they are recognized by the TCR of the CTL.

c. Which of the peptides above would best be classified as an "antagonist" peptide that might inhibit specific CTL activity? (3).

In order to inhibit CTL activity, you need a peptide that will saturate MHC sites but not activate the CTL. Peptide 4 still binds with high affinity to HLA-A2, but does not elicit CTL activity.

III. Explain, providing some molecular details, how a circulating naïve T cell enters a lymph node. (10).

1. **Rolling (1) – via interaction of L-selectin (1) with PNAd (1) on HEV**
2. **Chemokine secretion (1) activates chemokine receptors (e.g., CCR7 (1) on naïve T cell. This signaling thru G proteins activates integrins (1).**
3. **Sticking (1) – mediated by integrins (LFA-1 (1) with ICAM-1 (1)**
4. **Diapedesis (1)**

IV. A two step assay has been recently described for the activity of an enzyme "E" observed in some lymphocytes in the spleens of immunized animals. A 30-mer single stranded oligonucleotide containing a single C residue in position 13 (residues are

numbered 5' to 3') was labeled with radioactive phosphate at the 5' end. After incubation of this labeled oligonucleotide with purified "E", the DNA was then exposed to uracil N-glycosylase (UNG), an enzyme that excises U residues and hot alkali was added to cleave the single stranded DNA at the abasic site. The labeled DNA was separated on a gel.

a. What activity of "E" is being assayed here? (2)

deaminase activity (C → U) like AID

b. When the above assay was performed without the addition of UNG, a 30-mer labeled fragment was observed. What would be the size of the new labeled fragment generated if the assay was performed with all the ingredients mentioned above? (3).

Excision of the U would create a labeled fragment of 12 bases

c. What functions do you assume "E" mediates during an immune response? (5)

somatic hypermutation in B cell heavy chain and light chains as well as class switching