

HST 175: Midterm examination, October 10<sup>th</sup>, 2000

**Please read the questions carefully and answer all of them as concisely as possible. Please separate the answers for 1 and 2 from 3 and 4 and write your name on each answer sheet.**

1. W., a one year old child with a history of recurrent opportunistic infections, was investigated at a referral center. Investigations showed an unrelated congenital heart disorder, the presence of near normal numbers of IgM and IgD bearing lymphocytes, and decreased numbers of CD3<sup>+</sup> peripheral T cells. Peripheral T cells were found to be almost exclusively  $\gamma\delta$  T cells - a very small number of  $\alpha\beta$  T cells were found in the blood stream (reduced about 10, 000 fold compared to normal). The peripheral  $\gamma\delta$  T cells could however be efficiently triggered *in vitro* with activating anti-CD3 antibodies to proliferate and secrete cytokines.

During surgery to correct a congenital cardiac defect, a biopsy of the thymus was undertaken. The thymus was shrunken. A control thymic biopsy was obtained from another individual undergoing thoracic surgery. Thymocytes were stained simultaneously with anti-CD4 (labeled with fluorescein isothiocyanate, a green fluorescent tag) and anti-CD8 (labeled with phycoerythrin, a red fluorescent tag). Flow cytometry was performed on 10,000 cells from each stained preparation. Each dot represents a single cell. The horizontal line within each square below separates cells which express CD4 (above the line) from those that do not, and the vertical line separates cells which express CD8 (to the right) from those that do not.

Single cells were cloned from the upper left quadrant of the control, and from the same quadrant for patient W ("breakthrough" T cells). The "breakthrough" T cells in the patient expressed  $\alpha\beta$  T cell receptors, but intriguingly when rearranged TCR  $\beta$  chain genes were PCR amplified and sequenced, two productive in-frame rearrangements were found in a number of the cells from the UL quadrant in the patient, but never in control cells.

Graphs removed due to copyright reasons.

- a. The four "quadrants" LL (lower left), LR (lower right), upper left (UL) and upper right (UR), each contains a distinct T cell population. List the quadrant containing the least developed thymocytes first and then the subsequent quadrants in temporal order. (5) **LL, UR, then UL and UR.**

- b. At what transition is  $\alpha\beta$  T cell development blocked in patient W? (5) **DN to DP OR Positive selection I, OR beta selection, OR pre-T receptor checkpoint**
- c. What gene or genes do you think might be mutant in patient W? Explain why other potential candidate genes were excluded. (5) **pre-T $\alpha$  is the best answer. NOT TCR $\beta$ , NOT CD3, NOT signaling components like Zap-70**
- d. Derangement of a regulatory phenomenon led to the expression of two productively rearranged TCR $\beta$  chain genes in some of the rare breakthrough T cells in patient W. What is this phenomenon called? What receptor drives a similar phenomenon during B cell development? (5). **Allelic exclusion. Pre-B receptor.**

- 2. a. Very briefly (in no more than one or two sentences) state the 12/23 rule. (5)

**A 12 bp spacer containing RSS can only be joined to an RSS which contains a 23 bp spacer and vice versa.**

b. Name two immunoglobulin isotypes that contain a J chain. Use a simple diagram to describe an immunoglobulin molecule in which 2 immunoglobulin H-L units are held together by a J chain. Label the Fab and Fc regions and the J chain in your diagram. What isotype best fits the structure you have drawn? (4 + 9 + 2)

### **IgA and IgM. IgA**

3. George Tucker, a staff member in the Nader campaign, was seen at the General Hospital because of low grade fever and a persistent cough. He had been a Peace Corps worker in Nepal in the early 70s. A chest X-ray revealed a possible cavity in the right upper pulmonary lobe and a PPD test showed marked induration and swelling at 72 hours. A tentative diagnosis of pulmonary tuberculosis was made.

a. Mycobacteria typically reside in and activate macrophages. What cytokine/s do macrophages secrete in response to these bugs that contribute/s to the polarization of the helper T cell response? (5) **IL-12 and IL-18 (full marks for IL-12 alone as well)**

b. What is the key cytokine made by polarized helper T cells in this situation that can contribute to the activation of macrophages and induce them to kill some intracellular microbes. (5)  **$\gamma$ -interferon**

c. Soluble mycobacterial proteins that make up PPD are presented by professional antigen presenting cells to helper T cells. Provide a schematic view of the MHC protein that is critical during this activation process. Describe the antigen presentation pathway involved with the aid of a diagram. (5+15).

### **MHC class II**

**Diagram of MHC class II should show two TM chains**

**Diagram of pathway should mention or touch on endocytosis/internalization, invariant chain,  $\alpha 3\beta 3$  complexes, and HLA-DM**

4. Describe, in a point by point fashion, the events that regulate the entry of a naïve T cell into a lymph node (30).

**Should mention, High endothelial venules, Selectins, Chemokines, Inside-out signaling, Integrins; Rolling, Tethering, Firm adhesion, Diapedesis**

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