HST 175 Final Examination 12/16/2002

Please answer all questions. Be brief and to the point. Please write your name on the answer sheets .

1a. In mice that lack CD40L, as well as in boys with the X-linked hyper-IgM syndrome, a deficiency in cell mediated immunity is observed. *Pneumocystis carinii* pneumonia is a common presentation in patients. Why does the absence of the CD40L lead to a defect in CMI (5)?

Lack of "APC licensing". Activated CD4+ T cells can activate APCs via CD40L/CD40 interactions. This activation or "licensing" involves improved APC effector function (e.g., macrophage phagocytosis) and MHC presentation abilities as well as upregulation of the costimulatory B7 molecules, which help to activate CTLs. Thus, lack of this APC licensing leads to defects in CMI.

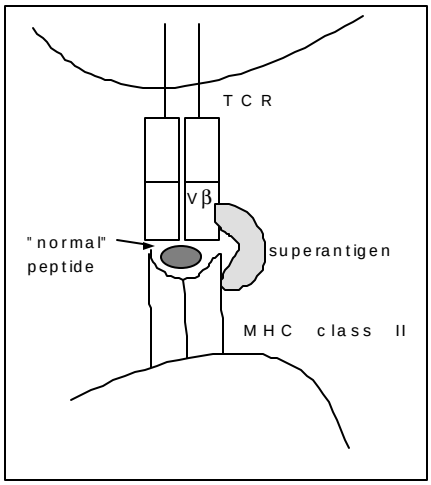
b. Explain with the aid of a diagram (showing what you know of the pathway involved) why a homozygous hypomorphic mutation in IKK-γ (IκB kinase γ, also known as NEMO) might contribute to a hyper-IgM like syndrome (10).

Upon binding of CD40L, CD40 signals for processes such as class switching and somatic mutation via the NF-kB pathway. Since NEMO is an essential part of this pathway, mutations in both NEMO and CD40L can result in a hyper-IgM syndrome. The pathway involves the following: CD40 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.

c. Children born with mutant AID develop a hyper-IgM like syndrome and fail to generate high affinity antibodies. What is the mechanistic basis for AID's presumed role in somatic mutation(5) ?

AID (activation induced deaminase) acts directly on DNA to catalyze C --> U transitions.

2. Superantigens can bind to a large number of Vβ regions on TCRs and simultaneously also associate with MHC class II molecules on APCs (see figure). A typical superantigen may crosslink MHC class II molecules to TCRs on as many as 5-20% of all CD4 T cells (which all share a subset of Vβ domains). Superantigens are distinct from "normal" antigens and they do not bind to the class II groove. They bind to structural determinants on class II molecules



and framework determinants on specific Vβ proteins, and can bypass the need for costimulation.

a. In the Toxic shock syndrome caused by the TSST1 toxin from *S. aureus*, TSST1 has been demonstrated to be a superantigen that causes high fever, shock, and a diffuse erythematous rash. It can be life-threatening. Given the above information, what do you think (in one or two sentences) is the pathogenic basis of this syndrome? (5)

Binding of the superantigen results in widespread activation of T cells, leading to a cytokine storm. These cytokines include IFNg, TNF, IL-1, IL-2, and IL-6, evoking a Th1-like response. IFNg leads to macrophage activation and subsequent tissue damage. This in conjunction with TNF-mediated inflammation can explain the rash. TNF also leads to vasodilation, thus causing the patient to be hypotensive and to go into shock. IL-1 induces the patient's fever.

b. A superantigen that binds to Vβ4 and Vβ11 containing murine TCRs was expressed transgenically in mice. Transgenic mice are viable. You are given monoclonal antibodies to all 20 murine Vβ family proteins (Vβ1 to Vβ20). Do you expect to see any differences in the immune system between transgenic and non-transgenic mice? Explain in no more than one sentence (5).

The transgenic mice will not have any CD4+ T cells with Vb4 and Vb11 because these T cells will be negatively selected in the thymus upon binding of the superantigen.

c. Explain briefly the function of HLA-DM (5).

HLA-DM is important for MHC II peptide presentation and acts to displace CLIP from the MHC II cleft, allowing extracellular peptide to bind. Remember that CLIP is the remnant of the invariant chain on MHC II molecules after proteolysis in the lysosome.

3. What is granzyme B's substrate specificity (5)? What exactly are caspases? (5). The cation-independent mannose-6-phosphate receptor (CI-M6PR) is a protein that can function as a cell surface endocytic receptor and the gene for this protein is frequently deleted in tumors. It is a tumor suppressor – loss of both alleles favors tumor progression. Why do you think loss of CI-M6PR favors tumorigenesis? (No more than two sentences please) (5).

Granzyme B directly activates caspase 3. Caspases are cysteine-requiring aspartate proteases, meaning that they use a cysteine residue in the active site to cleave after an aspartate residue.

Loss of the M6PR is thought to contribute to tumorigenesis because granzymes contain mannose-6-phosphate on their surface and are thought to be endocytosed

into target cells by the M6P receptor, thus, leading to CTL mediated killing. Mutations in M6PR thus lead to reduced immunity to tumors.

4. Describe in a point-by-point manner three ways in which certain proteins in tumors might be recognized as tumor antigens by host T cells (10).

-- Point mutations (e.g., oncogenes and tumor suppressor gene mutations that lead to non-self antigenic structures)
-- Viral peptides
-- Translocations (e.g., BCR-ABL in chronic myelogenous leukemia), resulting in new antigenic structures

5. Describe how cyclosporin A interferes with cytokine gene transcription (5).

Cyclosporin A binds to cyclophilin in the cytosol, and the complex inhibits calcineurin. Thus, the drug prevents the activation of NFAT.

6a. Direct immunization with plasmid DNA of bacterial origin induces immune responses. Assuming that the plasmid DNA is endotoxin-free, mention how you think "danger" is signaled by DNA of bacterial or plasmid origin (5).

umethylated CpG DNA binds to Toll-like receptor

b. How do signal one and signal two cooperate in the cytosol and nucleus to induce IL-2 transcription during T cell activation? Please be brief (10).

Signal 1: Zap-70 activates PLC gamma, leading to calcium release and activation of calcineurin. Calcineurin leads to NFAT activation. Zap-70 also activates the Ras-Erk pathway, leading to fos upregulation.

Signal 2: CD28 activates JNK, leading to the phosphorylation and activation of jun.

Jun and fos come together to form AP-1. AP-1 and NFAT together induce IL-2 transcription.

**** You should know all of the details of signaling that were discussed in the review session.**

7. Where is AIRE expressed and what is it's presumed function? (5)? Why do you think patients with AIRE mutations develop recurrent mucocutaneous candidiasis (5)?

AIRE is expressed in thymic epithelial cells and acts as a transcription factor that upregulates transcription of peripherally expressed, tissue-specific proteins. It is thought that these proteins are presented on MHC molecules on the thymic epithelia and induce central tolerance to these peripheral self-antigens.

Mutations in AIRE result in autoimmunity, since T cells that were meant to be negatively selected now survive and can recognize self-antigen. Thus, patients with AIRE mutations develop widespread autoimmunity leading to diabetes, adrenal insufficiency, and hypothyroidism. On the other hand, these patients also develop forms of immunocompromise (e.g., they are susceptible to mucocutaneous candidiasis, a type of yeast infection). This has to do with the fact that T cells undergo two selection processes: those with no affinity for MHC-peptide die by neglect. Those with very high affinity for MHC-peptide die during negative selection by apoptosis. Those with intermediate affinity survive. With regards to AIRE mutations, it is thought that some T cells that were meant to survive via intermediate MHC-peptide interactions now die by neglect. This creates a hole in the T cell repertoire, leading to susceptibility to candida.

H-A-P-P-Y H-O-L-I-D-A-Y-S!!!!!