Fall 2010 MCB 150 Midterm1 Name (last, first): _____

Please use PEN only. **Each page (total of 4 pages) must have your name on the top**.

1 a) (4 pts) What is the main difference between a lamprey variable lymphocyte receptors (VLR) and a human lymphocyte receptor?

Lamprey VLRs use Leucine Rich Repeats (LRR) while the human lymphocyte receptors use Ig domains to detect antigens.

b) (4pts) how is diversity generated in lamprey VLRs during development (please be specific)?

From the paper's abstract: "To generate diversity, the single lamprey VLR locus contains a large bank of diverse LRR cassettes that are available for insertion into an incomplete germline VLR gene"

2) (8 pts) The human/mouse immune system can clear a virus that infect dendritic cells BETTER than a virus that infects skin fibroblast cells. Why is this?

Dendritic cells are APC and express the B7 molecules that can provide the 2nd signals to directly stimulate killer T cells. Skin fibroblast cells don't have B7 and need to recruit T cell help to activate the killer T cells.

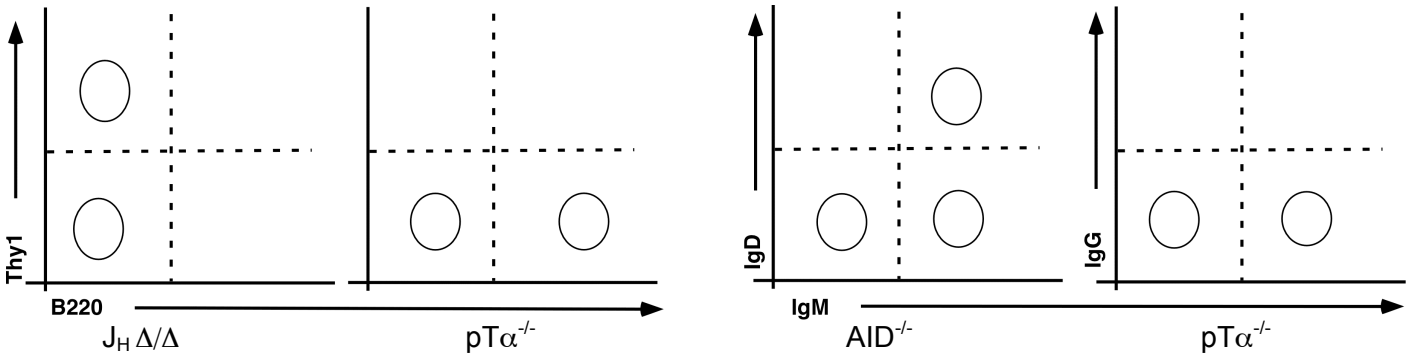
Acceptable answer: Dendritic cells express MHC class II and B7 molecules that can recruit CD4^{+} T cells to help killer T cells.

3) (16 pts). In a few words, describe the main phenotype and main cause of the following mice:

Mice:	Main phenotype/problem (2 pts, e.g. die, arrest at DN T cell stage):	Main cause (2 pts, be specific with the molecular mechanisms)
TLR4$^{-/-}$ mice infected with Salmonella (Gram^{-} bacteria)	*die, can't clear infection*	*TLR4 recognizes LPS present in Gram^{-} bacteria. Without it, mice are susceptible to Gram^{-} bacteria infection*
Human patients with CD40L mutation	*recurrent infection or Hyper IgM syndrome*	*B cells unable to switch to other isotypes due to lack of T cell help.*
Artemis$^{-/-}$ mice	*Partial or complete B and T cell development*	*Artemis is required to cut the hairpin generated by RAG1/2. Without it, VDJ recombination can't occur*
Ig$\alpha^{-/-}$ mice	*No B cells*	*Lack of pre-BCR leads to an arrest of B cell development.*

Acceptable if the students put the phenotype in the main cause or the other way around.

4) a) (12 pts) Draw the expected patterns of flow cytometric analysis using **spleen** from the different mutant mice below ($J_H\Delta/\Delta$= deletion of all the J_H gene segments, 3 pts each):



b) (12 pts). Briefly explain your answers for each of the FACS plot (e.g. no IRF8, block of $CD8^+$ dendritic cell development and hence no $CD8^+$ $CD11c^+$ cells).

$J_H\,\Delta/\Delta$ (Thy1 vs B220)

No J_H = No Ig heavy chain rearrangements = No $B220^+$ B cells.

pTα$^{-/-}$ (Thy1 vs B220)

No pTα= no preTCR = no $Thy1^+$ T cells

AID$^{-/-}$ (IgD vs IgM)

IgD isotype is not dependent on AID-mediated class switching and thus loss of AID has no effect on the IgM/IgD profile.

pTα$^{-/-}$(IgG vs IgM)

No T cells=No B cell class switching and thus no IgG^+IgM^- cells.

5) (16 pts) Indicate below the expected pattern of I-κB western blot of cells stimulated with the indicated ligands and whether the stimulation will produce lots (=wild-type levels), some or no cytokines.

cDC	Stimulation	I-κB	cytokines
		0' 30' post-stimulation	
TLR3$^{-/-}$ pDC	unmethylated CpG	____ (blank)(dotted line ok)	*lots*
TLR9$^{-/-}$ pDC	unmethylated CpG	____ ____	*No*
RIG-I$^{-/-}$ cDC	Newcastle Disease RNA virus	____ ____	*No*
MyD88$^{-/-}$ macrophages	LPS	____ – – –	*some*

6). In the class, we discussed HEL transgenic mouse experiments using rearranged κ and heavy chain DNAs from BALB/c HEL-specific hybridomas. Instead of Cδ, you decided to generate similar transgenic mice (Ig-HEL) with the Cγ1 attached at the end of the transgene (see below for maps, same κ). Transgenic mice were made in B6 mice. Let's assume that the rearranged heavy chain is $V_H2/D_H3/J_H1$ and κ gene is Vκ8/Jκ1. Below are the hypothetical germline sequences of Vκ8 and Jκ1:

Vκ8: ..TGTGGGTCCCACACAGTGATGAGGTGAGGCAATAAAATAGGCTATGG…

Jκ1: ..GCGTGTAATTTTTTGGCGGGTATGCTATGGGGGAGGGCACTGTGTGTGCGTCCTCCT….

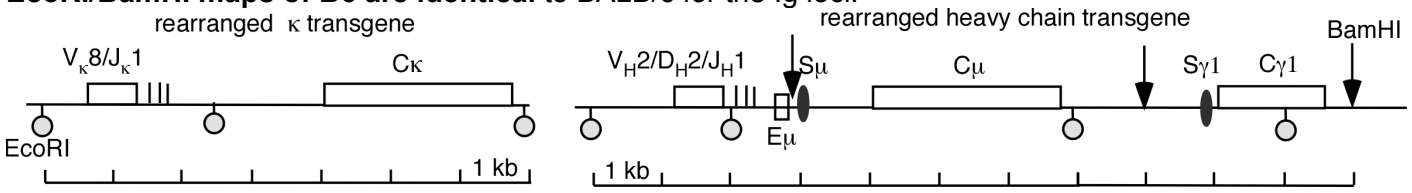
The rearranged Vκ8/Jκ1 contains the following junctional sequences:

…..TGTGGGTCCCA CA TGTGCGTCCTCCT....

V gene *P nucleotides* *J gene segment*

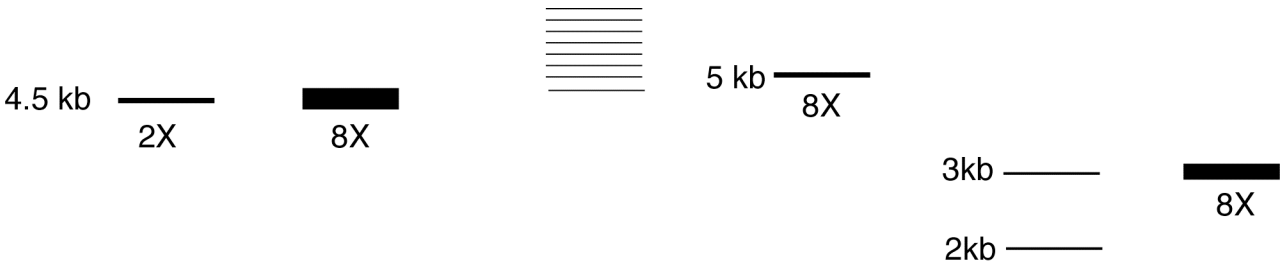
a) (10 pts) Please underline the heptamer-nonamer in both V and J (2 pts each) and delineate the junctional sequences above that correspond to the germline V, germline J, N and P nucleotides, if any (6 pts).

b) (18 pts) Below is a hypothetical map of the transgenes. You can assume that any polymorphism between BALB/c and B6 DNA wouldn't affect your Southern blot hybridization and **that the germline EcoRI/BamHI maps of B6 are identical** to BALB/c for the Ig loci.



Draw the expected southern blot patterns with Bone marrow (BM) or spleen DNA cut with EcoRI or BamHI from Ig-HEL and wild-type mice (indicate the size and copy number, if known). For Ig-HEL here, assume that there are **6 copies of the transgenes**)

Cκ as a probe		Eμ as a probe		Cγ1 as a probe	
EcoRI cut Bone marrow DNA		EcoRI cut Bone marrow DNA		BamHI cut Spleen DNA	
wild-type	Ig-HEL	wild-type	Ig-HEL	wild-type	Ig-HEL



c) (6 pts) Please explain the patterns when Eμ was used as a probe:

Smear for wild-type DNA due to rearrangements to J_H2-4 gene segments.
Allelic exclusion in Ig-HEL…no endogenous IgH rearrangements.

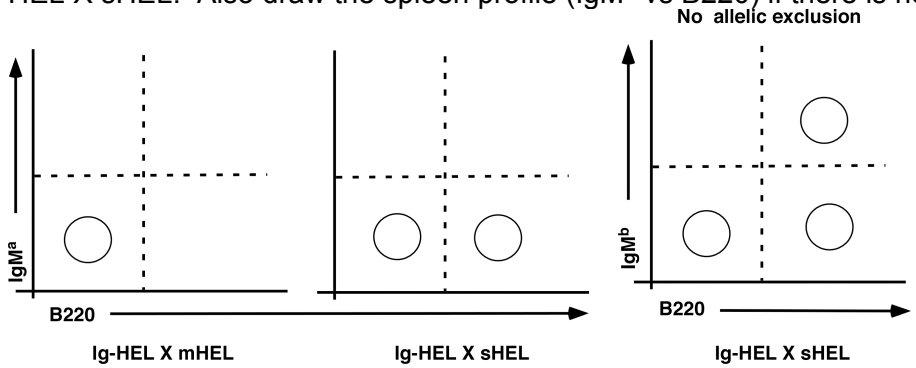
d) (6 pts) Please explain the patterns when Cγ1 was used as a probe:

3 kb is the germline Cγ BamHI band, it becomes 2 kb in IgG1 B cells (remember that many B cells in the serum/peripheral organs are IgG because these mice are not in germfree environment)
No class switching in unimmunized Ig-HEL monoclonal mice.

e) (15 pts) When these mice were crossed to mHEL (membrane bound HEL) or sHEL (soluble HEL) transgenic mice, predict the Northern blot patterns using the probes below (the size of the membrane μ RNA is 2.3kb. The size of the secreted μ RNA is 2 kb). All mice are unimmunized.

Cμ as a probe		Cμ as a probe		
bone marrow		spleen		
Non-Tg	Ig-HEL X mHEL	Non-Tg	Ig-HEL X mHEL	Ig-HEL X sHEL
2.3 kb _____	_____	2.3 kb _____ 2kb _____	(blank)	2.3kb _____

f) (9 pts) In the FACS below, draw the expected spleen profiles (IgM^a vs B220) of Ig-HEL x mHEL and Ig-HEL X sHEL. Also draw the spleen profile (IgM^b vs B220) if there is no allelic exclusion.



g) (4 pts) Briefly explain your reasoning for Ig-HEL X sHEL profile:

B cells are anergized and have internalized IgM (hence IgM^-) but $B220^+$ cells are present

h) (4 pts) Briefly explain your reasoning for Ig-HEL X sHEL (no allelic exclusion) profile:

No allelic exclusion leads to rearrangement of the endogenous Ig genes, resulting in the presence of $B220^+$ IgM^b positive cells. Down-regulation of IgM in anergic B cells or some endogenous B cells undergo class-switching can result in IgM^b negative B cells.

7) (6 pts) In March 2006, six young healthy volunteers who enrolled in a first phase clinical trial became violently ill immediately after taking TGN1412, a drug of humanized monoclonal antibodies against human CD28. Based on what you know about antibody structure, can you guess what might happen in this case?

These antibodies must have acted as an agonist due to their ability to cross-link CD28. Wholesale activation of all T cells led to cytokine storms, making these volunteers sick.

Acceptable answers: cross-linking of CD28 led to T cell activation.