Problem Set #3 Molecular Immunology Spring 2006

1) Estimate how many potential Ig molecules could be encoded by a heavy-chain locus with 100 VH, 13 DH, 4 JH gene segments, and a light chain locus with 100 Vκ and 4 Jκ gene segments? Is this an underestimate or an overestimate? Why?

100 x 13 x 4 = 5200 100 x 4 = 400 H/L pairing 5200 x 400= ~2 million underestimate due to the frequent occurrence of P-nucleotides and N-regions. Also, nucleotide deletions.

2) Why don't V_H gene segments rearrange with other V_H gene segments?

They all have the same spacer length in their RSSs (recall 12/23 rule)

3) What is a non-productive V(D)J rearrangement?

A rearrangement which does not preserve proper reading frame leading to a nonsense mutation.

4) Why does the Leukemia Society of America fund research into the mechanism of the V(D)J recombinase?

Because aberrant V(D)J recombination is likely involved in the generation of chromosomal translocations associated with most lymphoid malignancies. A proto-oncogene is rearranged to an Ig or TCR locus, dysregulating its expression and contributing to the series of genetic changes which result in malignant transformation.

5) Sketch the pathway of the V(D)J recombination reaction. Indicate where the pathway would be affected by homozygous null mutations in the following proteins:

-- directly from lecture notes diagram

a) terminal transferase (TdT)-- eliminates N-region addition to coding joints
b) RAG-2-- blocks recombination before the first step-- DNA nicking
c) DNA-PK-- interferes with coding joint formation; near normal signal joints
d) DNA polymerase α-- no known effect
e) Ku70-- interferes with both signal and coding joint formation.

6) Under what circumstances does V(D)J recombination result in a chromosomal inversion? (extra credit)

When the rearranging gene segments are in opposite transcriptional orientation to one another.

7) What is allelic exclusion? Why is it important for the regulation of antibody-mediated (humoral) immunity?

It assures that a single B cells expresses a single, bivalent antibody. Bivalency is important to assure a reasonable avidity of binding. Expression of a single specificity makes clonal selection more efficient.

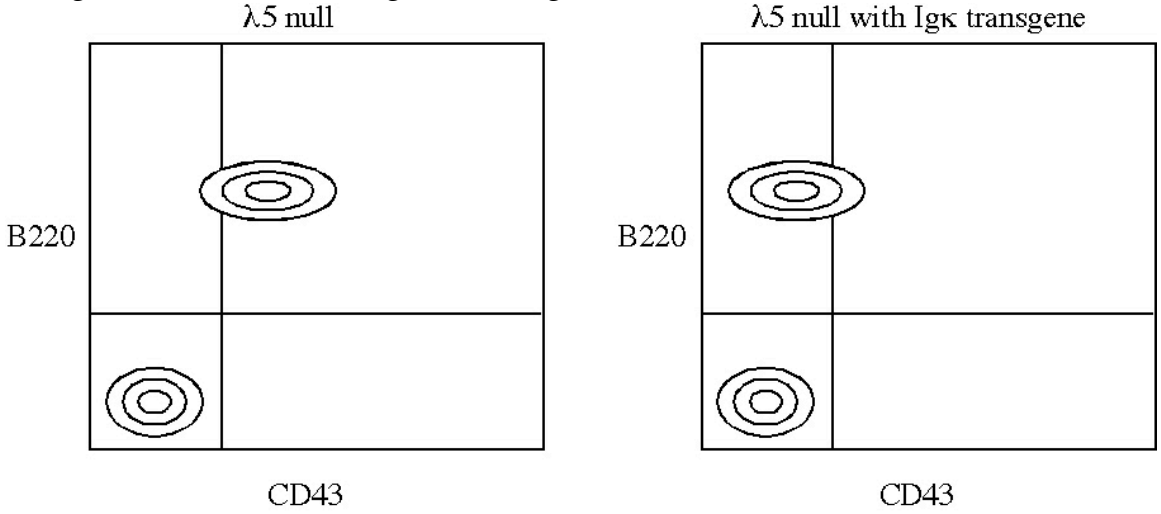
8) How would you distinguish a pro-B cell from a pre-B cell from a mature B cell?

By flow cytometry using anti-μ, anti-B220, and anti-CD43 antibodies. Pro-B is B220+, μ–, CD43+; pre-B is B220+, μ-, CD43-; mature B is B220+, μ+ (and δ+), and CD43-. You could also use immunofluoresence. Pre-B cells would be surface Ig-, but cytoplasmic μ +. You could also use gene expression markers such as TdT, RAG, λ5, germline transcripts.

9) What is the difference between transgenesis and "gene targeting.?"

Transgenes are inserted into the genome at random locations and do not disrupt or replace the corresponding endogenous gene. Good for gain of function studies or complementation studies. Gene targeting inserts a mutant gene in place of its endogenous counterpart. Can create null mutant mice.

10) Mice with a null mutation in the surrogate light chain gene λ5 show a block in development at the pro-B cell stage. Sketch what you think the FACS analysis of bone marrow from this mouse would look like when stained with anti-B220 and anti-CD43? What do you expect would happen to this pattern if you bred a productively rearranged Igκ transgene into the λ5-mutant genetic background?



11) What is receptor editing? Why is receptor editing more likely in the Igκ locus than in the IgHCμ locus?

Receptor editing is the secondary rearrangement of an already rearranged allele, deleting the previous gene and trying a new one. Happens most clearly in the Igκ locus. It is unlikely in the IgHCμ locus because all the D segments are deleted from fully rearranged gene and upstream VH genes have same RSS as downstream JH genes (rearrangement would violate the 12/23 rule)

12) What is clonal deletion? How does it differ from clonal anergy?

Immature B cells which undergo high avidity anti-self interactions are signaled to die via apoptosis in a process known as clonal deletion. Anergy, on the other hand, is associated with lower avidity self interactions and results in the biochemical inactivation, but not death, of the offending cell.