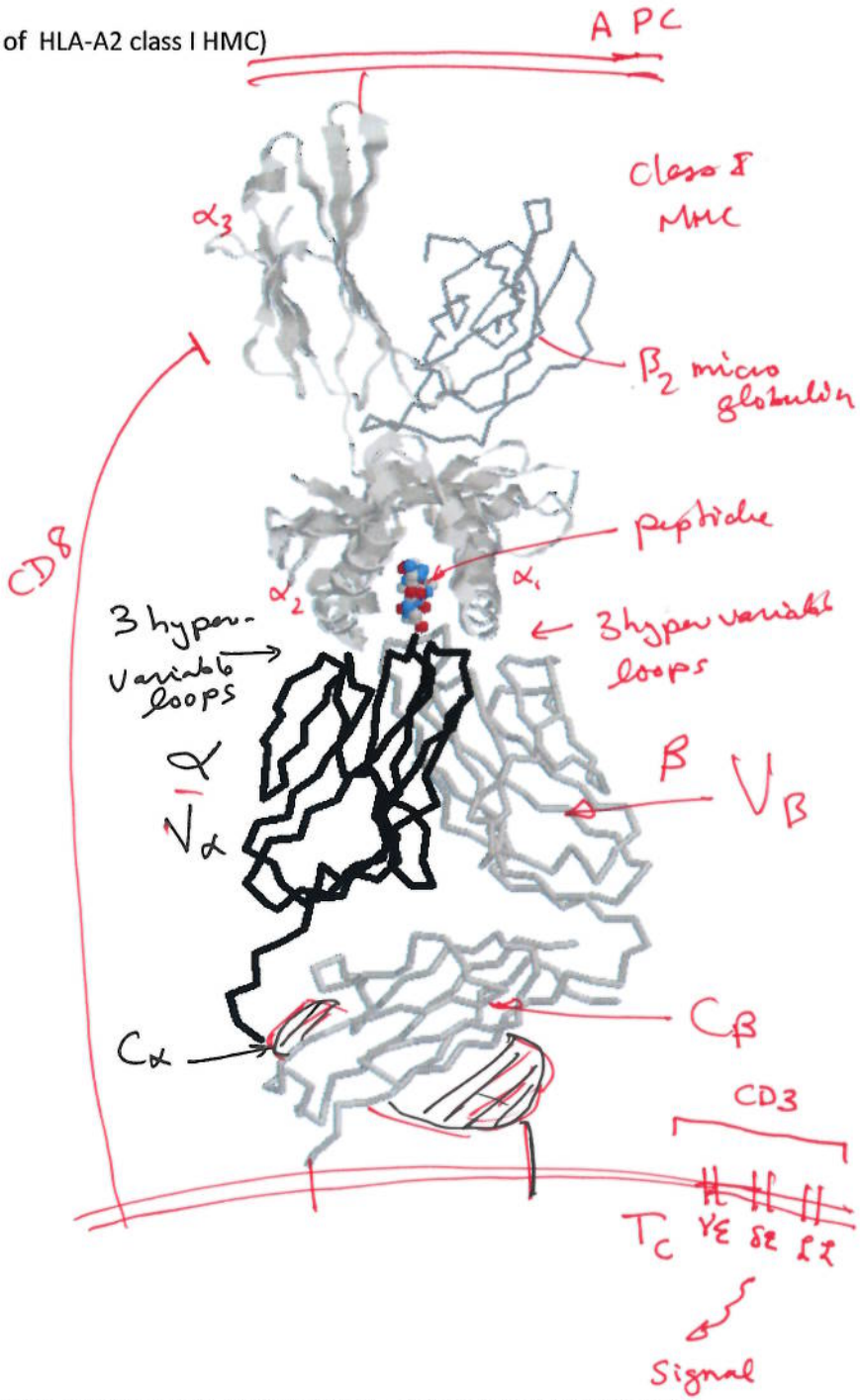
Lecture 13: TCR – Genetics and Structure (Suggested reading Chapter 10)

MHC/TCR/Peptide Complex: Garboczi et al, Nature (1996), 384, 134.
This is a ternary complex consisting of:

- Class I MHC: HLA-A2(A6) (6th allele of HLA-A2 class I HMC)
- HTLV-1 Tax peptide: human T-cell lymphotropic virus.
- αβ-TCR receptor that is specific for this peptide and this allele of HLA-A. Note that the structure of the constant domain of the α-chain was not obtained.

Individuals of this particular haplotype are subject to neurological impairment if infected with the HTLV virus because the nervous system is attacked. (An example where the ability to present antigen may not be a good thing!)



Components of the TCR:

αβ TCells:

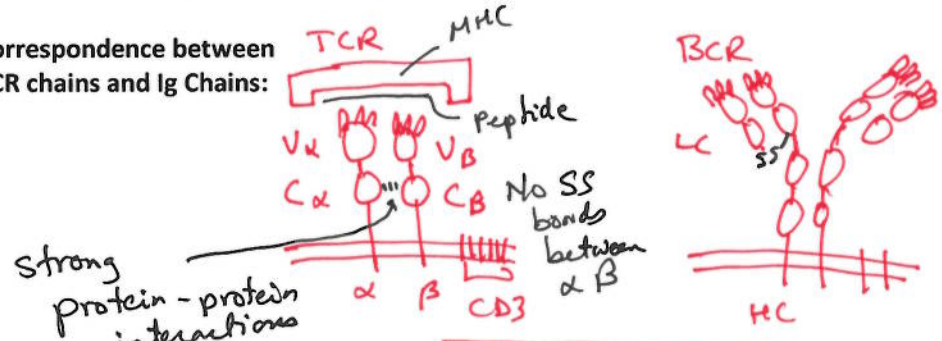
1. αβ-chains (recognize self MHC/peptide complex). Each chain consists of:

i) Variable region (amino terminus), with three hypervariable (also known as complementary determining regions, or CDR) loops. Similar level of diversity as BCR.
ii) Constant region (carboxy - terminus)
iii) Membrane anchor

2. Associated with phosphorylation signaling domain composed of γε δε heterodimer and ζζ homodimer. The collection of signaling chains is referred to as CD3, and is recognized by anti-CD3 antibodies (e.g BiTE antibodies).

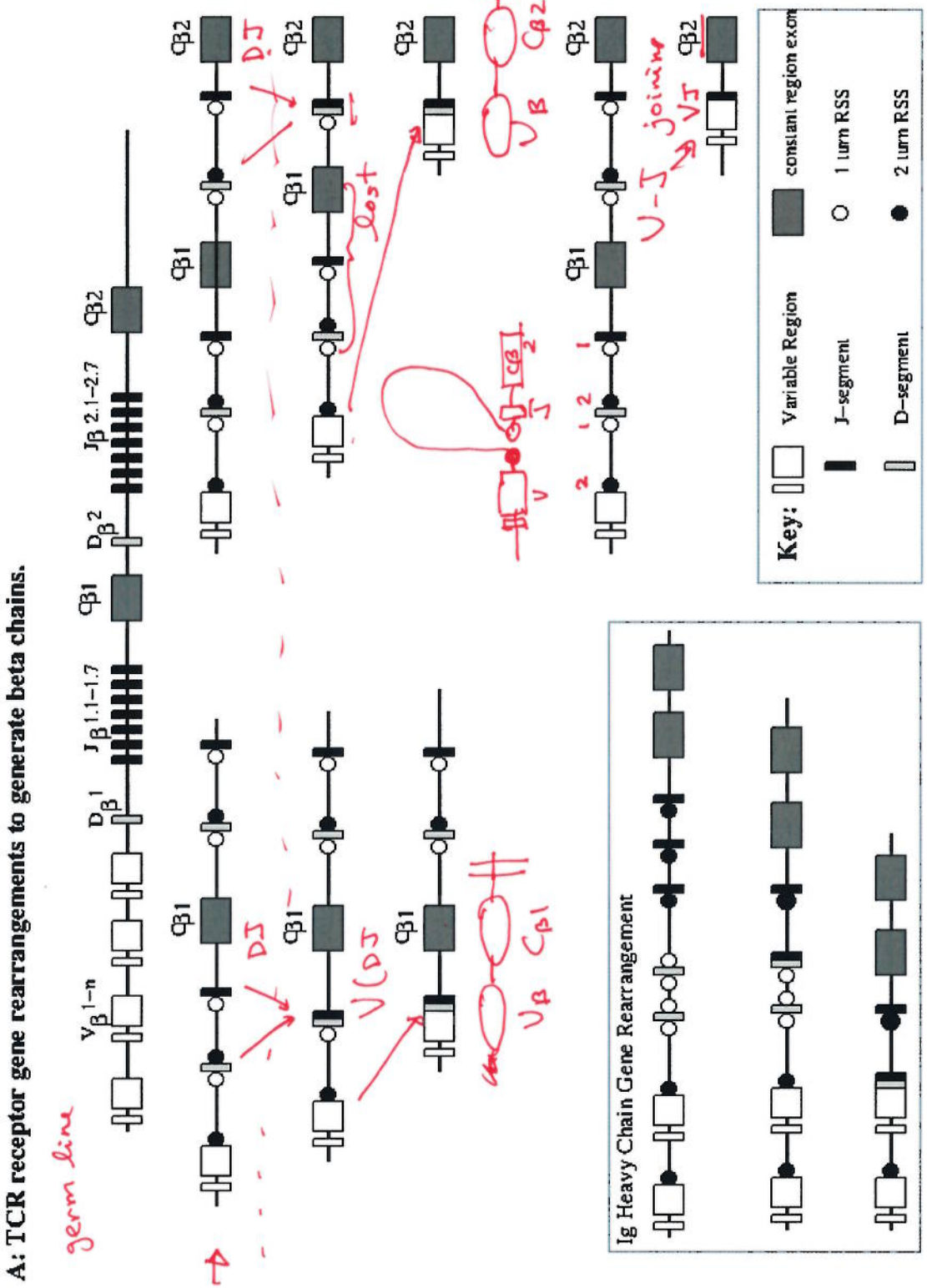
γδ TCells: Contain an alternate form of TCR. This TCR binds other antigens besides peptide-MHC, such as lipids. Although very prevalent in epithelial tissue, their function is not well understood but these may recognize lipid molecules presented on non-classical (e.g. non-peptide presenting) MHC.

Correspondence between TCR chains and Ig Chains:



Genetics of the T-cell Receptor : Joining of segments by RAG1 and RAG2.

Beta Chain: V(DJ) joining



How is the joining of the β Chain different than the Heavy chain (shown in the insert)?

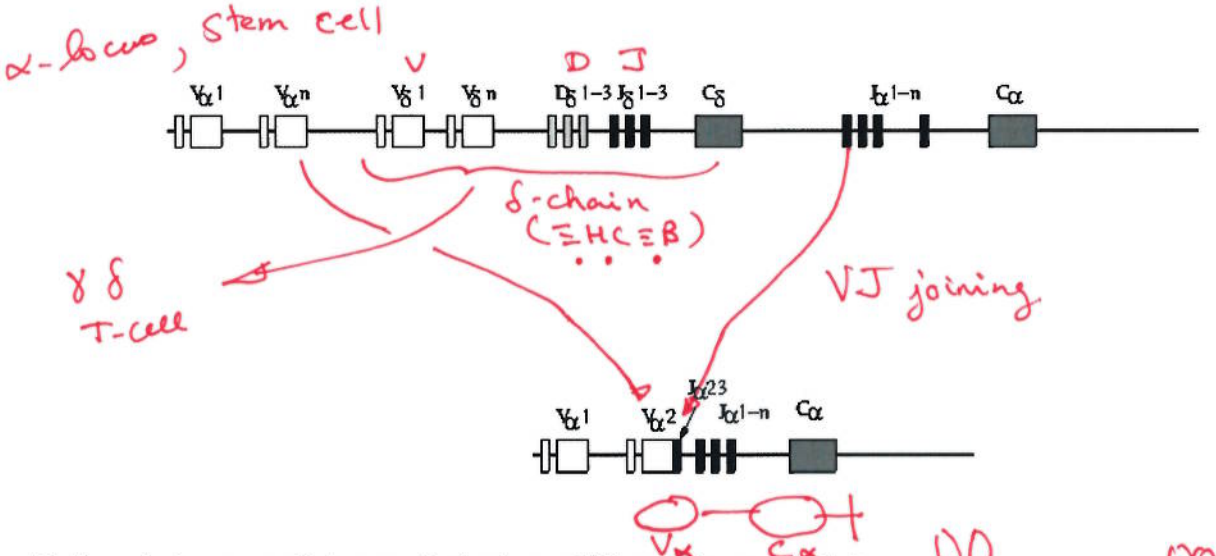
i) two constant. exons

ii) VJ joining is possible

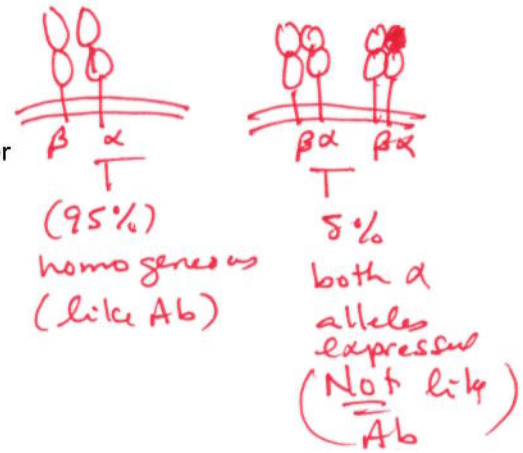
Alpha chain Rearrangement:

1. α and δ are found on the same chromosome. Productive rearrangement of α deletes δ constant region, an αβ T-cell cannot express a γδ TCR.
2. Large number of J segments on α-chain allow additional rearrangements of same allele, i.e. more than one VJ event can occur on one chromosome, increasing the chance that a functional alpha chain is produced.
3. N base addition occurs on the alpha (as well as the beta) chain.

TCR Rearrangements to generate Alpha Chain



Allelic exclusion generally insures that only one TCR receptor specificity is present on any given T-cell. This is not as stringent as in Ig gene rearrangements. There is a more gradual decrease in the activity of the RAG1/RAG2 recombinases such that it is possible to isolate a small number of T-cells with two different α-chains.

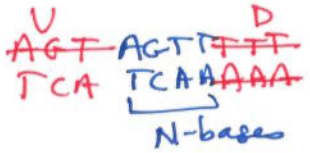


Class switching: No

Affinity Maturation: No

Diversity of the TCR is generated by:

- Combinatorial: Joining of any V to D to J (or V-J for α).
- Deletion of bases during joining by RAG-1/RAG-2 (Junctional flexibility)
- Addition of P-base & N-base nucleotides to ***both*** α- and β-chains.
- Association of almost any α-chain with almost any β-chain.



Comparison of Diversity in Immunoglobulins and TCR:

Mechanism	Immunoglobulins		αβ T-cell Receptor	
Gene Segments	H-chain	κ-Chain	β	α
V	300	300	50	100
D	12	-	2	-
J	4	4	13	60
Combinatorial V-J and V-D-J	300×12×4 $=1.4\times10^4$	300×4 $=1.2\times10^3$	50×2×13 $=1.3\times10^3$	100×60 $=6\times10^3$
Junctional Flexibility	+ (×9) VDJ	+ (×3) VJ	+ (×20) VDJ & VJ	+ (× 3) VJ
P-base addition	+ (×9)	+ (×3)	+ (×9)	+ (×3)
N-base addition (TdT)	+ (×9)	- (×1)	+ (×9)	+ (×3)
# Chains	~10^7	~10^4	10^6	~10^5
Estimated Diversity	10^{11}		10^{11}	

(×*n*) indicates the number of different sequences generated by this event, e.g. N-base addition is estimated to generate 9 different amino acid sequences to the Ig heavy chain.

TCR-HMC-Peptide Interactions: Crystal structure shows the following interactions between the TCR and MHC.

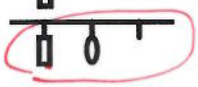
Very nice structure, but how do we prove that this structure is biologically relevant?

Testing the biological relevance of this model:

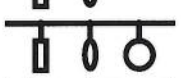
T_H-cell line was generated that was specific for moth cytochrome c (MCC) residues 88-103 in the context of IE^k. This T_H cell line would recognize the MCC peptide when bound to the IE^k allele of type E class II MHC. Alteration at positions 95, 99, or 102 within the peptide could not activate an immune response. How to determine which positions interact with the MHC and which interact with the two different chains of the TCR?

MCC Sequence: **88-K-F-D-103**

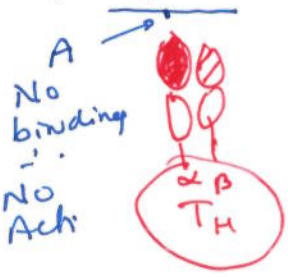
(1) **Testing MHC Interaction:** MHC binding measured by immobilizing IE^K in a plastic well. Radioactive MCC was added, along with other non-labeled peptides and the amount of bound radioactivity was measured. *Conclusion?*

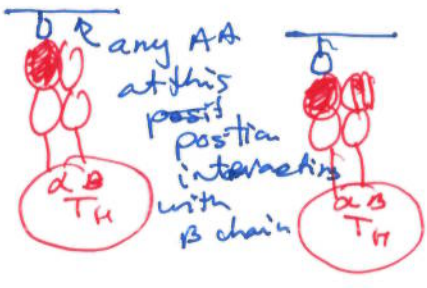
Peptide (unlabeled)		**Radioactivity**
None		100%
-K-F-D-(MCC)		10%
-A-F-D-		100%
-K-A-D-		10%
-K-F-A		10%

(2) **Testing TCR Interaction:** Transgenic mice, expressing *only* the α-chain of the TCR that recognized MCC-IE^k were produced. These mice produce T-cells with most of the α chain from the transgene. The β chain undergoes normal VDJ joining, i.e. there are 10^6 different β chains.

Peptide		**T-Cell Act.**
-K-F-D-(MCC)		100%
-K—A-D-		10%
-K-F-A-		**100%**
-K-F-W-		**100%**
-K-F-R-		**100%**

If you sequenced the **β-chain** gene from mice that were activated (last 3 sequences), what would you expect to find?





[redacted] HC AA AB BB BA (Same)

03 390 Immunology October 19, 2016

Oli Q5 Questions

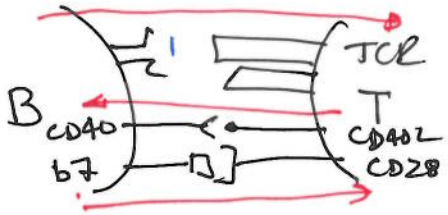
1. If allelic exclusion only happened for the light chain (i.e. both light chains are identical), but not the heavy chain, you would find 3 different antibodies on the surface of a B-cell.

2. DNA rearrangements can/may occur during what stages of B-cell development (select all that apply).

   A. ☑ VDJ joining in the heavy chain.
   B. ☑ VJ joining to generate a light chain that can bind to the heavy chain.
   C. ☑ Failure of self-tolerance to membrane antigens. (New L.C.)
   D. ☐ Affinity maturation. (Single site mutations)
   E. ☑ class switching.
   F. ☐ failure of self-tolerance to soluble antigens. (Anergic B-cell)

3. Which signal directly causes cytokine release from T_H cells, resulting in B-cell proliferation.

   A. ○ CD40 on the B-cell interacting with CD40L on the T-cell.
   B. ◉ B7 on the B-cell interacting with CD28 on the T-cell.
   C. ○ LFA on the T-cell interacting with ICAM on the B-cell.
   D. ○ MHC II-peptide complex binding to the TCR on the T_H-cell.
   E. ○ B7 on the B-cell interacting with LFA (leukocyte functional antigen) on the T-cell.



4. Which of the following items are important features of affinity maturation that select for B-cells that have a higher affinity antibody (select all that apply).

   A. ☑ increased capture of antigens by the BCR receptor.
   B. ☑ increased presentation of antigens on MHC II.
   C. ☑ increased stimulation of T_H cells.
   D. ☑ increased proliferation of B-cells.
   E. ☐ increased secretion of antibody by resultant plasma cells.

5. After production of a functional light chain, further rearrangements of light chain genes may occur

   A. ○ if the antibody recognizes soluble self-antigens in the bone marrow.
   B. ◉ if the antibody recognizes membrane associated self-antigens in the bone marrow.
   C. ○ during somatic hypermutation to increase the affinity of the antibody.
   D. ○ during class switching.

6. Once a B-cell produces antibodies of the IgG3 class, it can no longer produce antibodies of the IgA class. The order of heavy chain constant exons on the chromosome is: ~~IgM-IgD~~-IgG3-IgG1-IgG2b-IgG2a-IgE-IgA. (~~IgM-IgD~~ removed by 1ST class switch; IgA still on chromosome)

   A. ○ True
   B. ◉ False

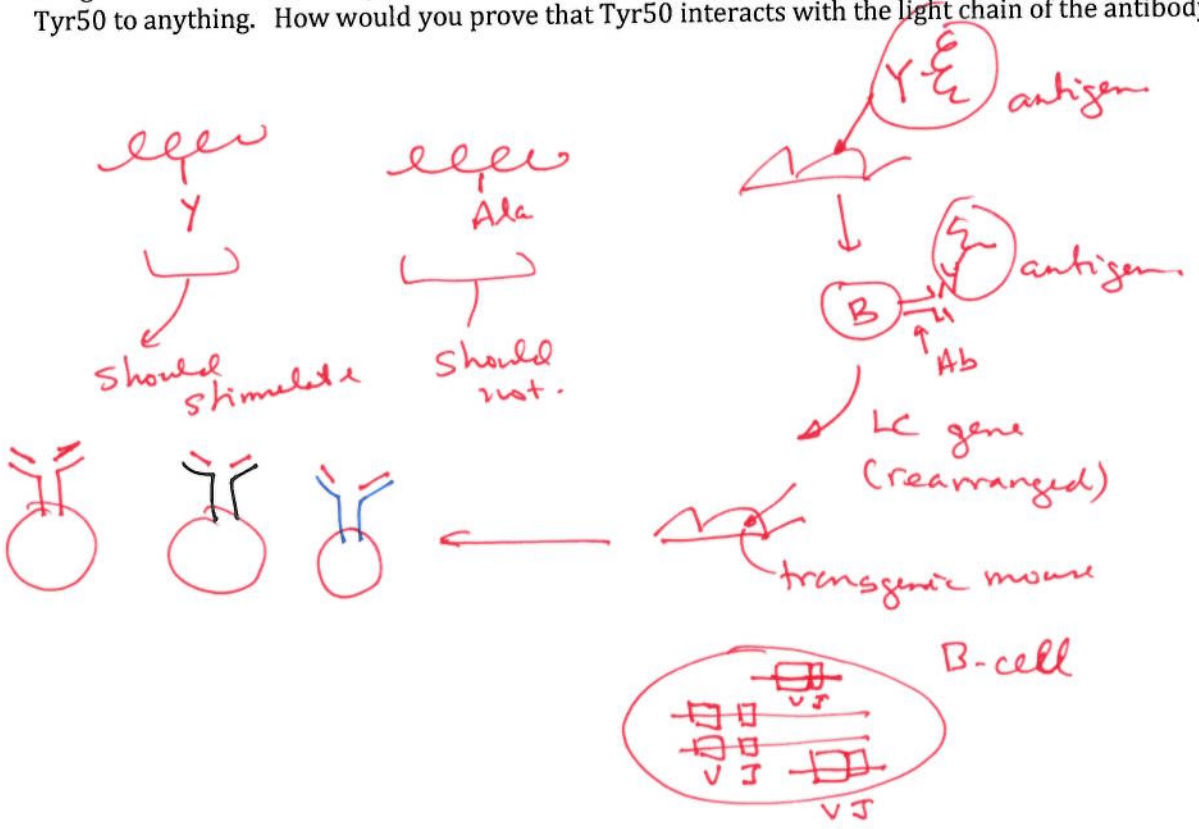
7. Affinity maturation (somatic hypermutation) is an important mechanism to increase the diversity of the immune response.

   A. ○ True
   B. ◉ False

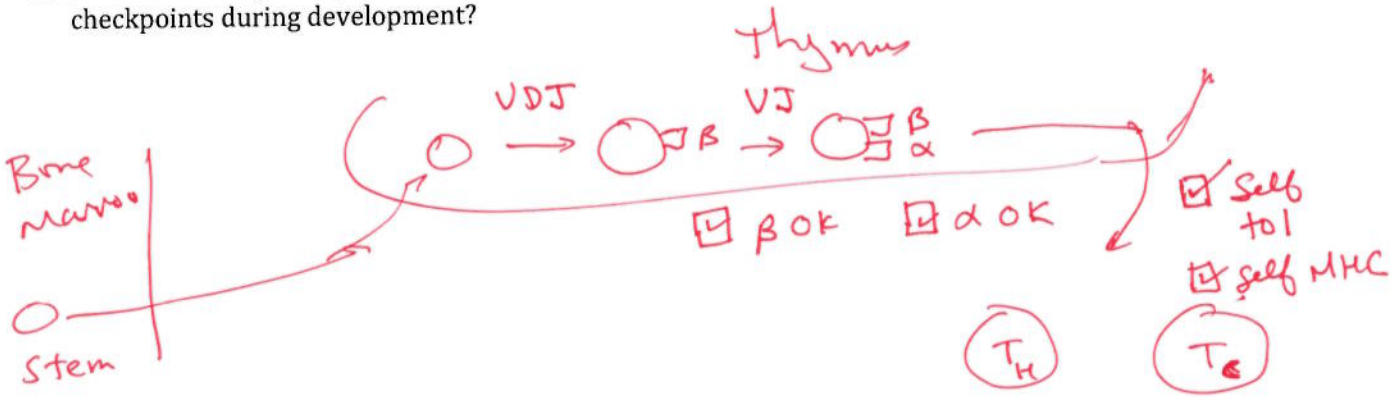
8. The interaction of CD40L on the T-cell with CD40 on the B-cell results in (select all the apply):

   A. ☐ Increased levels of MHC II on the surface of the B-cell.
   B. ☐ Increased levels of CD28 on the B-cell.
   C. ☑ Increased levels of B7 on the B-cell.
   D. ☐ Production of cytokine receptors on the T-cell.

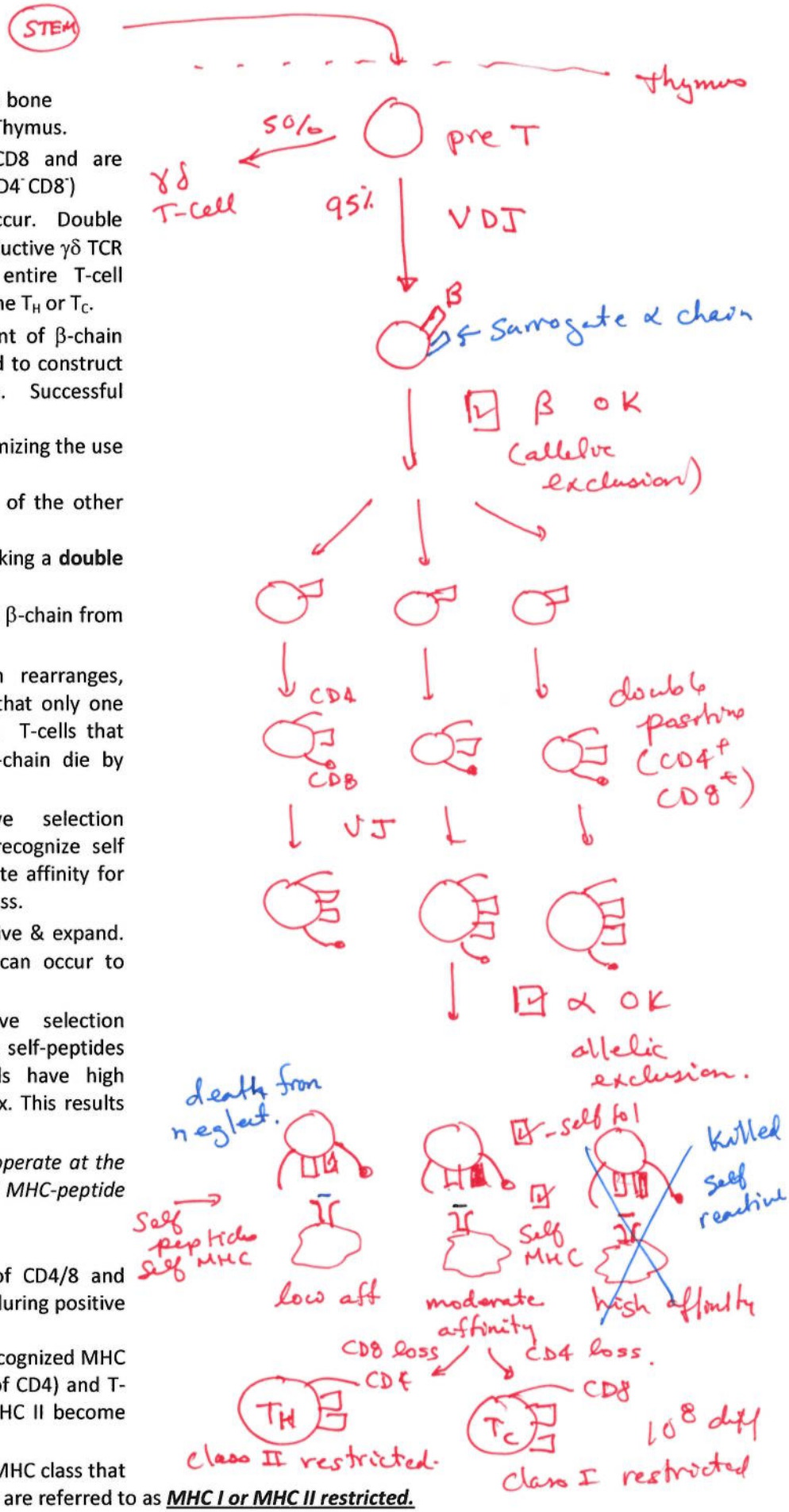
1. You are studying the binding of a protein antigen to an antibody. You suspect that tyrosine50 in your antigen interacts with the light chain. You express the antigen in bacteria so it is easy to change Tyr50 to anything. How would you prove that Tyr50 interacts with the light chain of the antibody?



2. What are the major steps in T-cell development? Where do they occur and what are the key checkpoints during development?



Steps in T-Cell Development



1. Immature T-cells are produced in bone marrow and then migrate to the Thymus.
2. Early T-cells lack CD4 and CD8 and are referred to as **double negative** ($CD4^- CD8^-$)
3. Rearrangement of γδ genes occur. Double negative T-cells that make a productive γδ TCR leave the thymus. ~5% of entire T-cell population, remaining 95% become T_H or T_C.
4. **First checkpoint**: Rearrangement of β-chain occurs. **Surrogate α-chain** is used to construct the TCR using the β-chain. Successful rearrangement of β causes:
   a) expansion of the clone, maximizing the use of a functional β–chain.
   b) inhibition of rearrangement of the other allele (**allelic exclusion**).
   c) Expression of CD4 & CD8 making a **double positive T-cell.**

   Failure to generate a functional β-chain from either allele leads to apoptosis.
5. **Second checkpoint:** α-chain rearranges, allelic exclusion usually insures that only one allele is successfully rearranged. T-cells that cannot produce a functional α-chain die by apoptosis.
6. **Third checkpoint:** Positive selection eliminates T-cells that cannot recognize self MHC – TCR has to have moderate affinity for self-MHC to allow T-cell to progress.

   T-cells that recognize self-MHC live & expand. Rearrangement of 2^{nd} α-allele can occur to rescue T-cell (receptor editing).
7. **Fourth checkpoint**: Negative selection eliminates T-cells that recognize self-peptides on self-MHC. Eliminated T-cells have high affinity for MHC-peptide complex. This results in ***self-tolerance***.

   Positive/Negative checkpoints operate at the same time, based on combined MHC-peptide affinity.
8. T-cells lose CD4 or CD8 by :

   Random model: random loss of CD4/8 and subsequent loss of the T-cell during positive selection if it can't bind MHC.

   Instructive model: T-cells that recognized MHC I become $CD8^+$ T_C cells (loss of CD4) and T-cells that recognized class MHC II become $CD4^+$ T_H cells (loss of CD8).

   Loss of CD4 or CD8 restricts the MHC class that T-cells can bind to, mature T cells are referred to as ***MHC I or MHC II restricted.***

Predict the outcome of the following experiments:

A. If the class I MHC genes are removed (knocked out), what type of T-cells will be produced? Why?

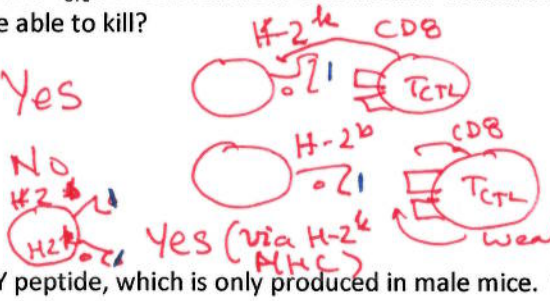
T_H produced
T_C lacking → no stimulation of $CD4^+CD8^+$ T cells that bind to MHC I

B. A H-2^k mouse is infected with a virus and T_{CTL} are isolated from that mouse. Which of the following infected liver cells would these T_{CTL} be able to kill?

i) Liver cells from an H-2^k mouse? Yes

ii) Liver cells from an H-2^b mouse? No

iii) Liver cells from an H-$2^{k/b}$ mouse? Yes (via H-2^k MHC)



C. A female mouse was injected with H-Y peptide, which is only produced in male mice. The *rearranged* genes for the αβ TCR from this mouse were inserted into a mouse embryo to make a transgenic mouse. These mice were bred to produce both male and females pups that expressed the H-Y peptide specific TCR.

i) How many different TCRs would you find on $CD4^+CD8^+$ cells in the pups (careful this is tricky)?

→ α β
homogenous, recognize H-Y peptide

ii) The table on the right shows the number of T-cells found in male and female pups, both early in T-cell development ($CD4^+CD8^+$) and those T-cells emerging from the thymus. Explain these data.

	Male	Female
$CD4^+CD8^+$ pre T-cells	+	+
$CD8^+$ mature T-cell	-	+

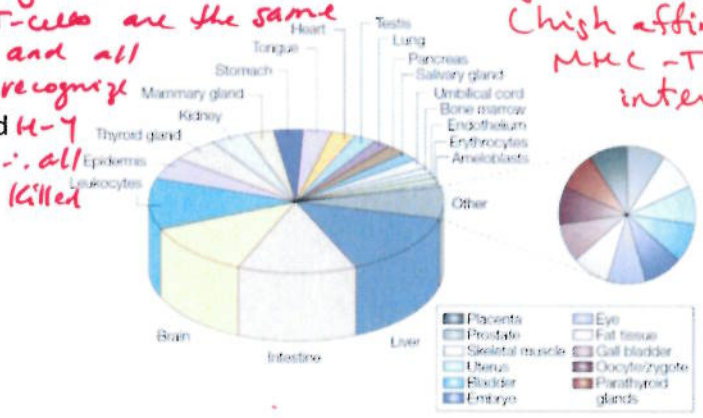
all eliminated because H-Y peptide + in males → self reactive T-cell (high affinity MHC-T cell interaction)

H-Y peptide is expressed on mTEC cells in male mice. Any T-cell that recognizes H-Y will be killed. In the transgenic mouse all T-cells are the same and all recognize H-Y ∴ all killed

How does self-tolerance occur? Medullary thymic epithelial cells (mTEC) express most of the proteins found in the entire organism, e.g. they produce insulin, which is usually produced only in the pancreas.

Fezf2 – classical transcription activator, activates many genes in mTEC cells.

Aire (Autoimmune regulator of expression) – binds to unmethylated histones to uncondensed chromatin.



The number of mTECs (~10^5) is small relative to the number of thymocytes that are processed daily. In addition, the level of antigen expression varies from one mTEC to another. *How are T-cells efficiently trained?*

- hand off of antigens from mTECs to local DCs that can present peptides to developing T-cells.
- torturous passage of developing T-cells through the thymus = interaction with many mTECs.