

## T Cell Differentiation

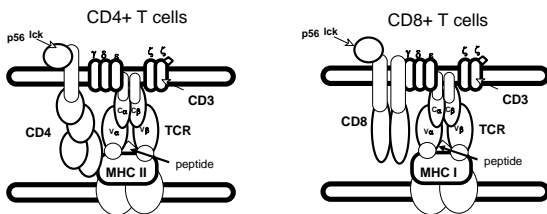
Ned Braunstein, MD

## MHC control of Immune Responsiveness: Concept

Whether or not an individual makes an immune response to a particular antigen depends on what MHC alleles an individual has.

- Example – Hepatitis vaccination
- Example – autoimmune disease – eg: RA

Today's Lecture is Sponsored by the Molecules CD3, CD4, CD8, TCR, & MHC



## MHC control of Immune Responsiveness: Mechanisms

MHC genes control immune responsiveness in 2 ways:

- Peripheral effects – peptide binding
- Central effects – repertoire selection in thymus

## Overview

## MHC control of TCR Repertoire Selection: Concept

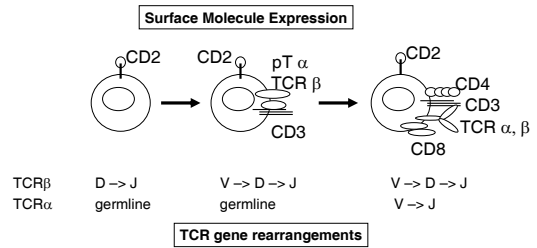
Individuals each express a unique combination of MHC alleles

These different MHC alleles constitutively bind and are expressed with different self-peptides

In the thymus, the individualized expression of MHC/self-peptide complexes results in the selection of an individualized repertoire of TCRs expressed by mature T cells

## Molecular Details

### Differentiation of $\alpha\beta$ T cells in the Thymus



### What happens in the thymus?

Ordered TCR gene rearrangement and TCR expression

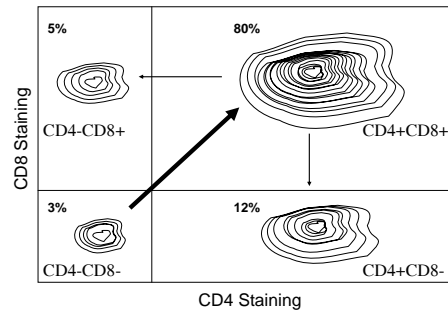
Ordered expression of surface molecules:

- CD2
- CD4 and CD8
- CD3 and the TCR

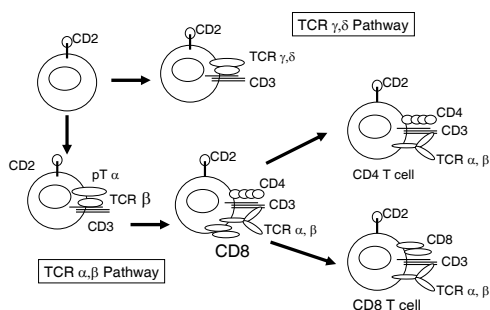
Thymocyte Education: Selection of the T cell repertoire

- Negative Selection
- Positive Selection

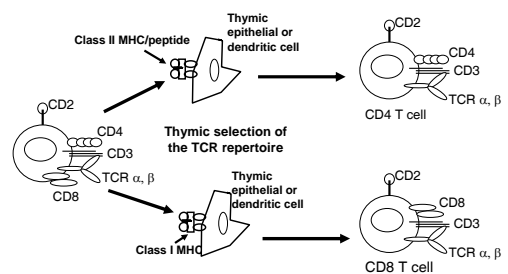
### TCR repertoire selection and thymocyte differentiation into CD4+ or CD8+ T cells



### Differentiation Pathways in the Thymus



### TCR repertoire selection and thymocyte differentiation into CD4+ or CD8+ T cells



### TCR repertoire selection and differentiation into CD4+ or CD8+ T cells

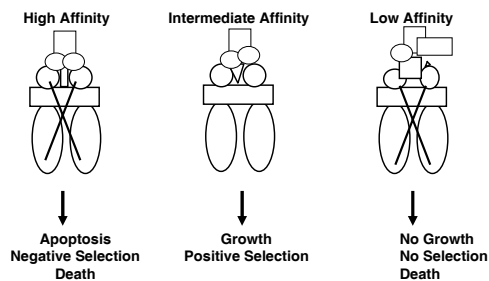
- Interaction of the TCR expressed on CD4+, CD8+ (double positive) thymocytes with MHC class I/peptide complexes or MHC class II/peptide complexes expressed on thymic epithelial or dendritic cells selects the TCR repertoire and dictates differentiation into either CD4+ or CD8+ (single positive) T cells
- High affinity Interactions of the TCR with MHC/peptide complexes leads to thymic cell apoptosis and death; very low affinity interactions does not give sufficient signals for differentiation and these thymocytes also die.
- The only double positive thymocytes that survive and further differentiate into CD4+ or CD8+ T cells are cells with TCRs which interact with intermediate affinity to epithelial or dendritic cell MHC/peptide complexes.

### Questions

How can we demonstrate that the MHC molecules in the thymus determine the repertoire of T cells that develop in the thymus?

- Bone marrow chimera experiments
- TCR transgenic mice

### Affinity of the interaction between TCR and the MHC/self-peptide complex dictates thymic selection



### Bone Marrow Chimeric Animals

- Irradiate host animal (1) and reconstitute with bone marrow from donor animal (2)
- T cells and APCs (B cells, DCs, macrophages) express MHC of the donor (2)
- Other cells (eg: thymic epithelium) express MHC of the host (1)

### Operational Demonstrations of Thymic Selection

### Question

Is the T cell repertoire determined by MHC genes expressed by bone marrow-derived cells or is it determined by MHC genes expressed in thymus?

**Thymic education:  
MHC genotype of host thymus determines  
the Immune response (Ir) repertoire of T cells**

Ir phenotypes of different experimental animals					
Antigen	A <sup>a</sup>	B <sup>b</sup>	(A x B)F <sub>1</sub>	(A x B)F <sub>1</sub> →A	(A x B)F <sub>1</sub> →B
1	++	---	++	++	---
2	---	++	++	---	++
3	++	++	++	++	++
4	---	---	---	---	---

++ = high responder, --- = low responder

\*Strain A and Strain B are highly inbred congenic mouse strains that differ only at the MHC allele expressed; A or B

**Question**

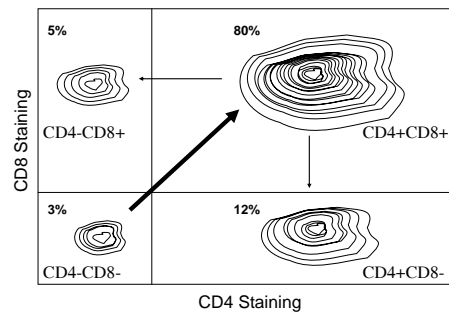
If we clone the DNA encoding the 2C TCR $\alpha\beta$  and inject the genes into eggs from H-2<sup>b</sup> mice, what happens to the T cells as they develop in the thymus of female mice?

*(Since the 2C T cell came from a CD8+ T cell in a female H-2b mouse, we would expect that the T cells should mature in the thymus and at least some would mature into CD8+ T cells. Do they all become CD8+ or do some also become CD4+?)*

**Use of TCR Transgenic Animals to Study Thymic Selection**

- Clone the rearranged TCR  $\alpha$  and  $\beta$  genes from a T cell.
- Inject the rearranged TCR genes into a fertilized egg from a mouse that has mutant rag genes.
- The mouse cannot rearrange its own TCR genes. All developing thymocytes will therefore express this TCR.
- Study how alterations in the thymic environment (different MHC genes or peptides) change the developmental fate of this T cell

**Thymocyte differentiation into CD4+ or CD8+ T cells in normal mouse**

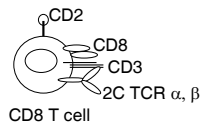


**The 2C cell line**

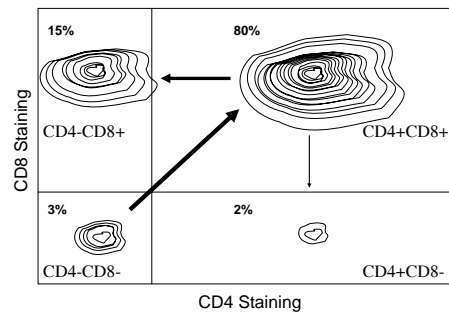
1. CD8 positive
2. Specific for the "H-Y" antigen

This antigen is a peptide derived from some molecule encoded on the Y chromosome

3. Derived from a female H-2<sup>b</sup> mouse by immunization with male cells
4. Restricted by D<sup>b</sup>



**Positive Selection: Differentiation of 2C TCR Tg<sup>ic</sup> Thymocytes in Female H-2<sup>b</sup> mouse**

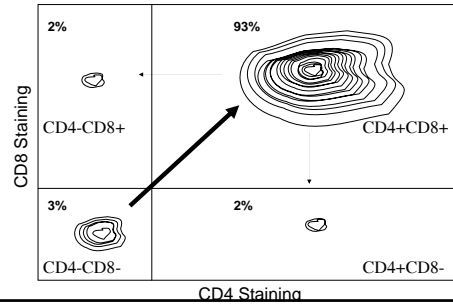


### Question

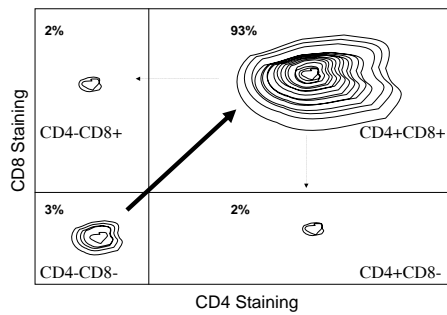
If we clone the DNA encoding the 2C TCR $\alpha\beta$  and inject the genes into eggs from H-2<sup>b</sup> mice, what happens to the T cells as they develop in the thymus of male mice?

*(Since the CD8+ 2C T cell responds to male cells from H-2<sup>b</sup> mice, we would expect that no CD8+ T cells mature. Do any CD4+ T cells mature?)*

### Absence of Positive Selection: No Differentiation of 2C TCR Tg'ic Thymocytes in Female H-2<sup>k</sup> mouse



### Negative Selection: No Differentiation of 2C TCR Tg'ic Thymocytes in Male H-2<sup>b</sup> mouse



### Question

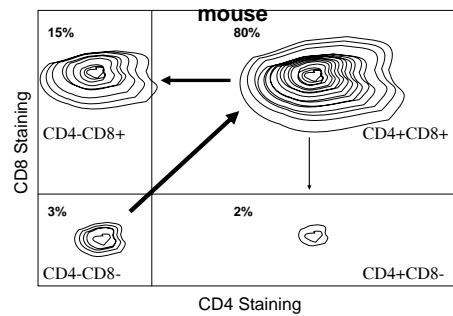
Is the absence of maturation beyond double-positive stage in H-2<sup>k</sup> mice due to negative selection or positive selection?

If we clone the DNA encoding the 2C TCR $\alpha\beta$  and inject the genes into eggs from H-2(k x b) F1 mice, what happens to the T cells as they develop in the thymus of female mice?

### Question

If we clone the DNA encoding the 2C TCR $\alpha\beta$  and inject the genes into eggs from H-2<sup>k</sup> mice, what happens to the T cells as they develop in the thymus of female mice?

### Positive Selection: Normal Differentiation of 2C TCR Tg'ic Thymocytes in Female H-2<sup>b x k</sup> mouse



## Interpretation of 2C Experiments

- In absence of H-2b MHC molecules, (DP) cells expressing the 2C TCR do not receive signals to allow further differentiation (non-selection)
- In the presence of H-2b MHC molecules and non highly-stimulatory self peptides, (DP) cells expressing the 2C TCR receive signals that allow further differentiation into SP cells (positive selection)
- In the presence of H-2b MHC molecules and highly-stimulatory self peptides, (DP) cells expressing the 2C TCR receive signals that cause apoptosis (negative selection)

## Implications of Positive/Negative Selection

- Individuals with different MHC alleles have different TCR repertoires
- T cells mature into CD4 or CD8 single-positive cells as a result of positive selection.

## Summary

1. Interaction of the TCR expressed on CD4+, CD8+ (double positive) thymocytes with MHC class I/peptide complexes or MHC class II/peptide complexes expressed on thymic epithelial or dendritic cells selects the TCR repertoire and dictates differentiation into either CD4+ or CD8+ (single positive) T cells
2. High affinity interactions of the TCR with MHC/peptide complexes leads to thymic cell apoptosis and death; very low affinity interactions does not give sufficient signals for differentiation and these thymocytes also die.
3. The only double positive thymocytes that survive and further differentiate into CD4+ or CD8+ T cells are cells with TCRs which interact with intermediate affinity to epithelial or dendritic cell MHC/peptide complexes.
4. The T cell repertoire is influenced by MHC haplotypes. These determine which peptides will be presented to T cells and the strength of the stimulus to the TCR; therefore they determine which T cells undergo positive or negative selection.