T Cell Differentiation

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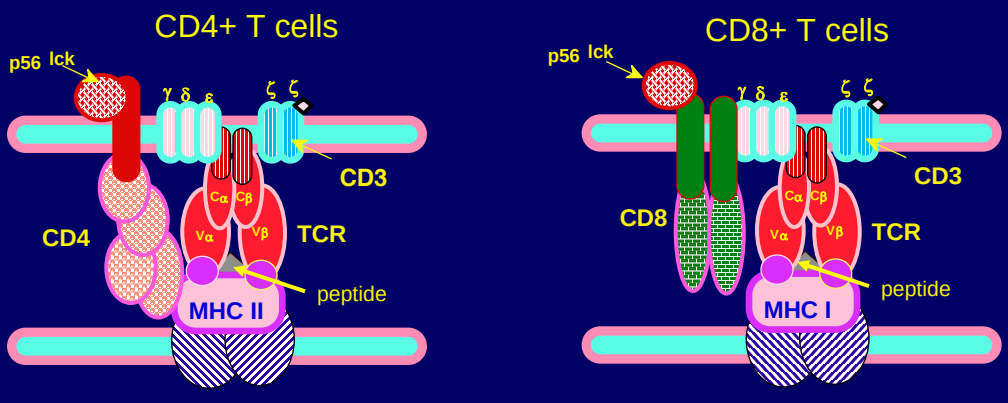
Today's Lecture is Sponsored by the Molecules
CD3, CD4, CD8, TCR, & MHC



Overview

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

MHC control of Immune Responsiveness: Mechanisms

MHC genes control immune responsiveness in 2 ways:

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

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

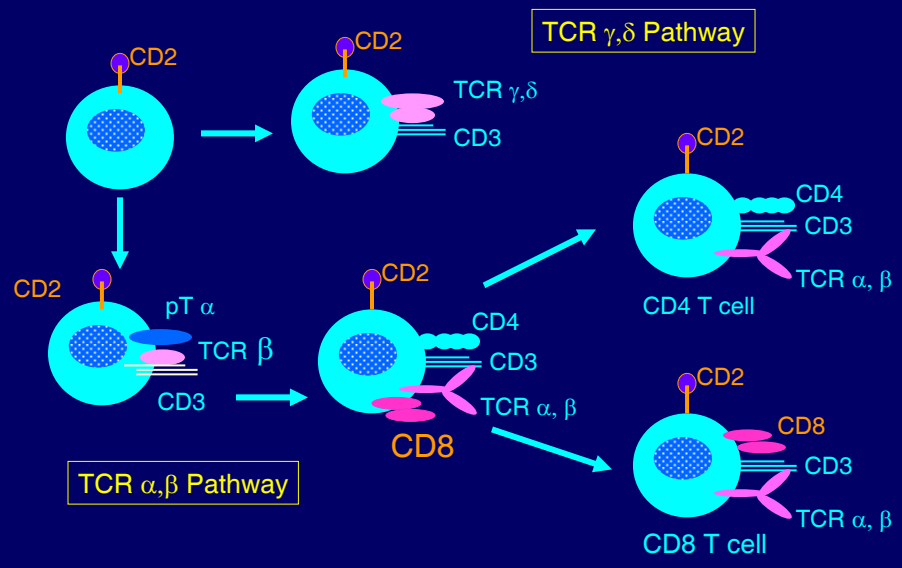
What happens in the thymus?

Ordered TCR gene rearrangement and TCR expression

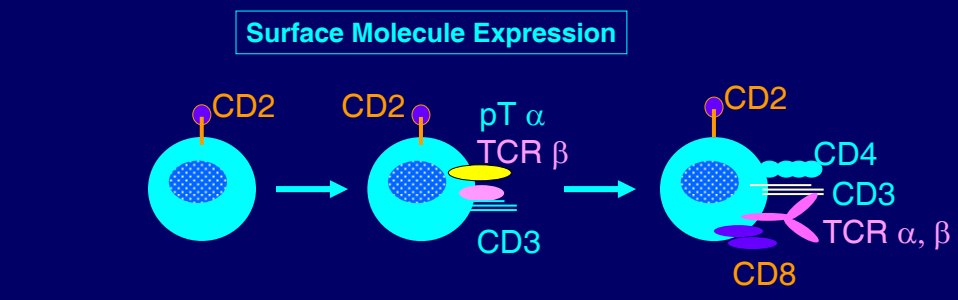
Ordered expression of surface molecules:
- CD2
- CD4 and CD8
- CD3 and the TCR

Thymocyte Education: Selection of the T cell repertoire
- Negative Selection
- Positive Selection

Differentiation Pathways in the Thymus



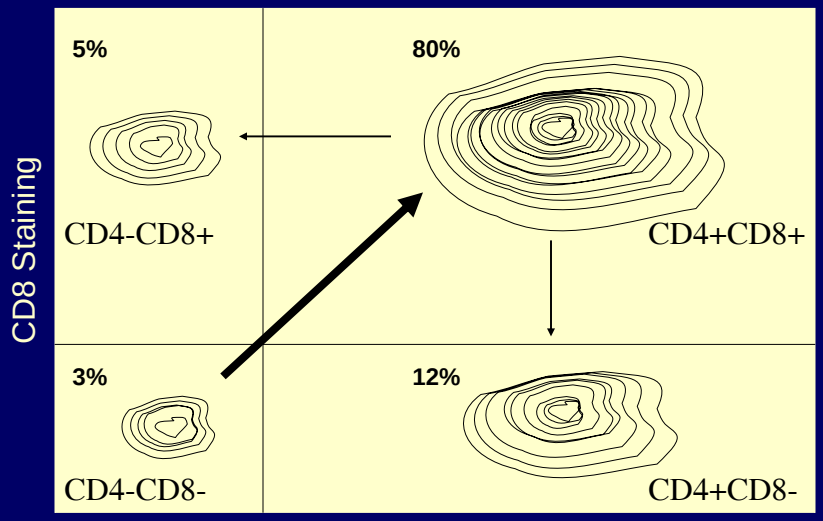
Differentiation of αβ T cells in the Thymus



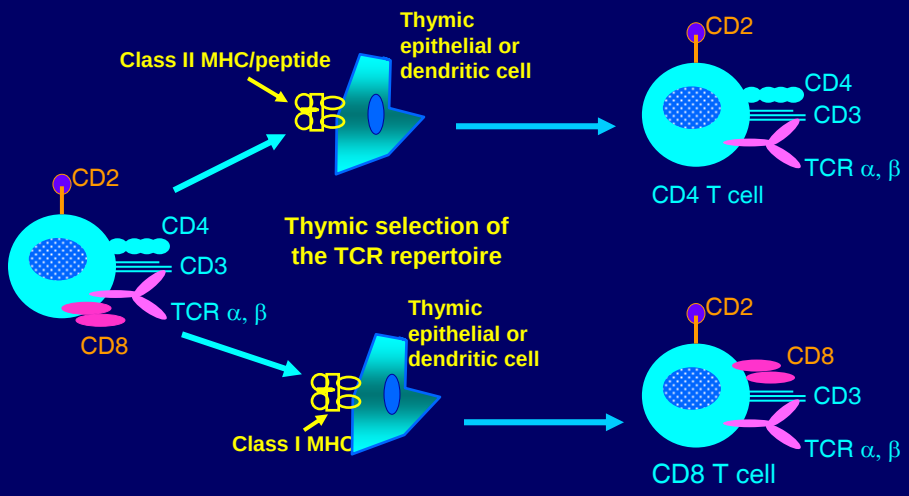
TCRβ	D –> J	V –> D –> J	V –> D –> J
TCRα	germline	germline	V –> J

TCR gene rearrangements

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



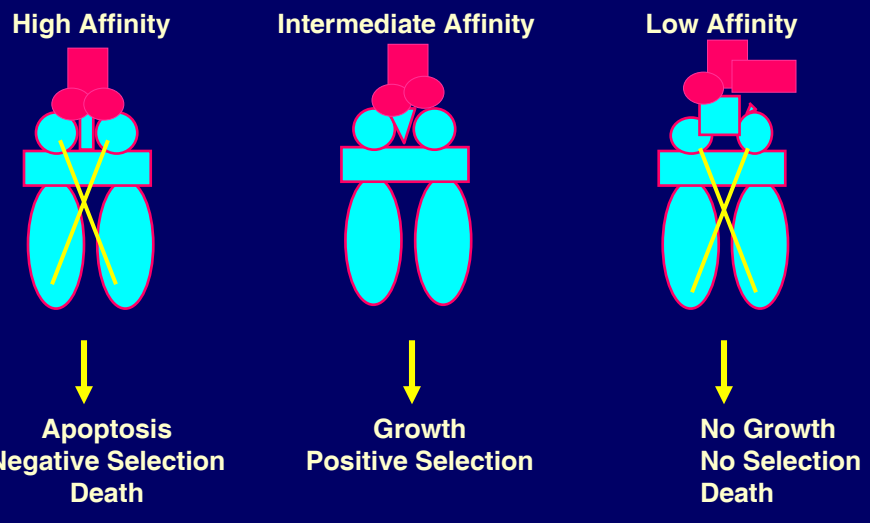
TCR repertoire selection and thymocyte differentiation into CD4+ or CD8+ T cells



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.

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



Operational Demonstrations of Thymic Selection

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

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 (2)

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*	B*	(A x B)F1	(A x B)F1–>A	(A x B)F1–>B
1	++	—	++	++	—
2	—	++	++	—	++
3	++	++	++	++	++
4	—	—	—	—	—

++ = high responder, — = low responder

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

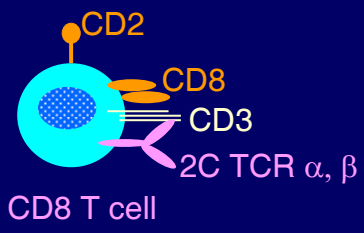
Use of TCR Transgenic Animals to Study Thymic Selection

- Clone the rearranged TCR α and β 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

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*^{b} mouse by immunization with male cells
4. Restricted by D ^{b}



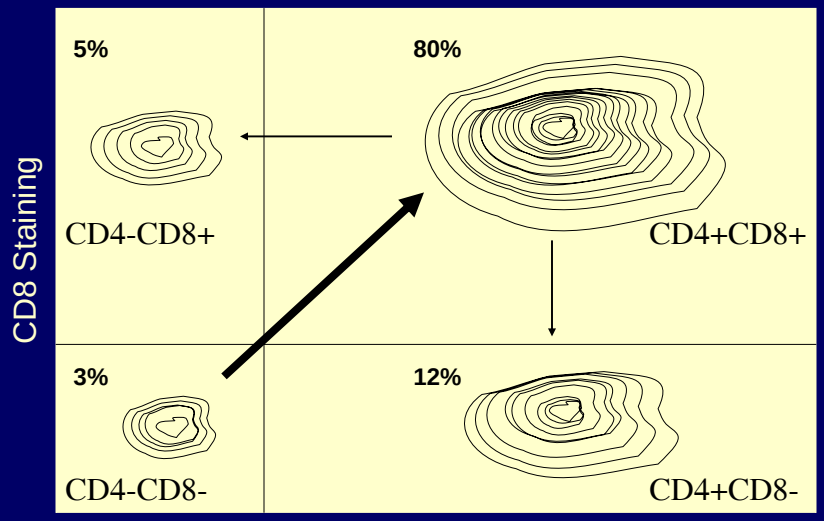
CD8 T cell

Question

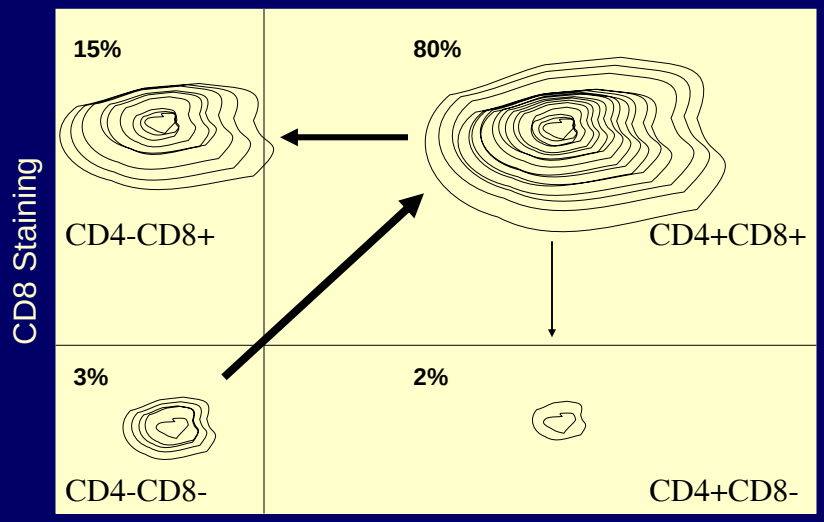
If we clone the DNA encoding the 2C TCRαβ and inject the genes into eggs from H-2^{b} mice, what happens to the T cells as they develop in the thymus of female mice?

(*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+?*)

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



Positive Selection: Differentiation of 2C TCR Tg'ic Thymocytes in Female H-2^{b} mouse

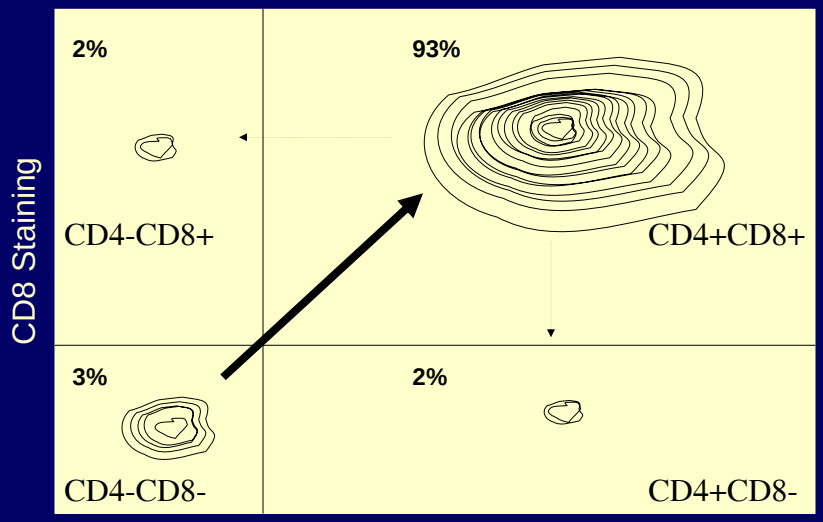


Question

If we clone the DNA encoding the 2C TCR$\alpha\beta$ and inject the genes into eggs from H-2^b 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^b mice, we would expect that no CD8+ T cells mature. Do any CD4+ T cells mature?*)

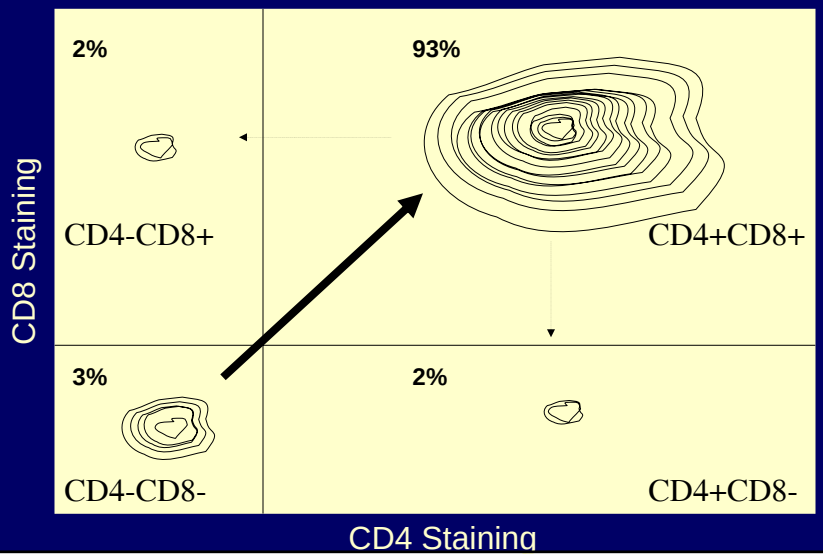
Negative Selection: No Differentiation of 2C TCR Tg'ic Thymocytes in Male H-2^b mouse



Question

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

Absence of Positive Selection: No Differentiation of 2C TCR Tg'ic Thymocytes in Female H-2^k mouse

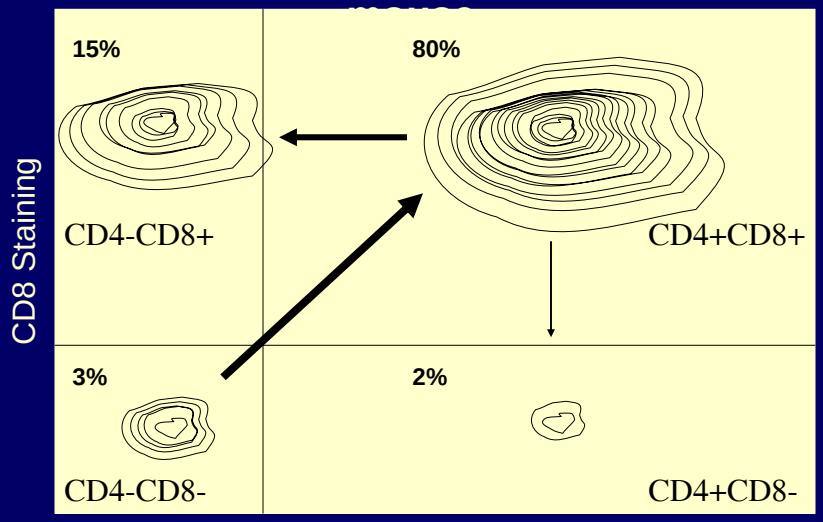


Question

Is the absence of maturation beyond double-positive stage in H-2^k 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?

Positive Selection: Normal Differentiation of 2C TCR Tg'ic Thymocytes in Female H-2^{bxk} 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.