T Cell Differentiation
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Concept – Ir genes
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

Mechanisms – Ir genes
MHC genes control immune responsiveness in 2 ways:
- Peripheral effects – peptide binding
- Central effects – repertoire selection in 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

Differentiation Pathways in the Thymus

Differentiation of αβ T cells in the Thymus

TCR αβ Pathway
TCR γδ Pathway

Surface Molecule Expression

TCR αβ D → J
V → D → J
germine

TCR αβ V → J
TCR repertoire selection and thymocyte 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 do 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

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 (e.g.: 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?

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-2b mouse by immunization with male cells
4. Restricted by D^b

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

Question

If we clone the DNA encoding the 2C TCRαβ and inject the genes into eggs from H-2b 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+?)
**Positive Selection: Differentiation of 2C TCR Tg'ic Thymocytes in Female H-2b mouse**

If we clone the DNA encoding the 2C TCRαβ and inject the genes into eggs from H-2b 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-2b 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-2b mouse**

If we clone the DNA encoding the 2C TCRαβ and inject the genes into eggs from H-2b 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-2k mouse**

Is the absence of maturation beyond double-positive stage in H-2k mice due to negative selection or positive selection?
Implications of Positive/Negative Selection

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