**T-cell Maturation**

What allows T cell maturation?
- Direct contact with thymic epithelial cells
- Influence of thymic hormones
- Growth factors (cytokines, CSF)

**The earliest T cell precursors in the thymus:**
- **Express Thy-1 (mice)**
- Have not yet rearranged TCR loci
- Do NOT express CD4 or CD8
- Do not express CD3
- Are called "double negatives"

**MARKERS:**
- **C-KIT**: Receptor for Stem Cell Growth Factor
- **CD44**: Adh. Molecule. Homing to thymus
- **CD25**: Alpha chain of IL-2 receptor

**Time Course of Appearance in Thymus**
- Most double negative thymocytes will give rise to αβ T cells (in mice and humans).
- Some (5%) will differentiate into γδ T cells.
- The developmental pathway of γδ T cells is not well defined.
- CD3 expression first appear between DN2 to DN3
- Double negative thymocytes (DN3 stage) undergo $\beta$ chain locus re-arrangement.
- The newly formed $\beta$ chain combines with the Pre-T$\alpha$ (surrogate chain) and CD3 to form the Pre-T cell receptor (Pre-TCR).

Why is Pre-TCR important?
1) Productive TCR $\beta$ chain re-arrangement
2) Signals for proliferation (similar $\beta$ chain) and maturation
3) Suppresses further $\beta$ chain re-arrangement (allelic exclusion)
4) Signals for TCR $\alpha$ chain re-arrangement
4) Induces development of CD4+8+ (double positive) stage

- After $\beta$ chain re-arrangement is completed the DN3 cells progress to DN4.
- Both CD4 and CD8 are expressed = double positive (DP) cells.
- Rapid proliferation occurs
- After proliferation of double positive cells stops, $\alpha$-chain locus rearrangement occurs.
- Good: clones with similar $\beta$ chain but potentially different $\alpha$-chain locus rearrangement occurs.
- If a productive rearrangement is made, an $\alpha\beta$ TCR is expressed on the cell surface.
- Cells undergo positive and negative selection.
- Those that fail either selection undergo apoptosis.
- Those that pass the selection step lose EITHER CD4 or CD8 becoming "single positives"
- These mature single positive cells leave the thymus.

Positive and Negative selection of T cells: GOAL—to recognize foreign Ag combined with self MHC molecules!!!
- Positive selection: occurs in the cortex and allows only those T cells that are able to bind to self-MHC molecules in the thymus to mature
- Negative selection: occurs in the medulla and removes T cells whose TCR strongly recognize (high affinity) self-MHC (with self-antigen). Die of apoptosis within the thymus.

Positive selection results in MHC restriction.

Negative selection results in self-tolerance (to some extent).

Positive selection:

Negative selection:
Summary:
MHC Restriction Self Tolerance

T cell maturation

THYMUS
- Thy-1, CD44, c-Kit, CD25
- CD3
- TCR β-chain
- Pre-Tαα αα αα
- TCR αα αα αα
- CD4
- CD8

Double Positive
- Expression of CD4 or CD8 is switched off randomly
- Only thymocytes with ON co-receptor and correct peptide+ MHC complex will mature

Summary of T cell maturation (αβ T cells only)
- Thymocytes enter the thymus as "double negative" (markers?)
- pre-Ta (surrogate chain) induces β-chain rearrangement (apoptosis of cells that fail to rearrange β chain correctly)
- Expression of pre-TCR (surrogate α chain)
- Expression of CD4 and CD6 (to form "double positive" thymocytes)
- Proliferation of similar β-chain clones with surrogate α-chain
- α-chain rearrangement (apoptosis of cells that fail to rearrange α correctly)
- Expression of mature αβ TCR
- Positive and negative selection (death of cells with too low or too high an affinity for self MHC...>99% of thymocytes die within the thymus)
- Loss of either CD4 or CD8
- Migration to periphery of cells that successfully complete these steps

Model to Explain CD4/CD8 Single Positive Cells

- Multiple interactions between TCR, CD8 or CD4 with MHC-I or MHC-II will deliver the "right signal" (strength?) and instructs the cell to differentiate.

TABLE 10-I
Effect of class I or II MHC deficiency on thymocyte populations

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Control mouse</th>
<th>Class I deficient</th>
<th>Class II deficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4⁺CD8⁻</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CD4⁺CD8⁺</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CD4⁺</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CD8⁺</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*Plus sign indicates normal distribution of indicated cell types in thymus. Minus sign indicates absence of cell type.
Activation

Signals through the TCR, CD3 and CD4/8 activate a protein tyrosine kinase (PTK) cascade

- **PTK** = enzyme that phosphorylates the amino acid **tyrosine (Y)** in ITAMs

- **PHOSPHATASES** = enzymes that remove inhibitory phosphates

![Diagram of TCR, CD3, and CD4/8 activation](image)

Signals through the TCR, CD3 and CD4/8 activate a PTK cascade

- CD4/8 are associated with a cytoplasmic tyrosine kinase enzyme – **Lck** (lymphocyte kinase)
1. TCR-MHC-Peptide activates the phosphatase **CD45**
2. **CD45** activates **Fyn** and **Lck**
3. Activated Fyn and Lck phosphorylate ITAMs in CD3 chains
4. Phosphorylated ITAM motifs on the CD3zz chains become a docking site for the PTK **ZAP-70**.
5. Binding of CD4 to MHC allows Lck to phosphorylate and activate ZAP-70 to become an active PTK.
- Activation of ZAP-70 initiates a cascade of events that results in phosphorylation of ADAPTOR molecules such as Linker of Activated T cells (LAT) activation and phosphorylation (activation) of phospholipase C (PLCγ1).

- PLCγ1 converts phosphatidylinositol 4,5-bisphosphate (PIP₂) to diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP₃):  
  \[
  \text{PIP}_2 \rightarrow \text{DAG and IP}_3
  \]

- IP₃ triggers the release of calcium from intracellular storage vesicles into the cytosol, thus raising cytoplasmic calcium levels.

**The calcium:**

1. synergizes with DAG to activate protein kinase C (PKC)
2. PKC activates the transcription factor NF-κB
3. and PKC activate IkB kinase (IKK), which phosphorylates IkB, releasing the transcription factor NF-κB – which translocates to the nucleus.
4. acts together with calmodulin to activate calcineurin (phosphatase)
5. Calcineurin activates the cytoplasmic component of the transcription factor NFAT (NFATc), causing it to translocate to the nucleus, where it combines with NFATn

**TRANSCRIPTION FACTORS:** lead to gene transcription, cell proliferation and differentiation.

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**ZAP-70 also phosphorylates and activates LAT and SLP-76**

- SLP-76 binds and activates PLC-γ, GEF and Tec Kinases
- GEF activates the Ras pathway resulting in formation of the Jun/Fos, a component of the AP-1 transcription factor.
- NFATn and Jun/Fos bind to sites in the regulatory region of the IL-2 gene and increase transcription of IL-2.
- The expression of >70 genes is increased within 4 hr of T cell activation.
- The potent immunosuppressive drugs cyclosporin and FK506 act by inhibiting the activation of calcineurin → X NF-AT!

**ADAPTOR PROTEINS - SLP76 (SH₂-domain containing leukocyte protein of 76 KDa and LAT (Linker of activated T cells) are phosphorylated by ZAP-70.**

**ADAPTOR PROTEINS:** 1) Serve as links for proteins, 2) Promote assembly of membrane proteins

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**The interaction of CD28 with B7 sends additional activation signals.**

- CTLA-4 is not initially expressed, but is expressed after T cell activation.
- CTLA-4 has a higher affinity for B7 than CD28.
- Interaction of CTLA-4 with B7 is thought to down-regulate T cell activation.
- There are actually two related molecules: B7.1 and B7.2
During activation of helper T cells by antigen presenting cells, many cell-cell interactions must occur.

- **Signal 1** is the interaction of the TCR with peptide antigen presented on class II MHC (and interaction of CD4 with class II MHC).
- **Signal 2** is the interaction between CD28 (and CTLA-4) on the helper T cell and B7 on the antigen-presenting cell. This provides a costimulatory activation signal.
- If T cells receive signal 1 only, they will NOT be activated by antigen. Instead, they will become anergic i.e. they will become refractory to any subsequent activation by antigen.
- If T cells receive signal 1 and signal 2, they will be activated to participate in an immune response to the antigen.

(If T cells receive signal 2 only, nothing happens.)

### Superantigens

- Proteins produced by pathogens
- Not processed by antigen presenting cells
- Intact protein binds to the \( \beta \) variable region on TCR of T cells and to MHC class II on antigen presenting cells (APC)
- Large numbers of activated T cells and APC release cytokines having pathological effects
**Consequences:**

- Because they cross-link the Vβ domain of the TCR with the Vα domain of the MHC-II, this results in non-specific proliferation and activation.
- Over production of Th cytokine leading to systemic toxicity (IFN-γ, TNF-α) and inflammatory mediators.
- Deletion (negative selection) of thymocytes bearing Vβ domains recognized by the super antigen---- beneficial?

**T cell differentiation:**

- Remember: Naïve T cells continually re-circulate between the blood and lymph system → search for appropriate antigen
- Once activated (Remember signal 1 and 2) → Primary response where T cells proliferate and differentiate into effector and memory T cells.
- CD4 effector T cells can form two subpopulations based on cytokine production: Th1 subset (IL-2, IFN-γ) and Th2 subset (IL-4, IL-5, IL-10)
- Th1: associated with cell-mediated functions inflammation (delayed-type hypersensitivity, activation of CD8 T cells); Th2: associated with B-cell activation.
Suppressor T cells

- The old questions revisited!
- First described in the 70’s made CD8+ the candidate for Ts cells
- Now it is believed that Ts cells are CD4+ cells with the phenotype CD4+CD25+.
- Several potential applications: 1) suppression→ tissue rejection; 2) treatment of allergies or autoimmune diseases; 3) enhance response to vaccines; etc

Cell death (apoptosis)

- Several apoptotic pathways
- Fas-FasL pathways
- Fas and its ligand FasL are induced upon T cell activation

GOAL:
1) Regulation of T cell numbers,
2) Removal of “turned off” T cells

The End