T cell development and TCR signaling
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Why do we need T cells?

Innate
Adaptive

Why do we need T cells?

CD4
CD8

TCR\(\alpha\beta\)

Cells involved in innate immunity (neutrophils & macrophages) can recognize only a limited number of pathogens that share common surface markers:
One cell for multiple pathogens

Cells that mediate adaptive immunity (B & T cells) can recognize a significant and diverse number of pathogens due to the ability to recognize an infinite number of surface markers on microorganisms:
One cell for each pathogen

T cell development

How do mature T cells recognize antigen?
How do T cells recognize antigen?

2 MHC classes to deal with specific pathogens
- Class I - those that replicate in the cytosol
- Class II - those that replicate in endosomes and lysosomes

Each interacts of a distinct subset of T cells

Relationship between TCR (CD3) and co-receptors CD4 and CD8

Interactions of TCR with peptide bound to MHC (Pink = TCR variable region)
- TCR composed of 2 polypeptide chains (α & β), whose variable region mediates binding to peptide/MHC complex
- α & β are coupled to the CD3 complex, which contains 3 dimers responsible for signal transduction
- ~ 30,000 TCR per cell

TCR-Coreceptors (CD4/8)
- CD4 - helper T cells
- CD8 - cytotoxic T cells

Strengthen TCR engagement with MHC/peptide complex
Reduce the amount of MHC:peptide complexes needed for optimal activation of T cells by ~100-fold

Where do T cells develop?

Arise from endo and ectodermal layers of the third pharyngeal pouch and branchial cleft
Colonization of the thymus by bone-marrow derived lymphoid progenitor cells occurs by 8th week of gestation
50 million new thymocytes generated each day, but only 1-2 million (2-4%) leave as mature single positive CD4+ or CD8+ T cells
Permit the developing immune system to recognize "self" to avoid mounting an immune response against them
Importance of thymus in T cell development demonstrated in DiGeorge's syndrome (lack of thymic development resulting in a paucity of T cells)

Cellular composition

Thymic epithelial cells
- express high density of MHC class I and II associated peptides
- role in positive (cortical epith. cells) and negative selection (medullary epith. cells)
- chemoattractant production for thymocyte migration

Thymic dendritic cells and macrophages
- mainly found in the medulla
- role in negative selection
Major Thymocyte Subsets

CD4-CD8- (Double Negative, DN) cells: 3-5% of total thymocytes.
- Contain least mature cells, considerable cell division.
- 2/3rd are triple negative (TN) based on TCR expression, can be further divided based on CD44 and CD25.
- TCRβγ rearrangement occurs at this stage.
- 1/3rd are TCRγδ.

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CD4+CD8- and CD4-CD8+ (Single positive, SP) cells: 5-10% of total thymocytes
- Most are mature cells with high levels of CD3 and TCRαβ.
- CD4:CD8 approx 2:1
- Most SP cells are functionally mature and destined to leave the thymus.

Thymocyte development can be followed by flow cytometry based on surface expression of CD4, CD8, CD44 and CD25.

Double negative (CD3-, CD4-, CD8-) cells are classified based on expression of CD44 and CD25.

DN 1 = CD44+/CD25–
DN 2 = CD44+/CD25+
DN 3 = CD44–/CD25+
DN 4 = CD44–/CD25–

Checkpoint #1 - TCRβγ gene rearrangement and expression of a functional β-chain protein

TCRβ- and β-chain genes are composed of discrete segments that are joined by somatic recombination during T cell development.

V: variable gene segment
D: diversity gene segment
C: constant gene

Omenn’s Syndrome

Omenn’s Syndrome is a rare, inherited disorder (AR) of the immune system that belongs to a family of similar immune deficiencies known as SCID, or Severe Combined Immunodeficiency. Some cases are caused by a lack of RAGs. Specifically affects three types of cells in our immune system: B lymphocytes, T lymphocytes and natural killer cells. Each of these cells plays an important role in fighting infection. Omenn’s Syndrome is the type of SCID that only affects B and T lymphocytes. Natural killer cells are normal in number and function. Fatal within the first few months of life.
preTCRβ signaling

- Involves several family of signaling molecules and ultimately results in cell survival, proliferation, induction of CD4 and CD8 gene expression, TCRβ gene rearrangement, and generation of CD4/CD8 DP thymocytes expressing a mature TCR/CD3 complex.

Lck (p56κ)

- Member of the Src-family of tyrosine protein kinases
- Primarily expressed in lymphoid cells (developing thymocytes and mature T cells)
- Associated with the plasma membrane due to palmitoylation / myristylation
- Known to co-constitutively associate with the cytoplasmic domains of CD4 and CD8
- Interaction with preTCR/CD3 complex
- Recruited to lipid rafts with complex
- Genetic deletion in mice results in a profound block in thymocyte development

Initiation of preTCR signaling

- PreTCR does not directly contribute to intracellular signaling. Role of associated η, δ, ζ chains that form the CD3 complex.
- These molecules form a nucleation center for recruitment of several signaling molecules as a result of phosphorylation of tyrosine residues contained within immunoreceptor tyrosine based activation motifs (ITAMS - YXX(L/V)X6-9YXX(L/V)).

ZAP-70 (Zeta-chain-associated protein)

- Member of the Syk-family of tyrosine protein kinases
- Primarily expressed in T cells (developing thymocytes and mature T cells) and NK cells
- Located in the cytosol and binds to ITAM-P residues of Zeta chains of the CD3 complex.
- Activated by Lck at specific tyrosine residues
- Genetic deletion in mice results in a profound reduction in DP CD4 or CD8 T cells, but normal number of DN and DP thymocytes (Nagai et al, Nature 1995)
- Lack of ZAP-70 and a related kinase Syk results in normal TCRβ chain rearrangement but no transition of DN to DP (Cheng et al, PNAS 1997)

Zap-70 deficiency

Zap-70 deficiency is a rare autosomal recessive form of severe combined immunodeficiency syndrome (SCID), characterized by the absence of CD8+ T cells and by the presence of CD4+ T cells in the peripheral blood that are unresponsive to T-cell receptor (TCR)-mediated stimuli (1-5). Peripheral T cells from affected patients demonstrate defective T-cell signaling and abnormal thymic ontogeny caused by inherited mutations in the TCR-associated protein tyrosine kinase (PTK) ZAP-70 (Elder ME, Science 1994)
Adaptor molecules

**Role:**
- Serve as molecular scaffolds for the recruitment and assembly of numerous intracellular molecules that must be integrated into a complex for successful interpretation of TCR-mediated signaling.

**Composition:**
- Contain modular domains or unique residues that permit protein-protein or protein-lipid interactions.
- **DO NOT** contain any enzymatic or direct effector function
- **DO** bring effectors into close proximity of their targets

**LAT (Linker for Activation of T cells):**
- Expressed in T cells (thymocytes and mature T cells), NK cells, mast cells, platelets.
- Palmitoylated, integral membrane protein that has multiple tyrosine residues that are phosphorylated by ZAP-70.
- Provides multiple docking sites for SH2-containing signaling molecules, targeting them to the plasma membrane.

**Thymocyte development**

**Checkpoint #2**
- 90% Expansion
- 90% Negative selection
- 4% Mature T-cell

**Double Positive (CD4⁺, CD8⁺)**
- Positive selection
- Negative selection
- CD4⁺ or CD8⁺

**Checkpoint #1**
- Molecules critical for completing CHECKPOINT#1
- Blocked by null mutations of genes encoding:
  - CD4⁺-PK (B2R)
  - RAG1 or RAG2
  - CD47 and FYN
  - LAT

**Expressed in T cells (thymocytes and mature T cells) NK cells, mast cells, platelets.**
CHECKPOINT #2: Positive and Negative Selection mediated by the mature TCR αβ Receptor

**Purpose:** To ascertain whether:
- α-chain is functionally rearranged.
- Mature TCR is self-MHC restricted.
- Mature TCR is auto-reactive.

**Consequences:**
- Maturation of thymocyte to functionally competent SP CD4 or CD8 T cell.
- Establishes a self-MHC restricted, non-auto-reactive TCR repertoire with appropriately matched co-receptors and functional potential.

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**Selection as Assessed with TCR Transgenic Mice**

Kisielow and von Boehmer 1988 - HY transgenic mice

HY transgenic mice made by isolating TCR α and β chain cDNAs from CD8+ clone derived from H-2Dβ mouse. This TCR recognizes a male-specific peptide bound to H-2Dβ (MHC class I). So transgenic CD8+ clone will kill H-2Dβ male cells but not H-2Dβ female cells. This CD8+ clone will not kill male cells from H-2Dβ because of MHC restriction. So the thymocyte from which the CD8+ clone was derived was “educated” in an H-2Dβ thymus.

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**Positive Selection vs Negative Selection**

**Avidity Model:**

Avidity depends on the affinity of the TCR-peptide/MHC interaction and the density of the peptide/MHC on the thymic epithelial cell. Avidity determines the strength of signal delivered which dictates the outcome. Stronger signals may mean longer signaling or additional signaling.

**Differential Signaling Model (based on altered peptide ligands)**

Different signals are generated by peptide/MHC complexes that interact with the TCR differently (qualitative vs quantitative).
Summary:

- T cell development and signaling are intricately linked as one cannot occur without the other.
- Developing T cells are programmed to undergo cell death in the absence of TCR signaling (fate for the majority of thymocytes). Thus, TCR signaling promotes survival by regulating gene expression; process that utilizes various intracellular adaptor molecules that localize signaling molecules in the vicinity of the TCR/CD complex.
- preTCR signaling that occurs during the DN to DP transition (checkpoint 1) does not require MHC presentation of antigen, while TCR signaling during the transition of DP to SP T cells (checkpoint 2) does require MHC presentation of self-antigen.
- Purpose of positive and negative selection is to assure that the TCR can distinguish between self antigens (you) and those found on pathogens (i.e. viruses).

Positive and Negative Selection
- Selects appropriate TCR repertoire
- Eliminates autoreactive cells
- Lineage committed to CD4 or CD8