T cell development and TCR signaling

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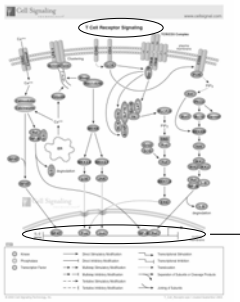
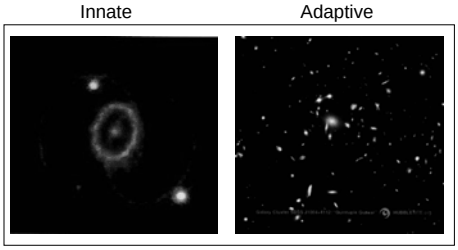


-T cell development
-survival
- effector function

Why do we need T cells ?

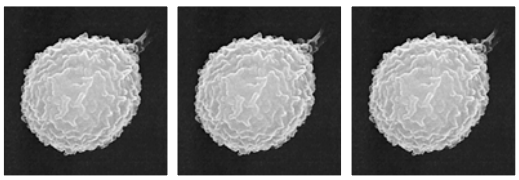
Innate Adaptive



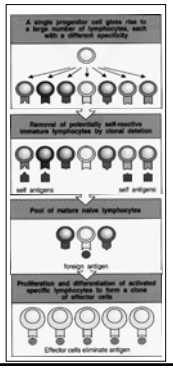
Why do we need T cells ?

TCR$\alpha\beta$ □□□□

CD4 CD8 TCR$\gamma\delta$



T cell development



Why do we need T cells ?

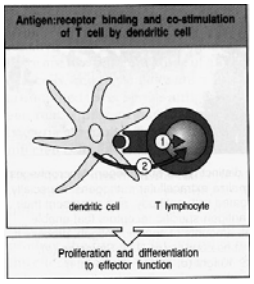
- Cells involved in innate immunity (neutrophils & macrophages) can recognize only a limited number of pathogens that share common surface markers.

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 surface markers on microorganisms .

One cell for each pathogen

How do mature T cells recognize antigen?



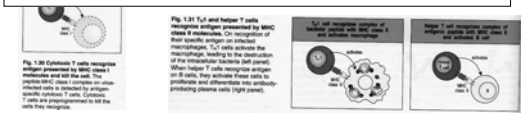
How do T cells recognize antigen?

2 MHC classes to deal with specific pathogens

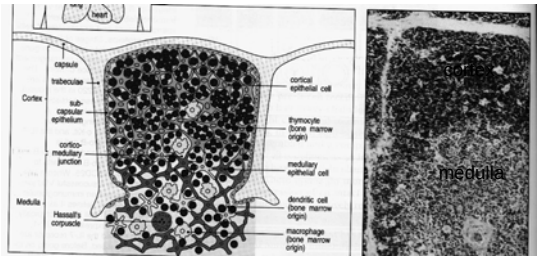
Class I - those that replicate in the cytosol

Class II - those that replicate in endosomes and lysosomes

Each interacts of a distinct subset of T cells



Thymic architecture



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

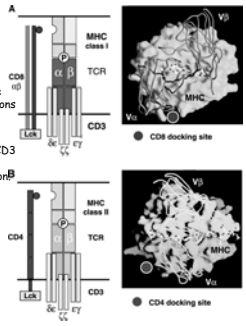
Interactions of TCR with peptide bound to MHC

(Pink = TCR variable region)

- TCR composed of 2 polypeptide chain (α & β), whose variable regions mediate 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.

Reduces the amount of MHC-pepide complexes needed for optimal activation of T cells by ~100-fold

Cellular composition

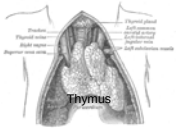
Thymic epithelial cells

- 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

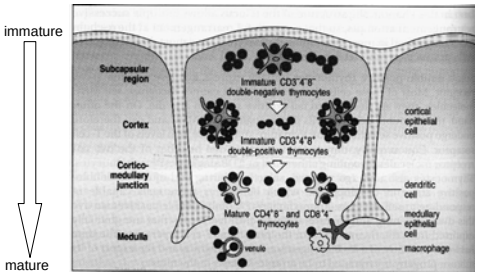
-mainly found in the medulla.
- role in negative selection.

Where do T cells develop?



- Arises 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.
- Permits 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 pausity of T cells)

Thymic architecture



Major Thymocyte Subsets

$CD4^-CD8^-$ (Double Negative, DN) cells: 3-5% of total thymocytes.

- Contain least mature cells, considerable cell division.
- 2/3rds are triple negative (TN) based on TCR expression, can be further divided base on CD44 and CD25.
- *TCR* β, γ and δ rearrangement occurs at this stage.
- 1/3rd are TCR $\gamma\delta^+$

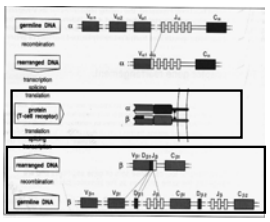
$CD4^+CD8^+$ (Double Positive, DP) cells: 85-90% of total thymocytes

- TCR α rearrangement occurs here.
- Most have rearranged TCR $\alpha\beta$ genes and express low levels of mature TCR
- Small subset has high levels of TCR (most mature, positively selected cells)
- Majority undergo apoptosis, with death by neglect accounting >95% of casualties.

$CD4^+CD8^-$ and $CD4^-CD8^+$ (Single positive, SP) cells: 5-10% of total thymocytes

- Most are mature cells with high levels of CD3 and TCR $\alpha\beta$
- CD4:CD8 approx 2:1
- Most SP cells are functionally mature and are destined to leave the thymus

CHECKPOINT #1 - TCRβ gene rearrangement and expression of a functional β-chain protein



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

Occurs in the thymus

Process identical to that for Ig genes.

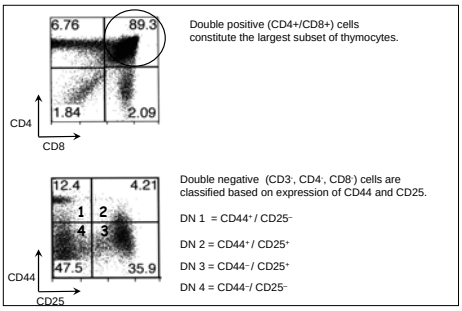
V = variable gene segment

D = diversity gene segment

J = joining gene segment

C = constant genes

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



Double positive ($CD4^+/CD8^+$) cells constitute the largest subset of thymocytes.

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 = $CD44^-$ / $CD25^+$

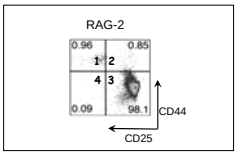
DN 4 = $CD44^-$ / $CD25^-$

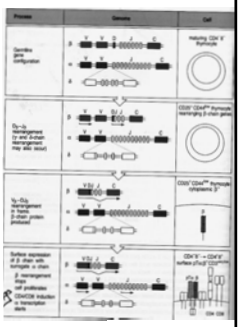
CHECKPOINT #1

TCRβ gene rearrangement and generation of a functional preTCR complex.

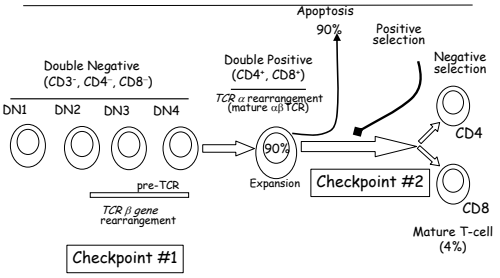
<u>TCRβ gene rearrangement precedes TCRα.</u>

- begins at the DN3 developmental stage
- regulated by helix-loop-helix (HLH) family of transcription activators / repressors (Engel & Murre. Nat. Imm 2001).
- involves the recombination activating genes (RAG)
- only in-frame version can pair with preTCRα□□□□□□□□□ in a cessation of β-gene rearrangement and generation of a functional TCR. If not - DEATH





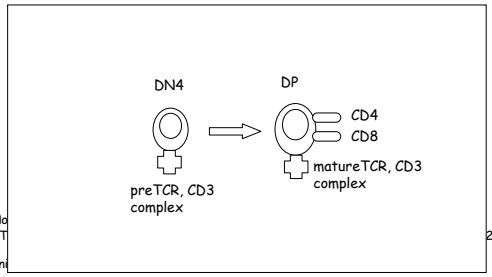
Thymocyte development



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 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 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 FEW FIRST MONTHS OF LIFE.

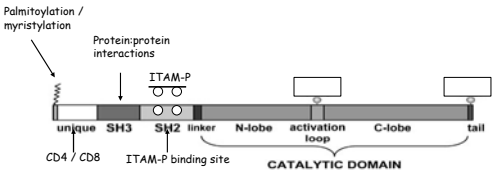
preTCRβ signaling



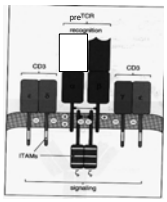
- No
preT 2000)
- Ini
- Involves several family of signaling molecules and ultimately results in cell survival, proliferation, induction of CD4 and CD8 gene expression, TCRα gene rearrangement, and generation of CD4/CD8 DP thymocytes expressing a mature TCR/CD3 complex.

Lck (p56^K)

- 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 non-covalently associate with the cytoplasmic domains of CD4 and CD8; Interaction with with preTCR/CD3 complex?? - recruited to lipid rafts with complex
- Genetic deletion in mice results in a profound block in thymocyte development (Molina et al. Nature 1992)



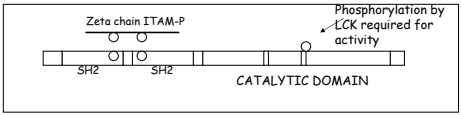
Initiation of preTCR signaling

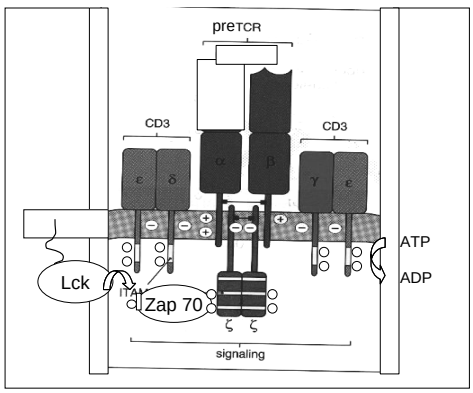


- PreTCR does not directly contribute to intracellular signaling. Role of associated ε, δ, ζ chains that form the CD3 complex.
- These molecules form a nucleation center for recruitment of several signaling molecules as a result of phosphorylation of tryosine residues contained within immunoreceptor tyrosine based activation motifs (ITAMS - YXX(L/V)X_{6-9}YXX(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 SP CD4 or CD8 T cells, but normal number of DN and DP thymocytes (Negishl 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
(Clements et al. Annu. Rev. Immunol. 1999)

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.

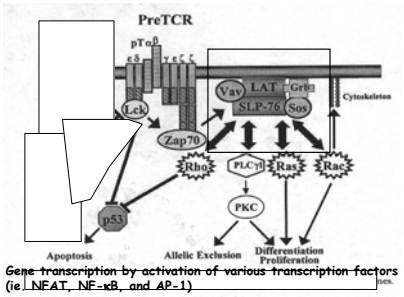
Composition:

- Contain modular domains or unique residues that permit protein-protein or Protein-lipid interactions.

DO NOT contain any enzymatic or direct effector function

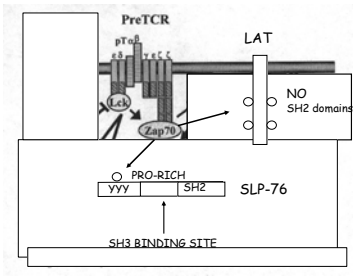
DO bring effectors into close proximity of their targets

Adaptor molecules

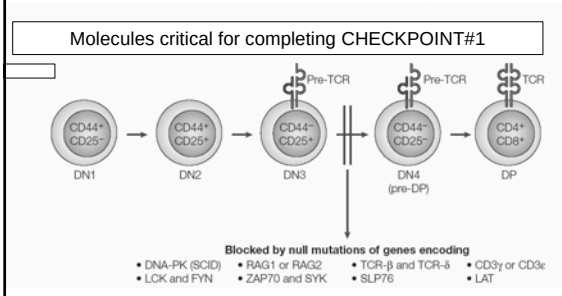


Gene transcription by activation of various transcription factors (ie NFAT, NF-κB, and AP-1)

Adaptor molecules
(Clements et al. Annu. Rev. Immunol. 1999)



Molecules critical for completing CHECKPOINT#1

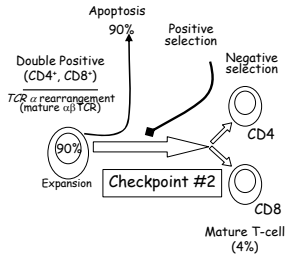


Adaptor molecules

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.
- Genetic deletion in mice results blockade of thymocyte development at the DN3 stage ($CD44^-CD25^+$) (Zhang et al. Immunity 1999).

Thymocyte development



CHECKPOINT #2: Positive and Negative Selection mediated by the mature TCR αβ Receptor

Purpose: To ascertain whether:
- alpha-chain is functionally rearranged.
- MatureTCR is self-MHC restricted.
- MatureTCR is auto-reactive.

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

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-2D^b mouse. This TCR recognizes a male-specific peptide bound to H-2D^b (MHC class I). So transgenic CD8+ clone will kill H-2D^b male cells but not H-2D^b female cells. This CD8+ clone will not kill male cells from H-2D^d because of MHC restriction. So, the thymocyte from which the CD8+ clone was derived was "educated" in an H-2D^b thymus.

CHECKPOINT#2: Positive and Negative Selection mediated by the Mature TCR αβ Receptor

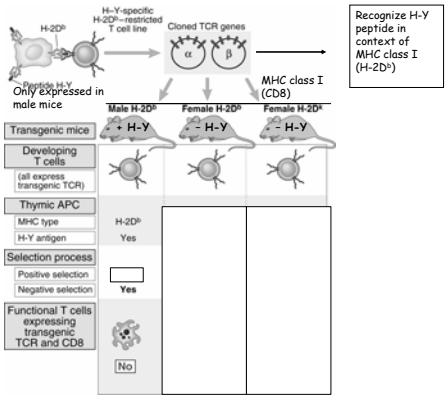
How is specificity of the TCR $\alpha\beta$ assessed?
- Requires peptide/MHC molecule interactions.

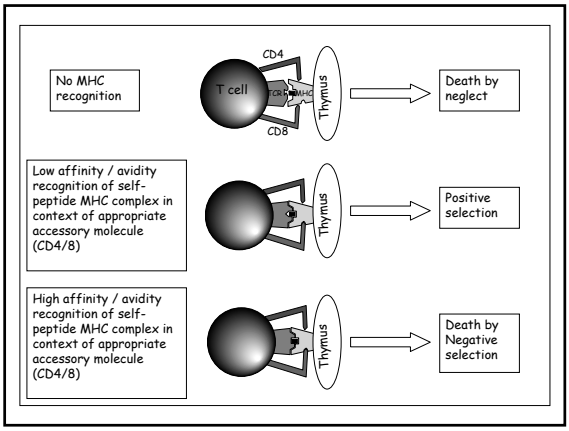
What happens if there is no interaction?
- Absence of interaction leads to apoptosis (death my neglect), most common fate.

Is there a time limit to this process?
- Yes, DP only live 3-4 days as this subset is exquisitely sensitive to apoptotic stimuli due to down regulation of specific survival pathways (i.e.BCL gene family).

If there is an interaction, what determines cell fate?
- distinct positive vs negative selection signals

What are the signals?
- Probably same requirements as pre-TCR.

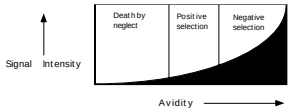




Positive Selection vs Negative Selection

Avidity Model:

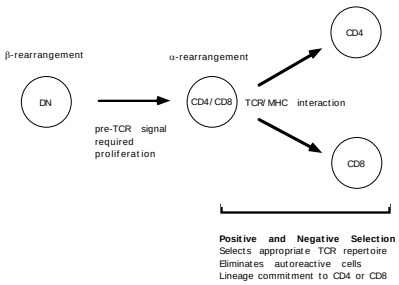
Avidity depends on the affinity of the TCR-peptide/MHC interaction and the density of the peptide/MHC on the thymic epithelial cell. Avidity determines the strength of signal delivered which dictates the outcome. Stronger signals may mean longer signaling or additional signaling.



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:



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).