T cell development and TCR Complex signaling

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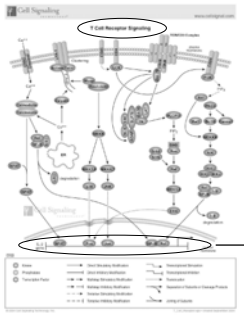
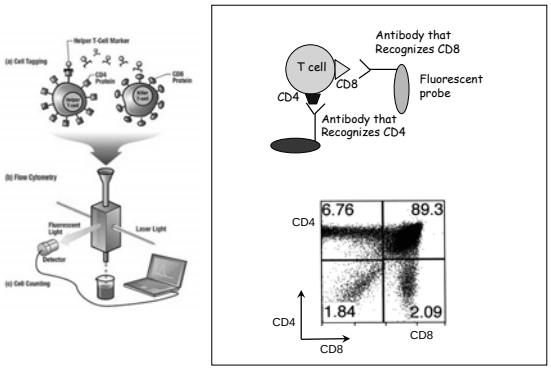


-T cell development
-survival
- proliferation
- effector function

Detection of T cells by flow cytometry



What is a T cell and why do I care?

A type of white blood cell that is of key importance to the immune system and is at the core of adaptive immunity, the system that tailors the body's immune response to specific pathogens

- Cells involved in innate immunity (neutrophils & macrophages) can recognize only a limited number of pathogens that share common surface markers (non-adapting). Cannot adequately defend against viral pathogens.

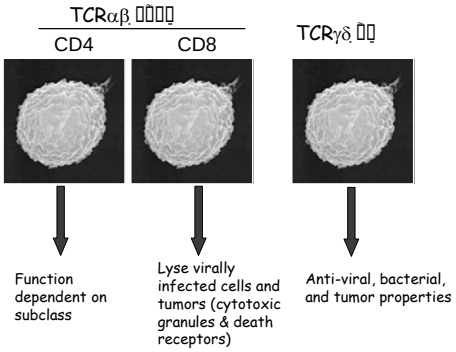
Innate: <u>One cell for multiple pathogens (one size fits all)</u>

- 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 (custom fit).

Specific subsets of T cells can:
Directly kill cells infected by pathogens (viruses)
Regulate B cells function (antibody production)
Regulate the extent of the immune response

Adaptive: <u>One specific T cell for each pathogen (individual fit)</u>

T cell subsets



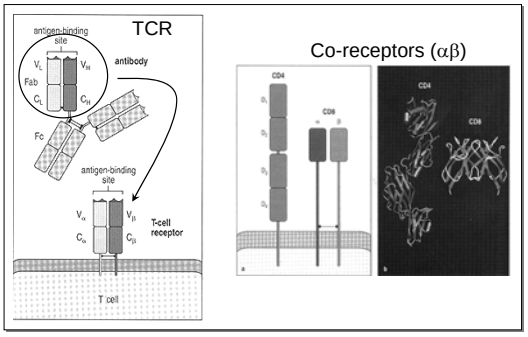
Function dependent on subclass

Lyse virally infected cells and tumors (cytotoxic granules & death receptors)

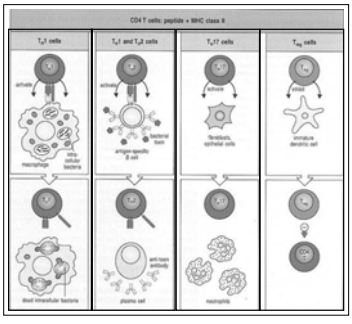
Anti-viral, bacterial, and tumor properties

How do we classify T cells ?

Based on expression of surface molecules unique to these cells



CD4 subsets



T_H1 - enhance macrophage killing of bacteria & stimulate antibody production by B cells

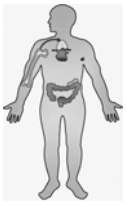
T_H2 - stimulate antibody production by B cells (IgE)

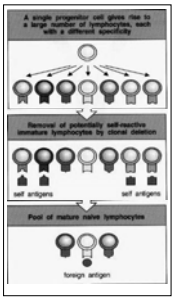
T_H17 - stimulate epithelial and stromal cells to produce chemokines that attract neutrophils to sites of infection

T_{reg} - suppress T cell activity and help prevent the development of autoimmunity

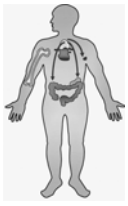
T cell development occurs in the thymus

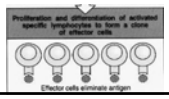
Common lymphocyte progenitor cell from BM





Seeding of secondary Lymphoid organs



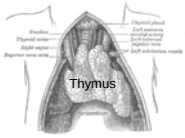


How do we know the thymus is important?

Digeorge syndrome
(abnormality in the development of the 3rd & 4th pouch/arch)

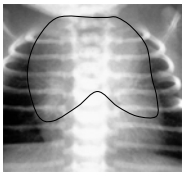
- Primary immunodeficiency disease (variable penetrance) caused by abnormal thymic development (low T cell numbers)
- Affects 1/4000 newborns
- >90% associated with small deletion in chromosome 22 (22q11.2)
- Increased susceptibility to infections caused by organisms typically associated with T-cell dysfunction is observed. These include systemic fungal infections, Pneumocystis jiroveci (previously Pneumocystis carinii) infection, and disseminated viral infections

T cell development occurs in the thymus

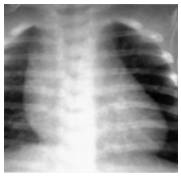


- Arises from endo and ectodermal layers of the third pharyngeal pouch and branchial cleft.
- Colonization of the thymus by bone-marrow derived, common lymphoid progenitor cells occurs by 8th week of gestation.
- 50 million new thymocytes generated each day, but only 1-2 million (2-4%) leave as mature single positive $CD4^+$ or $CD8^+$ T cells.
- Permits the developing immune system to recognize "self" to avoid mounting an immune response against the host.

Normal



Digeorge

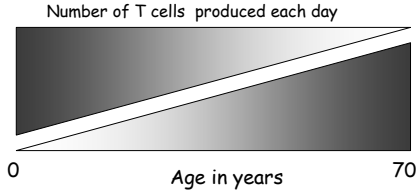


CD8 CD8

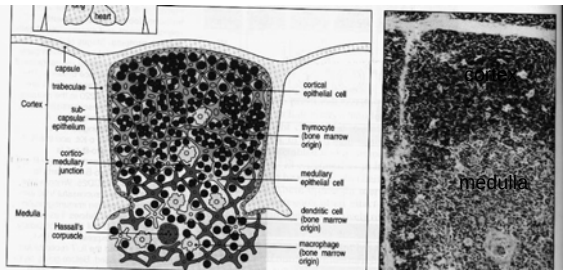
QuickTime™ and a decompressor are needed to see this picture.

CD4 CD4

Thymic atrophy occurs as we age, resulting in an age-related decrease in T cell production but not in total numbers of circulating cells



Thymic architecture



Cellular composition

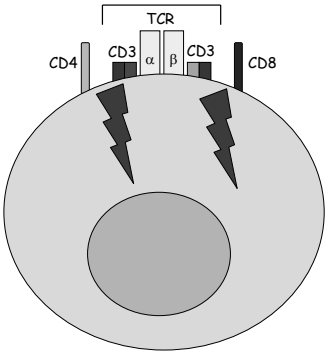
Thymic epithelial cells

- 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.

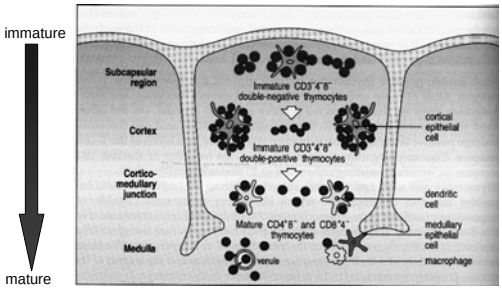
Thymic dendritic cells and macrophages

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

Expression of a functional T cell receptor complex and accessory molecules is critical for this process



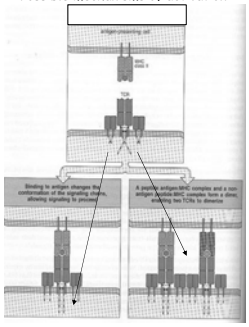
Thymic architecture



What causes the TCR complex to generate an intracellular signal ?

T cell receptor must engage the correct peptide:MHC complex in order for signal transduction to occur

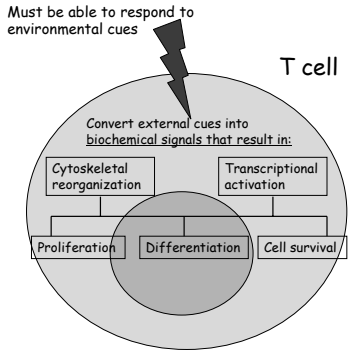
Possible mechanisms of activation



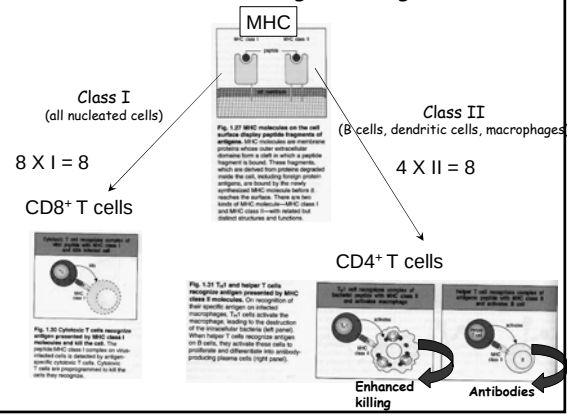
TCR interactions with self peptide:MHC complex results in conformational changes in cytoplasmic domain, which permits modification by cytoplasmic enzymes

TCR interactions with self peptide:MHC complex results in TCR receptor clustering, augmenting signal transduction

How do T cells develop in the thymus ?



How do T cells recognize antigen?



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

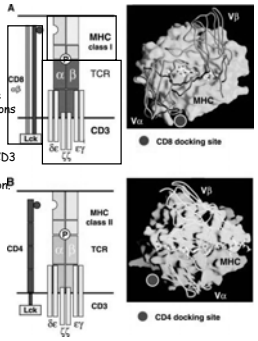
Interactions of TCR with peptide bound to MHC

(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

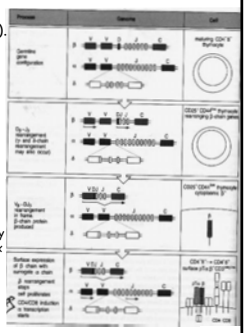
CD8 - cytotoxic T cells

Strengthen TCR engagement with MHC/peptide complex.

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

CHECKPOINT #1

TCRβ gene rearrangement and generation of a functional preTCR$\alpha\beta$ / CD3 complex (β-selection).

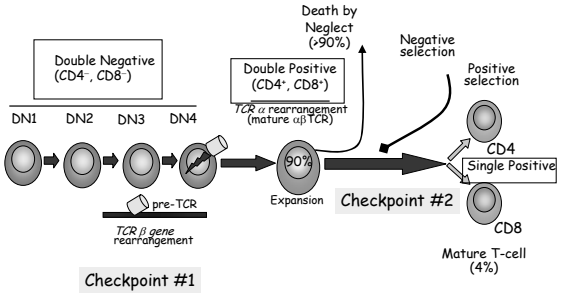


TCRβ gene rearrangement precedes TCRα.

- begins at the DN3 developmental stage.
- regulated by transcription activators / repressors (Engel & Murre. Nat. Imm 2001).
- involves the recombination activating genes (RAG).
- only a functional β-chain protein can pair with a constitutively expressed preTCRα chain, resulting in a preTCR$\alpha\beta$/CD3 complex that spontaneously signals. PreTCR$\alpha\beta$ prevents cell death, and promotes cell proliferation and differentiation.

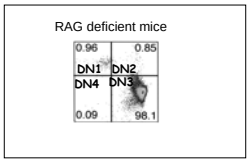
If no preTCR signaling - DEATH

Thymocyte development (3 major cell populations)



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.



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/CD3 expression.
- Rearrangement of TCR β chain gene and expression of a functional pre *TCR occurs*, an event essential for continuation of thymocyte development (checkpoint #1).

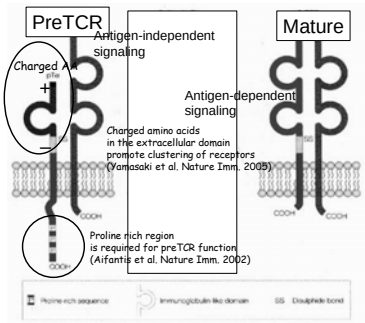
$CD4^+CD8^+$ (Double Positive, DP) cells: 85-90% of total thymocytes.

- TCR α gene rearrangement occurs and cells express both CD4 and CD8.
- Majority undergo cell death (>90%) because they cannot recognize MHC.
- Stage in which negative and positive selection occurs, an event critical for deletion of autoreactive T cells and MHC restriction (checkpoint #2).

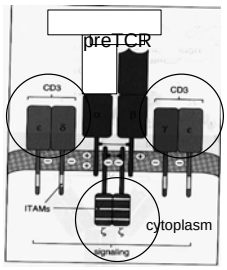
$CD4^+$ or $CD8^+$ (Single positive, SP) cells: 5-10% of total thymocytes.

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

How does the preTCR induce signaling ?



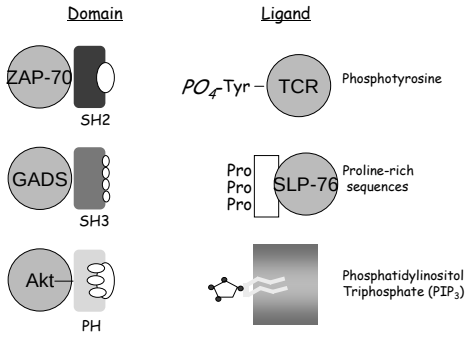
preTCR signaling occurs through the CD3 complex



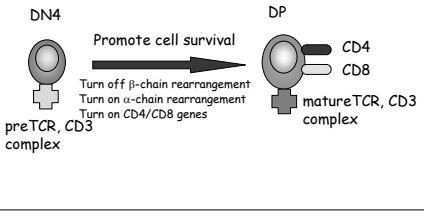
Phosphorylation of of specific tyrosines permits the TCR/CD3 complex to engage the cell's signaling machinery

- PreTCR does not directly contribute to intracellular signaling. Role of associated ε, δ, ζ chains that form the CD3 complex. DOES NOT RELY ON INTERACTIONS WITH MHC !
- 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 - $YXX(L/V)X_{6-9}YXX(L/V)$).

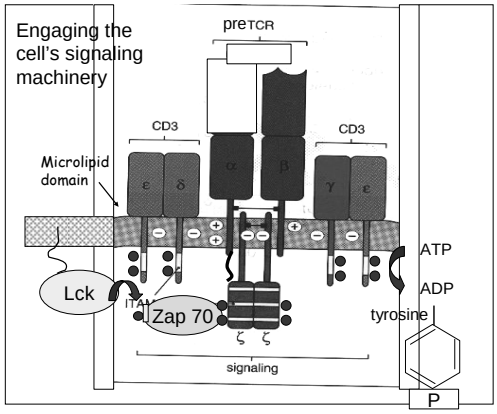
Interactions critical for T cell signaling



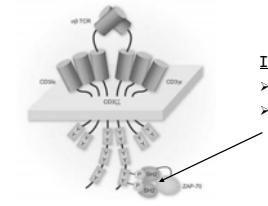
NET RESULT of preTCR signaling:
CD4/CD8 DP thymocytes expressing a mature TCRαβ/CD3 complex.



Engaging the cell's signaling machinery

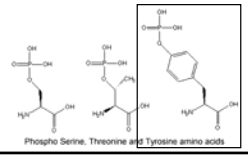


Phosphorylation of tyrosine residues on ITAMS by enzymes known as tyrosine protein kinases is a key event in multiple signal transduction pathways



Importance of tyrosine phosphorylation

- Activate the catalytic function of a protein
- Serve as a binding site for other proteins



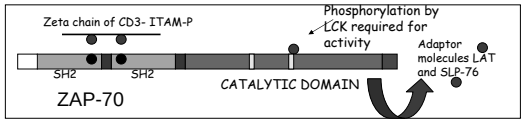
Lck

(lymphoctye-specific protein tyrosine kinase)

- Member of the Src family protein tyrosine kinases
- Phosphorylates ITAMS on the cytoplasmic tail of the CD3 complex as well as tyrosine contained within catalytic domains of other signaling molecules (ZAP-70).
- Primarily expressed in lymphoid cells (developing thymocytes and mature T cells).
- Physically linked to the plasma membrane and known to associate with the cytoplasmic domains of CD4 and CD8; Interaction with preTCR/CD3 complex?? Recruited to microdomains contained with the plasma membrane with complex.
- Genetic deletion in mice results in a profound block in thymocyte development (Molina et al. Nature 1992)

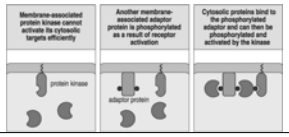
ZAP-70 (Zeta-chain-associated protein)

- Phosphorylates ITAMS on adaptor molecules that form the TCR/CD3 signaling complex.
- Primarily expressed in T cells (developing thymocytes and mature T 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 (SH2 domain)
- Genetic deletion in mice results in a profound reduction in SP CD4 or CD8 T cells (Negishl et al. Nature 1995). Combined deletion with Syk: block at DN3 stage



Adaptor molecules

(Koretzky and Myung. Nat. Rev. Imm. 2001))



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

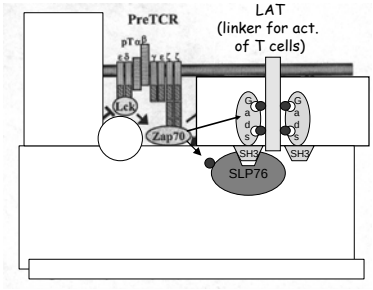
DO bring effectors into close proximity of their targets and can induce conformational changes in their binding partners, thereby regulating their activities (+/-)

Zap-70 deficiency in humans

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

(Koretzky and Myung. Nat. Rev. Imm. 2001)



Adaptor molecules

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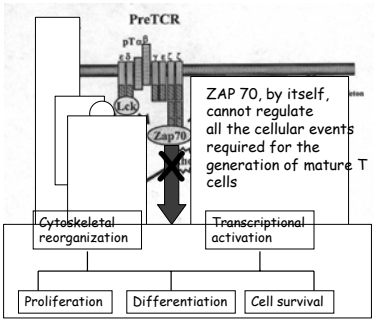


Adaptor molecules

(Koretzky and Myung. Nat. Rev. Imm. 2001)

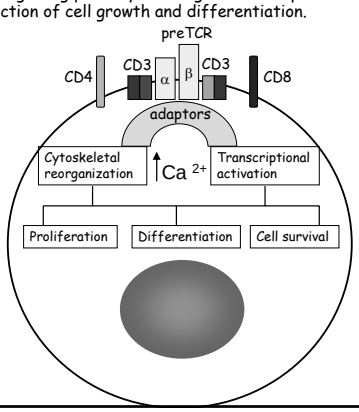
LAT (Linker for Activation of T cells)

- Phosphorylated by ZAP-70
- Provides multiple docking sites for SH2-containing signaling molecules and adaptors, targeting them to the plasma membrane (GADs, SLP-76, Grb2, PLCγ1, PI3K)
- Genetic deletion in mice results blockade of thymocyte development at the DN3 stage (Zhang et al. Immunity 1999).

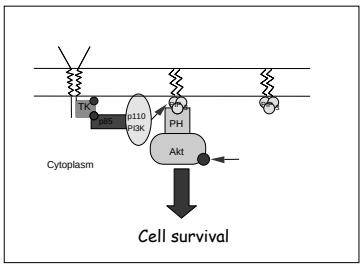
SLP76 (SH2-domain containing leukocyte-specific phosphoprotein)

- Phosphorylated by ZAP-70
- Contains an SH2-domain for interactions with phospho-tyrosines and a proline rich region that serves as a docking site for SH3-domain containing proteins such as Gads
- Genetic deletion in mice results blockade of thymocyte development at the DN3 stage (Clements et al. Science 1998).

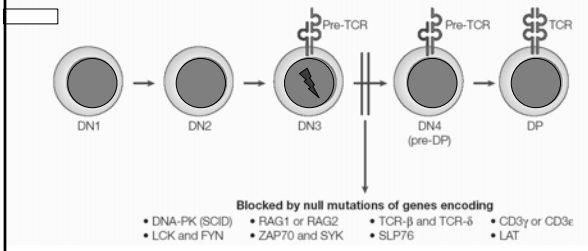
Stimulated antigen receptors utilize tyrosine kinases to induce intracellular signaling pathways leading to transcription factor activation and the induction of cell growth and differentiation.



PI3K/Akt signaling pathway plays a critical role in thymocyte development

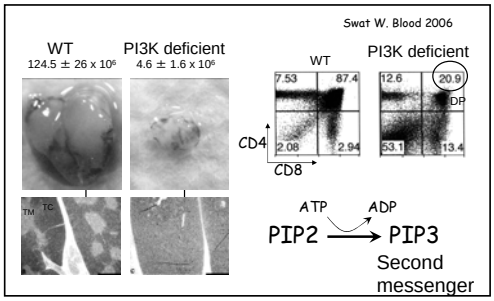


Molecules critical for completing CHECKPOINT#1



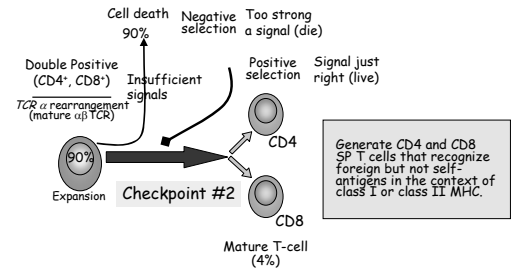
Staying Alive

Class I Phosphoinositide 3-Kinases (PI3K)



Thymocyte development (staying alive)

Class I PI3Ks critical for DP survival



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

Purpose: To ensure that:
- alpha-chain is functionally rearranged (preTCR downregulated).
- MatureTCR is self-MHC restricted.
- MatureTCR is NOT 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.

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

How is specificity of the TCR αβ assessed?

- Requires self-peptide/MHC molecule interactions with mature TCR. PreTCR is downregulated as mature alpha chain undergoes gene rearrangement.

What happens if there is no interaction?

- Absence of interaction leads to apoptosis (death by neglect), most common fate.

If there is an interaction, what determines cell fate?

- distinct positive vs negative selection signals
- intensity and duration of the signal

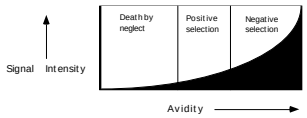
Too strong - die / Just right - live

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.

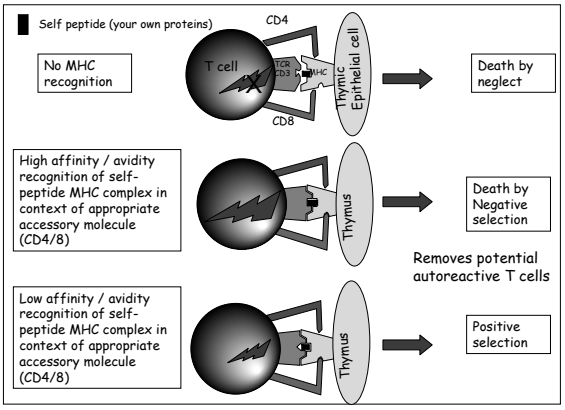
Positive Selection vs Negative Selection

Signal strength:(same signal, just different intensities and/or duration).
Strength of signal delivered which dictates the outcome.
Stronger signals may mean longer signaling or additional signaling.

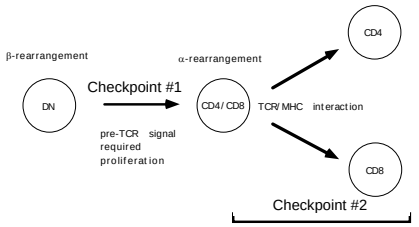


Differential Signaling Model (based on altered peptide ligands).

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



Summary:



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

MHC deficiencies (Bare lymphocyte syndromes)

- Rare autosomal recessive disorder that represents one form of severe combined immunodeficiency syndrome (SCIDS)

- Type I - lack MHC class I molecules
  - lack of positive selection for CD8 T cells
  - low C8 T cells numbers
  - repeated infections of sinuses, middle ear, lungs
  - normal humoral immunity but prone to necrotizing skin lesions by activated NK cells

- Type II - lack MHC class II molecules
  - lack of positive selection for CD4 T cells
  - low CD4 T cell numbers
  - severe defect in both cellular and humoral immunity
  - repeated and life threatening infections (viral, bacterial, fungal)

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/CD3 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).