Dr. Chiranjeeb Dey, Assistant Professor, Dept. of Zoology

T Cell Development

Generation of T Cells

T cell development occurs in the thymus; the thymic microenvironment directs differentiation as well as positive and negative selection. Lymphoid progenitors which have developed from hematopoietic stem cells in the bone marrow migrate to the thymus to complete their antigen-independent maturation into functional T cells. In the thymus, T cells develop their specific T cell markers, including TCR, CD3, CD4 or CD8, and CD2. T cells also undergo thymic education through positive and negative selection.

The thymus is a multi-lobed organ composed of cortical and medullary areas surrounded by a capsule. T cell precursors enter the subcapsular cortical areas, where they encounter networks of cortical epithelial calls (the thymic stroma) and undergo a period of proliferation. As they differentiate, they move from the cortex towards the medulla of the thymus; different microenvironments within the thymus direct T cell development. Most cells that enter the thymus die by apoptosis without successfully completing the steps required for becoming a mature naive T cell.

When progenitor cells begin to express CD2 but have not yet rearranged their TCR genes ($CD2^+$ $CD3^-$), they are double negative for CD4 and CD8 ($CD4^-$ $CD8^-$), the markers for Th and Tc lineages. Of the double negative cells in the thymus, about 20% have rearranged gd TCR, about 20% have very homogenous ab TCR, and 60% are committed to becoming the majority of mature ab T cells. These cells next express the adhesion molecule CD44, then the a chain of the IL-2 receptor (CD25). $CD44^{low}$ $CD25^+$ double negative T cells rearrange TCR b chain. b chain rearrangement begins with D-J joining, followed by V-DJ joining. The chances of successful b chain rearrangement are increased by the presence of two DJCb gene clusters. If rearrangement in the first cluster fails, rearrangement in the second can occur

Productive rearrangement of b chain is followed by its expression on the T cell membrane with CD3 and surrogate a chain, pTa (analogous to l5 in B cells). Signaling through the preT receptor causes the cells to stop rearranging b chain, undergo a period of proliferation, and begin to express both CD4 and CD8, becoming double positive T cells. Membrane CD25 is lost at this stage. Double positive cells re-express RAG-1 and RAG-2 to rearrange their a chain genes. a chain rearrangement can occur on both chromosomes and continue until the cell undergoes selection or dies, so T cells are not allelically excluded for a chain. However, even cells with two different TCR have only one which can bind self MHC with enough affinity to pass positive selection (one *functional* receptor specificity). Double positive ab T cells move into the cortico-medullary junction, where they undergo positive and negative selection and mature into Th and Tc cells.

T cell development is greatest during fetal development and before puberty. After puberty the thymus shrinks and T cell production declines; in adult humans, removal of the thymus does not compromise T cell function.

Thymic Selection:

Positive Selection of T Cells

Double positive ab TCR^{low} cells must successfully undergo positive and negative selection before they can leave the thymus. Cells which have successfully rearranged ab TCR will die in the thymus cortex if they do not bind self MHC within 3-4 days. **Positive selection** occurs when double positive T cells bind cortical epithelial cells expressing Class I or Class II MHC plus self peptides with a high enough affinity to get the survival signal. **Negative selection** occurs when double positive T cells bind to bone-marrow derived APC (macrophages and dendritic cells) expressing Class I or Class II MHC plus self peptides with a high enough affinity to receive an apoptosis signal. Note that selection occurs on *self* peptides in the thymus; MHC presents self peptides in the absence of pathogen.

The ability of the developing T cell to make several a chain rearrangements increases its chances of undergoing positive selection. It has been shown that approximately 1/3 of T cells express more than one TCR. However, because the probability of positive selection is so low, these T cells with two TCR idiotypes should still have only a single idiotype that can recognize peptide on self MHC and not violate clonal selection.

Positive selection also determines whether the T cell will become a helper or a cytotoxic T cell. Positive selection on Class I MHC will produce a CD8 Tc cell, while positive selection on Class II MHC will yield a CD4 Th cell.

The genetic mechanism by which a cell becomes either a Th or a Tc is still under intense study. According to the **instructive model**, signals received through CD4 shut off the CD8 gene and cause the cell to differentiate into a Th, while signals received through CD8 shut off CD4 expression and induce Tc differentiation. According to the instructive model, the cell could go equally easily down either pathway and the first strong enough signal decides its fate. In the **stochastic model**, the cell is somehow randomly committed to becoming either a Tc or a Th before positive selection. If it gets the correct signal during positive selection, it proceeds down its predetermined pathway; if it doesn't get signaled through the correct co-receptor, it dies.

Negative Selection of T Cells

T cells that survive positive selection migrate further into the cortico-medullary junction of the thymus where they encounter macrophages and dendritic cells, bone-marrow derived APC with high expression of MHC-self peptide complexes. T cells which bind self peptide-MHC with high affinity at this stage undergo **negative selection** and die by apoptosis. Transgenic mice have been used to demonstrate negative as well as positive selection. Since not all self peptides are expressed in the thymus, other mechanisms for inducing peripheral tolerance must also exist.

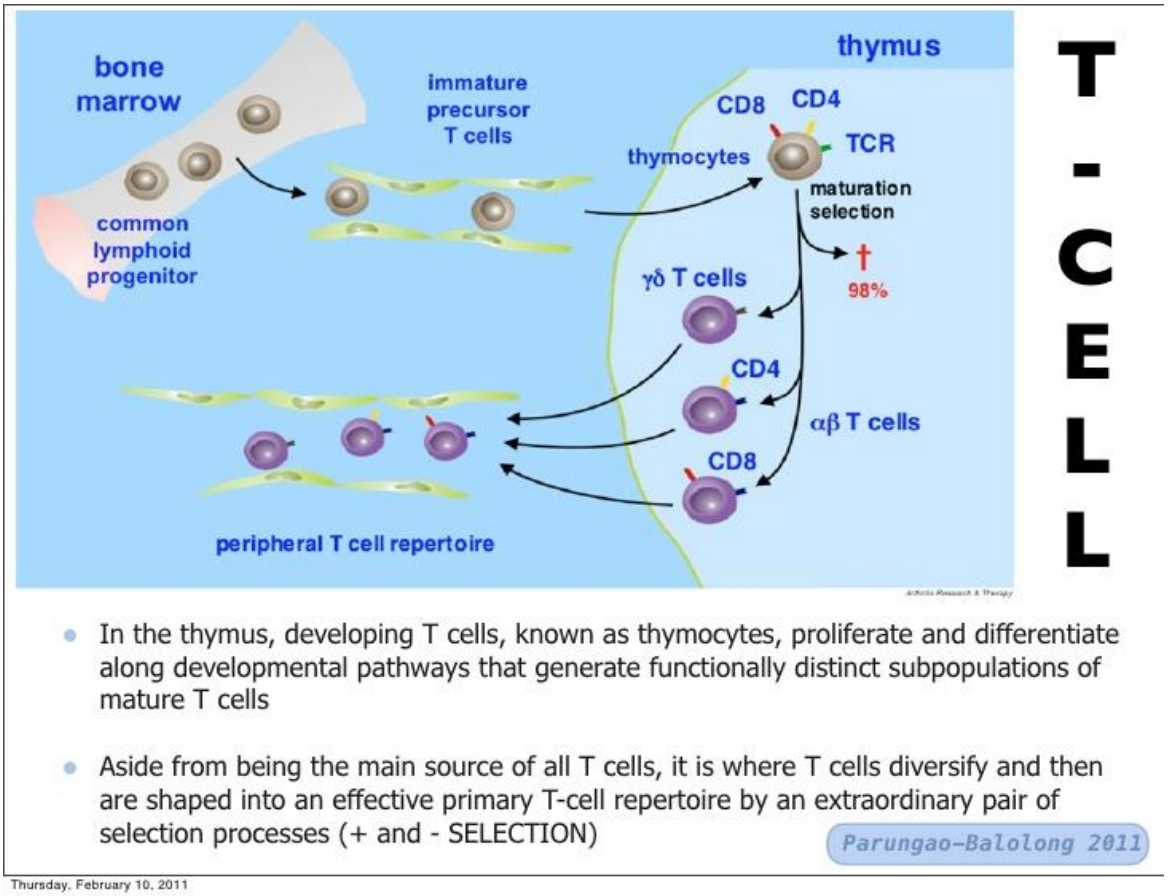
Negative selection to self antigen has been studied in mice expressing an **endogenous superantigen**. **Superantigens** bind TCR Vb region and MHC outside the normal peptide-binding site and send strong signals to mature Th cells, inducing cytokine secretion and shock.

The signals received during positive and negative selection must differ; otherwise all developing T cells would die before they leave the thymus. The **differential avidity hypothesis** proposes that the same peptide-MHC complex delivers both signals, but that the avidity of positive

selection is lower (less signal is required to save the cells from death), while the avidity of the negative selection signal is higher (more signal is required to kill them). The **differential signaling hypothesis** proposes that qualitatively (not just quantitatively) different signals are delivered during positive and negative selection.

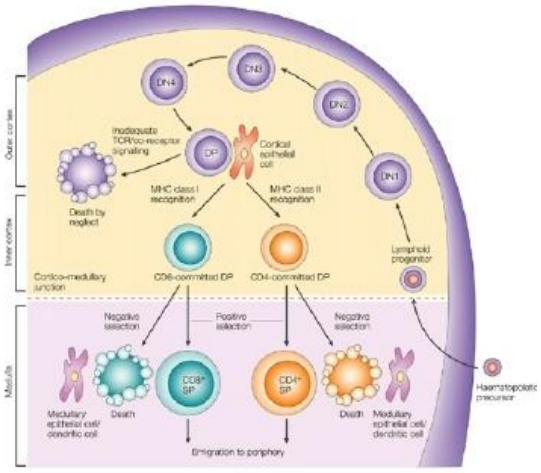
γδ T Cells

T cell development resembles that of B cells, with a few key differences. One difference is that while a given B cell expresses first IgM, then both IgM and IgD, and later IgG or IgA or IgE (all with the same light chain), T cells express either ab (95% of T cells) or gd TCR for their whole life span. The earliest T cells seen during fetal development express gd TCR. RAG-1, RAG-2, and TdT begin to rearrange g, d, and b gene segments nearly simultaneously and before a segments are rearranged. d gene segments are located within the a gene segment region. Immunologists believe that if g and d are productively rearranged first, the cell will probably become a gd T cell. If b is productively rearranged first and expressed on the membrane with surrogate a chain (pTa), the cell will usually go on to rearrange a chain gene segments and become an ab T cell.



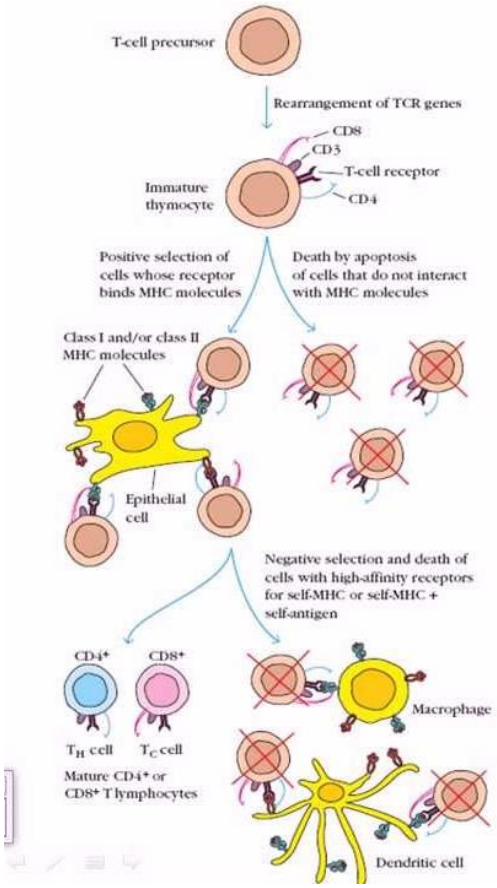
POSITIVE AND NEGATIVE SELECTION

- **positive selection**, permits the survival of only those T cells whose TCRs are capable of recognizing self-MHC molecules
  - It is thus responsible for the ***creation of a self-MHC-restricted repertoire of T cells***
    - Cells that fail positive selection are eliminated within the thymus by apoptosis
- **negative selection**, eliminates T cells that react too strongly with self-MHC or with self-MHC plus self- peptides
  - bearing high-affinity receptors for self-MHC molecules alone or self-antigen presented by self-MHC, which results in self-tolerance
  - It is an extremely important factor in ***generating a primary T-cell repertoire that is self-tolerant***



Parungao-Balolong 2011

Thursday, February 10, 2011



Thymic selection