T cell development
B and T cells develop from a common hematopoietic stem cell that lives in the bone marrow.
Lymphocyte development

- B cells develop in the bone marrow
- T cells develop from a progenitor cell that leaves the bone marrow and migrates to the thymus
- Developing B and T cells undergo ordered rearrangement of their antigen receptor genes
  - For B cells, heavy chain rearranges first, then kappa light chain, then lambda light chain
  - For T cells, beta rearranges first, then the alpha chain
- Lymphocyte development produces a heterogeneous population of cells with widely varying antigen receptors
- The population of developing lymphocytes must be purged of self-reactive cells
Goals of T cell development in the thymus

• Eliminate self-reactive T cells (tolerance)
• Ensure that the collection of TCRs favors binding to self-MHC molecules
• Ensure that cytotoxic T cells have TCRs specific for MHC class I (vice-versa for helper T cells and MHC class II)
Thymic organization

- Cortex
  - capsule
  - trabeculae
  - sub-capsular epithelium
  - cortico-medullary junction

- Medulla
  - Hassall's corpuscle
  - cortical epithelial cell
  - thymocyte (bone marrow origin)
  - medullary epithelial cell
  - dendritic cell (bone marrow origin)
  - macrophage (bone marrow origin)
Thymocyte subsets

- CD4+8+: TCRlow
- CD4-8-: TCRneg
- CD4+8+ & CD4+ & CD8+ & TCRhi
Thymic compartments

cortex

medulla

subcapsule

DN

DP

SP
Thymocyte profile (developing T cells)

Lymph node profile (mature T cells + other cells)
Summary of key points

• T cell development takes place in the thymus, and is dependent on Notch signaling
• The thymus is organized into compartments: subcapsule, cortex, and medulla
• Thymocytes develop from CD4-8- cells to CD4+8+ cells, and then to CD4+ or CD8+
• Thymocytes migrate from subcapsule to cortex to medulla as they differentiate
Regulation of thymocyte maturation

How is this regulated?
CD4/CD8 Thymocyte subsets
Regulation of T cell development

- Are CD4 and CD8 expression tied to TCR gene rearrangements and/or TCR protein expression, or vice-versa?

- Analyze T cell development in TCR alpha or TCR beta chain knockout mice...
Thymocytes from TCR $\alpha$ or TCR $\beta$ KO mice

- WT: $2 \times 10^8$ cells
- TCR$\alpha$-/-: $2 \times 10^8$ cells
- TCR$\beta$-/-: $1 \times 10^6$ cells
The Real Data

WT | TCRα−/- | TCRβ−/- | TCRα−/- | TCRβ−/- | TCRγδ

10^8 | 10^8 | <10^7 | <10^7 | 10^6

CD4 | CD8

Expressing TCRγδ
TCRβ is important for early step of T cell maturation

• Studies of TCRα and TCRβ KO mice
  – TCRβ−/−
    • 20-fold decrease in thymocyte numbers
    • Few CD4+8+ cells
  – TCRα−/−
    • Normal thymocyte numbers
    • Block at last stage of T cell development (CD4+8+ to CD4+ or CD8+)
The pre-TCR complex

- CD3 and $\zeta$
- Pre-Te
- TCR $\beta$
Pre-TCR-mediated differentiation events

• DN thymocytes differentiate into DP (CD4+8+) thymocytes
• Cell proliferation, leading to 100-fold expansion
• Activation of TCRα chain rearrangements
Pre-TCR signaling

CD4-8- rearrange TCR β proliferation and expression of CD4 and CD8
Pre-TCR signaling

• Many TCR and TCR-associated signaling proteins implicated in pre-TCR pathway
  – CD3ε, TCRβ, Pre-Tα
  – Tyrosine kinases: Lck/Fyn, Zap-70/Syk
  – Adapters: LAT, SLP-76

• Also
  – Rag-1 and Rag-2
Pre-TCR signaling

- TCRβ
- CD8
- CD4

CD4-8-

deficiencies in cytokines, cytokine receptors, and cytokine receptor signaling proteins
deficiencies in TCR gene rearrangements and TCR signaling proteins
Summary of key points

- Developing T cells rearrange TCR $\beta$ chain genes first.
- Productive (in-frame) $\beta$ chains pair with pre-T$\alpha$ chains to form pre-TCRs.
- The pre-TCR signals DN thymocytes to proliferate, differentiate into DP cells, and commence TCR$\alpha$ chain gene rearrangement.
- Deficiencies in TCR $\beta$ chain rearrangement, pre-TCR signaling, or cytokine receptor signaling block T cell development and lead to immune deficiencies.
TCR\(\alpha\) chain gene rearrangements occur at the CD4+CD8+ (DP) stage

- Each DP thymocyte rearranges and expresses a unique TCR \(\alpha\) chain
- Cells that fail to generate a TCR\(\alpha\) chain protein die at this stage of development
Development of CD4+ or CD8+ cells

• Do all CD4+8+ thymocytes develop into mature T cells?
• NO!!

• Two selection processes weed out unwanted TCRs
  – Negative selection (clonal deletion) results in elimination of self-reactive TCRs
  – Positive selection leads to maturation of CD4+8+ thymocytes with TCRs that have some affinity for self-MHC molecules
Consequences of positive and negative selection

• Creation of a repertoire of TCRs that suits each individual
  – TCR repertoire is depleted of receptors that could potentially lead to autoimmunity
  – TCR repertoire is enriched in receptors that are most likely to be useful during the recognition of pathogen-derived (foreign) peptides bound to self-MHC molecules
TCR Repertoire Selection

Number of thymocytes vs. TCR affinity.

- **Positive selection**
- **No selection**
- **Negative selection**
Positive selection

• Thymocytes “learn” to recognize MHC molecules present during their development in the thymus
• Positive selection definitively shown in TCR transgenic mice
TCRαβ transgenic mice

- Rearranged TCR α and β chain genes in the germline
- Inhibits rearrangement of endogenous TCR α and β chain genes
- Nearly 100% of thymocytes and T cells in these mice express the same TCR αβ receptor
  - Most TCR transgenic lines are “leaky”
  - If cross to RAG knockout, can achieve 100%
Generation of transgenic mice

Female mouse is injected with follicle-stimulating hormone and chorionic gonadotropin to induce superovulation, and then mated.

Fertilized eggs are removed from female. DNA containing the Eα gene is injected into the male pronucleus.

Injected eggs are transferred into uterus of pseudopregnant female.

Some offspring will have incorporated the injected Eα gene (transgene).

Mate transgenic animal to Eα− C57BL/6 mice to produce a strain expressing the Eα transgene.
Antigen-specific T cell lines

T cells from an immunized animal comprise a mixture of cells with different specificities.

The T cells are placed into culture with antigen-presenting cells and antigen. Antigen-specific T cells proliferate while T cells that do not recognize the antigen do not proliferate.

Antigen-specific T cells can be cloned by limiting-dilution culture in IL-2.

TCR transgenic mice are made with TCR α and β chain genes cloned from antigen-specific T cell lines.
Advantages of TCR$\alpha\beta$ transgenic mice

- Homogeneous population of developing T cells with a known TCR specificity
- Allows the study of T cell development and selection in the absence of antigen
- First two TCRs studied:
  - H-Y TCR: Male antigen + Db
    - Compare H-2b to H-2d
  - 2B4 TCR: moth cyt c + I-Ek
    - Compare H-2k to H-2b
  - Does the development of mature thymocytes depend on the presence of the “right” MHC?
Data from TCR transgenic mice

"right" MHC

H-Y

CD4

CD8

"wrong" MHC

2B4
Conclusions from TCR transgenic experiments

• Development of mature thymocytes depends on the presence of the specific MHC molecule

• TCR specificity for MHC class I determines differentiation into CD8 lineage, and specificity for MHC class II determines differentiation into CD4 lineage
TCR specificity determines CD4 vs. CD8 lineage

Transgenic receptor recognizing MHC class I

- Immature CD4$^+$8$^+$ double-positive T cells
- Only CD8$^+$ T cells mature

Transgenic receptor recognizing MHC class II

- Immature CD4$^+$8$^+$ double-positive T cells
- Only CD4$^+$ T cells mature
Lack of MHC class II expression prevents development of CD4 cells

WT

MHC Class II KO

Pretend data
Lack of peripheral CD4+ T cells in MHC class II-deficient mice
T cell immunodeficiencies

CD4+8+ thymocytes

MHC class II

CD4

CD4

MHC class I

CD8

CD8

CD8

CD8
Summary of key points

• At the CD4+8+ stage, thymocytes undergo positive selection for recognition of self-MHC/peptide complexes

• Positive selection also ensures appropriate coordination between CD4/CD8 differentiation and TCR specificity for MHC class I vs. II
Negative selection (clonal deletion)

- One important mechanism of self-tolerance is the elimination of self-reactive T cells during their development in the thymus
- Experiments demonstrating this required two technical advances
  - Discovery of superantigens and the generation of $V_\beta$-specific monoclonal Abs
  - TCR transgenic mice
Data from first TCR transgenic

- TCR from CTL clone specific for male antigen (H-Y) + Db (MHC class I)
- Compare thymuses of male versus female transgenic mice

Female: -Ag  Male: +Ag

~10^8 cells  ~10^7 cells

CD4  CD8
Importance of thymic negative selection: APECED

- Defects in thymic negative selection (the deletion of autoreactive T cells) occur in individuals lacking the protein AIRE
- In these individuals, autoimmune responses occur and are targeted against a wide variety of cells and tissues
- The AIRE protein is expressed in thymic medullary stromal cells
- AIRE may cause a variety of “tissue-restricted” proteins to be expressed in the thymus, leading to deletion of developing T cells reactive to these proteins
### APECED patients suffer a variety of autoimmune diseases and candidiasis

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<th>Frequency in Finnish patients (%)</th>
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<td>Intestinal malabsorption</td>
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Positive versus negative selection?

• Both outcomes depend on signaling through the TCR
  – Positive selection ---> differentiation
  – Negative selection ---> death

• Distinction depends on TCR affinity/avidity for MHC/peptide complexes in the thymus
  – High affinity ---> death
  – Low/medium affinity ---> differentiation
Positive vs. negative selection

What would happen if positive and negative selection had the same threshold for TCR signaling?

Actual case
Selection occurs as cells migrate through the thymus.
Conclusions

• Natural situation
  – Range of peptides on MHC molecules

• Likely mechanism
  – Outcome will depend on sum of signals arising from multiple TCR interactions of different affinities
Sum of signals leads to thymocyte selection

Positive selection

Negative selection

No selection
Sum of signals...

TCR signaling

Time

- Negative selection
- Positive selection
- Neglect
Conclusion

• T cell development produces T cells which express a single TCR$\beta$ chain, but may have more than one TCR$\alpha$ chain

• Process produces a repertoire of T cells that are depleted for self-reactive cells and enriched for cells with modest affinity for self-MHC

• T cells are matched for function (cytotoxic vs helper), co-receptor expression (CD4 vs CD8), and TCR specificity for MHC class II vs class I