Development of T cells

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The Thymus Gland
Lineage-minus cells...precursors are born in the bone marrow and “educated” in the thymus.
Figure 11.1. The multipotential hematopoietic stem cell and its progeny which differentiate under the influence of a series of soluble cytokines and growth factors within the microenvironment of the bone marrow.
Interaction between the two populations are essential for the function of the thymus.
**SCID mutation:**
Severe Combined Immunodeficiency (SCID)

Recombination-activating gene (RAG) mutation
V(D)J recombination

NO B or T CELLS

**nude mutation:**

FoxN1 mutation: transcription regulator for terminal differentiation of thymic epithelial cells

NO Thymus
Severe Combined Immunodeficiency (SCID): Bubble Boy

DiGeorge Syndrome: Chromosome 22q11 deletion.

- Thymus hypoplasia
- Defective thyroid development
- Cardiac anomalies
- Cleft palate
Age-related thymic involution

> 80% of thymic volume is lymphoid tissue at age 20, but this amount declines to ≈5% by age 40, replaced by infiltrating or aberrantly-differentiated adipocytes.

Numerous factors can affect the function of the thymus
T cells mature in the thymus but most die there.

98% of cells die in the thymus without inducing any inflammation or any change in the size of the thymus.

Thymic macrophages phagocytose apoptotic thymocytes.
Questions for the lecture

- How to generate a functional T-cell population with a diverse TCR repertoire?
- How to generate a T-cell population that is self-MHC restricted?
- How to prevent self-reactivity?
- How various T-cell lineages are specified?
Flow Cytometry Data

CD4 single-positive (SP)  Double-positives (DP)

Double-negatives (DN)  CD8 single-positive (SP)
DN1 cells in are not yet committed to become T lymphocytes

- TCR loci are in germline configuration
- Cell can differentiate to become a B lymphocyte, Natural Killer cell, or Dendritic cell

Location: Region 1 in the cortical region
The fate of DN2 cells are more restricted, but not totally committed.

- TCR loci are in germline configuration.
- DN2 cells can still differentiate to thymic dendritic cells, but no longer possess the capability to differentiate to the NK cells or B cells.

Location: Region 2 in the cortical region

CD4- CD44High
CD8- CD25+
CD3-

= proliferation

Germline configuration
DN3 cells are committed to the T lineage

- Downregulation of CD44 expression
- Upregulation of RAG genes
- V-D-J recombination of TCRβ chain locus
- Expression of pre-TCRα chain

Location: Region 3 & 4, subcapsular zone

CD4- CD44Low
CD8- CD25+
CD3-
The pre-TCR\(\alpha\) chain (DN3)

- Invariant Type I TM protein
- Contains only one Ig Domain
- Physically associated with TCR\(\beta\) chain
- Signaling appears to be ligand-independent
  - TCR\(\beta\) chain in this complex lacking extracellular domain is sufficient to allow progression to DP stage

From *Fundamental Immunology*, 4th ed. (Paul)
After Assembly of a Functional pre-TCR

- Shut down of TCRβ rearrangement; TCRβ allelic exclusion
- Onset of proliferation/expansion
- Differentiation to DN4, CD8ISP, and then DP
At the DP stage, TCRalpha rearrangement begins

<table>
<thead>
<tr>
<th>Process</th>
<th>Genome</th>
<th>Cell</th>
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<tbody>
<tr>
<td>$V_\alpha - J_\alpha$ rearrangement</td>
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<tr>
<td>surface expression of $\alpha:\beta$:CD3</td>
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<td>selective events begin</td>
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$V_\alpha - J_\alpha$ rearrangement

surface expression of $\alpha:\beta$:CD3

selective events begin

Figure 7-21 part 3 of 3 Immunobiology, 6/e. (© Garland Science 2005)
T Lymphocyte Maturation in the Thymus

DN1 → DN2 → DN3* → DN4 → CD8 ISP → DP# → CD4 SP

CD4- CD8- CD3- CD44hi CD25*
CD4- CD8- CD3- CD44hi CD25+
CD4- CD8- CD3- CD44lo CD25+
CD4- CD8+ CD3- HSAhi CD25-
CD4+ CD8+ CD3lo CD44- CD25-
CD4+ CD8hi

<-> = proliferation
*Commitment to αβ T lineage
#Positive and negative selection
Fates awaiting a DP thymocyte

- Negative Selection
- Default
- Death by Neglect (95%)
- CD4 SP
- CD8 SP
Positive selection ensures the usefulness of the developing T lymphocytes: self-MHC restriction
Thymic cortical epithelial cells mediate positive selection
Summary

Bone marrow chimeras show that MHC restriction is learnt in the thymus

T cells are ‘educated’ in the thymus to recognise antigens only in the context of self MHC

MHC restriction is learnt in the thymus by positive selection

The MHC haplotype of the environment in which T cells mature determines their MHC restriction element
How the thymus can effectively distinct self from nonself?

- Migratory thymic dendritic cells (CD11c+CD8α-SIRPα+) can acquire self-antigens from peripheral tissues as well as blood borne antigens, and present them to developing thymocytes.

- Residential thymic dendritic cells (following intrathymic differentiation pathway from DN2 cells, CD11c+CD8α+SIRPα-) can cross-presenting self-antigens in the thymic microenvironments.

- How about peripheral antigens that are expressed only in specific tissues, such as liver and pancreas?
Low levels of tissue restricted antigen (TRA) expression in medullary thymic epithelial cells
Genes expressed in pancreatic beta cells are transcribed in the medullary thymic epithelial cells.
Use the Cre-loxP System to generate mice with insulin deletion specifically in thymic epithelial cells (ID-TEC).
New born ID-TEC (insulin deletion in thymic epithelial cells) mice

Control  ID-TEC

Glucagon + Insulin + Nucleus

Normal pancreatic β-cell development
Insulitis of different severities were observed

Postnatal Day 14
Postnatal Day 21

ID-TEC mice develop spontaneous diabetes

**Graph:**
- **X-axis:** Postnatal Days (7, 10, 13, 16, 19, 22, 25, 28, 31, 34)
- **Y-axis (left):** Blood Glucose (mg/dl)
- **Y-axis (right):** Plasma insulin levels (ng/mL)

**Data Points:**
- **ID-TEC** shows a significant increase in blood glucose levels from Postnatal Day 13 onwards.
- **Control** remains stable and lower than ID-TEC throughout the observed period.
Specific loss of insulin secreting $\beta$-cells

Control

ID-TEC

Insulin + Glucagon
Use enzyme-linked immunosorbent spot (ELISpot) assay to detect the presence of insulin-specific autoreactive T-cells in ID-TEC mice.

1. Antibody Coating
2. Blocking
3. Cell Stimulation
4. Cytokine Capture
5. Detection Antibody
6. Detection Enzyme
7. Detection of Cytokines captured with Substrate
Presence of insulin-specific autoreactive T-cells in ID-TEC mice

- Insulin
- GAD65
- Ins B9-23
- GAD 206-220

Ratio of # of spots (ID-TEC/Control)

ID-TEC: [Image of spots]
Control: [Image of spots]

Fan et al. EMBO J, 2009
To transcribe tissue restricted antigens (TRAs), multiple fundamental rules of transcriptional regulation are violated by the mTECs:

- Tissue specificity.
- Lineage specificity.
- Developmental switches.
- Sex specificity
Two mechanisms were identified that can simultaneously mediate the transcription of a large number of TRAs in mTECs.
Aire binds to unmethylated lysine 4 in Histone 3 (H3K4) to open the chromatin for transcription
The \textit{Autoimmune Regulator} (Aire) gene regulates the tissue restricted antigen expression in the mTECs.

What is the pathological consequence of loss of TRA.
Aire-deficient mice show multiple organ autoimmunity
How the small number of mTECs (1-2x10^5) can screen 5x10^7 developing thymocytes?
“Thymic zone defense” from autoimmunity
Transcriptional factors regulate thymocyte lineage specification
Interesting Videos about T-cell Development (Courtesy of Dr. Giannoukakis)

https://youtu.be/9E_UxnC_L2o

https://laraslittlebcells.wordpress.com/?s=Thymus&search=Go