Lecture 21

Development of T cells

Yong Fan

Email: yongf@andrew.cmu.edu
Phone: (412) 359-6382
The Thymus Gland
The life cycle of T cells

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**

![Diagram](image-url)
Severe Combined Immunodeficiency (SCID): Bubble Boy

DiGeorge Syndrome: Chromosome 22q11 deletion.

- Thymus hypoplasia
- Defective thyroid development
- Cardiac anomalies
- Cleft palate
> 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.

Only need to know that microbial infectious (such as HIV) can affect thymus function.
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

NO need to remember the changes of surface markers (CD44CD25) during DN differentiation
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
During DN differentiation, DN cells undergo lineage restriction (become T cells), TCRβ rearrangement (no more 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

< = proliferation
The pre-TCRα chain (DN3)

- Invariant Type I TM protein
- Contains only one Ig Domain
- Physically associated with TCRβ chain
- Signaling appears to be ligand-independent
  - TCRβ 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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</thead>
<tbody>
<tr>
<td>$V_\alpha - J_\alpha$ rearrangement</td>
<td>$V DJ J C$</td>
<td>$\alpha - \beta$ CD3$^{low}$</td>
</tr>
<tr>
<td>surface expression of $\alpha:\beta$:CD3</td>
<td>$V J J C$</td>
<td>CD4$^+$ CD8$^+$</td>
</tr>
<tr>
<td>selective events begin</td>
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Figure 7-21 part 3 of 3 Immunobiology, 6/e. (© Garland Science 2005)
T Lymphocyte Maturation in the Thymus

- DN1: CD4^- CD8^- CD3^- CD44^hi CD25^*
- DN2: CD4^- CD8^- CD3^- CD44^hi CD25^*
- DN3*: CD4^- CD8^- CD3^- CD44^lo CD25^- 
- DN4: CD4^- CD8^- CD3^- CD44^- HSA^hi CD25^- 
- CD8 ISP: CD4^- CD8^+ CD3^- CD44^- HSA^lo CD25^- 
- DP#: CD4^- CD8^+ CD3^lo HSA^hi CD44^- CD25^- 
- CD4 SP: CD4^+ CD8^- CD3^hi 
- CD8 SP: CD4^- CD8^+ CD3^hi HSA^lo 

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

- **Negative Selection**
- **Default**
- **Positive Selection**
  - CD4 SP
  - CD8 SP

**Death by Neglect (95%)**
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?

No need to know the markers for thymic DC types. Only need to know that thymic DCs are important to present blood borne self-antigens, acquired peripheral self-antigens and thymic local self-antigens.
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

Derbinski et al. Nature Immunology, 2001
Use the Cre-loxP System to generate mice with insulin deletion specifically in thymic epithelial cells (ID-TEC)

No need to know the details of the technology
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
ID-TEC mice develop spontaneous diabetes

Blood Glucose (mg/dl)

Postnatal Day 21

Plasma insulin levels (ng/mL)

ID-TEC mice show a significant increase in blood glucose levels compared to controls, indicating the development of spontaneous diabetes.

Postnatal Days

ID-TEC

Control
Specific loss of insulin secreting $\beta$-cells

Control

ID-TEC

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

Antibody Coating

Blocking

Cell Stimulation

Cytokine Capture

Detection Antibody

Detection Enzyme

Detection of Cytokines captured with Substrate

No need to know the details of the technique
Presence of insulin-specific autoreactive T-cells in ID-TEC mice

<table>
<thead>
<tr>
<th>Ratio of # of spots (ID-TEC/Control)</th>
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<tbody>
<tr>
<td>Insulin</td>
</tr>
<tr>
<td>GAD65</td>
</tr>
<tr>
<td>Ins B9-23</td>
</tr>
<tr>
<td>GAD 206-220</td>
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No need to know the detail

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.

1. Fezf2, classic transcription factor action of large numbers of targets (>10,000)
2. Aire (far more famous and well studied) functions as a co-activator by changing the configuration of the chromatin to open status.
Aire binds to unmethylated lysine 4 in Histone 3 (H3K4) to open the chromatin for transcription

No need for details.
The *Autoimmune Regulator* (Aire) gene regulates the tissue restricted antigen expression in the mTECs.

Just need to know Aire is essential for thymic expression (at low levels) of a large number of tissue restricted antigens. Should be able to give out at least one example.

What is the pathological consequence of loss of TRA
Aire-deficient mice show multiple organ autoimmunity.

No need for details, but should know that defects in thymic TRA expression lead to peripheral autoimmunity.
How the small number of mTECs (1-2x10^5) can screen 5x10^7 developing thymocytes?

Three basic mechanisms of TRA presentation: 1) Direct presentation by mTECs; 2) mTECs express the TRAs and handover them to thymic DCs; 3) each mTEC expresses a subset of self TRAs; developing T-cells pass through these "zones" of self (mosaic) to establish self-tolerance.
“Thymic zone defense” from autoimmunity
MHC II (Major histocompatibility complex class II)

TCR (T-cell receptor)

Antigens (self- or non-self)

Death by neglect

Negative selection

Positive selection

Tregs have relative higher affinity to naive T cells

Nature Reviews | Immunology
Transcriptional factors regulate thymocyte lineage specification

No need to know details
Interesting Videos about T-cell Development  
(Courtesy of Dr. Giannoukakis)

https://youtu.be/9E_UxnC_L2o

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