T Cell Development I: The Generation of TCR+ Thymocytes

Questions for the next 2 lectures:

How do you generate a diverse T cell population with functional TCR rearrangements?

How do you generate a T cell population that is self-MHC restricted?

How do you ensure that those diverse T cell receptors are not-self reactive?

How do you coordinate lineage specification with MHC specificity and coreceptor expression?
  • αβ vs. γδ T cell
  • CD4 vs. CD8

The pathway of T cell development

T cells...precursors are born in the bone marrow and “educated” in the thymus.

• The thymus “involutes” with age
• DiGeorge Syndrome, Nude mice

Nude Mice

A. Human (mammal) skin after 60 days.
B. Cat (mammal) skin at 51 days.
C. Chicken (bird) at 32 days;
D. Chameleon (reptile) at 41 days.
E. Fence lizard (reptile) at 28 days.
F. Tree frog (amphibian) at 40 days.
The Thymus is required for T cell development

**scid mutation:**
DNA PK
no gene rearrangement

**nude mutation:**
transcription factor required for terminal epithelial differentiation (whn)

NO B or T CELLS

The Thymus is required for T cell development

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The epithelial cells & developing
T cell development can be characterized using flow cytometry

Real data

DN’s can be further subdivided into DN1 through DN4

“Gate” on CD4 CD8+, analyze for CD44 and CD25
The real data

CD44

CD25

The real data

CD4

CD8

Double negative cells appear in the fetal thymus before double positive cells

K. Hogquist

T cell populations during development and in the lymphoid tissue

Thymocytes at different developmental stages are found in distinct parts of the thymus

Maturation
Most cells fail to complete thymocyte maturation

Macrophages...garbage collectors, contain the dying cells

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T cell development involves the sequential generation, assembly and testing of the newly rearranged TCR
TCR rearrangement is very similar to Ig rearrangement.

**Figure 5-6**

First step: TCRbeta rearrangement

<table>
<thead>
<tr>
<th>State or process</th>
<th>Genome</th>
<th>Cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vβ-DJβ rearrangement in frame, β-chain protein produced</td>
<td>V β J C</td>
<td>CD25+ CD44low thymocyte expresses intracellular β chain</td>
</tr>
<tr>
<td>Surface expression of β chain with surrogate α chain - β rearrangement stops - cell proliferates - CD4/CD8 induction - α transcription starts</td>
<td>V β J C</td>
<td>CD4+8+ cell surface pTαβ CD3εlow</td>
</tr>
</tbody>
</table>

How do you test for successful TCRbeta chain rearrangements if you have not rearranged TCRalpha?

Pre-Talpha

- Inhibition of β chain gene recombination
- Proliferation of pre-T cells
- Stimulation of α chain recombination
- Expression of CD4 and CD8
TCR β chain is required for DN to DP transition:
Experimental Evidence

\[ \text{WT} \\
\text{TCR}\beta^{-/-} < 10^7 \\
\text{Expressing TCR}\gamma\delta \\
\text{TCR}\beta/\delta^{-/-} 10^6 \]

Total thymocyte numbers

Pre-TCR extracellular domains are not required!

\[ \text{WT} 1.7 \pm 0.2 \times 10^6 \\
\text{RAG-1}^{-/-} 1.8 \pm 0.7 \times 10^6 \\
\text{pTαβ}^{-/-} 1.4 \pm 0.4 \times 10^6 \\
\text{pTαβRAG-1}^{-/-} 2.49 \pm 0.6 \times 10^6 \]

(pTαβ;βT receptor restores CD4+8+ development and thymocyte expansion)

β-selection leads to proliferation

\[ \text{Gated on CD4/CD8 DNs} \\
\text{DN2} \quad 9.8 \\
\text{DN3} \quad 50 \]

Proliferating cells are bigger

Forward scatter (cell size)

Does the pre-TCR have a ligand?

Remove extracellular domains...
...and examine T cell development

(Work of Nigel Killeen, UCSF)
TCR β locus has two D-J clusters
Allows a 2nd rearrangement if 1st is nonproductive

Progression through development correlates with rearrangement

At the DP stage, TCRalpha rearrangement begins
TCR $\alpha$ locus has 50 $J\alpha$ segments

Mouse TCR $\alpha$-chain and $\delta$-chain DNA (chromosome 14)

(V$_{\alpha}$ = 100; V$_{\delta}$ = 10)

$J\alpha$ (n = 50)

Mouse TCR $\beta$-chain DNA (chromosome 6)

(V$_{\beta}$ = 20–50)

Mouse TCR $\gamma$-chain DNA (chromosome 13)

(V$_{\gamma}$, L, V$_{\gamma}, L$, C, J)

$\Phi =$ pseudogene

Repertoire


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DP thymocytes express surface TCR

CD3 lo cell-- pre-TCR

CD3 hi cell-- TCR

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TCR $\alpha$ locus-- replacement rearrangement

Repeated rearrangements can rescue unproductive $V\alpha J\alpha$ joints

Subsequent rearrangements bypass non-functional $V\gamma$ gene segment

Several rounds of rearrangement generate a functional $\alpha$ chain

Production of functional $\alpha$-chain mRNA

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TCR $\alpha$ chain is required for DP to SP transition: Experimental Evidence

WT        TCR$\alpha$-/-        TCR$\beta$-/-

$10^8$    $10^8$    <10$^7$

Expressing TCR$_{\gamma\delta}$

Total thymocyte numbers

CD4

CD8
Role of Lck in T cell development

RAG KO

RAG KO x Lck KO

Total thymocyte number

CD4

CD8

0.4 x 10^6

288 x 10^6

10 x 10^6

30-fold reduction in DPs!

Remember what Lck does

Thursday αβ vs γδ
A model for $\alpha\beta$ versus $\gamma\delta$ lineage commitment

Signals through the $\gamma\delta$ TCR switch off the $\gamma$-chain gene and commit the cell to the $\gamma\delta$ lineage.

Signals through the pre-TCR switch off the $\gamma$- and $\delta$-chain genes and commit the cell to the $\alpha\beta$ lineage.

Rearrangement of the TCR$\alpha$ chain creates the mature $\alpha\beta$ TCR receptor.

The $\gamma\delta$ T cell matures and migrates to the periphery.

TCR$\alpha\beta$ lineage comprises the majority of T cells

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Figure 7.24 Immunobiology, 4th ed. (c) Garland Science 2005.

TCR$\alpha$ rearrangement removes the delta locus

Process | Genome | Cell
---|---|---
V$_{\alpha}$ J$_{\alpha}$ rearrangement | surface expression of $\alpha\beta$:CD3 |

Figure 7.31 part 3 of 3 Immunobiology, 4th ed. (c) Garland Science 2005.

$\gamma\delta$ T cells are favored during early fetal development

CD3+ cells, %

Days of gestation

Figure 10.1 Adapted from Immunobiology, 4th ed. (c) Garland Science 2005.
Waves of $\gamma\delta$ T cells with specific TCR usage develop early gestation

Early waves of $\gamma\delta$ T cells

- $\gamma\delta$ cells bearing specific receptors end up in skin (V$\gamma$5), gut (V$\gamma$2), uterus (V$\gamma$6), etc.
- Limited junctional diversity: no N nucleotides (no TdT)
- The mechanisms controlling this limited rearrangement are poorly understood.

Waves of $\gamma\delta$ T cells home to different tissues

- $\gamma\delta$ thymocytes
- Stem cells in fetus
  - Days 14–18 of development
  - V$\gamma$1, 2, 4, 7
  - V$\gamma$5, 6, 7, 8
  - V$\gamma$1, 2, 4, 7J$\gamma$
- Stem cells in fetus and newborn
  - Day 17 of development to day 1 after birth (day 22)
  - V$\gamma$1, 2, 4
- Stem cells from neonate to adult
  - V$\gamma$1, 2, 4, 7J$\gamma$
  - V$\gamma$6, 7, 8
  - V$\gamma$1, 2, 4, 7J$\gamma$

$\gamma\delta$ T cells become established in epidermis

$\gamma\delta$ T cells become established in reproductive epithelium

Some $\gamma\delta$ T cells become established in intestinal epithelium; others are found in lymphoid organs

Antigen recognition by $\gamma\delta$ T cells is different than $\alpha\beta$ T cells

$\gamma\delta$ T cells are not MHC restricted!

Antigen is recognized directly, more like an antibody

In some cases ligands for the $\gamma\delta$ TCR are self proteins upregulated under stress conditions

In humans, circulating $\gamma\delta$ cells recognize a phospholipid antigen from *Mycobacterium tuberculosis*
Dendritic epidermal T cells (DETC)

$\gamma\delta$ T cells home to the skin and wedge among keratinocytes
Involved in wound healing as well as tumor protection

Roles of $\gamma\delta$ T cells in cancer

• $\gamma\delta$-knockout mice have a higher incidence of skin cancers induced by carcinogens, including DMBA/TPA (tumor initiator/promoter) (shown in figure) or methylcolanthrene (MCA).
• The protection is mediated by DETC resident in the mouse skin


DETCs promote wound healing

Appearance of tumor cells in knockout versus wildtype mice treated with carcinogen