T Cell Development II: Positive and Negative Selection

The two phases of thymic development:
- production of T cell receptors for antigen, by rearrangement of the TCR genes
- selection of T cells that can interact effectively with self-MHC with moderate affinity

Second phase of thymic development:
selection of T cells that can interact with self-MHC

- This applies only to αβ TCR-bearing cells (>95% of T cells). γδ T cells are not restricted to interactions with MHC class I or class II molecules.
- This phase of T cell development consists of two steps:
  - positive selection (TCR that can interact with self-MHC)
  - negative selection (eliminate self-reactive cells that are strongly stimulated by MHC + self)

The thymus subsets characterized by CD4 and CD8 expression

MHC Expression

<table>
<thead>
<tr>
<th>Cells</th>
<th>MHC Class I</th>
<th>MHC Class II</th>
</tr>
</thead>
<tbody>
<tr>
<td>T cells</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>B cells</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Macrophages</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Dendritic cells</td>
<td>+++</td>
<td>++</td>
</tr>
</tbody>
</table>

APCs
Positive selection refers to the selection of thymocytes that are able to bind to, and interact with, self-MHC molecules present on thymic cortical epithelial cells.

In positive selection developing thymocytes continue to live if they bind MHC well enough to receive a signal through their TCR. If they don’t bind they die by neglect (about 95% of them).

Positive selection leads to MHC restriction.

TCRα chain rearrangements can continue during positive selection.

The expression of either CD4 or CD8 is determined during positive selection.

Recruitment of the CD4 or CD8 coreceptors induces distinct signals that enable the differentiating cell to couple its TCR specificity with a program of maturation to an appropriate effector lineage.

The signal delivered by engagement of the CD4 coreceptor and the TCR appears to be stronger and/or longer lasting than that delivered through engagement of the CD8 coreceptor and the TCR.
Positive Selection

- Selection of thymocytes that interact with self-MHC molecules leads to MHC restriction.
- Thymocytes that do not interact with self-MHC die by neglect.
- Positive selection is mediated by the interactions of thymocytes with thymic cortical epithelial cells.
- Thymic cortical epithelial cells express both MHC class I and MHC class II molecules, complexed with self-peptides.

T cell receptor transgenic mice:

H-2^b restricted anti-H-Y TCR transgenes analyzed in a H-2^b female

Presence of a second MHC haplotype doesn’t prevent survival and selection

Non-selection: H-2^b restricted anti-H-Y TCR transgenes analyzed in a H-2^d female

If TCR can’t recognize self-MHC peptide complex, it dies by neglect.
CD4/CD8 FACS profile of thymocytes from a MHC-I deficient mice

Negative Selection

- Negative selection refers to the elimination of those thymocytes that bind to self-MHC molecules + self with high affinity.
- In negative selection developing thymocytes die if they bind MHC + self peptides too well (strongly enough so that they would be activated by this interaction, via signaling through their TCR).

What about genes that are not expressed in the thymus?

How do you get rid of T cells that recognize peptides derived from tissue specific proteins?
- Pancreas, eye, heart, intestine, etc...

Medullary thymic epithelial cells express many unexpected “tissue specific” proteins.

Expression of these genes requires a transcription factor named Aire.

Peptides from these proteins are presented to developing thymocytes and mediate negative selection of potentially self-reactive TCRs.

APECED (autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy): a human autosomal recessive disorder -> mutations in AIRE
Ignores the useless
Selects for the useful
Destroys the harmful

How does TCR signaling lead to either positive or negative selection?

Different peptides will interact with a given TCR with different affinities. The combination of this particular affinity and the number of peptides equals the avidity of the TCR for that particular peptide. The avidity between a particular TCR and different peptides will result in distinct signals. These signals lead to unique developmental outcomes — positive vs. negative selection. How exactly these differences are translated into different outcomes remains controversial and an area of intense research.

Fetal thymic organ culture
Fetal mouse
TCR transgenic mouse (class I restricted TCR)

- peptide
+ peptide
Dissociate thymic lobes and analyze cells

OT-1 TCR transgenic mice (anti-Ova-peptide + K\textsuperscript{b})

High affinity binding of DP TCR+ T cell to self-peptide MHC complex leads to negative selection.

SP cells which do exist are MHC class II restricted CD4+ cells.
Variants of Ova (SIINFEKL) + K\textsuperscript{b}

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Sequence</th>
<th>β\textsuperscript{2m}-</th>
<th>β\textsuperscript{2m}+/</th>
</tr>
</thead>
<tbody>
<tr>
<td>40 kd</td>
<td>GAYEFTTL</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>CDC</td>
<td>ASYEFTQL</td>
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<td>None</td>
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<tr>
<td>PolySER</td>
<td>SSYSYSSL</td>
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<td>None</td>
</tr>
<tr>
<td>P7</td>
<td>SIINFEPL</td>
<td>Weak Pos.</td>
<td>None</td>
</tr>
<tr>
<td>V-OVA</td>
<td>RGYNYEKL</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>K1</td>
<td>KIINFEKL</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>R4</td>
<td>SIINFEKL</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>E1</td>
<td>EINFEKL</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>OVAp</td>
<td>SIINFEKL</td>
<td>Negative</td>
<td>Negative</td>
</tr>
</tbody>
</table>

Hoquist & Bevan β\textsuperscript{2}-microglobulin-deficient TCR transgenic mouse.

Add different peptides + β\textsuperscript{2} microglobulin to thymic organ culture and assay for selection.

Selection correlates with TCR affinity for that peptide/MHC complex.

Low affinity peptides induce positive selection in FTOCs

Thresholds for positive and negative selection

Summary

The avidity of the interaction between a peptide and a TCR determines the outcome for cells expressing that TCR

Low avidity peptides lead to death by neglect

Intermediate avidity peptides lead to positive selection

High avidity peptides lead to negative selection

Alloreactivity

- Reaction of T cells against target cells from genetically non-identical individual of same species.
- VERY high frequency of allo-reactive T cells in unperturbed mice and humans (~1 in 10 T cells)!!!
- Why?
Alloreactivity = Cross-reactivity

- Repertoire selected for a certain range of affinity to self-MHC.
- T cell repertoire has “inherent” MHC reactivity.
- Alloreactive cells have not undergone negative selection against foreign MHC peptide reactivity.

Mixed lymphocyte reaction (MLR)

<table>
<thead>
<tr>
<th>Responder Splenocytes</th>
<th>Irradiated Tester</th>
<th>Proliferation</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-2^b</td>
<td>H-2^b</td>
<td>+++</td>
</tr>
<tr>
<td>H-2^d</td>
<td>H-2^b</td>
<td>-</td>
</tr>
<tr>
<td>H-2^b/d</td>
<td>H-2^b</td>
<td>+++</td>
</tr>
<tr>
<td>H-2^b</td>
<td>H-2^b/d</td>
<td>+++</td>
</tr>
</tbody>
</table>

Skin transplants require MHC identity

McDevitt’s and Gorer & Snells experiments mapping the genetics of transplantation led to the discovery of the MHC.