Immunological Tolerance

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Immunological Tolerance

Tolerance to **self**
- highly desired
- central tolerance
- peripheral tolerance
- prevention of autoimmunity

Tolerance to **foreign** antigens
- highly desired
- food antigens
- inhaled antigens
- fetal antigens
- commensal flora
- organ transplants

Tolerance to (altered) **self**
- undesired
- transformed cells
- tumors

Tolerance to **foreign** antigens
- undesired
- pathogens
- vaccines
Overview

1. “Basic” mechanisms of self-tolerance

- central tolerance
- peripheral tolerance

2. Mucosal immunology

- role of innate immunity in tolerance to foreign
- oral tolerance

3. Analyzing tolerance in clinical disease settings

- autoimmune disease and hypersensitivity reactions
- pathogenetic role of genetic and microbial factors
- where does tolerance fail and how can it be assessed?

Abbas 8th edition:

Chapters 8, 15

Chapter 14

Chapters 15, 19
1. “Basic” mechanisms of self-tolerance

<table>
<thead>
<tr>
<th>Feature</th>
<th>Functional significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specificity</td>
<td>Ensures that specific antigens elicit specific responses</td>
</tr>
<tr>
<td>Diversity</td>
<td>Enables immune system to respond to a large variety of antigens</td>
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<tr>
<td>Memory</td>
<td></td>
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<tr>
<td>Specification</td>
<td></td>
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<tr>
<td>Self-limitation</td>
<td></td>
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<tr>
<td>Nonreactivity to self</td>
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</tbody>
</table>

Diversity of Ig and TCR repertoires is generated by combinatorial associations of multiple germline genes.

Ig: $10^{11}$ specificities  
TCR$\alpha\beta$: $10^{16}$ specificities

also includes:  
receptors not capable of recognizing self-MHC  
receptors recognizing self-peptide-MHC complexes
Thymic selection processes

**positive selection:**
selective survival and expansion of thymocytes with TCR capable of recognizing self-MHC (with low avidity)

**negative selection (central tolerance):**
deletion of thymocytes with TCR capable of recognizing self-peptide-MHC complexes with high avidity
Thymic selection processes: Central tolerance

Klein et al., Nat Rev Immunol, 2009

only 1-5% of thymocytes survive both selection processes...

abundance of self-antigens, numbers of TCR engaged, overall avidity of bond...

central tolerance in B cells (bone marrow): receptor editing or deletion by apoptosis
Central tolerance: Exceptions to the rules

High avidity recognition of self-antigen by immature T cells in the thymus induces negative selection but can also promote the development of CD4⁺ CD25⁺ regulatory T cells ("agonist selection")

Klein et al., Nat Rev Immunol, 2014
Central tolerance: Location and timing

<table>
<thead>
<tr>
<th>Stage of maturation</th>
<th>Stem cell</th>
<th>Pro-T</th>
<th>Pre-T</th>
<th>Double positive</th>
<th>Single positive (immature T cell)</th>
<th>Naive mature T cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proliferation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Rag expression</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>TdT expression</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TCR DNA, RNA</td>
<td>Unrecombined (germline) DNA</td>
<td>Unrecombined (germline) DNA</td>
<td>Recombined β chain gene [V(D)J-Cγ]; β chain mRNA</td>
<td>Recombined [β, α chain genes [V(D)J-Cγ]; β and α chain mRNA</td>
<td>Recombined [β, α chain genes [V(D)J-Cγ]; β and α chain mRNA</td>
<td>Recombined [β, α chain genes [V(D)J-Cγ]; β and α chain mRNA</td>
</tr>
<tr>
<td>TCR expression</td>
<td>None</td>
<td>None</td>
<td>Pre-T receptor (β chain/pre-T α)</td>
<td>Membrane αβ TCR</td>
<td>Membrane αβ TCR</td>
<td>Membrane αβ TCR</td>
</tr>
<tr>
<td>Surface markers</td>
<td>c-kit+ CD44+ CD25+</td>
<td>c-kit+ CD44+ CD25+</td>
<td>c-kit+ CD44+ CD25+</td>
<td>CD4+CD8+ TCR/CD3γ</td>
<td>CD4+CD8+ or CD4+CD8+ TCR/CD3γ</td>
<td>CD4+CD8+ or CD4+CD8+ TCR/CD3γ</td>
</tr>
<tr>
<td>Anatomic site</td>
<td>Bone marrow</td>
<td>Thymus</td>
<td>Periphery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Response to antigen</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>Positive and negative selection</td>
<td>Activation (proliferation and differentiation)</td>
<td></td>
</tr>
</tbody>
</table>

cTEC: cortical thymic epithelial cell
mTEC: medullary thymic epithelial cell

Klein et al., Nat Rev Immunol, 2009
Central tolerance: Role for thymic epithelial cells (TEC)

cTEC: cortical epithelial cell → positive selection
mTEC: medullary epithelial cell → negative selection

Klein et al., Nat Immunol 2001

| Table 1. Summary of the cell type-specific pattern of promiscuous gene expression |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Table 1. Summary of the cell type-specific pattern of promiscuous gene expression |
|---------------------------------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| SAP | CRP | Complement C3 | α-fetoprotein | Albumin | Haptoglobin | α1-antitrypsin | FLJ | NOG | GAD67 | GAD65 | Somatostatin | Tryptsin2 | Blastase | Insulin | Tyrosinase | Gp100 | PI-A | Thyroglobulin | Lactalbumin | α1-crystallin | Retinal S antigen | NACHRc1 | IFN-β | Amylase1 |
| cTECs | −/+/+ | + | − | − | − | − | − | − | − | − | −/+/+ | − | − | −/+/+ | + | − | − | −/+/+ | + | − | −/+/+ | + | −/+/+ |
| mTECs | + | + | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | + | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ |
| DCs | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| Macrophages | −/+/+ | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |

Klein et al., Nat Immunol 2001

genes overexpressed in mTEC compared to cTEC

Kyewski et al., Nat Rev Immunol 2004
Central tolerance: Role for AIRE (autoimmune regulator)

An autoimmune disease, APECED, caused by mutations in a novel gene featuring two PHD-type zinc-finger domains

The Finnish-German APECED Consortium

Autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy (APECED) is the only described systemic autoimmune disease with established monogenic background, and the first autoimmune disorder localized outside the major histocompatibility complex (MHC) region. The primary biochemical defect in APECED is unknown. We have isolated a novel gene, AIRE, encoding for a putative nuclear protein featuring two PHD-type zinc-finger motifs, suggesting its involvement in transcriptional regulation. Five mutations in AIRE are reported in individuals with this disorder. This is the first report of a single-gene defect causing a systemic human autoimmune disease, providing a tool for exploring the molecular basis of autoimmunity.

mTEC express high levels of the transcription factor AIRE...

and

....exhibit macroautophagic activity (delivery of endogenous proteins to MHCII)

Aire−/−

reduced promiscuous gene expression in mTEC

multi-organ autoimmune disease!
Central tolerance: ...is not complete...

- not all tissue-restricted antigens are expressed in the thymus
- some tissue-restricted antigens are only expressed in adult life
- expression levels may be insufficient
- avidity-based selection is not “black and white”

• T cells with self-specific TCR can be isolated from most healthy individuals

↓

Peripheral tolerance mechanism must be operative!
Peripheral tolerance: Overview

- Anergy (functional unresponsiveness)
- Apoptosis / Deletion
- Suppression by $T_{reg}$
- (Immunological ignorance)
- (Immunoprvilege)
Peripheral tolerance: Anergy

alternative outcome: immunological ignorance

TCR tg cells can be re-stimulated ex vivo!

co-expression of viral antigen with B7, CD40L, TNFα

or: viral infection

autoimmunity
Peripheral tolerance: Anergy

immunizations:  

| none | • large dose  
|       | • in aqueous form  
|       | • no adjuvants  
|       | • i.v. or oral  
| • “immunogenic” dose  
|       | • in particulate form  
|       | • with adjuvants  
|       | • s.c. or i.d. 

(A) Naive  
(T) Tolerant  
(P) Primed

(B) Proliferation (counts per min) per antigen-specific T cell vs. Antigen concentration (µM)

© Elsevier. Abbas et al: Cellular and Molecular Immunology 6e - www.studentconsult.com
Peripheral tolerance: Anergy

Decision over immunity vs. tolerance lies with the antigen-presenting cells (importance of the "signal 2" concept!)

![Diagram showing antigen recognition and T cell response](https://www.studentconsult.com)
Peripheral tolerance: Anergy

The danger model:


decision over immunity vs. tolerance is determined by recognition of “danger” (DAMPs) rather by self versus non-self (PAMPs) discrimination

endogeneous factors can trigger inflammation

tissues take a critical role in transmitting signals to immune cells
Peripheral tolerance: Anergy

Anergy is induced and maintained actively by several mechanisms:

• Activation of protein tyrosin phosphatases (e.g. SHP-1 / SHP-2)

• Activation of cellular ubiquitin ligases (e.g. Cbl-b)

• Induction and engagement of inhibitory receptors (CTLA-4, PD-1)
Peripheral tolerance: Anergy

Two potential mechanisms behind CTLA-4 mediated immune attenuation:

**competition for B7**
(CTLA-4 has a higher affinity than CD28)

**cell intrinsic inhibitory signaling**
(phosphorylated ITIM motifs on CTLA-4 recruit phosphatases that dephosphorylate signaling molecules in the vicinity)
Peripheral tolerance: Anergy

Significance of CTLA-4:

- Loss of CTLA-4: Lymphoproliferation and fatal multiorgan tissue destruction. Critical regulatory role of CTLA-4!

Polymorphisms in the human CTLA-4 gene are associated with several autoimmune diseases.

CTLA-4-based therapeutics exist!

- CTLA-4 Ig fusion protein for rheumatoid arthritis and kidney transplant patients
- CTLA-4 neutralising antibody for melanoma patients "check-point inhibitor" (autoimmune side effects!)

Tivol EA et al., Immunity. 1995 Nov;3(5): 541-7
Peripheral tolerance: Deletion

Self-antigens cannot be eliminated \(\rightarrow\) chronic/repetitive stimulation

- Deprival of survival factors, activation of Bim
- Induction of Fas/FasL

\[\text{Apoptosis}\]

**Extrinsic Pathway**
- Expression of Fas and FasL
- Engagement of death receptors

**Intrinsic Pathway**
- Induction of pro-apoptotic proteins (e.g., Bim)
- Activation of Bim
- Induction of Fas/FasL

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Mice with mutations in either Fas (lpr/lpr), FasL (gld/gld) or Bim (Bcl2l11/-): autoimmune lymphoproliferative disorders!

increased cellularity  auto-antibodies  immune complex deposition

Peripheral tolerance: Deletion

Dominant Interfering Fas Gene Mutations Impair Apoptosis in a Human Autoimmune Lymphoproliferative Syndrome

(analysing five children with enlarged spleens and lymph nodes, autoimmune hemolytic anemia, thromocytopenia and glomerulonephritis)
Peripheral tolerance: Suppression by regulatory T cells ($T_{\text{regs}}$)

$T_{\text{regs}}$ come in different shapes and flavours (nTreg, Tr1, Th3 cells,...)

**natural $T_{\text{regs}}$**

- CD4$^+$ CD25$^+$ Foxp3$^+$
- Recognition of self antigen in thymus

**induced/adaptive $T_{\text{regs}}$**

- Lymph node
- Possible mechanisms:
  - Production of IL-10 and/or TGFβ
  - Consumption of IL-2
  - Competition for B7 molecules (high CTLA-4)
Peripheral tolerance: Suppression by regulatory T cells ($T_{reg}$)

Foxp3$^+$ T$_{reg}$s

THE master regulators of the immune system!

Sometimes I wish Trex, rather than Tregs, moderated the immune system.
Peripheral tolerance: Suppression by regulatory T cells \( (T_{\text{regs}}) \)

Evidence for a role of Foxp3 and \( T_{\text{regs}} \) in the maintenance of peripheral tolerance:

Disruption of a new forkhead/winged-helix protein, scurfin, results in the fatal lymphoproliferative disorder of the scurfy mouse.

Mary E. Brunkow\(^1\), Eric W. Jeffery\(^1\), Kathryn A. Hjerrild\(^1\), Bryan Paeper\(^1\), Lisa B. Clark\(^1\), Sue-Ann Yasayko\(^1\), J. Erby Wilkinson\(^2\), David Galas\(^3\), Steven F. Ziegler\(^4\) & Fred Ramsdell\(^1\)

The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of \( FOXP3 \)

IPEX is a fatal disorder characterized by immune dysregulation, polyendocrinopathy, enteropathy and X-linked inheritance (MIM 304930). We present genetic evidence that different mutations of the human gene \( FOXP3 \), the ortholog of the gene mutated in scurfy mice (\( Foxp3 \)), causes IPEX syndrome. Recent linkage analysis studies mapped the gene mutated in IPEX to an interval of 17–20-cM at Xp11.23–Xq13.3 (refs. 1,2).

Bennet et al. Nature Genetics 2001
Peripheral tolerance: Suppression by regulatory T cells ($T_{\text{regs}}$)

Further exemplary evidence for a role of $T_{\text{reg}}$ peripheral tolerance:

mouse models of chronic inflammatory diseases, e.g.

(Cutting Edge: Cure of Colitis by CD4$^+$CD25$^+$ Regulatory T Cells$^1$

Christian Motter,$^2$ Holm H. Uhlig,$^2$ and Fiona Powrie$^3$

J Immunol 2003)

(vice versa: induction of disease by $T_{\text{reg}}$ depletion)

human individuals

In vivo switch to IL-10–secreting T regulatory cells in high dose allergen exposure

("natural" desensitization to bee venom in beekeepers related to the induction of IL-10$^+$ $T_{\text{regs}}$)

Flurina Meiler, Judith Zumkehr, Sven Klunker, Beate Rücker, Cezmi A. Akdis, and Mübeccel Akdis

Swiss Institute of Allergy and Asthma Research, University of Zurich, 7270 Davos, Switzerland

Peripheral tolerance: Immune privileged sites

- physical barriers (e.g. BB-barrier)
- absence of lymphatic drainage (e.g. brain)
- low numbers of APC
- low MHC expression (e.g. fetal trophoblast)
- expression of FasL (e.g. testis)
- TGFβ-rich environment (e.g. eye)

sequestration of antigens (immunological ignorance)

induction of anergy or apoptosis

immune privilege must be considered as an active process rather than a merely passive state!