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 vs. DTH reactions
   • pathogenetic role of genetic and microbial factors
   • where does tolerance fail and how can it be assessed?
2. Mucosal immunology

**MALT**: mucosa-associated lymphoid tissues

**BALT**: bronchi-associated lymphoid tissues

**NALT**: nasopharynx-associated lymphoid tissues

**GALT**: gut-associated-lymphoid tissues

MALT of the urogenital tract

- barriers between internal and external environments

- confronted with a vast array of harmless and potentially harmful antigens

- contain lymphoid inductive and effector sites with specialized immune cells

> 200 m²
Mucosal immunology: Organization of the GALT
Mucosal immunology: The difficult task of the GALT

...when two supersystems are confronted....

(abortant) commensal microbes and food antigens need to be tolerated while (rare) pathogens have to be recognised and fought off. Collateral tissue damage is a “no go” in order to prevent loss of sequestration and a potentially deleterious uncontrolled influx of antigens.
Mucosal immunology: Multi-layered adaptations of the GALT

- intestinal epithelial cells
  - intestinal intraepithelial lymphocytes
- intestinal macrophages
- intestinal dendritic cells
- intestinal innate lymphoid cells
- intestinal B cells
- intestinal CD4 T cells

Tolerance mechanisms at the level of innate immunity also exist!
The first line of defense: the mucus layer

functions of mucus layer:

• outer layer: providing optimal symbiotic habitat
• inner layer: segregation of bacteria, local concentration of antimicrobial peptides and IgA
• small intestine: delivery of tolerogenic functions to dendritic cells

colon: two different mucus layers the inner layer is devoid of bacteria!

Muc2−/− mice: penetrance of bacteria spontaneous colitis!

Johansson et al. PNAS 2008
Multi-layered adaptations of the GALT: Intestinal epithelial cells

Production of anti-microbial proteins further limits bacterial-epithelial contact

- defective anti-microbial peptide expression in experimental models (IL-22\(^{-/-}\) or MyD88\(^{-/-}\) mice): increased susceptibility to intestinal inflammation

- ATG16L1 is a disease susceptibility locus in Crohn’s disease - disruption of ATG16L1 in mice results in a disturbed Paneth cell granule exocytosis
Multi-layered adaptations of the GALT: Intestinal epithelial cells

Highly regulated expression of TLRs during postnatal development:

Immediately after birth, IEC respond to LPS exposure but then transiently acquire endotoxin tolerance due to active downregulation of IRAK1.

First contact of neonatal IEC with colonizing bacteria is crucial to simultaneously allow for maturation of the intestinal immune system and to protect from inappropriate pro-inflammatory immune activation until vital structures have been established.

Lack of tolerisation of the neonatal epithelium in Caesarean section-born mice results in massive epithelial apoptosis after oral exposure to E. Coli.

Relevant in pathogenesis of necrotising enterocolitis in pre-term infants?
Multi-layered adaptations of the GALT: Intestinal epithelial cells

Highly regulated and compartmentalized expression of pattern recognition receptors also in mature enterocytes:

NLR family receptors for bacterial flagellin are located in the cytosol

TLR5 is only expressed on the basolateral, not the apical side

...allows for discrimination of commensal flora vs. invading pathogens!
...however, “tonic” TLR signaling is vital for intestinal homeostasis!

MyD88−/− mice suffer from an exaggerated DSS-induced colitis due to a lack of microbial TLR-induced tissue protective factors (and anti-microbial peptides) in the intestinal epithelium.

Mice with an epithelial-specific inhibition of NFκB develop spontaneous intestinal inflammation due to impaired antimicrobial peptide expression, increased epithelial cell apoptosis and increased translocation of bacteria.
Multi-layered adaptations of the GALT: Intestinal epithelial cells

by the production of a variety of cytokines and other factors, the intestinal epithelium can actively modulate immune responses and “educate” local immune cells towards tolerance.

e.g. production of:

- TGFβ
- IL-10
- TSLP
- retinoic acid
- April
- BAFF

modulation / conditioning of DC

bias of Th differentiation into Th2 / T_{reg}

promotion of B cell IgA switching

Artis D, Nat Rev Immunol 2008
Multi-layered adaptations of the GALT: Intestinal macrophages

...are continuously replenished from blood monocytes and educated by the local tissue environment (e.g. IL-10, TGFβ) to acquire the typical properties of resident intestinal MΦ: IL-10 production, highly efficient phagocytosis and bacterial killing in the absence of pro-inflammatory cytokine secretion!
Multi-layered adaptations of the GALT: Intestinal macrophages

Mutations in the human IL-10R gene: very-early-onset colitis

Moran CJ, Inflamm Bowel Dis 2013

IL-10-deficient (IL-10$^{-/-}$) mice: spontaneous intestinal inflammation

Interleukin-10 Receptor Signaling in Innate Immune Cells Regulates Mucosal Immune Tolerance and Anti-Inflammatory Macrophage Function

Dror S. Shouval,1,2,3 Amlan Biawas,1,2,3 Jeremy A. Goettel,1,2,3 Katelyn McCann,1,2,3 Evan Conaway,3 Naresh S. Redhu,1,2,3 Ivan D. Mascarenhas,4 Ziad Al Adham,5 Sydney Lavoie,1 Mouna Ibourk,1 Deanna D. Nguyen,67 Janneke N. Samsom,2,3 Johannes C. Escher,1,2,3 Raz Somech,10,12,13 Bata Weiss,11,12,13 Rita Beier,11,12,13 Laurie S. Conklin,14,15 Christen L. Ebens,15,16 Fernanda G.M.S. Santos,18,23 Alexandre R. Ferreira,18,23 Mary Sherlock,17,23 Atul K. Bhan,18,19 Werner Müller,20 J. Rodrigo Mora,20 Francisco J. Quintana,4 Christoph Klein,21,23 Alexio M. Muise,23 Bruce H. Horwitz,2,3,4 and Scott B. Snapper1,7,22,23,*

Macrophage-Restricted Interleukin-10 Receptor Deficiency, but Not IL-10 Deficiency, Causes Severe Spontaneous Colitis

Ehud Zigmond,1,2 Biana Bemshtein,1 Gilgi Friedlander,3 Catherine R. Walker,4 Simon Yona,1 Ki-Wook Kim,1 Ori Brenner,5 Rita Krauthammer,1 Chen Yarol,2 Werner Müller,20 and Steffen Jung1,24

Immunity 2014

Lack of IL-10 receptor signaling in intestinal macrophages prevents their anti-inflammatory conditioning & leads to intestinal inflammation.
Multi-layered adaptations of the GALT: Intestinal dendritic cells

- DC in Lamina Propria (LP), Mesenteric Lymph nodes (MLN), Peyer’s patches (PP)
- various subsets, two main subsets: CD103$^+$ vs. CD103$^-$ DC

LP-DC efficient inducers of Foxp3$^+$ T$_{reg}$s

capacity lies within the CD103$^+$ subset...

intestinal CD103$^+$ DC have a propensity to induce Foxp3$^+$ T$_{reg}$ and are critically required for induction of oral tolerance

...is associated with production of TGFβ and the vitamin A-derivative retinoic acid

Sun et al., JEM 2007
Coombes et al., JEM 2007
Mucosal immunology: Oral tolerance

The phenomenon:
Oral administration of antigen can lead to suppressed immune responses to peripheral challenge with the same antigen.

TGFβ appears to be involved

tolerance can be transferred by T cells
Mucosal immunology: Oral tolerance

The (likely) mechanism:

• (clonal deletion)
• induction of regulatory T cells

<table>
<thead>
<tr>
<th>Treg subtype</th>
<th>Induction</th>
<th>Cytokine</th>
</tr>
</thead>
<tbody>
<tr>
<td>nT_{reg} (Foxp3+)</td>
<td>IL-2, TGFβ</td>
<td>TGFβ, IL-10, cytokine-independent suppression?</td>
</tr>
<tr>
<td>Th3 (Foxp3+)</td>
<td>TGFβ</td>
<td>TGFβ, IL-10, IL-4</td>
</tr>
<tr>
<td>Tr1 (Foxp3-)</td>
<td>IL-10</td>
<td>IL-10, TGFβ, IFNγ</td>
</tr>
<tr>
<td>iT_{reg} (Foxp3+)</td>
<td>TGFβ, IL-2, retinoic acid</td>
<td>TGFβ, IL-10, cytokine-independent suppression?</td>
</tr>
</tbody>
</table>

feeding of ovalbumin (but not BSA) to Ovalbumin-TCR tg mice (lacking endogenous Tregs) de novo induces Foxp3^+ CD4^+ T_{regs} cells in the MLN Coombes et al., J Exp Med 2007
Mucosal immunology: Oral tolerance

Evidence exists that oral tolerance mediated by $T_{\text{regs}}$ is operative in humans.
Mucosal immunology: Oral (mucosal) tolerance

Allergen-specific immunotherapy / desensitization

- Repetitive administration of antigens via the oral, sublingual (nasal) route or the skin
- Inhibition of basophil and mast cell activation
- Skewing of Th2 cells to allergen-specific $T_{regs}$
- Shift from IgE to IgG4
Mucosal immunology: Oral tolerance does not apply to commensal flora

The systemic immune system is ignorant, rather than tolerant of the intestinal commensal flora!

Mucosal DC loaded with commensal bacteria do not traffic beyond the mesenteric lymph node!

MacPherson AJ et al., Science 2000

MacPherson et al. Nat Rev Immunol 2004