Mucosal Immunology

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Mucosal surfaces are the major portals of entry for antigen

Largest area of contact of the immune system with the environment.

Largest accumulation of lymphoid tissue in the body:
- \(6 \times 10^{10}\) antibody forming cells in mucosal tissues vs \(2.5 \times 10^{10}\) in lymphoid organs.

The gut associated lymphoid tissue (GALT) contains more lymphocytes than all of the secondary lymphoid organs combined!

Secretory IgA is produced at a rate of 40-60 mg/kg/day.
Antigens enter through mucosal surfaces

Lymphoid cells protect epithelial barriers at mucosal surfaces

From Gerald Pier, Channing Labs, HMS
The gut-associated lymphoid tissue contains both inductive and effector sites

Peyer’s patch (inductive)

Villus epithelium and lamina propria (effector)

The intestinal epithelium is self-renewing

Small intestine

Colon

Specialized protective adaptations of the intestinal epithelial barrier

1. Mucus
2. Anti-Microbial Peptides
3. Intercellular **tight junctions** that restrict the passage of even very small (2kD) molecules between cells
4. Secretory IgA
Mucus layers in the small intestine and colon

Small intestine

- Lumen
- Mucus layers
- Enterocyte
- Crypt
- Goblet cell
- Stem cell
- Paneth cell
- Mucus secretion, expansion and release
- Antibacterial products
- Diffusion

Colon

- Lumen
- Outer mucus layer
- Inner mucus layer
- Formation of loose mucus by endogenous proteolysis
- Mucus secretion, expansion and attachment

Anti-microbial peptides protect the intestinal epithelial barrier

Anatomy of the mucosal barrier: the intestinal epithelial junctional complex

The epithelial tight junction regulates mucosal homeostasis

IgA (not IgG) is the major isotype of Ig synthesized by the body! At least 80% of all plasma cells are located in the intestinal lamina propria and together produce more IgA than all other Ig isotypes combined. Class switching to IgA is regulated by TGF-β.
Models of J chain expression

Is J chain protein expressed in all plasma cells but degraded when it is not associated with secretory Ig?
Or is it absent from some B cells?

IgA binds to the polymeric Ig receptor (pIgR), also known as transmembrane secretory component (SC) on the basolateral surface and is transported to the apical surface. The portion of the pIgR attached to the Fc region of IgA is then enzymatically cleaved and stays bound to dimeric IgA as SC.

From Gerald Pier, Channing Labs, HMS
Secretory IgA has several functions at epithelial surfaces

**Figure 9.6 Principles of Mucosal Immunology (© Garland Science 2013)**
Mechanisms of antigen sampling in the small intestine

Schulz, O & Pabst, O. Trends Immunol 2013, 34: 155
The chemokine CCL25 (also called thymus expressed chemokine, TECK) is secreted only by epithelial cells in the thymus and the small intestine, and attracts developing CCR9+ T cells to these sites.

The dual expression of the intestinal homing receptor $\alpha 4\beta 7$ (an integrin which binds the mucosal vascular addressin MADCAM-1) and CCR9 allows for the selective homing of memory T cells to the intestinal lamina propria.

Enzymatically converted vitamins control lymphocyte migration in the skin and the gut

Dendritic cells (DCs) in the GALT express enzymes required for converting dietary vitamin A to retinoic acid (RA). T cell activation in the presence of retinoic acid induces the expression of the gut homing receptors CCR9 and α4β7.

DCs in the skin convert sunlight induced vitamin D3 to its active form 1,25(OH)_{2}D_{3}, which induces the expression of CCR10 on activated T cells, allowing their migration into the epidermis.

From Mebius, Nat. Immunol 2007, 8: 229
Memory T cells accumulate in the GALT

Memory T effector cells accumulate in the intestinal lamina propria, enabling the GALT to respond quickly and effectively to challenge with enteric pathogens.

Antigen challenge redistributes memory T effector cells to “man the barrier” for strategic mucosal defense
Intestinal intraepithelial lymphocytes

Atypical populations of T cells that reside between enterocytes above the basement membrane.

Express CD8 as a **CD8$$\alpha\alpha$$ homodimer** (rather than the CD8$$\alpha\beta$$ heterodimer expressed by CD8$$^+$$ T cells at other sites)

Many IEL are **constitutively cytolytic** directly ex vivo.

A large proportion of IEL bear $$\gamma\delta$$ (as opposed to $$\alpha\beta$$) T cell receptors.

From Karen Edelblum, U. Chicago
IEL act as sentinels to detect and repair damaged epithelium


Edelblum et al PNAS 2012, 109: 7097
How does the gut associated lymphoid tissue distinguish innocuous dietary antigens and commensal bacteria from pathogenic microbes 
....and mount an appropriate response to each?
Oral tolerance- induction of mucosal and systemic non-responsiveness to orally-administered antigens
Orally administered antigen ameliorates disease in a large variety of experimental models

<table>
<thead>
<tr>
<th>Disease model</th>
<th>Protein fed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Airway eosinophilia</td>
<td>OVA</td>
</tr>
<tr>
<td>Allergy</td>
<td>Derp I, cedar pollen</td>
</tr>
<tr>
<td>Anti-phospholipid syndrome</td>
<td>β2-glycoprotein</td>
</tr>
<tr>
<td>Arthritis (CIA, AA, AIA, PIA, SCW)</td>
<td>Collagen II, Hsp65, BSA</td>
</tr>
<tr>
<td>Atherosclerosis</td>
<td>Hsp65</td>
</tr>
<tr>
<td>Cardiac reperfusion injury</td>
<td>Troponin</td>
</tr>
<tr>
<td>Colitis</td>
<td>Colonic proteins, OVA</td>
</tr>
<tr>
<td>Diabetes (NOD mouse)</td>
<td>Insulin, GAD, OVA</td>
</tr>
<tr>
<td>Encephalomyelitis (EAE)</td>
<td>MBP, PLP, MOG, GA</td>
</tr>
<tr>
<td>Food hypersensitivity</td>
<td>aS1-casein</td>
</tr>
<tr>
<td>Myasthenia gravis</td>
<td>AchR</td>
</tr>
<tr>
<td>Neuritis</td>
<td>PNS-myelin</td>
</tr>
<tr>
<td>Nickel sensitization</td>
<td>Nickel</td>
</tr>
<tr>
<td>Sjögren’s syndrome</td>
<td>Ro peptides</td>
</tr>
<tr>
<td>Stroke</td>
<td>MOG</td>
</tr>
<tr>
<td>Thyroiditis</td>
<td>Thyroglobulin</td>
</tr>
<tr>
<td>Transplantation</td>
<td>Alloantigen, MHC peptide</td>
</tr>
<tr>
<td>Uveitis</td>
<td>S-Ag, IRBP</td>
</tr>
<tr>
<td>Nerve injury</td>
<td>MBP</td>
</tr>
</tbody>
</table>

OVA, ovalbumin; BSA, bovine serum albumin; MBP, myelin basic protein; MOG, myelin oligodendrocyte glycoprotein; AchR, acetylcholine receptor; MHC, major histocompatibility complex.

A multistep model of oral tolerance to dietary antigens

I. Antigen loaded CD103+ DC migrate to MLN
II. RA produced by DC and stromal cells in MLN induce homing receptors and favor TGF-β dependent conversion of Foxp3+ Tregs
III. Committed Tregs home back to LP
IV. Tregs expand under the influence of IL-10 produced by CX3CR1hi macrophages
V. Some Tregs exit mucosa via lymph or bloodstream to promote systemic tolerance

Pabst and Mowat, Mucosal Immunology 2012, 5: 232
Tolerance to dietary antigen requires the induction of a bacteria-induced barrier protective response

Stefka, Feehley et al. PNAS 2014, 111; 13145
Mucosal Vaccines

Mucosal (oral/nasal) vaccines are the preferred method for vaccination in the developing world.

Mucosal vaccines are easily administered (needle-free), non-invasive and cost-effective.

Only mucosal vaccination elicits a protective secretory IgA response.
### Currently licensed mucosal vaccines

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Trade name</th>
<th>Composition</th>
<th>Dosage</th>
<th>Immunological mechanism</th>
<th>Efficacy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rotavirus</td>
<td>Rotarix, RotaTeg</td>
<td>Live attenuated, monovalent or pentavalent</td>
<td>Oral, 3 doses</td>
<td>Mucosal IgA and systemic neutralizing IgG</td>
<td>Over 70%-90% against severe disease</td>
</tr>
<tr>
<td>Poliovirus</td>
<td>Orimune, OPV, Poliomyelitis</td>
<td>Live attenuated trivalent, bivalent, and monoavalent polioviruses</td>
<td>Oral, 3 doses</td>
<td>Mucosal IgA and systemic IgG</td>
<td>Over 90% in most of the world</td>
</tr>
<tr>
<td>Salmonella Typhi</td>
<td>Vivotif, Ty21A</td>
<td>Live attenuated <em>S. Typhi</em> bacteria</td>
<td>Oral, 3-4 doses</td>
<td>Mucosal IgA, systemic IgG, and CTL responses</td>
<td>Variable, but more than 50%</td>
</tr>
<tr>
<td><em>Vibrio cholera</em></td>
<td>Dukoral, ORC-Vax, Shanchol</td>
<td>Inactivated <em>V. cholera</em> O1 classical and El Tor biotypes with or without CTB</td>
<td>Oral, 2-3 doses</td>
<td>Antibacterial, toxin-specific, and LPS-specific IgA</td>
<td>Strong herd protection over 85%</td>
</tr>
<tr>
<td>Influenza type A and B virus</td>
<td>FluMist</td>
<td>Live viral reassortant with trivalent mix of H1, H3, and B strains of hemagglutinin and neuraminidase genes in an attenuated donor strain</td>
<td>Intranasal in young children, 2 doses</td>
<td>Hemagglutinin- and neuraminidase-specific mucosal IgA and systemic IgG responses</td>
<td>&gt;85% in children, variable in adults</td>
</tr>
</tbody>
</table>

Mucosal vaccination routes and compartmentalization of effector functions

Mucosal adjuvants

Adjuvants that are effective parenterally are generally toxic or unstable when given orally.

The tendency of the GALT to induce tolerance to soluble antigens has made identification of effective mucosal adjuvants difficult.

Microbial products such as cholera toxin, E. coli heat-labile toxin and oligodeoxynucleotides containing a bacterial CpG motif can act as effective mucosal adjuvants and induce both mucosal and systemic immune responses to co-administered protein antigens.
Pattern recognition receptors and their cellular location

Figure 16.1 Principles of Mucosal Immunology (© Garland Science 2013)
Bacterial TLR ligands
TLR expression in the intestinal epithelium

Pathogenic bacteria use various strategies to trigger a proinflammatory program in intestinal epithelial cells

Commensal bacteria populate our skin and mucosal surfaces and profoundly influence our health.

There are as many E. coli in our gut as there are people on earth!
We exist in a dynamic interrelationship with our commensal microbiome!

Healthy individuals “tolerate” their intestinal microbiota but are also constantly receiving signals from the microbiome that have a profound impact on both systemic and mucosal immunity.
The commensal microbiota confers many health benefits to the host.

- Synthesize essential metabolites such as vitamin K.
- Break down plant fibers in food to release small molecules that can be used in metabolism and biosynthesis.
- Inactivate toxic substances in food or made by pathogens, degrading toxins into harmless components.
- Prevent pathogens from benefiting from the resources of the human gut, limiting pathogen species to small numbers that are not harmful.
- Interact with epithelium to trigger development of secondary lymphoid tissue.

Cofactor for synthesis of clotting factors in the liver.
Release of small molecules that can be used in metabolism and biosynthesis.
Degradation of toxins into harmless components that can be used by human cells.
Limitation of pathogen species to small numbers that are not harmful.
Establishment of the gut-associated lymphoid tissue.

Figure 10.5 The Immune System, 4th ed. (© Garland Science 2015)
The 16S rRNA gene is highly conserved among bacterial species.

“Universal” primers target conserved regions of this gene and allow for amplification and sequencing of species specific hypervariable regions for bacterial classification.

Structure of 16S ribosomal RNA
The composition of the microbiota varies by anatomical site

The gastrointestinal microbiota changes throughout life

Newborn
- Initial gut bacteria (founder species) depends upon delivery mode
  - Vaginal delivery: Lactobacillus, Prevotella spp.
  - Vertical inheritance from mother
  - C-section: Staphylococcus, Corynebacterium, Propionibacterium spp.
  - Higher susceptibility to certain pathogens
  - Higher risk of atopic diseases

Early childhood
- New strains (less certain in origin) outcompete old ones
- Rapid increase in diversity
- Early microbiota development = high instability
- Shifts in response to diet, illness

Adult
- Highly distinct, differentiated microbiota
- Microbial community may continue to change, but at a slower rate than in childhood

Elderly
- Substantially different gut communities than in younger adults

Dominguez-Bello, MG et al, Gastroenterology 2011; 140:1713
Antibiotic use

Western high fat, low fiber diet

Elimination of entero-pathogens (H. pylori, helminths)

Vaccination/reduced exposure to infectious disease

Caesarean birth/formula feeding

“Diseases of Western Society”

Inflammatory Bowel Disease

Obesity

Food Allergy

Diabetes

Autism

Asthma

Alteration of commensal microbiota “dysbiosis”

genetically susceptible individual

Feehley et al Seminars in Immunopathology 2012, 34; 671
FOOD ALLERGIES IN THE U.S.

15 MILLION
Americans have food allergy, a serious medical condition.

People can be allergic to any food, but there are

**8 FOODS THAT CAUSE THE MOST REACTIONS.**

- Milk
- Eggs
- Peanut
- Tree Nuts
- Soy
- Wheat
- Fish
- Shellfish

Reactions can range from a mild response to **anaphylaxis**, a severe and potentially deadly reaction.

**Every 3 minutes** a food allergy reaction sends someone to the ER.

The number of people who have the disease is growing, increasing **50% among children** between 1997 and 2011.

It now affects **1 IN 13 children**
Germ free mice are a powerful tool to examine the role of the commensal microbiota in the regulation of health and disease.
Protection against allergic sensitization to food requires the induction of a bacteria-induced barrier protective response.
The composition of the fecal microbiota is altered in cow’s milk allergic infants

Berni Canani, Sangwan, Stefka et al ISMEJ 2016, 10; 742
modified from
M. Velasquez-Manoff,
Nature 2015, 518: S4