Why do we Need to Understand How the Mucosal Immune System Works?

- The mucosa is the major site of contact in the body for foreign antigens
- Gastrointestinal diseases kill more than 2 million people every year
- Lack of effective mucosal vaccines
The Mucosa is Bombarded by Foreign Antigens

- No Response (Tolerance)
- Response (Immune Activation)

• Eradication
• Containment
• Disease

mucosal barrier

Learning Objectives

• Identify the major populations of lymphoid cells in the intestinal tract.

• Describe the basis of lymphocyte migration (homing) into the intestinal mucosa.

• Describe secretory immunoglobulins and their transport at mucosal surfaces.

• Understand the concept of mucosal immunity versus oral tolerance.
The Human Gut Flora

- Rapidly colonises gut after birth
- Comprises more than $10^{14}$ organisms
- Weighs 1-2 kg
- More than 400 species
- An individual's flora is immunologically distinct
- Symbiotic relationship with host
- Probiotics

Bacteria of the Oral Commensal Flora

- The oral flora appears to be dominated by the a-haemolytic streptococci. However, many bacteria are not cultivable as pure strains in artificial culture.
- The oral flora demonstrates how many microbes live in complex mutually-sustaining communities.
- The flora of different anatomical sites can vary markedly, even though the sites may be in close proximity. This may be illustrated by observing the flora of the tongue and of the back surface of the teeth.
- On the soft tissue of the tongue, *Streptococcus salivarius* can be found in relatively high numbers. *Streptococcus mutans* on the other hand is rarely isolated from the tongue and, if it is found, then the relative numbers are low.
- In contrast, *Streptococcus mutans* is found in large numbers on the teeth but *Streptococcus salivarius* is rarely seen there.
Factors Controlling the Intestinal Microflora

- Saliva
- Stomach acid
- Bile
- Epithelial barrier
- Water & electrolyte secretion
- Mucus, antimicrobial products, IgA
- Peristalsis
- Bacterial flora
**Physiologic Functions of Intestinal Microflora**

- Intestinal microflora is not required for survival and health. Germ-free animals (mice, rats) are healthy and reproduce normally.

- Intestinal microflora is important for "colonization resistance", i.e., decreased susceptibility to infection with enteric pathogens.

- Intestinal microflora affects multiple metabolic and immunologic host functions.

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**Our Mucosal Flora Helps Prevent Colonisation by Pathogens**

- The colon is colonized by large numbers of commensal bacteria
- Antibiotics kill many of these commensal bacteria
- Clostridium difficile gains a foothold and produces toxins that cause mucosal injury
- Neutrophils and red blood cells leak into gut between injured epithelial cells

Fig 10.21 © 2001 Garland Science
Organisation of the Mucosal Immune system

- Organized mucosal lymphoid tissue
  - Tonsils
  - Adenoids
  - Peyer’s patches
  - Isolated lymphoid follicles
  - Appendix
- Intraepithelial lymphocytes
- Lamina propria lymphocytes

Three major lymphoid populations in the intestinal tract

1. Peyer's Patch
2. Intraepithelial lymphocytes (IEL)
3. Lamina propria lymphocytes (LPL)
Peyer’s patches

- Play a key role in the initiation of mucosal immunity
- PP differ from other lymph nodes in that they lack afferent lymphatics
- Efferent lymphatics drain into mesenteric LN
- Follicles contain B lymphocytes, DC, and macrophages
- Dome and parafollicular regions contain T cells
- B cells in germinal centers have switched to IgA
- Plasma cells are rare
Peyer's patch

Follicle-associated epithelium (FAE)

Dome

M cell

lumen

B

T

T

B

T

Initiation of Gut Responses

M cells are interspersed between enterocytes and in close contact with subepithelial lymphocytes and dendritic cells

M cells take up antigens from the gut lumen by endocytosis

Antigens are released beneath M cells and taken up by antigen-presenting dendritic cells

Fig 10.17 © 2001 Garland Science
Small Intestinal Villous

Intraepithelial lymphocytes (IEL) reside in the paracellular space between epithelial cells.

IEL

CD103

E-cadherin

Epithelial cell

Epithelial cell

Goblet cells

Dendritic cells

B lymphocytes

T lymphocytes

Plasma cells

Macrophages

 Mast cells

Eosinophils

Intraepithelial lymphocytes

Lamina propria
Intraepithelial lymphocytes (IEL)

- Lymphocytes located between intestinal epithelial cells. IEL are predominately T cells.
- The majority (80-90%) of IELs express the \(\alpha\beta\) TCR; 10-20% of IEL express \(\gamma\delta\) TCR. Most IEL are CD8+ and have an \(\alpha\alpha\) rather than the \(\alpha\beta\) form of CD8.
- IEL attach to epithelial cells through an interaction between the integrin CD103 on the IEL and E cadherin on the epithelial cell.
- IELs can produce cytokines (e.g., IFN-\(\gamma\), IL-5) and can be cytolytic but their specific functions are not well understood.
Lamina propria lymphocytes (LPL)

- The lamina propria (LP) contains a mixture of T cells, B cells, plasma cells, macrophages, dendritic cells, eosinophils, and mast cells interspersed in the vascular- and lymphatic-rich connective tissue.

- LP T cells: >95% αβ TCR, <5% γδ TCR, 70% CD4, 30% CD8
  Key in intestinal host defense and inflammation

- LP Plasma cells: 70-90% IgA-producing, 10-20% IgM, 2% IgE, few IgG-producing cells
  Responsible for local IgA and IgM production

CD8 T Cells Predominate in the Intraepithelial Region and CD4 T Cells Predominate in the Lamina Propria
Immunofluorescent stain showing IgA B Cells (yellow) in the Lamina Propria of Small Intestine

LYMPHOID MIGRATION AND HOMING IN THE INTESTINAL TRACT
LYMPHOCYTE TRAFFIC FROM PEYER’S PATCHES TO OTHER MUCOSAL SITES: THE “COMMON MUCOSAL IMMUNE SYSTEM”

LYMPHOCYTE MIGRATION INTO MUCOSAL SITES INVOLVES AN INTERACTION BETWEEN THE LYMPHOCYTE INTEGRIN α4β7 AND A MUCOSAL VASCULAR ADDRESSIN, MadCAM-1
Two special features of the intestinal immune system

- Predominance of secretory IgA in intestinal secretions
- Selective non-responsiveness ("oral tolerance") towards numerous ingested antigens

Synthesis of IgA exceeds that of all other immunoglobulins in the body
DEVELOPMENT OF IgA PRODUCING CELLS IN HUMAN SMALL INTESTINE

Schematic of sIgA Molecule
Structure of secretory IgA (sIgA)

- Dimer (MW ~380 kDa) composed of two monomer IgA subunits, secretory component (SC), and J chain
- IgA and J chain are synthesized by B cells/plasma cells.
- The polymeric immunoglobulin receptor (pIgR) is synthesized by epithelial cells
- One J chain per polymer; J chain important for formation of polymeric IgA, as well as polymeric IgM
- The pIgR is expressed on the basolateral side of epithelial cells and transports dimeric IgA across the epithelium into the lumen. It is cleaved and remains bound to the sIgA molecule where it is termed SC.

IgA transport into the intestinal lumen
There are 2 subclasses of Human IgA and differences in the Relative amounts of each present in serum and secretions

SERUM
IgA is mainly IgA1 and a monomer

IgA2 15%
IgA1 85%

MONOMER 85%
DIMER 15%

SECRETIONS
IgA is mainly a dimer
The ratio of IgA2:IgA1 increases in the more distal small intestine and colon

IgA1 50%
IgA2 50%

MOSTLY DIMER

Physiologic functions of secretory IgA

- Bind antigens in intestinal lumen (e.g., food antigens, bacterial and protozoan pathogens, viruses) to prevent interactions with, and uptake by, intestinal epithelial cells.
- Neutralization of viruses inside epithelial cells (e.g. polio).
- Protection of nursing newborn (breast milk contains secretory IgA).
- IgA is relatively non-inflammatory, since it does not fix complement by the classical pathway. This might counteract the pro-inflammatory functions of IgG and IgM in the intestine.
Ocular Immune Privilege

- Ocular immunologic privilege is the concept that foreign antigens and grafts placed in the anterior chamber of the eye do not induce an immune response.
- One mechanism for ocular immune privilege is termed Anterior Chamber Associated Immune Deviation (ACAID).
  - In ACAID, effectors of immunogenic inflammation (delayed hypersensitivity T cells, complement-fixing antibodies) are selectively suppressed, whereas other effectors (precursor cytotoxic T cells, non-complement fixing antibodies) are preserved.
  - ACAID is initiated by an antigen-specific signal generated within the anterior chamber via intraocular dendritic cells and macrophages.
  - Under the influence of immunoregulatory factors in aqueous humor, these cells capture antigen, process it uniquely, and migrate across the trabecular meshwork into the blood and thence to the spleen.
Table 3 | Soluble factors in aqueous humor that promote ocular immune privilege

<table>
<thead>
<tr>
<th>Factor</th>
<th>Activity</th>
<th>References</th>
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<tr>
<td>TGF-β2</td>
<td>Suppresses the activation of T cells, NK cells and macrophages;</td>
<td>71, 115</td>
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<td></td>
<td>confers tolerance-promoting properties on APCs</td>
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<tr>
<td>α-MSH</td>
<td>Converts IFN-γ-producing T cells into regulators;</td>
<td>85, 86</td>
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<td>Inhibits the activation of polymorphonuclear leukocytes</td>
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<td>VIP</td>
<td>Inhibits T-cell activation and differentiation</td>
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<td>CGRP</td>
<td>Inhibits macrophage activation and effector function;</td>
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<td></td>
<td>Impairs APC capacity to promote T&lt;sub&gt;+&lt;/sub&gt; cell differentiation</td>
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<td>TSP</td>
<td>Causes APCs to activate TGF-β and secrete CXCL2;</td>
<td>66, 116</td>
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<td></td>
<td>suppresses APC expression of IL-12 and CD40</td>
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<td>MIF</td>
<td>Inhibits NK-cell killing of target cells</td>
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<td>IL-1Ra</td>
<td>Inhibits pro-inflammatory effects of IL-1α/β</td>
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<td>CD46, CD55, and CD59</td>
<td>Inhibit complement activation</td>
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<tr>
<td>CD65L</td>
<td>Suppresses polymorphonuclear leukocyte recruitment and activation</td>
<td>119</td>
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Background Reading

- **Ocular Immune Privilege:**
    - Ocular Immune Privilege: Therapeutic Opportunities From An Experiment of Nature, J. Wayne Streilein
  - Immunological Reviews 213:23 (2006)
    - Ocular Autoimmunity: The price of privilege?

- **Oral Immunity:**
    - Protective and Destructive Immunity in the Periodontium: Part 1-Innate and Humoral Immunity
    - Protective and Destructive Immunity in the Periodontium: Part 2-T cell mediated immunity

<table>
<thead>
<tr>
<th>Sites</th>
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<tr>
<td>Eye: cornea, anterior chamber,</td>
<td>Eye: cornea, lens, pigment</td>
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<td>vitreous cavity and subretinal space</td>
<td>epithelium and retina</td>
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<td>Brain: ventricles and striatum</td>
<td>Brain and spinal cord</td>
</tr>
<tr>
<td>Pregnant uterus</td>
<td>Placenta</td>
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<td>Ovary</td>
<td>Ovary</td>
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<td>Testis</td>
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<tr>
<td>Adrenal cortex</td>
<td>Liver</td>
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<td>Hair follicles</td>
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<td>Hamster cheek pouch</td>
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<tr>
<td>Certain tumours</td>
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