Mucosal Immunology

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Gastrointestinal tract

Mucosal tissues

Mouth
Esophagus
Stomach
Small intestine
Large intestine, colon
Anus
Mucosal tissues
Tissues that are covered by epithelium

Mucosal tissues of the human body

These tissues are inside our body. But they have gateways to the outside. Mucosal tissues are exposed to the external environment.

Mucosal tissues

A target of invasion of pathogenic microorganisms
### Routes of infection for pathogens

<table>
<thead>
<tr>
<th>Route of entry</th>
<th>Mode of transmission</th>
<th>Pathogen</th>
<th>Disease</th>
<th>Type of pathogen</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mucosal surfaces</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Respiratory tract</td>
<td>Inhalation or ingestion of infective material (e.g. saliva droplets, sputum)</td>
<td>Measles virus</td>
<td>Measles</td>
<td>Paramyxovirus</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Influenza virus</td>
<td>Influenza</td>
<td>Orthomyxovirus</td>
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<td></td>
<td></td>
<td>Varicella-zoster</td>
<td>Chickenpox</td>
<td>Herpesvirus</td>
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<td></td>
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<td>Epstein-Barr virus</td>
<td>Mononucleosis</td>
<td>Herpesvirus</td>
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<tr>
<td></td>
<td></td>
<td>Streptococcus pyogenes</td>
<td>Tonsillitis</td>
<td>Gram-positive bacterium</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Haemophilus influenzae</td>
<td>Pneumonia, meningitis</td>
<td>Gram-negative bacterium</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Neisseria meningitidis</td>
<td>Meningococcal meningitis</td>
<td>Gram-negative bacterium</td>
</tr>
<tr>
<td>Spores</td>
<td>Bacillus anthracis</td>
<td>Inhalation anthrax</td>
<td></td>
<td>Gram-positive bacterium</td>
</tr>
<tr>
<td>Gastrointestinal tract</td>
<td>Contaminated water or food</td>
<td>Rotavirus</td>
<td>Diarrhea</td>
<td>Rotavirus</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Salmonella enteritidis, S. typhimurium</td>
<td>Food poisoning</td>
<td>Gram-negative bacterium</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vibrio cholerae</td>
<td>Cholera</td>
<td>Gram-negative bacterium</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Salmonella typhi</td>
<td>Typhoid fever</td>
<td>Gram-negative bacterium</td>
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<tr>
<td>Urogenital tract</td>
<td>Sexual transmission/infected blood</td>
<td>Hepatitis B virus</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Human immunodeficiency virus (HIV)</td>
<td>Acquired immunodeficiency syndrome (AIDS)</td>
<td>Retrovirus</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Neisseria gonorrhoeae</td>
<td>Gonorrhea</td>
<td>Gram-negative bacterium</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Treponema pallidum</td>
<td>Syphilis</td>
<td>Bacterium (spirochete)</td>
</tr>
</tbody>
</table>

### Worldwide deaths annually from mucosal infections

- **Acute respiratory infections (4 million)**
- **Diarrheal diseases (1.8 million)**
- **Tuberculosis (1.5 million)**
- **HIV (2.9 million)**
- **Measles (600,000)**
- **Hepatitis B (103,000)**
- **Whooping cough (294,000)**
- **Roundworm and hookworm (12,000)**
Gastrointestinal tract
A tube from mouth to anus

- We ingest diets (nonself)
- Pathogens (nonself) come in to the tract

Surface area corresponds to 1.5 tennis courts
10.97 m² ≈ 1.5

There are the highest numbers of immune cells in the intestinal mucosa

Mucosal immune system
Exposed to external environment.
Unlike immune cells residing in lymphoid tissues, immune cells residing in the mucosa are constantly exposed to nonself.
The intestinal mucosa establishes a unique immune system.
Topics

Mucosal immune system in the intestine

1. Barrier functions of epithelial cells
2. Intestinal environmental factors
3. Unique immune cells in the intestinal mucosa

Barrier functions of intestinal epithelia

Prevention of invasion of pathogenic microbes

Epithelial cells

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Barrier functions of intestinal epithelia

Cell adhesion complexes

Mechanical barrier:
There is no interspace between epithelial cells

Barrier functions of intestinal epithelia

Viruses  Bacteria

Mucus layer (100-300 µm)

Glycocalyx  Microvillus

Dietary compounds

Cell adhesion complexes
Major components of mucus

Mucins

“Slimy” material that coats epithelial surfaces

Mucus

There are many mucins (Muc1-21)

Membrane-bound and secretory forms

Barrier functions of intestinal epithelia

Secreted mucins

Apical membrane
Lateral membrane
Basal membrane
Basement membrane

Glycoprotein

Structure of mucins

Sugar chain, glycan

Transmembrane Region
Core Protein
Cytoplasmic Region

High viscosity prevents access of microbes to the epithelial cells
Barrier functions of intestinal epithelia

Intestinal lumen

Epithelia

Bacterial free space

Epithelial cells producing mucins

Goblet cell
Barrier functions of intestinal epithelia

In addition to goblet cells, there are other secretory cells in the intestinal tract.

Paneth cells

Located in the bottom of crypt secrete anti-microbial peptides.
Anti-microbial peptides

- Weak: Electrostatic interaction and Hydrophobic interaction
- Strong: Hydrophobic interaction

Mammalian cell membranes: Cholesterol Zwitterionic phospholipids
Bacterial cell membranes

Anti-microbial peptides (AMP)

- Electrostatic attraction and the transmembrane electric field bring the AMP into the lipid bilayer
- AMP peptides form a pore
Barrier functions of intestinal epithelia
Prevention of infection for pathogenic bacteria

Infectious diseases in the gastrointestinal tract

**Bacteria**
- *Staphylococcus aureus*  Food poisoning
- *Salmonella enteritidis*  Food poisoning
- *Salmonella typhi*  Typhoid fever
- *Shigella flexneri*  Shigelosis
- *Vibrio cholera*  Cholera
- *Enterohemolytic E. coli (O157, O111)*  Gastroenteritis

**Viruses**
- Rotavirus  Gastroenteritis
- Norovirus  Children’s common diarrheal diseases

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Gastrointestinal tract
Intestinal lumen
Commensal bacteria
Mucosal epithelium
Lamina propria

Intestinal epithelia cell
Mucus
Paneth cells
Crypt
Mast cell
Dendritic cell
Macrophage
T cell
B cell
Follicle
Peyer’s patch
IgA

Villus
Intraepithelial lymphocytes
Lymphatic drainage
Dendritic cell
Afferent lymphatic

Anti-microbial peptides

Barrier functions of intestinal epithelia

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Mucosal tissues in gastrointestinal tract

1. Barrier functions of epithelial cells
2. Intestinal environmental factors (commensal bacteria)
3. Unique immune cells in the intestinal mucosa
Intestinal environmental factors

Commensal bacteria

There are >1,000 species of bacteria in the intestine
Intestinal bacteria outnumber our cells
1kg intestinal bacteria in 60kg host

Stomach
10^1~10^3 CFU/mL
Lactobacillus
Candida
Streptococcus
Helicobacter pylori
Peptostreptococcus

Lactobacillus
Streptococcus

Jejunum/ileum
10^4~10^5 CFU/mL
Lactobacillus
Streptococcus
Clostridium
Bacteroides
Actinomycinae
Corynebacterium

Duodenum
10^1~10^3 CFU/mL
Lactobacillus
Streptococcus

Colon
10^10~10^12 CFU/mL
Clostridium group
Bacteroides
Bifidobacterium
Enterobacteriaceae

Mutual relationship between commensal bacteria and host

Provision of niche for colonization

Commensal bacteria

Host
Intestinal environmental factors
Commensal bacteria

Function of commensal bacteria

Production of metabolites
- Produce nutrients (short chain fatty acid, vitamin K, B12 etc)
- Digest polysaccharides (dietary fiber, cellulose)

Prevention of infection
- Create biological barrier preventing invasion of pathogenic bacteria
- Produce anti-microbial factors (lactic acid)

Development of immune cells
- Induction of IgA
- Induction of Th1/Th17 cells
- Maturation of lymphoid tissues

Infection by *Clostridium difficile*

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
Connective tissue degradation leads to colitis and pseudomembrane formation
Intestinal environmental factors
Commensal bacteria

<table>
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<th>Function of commensal bacteria</th>
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<tr>
<td><strong>Production of metabolites</strong></td>
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<tr>
<td>Produce nutrients (short chain fatty acid, vitamin K, B12 etc)</td>
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<tr>
<td>Digest polysaccharides (dietary fiber, cellulose)</td>
</tr>
<tr>
<td>Neonatal vitamin K deficiency: bleed easily</td>
</tr>
<tr>
<td><strong>Prevention of infection</strong></td>
</tr>
<tr>
<td>Create biological barrier preventing invasion of pathogenic bacteria</td>
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<td>Produce anti-microbial factors (lactic acid)</td>
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<td><strong>Development of immune cells</strong></td>
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</tr>
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<td>Induction of Th1/Th17 cells</td>
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<tr>
<td>Maturation of lymphoid tissues</td>
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</table>

Germ-free mice
Who have no microorganisms living in or on them.

Maintained in an isolator
Immunological defects in germ-free mice

Development of T cells
- Th1 cells: fewer
- Th17 cells: fewer
- Treg cells: fewer

Development of plasma cells
- IgA-secreting cells: fewer

Commensal bacteria are required for development of adaptive immunity

Intestinal environmental factors
Commensal bacteria

Good bacteria VS Bad bacteria

Precise pictures of commensal bacteria remained long unknown
Many commensal bacteria are obligate anaerobic, and hard to culture
“Next generation sequencer” enables analysis of commensal bacteria
Inflammatory Bowel Diseases (IBD) in humans

Crohn’s diseases
Ulcerative colitis

Intractable diseases with unknown etiology

Percentage of bacteria in gut habitat

Cell Host & Microbes 3, 417, 2008
Altered composition of commensal bacteria (dysbiosis) correlates with the development of several diseases:

- Allergy
- Autoimmune diseases (Multiple sclerosis, Arthritis)
- Inflammatory bowel disease (Crohn's disease, Ulcerative colitis)
- Metabolic disease (Obesity, Diabetes mellitus)
- Cancer (Colon cancer, Liver cancer)

Mucosal tissues in gastrointestinal tract:

1. Barrier functions of epithelial cells
2. Intestinal environmental factors (dietary compounds)
3. Unique immune cells in the intestinal mucosa
### Intestinal environmental factors

- **Dietary compounds**

### Oral tolerance

<table>
<thead>
<tr>
<th>Non-self</th>
<th>Protective immunity</th>
<th>Mucosal tolerance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathogen bacteria, viruses, toxins</td>
<td>Dietary compounds</td>
<td>Commensal bacteria</td>
</tr>
</tbody>
</table>

| Immune response | Yes | No | No |

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### Mucosal tissues in gastrointestinal tract

1. Barrier functions of epithelial cells
2. Intestinal environmental factors
3. Unique immune cells in the intestinal mucosa
Mucosal tissues in the gastrointestinal tract

Lamina propria: tissues existing just beneath the epithelia of mucosa

- Villus
- Lamina propria
- Intestinal lumen
- Mucosal epithelium
- Intestinal epithelia cell
- Mucus
- Peyer's patch
- Goblet cell
- Follicle
- IgA
- Crypt
- Anti-microbial peptides
- Dendritic cell
- Paneth cells
- Mucosal tissues in the gastrointestinal tract
- Anti-microbial peptides
- Goblet cell
- Paneth cells
- Commensal bacteria
- Lymphatic drainage
- Intraepithelial lymphocytes
- Dendritic cell
- Afferent lymphatic
- T cell
- B cell
- Macrophage
- Mast cell
- Plasma cell
- Image is protected by copyright.

Gastrointestinal tract
A tube from mouth to anus

- We ingest diets (nonself)
- Pathogens (nonself) come in to the tract
- Surface area corresponds to 1.5 tennis courts
- There are the highest numbers of immune cells in the intestinal mucosa

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**Mucosal tissues in the gastrointestinal tract**

Gut-associated lymphoid tissues (GALT)

- **Intestinal lumen**
- **Mucosal epithelium**
- **Lamina propria**

**Key Structures**

- **Intestinal epithelia cell**
- **Anti-microbial peptides**
- **Goblet cell**
- **Paneth cells**
- **Intraepithelial lymphocytes**
- **Villus**
- **B cell**
- **T cell**
- **M cell**
- **Dendritic cell**
- **Mast cell**
- **Plasma cell**
- **Commensal bacteria**

**Lymphoid tissue, Lymphoid organs**

- **Primary lymphoid organs**
  - Tissues where lymphocytes are generated (naïve lymphocytes)
  - (Bone marrow, thymus)

- **Secondary lymphoid organs**
  - Tissues where naïve lymphocytes (they are not exposed to antigens) are activated
  - (Lymph nodes, spleen, gut-associated lymphoid tissues)
Secondary lymphoid organs

Structure of lymph node

- Cortical sinus
- Secondary lymphoid follicle (with germinal center)
- Afferent lymphatic vessel
- Paracortical area (mostly T cells)
- Germinal center
- Primary lymphoid follicle (mostly B cells)
- Medullary cords (macrophages and plasma cells)
- Medullary sinus
- Artery
- Vein
- Efferent lymphatic vessel
- Senescent germinal center
- Marginal sinus

Gut-associated lymphoid tissues (GALT)

Peculiar lymphoid tissues present in the gastrointestinal tract

- Amygdala
- Tonsil
- Adenoid
- Peyer’s patch
- Isolated lymphoid follicle
- Cecal patch
- Mesenteric lymph node
Gut-associated lymphoid tissues (GALT)

**Waldeyer’s ring**

Pharyngeal tonsil (adenoid)

Tubal tonsil

Palatine tonsil

Lingual tonsils

An anatomical term for the annular arrangement of lymphoid tissues in the pharynx.

Gut-associated lymphoid tissues (GALT)

**Peculiar lymphoid tissues present in the gastrointestinal tract**

- Amygdala
- Tonsil
- Adenoid
- Peyer’s patch
- Isolated lymphoid follicle
- Cecal patch
- Mesenteric lymph node

Waldeyer’s ring in the pharynx
A typical gut-associated lymphoid tissue

Peyer’s patch

Scattered lymphoid cells
Lamina propria lymphocyte
Intraepithelial lymphocyte
Epithelium
Crypt
Peyer’s patch
Isolated lymphoid follicle
Lamina propria
Afferent lymphatic
To mesenteric lymph node
### Structural difference between lymph node and Peyer’s patch

<table>
<thead>
<tr>
<th>Peyer’s patch</th>
<th>Antigen uptake</th>
<th>Lymph node</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antigen</td>
<td>Villus</td>
<td>Lymphatic follicle</td>
</tr>
<tr>
<td>M cell</td>
<td></td>
<td>Lymphatic vessel</td>
</tr>
<tr>
<td>High endothelial venules</td>
<td></td>
<td>Primary lymphoid follicle</td>
</tr>
</tbody>
</table>

Antigens are directly captured from the intestinal lumen.

Antigens are delivered by dendritic cells, which capture them in the periphery and migrate via afferent lymphatic vessels.

### M cell (microfold cell)

Peyer’s patches are covered by an epithelial layer containing specialized cells called M cells, which have characteristic membrane ruffles.

![Image](image_url)
A unique mechanism for antigen uptake in the intestine

Direct antigen uptake by
1. M cells on Peyer’s patch
2. CX3CR1+ dendritic cells in the lamina propria

M cells take up intestinal antigens and deliver them to dendritic cells in GALT

M cells take up antigen by endocytosis and phagocytosis

Antigen is transported across the M cells in vesicles and released at the basal surface

Antigen is bound by dendritic cells, which activate T cells
A unique mechanism for antigen uptake in the intestine

A subset of dendritic cells extend their dendrites across the epithelial layer to capture antigen from the lumen of the gut.

Unique subsets of dendritic cells in the intestine

CD11b+ CX3CR1+ dendritic cell

TGF-β

T cell

IL-6

TGF-β

INF-γ

IL-17

CD103+ CX3CR1- dendritic cell

TGF-β

RA

Foxp3+ Treg cell
A unique mechanism for antigen uptake in the intestine

Direct antigen uptake by
1. M cells on Peyer’s patch
2. CX3CR1+ dendritic cells in the lamina propria

Lymphocytes residing in the intestine

T cells: Th1, Th17, Treg cells
Intraepithelial lymphocytes (IEL)
B cells: IgA-secreting cells
Recruitment of lymphocytes to the intestine

Naïve lymphocytes (B, T cells) ➔ Peyer’s patch ➔ Activation by Ag presentation ➔ Mesenteric lymph nodes ➔ Blood circulation via thoracic duct ➔ Recruitment to the intestinal lamina propria

Recruitment of lymphocytes to the intestine

- Vitamin A
- TSLP, other factors
- Dendritic cell
- MadCAM
- CCL25
- Lamina propria T cell or plasma cell
- Lamina propria venule
- Blood

Peyer’s patch or mesenteric lymph node

RALDH

Retinoic acid

Effector T or B cell

α4β7

Gut epithelial barrier

Gut epithelial cells express chemokines specific for gut-homing T cells

Gut-homing effector T cells bind MadCAM-1 on endothelium

Epithelium

Small intestine

Large intestine

Gut epithelial barrier

Blood vessel

Endothelium

CCL28

E-cadherin

α4β7

L-selectin

α4β7

MadCAM-1

Lamina propria

Small intestine

Large intestine

Gut-homing effector T cells bind MadCAM-1 on endothelium

Epithelium
Intraepithelial lymphocytes (IEL)

Lymphocytes called intraepithelial lymphocytes (IELs) lie within the epithelial lining of the gut.

90% of IEL are T cells, among them 80% are CD8-positive.
Intraepithelial lymphocytes (IEL)

- Kill viral-infected epithelial cells
- Kill stressed epithelial cells

Virus infects mucosal epithelium cell

Infected cell displays viral peptide to CD8 IEL via MHC class I

Activated IEL kills infected epithelial cell by perforin/granzyme and Fas-dependent pathways

Epithelial cells undergo stress as a result of infection, damage, or toxic peptides, and express MIC-A and MIC-B

NKG2D on IELs binds to MIC-A,B and activate the IEL. CD8α:α homodimers also bind to TL

Activated IEL kills the stressed cell via the perforin/granzyme pathway

Lymphocytes residing in the intestine

T cells: Th1, Th17, Treg cells
Intraepithelial lymphocytes (IEL)

B cells: IgA-secreting cells

Intestinal lumen
Mucosal epithelium
Lamina propria

Villus
T cells
B cell
Dendritic cell
Paneth cells
Goblet cell
Macrophage
Mast cell
Plasma cell

Intestinal epithelia cell
Mucus
Intestinal lumen
Commensal bacteria

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**Antibody (Immunoglobulin)**

- IgG
- IgD
- IgE
- IgA
- IgM

**Recruitment of IgA-secreting cells to the intestine**

- Luminal antigen
- M cell
- Mucus
- IgA
- Activated helper T cell
- Naive CD4+ T cell
- B cell
- CD40 ligand
- CD40
- NO
- TGF-β
- Dendritic cell
- IgA
- Via blood
- IgA-secreting plasma cell
- Peyer’s patch
- Lamina propria
IgA production in the gut
Polymeric immunoglobulin receptor (poly-Ig receptor)

- IgA-producing plasma cell
- J chain
- Dimeric IgA
- Poly-Ig receptor with bound IgA
- Endocytosed complex of IgA and poly-Ig receptor
- Secreted IgA
- Proteolytic cleavage

Function of secreted IgA

- IgG, IgM: complement activation
- Opsonization (Induction of phagocytosis)
- Induction of inflammation

- IgA does not induce inflammation

- IgA binds intestinal bacteria (commensal and pathogenic) and inhibits invasion of these bacteria into epithelial cells

- Prevention of intestinal infectious diseases
- Maintenance of commensal bacteria
Summary

Mucosal Immunology

Commensal bacteria
Dietary compounds
Mechanical barrier: Cell adhesion complex
Chemical barrier: Mucus layer
Anti-microbial peptides
Gut-associated lymphoid tissues (GALT)
Unique antigen uptake
Lamina propria lymphocytes (IEL & IgA)

Mechanical barrier: Cell adhesion complex
Chemical barrier: Mucus layer
Anti-microbial peptides
Gut-associated lymphoid tissues (GALT)
Unique antigen uptake
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