Human gut microbiota and mucosal immunity

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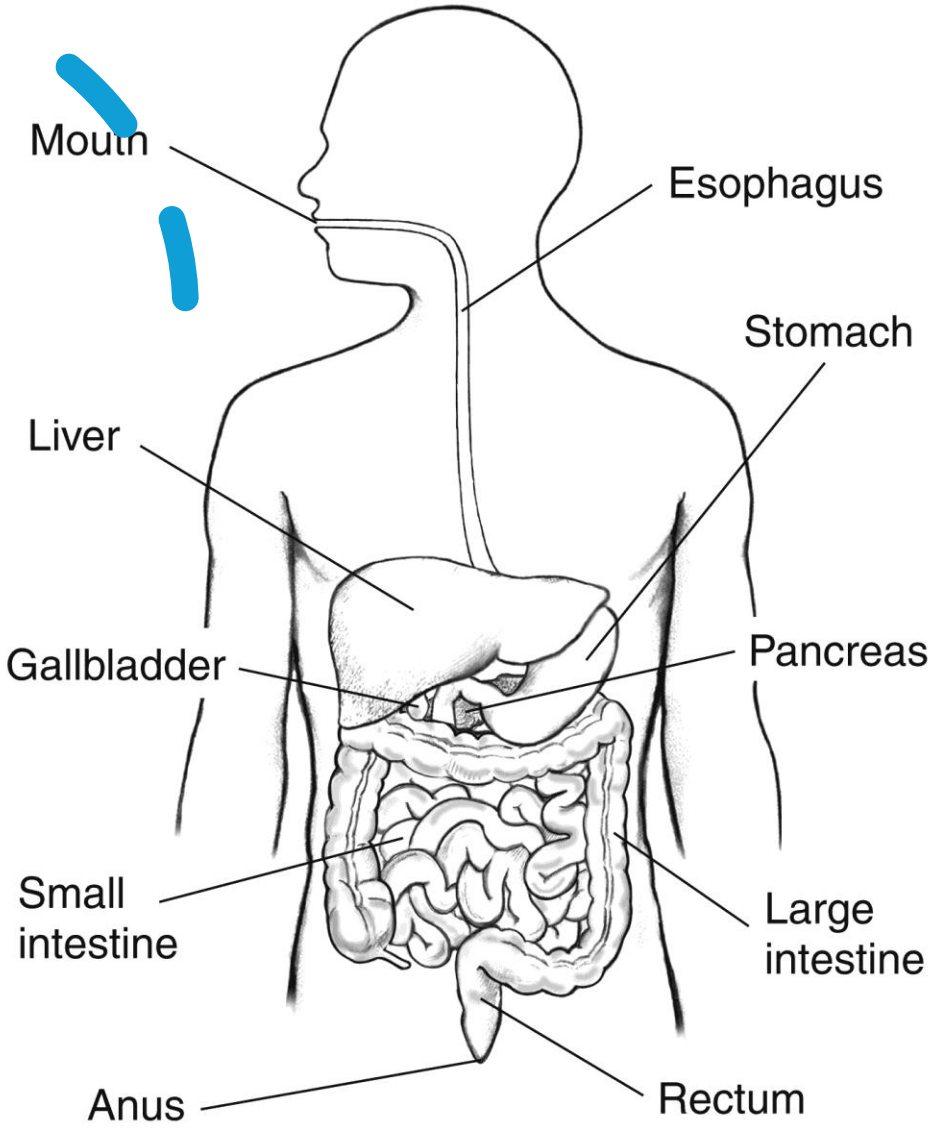
- The gastrointestinal tract is colonized by a dense and diverse microbiota that contributes to host nutrition, epithelial health, and immune regulation.
- The gut immune system must defend against pathogens while maintaining tolerance to commensal microbes and food antigens.
- Breakdown of this balance can lead to dysbiosis, infection, or inflammatory disease



National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health.

Learning objectives

- Define the normal gut microbiota and give representative examples.
- Explain how gut microbes promote health and resist pathogen colonization.
- Name the main epithelial, innate immune, and adaptive immune cells involved in GI immunity.
- Explain how mucosal immunity is induced and how tolerance is maintained.

- The gut is the largest and most continuously challenged mucosal surface in the body.
- It is exposed every day to food antigens, commensal microbes, and potential pathogens.
- Health depends on a balanced interaction between the **microbiota, epithelial barrier, and mucosal immune system.**

Gut microbiota

- The gut microbiota includes bacteria, archaea, viruses, bacteriophages, and fungi living in the gastrointestinal tract.
- **Bacteria** are the dominant members numerically and functionally.
- The **highest density is in the colon**, while the stomach contains far fewer organisms because of acidity.

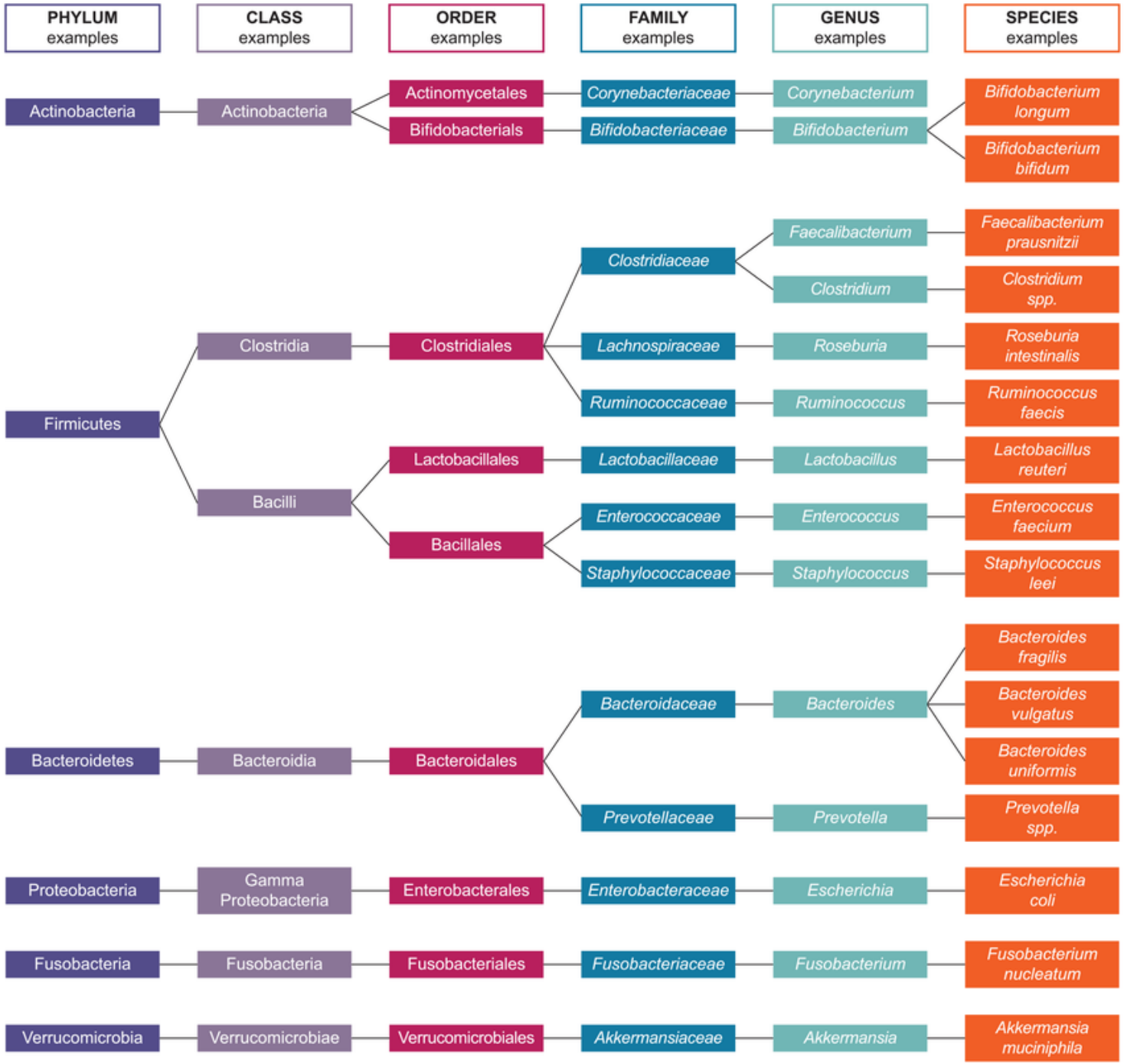
TABLE 6–3 Major Bacteria Found in the Colon

Bacterium[1]	Number/g of Feces	Important Pathogen
Bacteroides, especially *B. fragilis*	10^{10}–10^{11}	Yes
Bifidobacterium	10^{10}	No
Eubacterium	10^{10}	No
Coliforms	10^{7}–10^{8}	Yes
Enterococcus, especially *E. faecalis*	10^{7}–10^{8}	Yes
Lactobacillus	10^{7}	No
Clostridium, especially *C. perfringens*	10^{6}	Yes

[1] *Bacteroides, Bifidobacterium*, and *Eubacterium* (which make up more than 90% of the fecal flora) are anaerobes. Coliforms (*Escherichia coli, Enterobacter* species, and other gram-negative organisms) are the predominant facultative anaerobes.

Important gut bacteria

- **Bacteroides** species are major anaerobes in the colon and are important in carbohydrate metabolism.
- **Faecalibacterium** and other **Firmicutes** are linked to production of short-chain fatty acids.
- **Bifidobacterium** species are especially important in early life and are often considered beneficial commensals.
- **Akkermansia** is associated with the mucus layer and mucosal homeostasis.
- **Escherichia coli** can be a normal commensal, although some pathotypes are diarrheagenic.



The role of the gut microbiome in colonization resistance and recurrent Clostridioides difficile infection - Scientific Figure on ResearchGate. Available from: https://www.researchgate.net/figure/Composition-of-common-gut-microbiota-Taxonomically-bacteria-are-classified-into-phyla_fig1_365587433

Other members of the gut microbiota

- The gut **mycobiota** includes fungi such as **Candida**, **Saccharomyces**, **Malassezia**, and **Cladosporium**.
- The gut **virome** includes bacteriophages and eukaryotic viruses.
- Archaea are also present, especially **methanogenic archaea**.

Distribution along the GI tract

- The **stomach** has low microbial density because of acid.
- The **small intestine** has more microbes than the stomach but fewer than the colon because of bile, digestive enzymes, and motility.
- The **ileum** combines a relatively higher microbial load with organized lymphoid tissue such as Peyer patches.
- The **colon** contains the greatest microbial density and is the main site of host–microbiota interaction.

Role of gut microbiota

- Helps digest otherwise poorly digestible dietary components (e.g. ***bacteroides*** - complex polysaccharides).
- It contributes to vitamin production and metabolite generation (e.g ***Escherichia coli*** **-** vitamin K**)**.
- It promotes epithelial development and barrier integrity.
- It shapes immune maturation and basal immune tone (e.g. **Bifidobacterium**).
- It protects against pathogen colonization (e.g. normal gut flora competes with ***Salmonella*** or diarrheagenic ***E. coli*** for nutrients and attachment sites).

Colonization resistance

- Colonization resistance is the ability of the resident microbiota to prevent pathogens from establishing themselves in the intestine.
- Commensal microbes occupy niches, consume nutrients, and make the environment less favorable for invaders.
- This is one of the key reasons a healthy intestine resists infection.

Practical examples of colonization resistance

- Normal **E. coli** strains can help protect against harmful organisms by occupying ecological space in the intestine.
- **Bifidobacterium** is associated with early-life pathogen resistance and healthy microbiota development.
- Commensals in general reduce opportunities for pathogens to attach, expand, and dominate the lumen.
- Antibiotic disruption weakens this natural protection.

Gut dysbiosis

- Dysbiosis refers to disturbed microbiota composition or function.
- It can follow antibiotic use, acute infection, diet shifts, inflammation, or hospitalization.
- Dysbiosis can impair colonization resistance, weaken mucosal homeostasis, and increase susceptibility to enteric disease.
- After antibiotics, **Clostridioides difficile** can proliferate and produce enterotoxins that cause **antibiotic-associated colitis**.
- In **ulcerative colitis**, bacteria that **degrade mucin** may damage the protective mucosal barrier and trigger inflammation.

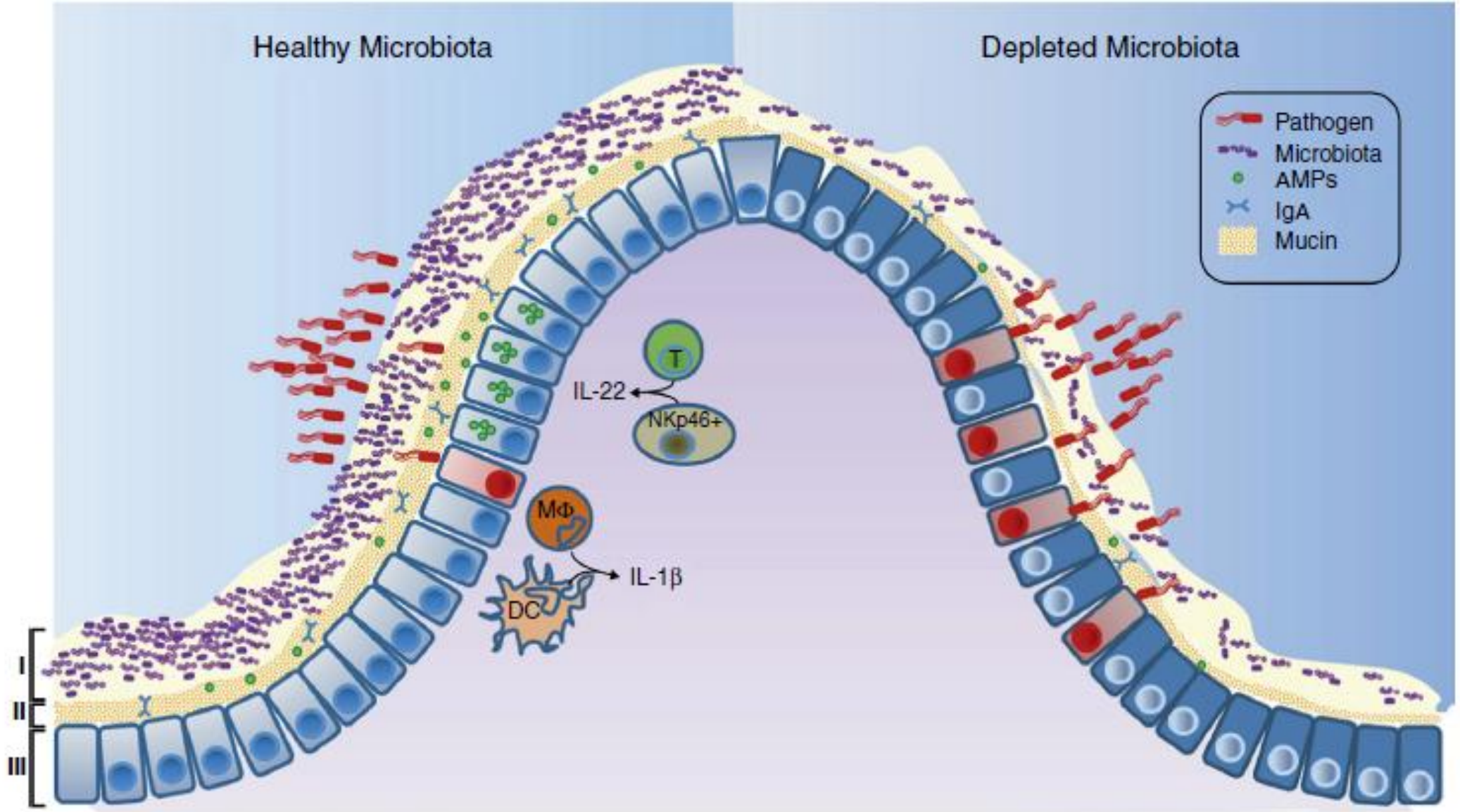


Fig. 2.2 Intestinal microbiota protection against enteric infections. (I) Saturation of colonization sites and consumption of nutrients limit pathogen access to host tissues; (II) the microbiota prime innate immunity by stimulating mucin production, immunoglobulin *(Ig)*A, and antimicrobial peptides *(AMPs);* and (III) the microbiota stimulate interleukin *(IL)*-22 expression, which increases epithelial resistance, and IL-1β production, which promotes recruitment of inflammatory cells. (From Khosravi, A., Mazmanian, S. 2013. Disruption of the gut microbiome as a risk factor for microbial infections. Curr. Opin. Microbiol. 16, 221–227.)

What happens after antibiotics?

- Broad-spectrum antibiotics can kill many commensal organisms in the colon.
- This reduces microbial competition and colonization resistance.
- Inappropriate bacteria can then expand more easily and cause disease.

Overview of the intestinal barrier

- The intestinal barrier consists of **mucus, epithelial cells, tight junctions, antimicrobial molecules, immune cells, and underlying lymphoid tissue**.
- It selectively permits nutrient absorption while limiting microbial penetration.
- Effective gut defense depends on both structure and immune function.

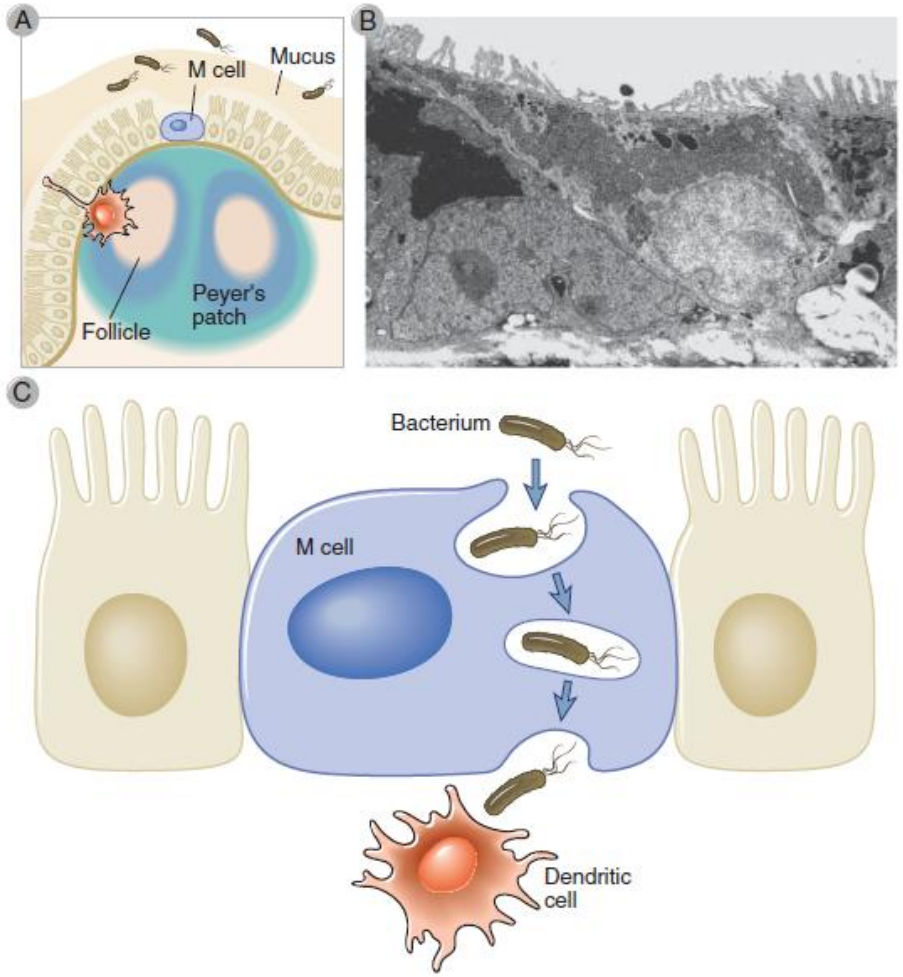
Epithelial cell types in the intestine

- **Enterocytes** are the main absorptive epithelial cells.
- **Goblet cells** secrete mucus maintaining spatial separation between microbes and tissue.
- **Paneth cells** secrete antimicrobial peptides, especially in the small intestine, that help control microbes near the epithelial surface.
- **Microfold cells** sample luminal antigens and transport them to immune cells.
- **Enteroendocrine cells** release signaling molecules that influence GI physiology.
- **Tuft cells** are specialized chemosensory epithelial cells that also participate in mucosal immune signaling.
- **Intestinal stem cells** replenish the epithelial lining.

TABLE 13–1 Features of Regional Immunity

Region	Special Challenges	Special Anatomic Features	Specialized Cells or Molecules: Functions
Gastrointestinal tract	Tolerance of food antigens Tolerance to commensal microbiota but responsive to rare pathogens Enormous surface area	Tonsils Peyer's patches, lamina propria follicles	Intestinal epithelial cells: mucus secretion M cells: luminal antigen sampling Paneth cells: defensin production Secretory IgA, IgM: neutralization of microbes in the lumen DC subsets: luminal antigen sampling; lamina propria antigen sampling; T cell tolerance induction; effector T cell activation; induction of B cell IgA class switching; imprinting gut-homing phenotypes of B and T cells

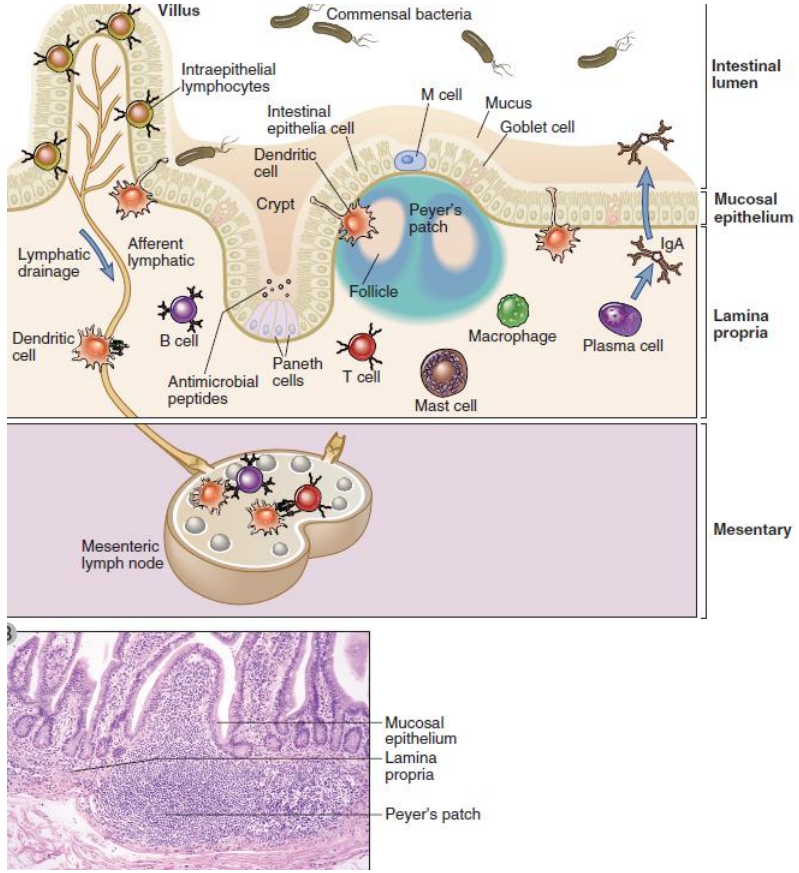
- **M cells**
- **Microfold cells, or M cells**, are specialized epithelial cells over Peyer patches.
- They take up luminal antigens by endocytosis and transcytosis.
- They deliver antigens to underlying **dendritic cells** and **lymphocytes**.
- M cells are essential for initiating mucosal immune responses.



GURE 13–3 M cells in the small intestine. M cells are specialized intestinal epithelial cells found in the small bowel epithelium over yer's patches and lamina propria lymphoid follicles **(A)**. Unlike neighboring epithelial cells with tall microvillous borders and primary absor nctions, M cells have shorter villi **(B)** and engage in transport of intact microbes or molecules across the mucosal barrier into gut-associated lymp sues, where they are handed off to DCs **(C)**. *(Electron micrograph from Corr SC, CC Gahan, and C Hill. M-cells: origin, morphology and role in mu mmunity and microbial pathogenesis. FEMS Immunology and Medical Microbiology 52:2-12, 2008.)*

Innate immune cells in GI immunity

- **Macrophages** are abundant in the intestinal lamina propria and help clear microbes and debris.
- **Dendritic cells** capture antigen and present it to T cells.
- **Neutrophils** are recruited rapidly during acute infection and inflammation.
- **Innate lymphoid cells**, especially **ILC3**, are important in mucosal barrier defense.
- **Mast cells** contribute to local inflammatory responses.
- **Eosinophils** are relevant particularly in helminth infection and some mucosal inflammatory settings.



- **Dendritic cells and macrophages**
- **Dendritic cells** are professional antigen-presenting cells that link innate and adaptive immunity.
- In the gut, they sample antigens delivered through M cells or directly from the mucosa.
- **Macrophages** phagocytose microbes and help maintain tissue homeostasis.
- Gut macrophages are functionally specialized because they must defend against pathogens without causing constant inflammation.

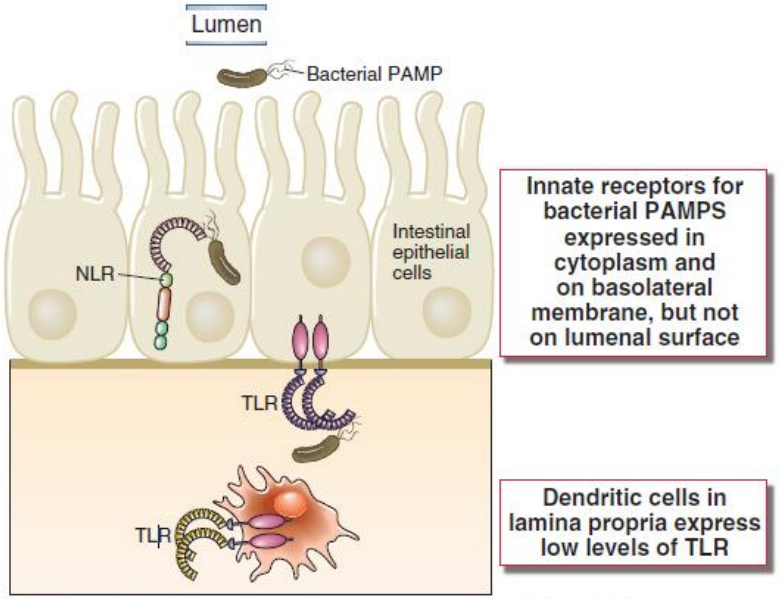
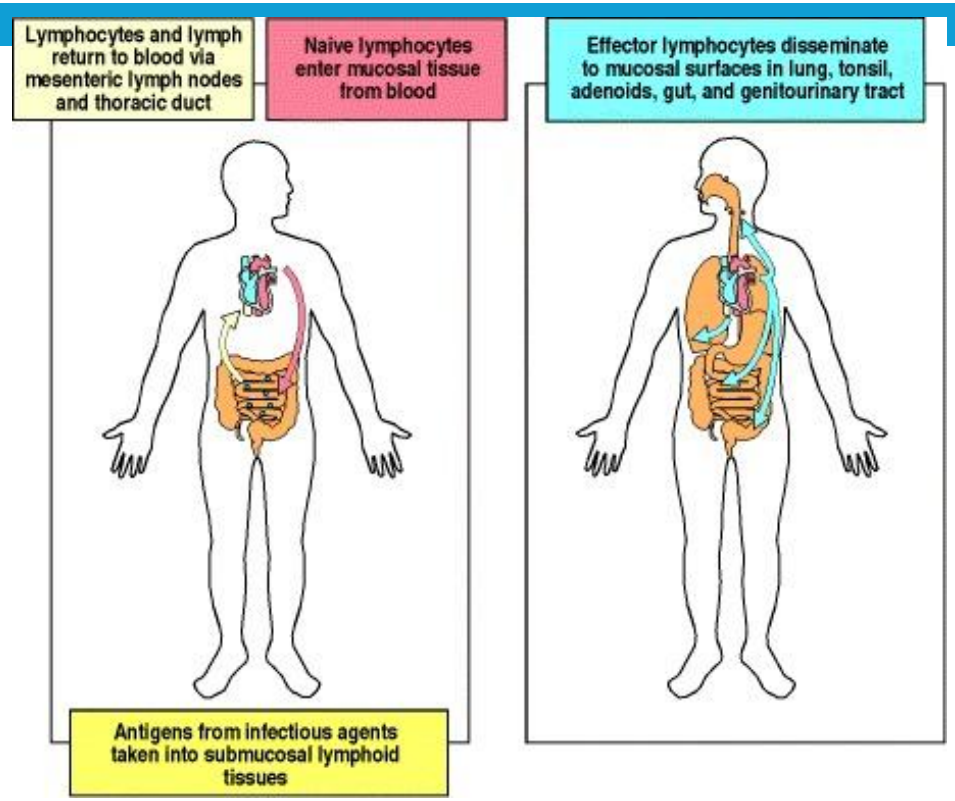


FIGURE 13–2 Mechanism of regulation of innate immune responses in the intestinal mucosa. Pattern recognition receptor expression and function in intestinal epithelial cells and lamina propria DCs minimize inflammatory responses to commensal bacteria in the lumen but promote responses to microbes that traverse the barrier and enter the lamina propria. *Top,* Pattern recognition receptors that recognize bacterial flagellin are compartmentalized in the cytosol (NLR) or basal membrane (TLR5) of intestinal epithelial cells but not on the apical/lumen membrane. *Bottom,*TLR4, which recognizes bacterial lipopolysaccharides, is expressed at low levels on intestinal epithelial cells and lamina propria DCs. TLR signaling does not induce inflammatory gene expression in lamina propria DCs because of more dominant effect of intracellular regulators of TLR signal transduction, such as TOLLIP and IRAK-M, compared with conventional DCs in other tissues.

- **Lymphoid tissue in the gut**
- The gut-associated lymphoid tissue, or **GALT**, includes **Peyer patches**, **isolated lymphoid follicles**, **mesenteric lymph nodes**, and diffuse immune cells in the lamina propria.
- These sites are where mucosal immune responses are induced and coordinated.
- The ileum is especially important because Peyer patches are prominent there.



Immunobiology: The Immune System in Health and Disease. 5th edition.
Janeway CA Jr, Travers P, Walport M, et al.
New York: Garland Science; 2001.

- **Adaptive immune cells in GI immunity**
- **B cells** in mucosal tissues differentiate into plasma cells that produce IgA.
- **Plasma cells** in the lamina propria are major sources of secretory IgA.
- **CD4+ T cells** help coordinate adaptive immune responses.
- **Regulatory T cells, or Tregs**, are important for maintaining tolerance.
- **CD8+ T cells** contribute to defense against infected cells.
- **Intraepithelial lymphocytes** are specialized lymphocytes located within the epithelial layer and contribute to barrier defense.

- **Secretory IgA**
- **Secretory IgA** is the dominant antibody class in the gut lumen.
- It is produced by plasma cells in the lamina propria and transported across epithelial cells into the lumen.
- IgA helps block microbial adherence and neutralize toxins.
- It protects mucosal surfaces while causing less tissue-damaging inflammation than many systemic immune responses

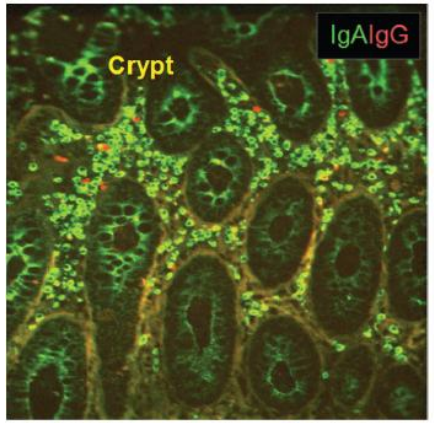
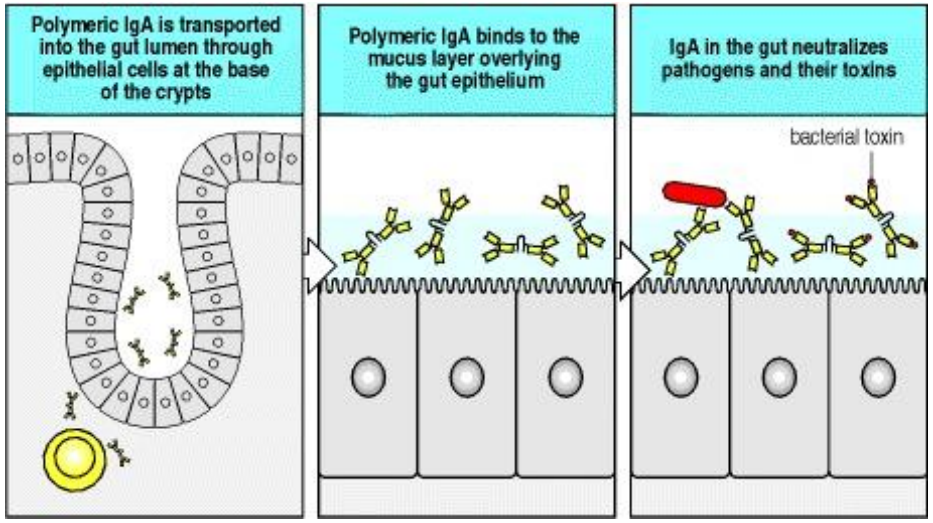


FIGURE 13–6 IgA-secreting plasma cells in the intestine. The abundance of IgA-producing plasma cells (green) in colon mucosa compared with IgG-secreting cells (red) is shown by immunofluorescence staining. IgA that is being secreted can be seen as green cytoplasm in the crypt epithelial cells. *(From Brandtzaeg P. The mucosal immune system and its integration with the mammary glands. The Journal of Pediatrics 156[Suppl 1]:S8-S16, 2010.)*



Immune tolerance in the gut

- The gut cannot respond aggressively to every antigen it encounters.
- Tolerance is maintained toward many food antigens and commensal microbes.
- **Regulatory T cells**, epithelial signaling, and controlled antigen presentation help maintain this balance.
- Failure of tolerance contributes to inflammatory disease.

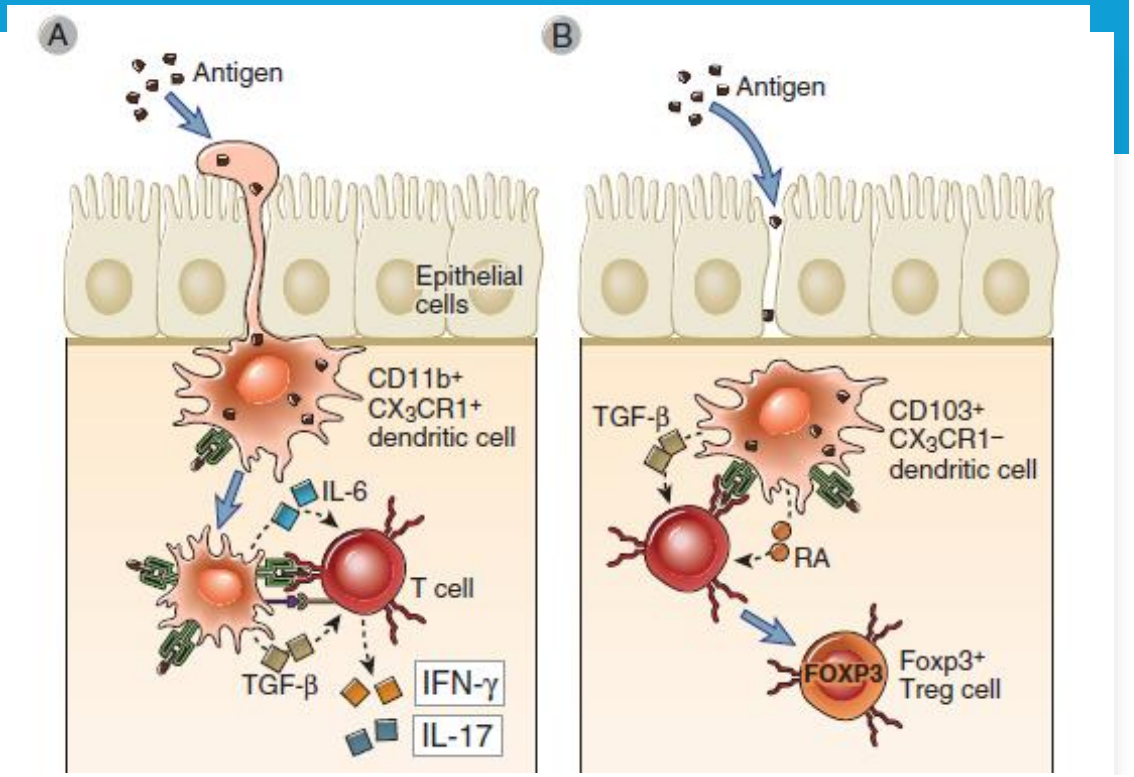


FIGURE 13–4 DCs in the intestinal mucosa. There are several different subsets of DCs constitutively present in the intestinal mucosa that are defined by cell surface molecules and function. Two such subsets are shown that are also present in other mucosal tissues. **A,** Antigen-sampling DCs extend dendritic processes between intestinal epithelial cells into the lumen to sample antigens and then migrate to mesenteric lymph nodes, where they initiate activation and differentiation of proinflammatory effector T cells. These DCs express the CD11b integrin chain and the CX_3CR1 chemokine receptor. **B,** Other DCs present in the lamina propria, which express the integrin CD103, present antigens to naive T cells and induce their differentiation of regulatory T cells, in part by secreting TGF-β and retinoic acid (RA). The regulatory function of these DCs depends on factors secreted by intestinal epithelial cells.

Probiotics

- Probiotics are mixtures of bacteria or yeast that when ingested colonize and proliferate, even temporarily, the intestine.
- Probiotics are commonly gram-positive bacteria (e.g., *Bifidobacterium, Lactobacillus*) and yeasts (e.g.,*Saccharomyces*).
- Consumers use probiotics to promote and maintain regular bowel function and improve tolerance to lactose.
- It has been used to treat *C. difficile*–associated diarrhea and inflammatory bowel disease, or to provide protection from *Salmonella* and *Helicobacter pylori* disease.
- Generally safe but many are ineffective or with unproven value.

Clinical case

- A **72-year-old hospitalized man** treated for pneumonia with Piperacillin-tazobactam developed **watery diarrhea on hospital day 6** after initial clinical improvement: **8–10 loose stools in 24 hours**, **crampy abdominal pain, low-grade fever, dehydration, leukocytosis, and rising creatinine**, suggesting clinically significant colitis.
- Antibiotics may reduce **Bifidobacterium**, commensal **E. coli**, and other protective microbes; loss of microbiota weakens colonization resistance.
- **Toxigenic C. difficile** acquired in the healthcare setting proliferate and produce toxins.
- Treatment options: fidaxomicin, vancomycin; metronidazole can be added in fulminant cases, or fecal microbiota therapy in recurrence.