

# The Front Line of Enteric Host Defense against Unwelcome Intrusion of Harmful Microorganisms: Mucins, Antimicrobial Peptides, and Microbiota

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## INTRODUCTION

The mucosal surface of the intestinal tract is the largest body surface in contact with the external environment (200 to 300 m<sup>2</sup>). It is a complex ecosystem combining the gastrointestinal epithelium, immune cells and resident microbiota (249). The mucosa of the intestinal tract is exposed to various microbial pathogens. These potentially harmful enteric microorganisms can hijack the cellular molecules and signaling pathways of the host and become pathogenic. In the first step in the infectious process, some enteric bacterial pathogens adhere to the brush border of intestinal cells (46, 380), enabling them to exploit the underlying signaling pathways. Moreover, some enteric microbial pathogens have developed specialized systems that, after this essential step of adhesion, produce virulence factors. After normal host-cell processes have been subverted, these systems enable the pathogen to cross the epithelial barrier (74). The host cell cytoskeleton is commonly used and targeted by enteric microbial pathogens during the cell penetration step; it is exploited for purposes that include gaining entry into cells, moving within and between cells, and forming and remodeling vacuoles in order to create a specialized niche, which enhances the pathogen's chances of survival (131).

The host is protected from attack by potentially harmful enteric microorganisms by the physical and chemical barriers created by the intestinal epithelium (Fig. 1). The surface of the

intestine is lined by a simple columnar epithelium that is folded to form a number of invaginations, or crypts, which are embedded in the connective tissue. The intestinal cells (192, 228, 271) that make up the epithelium provide a physical barrier that protects the host against the unwelcome intrusion of microorganisms (Fig. 1). The intestinal epithelium is a model of tissue renewal, since intestinal cells are constantly generated from a source of multipotent stem cells located in the crypts of Lieberkühn, and these provide new precursor cells permitting a high rate of cell turnover. In the intestinal villi, the polarized epithelial cells that form the epithelium separate two different compartments. This epithelial barrier is composed of four epithelial cell lineages, including the enterocytes, enteroendocrine, goblet, and Paneth cells present in the intestinal villi. In addition, M cells are present in the follicle-associated epithelia. The integrity of the layer of epithelial cells is maintained by intercellular junctional complexes composed of tight junctions (TJs), adherens junctions (AJs), and desmosomes, whereas gap junctions allow intercellular communication to occur. TJs, the most apical components of the junctional complex (9, 344), create a semipermeable diffusion barrier between individual cells, which can be regulated and serves as the permeability barrier. Forty different proteins have been shown to be located in TJs, including, for example, ZO-1, ZO-2, and ZO-3 proteins, members of the membrane-associated guanylate kinase protein family, occludin, claudins, cingulin, 7H6, and several unidentified phosphoproteins (59, 267). Interestingly, the biogenesis of the TJs appears to be regulated, in part, by classic signal transduction pathways, such as those involving heterotrimeric G proteins, Ca<sup>2+</sup>, and protein kinase C, and raft-like

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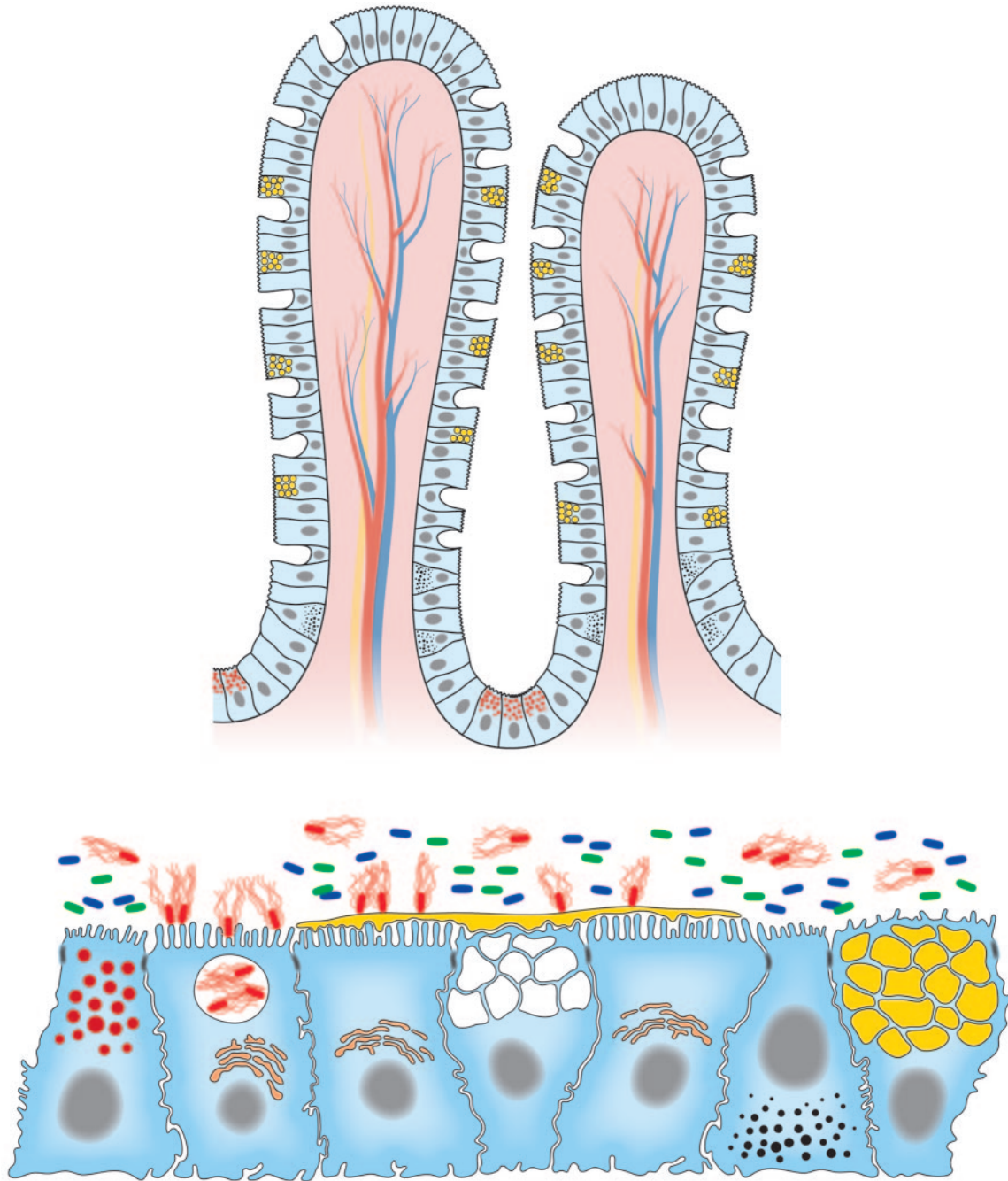


FIG. 1. Architecture of the intestinal epithelium lining the intestinal tract. (A) Crypt-villus cell organization. The cell renewal is achieved from the pluripotent intestinal stem cells up from the crypt base in the small intestinal and at the crypt base in the colon. Epithelial cells migrate up the crypt where they perform their differentiation, acquiring specific intestinal functions of absorption and secretion. Three cell types differentiate as they migrate: the predominant enterocytes, the mucus-secreting Goblet cells, and the peptide hormone-secreting enteroendocrine cells. Oppositely, the Paneth cells migrate down to the base of the crypt. (B) The assembly of the polarized epithelial-cell types results from an epithelium that provides a permeability barrier between the external and internal compartments. This barrier function is assumed by the junctional domain, including well-defined gap junctions, desmosomes, adherens junctions, and tight junctions. Four polarized epithelial cell lineages were present in the intestinal epithelium: the enterocytes expressing at the apical domain a dense, well-ordered brush border consisting of organized microvilli in the membrane of which oriented proteins support specific functions; the mucus-secreting goblet cells (cell with large yellow granules) producing membrane-bound mucins and containing mature storage granules in which secreted mucins are packaged; the enteroendocrine cells (cells with small, dark granules) containing small, oriented secretory granules in which different peptide hormones should be stored, although a same granule may store more than one peptide hormone; and the Paneth cells (cell with small, red granules) containing apically oriented granules in which AMPs and antimicrobial proteins were packaged as pro or mature forms. Enteric pathogens (red bacteria with flagella) interact with the intestinal epithelial cells, enter the cells, affect the cell architecture or organization, and disturb the cell functions. The commensal bacteria (blue and green bacteria) mainly reside in the lumen outside the mucus layer. Secreted mucins (in yellow, coating the epithelial surface) in association with the membrane-bound mucins act as a physicochemical barrier for the protection of the epithelial cell surface against undesirable harmful pathogens.

membrane microdomains that act as a platform for signaling molecules (81, 246, 286, 416). Downstream from the TJs are the AJs, composed of a cadherin-catenin complex and its associated proteins, and membrane and PDZ proteins (277, 377). In both TJs and AJs, interactions among their specific components seem to be dynamically regulated during the formation of the junctional complex in epithelial cells. Importantly, it has been noted that TJs and AJs play a pivotal role in maintaining cell polarization. Indeed, recent evidence suggests that cell polarization operates regardless of whether TJs are present, since they form an intramembrane barrier to diffusion that restricts mingling between the apical and basolateral membrane components (245, 382). It is of interest that many pathogenic enteric bacteria target and exploit the TJ domain to accomplish their pathogenic strategies by modulating intestinal permeability (105, 156). It has also been established that some enteric pathogens use the M cells overlying the organized mucosal-associated lymphoid system (204, 282) as a route of invasion and that after passing through these cells, the bacteria face phagocytic cells, particularly the macrophages that are present in the follicle dome (60, 186, 339).

Host defense systems against unwelcome intrusion of pathogenic enteric microorganisms include adaptive and innate immunity. Adaptive immune responses are typically observed 4 to 7 days after infection, and this mechanism involves the generation of immunological memory and the expansion of receptors with relevant specificities. In contrast, the innate immune system is mobilized within the first few days in order to control infection (258). Unlike the adaptive immune system, which uses a clonal, random, and highly diverse repertoire, the innate immune system uses nonclonal sets of recognition molecules. The intestinal epithelium provides a surface where the host can sense the microbial environment in order to trigger a strong defense response, when this is required, by releasing signaling molecules such as cytokines and chemokines. These in turn trigger the recruitment of leukocytes and initiate the attraction of immune cells. However, the intestinal epithelium, unlike that of the lung, tolerates bacterial colonization by members of the resident microbiota. Indeed, although consistently exposed to commensal bacteria, the normal mucosa exhibits only a minimal inflammatory status in response to the abundant products of the normal flora triggered by resident gram-negative and gram-positive bacteria. These products include substances such as lipopolysaccharide (LPS) (for gram-negative bacteria) and lipoprotein and peptidoglycan (for gram-positive bacteria). Investigating how the host gut distinguishes between its commensal microbiota and unwelcome enterovirulent microorganisms has revealed that hosts possess highly sophisticated systems for detecting antigens (6, 14, 89). The endogenous bacterial species of the microbiota all share "self" signature molecules, known as microbe-associated molecular patterns. In contrast, following infection, the host innate mucosal immunity response is activated mainly as a result of the specific recognition by pattern recognition receptors of conserved "non-self" molecular structures found in large groups of pathogens, known as pathogen-associated molecular patterns (180). For example, epithelial cells sense the environment within the gut by means of their pattern recognition receptors, which include Toll-like receptors (TLRs) and the NOD (nucleotide-binding oligomerization domain) proteins (93, 181, 191, 281, 310). TLRs are

evolutionary conserved proteins characterized by having an extracellular leucine-rich repeat domain involved in ligand recognition (1, 181, 257, 258) and an intracellular Toll/interleukin 1 (IL-1) receptor-like domain involved in signal transduction (4, 191). Moreover, two mammalian nucleotide-binding, leucine-rich, repeat proteins (NOD1 and NOD2) function as intracellular sensors of bacterial products in the induction of inflammatory responses (125, 155, 176). Biochemical studies have revealed that NOD2 is in fact a protein involved in the innate immune detection of bacterial products (201, 311). More specifically, NOD2 recognizes a fragment of peptidoglycan, known as muramyl dipeptide, which is found in the cell walls of both gram-negative and gram-positive bacteria.

The intestinal epithelium is not just a physical barrier that prevents unwanted bacteria from gaining access to essential organs; it also provides a surface covered by specialized cells producing mucus, antimicrobial peptides (AMPs), and antimicrobial molecules, such as lysozyme, which together with resident microbiota provide the front line of defense against pathogenic microorganisms (118). The aim of this review is to analyze what we know about this first line of defense. Our analysis focuses on the two cell lineages present in the intestinal epithelium: the goblet cells and the Paneth cells, both of which play a pivotal role in this front line of enteric defense (Fig. 1). We also discuss recent insights into the mechanisms by which the intestinal microbiota acts as a barrier to enteric pathogens.

## MUCUS

The intestinal mucosa has a surface coating of mucus that is secreted by the specialized goblet cells, also known as mucin-secreting cells (72, 108, 207).

### Mucin-Secreting Cells

Mucin-secreting cells have a polarized phenotype characterized by the fact that the apical and basolateral domains of the cell membrane are separated by TJs that are also involved in connections with adjacent cells (Fig. 1). In the apical domain, the mucin-secreting cells have a brush border, an ordered structure consisting of organized microvilli. As in the enterocytes, the microvillus of the brush border is organized by a cytoskeleton containing a bundle of actin filaments combined with various actin-bundling proteins, including villin and fimbrin. The cytoskeleton of the brush border plays a pivotal role in organizing and maintaining specialized intestinal functions in both enterocytes and mucin-secreting cells. The microtubule cytoskeleton localized within the cell is also specifically organized to facilitate vesicle trafficking between the Golgi network and the apical domain facing the luminal compartment (331), which is under the control of apical sorting signals. Mucin-secreting cells contain numerous high-electron-density, secretory granules containing packaged mucins located above the nucleus and below the brush border.

Mucin-secreting cells mature from the proliferative zone located at the base of the crypt, and they are all derived from stem cells located at the base of the crypt (18, 42, 241) (Fig. 1). Mucin-secreting cells mature as they migrate along the crypt-villus axis. The short-lived mucin-secreting cells ascend the

villus, differentiate, and then exfoliate into the lumen within ~5 to 7 days after they have been produced as a result of cell division, as do enterocytes and endocrine cells. Investigations of the regulation of stem cell proliferation and differentiation on the villus have revealed that they are controlled by several systems, including the Wnt and Hedgehog signaling pathways, the morphogenic proteins of bone, and intestinal transcription factors, CDX1, CDX2, and HNF1 (402, 403). For example, the canonical Wnt signaling cascade (312, 313) comprises 20 different secreted proteins, which interact with about 10 different Frizzled receptors. Wnt signaling is transduced via  $\beta$ -catenin/TCF4 (390) and is known to control multiple biological phenomena in vertebrates, including cell fate determination and maintaining stem/progenitor cells with predefined fates in specific compartments (241). Wnt signaling plays a key role in the intestinal epithelium (38, 202) in driving a stem cell/progenitor gene program that is crucial for maintaining undifferentiated progenitors near the bottom of the crypts of Lieberkühn. In addition, it has recently been reported that the Notch signaling (12) plays a critical role in intestinal development, since mucosecreting goblet cells are severely depleted in the double transgenic Rosa-Notch/Cre<sup>+</sup> mouse (110). Exfoliation of mature intestinal cells from the tip of the villi results from a particular cell death program, known as “anoikis,” that subject to both positive and negative control by focal adhesion kinase or  $\beta_1$ -integrin-related events, protein-kinase signaling pathways including phosphatidylinositol 3-kinase/Akt, mitogen-activated protein kinase, stress-activated protein kinase/Jun amino-terminal kinase, and certain Bcl-2 and Bcl-2-related proteins (113, 385, 423).

Changes in goblet cell function and in the chemical composition of the intestinal mucus have been detected in response to a broad range of luminal insults, including changes in the normal microbiota and the intrusion of harmful enteric pathogens, but the mechanisms involved are poorly understood (82). Studies have shown that germfree mice can exhibit changes in mucin gene expression, mucus composition, and mucus secretion in response to intestinal microbes or host-derived inflammatory mediators. For example, when germfree mice were conventionalized by the oral administration of microorganisms prepared from the feces of genetically identical mice, bacterial colonization led to a time-dependent change in the number of rectal goblet cells and mucin composition (115).

### Mucins

Mucin-type molecules consist of a core protein moiety (apomucin) within which a number of carbohydrate chains are attached to serines, prolines, and threonines by glycoside bonds. O-linked and N-linked oligosaccharides form up to 80% of the molecule, and the lengths of the carbohydrate side chains range from 1 to more than 20 residues (348). Mucin-type oligosaccharides play a pivotal role in their hydroscopic properties, by binding various small molecules and proteins, and in specific ligand-receptor interactions. Mucins are synthesized as nascent peptides and form oligomers in the endoplasmic reticulum (108). Core- and end-glycosylations occur in the Golgi apparatus, and the mature mucins are then moved from the condensing granules to mature storage granules. Mucins can be divided into three distinct subfamilies on the basis of

their structure: gel-forming, soluble, and membrane-bound mucins (Table 1). Moreover, the mucins secreted can be subdivided into two groups: gel-forming mucins and non-gel-forming mucins (51, 80, 121, 157). Eighteen genes encoding human mucin-type glycoproteins have so far been assigned to the MUC gene family, *MUC1*, *MUC2*, *MUC3A*, *MUC3B*, *MUC4*, *MUC5B*, *MUC5AC*, *MUC6* through *MUC13*, and *MUC15* through *MUC17*, with the approval of the Human Genome Organization Gene Nomenclature Committee (<http://www.gene.ucl.ac.uk/nomenclature>) (51, 79, 80, 269). A cluster of four mucin genes (*MUC2*, *MUC5B*, *MUC5AC*, and *MUC6*) located on chromosome 11p15.5 encodes secreted mucins. Nine genes, *MUC1* (1q21), *MUC3A* (7q22), *MUC3B* (7q22), *MUC4* (3q29), *MUC11* (7q22), *MUC12* (7q22), *MUC13* (3q13), *MUC16* (19p13.3), and *MUC17* (7q22), encode membrane-associated mucins. There are also some products of MUC genes, including those of *MUC7* (4q13 to 4q21), *MUC8* (12q24), *MUC9* (1p13), and *MUC15* (11p14.3), that do not fit well into either class.

The secreted mucins MUC2, MUC5AC, MUC5B, and MUC6 assemble via interchain disulfide-forming, disulfide-linked oligomers/multimers with molecular weights in the millions (307). They express specific mucin domains (51, 157), including VNTE (variable number of tandem repeat) domains that are rich in serine, threonine, and proline residues; VWD sequences homologous to von Willebrand factor D domains (which are thought to be involved in the oligomerization of mucin to form gel); C-terminal CK (Cys-rich [cystin-rich]/CK [Cystin-Knot]) domains (which are thought to be involved in the initial dimerization of apomucin monomers); and VWC domains (homologous to von Willebrand factor C domains), which are thought to be involved in binding trefoil factors (315, 338).

The mechanism(s) by which the apical exocytosis of granule content occurs has not been fully elucidated. It has been proposed that mucus exocytosis may develop after the granule and plasma membrane fuse to form a fusion pore and that an expulsive force then extrudes the viscous mucins from the granules into the luminal space. It has also been suggested that electrolyte secretion may provide the osmotic driving forces (140, 261, 262). There are two possible secretory pathways for secreted mucins in intestinal mucin-secreting cells (108, 206, 207). The first of these is the regular vesicular constitutive pathway of mucin exocytosis, also known as baseline secretion, in which no storage occurs, since the small vesicles transporting the mucins through the constitutive pathway are guided directly to the cell surface via microtubules and undergo immediate exocytosis of their contents. The second pathway for mucin exocytosis involves the packaging and storage of mucins in large vesicles, from which mucin release is regulated by specific stimuli involving the activation of signaling pathways by a number of secretagogues, including neuroendocrine mediators (such as acetylcholine, vasoactive intestinal peptide, and neurotensin [15, 44, 208, 314]), and inflammatory/immune mediators (such as interleukin-1 [184] and nitrite oxide [48]). Purinergic stimulation by extracellular ATP leads to an increase in mucin secretion (33, 138, 260). Both Ca<sup>2+</sup>- and cAMP-mediated second messenger cascades acutely regulate mucin secretion from human colonic epithelial cells (44, 47, 183). Cholera toxin that binds with high affinity to apically localized receptors on mucin-secreting cells (215) is a strong

TABLE 1. Membranous and secreted mucins

Gene	Type	Expression	Reference(s)	
			Mucins	Mucins/pathogens
<i>MUC1</i>	Membranous	Gastrointestinal epithelium, genitous tract, ocular, respiratory tract	51, 52, 182, 217, 222, 293, 356, 398, 405	52, 276, 396
<i>MUC3A</i>	Membranous	Gastrointestinal epithelium, genitous tract, respiratory tract	51, 75, 79, 205, 405, 411	210, 220, 231, 232
<i>MUC3B</i>	Membranous	Gastrointestinal epithelium, genitous tract, respiratory tract	51, 75, 79, 405, 411	210, 220, 231, 232
<i>MUC4</i>	Membranous	Gastrointestinal epithelium, genitous tract, ocular, respiratory tract	50, 51, 54, 79, 187, 270, 285, 308, 316, 356, 358, 410, 424, 427	220
<i>MUC12</i>	Membranous	Gastrointestinal epithelium, genitous tract	51, 220, 410	220
<i>MUC13</i>	Membranous	Gastrointestinal epithelium, genitous tract	51, 73, 412	
<i>MUC17</i>	Membranous	Gastrointestinal epithelium	51, 73, 134	
<i>MUC2</i>	Secreted	Gastrointestinal epithelium, genitous tract, respiratory tract	51, 79, 133, 356, 378	216, 231, 232
<i>MUC5AC</i>	Secreted	Gastrointestinal epithelium, genitous tract, respiratory tract	50, 79, 220, 287, 316, 350, 359, 388, 390	52, 64, 67, 90, 220, 276, 321, 388, 390
<i>MUC5B</i>	Secreted	Gastrointestinal epithelium, genitous tract, respiratory tract	51, 79, 220, 316, 359	
<i>MUC6</i>	Secreted	Gastrointestinal epithelium, genitous tract, respiratory tract	51, 79	52, 276

activator of mucin exocytosis (96, 214, 272, 273, 284, 332). In contrast, *Clostridium difficile* toxin A is able directly to affect the intestinal epithelial barrier function and down-regulates stimulated mucin exocytosis (48).

Membrane-bound mucins *MUC1*, *MUC3A*, *MUC3B*, *MUC4*, *MUC12*, *MUC13*, *MUC16*, and *MUC17* are associated with the cell membrane by an integral transmembrane domain and are characterized by having relatively short cytoplasmic tails that associate with cell cytoskeletal proteins. Membrane-bound mucins express specific mucin domains, including EGF (*Epidermal Growth Factor*)-like domains, the SEA (*Sea urchin sperm protein, Enterokinase, and Agrin*) domain, and the tandem repeat domain rich in serine, threonine and proline residues. Matsuo et al. (244) reported two distinct mucus layers. An elegant model of the functional organization of the mucus layer associating secreted mucins and membrane-bound mucins, has recently been proposed by Hollingsworth and Swanson (157). Membrane-bound mucins associate with the secreted mucins by both covalent and noncovalent bonds in order to create a high local concentration of specific molecular structures and to develop functions including binding sites for lectins, selectin, and adhesion molecules, stoichiometric power that enables them to exclude larger molecules and microorganisms, hygroscopic effects that influence the degree of hydration at the cell surface, ion exchange effects, and an area in which growth factors, cytokines, and chemokines are sequestered. Recent studies have also implicated membrane-bound mucins in cellular signaling, suggesting that they may have an important function as sensor mechanisms in response to invasion or damage of the epithelia (55). In this function, the cytoplasmic tails of membrane-bound mucins associate with adaptor proteins in the cytosol. For example, *MUC4* acts as a receptor ligand and *MUC1* as a docking protein for signaling molecules. *MUC1* has been found to be associated with lipid rafts that function as a platform for signaling molecules. It expresses a highly conserved cytoplasmic tail, which binds beta-catenin, a key component of adherens junctions and a regulator of transcription, in a process that is

tightly regulated by *MUC1* phosphorylation. *MUC4* is a novel intramembrane ligand for the receptor tyrosine kinase ErbB2/HER2/Neu, triggering specific phosphorylation of the ErbB2 in the absence of other ErbB ligands, and potentiating phosphorylation and signaling through the ErbB2/ErbB3 heterodimeric receptor complex that is formed in the presence of neuregulin.

Some of the *MUC7*, *MUC8*, *MUC9*, and *MUC15* mucins do not fit easily into either the secreted or membrane-bound class but do share some characteristics of these classes. For example, *MUC15* has a transmembrane domain and a cytoplasmic tail.

### Barrier Effect against Pathogens

For a long time, it was thought that the sole function of mucins was to protect and lubricate the epithelial surfaces (72); however, it has recently been established that they are also involved in other important functions, such as growth, and are directly implicated in fetal development, epithelial renewal, differentiation and integrity, carcinogenesis, and metastasis (71, 269). The mucus gel could be useful to enteric bacteria in at least two ways. First, the intestinal mucus offers numerous ecological advantages for both resident microbiotic bacteria and some pathogenic bacteria present within the lumen and in the intestinal epithelium, since it can provide nutrients for bacterial growth, thus promoting intestinal colonization by the adhering bacteria, which have the ability to survive and multiply in the outer regions of the mucus layer (11). Mucins do indeed provide a source of energy by producing the saccharides used for the sustained growth of both the indigenous enteric microbiota (27, 230) and the pathogens that adhere to the mucus (151, 197, 222, 323, 395). The second role played by the mucus layer is linked to its generally accepted role in cytoprotection (392). A discontinuous, thinner layer of mucus gel covers the epithelial cells that line the epithelium of the small intestine. Mucus thicknesses differ in the large intestine, gradually increasing from the colon to the rectum, and Peyer's patches apparently have no mucus covering (244). The mucus

layer creates a physical barrier that acts as a dynamic defense barrier against enteric microbial pathogens (Fig. 1) (268). Consistent with this, bacteria associated with the outer layer of mucus have been observed. Several gastrointestinal pathogens have developed specific pathogenic factors and/or ways of interfering with mucin production in order to enable them to cross the mucus barrier. The prototype of such pathogens is *Helicobacter pylori*, which colonizes the gastric mucous gel layer by means of a very close association with MUC5AC mucin (388, 389) and probably also with the membrane-bound mucin MUC1 (396). *H. pylori* uses its flagella for motility within the mucus layer in the acid-secreting stomach (296). In addition, *H. pylori* reduces mucin exocytosis (264), decreases gastric mucin synthesis by inhibiting UDP-galactosyltransferase (374), and causes an aberrant expression of the gastric mucins MUC1, MUC5AC, and MUC6 (52, 276). It is interesting that mucins play also a role in *Pseudomonas aeruginosa* pathogenesis since an upregulated transcription of the *MUC2* (216) and *MUC5AC* (90) mucin genes follows infection. The fact that upregulation of the *MUC5AC* gene can be mimicked by LPS indicates that there must be a general mechanism by which epithelial cells respond to the presence of bacteria by increasing mucin synthesis.

Secreted mucus has already been reported to act as a barrier to enteroinvasive *Yersinia enterocolitica* (239), rhesus rotavirus (58), and *Shigella flexneri* (287). It has also been reported that the bovine, mammary-associated, serum amyloid A3 increases the membrane-bound mucin MUC3, which in turn inhibits the adherence of enteropathogenic *Escherichia coli* (EPEC) (210). Resident intestinal bacteria are able to inhibit the adherence of pathogenic bacteria to intestinal epithelial cells as a result of their ability to increase the production of intestinal mucins. For example, *Lactobacillus plantarum* strain 299v increases the levels of expression of the mRNA of mucins MUC2 and MUC3, thus in turn inhibiting the cell attachment of EPEC, an effect that can be mimicked by adding purified exogenous MUC2 and MUC3 mucins (231, 232). Moreover, it has been observed that LPS of gram-negative bacteria increases the expression of the mRNA of MUC5AC and MUC5B and stimulates the secretion of MUC5AC and MUC5B mucins (359). It has been recently demonstrated that the secreted mucins including MUC5AC together with membrane-bound mucins, contributes to host defense by preventing bacterial invasion of the intestinal cells. Indeed, both in vivo (321) and in vitro (64) infections by the gram-positive, facultative intracellular human pathogen *Listeria monocytogenes* are associated with the massive release of mucus by goblet cells. This increase in mucin secretion develops through a listeriolysin-dependent mechanism that appears to be related to the binding of the toxin to multiple membrane-associated lipid receptors, which allows the toxin monomers to oligomerize and requires the toxin to be internalized through the caveolae (67). Listeriolysin also increases the transcription of the *MUC3*, *MUC4*, and *MUC12* genes that encode membrane-bound mucins (220). In contrast, the *MUC5AC* gene encoding a secreted mucin is not upregulated. Whereas secreted mucins or membrane-bound mucins alone were unable to prevent the cell entry of *L. monocytogenes*, both secreted and membrane-bound mucins have been shown to be necessary to inhibit cell entry (221). This is consistent with the fact that membrane-bound mucins, including

MUC3, MUC4, and MUC12, are associated with secreted mucins, in particular, with the gel-forming mucin MUC5AC, by both covalent and noncovalent bonds (157). The fact that the *MUC5AC* gene can be upregulated by LPS (90) but not by *L. monocytogenes* (220) suggests that for this *MUC* gene, epithelial cells respond to the presence of gram-negative bacteria by a general mechanism.

## ANTIMICROBIAL PEPTIDES

It is known that both nonvertebrates, such as insects and plants, and vertebrates, ranging from fish and frogs to humans, produce AMPs and that these peptides are the effectors of the innate immune response. The AMPs present in the gastrointestinal tract of the host constitute one of the partners involved in the front line of chemical defense against harmful microorganisms (Fig. 2) (35, 93, 117, 154, 172, 212, 235, 345, 379). This chemical antimicrobial defense system functions in the airways, gingival epithelium, cornea, reproductive tract, and urinary and gastrointestinal tracts. AMPs play a major role in the innate immune system, enabling it to respond in a matter of hours, well before the adaptive immune system can be sufficiently mobilized. The main advantage of the innate immune system is that it permits the host to curb, delay, or avoid the growth of undesirable intruding bacteria shortly after an infection, in a way that is not highly specific and does not involve memory. AMPs were first identified in polymorphonuclear neutrophils and macrophages. AMPs are gene-encoded peptides that have a broad spectrum of antibiotic activity.

### Intestinal Cells That Produce Antimicrobial Peptides

AMPs are produced by specialized cells known as Paneth cells (Fig. 1 and 2) (303, 347). These cells, one of the four major epithelial cell lineages present in the intestine, are present at the base of the crypts of Lieberkühn in mammals and play a pivotal role in the enteric defense against pathogenic harmful bacterial intruders. Paneth cells, like the enterocytes, goblet cells, and enteroendocrine cells, originate from intestinal epithelial stem cells (18, 42, 241). The maturation of Paneth cells has been investigated in mice. Recently, it has been demonstrated that the canonical Wnt signaling cascade (312, 313) plays a pivotal role in the maturation of Paneth cells (391). In addition, it has been reported recently that, consistent with the fact that Notch signaling (12) plays a critical role in intestinal development, the double transgenic Rosa-Notch/*Cre*<sup>+</sup> mouse exhibits compromised differentiation of the Paneth cells (110). Paneth cells are pyramid-shaped, columnar, exocrine cells, and they have been identified within a few days after birth in mice and as early as 24 weeks of gestation in humans. The ultrastructure of Paneth cells (317, 340) shows that they have a basally located nucleus with a nucleolus, a perinuclear region containing the rough endoplasmic reticulum and Golgi apparatus, and a supranuclear region containing numerous high-electron dense, apically located, eosinophilic secretory granules containing AMPs and other antimicrobial molecules, including lysozyme, phospholipase A<sub>2</sub>, α<sub>1</sub>-antitrypsin, and AMPs (91, 116, 212, 300) (Fig. 1). One of the functions attributed to Paneth cells is the control of the bacterial milieu in the intestine (16). It is possible that AMPs may influence the

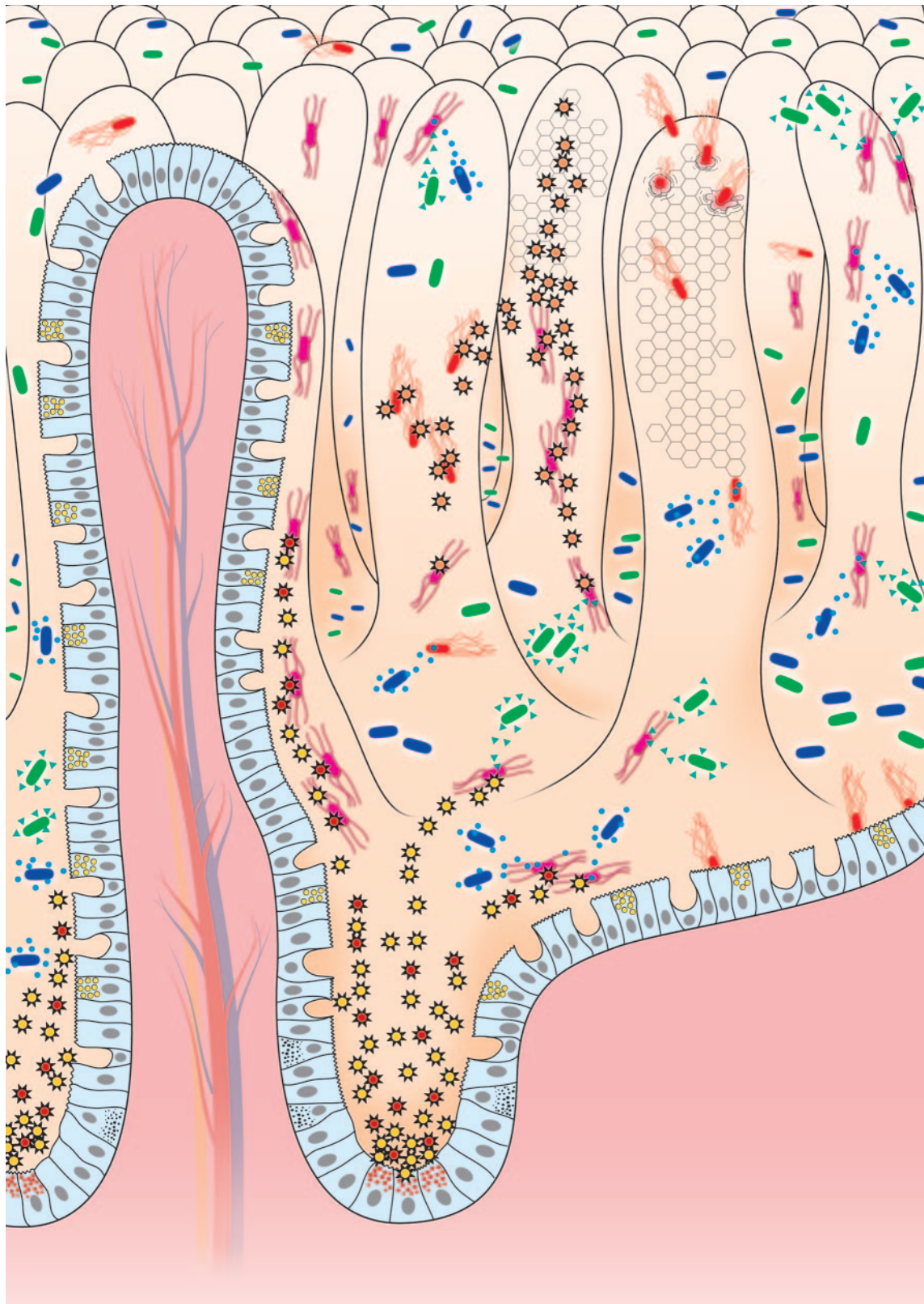


FIG. 2. The chemical front line of enteric host defense against unwelcome intrusion of harmful bacterial pathogens. Enteric invasive and noninvasive bacterial pathogens (red bacteria) expressing pathogenic factors (adhesive factors, invasins, and toxins, etc.) interact with the host epithelial cells lining the villi. At the base of the crypt, the Paneth cells containing antimicrobial-rich granules, released AMPs (red and yellow spike rings) upon exposure of intestinal epithelium to undesirable harmful pathogens and/or their bacterial products (LPS and toxins, etc.). Moreover, other intestinal cells lining the villi also secreted antimicrobial proteins (orange spike rings). In parallel, the commensal gram-negative (green bacteria) and gram-positive (blue bacteria) intestinal bacteria that reside in the lumen produced antibacterial molecules (green triangles and blue circles).

TABLE 2. Human intestinal AMPs

AMP	Family	Expression	Storage/processing	References
HD-5	$\alpha$ -Defensin	Paneth cells	Propeptide; during or after release	76, 77, 223, 318, 319
HD-6	$\alpha$ -Defensin	Paneth cells		36, 100, 223, 237, 409
hBD-1	$\beta$ -Defensin	Intestinal epithelial cells		26, 93, 188, 223, 294, 406, 425, 428
hBD-2	$\beta$ -Defensin	Intestinal epithelial cells	Mature peptide, endoplasmic reticulum?	21, 93, 100, 143, 223, 291, 294, 346, 372, 383, 400, 401, 404, 406–408
hBD-3	$\beta$ -Defensin	Gastrointestinal cells		120, 145
hLL37	Cathelicidin	Intestinal epithelial cells	Propeptide; during secretion process	22, 23, 148, 149, 152, 153, 295, 342, 417, 418

composition of the enteric microbial flora under physiological conditions, but this remains to be demonstrated (297). Moreover, because certain AMPs stimulate cultured epithelial cells to secrete the chloride ion, these peptides appear to be capable of interacting directly with the apical membranes of neighboring cells and, perhaps, of influencing crypt physiology (298). Under physiological conditions, the continual release of preformed AMPs allows the chemical defense system to contribute directly to the innate immunity of the crypt microenvironment, and it probably also does this by diffusing the peptides secreted into the lumen (Fig. 2). Interestingly, it has been reported that AMP activity can be compromised by inadequate dissolution of Paneth cell granules within the crypt lumina (61). Moreover, the maintenance of the release of granule constituents into the lumen of the crypt is important, since it has been recently demonstrated that compromised Paneth cell function is detrimental to host defense against *E. coli* infection in the neonatal small intestine (351).

Other observations have suggested that AMPs could be produced by intestinal cells other than Paneth cells lining the epithelium (Fig. 2). Cunliffe et al. (77) have identified dispersed epithelial cells expressing AMPs that resemble goblet cells. Little is known about the relationship between the expression of AMPs and the differentiation of polarized intestinal epithelial cells. Alteration in enterovirulent, diffusely adhering *E. coli* C1845 has been observed following the infection of human enterocyte-like Caco-2 and HT-29 Glc<sup>-/+</sup> cells (obtained by culturing the parental HT-29 cell line in culture medium deprived of glucose and then being adapted for growth in the presence of glucose), whereas this phenomenon is not observed in infected human, embryonic undifferentiated INT407 cells (31). Hase et al. (148) reported that hLL-37 mRNA and protein expression paralleled the spontaneous differentiation of Caco-2 human colon epithelial cells. Moreover, in HCA-7 human colon epithelial cells treated with the cell differentiation-inducing agent sodium butyrate, there is an increase in the expression of hLL-37 mRNA and protein (148). Similarly, sodium butyrate increased the level of hLL-37 transcripts in both colon and epithelial SW620 and SW480 cells, that do not express hLL-37, and in colon carcinoma Gek-12 and HT-29 cells, which do exhibit a basal level of hLL-37 expression (342).

### Antimicrobial Peptides

AMPs are small peptides, 20 to 40 amino acids in length (Table 2). Two major families of AMPs have been identified: the defensins (119, 213) and the cathelicidins (419). Defensins

were first identified by Ouellette (298) in mouse small intestinal cells. The mouse cryptin gene family encodes at least 19 different cryptdin proteins. The first murine Paneth cell defensin, known as cryptdin-1, which displays anti-*Salmonella* activity, has been identified by Ouellette (301). The mouse cryptdins (cryptdin-1 to cryptdin-5 and cryptdin-16) have been particularly investigated (167, 297–299, 302). In mammals, defensins are found in the phagocytic leukocytes and in various epithelial cells, including Paneth cells (35, 37, 41, 116).

AMPs have been classified on the basis of their secondary structure. Magainins and numerous cathelicidins (419) contain an  $\alpha$ -helical structure ( $\alpha$ -defensins), other AMPs have a  $\beta$ -sheet that contains three disulfide bonds ( $\beta$ -defensins), and the first circular AMP has recently been identified ( $\theta$ -defensins) (Table 2). Cathelicidins comprise mammalian proteins that are expressed by mammalian leukocytes (23, 203, 212, 324, 355, 418). The cathelicidin-derived AMPs are generally characterized by conserved propeptide sequences, include  $\alpha$ -helical, proline-rich, disulfide bonds, and/or a  $\beta$ -sheet, and tryptophan-rich peptides, but cathelicidins themselves have a linear, non- $\alpha$ -helical structure (Table 2) (212, 418).

To date,  $\alpha$ -defensins (HD) and  $\beta$ -defensins (hBD) (35, 76, 117), as well as cathelicidins (23, 212, 418–420), in humans have been identified (Table 2). In contrast,  $\theta$ -defensins are not expressed, although humans express mRNA encoding  $\theta$ -defensin orthologs and mutations that introduce stop codons abolish peptide production. Certain defensin genes are expressed in phagocytic cells of hematopoietic origin, whereas others are expressed in Paneth cells, and in the epithelial cells of the small intestine. The genes encoding the  $\alpha$ - and  $\beta$ -defensins are located in a cluster at chromosome 8p23 (223). For example, the gene encoding hBD-1 has been mapped to chromosomal region 8p23.1–8p23.2, which is in close proximity (within 100 to 150 kb) to the gene for the human neutrophil  $\alpha$ -defensin HNP-1 (224).  $\alpha$ -Defensins are small polypeptides with 29 to 35 residues and a six-cysteine motif that forms three intramolecular disulfide bonds (Cys1-Cys6, Cys2-Cys4, and Cys3-Cys5). Among the six  $\alpha$ -defensins identified, four designated as human neutrophil peptides (HNPs) 1, 2, 3, and 4 form part of the armory of the neutrophils, where they are involved in systemic innate immunity. The remaining HDs (HD-5 and HD-6) are expressed in intestinal cells and contribute to the innate defense of the intestinal mucosal surface. The levels of HD-5 and HD-6 transcripts are not high in the duodenum and increase distally (36). HD-5 is expressed in Paneth cells and also in some villous epithelial cells in healthy duodenum, jejunum, and ileum, but in contrast, it is not expressed in the healthy



stomach or colon (318).  $\beta$ -Defensins differ from  $\alpha$ -defensins in size (38 to 42 amino acid residues) and cysteine motifs (Cys1-Cys5, Cys2-Cys4, and Cys3-Cys6). Six  $\beta$ -defensins (hBD-1 to hBD-6) have been identified in humans. Human  $\beta$ -defensin-1 (hBD-1), consisting of a short basic peptide of 36 amino acid residues containing six cysteines forming three intramolecular disulfide bonds, has been found in epithelial cells of the small and large intestine (425).  $\beta$ -Defensin-2 (hBD-2) has been isolated from the skin and is expressed mainly in the respiratory tract (146) but also in the epithelial cells of both the small and large intestine (21).  $\beta$ -Defensin-3 (hBD-3), which exhibits microbicidal activity against *E. coli*, has been detected in the epithelia of the gastrointestinal tract (120, 145). Consistent with the fact that the  $\alpha$ - and  $\beta$ -defensins are located in a cluster at chromosome 8p23 (223),  $\beta$ -defensin-4 (hBD-4) has been recently identified by screening genomic sequences and found to be highly expressed in the testis and gastric antrum (120). In addition, it has recently been reported that polypeptides have been isolated from the human colon: three antimicrobials had previously been identified as ribosomal polypeptides (L30 and ubiquicidin), and two were members of the histone family (H1.5 and H2B) that exhibited bactericidal activity against *E. coli* (168). The levels of HD-5 and HD-6 transcripts are not high in the duodenum and increase distally. Both hBD-1 and hBD-2 mRNAs have been detected in some, but not all, biopsy specimens from healthy small intestines (86). HD-5 is expressed in Paneth cells and also in some villous epithelial cells in healthy duodenum, jejunum, and ileum, but in contrast, it is not expressed in the healthy stomach or colon (318). The cathelicidin hLL-37 has been shown to be expressed within epithelial cells located at the surface and upper crypts of healthy human colon (148) and gastric cells (149).

All AMPs are generated as prepropeptides, and all need to be processed to be activated. However, some are processed intracellularly and packaged in their processed forms, while others are processed after being secreted. It has been reported that some AMPs need to be processed to be activated. For example, HD-5 is present in Paneth cells only in the form of a precursor that does not have any antimicrobial activity against a defensin-sensitive *Salmonella* sp. and is processed to reach its mature form by a trypsin-dependent mechanism during and/or after being secreted inside Paneth cells (77, 124). Like defensins, some cathelicidins are fully processed before storage, whereas others are stored as precursors that still require further processing (212). Indeed, some cathelicidins are produced as inactive precursors containing a C-terminal cationic antimicrobial domain that becomes active after being freed from the N-terminal cathelin portion of the holoprotein. Signal peptidase removes the N-terminal signal sequence, whereas peptidylglycine  $\alpha$ -amidating monooxygenase often amidates and cleaves the C-terminal region. Removal of the cathelin domain liberates the active antimicrobial peptide. The hLL-37/hCAP-18 propeptide is present in the secondary granules, specific to neutrophils, and its C-terminal antimicrobial peptide, hLL-37, is liberated by proteinase 3 during degranulation and secretion. The bactericidal activity of cryptidins requires proteolytic activation of precursors by matrix metalloproteinase-7 (matrilysin), which is present in Paneth cells and known to be involved in innate host defense, since matrilysin-null mice have an impaired ability to

activate prodefensins and to kill exogenous bacteria in their small intestines (413).

### Antimicrobial Activities

AMPs have a wide spectrum of microbicidal activities against a wide variety of gram-negative and gram-positive bacteria, fungi, protozoa, and even enveloped viruses. AMPs either induce membrane damage that is a lethal event for target bacteria or bind to several targets in the cytoplasmic region of the bacteria. All the evidence indicates that the action of the AMPs does not involve stereospecific protein-receptor recognition, since the interactions of AMPs with their targets are generally considered to be nonspecific. To a large extent, biophysical studies have been performed using membrane model systems demonstrating that AMPs use several distinctive different mechanisms to kill bacteria (185, 226, 305). The amino acid composition, amphipathicity, cationic charge, and size of AMPs allow them to attach to and insert into membrane bilayers to form pores by "barrel-stave," "carpet-like," or "toroidal-pore" mechanisms. It has been demonstrated that the trisulfide structure of mature  $\alpha$ - and  $\beta$ -defensins was essential for the microbicidal activity of the folded molecules. These defensins are microbicidal at concentrations in a range of 0.5 to 5  $\mu$ M. Various isoforms of hBD-1 showing bactericidal or bacteriostatic activities exist. Studies of the microbicidal effect of  $\alpha$ -defensins HNP1 to HNP3 have provided evidence that bacterial inner and outer membranes are permeabilized as the consequence of voltage-dependent channels created by the AMP. LL-37, a cationic, amphipathic  $\alpha$ -helical AMP, targets the bacterial membrane, destroys the chemical gradients over the membrane by forming stable or transient pores (152, 153) and produces a detergent-like effect via a "carpet-like" mechanism (295). However, it has recently been speculated that transmembrane pore formation may not be the only mechanism by which AMPs kill microbes. In fact, several observations suggest that translocated AMPs can alter cytoplasmic membrane septum formation, reduce the synthesis of the cell wall, nucleic acid, and protein, and inhibit enzymatic activity. It should be noted that some AMPs also display lytic activity against various eukaryotic cells, but these AMPs have two distinct physical states of binding to lipid bilayers (169).

Recent observations indicate that in response to attack by pathogenic bacteria, the host engages its front line of chemical defense by increasing the production of AMPs, such as the  $\alpha$ - and  $\beta$ -defensins (16, 17). Ayabe et al. (17) report that LPS, LTA, lipid A and muramyl dipeptide were all able to elicit cryptidin secretion. In HD-5 transgenic mice, in which endogenous enteric defensin gene expression has been found in Paneth cells, there is a marked resistance to an oral challenge with virulent *S. enterica* serovar Typhimurium (337). It has been recently reported that expression of LL-37/hCAP-18, a human cathelicidin antimicrobial peptide, by gene transfer into C57BL/6 mice results in an increase in the innate immune response, providing support for the hypothesis that vertebrate antimicrobial peptides provide protection against microorganisms in vivo (22). The cathelicidin-related antimicrobial peptide, the only murine cathelicidin to be expressed in the intestinal tract, displays antimicrobial activity against the murine enteric pathogen *Citrobacter rodentium*, which produces lesions

in the intestinal cells similar to those produced by EPEC and enterohemorrhagic *E. coli* (EHEC) (173). Indeed, greater penetration of *C. rodentium* into the colonic mucosa occurs in cathelicidin-knockout mice. Moreover, infection of HCA-7 cells with *S. enterica* serovar Dublin or enteroinvasive *E. coli* modestly upregulated hLL-37 mRNA expression (148). The expression, regulation, and production of AMPs in human intestinal epithelial cells are modulated in response to LPS and enteric pathogens. Although TLR-mediated  $\beta$ -defensin expression has been best investigated in lung tissues (114), LPS- and peptidoglycan-stimulated hBD-2 production by activation of TLR4 and TLR2 in cell lines that constitutively or transgenically express TLRs has been reported (399). Moreover, *S. enterica* serovar Enteritidis flagellin using TLR5 and gangliosides as coreceptors increases hBD-2 expression in Caco-2 cells (291, 292, 372). A mutation in the NF- $\kappa$ B or AP-1 site within the hBD-2 promoter eliminated this response. In addition, inhibition of Jun kinase prevents the up-regulation of hBD-2 protein expression in response to LPS. It has been found that human colon epithelial cell lines constitutively express hBD-1 mRNA and protein but not hBD-2 (294). In contrast, the expression of cathelicidin hLL-37 mRNA is not upregulated in response to tumor necrosis factor alpha (TNF- $\alpha$ ), IL-1 $\alpha$ , gamma interferon, LPS, or IL-6 (148). Caco-2 cells produce two hBD-1 isoforms and an hBD-2 peptide that is bigger than previously reported hBD-2 isoforms. Interestingly, hBD-2 expression is rapidly induced by infecting human colon epithelial Caco-2 cells with *S. enterica* serovar Enteritidis, *S. enterica* serovar Typhimurium, and *S. enterica* serovar Typhi. *S. enterica* serovar Dublin induced hBD-2 mRNA expression in human carcinoma cells, and hBD-2 expression, but not hBD-1, is up-regulated in xenografts infected intraluminally with *Salmonella* (291, 294). The flagellar filament structural protein FliC of *S. enteritidis* has been identified as inducing hBD-2 expression in Caco-2 cells via NF- $\kappa$ B activation (291, 292, 372). The myeloid ELF-1-like factor (MEF) is involved in innate immunity responses, such as the activation of perforin and lysozyme transcription (368, 370), and also increased the level of endogenous hBD-2 transcription (229, 369). In addition, it is interesting that elevated levels of hBD-2 and hBD-3 transcripts have been found in *Helicobacter pylori*-infected gastric cells (20, 122, 143, 400, 401, 408).

Enteric pathogens have developed sophisticated strategies to survive in the gastrointestinal tract by evading the host defenses. It is significant that some of the major enteric pathogens have developed resistance to AMPs as a way of evading innate mucosal defenses. Bacterial pathogens have evolved counter-measures to limit the effectiveness of AMPs, including the repulsion of AMPs by reducing the net negative charge of the bacterial cell envelope through covalent modification of anionic molecules; expelling AMPs by means of energy-dependent pumps; altering membrane fluidity; and cleaving AMPs with proteases (309). Oral inoculation of mice with wild-type *S. enterica* serovar Typhimurium results in a decrease in the expression of  $\alpha$ -defensins and lysozyme (336). Moreover, the expression of antibacterial peptides LL-37 and hBD-1 has been found to be reduced in biopsy specimens from patients with bacillary dysenteries and in *Shigella*-infected cultures of epithelial cells (177). Moreover, the intracellular survival of *Salmonella* depends on the bacterium's ability to resist the activity

of cationic AMPs within the phagolysosome (128, 137, 266). Indeed, *S. enterica* serovar Typhimurium can sense sublethal concentrations of AMPs and induces various mechanisms of AMP resistance. The *Salmonella* PhoP/PhoQ regulators sense host environments to promote remodeling of the bacterial envelope that results in the modification in LPS-promoting bacterial survival by increasing resistance to AMPs, and by altered host recognition of LPS (97, 135, 136, 138, 139, 279, 353, 354). In particular, it has been observed that sublethal concentrations of AMPs activate the PhoP/PhoQ and RpoS virulence regulons, while repressing the transcription of genes required for flagellum synthesis, for the invasion-associated, type III secretion system, and for inducing RpoS-dependent protection against hydrogen peroxide (19). It should be noted that the intestinal production of the antimicrobial agent nitric oxide (104) generated by the inducible nitric oxide synthase that mediated the conversion of L-arginine to L-citrulline (95) is stimulated following infection by certain enteric pathogen including invasive *E. coli* and *S. enterica* serovar Dublin (414). Interestingly, it has been recently demonstrated that EPEC infection in Caco-2 cells can inhibit the inducible nitric oxide synthase expression at transcriptional and posttranscriptional levels by direct and indirect type III secretion system-dependent mechanisms (240).

## RESIDENT MICROBIOTA

The gastrointestinal tract is a complex ecosystem that associates a resident microbiota (27, 230, 422) and cells of various phenotypes lining the epithelial wall (Fig. 1). The term "microbiota" was defined by Savage (341) to describe the collective societies of bacteria assembled on the mucosal surfaces of an individual. Mammals are born without these microorganisms (233).

### Species Composition

The resident microbiota in the digestive tract constitutes a heterogeneous microbial ecosystem containing up to  $1 \times 10^{14}$  CFU of bacteria (27, 144, 230, 274, 376, 394). Resident bacteria localize "off-shore" from the epithelial cells within the mucus and seem to be content to catabolize mucin components (Fig. 1). Aerobic, facultative, and anaerobic bacteria all form part of the gastrointestinal microbiota. The microbial profile of the digestive tract is typified by the absence of anaerobic microorganisms in the stomach and, conversely, their overwhelming predominance in the distal colon. The proportion of anaerobic bacteria gradually increases from the proximal to distal regions, and 99% of the inhabitants located in the large intestine are anaerobes. Moreover, facultative anaerobes tend to associate along the epithelial layer, where oxygen diffusing from the tissues can be efficiently utilized. This is crucial for *E. coli* and probably also for other organisms. Different microbial communities may be located in the intestinal lumen, in the mucus covering the epithelium, in the crypt spaces and in the various cells lining the epithelium, and in addition, some species adhere, whereas others do not. It has been estimated that there are about more than 400 bacterial species in the intestinal microbiota. Currently, only 20 to 40% of the bacterial species present in the gastrointestinal tract have been cultured

or characterized due in particular to the precise oxygen requirements of some species, the largely unknown nutrient requirements for growth and the fact that some species develop for growth a high level of mutualism, since in the microbiota, they live in close proximity and benefit from one another. Molecular biological methods help in analyzing the structural and functional complexity of the microflora and in identifying its components. Identification of the species present in the gastrointestinal microbiota is in progress, as a result of the introduction of higher resolution molecular techniques based on 16S rRNA or rRNA genes, and of technological innovations, such as the selective media that now make it possible to grow bacteria that could not previously be cultured (243, 376).

The colonization of gastrointestinal tract starts immediately at birth. In adults, the intestinal microbiota consists of an enormous biomass of >100,000 billion bacteria. The compositions of the bifidobacterial microbiotas differ in infants and adults and indeed during other stages in the host's life (166). For example, the fecal microbiota of children has been found to be bacteriologically less complex, whereas advancing age is associated with a decrease in bifidobacteria and increasing species diversity of the *Bacteroides* genus. It has been postulated that changes in the microbial composition of the gut with age may alter the metabolic capacity of the gut microbiota and that this has important implications for the occurrence of disease. The intestinal microbiota, which can be considered to be a postnatally acquired organ, is composed of a wide diversity of bacteria that perform important functions for the host and can be modulated by environmental factors, such as nutrition (40, 87, 94, 103). The first bacteria to colonize the gut originate in the birth canal, and include both aerobic and anaerobic bacteria, such as *E. coli*, *Clostridium* spp., *Streptococcus* spp., *Lactobacillus* spp., *Bacteroides* spp., and *Bifidobacterium* spp. The upper part of the small intestine has relatively low bacterial densities and the distal portion of the small intestine, the ileum, shows higher bacterial densities. The lower intestine is colonized predominantly by anaerobes, particularly the *Bacteroides* spp., bifidobacteria, fusobacteria, and peptostreptococci, and aerobes and facultative aerobes such as *Enterobacteriaceae* and lactobacilli are present at moderate densities. Analyzing the *E. coli* commensal microbiota, Escobar-Paramo et al. (98) have observed that the *E. coli* isolates of intercontinental populations distribute into the four phylogenetic groups A, B1, D, and B2 with major differences between the geographical populations. *Lactobacillus* and *Bifidobacterium* spp., all of which are autochthonous species in the intestinal microbiota, have attracted interest (248, 274, 375). Reuter (326) has recently gained new insights into the species of these microorganisms that are present within the human intestinal microbiota. In humans, the autochthonous *Lactobacillus* and *Bifidobacterium* remain stable throughout life. *Lactobacillus gasseri* and *L. reuteri* are predominant autochthonous *Lactobacillus* species in both infants and adults. Marked interindividual variations have been found in microbial composition at the genus and species levels (166). The compositions of the bifidobacterial microbiota differ in infants and adults and during different stages of the host's life (326). Species typically found in infants are *Bifidobacterium bifidum*, *B. infantis*, *B. breve*, and *B. parvulorum*. According to Matsuki et al. (243), the *Bifidobacterium catenulatum* group is the most commonly found taxon, fol-

lowed by *B. longum* and *B. adolescentis*, in the adult intestinal bifidobacterial flora, and *B. breve*, *B. infantis*, and *B. longum* are frequently found in the intestinal tracts of infants.

### Intestinal Functions

The intestinal microbiota plays an important role in normal gut function and in maintaining host health. All the components of the gastrointestinal ecosystem seem to be necessary for the gut to develop its specific intestinal functions (249, 422). Little is known about how members of the indigenous microbiota interact with their mammalian hosts to establish mutually beneficial relationships. Midtvedt et al. and Gordon et al. (49, 102, 158–165, 227, 365, 415) have recently gained important new insights into the mechanism by which members of the intestinal microbiota influence intestinal functions by means of cross talk with epithelial cells. For example, some observations lend support to the hypothesis that the capacity for synthesizing diverse carbohydrate structures may have arisen in part from our need both to evade pathogenic relationships and to coevolve in symbiotic relationships with our nonpathogenic resident microbes (161). The intraluminal microbiota influences the release of biologically active gastrointestinal peptides, and contributes to regulating gastrointestinal endocrine cells and the epithelial structure (384). *Bacteroides thetaiotaomicron* is one such bacterial symbiont that is a dominant member of the intestinal microbiota of mammals, including human beings (70, 159). Colonization of germfree mice by *B. thetaiotaomicron* VPI-5482, a component of the intestinal flora, has revealed that this commensal bacterium modulates the expression of genes involved in several important intestinal functions, including nutrient absorption, mucosal barrier fortification, xenobiotic metabolism, angiogenesis, and postnatal intestinal maturation (160, 164). The colonization of germfree mice with the VPI-5482 strain of *B. thetaiotaomicron* restored the fucosylation program, whereas an isogenic strain carrying a transposon insertion that disrupts its ability to use L-fucose as a carbon source did not (49, 165). Colonization of germfree mice with *B. thetaiotaomicron* has shown how this anaerobe modifies many aspects of intestinal cellular differentiation/gene expression to the benefit of both the host and the microbe (162). In line with this observation, comparison of gut glycosylation patterns in germfree and conventional mice have revealed both quantitative and qualitative differences in the cellular and subcellular distribution of glycans (111). It has been observed that this strain also has the capacity for changing the galactosylation process in cultured human mucin-secreting HT29-MTX cells as a result of posttranslational regulation, via a mechanism that involves a soluble, heat-labile, low-molecular-weight factor (112). Interestingly, in colonized germfree mice, a strain of *B. thetaiotaomicron* increased the production of matrilysin (227), a matrix metalloprotease expressed in Paneth cells and shown to be involved in innate host defense, as matrilysin-null mice have an impaired ability to activate prodefensins and to kill exogenous bacteria in their small intestines (413). It has also been reported that the normal colonization of the mammalian intestine with commensal microbes influences the development of the humoral and cellular mucosal immune systems during neonatal life and maintains the physiologically normal steady state of inflammation in the gut

throughout life (56, 373). In connection with microbiota, it has been observed that the introduction of germfree mice into a conventional environment results in the enhanced expression and secretion of the goblet cell-specific protein RELM $\beta$ , providing evidence that colon-specific gene expression can be regulated by colonization with normal enteric bacteria (150).

The host is highly adapted to the presence of commensal intestinal bacteria by a phenomenon termed "mucosal immune adaptation." In addition, a second adaptive phenomenon termed "systemic immune ignorance" has been investigated (196, 251, 253, 254). McPherson and Uhr (256) have showed that commensal bacteria are rapidly killed by macrophages and intestinal dendritic cells (DCs) can retain small numbers of live commensals for several days. This allows DCs to selectively induce immunoglobulin A through a pathway that was independent of T-cell help and of follicular lymphoid tissue organization, which helps protect against mucosal penetration by commensals and the specific anticommensal immunoglobulin A induction (251, 256). Because DCs loaded with commensal bacteria do not penetrate further than the mesenteric lymph nodes, immune induction to commensals is confined to the mucosa, which ensures that immune responses to commensal bacteria are induced locally, without potentially damaging systemic immune responses (252, 255). However, the resident microflora contains a number of components able to activate innate and adaptive immunity (288). In consequence, immune responses to mucosal microbiota require a precise regulatory control and unlimited immune activation in response to signals from commensal bacteria could pose the risk of inflammation (193, 194, 196, 250). Importantly, resident microbiota bacteria are recognized to suppress unnecessary inflammatory response, thereby helping to maintain immune homeostasis (194). An improved understanding of commensal bacteria-host interactions has been obtained employing germfree animal models with selective colonization strategies combined with modern molecular techniques. For example, the potential role of the intestinal microbiota in facilitating the development of tissue injury and systemic inflammation has been examined by Souza et al. (360) showing that there was marked edema formation, hemorrhage, and production of tumor necrosis factor alpha (TNF- $\alpha$ ) and monocyte chemoattractant protein 1 in intestine of conventional mice compared with germfree mice. Moreover, pathogenic *E. coli* organisms, including EPEC (426), enteroaggregative *E. coli* (147, 198), and EHEC (28, 198), and nonpathogenic organisms, including diffusely adhering *E. coli* (34), commensal *E. coli* strain MG1655 (24), and *B. vulgatus* (142), have been observed to be able to promote activation of NF- $\kappa$ B nuclear translocation and, thereafter, proinflammatory gene expression in intestinal cells. Generally, only *Lactobacillus* spp. were not able to promote proinflammatory response; however, in the presence of underlying leukocytes, challenge of Caco-2 cells with *L. sakei* induces expression of IL-8, monocyte chemoattractant protein 1, IL-1 $\beta$ , and TNF- $\alpha$  mRNA (141). Interestingly, it has been recently demonstrated that commensal bacteria could inhibit the proinflammatory responses (40, 343). For example, Kelly et al. (193, 195) have shown that *B. thetaiotaomicron* inhibits proinflammatory cytokine IL-8 expression (195) and attenuates the flagellated pathogen-induced proinflammatory cytokine expression by promoting nuclear export of NF- $\kappa$ B subunit RelA, through a

peroxisome proliferator-activated receptor- $\gamma$ -dependent pathway (193). Similar inhibition has been observed with the *Lactobacillus acidophilus* strain LB of intestinal microbiota origin against the *Salmonella*-induced IL-8 expression (66). Moreover, the *B. breve* strain BbC50 isolated from the fecal flora of a healthy breast fed infant has been found able to display a TNF- $\alpha$  inhibitory capacity (259). The host appears also adapted to the deleterious effects promoted by commensal intestinal bacteria. Indeed, alterations in the intestinal barrier that resemble those promoted by enteric pathogens have been observed induced by species of the intestinal microbiota. For example, the *E. coli* strain EM0, a human fecal strain expressing hemolysin and cytotoxic necrotising factor, induced a lytic effect against cultured human intestinal cells (170). The prototype translocating *E. coli* strain C25 isolated from human feces, induces a loss of transepithelial electrical resistance, changes in distribution of TJ-associated proteins ZO-1 and claudin-4, and vacuolation of mitochondria (421). Observation that these deleterious effects were not promoted by the commensal *E. coli* strain F18 (421) is indicative that only certain strains of the intestinal microbiota have the capacity for developing pathogen-like effects. It is possible that species of the intestinal microbiota, including *Lactobacillus*, function as regulators against the pathogen-like commensal strains since, as described below, they have the capacity for blocking the pathogen-induced deleterious effects in host cells.

#### Barrier Effect against Pathogens

One of the basic physiological functions of the resident microbiota is that of providing a microbial barrier against microbial pathogens (Table 3). For example, Nicaise et al. (283) have recently documented the mechanism(s) of the immune response of the intestinal microbiota by examining the regulation of interleukin-1 (IL-1), IL-6, TNF- $\alpha$ , and IL-12 production in macrophages from germfree and from flora-associated mice, conventional, conventionalized and *E. coli*-monoassociated mice. The findings show that the intestinal flora can modulate bone marrow and spleen macrophage cytokine production in a differential manner. Enhanced IL-12 production in the spleen by the intestinal flora is also potentially important, since this cytokine is implicated in determining the relative levels of Th1 and Th2 responses, and plays a pivotal role in defending the host against intracellular microorganisms. Recent reports have provided new insights into how members of the intestinal microbiota develop a barrier effect and produce antimicrobial activity against enteropathogens.

Cecal microflora of hamster is able to develop an anti-*C. difficile* barrier effect (367). Interestingly, a *C. cocleatum* strain has been found involved in this anti-*C. difficile* barrier effect (45). Ramare et al. (325) have observed that when a human intestinal strain of *Peptostreptococcus* colonized the gut of gnotobiotic rats, it produced an antibacterial substance that was active against several gram-positive bacteria, including potentially pathogenic *Clostridium* spp. such as *C. perfringens*, *C. difficile*, *C. butyricum*, *C. septicum*, and *C. sordellii*. Similarly, the E1 strain of *Ruminococcus gnavus*, a gram-positive strictly anaerobic strain isolated from a human fecal sample, was able to produce an antibacterial substance, known as ruminococcin A, that is also active against various pathogenic clostridia (78,

TABLE 3. Bacterial strains of microbiota origin exerting antibacterial effects against intestinal pathogens

Strain(s)	Reference(s)
<i>E. coli</i> Nissle 1917 producing microcins.....	7, 43
<i>E. coli</i> strains producing microcins .....	2, 25, 39, 84, 320, 328, 333, 335, 397
<i>E. coli</i> strains not producing microcins .....	170
<i>Peptostreptococcus</i> strain.....	325
<i>Ruminococcus gnavus</i> E1.....	78, 126
<i>Clostridium cocleatum</i> .....	45
<i>L. acidophilus</i> LB .....	57, 62, 63, 65, 66, 68, 219
<i>L. johnsonii</i> La1.....	30, 32, 106
<i>L. casei</i> DN-114001.....	106, 132, 175, 304
<i>L. casei</i> Shirota YT9029 .....	13, 85, 106, 289, 290
<i>L. rhamnosus</i> GG .....	106, 171, 211, 232, 357
<i>L. plantarum</i> 299v.....	232, 238
<i>L. acidophilus</i> HN017 .....	127
<i>L. rhamnosus</i> DR20 .....	127
<i>Bifidobacterium</i> strains.....	29, 69, 218

126). As previously reported for some AMPs, including HD-5 (77, 124) and some cathelicidins (212), it is interesting that two antibacterial substances produced by bacterial species in the intestinal microbiota, *Peptostreptococcus* sp. (325) and the *R. gnavus* E1 strain (78, 126), require processing to be activated, after the proforms have been cleaved by trypsin.

It has been demonstrated that strains of *E. coli* of intestinal microbiota origin have the capacity for protecting mice against bacterial infection (Table 3). *E. coli* contributes to the antibacterial defense by producing antibacterial proteins, known as colicins and microcins (83, 327). Microcins are a miscellaneous group of low-molecular-mass antibiotics (molecular mass less than 10 kDa), whereas colicins are much bigger, from 25 to 80 kDa. All colicins and some microcins are encoded by gene clusters organized in operons, whereas other microcins are encoded on the chromosome of produced bacteria (275). All the bacteria encoding microcins or colicins have immunity towards the antibiotics that they produce. Colicin immunity is specific, but in some cases, other mechanisms are also involved, such as pumping microcin out of the cells. The bactericidal spectrum of activity was found to be restricted to *Enterobacteriaceae* and specifically directed against *Escherichia* (333) and *Salmonella* (320, 397) species. The microcin inserts into the inner membrane, whereupon the potential becomes destabilized due to pore formation that leads to depolarization and permeabilization of the *E. coli* cytoplasmic membrane (25, 84, 328). Another mechanism of antibacterial activity has been reported for *E. coli* strain Nissle 1917 (129) that produces microcins (7). This *E. coli* strain induces the expression of hBD-2 in Caco-2 intestinal epithelial cells in a time- and dose-dependent manner (407). This induction results of the activation of the hBD-2 promoter involving functional binding sites for NF- $\kappa$ B and AP-1 via a signaling pathway involving c-Jun N-terminal kinase, p38 mitogen-activated protein kinase, and signal-regulated kinase 1/2. It is interesting that, as reported above for AMPs, microcins have generated mechanisms of resistance in *Salmonella* (53, 109). It should be noted that Hudault et al. (170) have shown that resident *E. coli* that did not produce microcin had also a barrier effect when colonizing the gut of gnotobiotic C3H/He/Oujco mice orally infected by a lethal strain of *S. enterica* serovar Typhimurium.

*Lactobacillus* and *Bifidobacterium* spp. of human intestinal microbiotic origin produce antimicrobial substances that are active in vitro and in vivo against enterovirulent microorganisms involved in diarrhea disorders (Table 3) (349). For example, *Lactobacillus acidophilus* LB, *L. johnsonii* La1, *L. rhamnosus* GG, *L. casei* Shirota YT9029, *L. casei* DN-114 001, *L. acidophilus* HN017, and *L. rhamnosus* DR20 strains produced antibacterial components that are active against a wide range of gram-negative and gram-positive pathogens, such as EPEC, EHEC, *L. monocytogenes*, *S. enterica* serovar Typhimurium, and *S. flexneri* (32, 63, 65, 106, 127, 171). Moreover, antibacterial components produced by *L. acidophilus* strain LB were able to inhibit the growth of *S. enterica* serovar Typhimurium residing intracellularly in a vacuole in infected intestinal Caco-2 cells (66). These components, although not characterized at the molecular level, do not share the characteristics of bacteriocins and are different from lactic acid (106). *L. rhamnosus* GG secretes a low-molecular-mass, heat-stable, inhibitory substance which is distinct from lactic and acetic acids (357). The molecules that support the antibacterial activity of *L. acidophilus* LB and *L. johnsonii* La1 have a low molecular mass and are heat stable and insensitive to proteases (32, 65). An antibacterial component produced by human *Bifidobacterium* sp. CA1 and F9 strains has been found to consist of one or more lipophilic molecule(s) with a molecular mass of less than 3,500 Da (218). A mechanism by which non-lactic acid molecules secreted by *Lactobacillus* may kill gram-negative pathogens has recently been identified (68). Evidence showing that the bacterial membrane damage induced by the nonbacteriocin, non-lactic acid molecule(s) produced by the *L. acidophilus* LB of human intestinal microbiotic origin are lethal for *S. enterica* serovar Typhimurium has been provided. The mechanism of action includes (i) the depletion of intracellular ATP, (ii) an increase in membrane permeabilization, (iii) the release of LPS from the bacterial membrane, and (iv) the sensitization of the bacterial membrane towards the lytic action of detergent. The mechanism by which *L. acidophilus* LB kills *S. enterica* serovar Typhimurium resembles the mechanism by which AMPs and several classes of antibiotics kill bacteria. Indeed, intracellular K<sup>+</sup> and ATP depletion have also been observed in EHEC strain O157:H7 subjected to AMPs (10). Moreover, it has been reported that a release of LPS from the membrane of gram-negative pathogens is triggered by several antibiotics (99, 179, 278, 381, 393). Since AMPs are discharged from Paneth cells at effective microbiocidal concentrations into the small intestinal crypts (116–118), it is tempting to suggest that some commensal intestinal bacteria, including *E. coli* and *Lactobacillus*, may discharge antimicrobial substance(s) into ecological niches within the intestine and thus also contribute to the front line of the chemical defense against enteric pathogens. In addition, metabolic end products of resident microbiotic bacteria could have an antimicrobial effect and so may potentiate the effects of other enteric antimicrobial substances, such as those produced by members of the microbiota and/or AMPs.

Importantly, it has been demonstrated that *Lactobacillus* and *Bifidobacterium* strains of intestinal microbiota origin that exert in vitro antimicrobicidal activities have the capacity for combatting infection in rodent models infected with human enterovirulent bacteria. The first model used is that of gnoto-

biotic mice, in which the microbiota is missing and the epithelium is not fully differentiated. The *L. johnsonii* La1 (32) and GG (171) strains, which colonize the gut of gnotobiotic C3H/He/Oujco mice, develop antibacterial activity when the mice are orally infected by *S. enterica* serovar Typhimurium C5, and this increases the survival of the mice. The human *Bifidobacterium* sp. CA1 and F9 bacteria that colonize the intestinal tract of axenic C3/He/Oujco mouse protect the mice against a lethal infection of *S. enterica* serovar Typhimurium C5 (218). The second model used is that of conventional mice, which have both a microbiota and a fully differentiated epithelium. In this mouse model, the spent culture supernatant of the human *L. acidophilus* strain LB, which contains an antibacterial molecule(s), given daily following infection is active against *S. enterica* serovar Typhimurium C5 infection in conventional C3H/He/Oujco mice, reducing the levels of viable *Salmonella* in the feces (65; D. Fayol-Messaoudi, M.-H. Coconnier-Polter, V. Lievin-Le Moal, C. N. Berger, and A. L. Servin, unpublished data).

#### Inhibition of Pathogen-Host Cell Interactions and Pathogen-Induced Cell Injuries

Bacteria that originated from the intestinal microbiota have the capacity for interfering with or block the process of pathogenicity of enteric bacterial pathogens. *E. coli* strain Nissle 1917 that produces microcin, was able to inhibit invasion of epithelial intestinal INT407 cells by *Salmonella* spp., *S. flexneri*, and *L. monocytogenes* without affecting the viability of the invasive bacteria (7). This *E. coli* strain, independently of the microcin production, is able to block the invasion process of the Crohn's disease-associated adherent-invasive *E. coli* LF82 (43). Similarly, the DN-114 001 strain of *L. casei*, independently of this bactericidal effect, strongly inhibits interaction of adherent-invasive *E. coli* LF82 with intestinal epithelial cells (175). It has been reported that *Lactobacillus* inhibited the internalization of *S. enterica* serovar Typhimurium within human intestinal cells, and this effect had been attributed to a secreted molecule(s) that could affect the virulence factors involved in cell entry and/or block the host cell signal transduction necessary for bacterial internalization (32, 63, 65, 66). An identical effect has been reported for *Bifidobacterium* strains isolated from stools of infants (29, 218). The mechanism by which some of the molecules produced by *Lactobacillus* strains impair the internalization process has been recently identified (Fayol-Messaoudi et al., unpublished). Indeed, compound(s) secreted by *L. johnsonii* La1 and *L. casei* Shirota YT9029 strains impair flagellum motility function in *S. enterica* serovar Typhimurium, and this in turn reduces the capacity of the pathogen to penetrate into human intestinal cells. This finding is consistent with the fact that the flagella that provide the motility of *Salmonella* (5, 234) have been found to be involved in bacterial internalization within eukaryotic cells (88, 189, 209, 225, 306, 329, 330, 386, 387). It has been observed that the expression of the FliC protein composing the full-length filament of the flagellum (234) is not modified, suggesting that the molecule(s) produced by *Lactobacillus* affect(s) the functionality of *S. enterica* serovar Typhimurium flagella. Interestingly, the *Salmonella* flagellum-dependent production of

the proinflammatory cytokine IL-8 (88, 92, 123) is blocked by *L. acidophilus* LB.

*Lactobacillus* strains of intestinal microbiota origin have the capacity for inhibiting the cellular lesions induced by enteric pathogens within the intestinal epithelial barrier. For example, *L. acidophilus* LB (219) and *L. helveticus* R0052 (352) antagonized the cytoskeleton rearrangements produced by enterovirulent *E. coli* in T84 and Caco-2 cells. The decrease in brush border expression of sucrase-isomaltase, dipeptidylpeptidase IV, alkaline phosphatase, and fructose transporter induced by the diffusely adhering Afa/Dr *E. coli* C1845 in Caco-2 cells was inhibited by the *L. acidophilus* strain LB (219). *L. helveticus* strain R0052 (352), *L. plantarum* 299v (238, 263), and *L. casei* DN-114 001 (304) all reduce the pathogen-induced drop in transepithelial electrical resistance in cultured colonic T84 cells forming monolayer infected by EHEC and EPEC.

#### CONCLUDING REMARKS

Mucins, AMPs, and members of the intestinal microbiota all separately provide an effective front line of intestinal defense against unwelcome harmful microorganisms (Fig. 1 and 2). Mucins create a dynamic physical barrier, while Paneth cells and intestinal microbiota produce AMPs and antimicrobial molecules, respectively, which have the effect of killing enteric pathogens and inhibiting pathogen-host cell interaction. Whether these systems of defense act in partnership, and whether they function synergistically to provide the host with an efficient front line of defense against harmful, enteric pathogens, remains currently poorly documented.

It is conceivable that the resident intestinal bacteria may affect goblet cell dynamics and the mucus layer both directly, via the local release of bioactive factors, and indirectly, by activating host cells. This hypothesis has been investigated in a few studies. For example, it has been observed that the LPS of gram-native bacteria increases the expression of the mRNA of MUC5AC and MUC5B and stimulates the secretion of MUC5AC and MUC5B mucins (359). The quorum-sensing signal molecule [*N*-(3-oxododecanoyl) homoserine lactone (3O-C<sub>12</sub>-HSL)] of gram-negative bacteria could stimulate the production of a major mucin core protein, MUC5AC (174). Moreover, *Lactobacillus* organisms, subdominant species of the microbiota, increase the levels of expression of the mRNA of mucins MUC2 and MUC3 (231, 232). It seems likely that the AMPs may influence mucin secretion, since AMPs stimulate the secretion of chloride ions (298), and both chloride secretion and mucin exocytosis have been observed to be stimulated in mucin-secreting cells (140, 260, 262).

How the microbiota can influence AMP production remains controversial. Some reports suggest that in fact the microbiota has no influence. Indeed, in the intestine of germfree mice, the same set of mature enteric defensins (defensins 1, 2, 3, 4, and 6) has been found as in mice colonized by a normal microbiota (322). Moreover, the expression of cathelicidin hLL-37 by the colonic epithelium does not require the presence of commensal bacteria, since the peptide is produced with a similar pattern of expression by epithelial cells in human colon xenografts that have no luminal microbiota (148). In contrast, it has been reported that the intestinal commensal bacteria can influence gut microbial ecology and shape innate immunity. Indeed, an-

giogenin-4, a molecule with bactericidal activity produced by mouse Paneth cells, is induced by *B. thetaiotaomicron*, a dominant member of the gut microbiota (163). The *B. thetaiotaomicron* strain that colonizes the intestine of germfree mice increased the production of matrilysin (227), a matrix metalloprotease expressed in Paneth cells, and has been shown to be involved in innate host defense (413). It has recently been observed that *Bifidobacterium* or its cell wall proteins can induce AMP hBD-2 gene expression in cultured human intestinal epithelial cells (404). In response to components of gram-negative bacteria, such as LPS and peptidoglycan, hBD-2 expression is increased (399). Moreover, the flagellum filament structural protein FliC of gram-negative bacteria has been found to induce hBD-2 expression via an NF- $\kappa$ B-dependent mechanism (291, 292, 372). It remains to be determined whether activity by LPS or flagella of the resident intestinal *E. coli* could contribute to activating the production of AMPs, in turn regulating the intestinal microbiota.

It has recently been established that gram-negative pathogenic bacteria are able to sense both the cell density and the metabolic potential of their environment by a quorum-sensing system, which is a cell density-dependent signaling system used by bacteria to coordinate gene expression within a population (3, 101, 265, 371). In particular, in pathogenic *E. coli*, quorum-sensing involves a transcription regulator (LuxR homologue) and an autoinducer, either AI-2 or AI-3, depending on the function encoded by the *luxS* gene (8, 130, 190, 361–364). A quorum-sensing system in gram-positive bacteria has been identified (366). Autoinducing peptides are involved in intercellular communication in gram-positive bacteria, and many of these peptides are exported by dedicated systems and finally sensed by other cells via membrane-located receptors. The production-biosynthesis, maturation, and secretion of colicins or microcins (83, 327) into the medium by *E. coli* are encoded by gene clusters organized in operons that are silent/repressed during exponential growth and are induced/derepressed when cells sense nutrient starvation and stop their exponential growth (275). It could be of interest in the future to find out whether the *E. coli* strains that are resident in the intestinal microbiota possess a quorum-sensing system that senses the presence of these pathogens and in turn controls the production and secretion of antimicrobial molecules. For gram-positive bacterial members of the intestinal microbiota an identical investigation is of interest considering that the production of bacteriocins, a type of bactericidal proteinaceous molecules produced by *Lactobacillus* (178, 199, 247, 280, 334), has been found to be regulated at the transcriptional level in a manner dependent on cell-density (200) and appears to be controlled by a peptide-based, quorum-sensing system that drives strong, regulated promoters (236, 242). In addition, it has been reported recently that the production of nonbacteriocin, non-lactic acid antibacterial molecules that are active against gram-negative enteric pathogens is temperature controlled (106).

Further progress is required to enable us to understand how the three components of the front line of defense against enteric pathogens are coordinated and whether the various components modulate the functions of the others and/or act synergically.

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