

The intestine and its microflora are partners for the protection of the host: report on the Danone Symposium “The Intelligent Intestine,” held in Paris, June 14, 2002^{1,2}

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ABSTRACT The intestine is an extremely complex living system that participates in the protection of the host through a strong defense against aggressions from the external environment. This defensive task is based on 3 constituents that are in permanent contact and dialog with each other: the microflora, mucosal barrier, and local immune system. We review herein current knowledge about these important functions. The gut microflora play a major role against exogenous bacteria through colonization resistance, but the mechanism of action is not yet established, although it is linked to the bacteria colonizing the gut. This colonization involves bacteria-bacteria dialog, bacteria-mucins interactions, and bacteria-colonocytes cross-talk associated with environmental factors. The intestinal mucosa is a cellular barrier and the main site of interaction with foreign substances and exogenous microorganisms. It is a complex physicochemical structure consisting of a mucous layer linked to cellular and stromal components that participate in the defense of the host through mucosal blood flow, mucosal secretions, epithelial cell functionals, surface hydrophobicity, and defensin production. The intestine is the primary immune organ of the body represented by the gut-associated lymphoid tissue through innate and acquired immunity. This immune system can tolerate dietary antigens and the gut-colonizing bacteria and recognizes and rejects enteropathogenic microorganisms that may challenge the body's defenses. In cooperation with these endogenous barriers, some in-transit bacteria, such as probiotics, can act as partners of the defense system of the intestine. *Am J Clin Nutr* 2003;78:675–83.

KEY WORDS Gastrointestinal tract, microflora, gut colonization, quorum sensing, mucins, cross-talk, mucosal barrier, defensins, gut-associated lymphoid tissue, innate immunity, acquired immunity, toll-like receptors, probiotics, host defense, lactic acid bacteria, intestinal epithelium

INTRODUCTION

Consider Alfred Binet's definition of intelligence, given at the end of the 19th century: “intelligence is the range of processes involved in adapting to the environment.” If the intestine is considered “intelligent,” it means that it must be able to send, receive, and understand messages. How does the gut do this?

The intestine is an extremely complex ecosystem composed of 3 main components that are permanently in contact and interact with each other: host cells, nutrients, and microflora. Microflora

are an important living biomass that plays various physiologic roles in the host, in partnership with the other components.

Intestinal functions include the well-known processes that lead to the digestion of food, the absorption of nutrients, and a series of activities aimed at establishing a strong defense against aggressions from the external environment. This important defensive task of the intestine is based on 3 essential constituents: the microflora, the mucosal barrier, and the local immune system (ie, the gut-associated lymphoid tissue; GALT) as represented in **Figure 1**.

WHAT ROLE DOES THE NORMAL MICROFLORA PLAY IN THE BODY'S DEFENSE SYSTEM?

First, it should be understood that the microflora in the adult human body consist of an enormous biomass of > 100 000 billion bacteria of > 400 different species, which generate intense metabolic activity, mainly in the colon, and play an important physiologic role in the host (1). However, because they have a variety of physiologic conditions to contend with in the different sections of the gastrointestinal tract, the bacteria are not evenly distributed throughout it.

There is a very low population of bacteria in the stomach because it is highly acidic (pH of ≈ 1), and the acidity destroys almost all of the bacteria that pass through it. Nonetheless, gastric emptying allows many bacteria to survive and pass through the pyloric sphincter into the duodenum. Consequently, only a limited number of live bacteria reach the duodenum. When they arrive in this neutral (pH of ≈ 7) environment, they are subject to attacks from bile salts and pancreatic secretions.

Bacteria that can resist this type of attack significantly increase in number by the time they reach the jejunum and cross the ileocecal valve to reach the colon (2). At this point, the magnitude of

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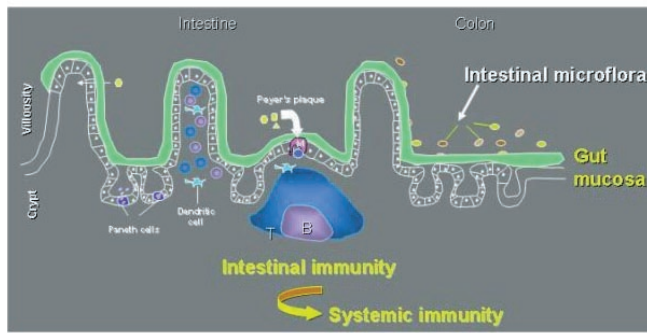


FIGURE 1. Natural defenses of the gut.

the transit times through the various sections of the intestine should be noted.

In healthy persons, the transit time from mouth to anus is between 55 and 72 h, of which 4–6 h is from the mouth to the cecum and 54–56 h is in the colon (3). It is understood that the short transit time through the small intestine does not allow bacterial growth, whereas, in the colon, the much longer transit time (among other factors) enables bacteria to reach considerable population levels [$\approx 10^{11}$ – 10^{12} colony forming units (CFU)/g].

The population consists of predominant bacteria ($> 10^9$ CFU/g), subdominant bacteria (between 10^6 and 10^9 CFU/g)—also called repressed bacteria, which are the endogenous or resident gut flora—and bacteria in transit ($< 10^6$ CFU/g). Depending on the number of bacteria ingested, the transit bacteria will be present in greater or lesser numbers. Most of the bacteria that manage to cross the barriers of the stomach and the small intestine will be alive and metabolically active. Some of these bacteria are useful to the host, such as bacteria of the genera *Bifidobacterium* and *Lactobacillus*. Other bacteria, to the contrary, are potentially pathogenic and can be dangerous to the host (4; Figure 2).

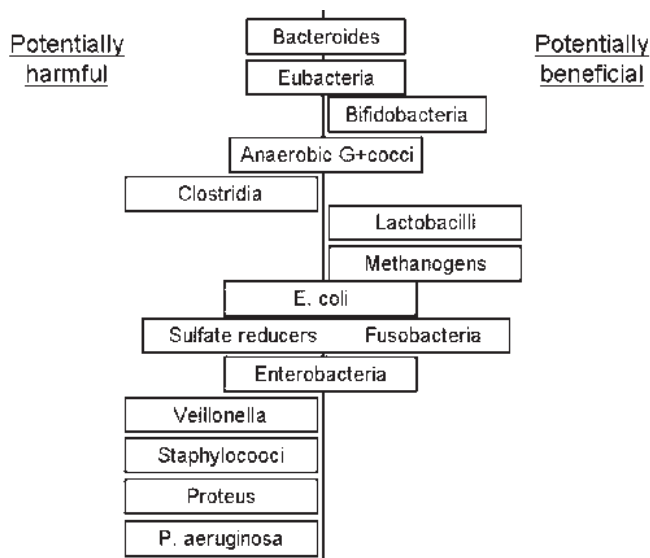


FIGURE 2. Potentially harmful and potentially beneficial bacteria. *P.*, *Pseudomonas*; *E.*, *Escherichia*.

What constitutes the normal flora?

The normal flora are an exceedingly complex equilibrium between the microorganisms that normally reside in the gastrointestinal tract, which play an important role in nutrition, physiology, and the regulation of the host's immune system. The equilibrium of the normal flora is now believed to vary from person to person. Although it is easy to identify the genus of bacteria in the normal flora and to compare bacterial populations between persons, it is very difficult to identify and thus to compare the populations of species or strains within the species.

Additional research using specific probes to identify the species, and possibly the strain, of bacteria is required to further elucidate the complex ecosystem that has such a vital role to play in humans. The species that make up the intestinal flora do not all have the same effects on the host's physiology, and the optimum effect is very closely linked to the qualitative and quantitative composition, in other words, the equilibrium. If the equilibrium is good, the host enjoys optimum conditions. If the equilibrium is disturbed, the conditions will be disturbed as well (5).

How do the bacteria proliferate?

A bacterium requires energy-giving nutrients to grow and reproduce. What nutrients does a bacterium find in the various portions of the gastrointestinal tract? Clearly, bacteria do not multiply in the stomach and multiply to only a small extent in the small intestine, mainly because of the short transit time and physiologic barriers.

When bacteria reach the colon, they have access to the nutrients they require to proliferate. The nutrients include all the food eaten but not absorbed by the host that has crossed the ileocecal valve (fiber and indigestible sugars), materials from the host (mucus and dead cells), and metabolites coming from bacterial enzyme activity—particularly carbohydrate digestion. The contact between bacteria and food in the colon causes fermentation, which produces numerous metabolites, which vary depending on the substrates available.

If the substrate is a carbohydrate, local production of short-chain fatty acids will occur, mainly giving rise to acetic, propionic, and butyric acids, which are responsible for physiologic effects in the host. In particular, the acids affect colonic metabolism, the hepatic regulation of lipids and sugars, and the supply of energy to cells (eg, butyric acid is an energy substrate for colonocytes) (6). Other beneficial effects include lipid hydrolysis, protein breakdown (which produces peptides and amino acids), and vitamin production. However, the fact that proliferating bacteria can also induce adverse effects should not be overlooked. Such effects include the production of potentially carcinogenic substances and toxic metabolites and the inactivation of certain drugs (7).

Are the intestinal microflora necessary for the host?

No, they are not. Germ-free animals exist and survive without any bacterial contamination; however, humans do not live in a sterile world. The presence of an appropriate intestinal microbial flora in the human body plays a very important protective role. Moreover, the comparison of germ-free animals with healthy animals shows significant differences that are related to the presence of the microflora. In germ-free animals, the cecum is markedly distended and the mucosa is much thinner. When such animals are administered normal flora, their condition and the anatomy of the cecum return to normal (8, 9).

Histologic findings show that the mitotic index and cell turnover rate are significantly lower in animals devoid of flora. In

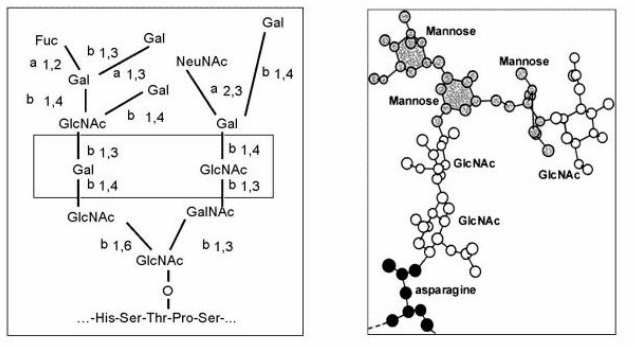


FIGURE 3. Simplified structures of glycoconjugates (M Freitas, PhD Thesis, 2001). GlcNAc, *N*-acetylglucosamine; NeuNAc, *N*-acetylneuraminic acid.

addition, intestinal transit times are prolonged when the microflora are absent. Moreover, anaerobic bacteria in the flora produce short-chain fatty acids, particularly butyric acid, which is used as an energy substrate by colonocytes (8).

How is the intestinal tract of newborn babies colonized and what mechanisms are involved?

Infants are born with a practically sterile gut, which is quickly colonized. The predominant source for initial colonization is maternal flora, followed by environmental flora. During the first 12–24 h of extrauterine life, the first colonizing bacteria appear: *Escherichia coli* and *Enterococcus* sp. Subsequently, obligate anaerobes appear very rapidly. The latter mainly consist of *Bifidobacterium* sp., together with some *Bacteroides* sp. if the baby is breastfed. In babies fed formula milk, the opposite occurs: *Bacteroides* sp. predominate and are accompanied by some *Bifidobacterium* sp. The infantile flora evolve toward a normal adult flora over the first 24 mo of life, but the time course depends on the infant's diet (9, 10).

How can colonization be explained?

The best way to proceed is to analyze what happens when bacteria come into contact with the host's tissues. This is undoubtedly the first type of interaction or communication between the various partners in the intestine.

A protective layer of mucus is found on the surface of all the body's mucous membranes. Mucus is continuously produced by cells. There are 2 types of mucus in the intestine: 1) an insoluble gel, which adheres strongly to the cells; and 2) a viscous layer, which is soluble in water, and covers the gel.

Mucus is made up of mucins, ie, native glycoproteins whose structure forms the gel. The latter traps bacteria by using a specific mechanism or simply because of its nature. Carbohydrates constitute the polysaccharide components of mucins. Only 5 different types of carbohydrate are involved: galactose, fucose, *N*-acetylglucosamine, *N*-acetylgalactosamine, and sialic acids. However, these carbohydrates combine to form a large number of different structures by linking in various ways. The polysaccharide structures come into direct contact with the bacteria and are now known to play an important role in cell-cell recognition processes (11).

Two simplified mucin structures are shown in **Figure 3**. The structure of a glycoconjugate bound to the protein core by an oxygen

bond and various configurations is shown in the left panel; the box contains a potential adhesion site for certain species. This type of structure is typical of free mucins. The panel on the right shows another type of glycoconjugate that occurs on the surface of the cell membrane. In this type of conjugate, the bond to the protein core is a nitrogen bond and another sugar—mannose—is also present.

The intestinal lumen is literally lined with carbohydrates. Thus, there is an incredibly wide repertoire of potential adhesion sites for bacteria with extra surface structures called adhesins when they come into contact with them. This repertoire of adhesion sites is genetically controlled by the host; therefore, the host's genome controls the behavior of the intestinal microflora, principally bacterial adhesion.

Bacterial adhesion occurs first and foremost at the mucin sites that act as soluble or insoluble layers. Under normal conditions, adhesion to cells does not occur. This has been confirmed by a whole series of experiments that show that the normal flora always remain on the surface of the mucus, at the entrances of the villi, but never inside the crypts.

Is there a regulatory mechanism for the repertoire of adhesion sites?

Initially, in neonates, the repertoire is genetically coded and is thus an "innate repertoire." However, as floral colonization of the gastrointestinal tract progresses, partial or total deterioration of the structures occurs over time, under the action of bacterial glycosidases (12), until the adult repertoire emerges. Thus, the first bacteria to colonize the neonate's gastrointestinal tract depends on the innate repertoire, which varies depending on the individual person's genome. However, if the initial bacteria are endowed with glycolytic potential, the repertoire evolves and new species bind to the mucins as new sites appear. For that reason, every person has his or her own intestinal flora.

Another regulation process, cross-talk (ie, dialogue between the bacteria and host), was also recently demonstrated. Hooper and Gordon (13) showed that resident bacterial flora send messages to host cells. The messages interfere with the expression or activity of cell glycosyltransferases, which induces changes in the carbohydrate repertoire of mucins. If the new sites allow nonpathogenic bacteria to adhere, the effect may be considered beneficial for the host. In contrast, the effect will be detrimental if enteropathogenic bacteria can adhere to the sites.

There are still many unknowns in this area, and a considerable amount of microbiological, cellular, and molecular biological research remains to be done. As a result, a new discipline emerged a few years ago—cellular microbiology. Many key issues have yet to be resolved: the nature of the signal, the mechanism of action, the number of bacteria needed to establish cross-talk, and the influence of other bacteria and secretions.

Behavior of bacteria within the ecosystem

Given the number of bacteria present in the colon and the existence of dominant and subdominant bacteria, it is obvious that interactions must occur within the bacterial community. Much less is known about the interactions occurring during communication between bacteria; however, some evidence is available. The ability of a bacterium to communicate with its environment and, in particular, other bacteria is known as "quorum sensing" (14). This ability could be beneficial for the bacteria that produce the signal and could have important consequences concerning, for example, the competition for nutrients or the production of antibiotic molecules.

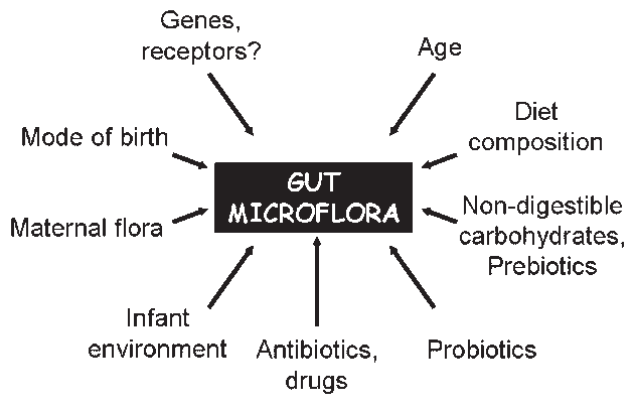


FIGURE 4. Factors influencing colonization.

Communication is based on the production of a signal molecule by certain species. The signal molecule is released into the environment. The more the bacteria proliferate, the greater the number of signals released, up to a threshold. If the signal molecule targets a gene regulation system in the receptive bacteria and if the cofactors are active, then the regulation system responds by activating, repressing, or derepressing a gene (14).

Different types of signals have been described and there are several types of molecules involved in bacterial signaling. The most extensively characterized molecule that occurs in gram-negative bacteria is *N*-acylhomoserine lactone. Numerous other examples exist, such as the pheromone peptides found in gram-positive bacteria or the proteins produced by genes belonging to the *Lux S* family, which appear to be produced by both gram-positive and gram-negative bacteria (14). Currently, it seems perfectly possible for a given bacterium to use several signal molecules belonging to the same or different chemical classes to communicate with other bacteria.

Functions contributing to the body's defense mechanisms

It is now well established that one of the essential functions of the colonic microflora is its ability to resist colonization by any new strain of bacteria from the exterior. This applies to both pathogenic and nonpathogenic bacteria. Given the complexity of the intestinal ecosystem, the mechanisms have yet to be fully elucidated. Several mechanisms appear to be involved and to act separately, sequentially, or together (15) and include 1) exhaustion or competition for the same substrate; 2) competition for mucin adhesion receptor sites; 3) production of a physiologically restrictive environment, for instance, with respect to pH, redox potential, hydrogen sulfide production, or production of metabolites toxic to other bacteria; 4) *in vivo* production of antibiotic substances such as bacteriocins, provided that they are not destroyed by intestinal secretions; and 5) production of a signal molecule that acts on the genes encoding for survival. This mechanism has yet to be investigated but has considerable potential given the research conducted on quorum sensing and on sequencing bacterial genomes.

Factors influencing colonization

A vast number of factors influence colonization. Only a few of these factors will be addressed (Figure 4). First, the effects of

breastfeeding and feeding infants with formula milk on the composition of the infantile flora are to be noted (16). It is now a firmly established fact that the flora of breastfed neonates, unlike the flora of infants who are being fed formula milk, are quickly dominated by bifidobacteria, in contrast with that of infants fed formula milk. In breastfed infants, the flora are not only much richer in bifidobacteria but they also include far fewer species liable to be pathogenic.

An age-related effect can be observed. The composition of the flora evolves over time, depending on the diet that the infants receive, until it resembles the flora of adults. As the body ages, other significant changes also occur. In particular, there is a considerable increase in the proportion of *Bacteroides* sp. and a decrease in lactic acid bacteria. Some authors maintain that this modification could, in part, be due to a partial or total loss of the gastric acid barrier (5).

The effect of diet on the intestinal flora is much more difficult to study in humans than in feces. However, studies of ileostomy effluents have shown that an increase in dietary fiber induces an increase in a whole range of bacterial species. An increase in fat induces an increase in nonsporulating anaerobic bacteria and a decrease in lactic acid bacteria. An increase in protein induces an increase in lactic acid bacteria (17, 18).

The most spectacular effects of diet on flora are obtained by using substrates that are specific to a certain genus or species. For instance, if fructooligosaccharides, which are specifically used by *Bifidus* bacteria, are added to the diet, there is a very marked increase in the concentrations of *Bifidobacterium* and a corresponding decrease in *Bacteroides* (19). Such substrates, also known as *prebiotics*, thus modify the composition of the intestinal flora.

Antibiotic intake is associated with impairment of the flora, of variable severity, often resulting in loose stools or diarrhea. Probiotic intake also influences the flora, which is considered in more detail below.

WHAT ROLE DOES THE MUCOSAL BARRIER PLAY IN THE BODY'S DEFENSES?

The intestinal mucosa has a very large surface area (> 300 m²), which constitutes the main interface with the external environment of humans. This interface is perfectly adapted to the primary function of the intestine—the digestion of food and the absorption of nutrients. However, the intestinal mucosa is also a major site of interaction with foreign substances and microorganisms from the external environment. A large variety of bacterial populations, including potentially pathogenic microorganisms and their products, and a marked concentration of endogenous and exogenous toxins (eg, bile acid conjugates and genotoxic compounds) constantly challenge the structure and function of the mucosal surface, particularly in the colon, because, as already indicated, the slow motility patterns of that organ allow bacterial proliferation to high densities and intense lumen-mucosal interactions.

What is the mucosal barrier?

The mucosal barrier is a complex physicochemical structure that separates the tissues from the luminal environment. Physically, the barrier consists of cellular and stromal components—from the vascular endothelium to the epithelial cell lining—and the mucous layer, a gel formed by the interaction of various mucosal secretions, including mucin glycoproteins, trefoil peptides, and surfactant phospholipids (20–22).

Mucosal barrier function ultimately depends on the physical integrity of the mucosa and the reactivity of dynamic defensive factors, such as mucosal blood flow, mucosal secretions, and epithelial cell functionals (eg, closure of tight junctions, cell turnover, recognition, and processing of foreign compounds).

Goblet cells secrete a combination of trefoil peptides and mucin glycoproteins to form a continuous gel on the mucosal surface (20, 21). The importance of mucin glycoproteins, as indicated above, has long been recognized, whereas trefoil peptides are a family of peptides described in recent years. Characteristically, these peptides exhibit a trefoil domain that renders them resistant to digestion by proteases. They are among the most abundant products of the gastrointestinal mucosa and, in conjunction with mucins, confer the viscoelastic properties of the gel.

The mucus layer constitutes a physical separation between the lumen and epithelium and an important framework for host-bacteria and bacteria-bacteria interactions, as previously indicated. In addition, a strong hydrophobic surface of the mucus layer prevents the influx of water-soluble toxins into the epithelium (23).

Surface hydrophobicity is due to a layer of surfactant lipids that are secreted by epithelial cells and coat the top of the mucous gel (22). In several mammalian species, including humans, the surface hydrophobicity is very marked for the gastric and colonic mucosa, whereas it is much less marked throughout the small intestine, an absorptive surface. The hydrophobicity and thickness of the mucus increases from the proximal to the distal part of the colon (22, 23). Ample experimental evidence shows that the mucous layer is an important defense mechanism, both protecting the mucosa from injury and facilitating lesion repair (20–23).

What are defensins?

Another important defense mechanism in the mucosal barrier consists of the production of endogenous antimicrobial peptides, known as defensins. These peptides are secreted into the lumen by a specialized cell type, Paneth cells (24, 25). Paneth cells develop early after birth, conferring innate resistance to environmental and pathogenic microorganisms on neonates. Considerable quantities of these cells are present in the small intestine, where the number of bacteria is low compared with the number in the colon. Paneth cells are found at the bases of the intestinal crypts.

Paneth cells originate from intestinal stem cells located at the interfaces between the villi and crypts and, unlike enterocytes and goblet cells (which migrate toward the top of the villi), Paneth cells migrate downward to the bottom of the crypt. On average, 5–15 Paneth cells are present in the base of each crypt (Figure 5).

Paneth cells produce and store several antimicrobial peptides in apical cytoplasmic granules. The peptides act synergistically and have specialized activities against different microorganisms. Paneth cells also contain lysozymes, phospholipase-A₂, DNAses, and trypsin (24, 25).

Secretion may be stimulated by gram-negative and gram-positive bacteria and by bacterial products (eg, lipopolysaccharide, lipoteichoic acid, and muramyl dipeptide) (26). Live fungi and protozoa, however, do not stimulate the degranulation of Paneth cells. In addition, other stimulatory pathways have been described and include cholecystokinin, cholinergic agonists, and cytokines such as tumor necrosis factor α and interferon α . Propeptides are released into the gland lumen and are activated by the metalloproteinase matrilysin.

Paneth cells have a broad-action spectrum and exert effective antimicrobial activity vis-à-vis both gram-positive and gram

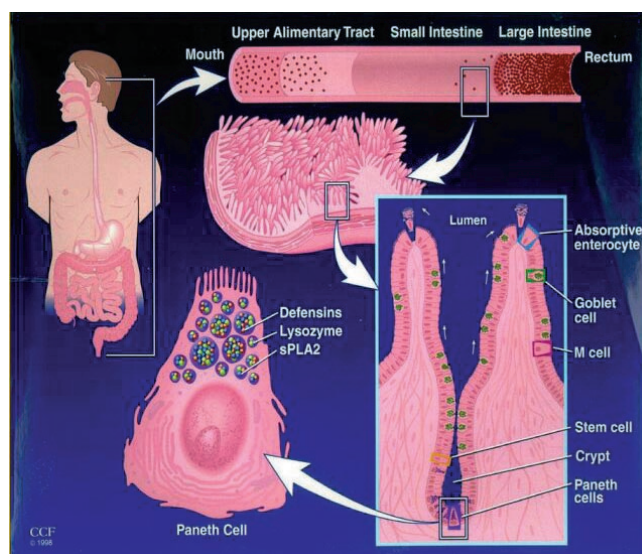


FIGURE 5. Mucosal immunity in the small intestine. Reprinted with permission from reference 26.

negative-bacteria. All the defensive mechanisms that contribute to the normal function of the mucosal barrier are components of a person's innate immune system and are nonspecific innate defensive mechanisms. However, the immune system also includes specific defensive mechanisms, ie, mechanisms directed against a particular agent. Specific immune mechanisms may be innate or acquired. In the latter case, they involve memory of previous exposure to the same agent. Memory mechanisms require the synergistic activity of the specialized cells that constitute the immune system.

WHAT ROLE DOES THE INTESTINAL IMMUNE SYSTEM PLAY IN THE BODY'S DEFENSES?

The intestine is the primary immune organ in the body because it contains $\approx 60\%$ of the total immunoglobulins and $> 10^6$ lymphocytes/g tissue (4). GALT contains the largest pool of immunocompetent cells in the human body. Experiments that compared specific germ-free and normal mice and rats have shown the strong influence of the presence of intestinal flora on the maturation and development of local and systemic immunity and on the regulation of immune functions (27–29).

The gut has a very complex mucosal immune system, which makes it possible to tolerate the massive load of dietary antigens and the commensal microorganisms that colonize the gastrointestinal tract. At the same time, however, the mucosal immune system is able to recognize and reject enteropathogenic microorganisms that may challenge the body's defenses.

What are the constituents of GALT?

GALT is divided into 2 areas (30–32): organized and diffuse lymphoid tissues. Peyer's patches (aggregate glands) and the mesenteric lymph nodes provide an appropriate microenvironment for the induction of intestinal immune responses. Peyer's patches are more permeable to microorganisms than are other epithelial areas of the gastrointestinal tract, because they have a lower number of mucus-producing cells. Specialized transport cells, called M cells, and dendritic cells (macrophage type) are found in the

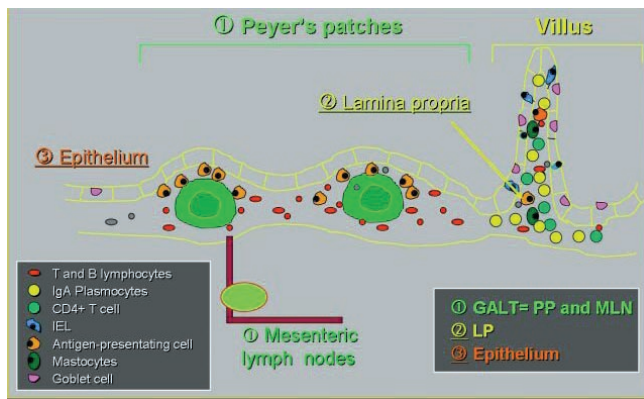


FIGURE 6. Intestinal immune system. IgA, immunoglobulin A.

epithelial layer of the patches. These cells can phagocytose soluble antigens and microorganisms that are presented to immunocompetent cells behind the surface epithelial layer. Organized lymphoid tissues are thus crucial for antigen sampling and recognition to induce specific immune responses.

Diffuse lymphoid tissues are composed of immunoglobulin A (IgA)–producing plasma cells and mature T cells. Effector cells are scattered through the intestinal mucosa. T lymphocyte subsets are not homogeneous but are classified as CD4⁺ (helper or inducer) and CD8⁺ (suppressor or cytotoxic) cells on the basis of their distinct functions. Most of the intraepithelial T cells have the CD8⁺ phenotype; however, cells of the lamina propria have a CD4⁺ phenotype.

The lamina propria also contain lymphocytes of the B cell line (memory cells and plasma cells), among which 70–90% produce type A immunoglobulins. Type A immunoglobulins are secreted into the intestinal lumen, where they resist proteolysis by digestive enzymes and play an important defensive role by blocking potentially offending organisms. The function mediated by IgA antibodies is called immune exclusion. Secretory antibodies prevent epithelial colonization by pathogens and inhibit penetration of harmful foreign material (30). Most bacteria in human feces are coated with specific IgA units. The inducer and effector sites are linked by blood and lymph vessels (29) (**Figure 6**).

What is the relation between innate and acquired immunity?

The immune response to microorganisms relies on both acquired components of immunity, which require memory of previous exposure, and innate mechanisms. Innate responses are mediated by white blood cells (eg, neutrophils and macrophages) and by intestinal epithelial cells. White blood cells can phagocytose and kill pathogens. Epithelial cells coordinate host responses. Intestinal epithelial cells can synthesize a wide range of inflammatory mediators and transmit signals to underlying cells in the mucosa.

The innate immune system has to discriminate between potential pathogens and commensal bacteria with the use of a restricted number of preformed receptors. Mammalian cells express a series of toll-like receptors that recognize motifs conserved by bacteria but that are not found in higher eukaryotes (33).

The system allows immediate recognition of bacteria to rapidly respond to any challenge. Immediate protection is conferred by activation of the innate immune cells (macrophages, dendritic cells, polymorphonuclear cells, and epithelial cells) via different

toll-like receptors that recognize critical molecules on the bacterial surface. This effect has been shown to involve the nuclear transcription factor κ B, which binds to specific promoter sequences and activates the transcription and expression of a series of genes that encode proinflammatory cytokines and inducible proinflammatory enzymes (34).

Activation of the nuclear transcription factor κ B is induced by transmembrane signals generated by toll-like receptors on the outer cell surface. The inflammatory response includes the release of soluble mediators with potent chemoattractant activity for white blood cells (particularly tumor necrosis factor α and interleukin 8) but also the generation of large amounts of nitric oxide, which has potent antimicrobial activity. Pathogens with enteroinvasive abilities trigger full responses.

However, different bacteria may induce different responses. A recent study showed that nonpathogenic bacteria also elicit different types of cytokine responses from epithelial cells, which are transduced to the underlying tissue and promote changes in the phenotype of lamina propria lymphocytes (35).

The complex interaction between the innate and acquired immune mechanisms protects the host against pathogens, which preserves intestinal mucosal structure and function and avoids entry and systemic spread of challenging agents. Precise regulatory mechanisms ensure that no inappropriate inflammatory reactions emerge and that nonpathogenic bacteria are tolerated at local and systemic levels (36, 37).

What mechanisms are triggered when an antigen is ingested orally?

Stimulation of an immune response and rejection of the antigen occurs via the following mechanisms:

- 1) capture of intraluminal antigens, preferentially via the epithelium of Peyer's patches (M cells) and presentation to specialized cells in Peyer's patches and to the mesenteric lymph nodes;
- 2) circulation of the sensitized B- and T blast cells via a hemolymphatic cycle that releases mature specific IgA, plasma cells, and T cells throughout the gastrointestinal system;
- 3) involvement of IgA, which is active against the enteropathic microorganisms after specific transport through the epithelium by transcytosis; and
- 4) involvement of mature intestinal T cells that are active against enteropathogenic microorganisms, either in the lamina propria, via cytokine secretion by CD4⁺ T cells and via macrophage, polymorphonuclear cell, and mast cell recruitment or in the epithelium via an unusual subassembly of T cells that carry receptors conferring innate and acquired immune-ability functions. Alternatively, an induction of an active response to tolerance may occur by activating regulatory T cell subsets that maintain tolerance toward dietary antigens and endogenous microflora. This process may be impaired in allergy and inflammatory bowel disease.

WHAT ROLES CAN TRANSIT PROBIOTIC BACTERIA PLAY IN THE BODY'S DEFENSES?

What is a probiotic?

The definition of *probiotic* has changed over time (38), but there appears to be a consensus on the definition recently approved by the National Yoghurt Association and the International Life

Science Institute in the United States: “Probiotics are living micro-organisms which, upon ingestion in sufficient numbers, exert health benefits beyond basic nutrition.” Thus, a probiotic food is a food that contains live probiotics in a sufficient quantity until the end of the product’s shelf life and that exerts health benefits to its host. The most commonly used and researched species belongs to the genera *Lactobacillus*, *Bifidobacterium*, and *Saccharomyces*, particularly *L. casei*, *L. rhamnosus*, *L. acidophilus*, *L. plantarum*, *B. longum*, *B. breve*, *B. bifidum*, and *S. cerevisiae boulardii*.

Probiotics and immunity: some important questions

Although there is an immense amount of literature on the subject of probiotics and immunity, a clear picture has not yet fully emerged because of the diversity of microorganisms and the experimental models used but also because of the type of immune response studied (intestinal or systemic) in the body fluids or the cells—innate or acquired. The significance of the results varies widely. Moreover, with fermented milk products, the effects are known to depend on the strain used, whereas the metabolites produced by the fermentation process may also exert immunomodulatory activity, which further complicates the research.

Probiotics remain in transit in the gastrointestinal system for a variable length of time, and it is common knowledge that the probiotic effect will be more likely to occur if the bacteria remain alive for as long as possible and are present in a sufficiently large quantity. To achieve and maintain an effect, the probiotic must be repeatedly administered to ensure a sufficient population level over time.

Effect of normal flora on the induction of immunity

As already indicated, the equilibrium of the intestinal flora is an important factor in maintaining the host’s good health. This is particularly true for neonates whose immune system must become functional as soon as possible to prevent food allergies and to ensure defense against enteropathogenic microorganisms such as *Rotavirus*, to which infants are particularly sensitive.

Moreover, the incidence of food allergies and enteropathies is highest between the ages of 0 and 2 y, a time during which the intestinal flora are fragile and not well diversified and, thus, are readily subject to disturbances and imbalances. Under such circumstances, living microorganisms ingested with certain foods, such as probiotics, exert beneficial effects. Probiotics have a palliative effect in settings where the resident flora are insufficient but only if present in sufficient quantities in the diet ($\geq 10^8$ cells/g) and if the microorganisms have immunomodulatory properties that are equivalent to those of the resident intestinal bacteria (39–41).

Circumstances in which probiotics can have an effect in healthy humans

The flora of infants are very different from those of adults. Qualitatively, few species are present in the flora of infants; therefore, infants are considered to be “immunocompromised” and are more likely to develop gastrointestinal infections and food allergies. The quality of the resident intestinal flora has a crucial effect on the intestinal immune system. The presence of *Bifidobacterium* sp. in the fecal flora of breastfed children is associated with strong stimulation of the antirotavirus IgA response compared with what is observed in formula-fed children. It is fully possible that the immunostimulant effect of ingesting probiotics may occur concomitantly with the emergence of resident bacteria, procuring the same effect (4).

In healthy adults, the complex flora normally induce an immunostimulant effect on all the host’s immune functions and thus play a part in maintaining homeostasis. The stability of the flora in adults is greater than that observed in children, but little is known about the influence of lifestyle factors, such as stress, drug treatment, or even changes in diet, on that equilibrium. To date, research has only addressed antibiotic treatment. The studies show that antibiotic treatment has a considerable effect on the equilibrium of the intestinal flora. Regular doses of probiotics could alleviate the detrimental effect by constantly supplementing the resident intestinal flora, thus procuring a beneficial effect on the immune system and maintaining the patient’s well-being. Even though this idea is empirical at present, it should not be dismissed because repeated modifications of the intestinal bacterial equilibrium can impair homeostasis with, as a consequence, immune imbalance and the emergence of infectious inflammatory or allergic diseases (42).

With regard to the elderly, limited data on the relations between intestinal flora and immunity during the aging process are available. The intestinal bacterial equilibrium seems to undergo modifications, ie, a reduction or change in the resident *Bifidobacterium* species and a parallel decline in the immune response. However, few studies of the effects of probiotics in the elderly are currently available.

Immunomodulatory effects of probiotics

Children

There is a consensus that certain probiotics [eg, *L. casei rhamnosus* (43), *L. casei paracasei* (44), *L. reuteri* (45), *B. bifidum*, and *Streptococcus thermophilus* (46)] reduce the duration and severity of rotavirus-related diarrhea. Studies have shown the stimulation of antirotavirus IgA antibodies (43). A protective role against bacterial diarrhea has been less well established. An effect has been observed in some forms of persistent diarrhea and withdrawal diarrhea, but the etiology of these types of diarrhea has not been determined (47).

Adults

Most of the research in adults addressed traveler’s diarrhea and diarrhea related to antibiotic treatment, in which *Clostridium difficile* is sometimes involved (47–49). The effects on the prevention of antibiotic-associated diarrhea are consistent, and a recent meta-analysis of clinical studies concludes that most probiotics can be used for this indication both in children and adults (50). However, only *Saccharomyces boulardii* and, to a lesser degree, *L. casei rhamnosus* (GG) have been shown to protect against *C. difficile*. The effects on other gastrointestinal infections, *Helicobacter pylori* in the stomach and *Cryptosporidium* in AIDS patients, are currently under investigation. The results are contradictory but worth following up.

Effects on food allergies

The results of some clinical trials have shown a beneficial effect of *L. casei rhamnosus* (GG) intake in cases of allergy to cow-milk proteins. In addition, probiotic therapy has also proved useful in the prevention and treatment of atopic dermatitis. Early administration of the probiotic under study prevented disease emergence by restoring the equilibrium of the intestinal flora (51–55).

Effects on inflammatory bowel diseases

Several large trials are currently investigating the effect of certain probiotics (*L. plantarum* 299v, a VSL-3 preparation containing 8 strains of lactic acid bacteria) in inflammatory diseases of

the digestive tract (eg, Crohn disease and ulcerative colitis), even though the etiologies of these diseases have yet to be fully elucidated (56, 57).

Conclusions

Although the immunomodulatory effects of certain strains of probiotics have been clearly shown, it is important to not confuse a probiotic with a drug. Probiotics are used mainly for prevention, whereas drugs are used to cure disease. A probiotic may or may not affect a healthy person's immune system, but these effects are often undetectable with our current markers of mucosal immune function. To determine the "health-giving" activity of a probiotic, it is usually tested in the setting of disease prevention, so that the extent to which the probiotic under study is able to prevent the emergence of the disease and the circumstances under which it exerts that action can be assessed. In summary, a probiotic should enable optimization of immune functions when this is not being achieved by the resident flora, rather than needlessly overstimulating those functions.

OVERALL CONCLUSIONS

The gastrointestinal tract is a highly complex ecosystem that science has learned to view in a new light. The gastrointestinal tract is not an organ whose only function is to digest food and absorb nutrients. Rather, it is an intelligent organ that allows dialogue between bacteria and between bacteria and their host and is a formidable barrier to pathogens because of its 3 lines of defense: the intestinal microflora, intestinal mucosa, and intestinal immune system.

However, despite the recent excellent results obtained with probiotics in certain circumstances (eg, lactose digestion, immune stimulation, rotavirus diarrhea, and antibiotic-associated diarrhea) and based on basic research on the gastrointestinal tract, the elucidation of its vast functional complexity, still needs to be implemented. Two ways of research are identified: basic research on one hand and applied research on the other hand. Regarding basic research, numerous issues have still to be addressed to elucidate the mechanisms of the amazing ecosystem. They concern the flora, the host, and the diet.

Research on flora consists of


- 1) knowing the molecular characteristics of the species of intestinal microflora better,
- 2) evaluating the behavior of flora toward the host (adhesive and nonadhesive species),
- 3) determining the in vivo metabolic activity of flora,
- 4) characterizing the signaling mechanisms of flora, and
- 5) determining the flora's useful and harmful antigens.

Research on the host could focus on

- 1) the study of the effect of the genotype and gene expression in the intestinal tract environment,
- 2) the cross-talk study with microflora,
- 3) homeostasis maintenance, and
- 4) the tolerability after oral administration.

Research on diet could focus on its effects, particularly the effects of indigestible carbohydrates and other factors that contribute to the impairment of gastrointestinal tract functions.

Considerable applied research is required to further elucidate the role of the ecosystem in the body's defenses and the role and additional effects of probiotic-type bacteria during gastrointestinal transit. Research is required to

- 1) develop and validate experimental models in animals and in vitro models;
- 2) characterize and validate markers of physiological effects, such as barrier function, inflammatory and immune responses, and mucosal integrity;
- 3) define the specific characteristics of the strains to be selected on the basis of the required results (eg, production of enzymes, production of antiinflammatory and immunologic mediators, and regulation of the host's genes) and potential targets (eg, children, the elderly, pregnant women, and immunocompromised patients);
- 4) determine the assessment variables (eg, live strains, associated strains, survival in the gastrointestinal tract, and interactions with other bacteria and with the host) in view of the projected effects;
- 5) further elucidate the mechanisms of action of probiotics and their conditions of use, particularly the dose-effect relation; and
- 6) develop clinical trials to study the effects of probiotics on health in general and on well-being and to study the role of probiotics in the prevention of food allergies in infants, of relapse in inflammatory bowel diseases, of some neoplastic diseases, and of *H. pylori*-related diseases. 

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