

# Nutrition and Intestinal Microflora

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**Abstract:** The intestinal microflora is a complex ecosystem composed of numerous genera, species and strains of bacteria. This enormous cell mass performs a variety of unique activities that affect both the colonic and systemic physiology. The gut is colonized by a small number of bacterial species; *Lactobacillus* and *Bifidobacteria* spp. are seldom, if ever, identified. The predominant species are *Enterococcus faecalis*, *E. coli*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Staphylococcus epidermidis* and *Staphylococcus haemolyticus*. Hygienic conditions and antimicrobial procedures strongly influence the intestinal colonization pattern. But, when large numbers of bacteria colonize the small intestine, a syndrome known as small intestinal bacterial overgrowth (SIBO) occurs. Nutrient malabsorption is a hallmark of the disorder and can result in a multitude of problems for the host. New links between SIBO and disease entities such as irritable bowel syndrome (IBS), provide intriguing new insights into the pathophysiology of the syndrome. On the other hand, in addition to its role in digestion of food in the gastrointestinal tract, intestinal microflora are also capable of biotransforming numerous drugs. Likewise, intestinal microflora may significantly modulate xenobiotic-induced toxicity by either metabolically activating or inactivating xenobiotics. We herewith present a review of the research on the importance of intestinal microflora and nutrition. Probiotics can introduce missing microbial components with known beneficial functions to the human host. Prebiotics can enhance the proliferation of beneficial microorganisms or probiotics, to maximize sustainable changes in the human microbiome. In addition, among the numerous purported health benefits attributed to probiotic bacteria, their capacity to interact with the host's immune system is now supported by an increasing number of experiments. In addition to these, a few trials aimed at preventing chronic immune dysregulation have been reported. The identification of major immunomodulatory compounds in probiotics, and their interaction with immunocompetent cells as well as the role of secretory IgA in gut homeostasis are also evoked.

**Keywords:** Intestinal microflora, *Bacteroides*, *Bifidobacterium*, probiotic, prebiotic.

## 1. INTRODUCTION

Although most have yet to be identified, more than 1000 species of bacteria exist in the human intestine, a nutrient-rich environment packed with approximately  $10^{14}$  microorganisms and whose accumulated genomes may have 100 times more genes than the human genome. In 2007, the National Institutes of Health (NIH) initiated the Human Microbiome Project (HMP) as one of its Roadmap initiatives, to provide resources and build the required research infrastructure. To date, 239 genomes including 61 genomes at various stages of upgrading have been described by the Jumpstart Consortium and released into public databases. At the time of manuscript preparation, 178 had been completely annotated. One hundred fifty-one strains sequenced to date are distributed in the gastrointestinal tract [1].

In this perspective, gut microbiota can be viewed as an organ that regulates its host's metabolic and immune systems. Although definitive proof remains to be provided, growing evidence indicates that

gastrointestinal (GI) microbiota play a crucial role in the progress of human disease, particularly in the metabolic syndrome (MS), characterized by obesity, diabetes and hypertension [2]. On the other hand, some of these microorganisms are able to metabolize xenobiotics. More than 99% of the potentially culturable fecal microbiota is represented by 30–40 bacterial species [3].

This ecosystem is composed predominantly of bacteria belonging to the *Bacteroidetes* (mainly *Bacteroides*), *Firmicutes* (mainly *Eubacterium*) and *Actinobacteria* (mainly *Bifidobacterium*) phyla and it is estimated that the majority of species comprising this community (~80%) remain uncultured [4, 5]. Little is known regarding the pool of mobile genetic elements associated with the human gut's internal ecosystem (gut microbiome). Some plasmids or plasmid families are present in the gut microbiomes of geographically isolated human hosts, with a broad global distribution (America, Japan and Europe), and are potentially unique to the human gut microbiome. Further investigation of the plasmid population associated with the human gut is likely to provide important insights into the development, function and evolution of the human gut microbiota [6].

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There has been an increased interest in the study of the intestinal microbial balance, which is enforced by increasing species diversity and the role of some bacteria in resistance to disease. Normal microflora represents the first line of defense against several potential pathogens by direct interference with the settlement of newly arrived species, and due to its close contact and balance with the host's defense systems [7].

Since the beginning of the last century, efforts have been devoted to describe bacterial succession in the gut of newborns by means of microbiological analysis of the infants' stools. Plate counts on selective media have yielded what is believed to be solid knowledge on the formation of this ecosystem [8]; however, in the last few years, a number of articles have reported results on neonates' microflora obtained by means of culture-independent analysis, such as fluorescent in situ hybridization (FISH) or denaturing gradient gel electrophoresis (DGGE), 16S RNA cloning and sequencing as well as real-time PCR.

New data on the accurate composition of infant microflora will allow improved nutritional strategies and a better understanding of host-bacterial relations. In addition, as a consequence of the availability of new diagnostic techniques, a revision of the actual composition of the microflora of breast-fed babies, which is believed to be the "golden standard", is probably mandatory [9].

Intestinal microbiota display an intense metabolic activity that serves to maintain symbiotic or commensal relationships with the host. In this context, the administration of selected bacteria (probiotics) and non-digestible carbohydrates that stimulate the growth of desirable bacterial groups (prebiotics) through foods (e.g. dairy products) or dietary supplements, constitutes an attractive alternative for modulation of the composition of the intestinal microflora and promotion of their beneficial metabolic functions [10].

Synbiotics are synergistic combinations of pro- and prebiotics [11].

The term "nutraceutical" was coined from "nutrition" and "pharmaceutical" in 1989 by Stephen DeFelice, MD, founder and chairman of the Foundation for Innovation in Medicine (FIM), Cranford, NJ. DeFelice proceeded to define nutraceutical as, "a food (or part of a food) that provides medical or health benefits, including the prevention and/or treatment of a disease" [12].

## 2. ROLE OF THE INTESTINAL MICROFLORA

### Colonic Microflora

In healthy humans, intestinal microorganisms are mostly confined to the distal ileum and the colon. It is believed that 50 genera of bacteria reside in the colon, comprising several hundred species. Microorganisms resident in the proximal colon tend to grow at a rapid rate, because they have a plentiful supply of dietary nutrients. In the distal colon, substrate availability is much lower, so bacteria grow more slowly. Growing evidence supports a role of colonic microflora and their fermentative end-products [short-chain fatty acids (SCFA)] in the mediation of host health and disease. Therefore, various methods to alter these bacteria and their resultant SCFA are being increasingly studied, including prebiotics [13].

During infancy, gastrointestinal tract colonization by normal flora is a gradual and slow process lasting several years and that translates into a stable autochthonous flora constituting a true intestinal ecosystem. The **first phase** is characterized by the initial acquisition of microorganisms. In utero, the fetal digestive tract is sterile until the rupture of amniotic membranes.

The stool of one-day old infants has yielded high levels of *Enterococcus* and moderate levels of *Staphylococcus*. No anaerobes such as *Bifidobacterium*, *Lactobacillus* or *Bacteroides* are found. On the second day of life, half of all studied infants were colonized by *Bifidobacterium* and by the third day, they were all colonized by this microorganism. *Bacteroides* microorganisms are also apparent at that point [14]. By the third day of life, microflora is composed of high levels of enterobacteria, *Enterococcus* and *Bifidobacterium*. *Bacteroides* species are abundant in more than half of the children [15].

In general, the mother's flora is established more firmly in the newborn than environmental flora. *E. coli* strains are transmitted vertically. The transmission of enterobacteria such as *Klebsiella*, *Enterobacter* and *Citrobacter* is rare due to the low prevalence of this flora in adults. Vaginal delivery is an important factor in intestinal colonization by *Lactobacillus* and *Bifidobacterium* [16]. Colonization of infants delivered by Cesarean section is influenced by the hospital's environmental microorganisms, where horizontal transmission of *E. coli* among newborns can occur through the nursing personnel's hands.

The **second phase** consists of the initial acquisition of microorganisms. In utero, the fetus' digestive tract is sterile until membrane rupture. During maternal lactation, certain characteristics of breast milk may influence colonization by intestinal flora. Oligosaccharides, including n-acetylglucosamine, glucose, galactose, fucose oligomers and certain glycoproteins act as growth factors for *Bifidobacterium*.

As of the sixth day of life, most infants are colonized by *Bifidobacterium*, the prevailing microorganism in a 1,000 to 1 ratio, when compared with enterobacteria [17]. *Bifidobacterium* is isolated in up to 84% of breast-fed infants. No significant differences have been observed in the anaerobe intestinal microflora between breast-fed infants and those fed artificial milks. This may be due to the milk formula's composition that is currently more similar to maternal milk.

The **third phase** begins approximately by six months of age, when complementary food is introduced. When breast-feeding is supplemented with solid foods, the intestinal microflora changes. *Enterococcus* and *Bacteroides* increase whereas enterobacteria and *Bifidobacterium* remain constant. The mean number of children colonized by *Lactobacillus* is high but the numbers of this microorganism found in the stools are low. All these changes in flora characteristics are closely related to an increase in fecal pH. Artificially fed children undergo minimal changes in their internal flora composition since they already have large amounts of aerobic bacteria and *Bacteroides* [16].

The **fourth phase** begins when the child is weaned or breast-feeding is discontinued and a more adult-oriented diet is introduced. Intestinal flora has been studied at 10 – 18 months of age and it is different to the adult's and characterized by the presence of more *Bifidobacterium* than *Bacteroides*, high levels of enterobacteria and *Enterococcus*, as well as *Lactobacillus* in a low percentage of children [18].

Although prebiotics have been broadly investigated in adults and in the elderly, relatively little work has been done to elucidate the effects of dietary components on the colonic microflora of infants. Previous studies indicate that breast-fed infants have a higher proportion of fecal bifidobacteria and a lower proportion of fecal pathogenic bacteria compared with those found in the feces of formula-fed infants [19].

It is likely that differences in SCFA production may be attributed at least in part, to differences in the

bacterial types present in the inoculum. An understanding of the effect of diet on intestinal bacterial populations and on the quantity and proportions of SCFA produced during fermentation, will assist in the formulation of infant foods that may optimize intestinal health and well-being [20]. Symbiotic microflora prevents contamination of the human intestine with foreign microflora; microbial enzymes split cellular tissue, proteins, fat, starch, deconjugated bile acids, synthesize B vitamins, amino acids, cholesterol and other substances. Microbial metabolism products--SCFA--stimulate intestinal motility and are useful and effective in some intestinal diseases. In the future, probiotics will be employed in the prevention and treatment of many human diseases. New probiotics and other forms of biopreparations should be designed based on various species of symbiotic intestinal microflora [21].

Human intestinal microbiota from children fed a modern western diet [European children (EU)] was investigated and compared with that of children on a rural diet [children from a rural African village in Burkina Faso (BF)]. Firmicutes were twice as abundant in the EU children, suggesting a dramatically different bacterial colonization of the human gut in the two populations. It was noteworthy that in BF, children were found to have a significantly higher amount of total SCFA compared with EU children. Gut microbial richness could have several health-related effects. The SCFA-producing bacteria that are abundant in the BF children's gut could possibly help to prevent the establishment of some potentially pathogenic intestinal microbes causing diarrhea, as suggested by the fact that *Shigella* and *Escherichia* were significantly underrepresented in BF when compared to EU children. Increased gut microbial diversity and reduced quantities of potentially pathogenic strains in BF would agree with the "old friend" hypothesis, indicating a role of microbiota in protecting children from pathogens as well as from gastrointestinal diseases, and underscoring the importance of preserving this treasure of microbial diversity from ancient rural communities worldwide [22].

In general, the intestinal microflora not only produces toxic or carcinogenic metabolites, but also forms metabolites not present in host tissues [23]. In addition, the intestinal microflora may either activate or inactivate drugs and/or toxins through metabolism.

Our understanding of intestinal microorganism-host interactions and their symbiosis is growing, but it is still

in its infancy. The role of bifidobacteria in intestinal microbiota is not known, but studies have suggested a likely beneficial role for the host. The role of bifidobacteria in controlling the levels of undesirable bacteria, such as clostridia and *Escherichia coli* in the intestine, is inferred from the reverse correlation of bifidobacterial numbers and the bacteria detected in clinical feeding studies [24]. This correlation is also evident in the intestinal microbial changes that occur as people grow older: specifically, the numbers of bifidobacteria decrease significantly while the numbers of other bacteria, including clostridia and *E. coli*, increase [25].

Successful microorganisms in any habitat, including the large intestine, must evolve efficient mechanisms for utilizing available nutrients. Most of the easily digestible simple sugars are generally absorbed or metabolized in the upper gut, and the host-indigestible complex carbohydrates (plant-derived dietary fibers, host-derived glycans, oligosaccharides, resistant starch, cellulose, hemicellulose, xylan, arabinofuran, arabinogalactan, pectins, and gums) and poorly metabolized polyols, are utilized by intestinal bacteria in the lower gut [26]. The sequenced bifidobacterial genomes contain genes predicted to encode many complex carbohydrate-metabolizing enzymes, consistent with the expected biology of these bacteria. While bifidobacteria do not encounter temperature stress in their normal intestinal environment, they are subjected to temperature stress when utilized in probiotic applications. All nine complete genome sequences of bifidobacteria carry several temperature stress-related gene analogs.

The microbial population of the human large intestine is limited primarily to host-indigestible nutrients, such as plant-derived complex carbohydrates and polyols. In this habitat, intestinal bifidobacteria are efficient metabolizers of these types of nutrients and a significant part of their genomes is dedicated to these tasks and underscore their adaptation to this habitat [27]. Mammals, including humans, rely primarily on milk for dietary purposes during the infant stage of life. The genomic sequence of *Bifidobacterium longum* revealed approximately 700 genes that are unique to infants, including a variety of co-regulated glycosidases relative to other Bifidobacteria, implying a co-evolution of human milk oligosaccharides and the genetic capability of select intestinal bacteria to utilize them [28]. Fecal culture studies of breast-fed human infants typically show a flora high in bifidobacteria [29].

On the other hand, it was recently discovered that the toxicological actions of many xenobiotics present in foods or medicinal plants, can be metabolically modulated by human intestinal microflora. These include arbutin, baicalin, geniposide and butyl paraben (Figure 1), [30, 31].

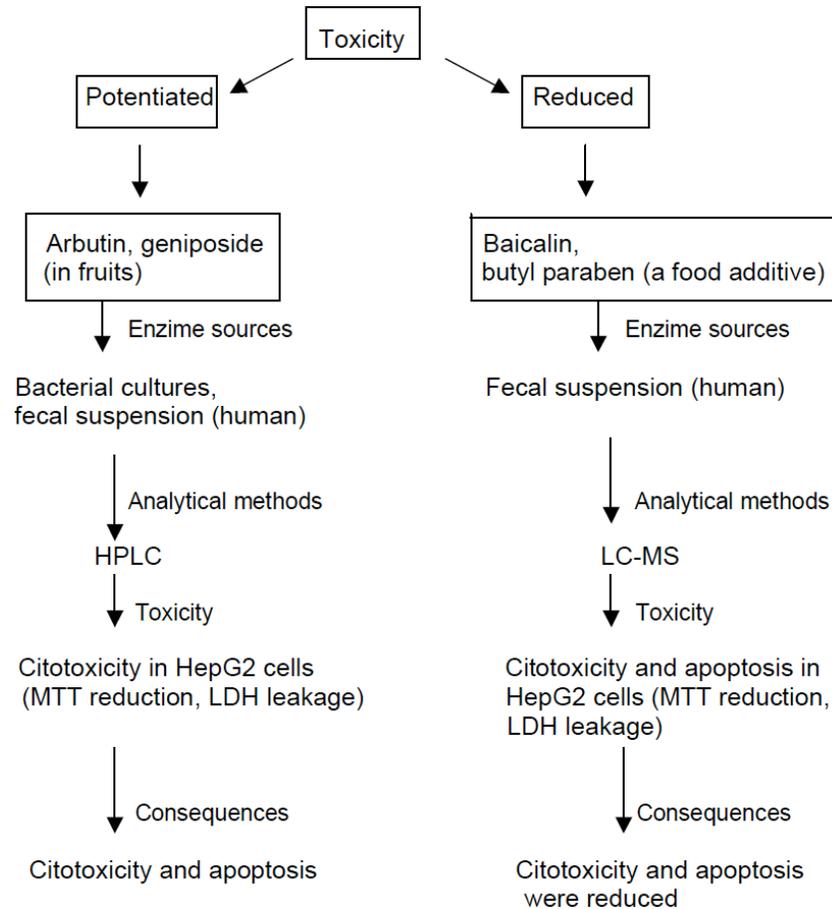
### 3. SMALL INTESTINAL MICROFLORA

The normal intestinal microflora (microbiota) represents a complex, dynamic, and diverse collection of microorganisms, which usually inhabit the gastrointestinal tract. Normally, between this flora and the human host, a mutually beneficial long-term symbiotic relationship is established, where the host contributes essential nutrients necessary for the survival of the microbiota and the latter fulfills multiple roles in host nutrition and development.

Small intestinal bacterial overgrowth (SIBO) is an often neglected mechanism in impaired nutrition. Normally, only small numbers of bacteria are found in the small intestine when compared with those found in the colon [32].

Since the small intestine is the site of digestion and food absorption, bacterial flora is excluded from the small intestine to prevent unwanted competition with the host as well as the abnormal entry of bacteria into the host. The dramatic changes in the bacterial flora of the small bowel that occur in SIBO have a multitude of effects on nutritional status. The bacterial flora may compete with the host for critical nutrients, alter host metabolism, directly damage the absorptive mucosa of the host, and produce gastrointestinal symptoms that reduce or alter food intake by the host. New studies suggest that changes in the microbiota could be linked to the etiopathogenesis of various diseases (celiac disease, irritable bowel syndrome, obesity, colorectal cancer, allergic disorders, and especially inflammatory bowel diseases) [33]. These outstanding findings could be used as new diagnostic tools and/or therapy. The diarrhea experienced by patients with SIBO may be a result of excess fecal loss of water and electrolytes or the elaboration of gases produced by intraluminal bacteria. The former condition is in part due to an increased delivery to the small intestine of osmotically active carbohydrate fragments resulting not just from mucosal injury, but also from disaccharidase deficiency [34].

The influence of the enormous population of microorganisms colonizing the intestinal lumen is



**Figure 1:** The role of metabolism by the intestinal microflora in xenobiotic-induced toxicity.

HPLC: High-performance liquid chromatography; LC-MS: Liquid chromatography-mass spectrometry; HepG2: Hepatocellular carcinoma, human; MTT: 3-(4,5-dimethyl thiazol-2-yl)-2,5-dipheniltetrazolium bromide; LDH: Lactate dehydrogenase.

important not only as a protective barrier but also because of the nutritional functions they fulfill. During bacterial metabolic processes, several substances are produced and are taken advantage of by the host; a) Vitamin synthesis, b) Enzyme production, c) Biliary salt transformation, d) Nutrient digestion and absorption and e) Nutrition of the intestinal epithelium.

#### 4. INTESTINAL FLORA AND IMMUNE SYSTEM

The normal intestinal tract remains in a state of *physiological inflammation* due to the intestinal flora. The presence of bacteria within it, induces activation, proliferation and the development of a complete immune system characterized by the presence of IgA immunoglobulin in the lamina propria, that is produced by plasma cells and secreted by epithelial cells into the mucosal surface; this leads to the formation of a protective barrier against foreign antigens.

In order for the immune system to exert its “regulatory” function and maintain homeostasis within that “physiological inflammation” milieu, the

gastrointestinal tract has specific tissues that can recognize unusual configurations and are known as lymphoid tissue and specifically, as gut-associated lymphoid tissue (GALT); it is responsible for maintaining that equilibrium and it is composed of four main components:

1. Lymph nodes in the lamina propria, including Peyer’s patches and colonic lymph nodes.
2. Lymphoid cells diffusely distributed in the lamina propria.
3. An intra-epithelial compartment in which lymphocytes are scattered between epithelial cells.
4. Lymphoid tissue associated to glands involved in digestion, such as salivary glands and those of the hepatobiliary system.

This organization fosters the activation of the appropriate immune responses, either humoral or

cellular, depending on the type of antigen inducing them [35].

The GI mucosa is in constant contact with a great number of antigens (either from nutrients or from microorganisms) as well as in perfect symbiosis with a large number of bacteria (with surface antigens foreign to the host) that coexist in delicate symbiosis and whose main objective is the prevention of the proliferation of pathogenic microorganisms that eventually may lead to infection and trigger immune responses; the immune system must distinguish between the types of immune responses, cellular or humoral, local or generalized, and even the development of tolerance to antigens in the diet that leads to the body's survival.

Food tolerance was empirically described since 1829, the year that Dakin described the habit of Northamerican natives of eating poison ivy to prevent dermatitis when coming in contact with the plant; they thus induced a tolerant state by orally administering the antigen. In 1919, Besredka showed that rabbits that were orally exposed to inactive *Shigella spp.* were protected against death due to shigellosis but without one of the characteristics of the immune response: serum transfer. The oral administration of a sensitizing agent was described by Chase in 1946 when upon adding picryl chloride to guinea pig diets, they were unable to mount a delayed hypersensitivity reaction when picryl was exposed to their skin, as did the guinea pigs whose diet had not included picryl chloride [36,37].

With these examples, the importance of orally activated immune responses is evident and occurs throughout the digestive tract. The presence of infectious agents in mucosal surfaces trigger local and systemic responses whereas if these same pathogens are enterically administered in their inactive form, only local responses develop that are in part, responsible for the chronic inflammatory state of the GI tract. In support of this statement, the importance of factors in human milk in the development of the immune and gastrointestinal systems has been clearly proven; in contrast, neonates fed milk formula orally ingest nucleotides that can be endogenously synthesized and are therefore, not considered essential nutrients. However research in humans and animal models have suggested that a nucleotide-rich diet has a significant effect on the immune and gastrointestinal systems, coining the terms "semi-essential" and "conditioning"

that are currently used when describing the role of nucleotides in human nutrition [38].

As previously described, normal intestinal flora plays a key role in the normal function of the GI tract and the host's health. This develops since birth and is crucial to the development of the immune system and the mucosa-associated immune system [39].

Different microflora factors play a role in organism protective functions such as:

- a) The maintenance of a physical barrier against colonization or invasion by pathogens.
- b) Facilitate digestion and nutrient assimilation.
- c) Provide appropriate immune signals to the mucosal/intestinal lumen interface.

The greatest number of bacteria living in human beings, such as normal flora, is in the colon [40, 41]. It is within that anatomical site where several species of *Bacterioides sp.*, such as *B. thetaiotaomicon*, display intense metabolic activity, releasing and producing in the process, simple carbohydrates, vitamins and amino acids that can be used by other microorganisms; this metabolic characteristic of *Bacterioides* species acts as a source of nutrients for other intestinal bacteria that further contribute to the establishment of normal colonic flora [42].

Intestinal *Bacterioides* strains have been shown to modulate enterocyte function by controlling the expression of various genes associated with nutrient absorption, strengthening the intestinal mucosa, producing angiogenic factors and defensins with antibiotic activity against a wide range of bacteria and other microorganisms [43, 44]. The genus *Bacterioides* has the specific function of activating and/or stimulating immune responses since its capsule polysaccharides are zwitterionic as in the case of *B. fragilis*, in which they activate CD4<sup>+</sup> T lymphocytes as well as the thymus-dependent response [41].

Evidently, this fragile balance between commensal flora that is beneficial to the body and its potential transformation into pathogens, may be due to the incorporation of genes encoding virulence factors, particularly genes encoding adhesion and tissue invasion proteins, toxin production, histolytic enzyme synthesis, superoxide dismutase and catalase production, procoagulant factors as well as bacterial surface components such as capsular polysaccharides

and lipopolysaccharides. Most of these factors can be found in different *Bacteroides* species but *B. fragilis* is the most virulent which correlates with the fact that although it is not the most prevalent in normal flora, it is the genus species most commonly isolated in human infections [45].

What are the interaction mechanisms between nutrition, infection and activation of the Neuro-Immuno-Endocrine System (NIES) that maintain the Body's Homeostasis upon detection of an agent, either from nutrients or infectious, that tends to specifically dysregulate the gastrointestinal tract's balance?

Intestinal flora is key in this regulation, but our current understanding of factors that regulate immune function have supported the close interdependence between the host's nutritional and immune status.

The mammalian immune system consists of innate immunity and adaptive mechanisms and both protect the host from environmental pathogens. However, innate immunity is the first line of defense against a broad variety of pathogens, regardless of previous exposure; it responds immediately and with no immunological memory unlike acquired immunity. The innate immune response recognizes pathogenic microorganisms such as bacteria, fungi and parasites through their individual components, such as polysaccharides that are recognized by receptors in the host's cells. These molecular patterns associated to pathogens are known as "pathogen-associated molecular patterns" (PAMPs) and the receptors on immune cells with which they interact are known as "pattern-recognition receptors" (PRR). Recognition of these PAMPs by the PRRs leads to the activation of intracellular signaling pathways that culminate in the production of inflammatory cytokines, chemokines and interferons and alert the body to the presence of infection [46].

There are various PRRs including the TLRs (Toll-like receptors), the nucleotide-binding oligomerization domain receptors (NOD-like receptors, NLRs) and the retinoic acid-inducible gene-like helicases (RIG-like helicases, RLHs) that are crucial to host protection against microbial infection and homeostasis maintenance of the colonizing flora.

In the intestine, microflora is in permanent contact and reciprocal interaction with the host's cells and with nutrients, resulting in a complex and highly regulated ecosystem.

Every Peyer patch has a dome region under the follicle-associated epithelium (FAE). This dome consists of T and B lymphocytes, macrophages, dendritic cells (DC) and follicles with germinal centers. The presence of all three types of antigen-presenting cells (APC) in the dome such as Memory B cells, macrophages and dendritic cells makes it likely that antigen uptake occurs immediately after release from M cells. M cell pockets in Peyer's patches contain approximately equal numbers of T and B cells, but fewer macrophages. Approximately 75% of the T cells are T helper (Th) cells.

The fact that intestinal microflora plays a relevant role in normal intestinal function and host health must be emphasized. This flora colonizes the intestine almost immediately after birth and is essential to immune system sensitizing during the ontogenesis, development and maturation of the immune system in mucosae as well as systemically [47, 48].

Different factors contribute to the intestinal microflora's protective function, including:

1. Maintenance of a physical barrier preventing colonization or invasion by pathogens.
2. Facilitation of digestion and nutrient assimilation.
3. Provides survival immune signals at the intestinal mucosa-lumen interface.

Microflora consists of potentially pathogenic bacteria and beneficial, non-pathogenic microorganisms. In order to control colonization by microflora and pathogens, the body has developed a wide range of defense mechanisms that in most cases prevent the development of invasive disease due to microorganisms. Commensal flora has been part of the human microecology for thousands of years but these "good bugs" are now less common or absent in the microbial milieu due to the industrialization of our cities. This is exemplified by the increased incidence of allergies (Th2-dependent pathology) and the modern style of hygiene that has led to the "Hygiene hypothesis" that dysregulates the Th1/Th2 balance but does not explain the increased incidence of immunological disorders such as inflammatory bowel disease (IBD), multiple sclerosis, type 1 diabetes mellitus and obesity, all primarily Th1 lymphocyte-dependent entities. It has recently been suggested that regulatory T lymphocyte induction by certain microorganisms may prevent or ameliorate this type of diseases [49]. However, defects in immunoregulatory

mechanisms, such as tolerance against commensal microflora, have proven to be associated to the pathogenesis of IBD [50]. On the other hand, a loss of function of IL-10 and IL-10 receptors (IL-10R) in patients with very early onset IBD has also been identified. These findings indicate that infantile IBD patients with perianal disease should be screened for IL-10 and IL-10R deficiency and that allogenic hematopoietic stem cell transplantation (HSCT) can induce remission in those with IL-10R deficiency [51]. Rupture of the intestinal mucosal barrier may allow the exposure of PAMPs or of commensal flora antigens that interact with the TLR expressed on immune cells and lead to an enhanced, severe inflammatory response [52]. The GI system's commensal flora may contribute to immune homeostasis by maintaining microbial balance and regulating the intestine's immune system because the relevance of TLR signaling has been proven. Recognition of commensal microflora by TLRs is necessary to decrease the physiological inflammation status present in homeostatic conditions; hence, any imbalance in this signaling pathway may lead to intestinal inflammatory disease [53]. Although the interaction of commensal and/or pathogenic microflora with intestinal epithelial cells and the activation of acquired or innate immunity has many other implications; the importance of intestinal microbiota in the development of inflammatory disease is evident. However, intestinal microbiota has numerous beneficial effects on health, such as nutrient extraction, a role in the development and maturation of the intestinal immune system and the regulation of fat storage in adipocytes that may play a role in metabolic disorders such as obesity in which the flora is abnormal.

Finally, the role of the adaptive or innate immune responses that in both cases would begin by recognition by TLR, is pivotal to the maintenance of balance in the intestinal milieu and hence, to the health and/or homeostasis of the human body.

## CONCLUSION

Bifidobacterial genome sequences have revealed genomes with extensive features that can be predictably important to survival and competition in the large intestine, such as complex carbohydrate utilization and competitive features protecting bifidobacteria from bacteriophages and other bacteria. The lack of knowledge of the molecular mechanisms of the proposed probiotic benefits of bifidobacteria greatly weakens the scientific credibility of current health

claims. The current explosion in the availability of genome sequences and molecular tools for bifidobacteria analysis may provide further clarification.

Despite its importance, the presumed role of intestinal microflora metabolism in xenobiotic-induced toxicity has not been thoroughly studied. Therefore, it is appropriate to briefly review our current situation, and state what type of research in xenobiotic metabolism by intestinal microflora, particularly in the field of toxicology, is needed.

On the other hand, with a high degree of suspicion and the use of the lactulose breath test, small intestinal bacterial overgrowth can be readily identified as a contributing factor to the patient's gastrointestinal symptoms and malnutrition. Eradicating SIBO will lead not just to dramatic improvement in symptoms, but will correct the multi-faceted adverse effect of bacterial overgrowth on nutrition.

It has been suggested that changes in the microbiota could be linked to the etiopathogenesis of several diseases. These outstanding findings could be used for the development of diagnostic tools and/or therapy. An essential probiotic function is the support of a normal immune system. In the future, probiotics will be employed in the prevention and treatment of many human diseases.

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