Host-Intestinal Microbe Interactions in Human Health and Disease

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Abstract

The human intestine harbours a complex community of microorganisms, known as the gut microbiota. Significant progress in microbiota research has not only provided knowledge into its composition and abundance, but has also elucidated its crucial functions and potential applications to human health and disease. This review will summarize the key understandings of the gut microbiota to date, with particular emphasis on gut microbiota development and the mechanisms for microbial interactions with the host immune system and the intestinal epithelium. Finally, it will explore the potential microbiota therapies currently being researched, and discuss some of the challenges of translating the current evidence from basic science to human trials.

Introduction

Host-microbe interactions are paramount to human health and disease – an idea that was first suggested by Louis Pasteur during the 19th century. When studying the anthrax epidemic, the prominent French microbiologist noted that the growth of *Bacillus anthracis* was reduced by the presence of other microorganisms – what he described as 'la luttte pour la vie', or 'the battle for life'.[1] Pasteur predicted that certain microbes in foods may be beneficial for health, and this was supported in 1907 when Eli Mechnikov made the seminal observation that peasants who regularly consumed lactic acid bacteria from fermented dairy products lived longer and healthier. Mechnikov reasoned "the dependence of the intestinal microbes on the food makes it possible to adopt measures to modify the flora in our bodies and to replace the harmful microbes with useful microbes".[2] This concept of balancing 'harmful' and 'useful', or the 'good' and the 'bad' microbes in relation to human health is the basis of the current understanding of host-microbe interactions in humans.

Every one of us harbours a complex community of microorganisms in our intestinal tract – collectively referred as the "microbiota". The gut microbiota numbers over 10^{14} microbial cells (10 times that of human somatic and germ cells) spread between 500 and 1,000 bacterial types that collectively contain at least 150 times more genes than their human host.[3] With the advancement of more precise sequencing technology in recent years, great strides have been taken not only to determine the diversity and abundance of the microbiota, but also to uncover its functions and therapeutic potential.

Mounting evidence has shown that the human microbiota engages in extensive cross-talk with the host immune system. The precise effect on the host consists of multiple interactions from different genera, different species and through different mechanisms.[4, 5] Therefore, while we are traditionally inclined to ascribe infectious diseases to a single causative agent, as is the case for *Helicobacter pylori*-induced gastritis or Salmonella-induced enteritis, gut microbial modulation of host health and disease consists of interactions between entire bacterial communities, host immunity, host genotypes and external factors such as diet and environment. Further knowledge about these interactions is necessary before adopting host-microbe manipulation as a means to reduce disease burden in patients. This review provides an overview of what is currently known about the microbiota, its development and how this understanding is being harnessed for therapeutic applications to manage human health and disease.

Development of the Gut Microbiota

In newborns, initial acquisition of the microbiota is highly dynamic and follows a step-wise progression at birth (Figure 1).[6] Conventionally, the fetus is believed to be sterile, and microbial colonization begins once the baby is exposed to the non-sterile extra-uterine environment during delivery. Contact with maternal tissues, feces and the hospital environment directly evoke massive bacterial colonization.[7, 8] Factors such as modes of delivery (caesarean section versus vaginal birth),[9] antibiotics administered to either mother or child,[10] resident microbes in the maternal birth canal[11] and the immediate environment[12] all have major influences on the initial microbial colonization in the intestinal tract. For instance, vaginally-delivered babies are predominantly colonized by Lactobacilli, which are lactic-acid producing bacteria which inhibit the growth of pathogenic bacteria, whereas caesarean-delivered babies are colonized by potentially pathogenic microbes, such as Staphlyococci and Actinobacteria. Aside from composition, caesarean-delivered babies also have lower gut microbial diversity and, interestingly, have a higher risk for developing allergic diseases later in life.[13] One explanation is the microbial facilitation of mucosal immune development. In early-life, the immature gut immune system samples bacterial antigens for the first time, and aids the development of immune tolerance by exposure to these different antigens.

Consistent with this notion, studies have reported that a low microbial diversity during the first year is associated with the development of atopic diseases such as eczema and asthma in infancy and childhood.[14, 15]

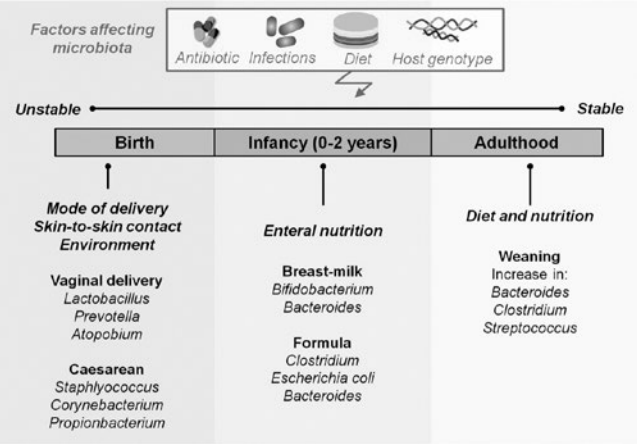

Figure 1. Step-wise development in microbial composition throughout life. Initial colonizers in a newborn's GI tract are largely influenced by modes of delivery and the nutritional profiles. During this initial colonization period, the microbiota is very unstable, and does not stabilize to adult profile until weaning. However, various external factors can invariably affect microbiota compositions throughout life, such as antibiotics, infections, diet, nutrition and host genotype.[5]

Following birth, bacterial colonization of the gut is influenced by nutrient intake of the infant (Figure 1). Human breast milk, in addition to being a complete food for the infant's energy metabolism, also contains growth factors, immunoglobulins, oligosaccharides, and various live bacteria such as Staphylococci, Streptococci, Bifidobacteria, and lactic acid-producing bacteria.[16] Human milk oligosaccharides (HMO) are non-digestible carbohydrates that stimulate the growth of selective beneficial bacteria, such as Bifidobacteria. Although the health advantages of Bifidobacteria abundance in early development is not entirely known, it appears that greater initial Bifidobacteria colonization is associated with less disease. This protection is speculated to be due to growth inhibition of pathogenic bacteria, interaction with intestinal epithelial cells (IECs) and stimulation of the innate and adaptive immune system.[6] Interestingly, in comparison to formula-fed infants, studies show that at one month of age, breastfed babies have higher numbers of Bifidobacteria, less *Escherichia coli*, Clostridium species, and Bacteroides as part of their colonic microbiota. Another study comparing fecal microbiota profiles at 18 months of age found that breastfed infants had almost double the proportion of Bifidobacteria with more diverse Bifidobacteria species than formula-fed infants.[17] Thus, nutrient intakes, such as HMO found in breast milk but not in formula, contribute to individual variation in gut microbial composition.[18] A recent biochemical comparison of HMO composition showed remarkable differences in abundance and carbohydrate diversity across mothers, suggesting that not all breast milks are created the same, and these differences in sugars may well account for variations in microbial composition during breastfeeding.[19]

With weaning and the introduction of solid foods (~4-6 months is the current norm for breast fed infants in North America), the infant microbiota shifts towards a more adult composition with increased counts of Bacteroides, Streptococci and Clostridia.[20] However, the establishment of a stable adult microbial community in the gut is not complete until around 2-3 years of age.[21]

Microbiota and Health

Shifts in the composition of the gut microbiota continue through childhood and into adulthood under the influence of diet, exposure to antibiotics, and intercurrent infections (see Figure 1). Compositional changes in microbiota, or "dysbiosis", profoundly shift the balance in microorganisms and thereby, allow for colonization and overgrowth by opportunistic enteric pathogens.[12] Dysbiosis is associated with decreased biodiversity, lower species representation and a reduced bacterial biomass. These conditions can be exploited by pathogens, allowing them to outcompete commensal bacteria for nutrients, actively colonize the gut and ultimately alter host susceptibility to infections. For instance, the use of antibiotics in early life reduces microbial diversity, and is correlated with the increasing incidence of asthma and other autoimmune diseases like chronic inflammatory bowel diseases (ulcerative colitis and Crohn's disease), obesity, and type 2 diabetes later in life.[23-26] Some examples of diseases with gut microbiota alterations are summarized in Table 1. However, the manner by which dysbiosis contributes to various disease phenotypes is still not fully understood. Most data today focuses on microbial modulation of the host immune system.

Table 1. Gut Microbiota Alterations in Various Diseases*

Diseases	Changes in microbial composition	Ref
Irritable bowel syndrome	Increased Firmicutes (Ruminococcus, Clostridium and Dorea species) Decreased Bifidobacterium and Faecalibacterium species	23
Inflammatory bowel diseases	Increased Gammaproteobacteria, adherent and invasive *Escherichia coli,* Clostridium Decreased *Faecalibacterium prausnitzii* and butyrate-producing bacteria like Roseburia and *Phascolarctobacterium*	24
Ulcerative colitis	Increased Proteobacteria, Fusobacteria and Spirochaetes Decreased Firmicutes, Lentisphaerae and Verrucombicroa	29
Colorectal cancer	Increased Fusobacterium members Altered Coprococcus*, Eubacterium rectale,* Roseburia and *Faecalibacterium prausnitzii*	29
Obesity	Increased Lactobacillus species, *Methanobrevibacter smithii, Faecalibacterium prausnitzii* Decreased Bacteroides	25
Type 2 diabetes	Increased Clostridium*, Akkermansia mucinphila,* Bacteroides and Desulfovibrio	26

**Table adapted from reference 29*

Effects of the Gut Microbiota on Host Immunity

The intestinal environment is constantly exposed to bacteria and dietary antigens. In order to mount an appropriate response, the gut immune system must be able to discriminate between offending pathogens and the commensal microbiota. Studies in germ-free animals demonstrate that the absence of microbes results in major developmental defects in immune structures.[22] Germ-free mice have less intestinal Peyer's patches and smaller and fewer lymphoid follicles. These animals also have structural defects in the spleen and lymph nodes with poorly formed B and T zones. Mesenteric lymph nodes contain a reduced number of plasma cells. Moreover, germ-free animals are more susceptible to infections; for instance, when challenged with the bacterium *Listeria monocytogenes* they have reduced bacterial clearance compared to bacterially colonized animals.[27] One explanation is the inability for T-cells to be recruited to sites of inflammation in these animals.

Insights provided from studies using germ-free mice highlight the complex interrelationship between the gut microbiota and host immunity (Figure 2). In rodent models of 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced colitis, the probiotic (live microorganisms which when administered in adequate amounts confer a health benefit on the host)[3] *Lactobacillus rhamnosus* GG reduces colonic inflammation by inducing T_{Reg} activation.[24] Another probiotic, *Bifidobacterium infantis* strain 35624, protects against Salmonella-induced enteritis through induction of T_{Reg} and suppression of NF-κB activation. Combination preparations, such as VSL#3 (containing 8 probiotic strains) are also anti-inflammatory in animal models of colitis.[28] Interestingly, depletion of CD4+ T_{Reg} from mice prior to probiotic administration abolishes the protective effect. Collectively, these studies indicate that probiotics can directly modulate host immunity via stimulation and induction of regulatory immune cells.

Effect of Gut Microbiota on the Intestinal Epithelium

Besides immunomodulation, another well characterized host-microbe interaction is modulation of intestinal epithelial barrier function (see Figure 2). The intestinal epithelial barrier consists of a single layer of intestinal epithelial cells (IECs) tightly sealed together by apical junction complexes. IECs consist of several cell types, including enterocytes (nutrient absorptive cells), goblet cells (mucus production), enteroendocrine cells (produce a variety of gut hormones, including those that regulate appetite), Paneth cells (produce anti-microbial peptides), and M cells which sample luminal antigens and present the antigen to immune cells.[29] Together, IECs are tasked with the responsibility of separating internal tissues from the luminal environment. This is accomplished through numerous functions: tight junctions tightly seal the IEC to forge a physical barrier against luminal contents; goblet cells secrete mucus to entrap microbes; and Paneth cells produce anti-microbial peptides such as defensins, lysozymes and regenerating islet-derived protein 3γ (REGIIIγ).

Beneficial microbes enhance epithelial barrier integrity through several mechanisms (Figure 2). Certain species, such as *L. acidophilus*, directly phosphorylate the intercellular tight junction proteins occludin and actinin, which can prevent invasion of enteroinvasive *Escherichia coli*.[31] Certain probiotics increase goblet cell production of mucus by upregulating the expression and production of mucin.[32] Other probiotics, such as *Lactobacillus fermentum* and *E. coli* Nissle 1917, stimulate the expression of anti-microbial peptides in Paneth cells.[33] One interesting mechanism was recently described for *Lactobacillus rhamnosus* GG, which releases a soluble protein (p40) that preserves tight junction integrity and prevents cytokine-induced apoptosis by activating the epidermal growth factor receptor (EGFR) on intestinal epithelial cells.[34, 35] Collectively, these studies demonstrate diverse modes of action of microbial modulation impacting IECs.

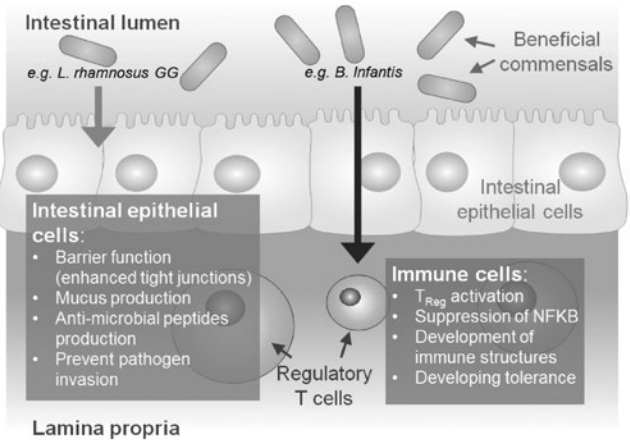

Figure 2. General mechanisms of microbiota in modulating host physiology. The majority of studies to date have generated insights between commensal bacterial and host immune cells and intestinal epithelial cells. Some of these mechanisms (highlighted in boxes) are hypothesized to contribute to intestinal homeostasis.

Microbiota Derived Therapies

The implications of the impact of gut microbiota on host physiology have generated increasing interest in potential therapeutic applications. Both probiotics and prebiotics (non-digestible food ingredients that stimulate the growth or activities of beneficial microbes) are potential options under active investigation to beneficially alter the intestinal microbiota. To date, clinical trials report benefits of probiotics in improving a wide range of diseases[36] such as intestinal inflammation, allergies, atopic eczema,[37] acute diarrhea,[38, 39] and necrotizing enterocolitis.[40, 41] However, these positive findings have not always been replicated,[42, 43] hence the subject of dose, formulation and choice of bacterial strain or strains to employ as a probiotic are under active investigation. For example, *Lactobacillus reuteri* strain DSM 17938 has been proposed for the treatment of infantile colic,[44] but reported benefits have not been replicable across different studies.[45, 46] Variability in outcomes may be due to several reasons. Firstly, some clinical trials test healthy individuals without any background of intestinal injury or insult, making it difficult to assess for pro-

biotic modulation of host phenotype. Secondly, the effects of probiotics are both species-specific and strain-specific. For example, *Lactobacillus acidophilus, Lactobacillus rhamnosus* and *Lactobacillus casei*, are all part of the same genus, but their biological effects are quite different.[47] Interestingly, variation in biological effects may also occur in the same species due to differences in strains and sources, as is the case for *Bifidobacterium lactis*, strain BI-04 versus *Bifidobacterium lactis*, strain Bi-07.[48] Thirdly, probiotics' effects may also differ given their ability to act either synergistically or antagonistically with gut microbes.and the variability in existing microbiota between individuals. Therefore, when evaluating potential benefits of probiotics, it is important to realize that the ingested bacterium must engage in complex interactions with host immunity, existing microbes, and external factors including dietary components. Drastic differences in any of these parameters could affect the expected benefit of the probiotic.

On the other hand, commonly used prebiotics such as inulin-type fructans; inulin, fructo-oligosaccharides and galacto-oligosaccharides[49] have the ability to affect the activity of beneficial bacteria. As substrates for microbial growth, ingested prebiotics can selectively stimulate the growth of Bifidobacteria and Lactobacilli in both animals and humans. While prebiotics can clearly modulate intestinal microbiota, their range of therapeutic efficacy still needs to be elucidated.

Future Prospects

Recent progress in gut microbiota research has dramatically enhanced understanding of host-microbe interactions, and has built substantial evidence for the use of probiotics in certain human diseases. Nevertheless, our current understanding of how microbes work is still relatively limited. As a result, it is too early to start routinely recommending probiotics as a therapy for the broad range of conditions which have been associated with intestinal dysbiosis. Moreover, the impact of diet in modulating the microbiota has recently gained prominence, where a tripartite interaction involving diet, immunity and the gut microbiota is hypothesized to facilitate host susceptibility to a variety of infectious and chronic, non-communicable diseases. This is an exciting time to be engaged in microbiota research as we begin to slowly tap into the complexities of host-microbe interactions.

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Conflicts of Interests

P. Sherman holds a research contract from Lallemand Human Nutrition (Montreal, Quebec), serves on the Research Advisory Board and is a stockholder of Antibe Therapeutics (Toronto, Ontario) and has received honoraria in the past five years for CME (Continuing Medical Education) presentations on prebiotics and probiotics from Abbott Nutrition, Mead Johnson Nutrition, Nestle Nutrition, and Procter & Gamble.

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