The Microbiome in Infectious Disease and Inflammation

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Abstract

The mammalian alimentary tract harbors hundreds of species of commensal microorganisms (microbiota) that intimately interact with the host and provide it with genetic, metabolic, and immunological attributes. Recent reports have indicated that the microbiota composition and its collective genomes (microbiome) are major factors in predetermining the type and robustness of mucosal immune responses. In this review, we discuss the recent advances in our understanding of host-microbiota interactions and their effect on the health and disease susceptibility of the host.

INTRODUCTION

The tissues of the gastrointestinal tract have the unique property of harboring an enormous number of microbes within the lumen. The concentration of microbes that reside in the small intestine is estimated to be from 10^3 to 10^9 cells/ml, whereas the large intestine contains abundant bacteria, which achieve a concentration of up to 10^{11} or 10^{12} cells/g of luminal contents (1). This concentration is similar to or even higher than that achieved in colonies growing under optimum conditions on laboratory plates, indicating that the colonic lumen provides a safe and nourishing environment and represents an extremely efficient natural bioreactor for bacteria. The estimated total number of bacteria carried by a healthy human in the gut is 10^{14}, which, as a whole, constitutes the microbiota (also referred to as the microbial flora), an ecosystem in dynamic equilibrium. The members of the intestinal microbiota can be categorized as either allochthonous or autochthonous (1, 2). Allochthonous bacteria are only transiently present, whereas autochthonous bacteria are indigenous and preferentially colonize physical spaces or niches in particular animal species. In most cases, indigenous bacteria can attach to the epithelium or mucus layer and form a biofilm, and thereby significantly affect host development and physiology.

The microbiota allows for optimal breakdown of foods, uptake of nutrients, and enhancement of intestinal development, which have led to diet diversification and increased evolutionary fitness. Beyond digestion and metabolism, the microbiota also contributes to development and maintenance of the intestinal epithelial barrier, development of the immune system, and competition with pathogenic microorganisms, thus preventing their propagation. Indeed, studies of germfree (GF) animals indicate that intestinal microbes profoundly affect the development of the mucosal immune system in terms of the organization of Peyer's patches (PPs) and isolated lymphoid follicles (ILFs), secretion of antimicrobial peptides by epithelial cells, and accumulation of various immunocytes at mucosal sites (3–7). Collectively, the gut microbiota provides an indispensable internal ecosystem for numerous host physiological processes and can be considered to have coevolved with the host to form a superorganism (8).

The collective genome of intestinal microbes, termed the microbiome, is estimated to contain at least 100 times more genes than our own genome (9). Unlike the human genome, which is rarely altered by xenobiotic intervention, the gut microbiota composition is readily changeable by diet, antibiotic ingestion, infection by pathogens, and other life events. The plasticity of the microbiome has been implicated in numerous disease conditions, and an unfavorable alteration of the gut microbiota composition is called dysbiosis, which includes an outgrowth of potential pathogenic bacteria (pathobionts) and a decrease in the number of beneficial bacteria (10, 11). Multiple recent reports have shown a link between dysbiosis and immune disorders. Crohn's disease and ulcerative colitis are two chronic intestinal inflammatory conditions referred to as inflammatory bowel disease (IBD). IBD is a disease with an elusive etiology, and although many potential triggers have been invoked, one attractive hypothesis is that IBD may be a result of dysbiosis in the intestinal microbial community that promotes the overgrowth of bacteria that aberrantly stimulate the intestinal immune system. Indeed, many reports have shown that the microbial populations in the intestine of IBD patients are different from those of healthy individuals (12–14). Accumulating evidence suggests that a change in the gut microbiota composition has a key role not only in IBD, but also in the development of systemic immune diseases, such as rheumatoid arthritis (15, 16), encephalomyelitis (17, 18), type 1 diabetes (19, 20), and allergic diseases (21, 22).

The microbiota affects the host immune system through multiple factors, which include microbial components and their metabolites. The immune system recognizes these factors mostly through innate immune receptors. Constitutive signaling induced by the microbiota

GF: germfree

PP: Peyer's patch

ILF: isolated lymphoid follicle

Microbiome: a genetic catalog of the microbial species that inhabit a defined environment such as the human body

Dysbiosis: a condition with imbalance in the composition of the bacterial microbiota; this includes an outgrowth of potentially pathogenic bacteria and/or a decrease in bacterial diversity and bacteria beneficial to the host

Pathobiont: a symbiont or a commensal that is able to promote pathology only when genetic or environmental conditions are altered in the host

IBD: inflammatory bowel disease

keeps the intestinal mucosa in a state of physiological inflammation, with continuous production of tissue repair factors, antimicrobial proteins, and immunoglobulin A (IgA) that, together, maintain intestinal barrier integrity and provide beneficial functions to the microbiota (23–25). Without constitutive innate signaling, intestinal barrier injury and bacterial translocation may occur. Innate immune recognition of the microbiota leads to the establishment of an arsenal of unique and diverse intestinal immune cell populations. IgA-producing plasma cells, intraepithelial lymphocytes (IELs), and T cell receptor (TCR) γδ-expressing T cells (γδ T cells) are the classically known lymphocytes unique to the mucosa. Recent studies have shown the presence of innate immune lymphocytes, such as $CD4^+CD3^-$ lymphoid tissue–inducer cells (LTi cells) and interleukin (IL)-22-producing natural killer (NK)-like cells (26, 27). Furthermore, $CD4^+$ T cells in the intestinal mucosa comprise significant numbers of IL-17-expressing cells (Th17 cells) and forkhead box P3 (Foxp3)-expressing regulatory T cells (Treg cells) (28). All these cells are particularly abundant in the intestinal mucosa, even under steady-state conditions, and their accumulation and function are deeply affected by the presence of the microbiota.

Although it is not fully understood why and how the intestinal microbiota generates such a large variety of immune cell populations, recent studies using gnotobiotes (animals with a defined microbiological status) have suggested that specific components of the microbiota induce specific populations of immune cells. Below, we summarize the recent findings on how members of the microbiota provide an intestinal environment uniquely suited for the well-balanced development of the innate and adaptive immune system and discuss the role of the microbiota in infectious diseases and inflammation.

COMPOSITION OF THE MICROBIOME

The establishment of the intestinal microbiota occurs progressively, beginning immediately after birth. The initial infant gut microbiota has a relatively simple composition, which is affected in large part by the maternal microbiota. Vaginally delivered infants acquire bacterial communities resembling their own mother's vaginal microbiota, dominated by *Lactobacillus*, *Prevotella*, or *Sneathia* spp., whereas infants delivered by Cesarean section harbor bacterial communities similar to those found on the skin surface, dominated by *Staphylococcus*, *Corynebacterium*, and *Propionibacterium* spp. (29). These pioneer bacteria may affect the composition of adult flora. After the weaning period, however, the microbiota markedly changes, and obligate anaerobes become prominent, with much lower numbers of facultative anaerobes.

Only four phyla dominate adult human intestinal habitats (30). Most (>90%) of them belong to Bacteroidetes (including *Bacteroides*) and Firmicutes (including *Clostridium*, *Lactobacillus*, and *Bacillus*). Firmicute bacteria in the gut include two major clostridial groups, namely the clostridial clusters IV and XIVa that comprise the Lachnospiraceae. Lower-abundance phyla are mainly composed of Proteobacteria (including *Escherichia*) and Actinobacteria (including *Bifidobacterium*). The mouse intestinal microbiota is similar to the human microbiota in broad terms. Such limited phylum predominance suggests the presence of strong selective forces over thousands, perhaps even millions, of years of coevolution. Notably, certain members of the Firmicutes, such as *Clostridium* and *Bacillus* genera, are found in a state of vegetative growth or as spores. The ability to make spores may be of ecological advantage to the organism as it enables it to survive under adverse conditions to efficiently colonize the intestine.

At lower taxonomic levels, there is considerable interindividual variation. Metagenomic approaches using massive parallel sequencing allow for the direct enumeration of the microbiota without having to isolate and cultivate bacteria. Using this technology, the international MetaHIT (Metagenomics of the Human Intestinal Tract) project has recently reported that each human individual carries on average 540,000 common genes in the intestine (9).

LTi: lymphoid tissue inducer

Gnotobiote: gnotobiotic comes from the Greek "known life" and refers to animals with defined microbiological status

MetaHIT (Metagenomics of the Human Intestinal Tract) consortium: the MetaHIT project aims to understand the role of the human intestinal microbiota in health and disease; the consortium involves 13 research centers from eight countries

This estimate suggests that only approximately 35% of bacterial genes are shared between individuals. Interestingly, the results from the MetaHIT consortium also suggested the existence of at least three enterotypes in the human population (31). Enterotypes, which can be compared to blood types, are defined by characteristic populations of bacterial species and the genes that they encode. It is not yet known how enterotypes affect metabolism or immune system homeostasis in the host.

The microbiome is adaptable to environmental changes and host genotypes. Recent studies have shown that community membership and function of the microbiota can change owing to numerous variables including lifestyle, hygiene, diet, and use of antibiotics (32). Furthermore, it has recently become clear that the composition of the microbiota can influence onset and/or progression of several diseases. Indeed, the respective levels of the two main intestinal phyla, the Bacteroidetes and Firmicutes, are linked to obesity and metabolic disorders, both in humans and mice (33, 34). There has also been a substantial increase in the number of reports showing the relationship between the microbiota composition and the incidence of chronic inflammatory disease, including allergic conditions and autoimmune disorders (15–22). Furthermore, transplantation experiments in which the microbiota of diseased animals is grafted into healthy recipients have demonstrated the transfer of several disease phenotypes. These include obesity, metabolic disorders, and chronic colitis (35–37), all of which have complex etiologies affected by host genetic and environmental factors. Therefore, a better understanding of the functional properties of individual members of the microbiota is increasingly relevant to the treatment of complex chronic diseases.

Factors that Affect Community Membership of Microbiota

Diet. Diet is one of the most important factors shaping microbial diversity in the gut. Because members of the microbiota have their own substrate preference and there is intense competition for resources, alterations in the components of the diet, particularly the type and quantity of fat and polysaccharides, result in changes in community composition and function of the microbiota. Mouse studies revealed that feeding mice with a high-fat and high-carbohydrate diet (Western diet) resulted in an increase in the number of bacteria of the Firmicutes phylum and a decrease in that of bacteria of the Bacteroidetes phylum (38, 39). This increase in the number of Firmicutes was mainly due to the proliferation of the Erysipelotrichaceae family (38, 39). The abundance of this family of bacteria immediately diminished when the diet was changed to a diet low in fat and rich in plant polysaccharides. The decrease in the proportion of Firmicutes after a low-calorie diet was similarly observed in humans (40). Another human study of 19 obese volunteers showed that a decreased carbohydrate intake led to a decrease in the number of bacteria within a specific group of Firmicutes that included *Roseburia* spp. and *Eubacterium rectale* (41). Diet also influences fecal community enterotypes in human subjects (42). Individuals with long-term diets rich in protein and animal fat had an enterotype dominated by *Bacteroides*, whereas those on high carbohydrate diets had a prevalence of *Prevotella*. Although change in diet resulted in a rapid change in microbiota, this was not sufficient to shift the enterotype during a 10-day course. A similar distribution of fecal enterotypes was observed in a comparison of European and African children, who have diets rich in protein/animal fat and carbohydrates, respectively (43). Whether enterotypes associated with long-term diets can be reversed by changes in the diet remains to be determined.

Changes in the diet and accompanied alterations in community membership of the microbiota, whether chronic or short-term, lead to changes in the gene expression profiles of the microbiota. For instance, alterations in availability of diet polysaccharides result in changes in the expression of genes for carbohydrate active enzymes (CAZymes), including glycoside hydrolases and polysaccharide

lyases, in members of the microbiota, such as *Bacteroides thetaiotaomicron* (44). Seaweeds are major components of the Japanese diet; *Bacteroides plebeius* residing in the gut of Japanese individuals acquired the genes of enzymes that can metabolize the sulfated polysaccharide porphyran of marine algae through the horizontal transfer from marine bacteria naturally colonizing dietary seaweeds (45). These changes in gene expression of constituents of the microbiota likely ensure that the microbial community can adapt to dietary changes and maximize energy harvest, while contributing to host fitness.

In some cases, changes in the diet and subsequent alterations in the microbiome may exert a detrimental effect on host physiology. Indeed, in individuals having a high-fat and high-carbohydrate diet, the microbiota is more heavily enriched with bacteria of the phylum Firmicutes and less with those of the phylum Bacteroidetes, and this condition may be more efficient at extracting energy from a given diet compared with the microbiota of lean individuals (33). This suggests a positive-feedback mechanism, in which an obesity-inducing diet can change the composition of the gut microbiota, which results in a shape of the microbiome more capable of extracting energy from the diet, thereby helping perpetuate obesity. It has been postulated that changes in the diet and associated changes in the gut microbiota may also lead to immune disorders (46). In fact, allergies and asthma are almost completely nonexistent in certain rural African communities, where people eat diets low in protein and animal fat and high in plant polysaccharides. Actinobacteria and Bacteroidetes are more abundant in African (Burkina Faso) than in EU children's microbiota, whereas Firmicutes and Proteobacteria are more abundant in EU than in African children (43). Importantly, the microbiome of African children exhibits a higher microbial richness and biodiversity than that of EU children. The African samples, but not the EU samples, contain two bacterial genera, *Prevotella* and *Xylanibacter* (43). These findings are consistent with the above-described diet/enterotype concept (42) and suggest that the African microbiomes are dominated by the *Prevotella* enterotype driven by the low-fat/high-fiber diet. *Prevotella* and *Xylanibacter* have enzymes necessary for the hydrolysis of cellulose and xylan and contribute to the production of high amounts of short-chain fatty acids (SCFAs) (43). As discussed below, SCFAs contribute to the maintenance of immune homeostasis in the intestine. Therefore, changes in the diet and gut microbial ecology are likely to affect the metabolic predisposition and immune status of the host.

Inflammation. Alterations in community membership can also be induced by inflammation. In mice, enteric pathogens such as *Citrobacter rodentium* and *Salmonella enterica* subspecies 1 serovar Typhimurium actively induce intestinal inflammation, which then alters the composition of indigenous microbiota, reducing the number of strict anaerobes such as the Firmicutes and allowing proteobacteria to proliferate (47, 48). Intestinal inflammation caused by either a chemical inducer, such as dextran sulfate sodium (DSS), or a genetic deficiency, such as *Il10* deficiency, can also alter the composition of the intestinal microbiota, resulting in a reduction in both the total number of resident intestinal bacteria and bacterial diversity (47). Studies have shown a change in composition of the microbiota in T-bet$^{-/-}$ Rag2$^{-/-}$ ulcerative colitis (TRUC) mice, which have a T-bet deficiency in the innate immune system and develop spontaneous colitis with high penetrance on a BALB/c background (36, 49). TRUC colitis is attributed to the hyperproduction of tumor necrosis factor (TNF)-α by dendritic cells (DCs), because T-bet is a negative regulator of TNF-α transcription (36). The colitis in TRUC mice is accompanied by a considerable alteration in microbial composition. TRUC colitis can be transmitted to wild-type mice when they are cross-fostered or cohoused with TRUC mice (36). Detailed analysis of altered microbiota has revealed a lower proportional representation of Firmicutes,

SCFA: short-chain fatty acid

DC: dendritic cell

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