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Fine-tuning of the mucosal barrier and metabolic systems using the diet-microbial metabolite axis

Motoyoshi Nagai [a], Yuuki Obata [a,b], Daisuke Takahashi [a], Koji Hase [a,b,*]

[a] Division of Biochemistry, Faculty of Pharmacy, Keio University, 1-5-30 Shibakoen, Minato-ku, Tokyo 105-8512, Japan
[b] International Research and Developmental Center for Mucosal Vaccines, The Institute of Medical Science, The University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan

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ABSTRACT

The human intestinal microbiota has profound effects on human physiology, including the development and maintenance of the host immune and metabolic systems. Under physiological conditions, the intestinal microbiota maintains a symbiotic relationship with the host. Abnormalities in the host-microbe relationship, however, have been implicated in multiple disorders such as inflammatory bowel diseases (IBDs), metabolic syndrome, and autoimmune diseases. There is a close correlation between dietary factors and the microbial composition in the gut. Long-term dietary habits influence the composition of the gut microbial community and consequently alter microbial metabolic activity. The diet-microbiota axis plays a vital role in the regulation of the host immune system, at least partly through altering microbial metabolism. In this review, we will describe the current findings regarding how dietary factors and microbial metabolites regulate the host immune system.

1. Introduction

Human beings coexist with an enormous number of microorganisms throughout their lives. These microorganisms, which are mainly bacterial species, colonize the body surface and mucosa to form a complex community, called the microbiota. The population of the human microbiota outnumbers the host cell population by tenfold. The collective genome of the human microbiota is termed the microbiome, which contains at least 100-fold more genes than the human genome [1]. The lumen of the gastrointestinal tract serves as a major habitat for this microbial consortium, forming a specialized ecosystem [2]. Under physiological conditions, the intestinal microbiota stays in the intestinal lumen without inducing pathogenicity. The microbiota has beneficial effects on the host physiology through nutrient supplementation and protection against pathogenic organisms. However, certain pathological conditions such as barrier disruption cause microbial translocation into the body, resulting in the development of systemic inflammation. Furthermore, disturbance of the microbiota-host relationship potentially leads to a chronic inflammatory status, represented by inflammatory bowel diseases (IBDs) [3] and metabolic syndrome [4]. Therefore, establishment of a symbiotic relationship between the microbiota and host is essential to maintain immunological homeostasis.

2. Characterization of the intestinal microbiota

The intestinal microbiota has been poorly characterized due to its complexity and the technical difficulty in culturing individual bacterial species. In 1977, C. R. Woese proposed a 16S ribosomal RNA (rRNA)-based phylogenetic taxonomy [5]. Because mutations of the bacterial rRNA gene are infrequent over the course of evolution, 16S rRNA sequences reflect the relevance of different bacterial species. This information is therefore utilized as bacterial fingerprints to distinguish each species. Recent advances in next-generation sequencing (NGS) technologies have enabled the analysis of microbial compositions by sequencing the 16S rRNA gene of whole microbial community. In addition to the meta-16S rRNA gene sequencing method, whole-genome-sequencing of the microbiome, termed "metagenome analysis," was introduced to comprehensively analyze bacterial genes. In 2008, two international consortiums, the Human Microbiome Project (HMP) and Metagenomics of the Human Intestinal Tract (MetaHIT), were established to characterize the human microbiome. Both the HMP and MetaHIT successfully uncovered an array of human microbiota genome sequences (http://hmpdacc.org) (http://www.metahit.eu) [2,6]. In healthy adults, the concentration of microbes in the large intestine is estimated to be 10^{11}–10^{12} cells/g of luminal content [7], with more than 1000 species identified to date [6]. The healthy human intestinal microbiota consists of two major phyla: Bacteroidetes and Firmicutes, with Proteobacteria and Actinobacteria at lesser frequencies [8]. The Firmicutes/Bacteroidetes ratio in the human microbiota is highly variable among individuals [8]. In addition, individual microbiomes are classified into three distinct enterotypes based on

* Corresponding author at: Division of Biochemistry, Faculty of Pharmacy, Keio University, 1-5-30 Shibakoen, Minato-ku, Tokyo 105-8512, Japan.
E-mail address: hase-kj@pha.keio.ac.jp (K. Hase).

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Table 1
Characterization of gut microbiota during early and adult stages.

	Immediately after birth	Infancy	Adulthood
Traits of gut microbiota	➢ Reflecting the maternal hand-over ➢ Highly susceptible to external factors ➢ Containing facultative anaerobic bacteria	➢ High inter-individual difference in taxonomic compositions ➢ Less stable over time ➢ High relative abundance of Actinobacteria	➢ Dominated by major four phyla: Bacteroidetes, Firmicutes, Proteobacteria and Actinobacteria ➢ High stability of metabolic pathways among healthy individuals
Factors	➢ Delivery mode ➢ Maternal health conditions ➢ Hygiene ➢ Gestation period ➢ Developmental stage of intestinal barrier function and immune system	➢ Breast-feeding period ➢ Hygiene ➢ Gestation period ➢ Developmental stage of intestinal barrier function and immune system	➢ Dietary habit ➢ Dietary factors (e.g., food additives) ➢ Obesity ➢ Disorders (e.g., IBDs, and metabolic diseases)
Representative microbes	Vaginal delivery Maternal vaginal microbes (e.g., *Lactobacillus*, *Prevotella*, and *Sneathia* spp.) Caesarian section Maternal skin microbes (e.g., *Staphylococcus*, *Corynebacterium*, and *Propionibacterium* spp.)	Actinobacteria (e.g. *Bifidobacterium longum* and *B. bifidum*) Firmicutes (e.g. *Lactobacillus* spp.)	Symbiosis Bacteroidetes (e.g., *Bacteroides* and *Prevotella* spp.) Firmicutes (e.g., *Clostridium*, *Eubacterium*, *Lactobacillus* and *Ruminococcus* spp.) Actinobacteria (e.g., *Bifidobacterium* spp.) Dysbiosis Proteobacteria (e.g., *Escherichia coli* and *Yersinia enterocolitica*) Pathogenic bacteria (e.g., *C. difficile*)

the relative abundance of three genera: *Bacteroides*, *Prevotella*, and *Ruminococcus* [9]. Interestingly, functional analyses of whole-microbiome genes revealed that the metagenomic carriage of the metabolic pathway is quite stable among individuals despite high inter-individual variation in taxonomic compositions. These metagenomes encode the full set of genes, which are essential for the biodegradation of complex sugars and glycan as well as the biosynthesis of short-chain fatty acids (SCFAs), essential amino acids, and vitamins [2,6]. Thus, colonization by a well-balanced whole microbiota rather than with one or several particular bacterial species is critical for the microbiota to exert its beneficial functions and to maintain a well-balanced microbial community. On the contrary, an alteration in the microbial community, such as an outgrowth of potentially hostile bacteria as well as a decrease in beneficial species and bacterial diversity, has been termed "dysbiosis" [10]. Compelling evidence indicates the pathological relevance of dysbiosis in multiple diseases such as IBDs [3], diabetes [11,12], obesity [13], and allergic diseases [14]. These findings raise the possibility that the intestinal microbiota could be not only a significant diagnostic marker but also a novel therapeutic target to cure metabolic and chronic inflammatory diseases.

3. Initial formation process of the gut microbiota

Mammalian fetuses are maintained under sterile conditions in utero; however, many different microorganisms colonize immediately after birth and eventually constitute a unique ecosystem on the human body surface and mucosa. As mentioned above, Bacteroidetes and Firmicutes dominate the intestinal habitat in healthy adults [8]. However, at the earliest stage of human life, the majority of gut-residing microbes are facultative anaerobes, including *Staphylococcus* and *Streptococcus* spp. [15,16]. Palmer et al. analyzed the fecal microbiota of full-term newborn babies from healthy mothers and followed up for 1 year to observe dynamic changes in the infant gut microbiota using species-specific DNA probes [15]. This follow-up study revealed that the microbial compositions of infants are highly variable among individuals and less stable over time. The intestinal microbiota during infancy appears to undergo a population rearrangement characterized by a significant reduction in facultative anaerobes and increases in *Clostridium* and *Eubacteria* spp. around 5 days after birth. Colonization by *Bacteroides* spp. was observed in all infants at 1 year, after which the microbial compositions become stable over time [15]. Accordingly, it is estimated that strong selective pressures operate to shape the functionality of the microbial community during the maturation process. In infancy, there are possible variables such as delivery mode, maternal health conditions, hygiene, gestation period, and breast-feeding period that shape the gut microbiota. For example, the gut microbiota of an infant delivered through the vagina is mainly populated by *Lactobacillus*, *Prevotella*, or *Sneathia* spp., which are mainly derived from the maternal vaginal microbiota. On the other hand, the microbiota of an infant delivered by Caesarean section is dominated by *Staphylococcus*, *Corynebacterium*, and *Propionibacterium* spp., which are found on the skin surface [17]. (See Table 1.)

It has been well documented that the relative abundance of Actinobacteria, especially *Bifidobacterium*, is significantly high in the infant gut microbiota during the breast-feeding period according to culture-based studies [18,19]. This microbial trait was also confirmed by species-specific DNA probe-based and metagenome sequencing-based studies [15,20]. The overrepresentation of *Bifidobacterium* is widely observed in breast-fed children in different countries and regions with distinct dietary habits, such as rural Africa and Florence, Italy [21], suggesting the significance of breast-feeding in the development of the infant gut microbiota. Furthermore, epidermal growth factor (EGF), IgA, lactoferrins, and various cytokines in breast milk potentially contribute to development of the infant gut immune system and microbiota [22–24]. The maternal skin microbiota and breast milk-derived microbes also affect the formation of the intestinal microbiota in the child [25]. (See Table 1.)

4. The influence of daily diet on the intestinal microbiota

Aside from breast milk, the overall diet is a major factor that influences the composition of the intestinal microbiota. A comparative study on intestinal microbiota between children in a rural village in Burkina Faso and Florence in Italy confirmed the importance of daily diet in determining the composition of the gut microbiota [21]. The diet of the African children was low in fat and animal protein and rich in starch fiber and plant polysaccharides. In sharp contrast, the diet of the European children was high in animal protein, sugar, starch, and fat and low in fiber. The microbiota of the African children had a higher microbial diversity than that of the European children [21]. The frequencies of *Prevotella* and *Xylanibacter*, both of which belong to the phylum Bacteroidetes, were predominant in the microbiota of the African children. These species possess a set of genes for cellulose and xylan hydrolysis and facilitate energy extraction from these polysaccharides [21,26]. A cross-sectional analysis of healthy volunteers revealed a correlation between food frequency questionnaire (FFQ)-based long-term dietary patterns and microbial compositions [27]. For instance, a diet containing a high amount of animal protein, amino acids, and saturated fatty acids

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