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Current Status and Prospects of Intestinal Microbiome Studies

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The incidence and prevalence of inflammatory bowel disease (IBD) in Asia has witnessed a rapid increase within a few decades. The genetic susceptibility and epidemiologic backgrounds in the Asian population have been found to be different from that of Western populations. There is an extensive crosstalk between gut microbiota and human hosts, with evidence of reciprocal interactions. It is well known that gut microbiota can affect the host immune system and in turn, host genetic backgrounds can affect gut microbiota reciprocally. Evidences have implicated gut microbes in the development of IBD, but no causative microorganisms have been identified. Recent advances in sequencing technology and computational analysis have now made identification of complex gut microbiomes accessible. Further research targeting gut microbiota could help in identifying biomarkers to predict clinical response, and therapeutic modalities that might affect their resilience. **(Intest Res 2014;12:178-183)**

Key Words: Inflammatory bowel disease; Microbiota; Host microbial interaction

INTRODUCTION

There are many microbes forming communities inside the intestine, maintaining homeostasis, and performing other functions within their hosts.[1,2] Inside a human body, there are ten-fold more microbiota than human cells. Why did microbiota come to exist in such large numbers? Gut microbiota would provide a beneficial symbiotic relationship with human hosts. It is possible that humans evolved to cooperate with the microbiota to share micronutrients in this symbiotic relationship. The gut microbes play a role in human metabolism and the immune system, protecting hosts from pathogenic bacteria, and reacting to medical therapies. A change in major phyla in the intestine is known to be associated not only with IBD, but also with obesity, allergy, autism, and many other diseases. This article reviews important facts about gut microbiota, highlighting both the current status and future prospects of microbiome research.

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ANALYSIS OF INTESTINAL MICROBIOME

The gut microbiome is a community of microbes, which lives in the gastrointestinal tract, the microbiome being the sum of all microbial organisms that live in or on the host. It includes bacteria, viruses, fungi, and archaea. Metagenomic analysis refers to the structural and functional studies of this gut microbial society.[2] Early efforts in characterizing the gut microbiome were limited to those microbes that were cultivable *in vitro*, limiting the verifiable bacteria to few hundreds. Recent advances in sequencing technologies and computational biology have led to easier use of metagenomic approaches for characterizing microbial communities, which led to profound activities for microbiome or metagenomic researches in the gut. These advances allowed for large-scale studies, such as the Metagenomics of the Human Intestinal Tract (MetaHIT) project,[3,4] and the Human Microbiome Project (HMP).[5,6] These studies were instrumental in in-

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forming us of diverse composition of microbes in different human communities and their biological functions. There is significant inter-individual and interspecies variability in the host microbiome, but there had conserved metabolic pathways.[3] Also "core microbiome" is existed in topographically different places in each. Over 57 species were demonstrated in most of the study cohort.[7]

Rapid development of sequencing technologies and computational biology aids not only in understanding of the importance of compositional changes of the microbiome in various disease, but also in functional analyses of the microbiome. Although system biology can aid in such analyses, its relatively recent and rapid development makes it unfamiliar or even strange for many clinicians. In the last decade, we learned about microbial communities, their diversity, and correlation with diseases. The current popular method based on 16S rRNA sequencing is confined to analyzing bacterial composition and distribution in a specific community.[8] Although a database based on most of the species detected by 16S rRNA has been established, technical limitations in identifying bacteria prevents analysis at the species level, which is necessary. Hence, analyzing detailed physiological, genetic, or functional characteristics of bacteria in a certain communities is controversial. Additionally, 16S rRNA-based PCR as a technique limits the analysis of communities solely to the identification of bacteria. A new method that has received considerable attention, named short-gun sequencing, sequences DNA directly, allowing microorganisms other than bacteria to be identified. This method can directly analyze the bacterial protein-encoding region from the acquired DNA and analyze the genus level, with results depending on sequencing depth and complexity of the communities. This method requires an expensive and sophisticated computer program that can handle large datasets, requiring technical expertise.[9,10] Meta Genome Rapid Annotation using Subsystem Technology (MG-RAST) (http://metagenomics.nmpdr.org), Quantitative Insights Into Microbial Ecology (QIIME) (http://qiime.sourceforge.net/), and mothur are widely used in 16S rRNA studies. MG-RAST is a free program that can align DNA sequence and assign functional phylogenetics.[11] QIIME uses a barcode against sample specimens to tag the 16S rRNA gene and sequence it. Then, it aligns the sequences before making phylogenetic tree or clustering by locating taxon specific regions, which allows high resolution taxonomic classification (especially accurate up to the family level).[12] Mothur, an open-source, platform-independent, community-supported program for describing and comparing microbial communities aims to be a comprehensive software. It can analyze unique sequences and can apply sparse metrics.[13] There are multiple methods for comparing communities. Changes in composition of the community can be checked by 16S rRNA based gene analysis, showing alpha or beta diversity. For 16S rRNA community analysis by sequencing, alpha diversity represents the richness of microbes in the community and is expressed as an abundance-based coverage estimator or the inverse Simpson index.[10,14,15] Beta diversity estimates the differences between two groups in the community using a principle coordinate analysis graph. For the analysis of short-gun sequencing datasets, Metagenomic Phylogenetic Analysis (MetaPhlAn), The HMP Unified Metabolic Analysis Network (HUMAnN), and other softwares are widely used for analysis. MetaPhlAn is a computational method for profiling the composition of microbial communities from metagenomic shotgun sequencing data.[16] It relies on unique, clade-specific marker genes identified from 3,000 reference genomes. HUMAnN can analyze metagenomes based on results from HMP.[17] MetaPhlAn and HUMAnN are widely used to analyze short-gun sequence. Different softwares should be used for analysis, regardless of whether result sequences are assembled or not.[10] Kyoto Encyclopedia of Genes and Genomes (KEGG) is a large scale molecular database resource for understanding high-level functions and utilities of biological systems, such as a cell and an organism, generated by genome sequencing and other high-throughput experimental technologies. This is generated by organizing and computing metabolic pathway data in terms of binary relations.[18,19] It has been used as a standard reference for functional studies. Changes in composition of microbiota and its metabolic functions are crucial for homeostasis of the immune system, and development of IBD. Functional analysis of the microbiome can be achieved using metabolomic analysis by chromatography and mass spectroscopy. It can facilitate understanding of microbial and host metabolic profiles, and is easy to perform even with small amount of samples. However, there is a lot of ambiguity in database repositories, a tedious process of identification, and no standardized protocols for the same.[20]

CROSSTALK BETWEEN MICROBIOTA AND HOST

Gut microbiota provide beneficial effects to the human body,[21] being implicated in short chain fatty acid metabolism, immune network development, protecting the host against pathogens, and in affecting drug metabolism such as anti-cancer drugs. The human host maintains distinct microbiota in different areas. The intestinal epithelium

separates the host from microbiota in the lumen, utilizing mucins, secretory IgA, antimicrobial peptides, and a variety of cytokines.[1] There is a thick mucus layer above the intestinal epithelial layer. Although there is a difference between the small intestine and colon, RegIIIγ is expressed in epithelial cells and is involved in constructing compartments to limit bacterial penetration, thus restricting contact between bacteria and host.[22,23] The mucosal immune system maintains homeostasis as physiologic inflammation by control of interleukin-10 (IL-10) and transforming growth factor-β, which are produced from response to microbial stimuli. The intestine of newborns stays sterile and its colonization begins by maternal feeding and environmental stimuli.[24] The functional maturation of the gut microbiome has been identified during the first 3 years of life, and the gut microbiome has been observed to converge to more similar phyla at later stages. Along with age-associated changes in the genes involved in vitamin biosynthesis and metabolism, bacterial assemblages and functional gene repertoires were found to be different between populations. Its features are evident in early infancy as well as adulthood.[25] Antibiotics also alter microbiota in the intestine, affecting metabolic and immune regulation.[26]

Intestinal microbiota has a homeostasis with human hosts. The host-microbial cross-talk plays a critical role in the maturation of the host immune system. It is well known that there is a delayed development of Peyer's patches in the small intestine, and an altered secretory IgA response in germ-free animals.[27] A genome-wide association study revealed many genetic loci underlying IBD susceptibility, and most of them were found to be associated with a host response to bacteria, such as pattern recognition or antigen processing.[28]

NOD2 knockout mice have an impaired function for antimicrobial defense by paneth cells of the small intestine.[29] This impairment loosens the epithelial barrier and allows translocation of pathogenic bacteria. In addition, colonization with segmented filamentous bacteria resulted in polarization of T helper (Th) 17 cells within the lamina propria of the intestine.[30,31] Polysaccharide A from *Bacteroides fragilis* induces IL-10 from T cells, and prevents the expansion of mucosal Th17 cells.[32] Intestinal microbiota decay dietary fiber, which humans cannot digest, and produce short chain fatty acids (SCFAs) important for epithelial cell survival. Colonic regulatory T cells are critical for controlling intestinal inflammation. These cells depend on microbiota-derived signals for development and maintenance.[33,34] SCFAs are also related with regulatory T cells in the intestinal mucosa and affects their activity.[35] The change in SCFA also affects systemic regulatory T cells. On the other hand, the host phenotype also affects intestinal microbiota. Mice lacking NLRP3 exhibit a low-grade intestinal inflammation and it depends on overgrowth of *Prevotellaceae* and *Porphyromonadaceae* of *Bacteroidetes* phyla.[36] The mucosal immune systems allows commensals become tolerant to systemic immune reactions.

It is well known that the intestinal microbiota can control the mucosal immune system and maintain its functional integrity. The microbiome controlled host phenotypes, while itself being modified by host phenotype. The intestinal microbiota had profound impacts on a mammalian host and its metabolism.

MICROBIOTA AND IBD

The importance of microbiota in IBD has been continuously highlighted over the years. A hypothesis suggesting the similarity in CD and Johne's disease, with colitis in ruminants was proposed, and another hypothesis suggesting a role for some microbes in the development of IBD.[37] It was well known that IBD might result from an uncontrolled immune response to exaggerated stimuli from commensal microbiota of the intestine in genetically susceptible hosts. Intestinal bacteria play roles in driving and perpetuating IBD.[38] For instance, the change of fecal stream, like that from a colostomy conduit, has been reported to improve colitis. Additionally, antibiotics, such as rifaximin, are reported to attenuate inflammation in refractory pouchitis.[39] A large epidemiologic survey showed that prevalence of CD increased with antibiotic use during infancy.[40] Bacteria also play a role in animals. Although colitis occurs in IL-10 knockout mice, (known as an animal colitis model under specific pathogen-free conditions), it does not occur in germ-free condition.[41] A similar phenomenon is observed in human leucocyte antigen-B27 transgenic rats. When antibiotics were applied to myeloid differentiation factor 88 (Myd88) knockout mice, spontaneous colitis was reported to be exacerbated.[42] Polysaccharide A from *Bacteroides fragilis* was observed to induce IL-10 from T cells, thereby preventing the expansion of mucosal Th17 cells.[32] This means that the microbiota affects the host immune system directly or indirectly. It is not certain whether intestinal microbiota evokes intestinal inflammation directly and eventually leads to development of IBD. Garrett et al. confirmed that a similar form of colitis occurs when the microbiome from T-bet –/– Rag2 –/– ulcerative colitis (TRUC) mice is transplanted in regular mice lacking

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genetic susceptibility (wild type fostering mice). *Enterobacteriaceae* were reported to act in concert with the endogenous gut microbiota to induce spontaneous and maternally transmitted colitis.[43]

The incidence of IBD has risen rapidly not only in Korea, but also in the rest of Asia, with these changes occurring over a short period of time. There have been dramatic changes in lifestyles brought about by many factors, including refrigerator use, a Westernized diet, a nation-wide parasite eradication program and the wide use of antibiotics in recent decades.[44] This could indicate that, apart from factors such as genetic susceptibility being important for development of IBD, environmental changes like the microbiota could be more important as causative factors for the development of IBD. It is interesting to note that known IBD susceptibility genes like *NOD2* and *ATG16L1* were absent in Koreans, Japanese, and Chinese. A male preponderance, frequent perianal involvement, and frequent ileocecal involvement were noted. There was same pattern of change in the intestinal microbiota in Korean CD, having a reduced diversity, a decrease in *Firmicutes*, *Bacteroidetes*, and an increase in *Proteobacteria* and *Fusobacteria*.[45] Even across different epidemiologic backgrounds and despite having different genetic susceptibility genes, microbial communities have been reported constant within certain Western populations. Consequently, microbial changes affected by foods or other environmental factors are more important in development of IBD.

Firmicutes and *Bacteroidetes* constitute most of the microbiota in healthy people. The human gut contains few bacterial phyla, but is extremely diverse at the strain and subspecies levels. Microbial diversity is decreased in CD patients.[46] There are differences evident in the composition of microbiota between stool and tissue specimens. The core phyla, *Firmicutes* and *Bacteroidetes*, are decreased and *Proteobacteria* increases exceptionally in CD patients.[47] *Fecalibacterium* and *Roseburia* were decreased. Some potentially anti-inflammatory species, such as *Faecalibacterium prausnitzii*,[48] are also reduced. By contrast, a greater abundance in *Enterobacteriaceae*, particularly *Escherichia coli* species, has been observed in mucosal tissues of CD patients.[49]

Adherent invasive *E. coli* has been isolated from biopsied samples and the changes are more remarkable in mucosal specimens than in fecal samples.[50] Mesalamine decreased intestinal inflammation, and is associated with a reduction in *E. coli* and *Shigella*. There was also a shift in oxidative pathways in CD.[51]

The role for compositional changes of the microbiota in IBD subsets still remains unclear and there is no distinct evidence on whether specific strains induce IBD. Changes in composition of microbiota in the intestine may also contribute to clinical courses of disease, disease severity, and drug responses. Therefore, a comprehensive understanding about the gut microbiome should be acquired to pinpoint its precise role in the disease context.

CONCLUSIONS

The intestinal microbiota play important roles in maintaining metabolic function and immunologic homeostasis in the human body. Recent animal studies showed colitic microbiota could be transmitted to hosts with normal immunological backgrounds. By high-throughput pyrosequencing and computational biology, we learned about compositional changes of microbiota and its metabolic pathways in the intestines. Currently, knowledge from microbiome research could be applied to develop biomarkers as predictors of treatment responses or risk stratification factors for prognosis, and not only as a causative agent of IBD. Like fecal transplantation, which was proven to be an effective treatment modality for recurrent *Clostridium difficile* infection,[52] treatment modalities using fecal microbiota could be another option for the treatment of IBD.[53] However, there is a long way to go, in terms of our understanding the exact roles of intestinal microbiota in the human body. Using only limited data from genotoxic animals, the gastroenterologist should understand recent metagenomic study results related with IBD. Therefore, a comprehensive understanding of composition of microbiota and functional analysis of metagenomes might lead us into a new era of knowledge about the pathogenesis of IBD.

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