

# Potential Impacts of Gut Microbiota on Immune System Related Diseases: Current Studies and Future Challenges

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## ABSTRACT

Human microbiota includes trillions of cohabitant microorganisms in a symbiotic relationship. They directly or indirectly communicate with immune system. Human microbiome profiling studies has accelerated microbiota studies and interest to microbiota and disease relationship . Some metabolic activities of human microbiota were known, but in recent years many different roles in addition to metabolic activities, have been shown. It can affect systems and mechanisms, and most importantly clinical course of diseases in dysbiosis condition, and reduces most symptoms when symbiosis is provided. These features make microbiota a potential therapeutic tool or biomarker for a spate of disease in clinic applications. This review summarizes host-gut microbiota interaction, role of microbiota in immune-related diseases, and potential therapeutic approaches.

Keywords: Gut microbiota, microbiome, immune system related disease

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## INTRODUCTION

The human microbiota consists of bacteria, archaea, viruses and eukaryotic microbes that live in and on our bodies, and corporate genome of our microbial symbionts, commensal and pathobiont (potential pathogenic microbes) are called as microbiome [1]. Population of diverse microbial species on or in human body is about one-tenth of the number of human cells [2]. Their total genes are more than 100 times that our own genes, and they have more diverse genomic capacity than their host [3-4]. Therefore, these microbes have enormous possibility to affect our physiological conditions in the case of both health and disease. They have critical roles on metabolic functions, protection against pathogens, and education of our immune system. Also, our physiological capacities are influenced by these essential functions straightforwardly or indirectly [5]. Increase in knowledge of advance molecular biology and recent technological advancements for performing culture-independent analysis help to show great progress in microbiome researches (Figure 1). The Human Microbiome Project (HMP) several complementary analyses such as 16S rRNA gene sequence (16S), gene including regions

of highly conserved sequence, and taxonomic profiles, whole genome shotgun (WGS) or metagenomics sequencing of whole community DNA, which make easy to study microbiome. Also, reference microbial genome from human body is formed by HMP. In addition to HMP, the MetaHIT project used WGS data in order to examine the gut microbiome in a cohort with different health status and physiological characteristics [6-7]. Optimized sequencing protocols, increased knowledge of taxonomic classification, ability to form operational taxonomic unit (OTU)-based community structure decreased bias generated by these studies. Therefore, in order to understand host-microbiota interactions and to explain potential impact of microbiota on disease, interest to microbiome researches has increased incrementally [4] (Figure 1). According to HMP data, working on soft tissue (mid vagina, anterior nares, and throat) and saliva is challenging because of higher human genome contamination, while studying gut microbiome is more preferable due to a relatively low abundance of human reads (up to 1%) in total reads.

Immune system and gut microbiota relationship

is one of the well-studied area among microbiome studies, however the effects of microbiota or dysbiosis (microbiotic changes in systems and host's negative response to these change) on immune-related and auto inflammatory diseases has

been trying to explore. In this review, we highlighted researches on relationship between microbiota and immune diseases mainly inflammatory conditions. Moreover, we discussed current and potential therapeutic approaches

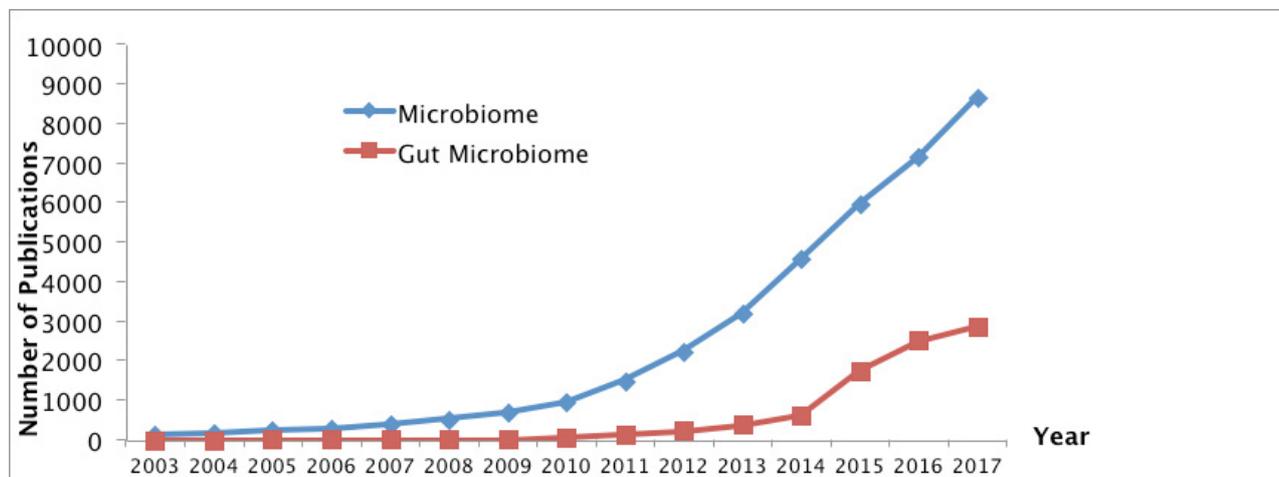


Figure 1. Number of publications on microbiome and gut microbiome from 2003 to 2017.

### Factors affecting gut microbiota

Gut microbiota is not a stable organ which is affected by a lot of factors result in deviation from core gut microbiome (Figure 2). The microbial composition of the gastrointestinal tract in humans shows noteworthy changes through the lifespan (Figure 3). During childbirth the gastrointestinal tract of infants is seen to be sterile. However, during or following birth, microbiota forms quickly by bacteria transferred from mother. Infant feeding method and mode of delivery shape the microbiota in infants. Breast fed babies sustained children have a tendency to basically support Bifidobacteria in their gastrointestinal tract while those of bottle-fed babies have a more various bacteria population [8]. Transition to solid food helps to increase microbial composition of the gut of infants like

adult gut microbiota diversity in the way of increase in the abundance of anaerobic Firmicutes [9-10]. In the healthy state, Firmicutes, Bacteroidetes and Actinobacteria are dominant bacterial phyla in post weaned children and adults [9-11]. Aging also changes the microbiota composition of gut. Numbers of facultative anaerobes show increasing trend, on the other hand, gram-negative bacteria (mainly Enterobacter) and number of good bacteria like Lactobacilli and Bifidobacteria show decreasing trend with age [12-13]. In addition to age, diet and lifestyle, host genetics, environmental contact, infection, pharmaceuticals, hygiene conditions, relationship between microbiota and immune system, even geography cause variation of gut microbiota (Figure2) [9-10].

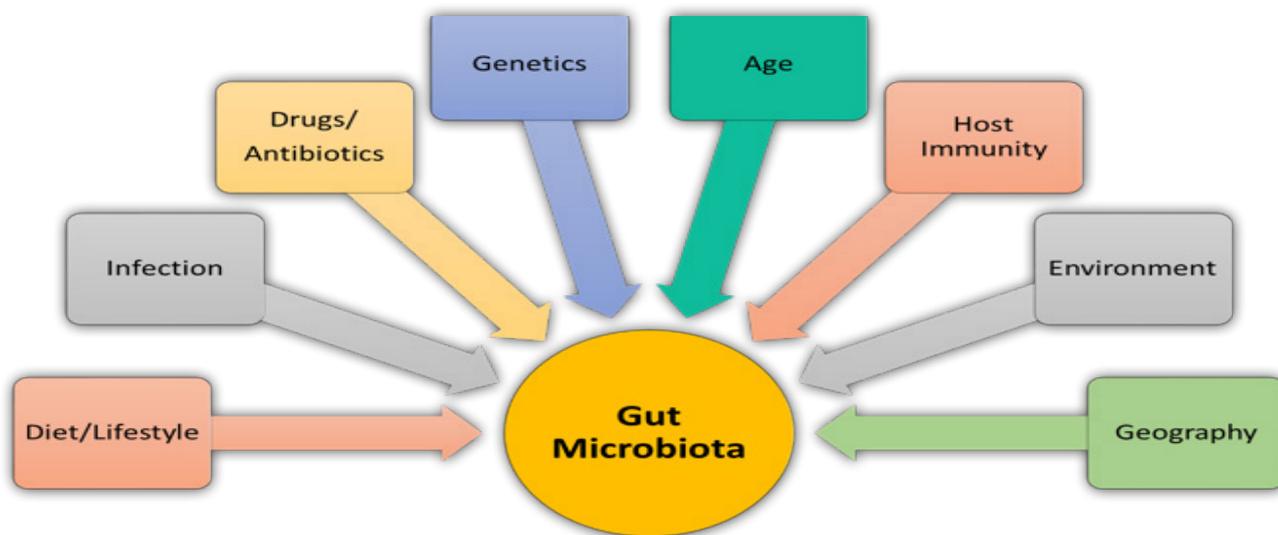


Figure 2. Factors affecting gut microbiota composition.

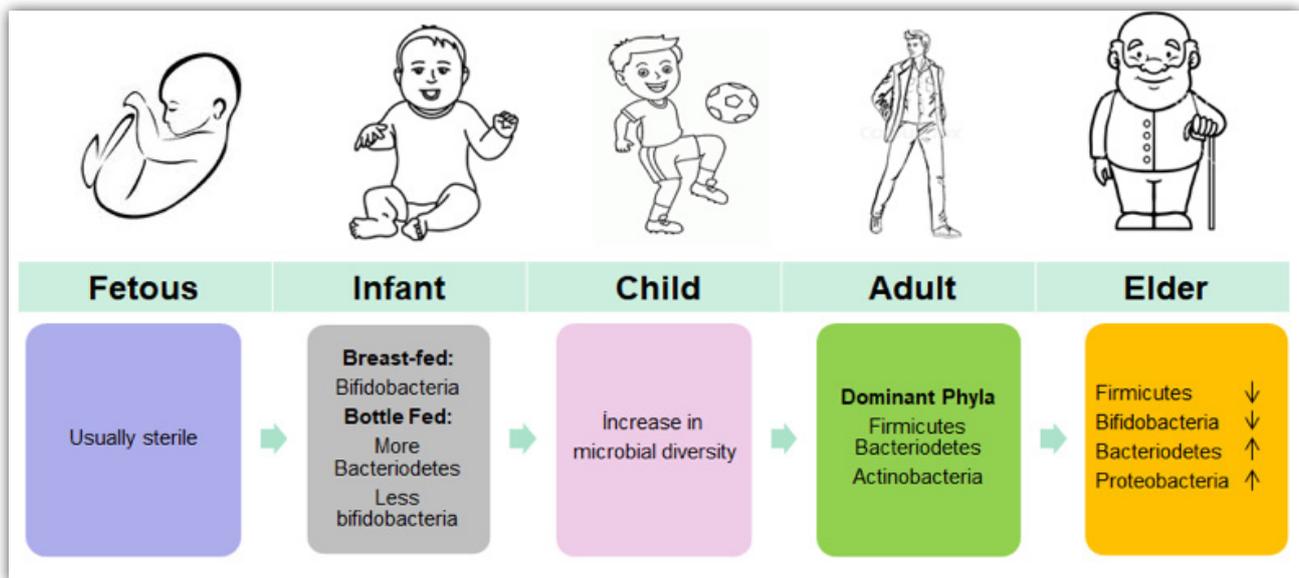


Figure 3. Formation and alteration of microbiota during lifespan.

### Metabolic activities of gut microbiota

Gut microbiota have functional activities like food metabolism, xenobiotics and drug metabolism, anti-microbial protection and immunomodulation. First of all, symbiont bacteria and pathobiont bacteria live together in gut and some symbiont bacteria degrade indigestible fibers which human digestive system do not. Xylosglucan, a type of high branched plant fiber, can be converted to xylose which can be absorbed by epithelial cells with the help of rare *Bacteroides* strain [14]. This example also indicates how our gut microbiota and human forms are in a mutual relationship. Gut microbiota is capable of production of short chain fatty acids (SCFAs)

from indigestible food components. These SCFAs vary but most abundant ones are butyrate, acetate and propionate in gut. These SCFAs lower cholesterol level and control blood glucose level since they are important for energy metabolism. Cholesterol homeostasis in the peripheral tissue is controlled by acetate and propionate which are substrates for gluconeogenesis in the liver. On the other hand, colonocytes use butyrate, a mediator of cell proliferation as an energy source [15,16].

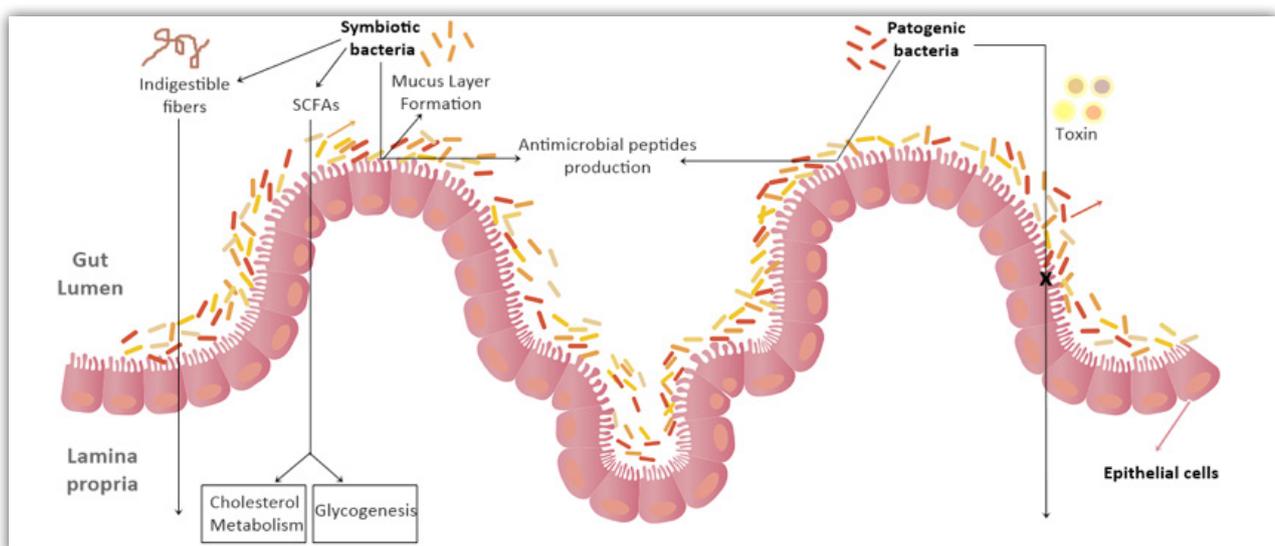


Figure 4. Roles of microbiota in the gut.

Crosstalk between Gut Microbiota and Immune System  
Gut microbiota undertake an essential role in the maturation and development of adaptive and innate immune system. Symbiotic bacteria convert diet fibers to fucose which is important for regulation of virulence factor [17]. Healthy gut epithelium surrounded by a layer composed of mucin glycoproteins that are secreted by the intestinal goblet cells. Mucus layer can be extended up to 150  $\mu\text{m}$  away from the colonic epithelium. Signals coming from bacteria in gut lumen promote cells to make mucus synthesis, important for epithelial barrier function [18-19]. Genetic deletion in mucin 2 gene causes colitis, which shows the important role of mucus production stability of physical barrier of gut [20]. In addition to mucin proteins, to maintain barrier integrity, goblet cells also produce resistin-like molecule- $\beta$  (RELM- $\beta$ ) [21]. Interleukin-22(IL-22) induces the production of the antimicrobial peptides, such as REGIII $\beta$  and REGIII $\gamma$ , from intestinal epithelial cells, which is a critical role on defending against pathogens. These peptides can affect not only pathogens but also symbiotic microbiota. Gut microbiota has a critical role on regulation and development of specific lymphocyte subsets. T helper 17 (TH17)-a type- helper CD4+ T cells- are critical for cellular defense against pathogens and secreting interleukin (IL)-17A, IL-17F, IL-21, and IL-22 [22]. Most commensals live in the gut lumen, isolated from the host mucosal immune system, but some can reach epithelial cells and can modulate host immunity and disease status [23]. For example, segmented filamentous bacteria (SFB), which can breach the intestinal mucus layer and attach to intestinal epithelial cells, are potent inducers of lamina propria T helper 17 (TH17) cells. Furthermore, *Bifidobacterium adolescentis*, a single species of human commensal, is sufficient to induce TH17 cells[24-26]. Gut microbiota and immune system relationship has complex mechanism and this relationship have not fully understood yet. Further information on these mechanisms can help to solve challenging steps of host-microbiome interaction.

### **Immune system related diseases and gut microbiota familial mediterranean fever (FMF)**

MEFV which encodes pyrin, cause familial Mediterranean fever (FMF; MIM249100), the most common auto-inflammatory disorder which is characterized by recurrent serositis or arthritis attacks and, in some patients, chronic sub-clinical inflammation that predisposes to secondary amyloidosis [27,28]. In 2008, Z.A. Khachatryan et al have studied microbiome profile of 15 FMF patients (12 patients in remission and 3 patients in attack periods) and 7 healthy individuals by using 16S rDNA library. The results of this study showed that cluster IX of Propionate-producing bacteria's proportion was found higher compared to healthy controls, and also this bacteria's tendency was toward disappearance during the attack period. The butyrate producing- *Faecalibacterium* group and *Acidaminococcaceae* subgroup was higher in active FMF compared to both FMF remissions and controls [29]. Further study of Z.A.

Khachatryan and his group did microbiome research on 19 FMF patients and 8 healthy individuals by sequencing 16S rDNA and by doing specific probe based fluorescence in situ hybridization (FISH) analysis. During attack period of FMF patients, total number of bacteria, and its diversity decreased significantly and major shifts in bacterial populations within the Bacteroidetes, Firmicutes and Proteobacteria phyla. In remission period, gut bacterial diversity with some deviations was close to the control group [30]. Therefore, MEFV mutations changes host-microbiota interactions and diversity of gut microbiota in different periods of the disease.

### **Inflammatory bowel disease (IBD)**

Inflammatory bowel disease (IBD), including Crohn's disease and ulcerative colitis, is characterized by chronic relapsing intestinal inflammation with increasing incidence worldwide. Etiology of IBD remains largely unknown, but cumulative effect of genetic, immunological and microbial activities cause inflammatory bowel disease [23]. Ulcerative colitis (UC) affects the entire large intestine (colon), but Crohn's disease can affect any part of digestive tract from mouth to anus. A large number of study showed that UC is a result of the immune cell response to the constant antigenic stimulation of intestinal microbiota and the corresponding metabolites [31]. According to study with a group of patient (35 UC patients and 60 patient with no IBD) in 2017, Zamani et al. found that Enterotoxigenic *Bacteroides fragilis* (ETBF) strains are important intestinal bacteria and can be related with development of UC and a causative effect for the formation of diarrhea in these patients [32]. The disease state was associated with increased abundance of Enterobacteriaceae, Fusobacteriaceae, Pasteurellaceae, and Bifidobacteriaceae[33]. In bacteria level, adherent-invasive *Escherichia coli*, *Bifidobacterium* abundance increased in CD and *Odoribacter*, *Roseburia*, *Faecalibacterium prausnitzii* abundance decreased[33-36]. Understanding the detailed microbial dynamics in gastrointestinal tract may help to further therapeutic applications for IBD.

### **Behçet's syndrome (BS)**

Behçet's syndrome, disease with dysimmune inflammatory mechanisms with a genetically susceptible background, is characterized by ocular manifestations, skin and/or gastro-intestinal involvement [37-38]. In 2008, C. Consolandi et al. did microbiome profiling study in a group of people (22 patients with Behçet's syndrome and that of 16 healthy cohabiting controls, sharing the same diet) by using pyrosequencing of 16S rDNA library and biochemical analysis. Genera *Roseburia* and *Subdoligranulum* decreased in Behçet's patients. In addition, significant decrease of butyrate production was also shown. Butyrate production is important for both reduced Treg responses and activation of immuno-pathological T-effector responses[38]. This study indicated that microbial changes in gut is very important in Behçet's Disease pathogenesis.

## Spondyloarthritis (SpA)

Spondyloarthritis (SpA) is a family of immune-mediated inflammatory rheumatic diseases with inflammatory back pain, axial skeleton arthritis, uveitis, gastrointestinal inflammation, and dermatological involvement, as well as a genetic association with the HLA-B27 allele and that includes ankylosing spondylitis (AS), psoriatic arthritis (PsA), juvenile spondyloarthritis (JSpA) and acute anterior uveitis [39-41]. According to study with a group of spondyloarthritis (SpA), approximately half of SpA patients had gut inflammation and further studies showed that there was an expansion of a pathobiont which is shown by using 16S rDNA sequencing [42]. In another microbiome study in SpA patient showed number of Firmicutes number decreased in gut microbiota and like most of inflammatory disorders, *Faecalibacterium prausnitzii* and *Clostridium leptum* have been found in decreasing trend [39]. In order to understand underlying mechanisms of SpA in microbial level, animal models (HLA-B27 transgenic rats) which can mimic the disease were used and as a result of these studies, probiotic or prebiotic treatment and fecal transplantation have become more important besides diagnosis of disease and specific antibiotic treatment.

## Therapeutic approaches

Most common therapeutic approach to dysbiosis and inflammation in gut is the use of anti-microbial drugs. These drugs can affect immune system, suppression of diversity of harmful bacteria, protection against bacterial invasion and blocking the transportation of bacterial metabolites [43]. For example, Sulphasalazine is a type of anti-rheumatic drug used in the treatment of IBD, SpA [43]. The antibiotics; macrolides, levofloxacin, dapson, ceftriaxone and metronidazole, are used in therapy of autoimmune disorders [15]. However, adequate amount of antibiotics usage is important for recovery of gut microbiota and turning the tables on symbiosis.

Probiotics are microorganisms, which have useful health effect on the host and the maintenance of positive correlation in gut. Probiotics and its relationship with gut health have been studied very often in recent years, thus research on the effect of probiotics on disease treatments has accelerated. A.Z. Pepoyan et al. study done in 2017 in a group of FMF patient, Narine, a type of commercial probiotic, composing of *Lactobacillus acidophilus* INMIA 9602 Er-2 strain 317/402 helps to be grown gut commensal *Escherichia coli*. Bacteriocin acidocin LCHV, a small anti-microbial peptide, is produced by this strain and it has a broad spectrum of activity against human pathogens, including methicillin-resistant *Staphylococcus aureus* and *Clostridium* [44-46]. Another study on 10 Crohn's Disease (CD) patients (5 patient in remission and 5 patient with active disease period), showed that,

*B. pullicaecorum* 25-3T and the mix of six butyrate-producers treatment helped to boost intestinal health by improving barrier integrity. This study led up to that butyrate-producing bacteria has a therapeutic potential in CD as probiotic after enlightening the mechanisms of action and identify the metabolite/s or bioactive compound/s that helps to promote intestinal epithelial barrier integrity [47].

Another therapeutic approach is fecal microbiota transfer (FMT) or fecal bacteriotherapy which is the delivery of stool from a healthy donor into a patient, either by enema, colonoscopy, or via the upper gastrointestinal (GI) tract (oral capsules, nasogastric, nasoduodenal or nasoenteric tube, or endoscopy) [48]. First reported FMT therapy was done in IBD in 1989 by a doctor who was suffering from UC and being symptom free at 3 and 6 months post transplantation without medication was reported. Patient with early phase refractory CD showed clinical recovery, but it was last up to 18 months. After 18 month, continuous or repeated FMT can be needed in order to maintain the therapy. FMT-related mechanisms have not been fully identified. Therefore, scientists are looking for a safer method of FMT or alternative techniques that can be used instead of FMT in therapy. New approach to overcome the problems is the use of synthetic microbiota, a mixture of selected bacteria, which can be efficient in treatment for some well-studied diseases. These can pave the way for elimination of the risk of transmissible disease which is the potential side effect of FMT treatment [49].

## CONCLUSION

Studies in the human gut microbiota have changed how researchers consider pathophysiology of most of disorders. Although available sequencing tools are still not perfect, 16S sequencing gives us data in genus level, and species and even strain level data can be obtained by whole bacterial genome sequencing or metagenomics approaches. Effective tools used to pull out consequential patterns from wealth of data. Technical achievements in sequencing make identification of host regulatory or inflammatory pathways that are modulated by the microbiota feasible. Furthermore, a deeper understanding of the efficacy of prebiotic like supplements, probiotics and fecal microbiota transfer to maintain gut microbiota make important contribution to therapeutic tools in human immune system related diseases.

## CONFLICT OF INTEREST

The authors have declared that no conflict of interest exist.

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