Short Review

Methods to Prevent or Treat Refractory Diseases by Focusing on Intestinal Microbes Using LPS and Macrophages

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Abstract. *Intestinal microbes are known to influence host homeostasis by producing various substances. Recently, the presence of a diverse range of intestinal microbiota has been shown to play a key role in the maintenance of health, along with influencing the host's innate immunity towards various diseases. For example, fecal microbiota transplantation (FMT) from healthy individuals was remarkably effective in cases of refractory Clostridium difficile colitis. Conversely, decreased number of intestinal microbes resulting from the oral administration of antibiotics reportedly suppressed the antitumor effects of immunotherapy or anticancer drugs. Furthermore, it has been shown that a change in the intestinal environment triggered by oral administration of antibiotics resulted in increased number of drug-resistant microbes causing nosocomial infections. Intestinal microbes are also shown to be effective in cancer treatment as they activate macrophages at the site of cancer. One of the effects of intestinal microbes on hosts that has been gaining increasing attention is the biological regulation caused by the lipopolysaccharides (LPS) produced by Gram-negative bacteria. Among the intestinal microbiota present in the host, Gram-negative bacteria form the most dominant flora. The administration of antibiotics leads to a decreased number of intestinal microbes, as well as to suppression of cancer immunotherapy effects or anticancer drug effects, and this deterioration has been shown to be improved by oral administration of LPS. In this article, we discuss the functions of intestinal microbiota, that is currently undergoing a paradigm shift in relation to maintenance of health and the validity of LPS as a possible target for bio-treatment in the future.*

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The Diversity of Intestinal Microbes Helps Maintain Health

In recent times, intestinal microbes have been attracting considerable attention in the scientific community (1-4). Recently, it was estimated that there are approximately 500-1,000 different types of intestinal microbes present with a total number of 100 trillion and a weight of approximately 1 kg (5). Although their physiological functions remain uncertain, the association of intestinal microbiota with maintenance of health has been reported in many articles, with probiotics being a common example. As intestinal microbiota consist of diverse genera, they are truly characterized by their complexity. The relationship between the health status of an individual and the intestinal microbiota, as inferred from the aforementioned factors, suggests the presence of an ordered status, which is expressed as a result of an interaction between human complexity and intestinal microbiota complexity. Therefore, it is probably safe to assume that parts of the maintenance of health or disease prevention may be attributed to this complexity. For this reason, we expect that methods that consist of many components and deal with this complexity will gain importance in the future. Intestinal microbes have functions, such as the synthesis of vitamin B group and K (6), and have long been considered to impact health. However, their significance has not been fully elucidated and we believe that further investigation in this area may lead to development of novel bio-treatment methods.

Treatment of *Clostridium difficile* Colitis by Intestinal Microbiota Transplantation from a Healthy Individual

When considering the function of healthy feces containing intestinal microbiota in the maintenance of health, its effect on *Clostridium difficile* colitis has been noted (7). *Clostridium difficile* colitis is a refractory disease that often occurs in the elderly (8). *Clostridium difficile* (CD) is an anaerobic, indigenous microbe; however, when the administration of antibiotics destroys the normal microbiota, drug-resistant CD undergoes abnormal proliferation and causes serious enteritis accompanied by severe diarrhea with the resulting intestinal perforation that may be fatal (9). Metronidazole, vancomycin and fidaxomicin are normally administered to treat *Clostridium difficile* colitis (10). However, as the efficacy of these treatments is low and there is a high risk of recurrence, *Clostridium difficile* colitis is considered to be incurable. Fecal microbiota transplantation (FMT), where feces from a healthy person are injected into the duodenum of a patient, has been considered to be effective in treatment of CD infections. Several clinical tests investigating this method have been conducted by medical doctors in Europe and, among them, a study conducted by a group of researchers from the University of Amsterdam, Netherlands, compared the efficacy of fecal microbiota transplantation (FMT) with the administration of vancomycin (11). In that study, the patients who had been treated with sufficient antibiotics, but experienced the recurrence of CD infection, were administered with either vancomycin or underwent FMT. The therapeutic efficacy of vancomycin administration was only 31%, whereas that of FMT was 94%, thus indicating that FMT from a healthy person is extremely effective against CD infections. As the diversity of intestinal microbiota in patients with CD infection clearly decreased compared to that in healthy persons, the importance of this diversity becomes apparent.

FMT in healthy individuals is currently being investigated to better understand its application in digestive disorders, such as idiopathic constipation, inflammatory bowel disease (12), Crohn's disease and irritable bowel syndrome (13), as well as other diseases, such as autism (14), chronic fatigue syndrome (15), diabetes and Parkinson's disease (16). Furthermore, a stool substitute mixture (mixture of several species of microbes) has also been tested as a replacement for fresh feces in this treatment method. According to Atarashi *et al*. (17), when germ-free mice were administered with the feces from a healthy person, T-cells (Treg), which suppress inflammation (18), were found to be enhanced. When the intestinal microbiota was analyzed to identify the microbes involved in the suppression of inflammation, 17 *Clostridium* species were shown to have inflammation-suppressive effects. Although *Clostridium* triggers enteritis, it also induces the suppression of inflammation. It appears that the regulation of hosts by intestinal microbiota is yet to be fully understood. Based on the current understanding, it would be shortsighted to conclude that certain microbiota improve or worsen the intestinal environment.

The Relationship of Intestinal Microbiota with Antitumor Effects and LPS

Iida *et al*. (19) reported a very interesting finding involving the association of intestinal microbes with antitumor effects. In a type of immunotherapy, where the mice transplanted with cancer cells were intraperitoneally administered with anti-interleukin-10 receptor (IL-10R) followed by CpG-oligoDNA, a TLR-9 agonist (20) administered was within the tumor, it was seen that tumor necrosis factor (TNF) induction at the cancer site increased significantly, thereby suppressing tumor proliferation. This antitumor effect did not change even when T-cells or B cells were deleted, thus suggesting the involvement of the innate immune system. When an antibiotic cocktail (vancomycin, imipenem and neomycin) was administered to this model three weeks prior to drug administration, TNF production within the tumor significantly decreased and the antitumor effect of immunotherapy declined. Additionally, no effects of anti-IL10 antibody and CpG antitumor treatment were seen in germ-free mice. The administration of antibiotics clearly showed a quantitative decrease in intestinal microbes indicating the possible importance of the amount of intestinal microbes in the maintenance of biological functions. Furthermore, the inhibitory effects of antibiotic administration on immunotherapy were shown to be prevented by orally administering Gram-negative microbes (*e.g*., *Alistipes shahii*) or LPS. This preventative effect was not obtained with the Gram-positive microbe *Lactobachillus fermentum*. In their report, they also observed similar antitumor effects of intestinal microbes on chemotherapeutic agents. A part of the antitumor effect of oxaliplatin (21) is considered to be due to reactive oxygen species (ROS) induction (22). However, when the number of intestinal microbes declined as a result of the antibiotic cocktail, insufficient ROS was induced at the site of tumor resulting in decreased antitumor effects. The oral administration of LPS was also shown to prevent such antibiotic-triggered decreases in the efficacy of treatment.

Antibiotic-induced Aggravation of Vancomycin-resistant *Enterococci* and its Prevention by LPS

The long-term administration of antibiotics triggers the onset of vancomycin-resistant enterococcus (VRE) and the resulting nosocomial infections have become a social problem (23). The proliferation of intestinal VRE is

normally suppressed by defensins (24), which are produced by Paneth cells (25). However, the long-term oral administration of antibiotics decreased intestinal Gram-negative microbes and the supply of LPS was reduced. This led to the production of defensins and a decline in RegIII-γ, which in turn caused the proliferation of VRE and increased the risk of infection. However, when LPS was orally administered under such circumstances, it was shown to induce RegIII-γ production and suppress the onset of VRE (26). Paneth cells are present in the intestinal crypt and they bind to LPS with their Toll-like receptor 4 (TLR-4) and produce anti-microbial peptides, such as β defensins (27), in addition to RegIII-γ (26). Several types of defensins regulate the proliferation of intestinal microbes and they are reported to selectively suppress pathogens. Within the intestines, LPS derived from food or intestinal symbiotic Gram-negative microbes facilitate the production of anti-microbial peptides by Paneth cells (25) and help maintain the intestinal environment. However, antibiotic intake decreases the number of intestinal microbes and the LPS supplied from Gram-negative microbes declines, thus preventing the stimulation of Paneth cells and causing the suppression of the production of anti-microbial peptides (27).

Moreover, other reports have suggested that RegIII-γ works to physically protect against infection (28). RegIII-γ produced from Paneth cells has been shown to create an aseptic space of 50 μm thickness on the surface of the gastrointestinal cavity (29). This physical barrier may prevent direct contact of the living organism with bacteria, thereby preventing infection.

Conclusion

It is difficult to study the hierarchy of individuals because it involves the consideration of complex methodologies. However, the prevention of diseases, such as dementia, sarcopenia and locomotive syndrome, along with incurable diseases, such as cancer, currently considered to be serious challenges, are likely to be deeply associated with the mechanisms that maintain order on an individual level. If such is the case, further research and clinical studies are required on this perspective. We believe that an investigation of the validity of intestinal microbiota regulation or LPS, which partially substitutes the function of intestinal microbiota, is important as it will help expand our current knowledge, as well as allow for the optimization of developed drugs on an individual level.

Moreover, tissue macrophages (30), which are the typical targets of intestinal microbiota or LPS, are also noteworthy. Recently, some of the tissue macrophages were revealed to have been derived from the yolk sac instead of monocytes and the indigenous tissue macrophages and permeated monocyte-derived macrophages were shown to have distinct functions (31). Tissue macrophages are involved in the secretion of xenobiotic substances that are produced during biological activity in order to maintain healthy status. Therefore, we believe that, when evaluating intestinal microbiota or LPS in relation to maintaining health, it is useful to investigate the function of phagocytes, including tissue macrophages, and the results of such research could contribute to the analysis of "functional foods."

Conflicts of Interest

The Authors have no financial conflicts of interest.

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