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Gut Microbiota in Health and Disease

Yuichiro Yamashiro

Probiotics Research Laboratory, Juntendo University Graduate School of Medicine, Tokyo, Japan

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Gut microbiota · Probiotics · Gut dysbiosis · Bacteremia · Bacterial translocation

Abstract
Intestinal regulatory T (Treg) cells are critical to maintaining immune tolerance to dietary antigens and gut microbiota. This paper reviews several papers on this topic that were recently published by Japanese researchers. Specifically, Prof. K. Honda and his group have found that commensal microbiota capable of metabolizing butyrate induces the differentiation of colonic Treg cells. In a separate work, Prof. Y. Yokoyama and his group used a novel, culture-independent analytical method (the Yakult Intestinal Flora-Scan) for detection of bacteria in the bloodstream. Their work revealed that bacteremia in invasive surgery patients was ameliorated by synbiotic supplementation; similar results were reported in pediatric surgical cases by Dr. T. Okazaki and his group. This cutting-edge method may lead to the evolution of an altered disease concept; an example of this change is provided by the description of bacteremia in patients with type 2 diabetes, as reported by Dr. J. Sato and her group. In a similar work, Prof. Y. Yamashiro and his group found that infants born by cesarean (C)-section, who typically have gut dysbiosis, exhibit higher carriage of toxigenic *Clostridium perfringens*. The finding suggests that C-section-born infants may serve as a potential reservoir of this opportunistic pathogen. Another separate work by the laboratory of Dr. K. Yamashiro has revealed that gut dysbiosis is associated with altered metabolism and systemic inflammation in patients with ischemic stroke. These papers are consistent with a study by Prof. N. Sudo and his group, who have made significant progress in research on interaction among the microbiota, gut, and brain.

Introduction

Since the end of the 19th century, microbiological research in Japan has strongly contributed to the development of the field. Japanese researchers have been involved since the dawn of modern microbiology, as evidenced by Shibasaburo Kitasato whose collaboration with E.A. von Behring in 1890 established the use of serotherapy for diphtheria and antiserum studies of tetanus, and by Kiyoshi Shiga who in 1898 discovered the causative organism of dysentery. The study of gut microbiota and probiotics in Japan goes back over 80 years to Dr. M. Shirota's iden-

E-Mail karger@karger.com
www.karger.com/anm

Yuichiro Yamashiro
Probiotics Research Laboratory
Juntendo University Graduate School of Medicine
3rd Floor, Hongo-Asakaze Bldg., 2-9-8 Hongo, Bunkyo-ku, Tokyo 113-0033 (Japan)
E-Mail yamasiro@juntendo.ac.jp

tification in 1930 of *Lactobacillus casei* Shirota as a beneficial bacterium, which led in turn to the founding in 1935 of the Yakult company, which to this day produces probiotics containing *L. casei* Shirota.

Japanese research activities on gut microbiota, built on the work of the pioneers, have made remarkable scientific achievements over the years. "Japan_topic," a special issue of *Annals of Nutrition and Metabolism*, provides a brief review of several papers, recently reported by Japanese researchers, and this may provide new insights into gut microbiota and probiotics. The following papers are expected to help readers to understand recent advances and current research activities in this field.

Studies on Regulatory T Cells in the Colon by Prof. K. Honda's group

Prof. Kenya Honda (Keio University School of Medicine, Tokyo) and his research group [1] reported evidence of the induction of regulatory T (Treg) cells in the colon, as reported by Furusawa et al. [2] in *Nature* and summarized below.

Gut commensal microbes shape the mucosal immune system by regulating the differentiation and expansion of several types of T cells. Clostridia, a dominant class of commensal microbe, can induce colonic Treg cells, which play a central role in the suppression of inflammatory and allergic responses. However, the molecular mechanisms by which commensal microbes induce colonic Treg cells have been unclear. Here we show that a large-bowel microbial fermentation product, butyrate, induces the differentiation of colonic Treg cells in mice. A comparative NMR-based metabolome analysis suggests that the luminal concentrations of short-chain fatty acids positively correlate with the number of Treg cells in the colon. Among short-chain fatty acids, butyrate induced the differentiation of Treg cells in vitro and in vivo, and ameliorated the development of colitis induced by adoptive transfer of CD4(+) CD45RB(hi) T cells in Rag1(–/–) mice. Treatment of naive T cells under the Treg-cell-polarizing conditions with butyrate enhanced histone H3 acetylation in the promoter and conserved noncoding sequence regions of the Foxp3 locus, suggesting a possible mechanism for how microbial-derived butyrate regulates the differentiation of Treg cells. Their findings provide new insight into the mechanisms by which host-microbe interactions establish immunological homeostasis in the gut.

These authors also reported related work on Treg cells [1] and Th17 cells [3].

A Cutting-Edge Analytical Method for Detection of Bacteria in the Bloodstream Leading to Evolution of an Altered Disease Concept

A highly sensitive microbial analytical system, the Yakult Intestinal Flora-Scan, has been developed to quantify the intestinal microbiota [4]. Targeting ribosomal RNA molecules, the Yakult Intestinal Flora-Scan is (at a minimum) hundreds of times more sensitive (detection limits: 10^{2-3} cell/g feces, 1 cell/mL blood) than conventional DNA-based methods. This high analytical sensitivity enables more accurate and reliable detection (compared with conventional culture methods) of microorganisms that have migrated into the bloodstream, a condition known as bacteremia. Using this method, bacteremia was detected far earlier, and in some cases unexpectedly, in individuals, including adults subjected to invasive abdominal surgery [2] and pediatric surgical patients [5]. The analytical method was found to be useful for the detection of bacteremia in pediatric patients with febrile neutropenia (mucositis) [6], type 2 diabetes [7], and ischemic stroke [8].

The 2 papers listed below are examples of the application of this analytical method.

Bacteremia in Highly Invasive Abdominal Surgery and Its Prevention

Invasive surgery of the gastrointestinal system entails a high risk of postoperative infectious complications, a condition to which alterations of the intestinal microenvironment could contribute. Prof. Yukihiro Yokoyama (Department of Surgery, Nagoya University, Nagoya) and his group reported bacteremia as a postoperative infectious complication; notably, their laboratory demonstrated clearly that the use of synbiotics prevented these complications. This study group also demonstrated these findings in the context of dysbiosis-related deteriorations of the gut environment [9]. One of the excellent papers of Prof. Y. Yokoyama's group [5] is summarized below.

The impact of perioperative synbiotics on bacterial translocation and subsequent bacteremia after esophagectomy is unclear. This study investigated the effect of perioperative synbiotic administration on the incidence of bacterial translocation to mesenteric lymph nodes (MLNs) and the occurrence of postoperative bacteremia. Patients with esophageal cancer were randomized to receive perioperative synbiotics or no synbiotics

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(control group). MLNs were harvested from the jejunal mesentery before dissection (MLN-1) and after the restoration of digestive tract continuity (MLN-2). Blood and feces samples were taken before and after operation. Microorganisms in each sample were detected using a bacterium-specific ribosomal RNA-targeted reverse transcriptase-quantitative polymerase chain reaction (RT-qPCR) method. Some 42 patients were included. There was a significant difference between the 2 groups in detection levels of microorganisms in the MLN-1 samples. Microorganisms were more frequently detected in MLN-2 samples in the control group than in the synbiotics group (10 of 18 vs. 3 of 18; $p = 0.035$). In addition, bacteremia detected 1 day after surgery using RT-qPCR was more prevalent in the control group than in the synbiotics group (12 of 21 vs. 4 of 21; $p = 0.025$). Neutrophil counts on postoperative days 1, 2, and 7 after surgery were all significantly higher in the control group than in the synbiotics group. Perioperative use of synbiotics reduces the incidence of bacteria in the MLNs and blood. These beneficial effects probably contribute to a reduction in the inflammatory response after esophagectomy.

Working with pediatric cases, Dr. Tadaharu Okazaki (Associate Professor of Pediatric Surgery, Juntendo University Urayasu Hospital, Chiba) and his group [10] reported findings similar to those of Prof. Y. Yokoyama and his group.

Bacteremia in Type 2 Diabetes

Gut dysbiosis in type 2 diabetes is now well accepted, following the 2010 report of Larsen et al. [11]. Dr. Junko Sato (Department of Metabolism & Endocrinology, Juntendo University, Tokyo) and her group [7] recently provided the first report demonstrating a high prevalence of bacteremia in patients with type 2 diabetes.

Mounting evidence indicates that the gut microbiota is an important modifier of obesity and diabetes. However, so far there has been no information on gut microbiota and "live gut bacteria" in the systemic circulation of Japanese patients with type 2 diabetes. Using a sensitive RT-qPCR method, we determined the composition of fecal gut microbiota in 50 Japanese patients with type 2 diabetes and 50 control subjects, and its association with various clinical parameters, including inflammatory markers. We also analyzed the presence of gut bacteria in blood samples. The counts of the *Clostridium coccoides* group, *Atopobium* cluster, and *Prevotella* (obligate anaerobes) were significantly lower ($p < 0.05$), while the counts of total *Lactobacillus* (facultative anaerobes) were significantly higher ($p < 0.05$) in fecal samples of diabetic patients than in those of control subjects. Especially, the counts of *Lactobacillus reuteri* and *Lactobacillus plantarum* subgroups were significantly higher ($p < 0.05$). Gut bacteria were detected in blood at a significantly higher rate in diabetic patients than in control subjects (28 vs. 4%, $p < 0.01$), and most of these bacteria were gram-positive. This is the first report of gut dysbiosis in Japanese patients with type 2 diabetes as assessed by RT-qPCR. The high rate of gut bacteria in the circulation suggests translocation of bacteria from the gut to the bloodstream.

Dysbiosis in Infants Born by Cesarean-Section

Infants born vaginally acquire bacterial communities that resemble the vaginal microbiota of their mothers, while those delivered by cesarean (C)-section acquire communities similar to the microbiota found on the surface of the skin of their mothers and those of the hospital staff. Prof. Yuichiro Yamashiro (Probiotics Research Laboratory, Juntendo University, Tokyo) and his group [12] found that the delivery-imparted effects on the gut microbiota (dysbiosis) persisted at 6 months and even up to 19 years of age, including a significantly lower level of *Bacteroides* in infants and young adults born via C-section delivery. Their paper [13] represents the first-ever report of these patterns. It is now known that C-section delivery is a risk factor for noncommunicable diseases, including obesity, in the offspring [14]. C-section deliveries have increased beyond the recommended level of 15% in the world, almost doubling (according to the WHO) in the last decade [15].

Prof. Y. Yamashiro and his group [8] also reported intriguing results as shown below.

Toxigenic *Clostridium perfringens* is a widespread opportunistic pathogen linked to numerous diseases. This study revealed that, compared to vaginally born infants, C-section-born infants had a higher carriage of *C. perfringens* (alpha-toxigenic and enterotoxigenic) and lower fecal organic acids during the first 6 months of postnatal life. Higher carriage of toxigenic *C. perfringens* in C-section-born infants means that these babies may serve as a potential reservoir of this opportunistic pathogen, and that they may be more prone to associated illnesses. The study by Nagpal et al. [13] is the first to demonstrate this fact.

Ischemic Stroke and Gut Dysbiosis

Ischemic stroke is associated with metabolic diseases including obesity, type 2 diabetes, and dyslipidemia. Systemic low-grade inflammation is closely linked to metabolic disorders [16], to which dysbiosis of gut microbiota with lipopolysaccharide-containing microbiota contributes [17]; such low-grade inflammation also plays a substantial role in the pathogenesis of cardiovascular diseases, including ischemic stroke [18]. Dr. K. Yamashiro (Department of Neurology, Juntendo University, Tokyo) and his group [9] showed, for the first time, the presence of gut dysbiosis with altered organic acid production in patients with ischemic stroke.

We analyzed the composition of the fecal gut microbiota and the concentrations of fecal organic acids in 41 ischemic stroke patients and 40 control subjects via 16S and 23S rRNA-targeted qRT-PCR and high-performance liquid chromatography analyses, respectively. It was found that although only the bacterial counts of *Lactobacillus ruminis* were significantly higher in stroke patients compared to controls, multivariable analysis showed that ischemic stroke was independently associated with increased bacterial counts of the *Atopobium* cluster and *L. ruminis*, and decreased numbers of *Lactobacillus sakei* subgroup, independent of age, hypertension, and type 2 diabetes. Changes in the prevalence of *L. ruminis* were positively correlated with serum interleukin-6 levels. In addition, ischemic stroke was associated with decreased and increased concentrations of acetic acid and valeric acid, respectively. Meanwhile, changes in acetic acid concentrations were negatively correlated with the levels of glycated hemoglobin and low-density lipoprotein cholesterol, whereas changes in valeric acid concentrations were positively correlated with the level of high-sensitivity C-reactive protein and with white blood cell counts. Together, their findings suggest that gut dysbiosis in patients with ischemic stroke is associated with host metabolism and inflammation.

Interaction among the Microbiota, Gut, and Brain

Prof. Nobuyuki Sudo (Department of Psychosomatic Medicine, Kyushu University School of Medical Science, Fukuoka) and his group [19] are well-known for their research on the brain-gut axis, and one of their papers was published in *Neurogastroenterology and Motility* .

Conclusion

The human gut microbiota forms a complex ecological community that influences normal physiology and susceptibility to disease through its collective metabolic and immunological activities and host interactions. Research activity on gut microbiota by Japanese scientists is expected to continue to facilitate further development of this field.

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