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Yamashita, Tomoya

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Intestinal Immunity and Gut Microbiota in Atherogenesis

Tomoya Yamashita

Division of Cardiovascular Medicine, Department of Internal Medicine, Kobe University Graduate School of Medicine, Kobe, Japan

Atherosclerosis is a chronic inflammatory disease. Interventions targeting the inflammatory process could provide new strategies for preventing atherosclerotic cardiovascular diseases (CVD). Previously, we have reported that oral administration of anti-CD3 antibodies, or active vitamin D₃, reduced atherosclerosis in mice via recruiting regulatory T cells and tolerogenic dendritic cells to the gut-associated lymphoid tissues. From this, it is reasonable to propose that the intestine could be a novel therapeutic target for prevention of atherosclerotic CVD. Recently, the association between cardio-metabolic diseases and gut microbiota has attracted increased attention. Gut microbiota, reported to be highly associated with intestinal immunity and metabolism, were shown to aggravate CVD by contributing to the production of trimethylamine-N-oxide (TMAO), a pro-atherogenic compound. We have also previously investigated the relationship between patient susceptibility to coronary artery disease (CAD) and gut microbiota. We found that the order *Lactobacillales* was significantly increased and the phylum *Bacteroidetes* was decreased in CAD patients compared with control patients. In this review article, we discuss the evidence for the relationship between the gut microbiota and cardio-metabolic diseases, and consider the gut microbiota as new potential diagnostic and therapeutic tool for treating CVD.

Key words: Intestinal immunity, Regulatory T cell, Tolerogenic dendritic cell, Gut microbiota, TMAO

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Introduction

Atherosclerosis and resulting cardiovascular diseases (CVD) are the leading causes of mortality in many developed and developing countries. Clinical studies and animal experiments have demonstrated that elevated plasma cholesterol, mainly transported by low density lipoprotein (LDL), promotes CVD, including coronary artery disease (CAD). On the basis of this finding, subsequent studies have shown that statin-based lipid lowering therapies reduce CV events. However, several clinical trials have revealed that more than 50% of residual cardiovascular risk remains, even after the aggressive reduction of LDL cholesterol¹⁾. Atherosclerosis is considered a chronic

inflammatory disease in which both innate and acquired immunity are involved²⁻⁶⁾. Inflammation of the vessel walls is an important feature of atherosclerosis, and contributes to both instability of plaques and thrombotic occlusion of arteries, resulting in CV events such as acute coronary syndrome and stroke. As a next-generation treatment, many researchers, including us, have been interested in anti-inflammation therapy for atherosclerotic CVD²⁻¹²⁾. We subsequently proposed that the intestine could be a novel therapeutic target for prevention of atherosclerosis and treating CVD, and have focused our research on intestinal immunity^{11, 12)}.

The gut mucosa is one of the largest immunologically active organs in human body. It protects the host from invading microorganisms, and harbors several hundred trillion bacteria, which are collectively referred to as the "gut microbiota." Fortunately, majority of these microorganisms are not harmful to the host, and in fact contribute to the maintenance of health. However, if disrupted, they have the potential to drive gastrointestinal and extragastrointestinal dis-

Address for correspondence: Tomoya Yamashita, Division of Cardiovascular Medicine, Department of Internal Medicine, Kobe University Graduate School of Medicine 7-5-1, Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan.

E-mail: tomoya@med.kobe-u.ac.jp

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orders¹³). Over the past decade, the expanded use of a mouse model lacking gut microbiota, known as “germ-free (GF) mice”, as well as the development of various omic technologies, including genomics, transcriptomics, proteomics, and metabolomics, have enriched our understanding of an ecological system of commensal bacteria in the intestine. Recent studies have demonstrated that gut microbe-derived factors may actually lead to many metabolic and digestive tract diseases. In this review, we describe how specific changes in the gut microbiome could affect host metabolism and immune regulation, and how these findings can lead to novel therapeutic targets for CVD and metabolic disorders.

The Intestine as a Therapeutic Target for Preventing Atherosclerosis

The intestinal immune system differentiates potentially harmful foreign antigens from harmless ones. The gut tolerates harmless antigens, but remains able to eliminate harmful pathogens. To accommodate the exposure to harmless antigens, including food components and commensal gut bacteria, the gut has evolved an anti-inflammatory environment. Recent research revealed that tolerogenic dendritic cells (DCs) in the gut present food antigens to T cells as tolerogens and induce antigen-specific immune suppression¹⁴. The oral tolerance is shown to involve both anergy/apoptosis of CD4⁺ effector T cells and induction of regulatory T cells (Tregs) which appear to come in several different forms. The naturally occurring Tregs (nTreg), originally described as CD4⁺CD25⁺ cells generated in the thymus, express the transcription factor Foxp3 and are involved in maintaining systemic homeostasis and preventing autoimmunity. Immunosuppressive mediators of Tregs include inhibitory molecules such as cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), immunoregulatory cytokine interleukin-10 (IL-10), and transforming growth factor- β (TGF- β)¹⁴.

CTLA-4 is a co-inhibitory molecule mainly expressed in CD4⁺Foxp3⁺ Tregs. It binds to CD80/CD86 on the DCs to suppress their function. IL-10-producing type 1 regulatory T (Tr1) cells, which do not express Foxp3, have been observed in Peyer's patches of mice fed a low dose of β -lactoglobulin, and produce high amounts of IL-10¹⁴. T helper 3 (Th3) cells are TGF- β -producing LAP (latency-associated peptide) ⁺CD4⁺ T cells originally isolated from mesenteric lymph nodes of orally tolerant mice. TGF- β induces expression of Foxp3 in naive CD4⁺ T cells, and Th3 cells can influence Treg development in neighboring cells. Importantly, retinoic acid promotes

the conversion of naive CD4⁺ T cells into Foxp3⁺ peripherally inducible Treg (pTreg) with the help of TGF- β in the gut, suggesting significant roles for intestinal DCs that produce retinoic acid (**Fig. 1**)¹⁴.

Oral tolerance is locally induced in an antigen-specific manner, but its effects are not constrained to the local immune response within the gut. Anti-inflammatory immune responses could be seen in other tissues and organs including distal non-lymphoid organs. From this, we hypothesized that modulation of intestinal immunity or induction of oral tolerance must affect the systemic immune responses, including vessel walls, and might prevent atherosclerosis.

Recent studies have shown that the oral anti-CD3 antibody is biologically active and induces TGF- β -producing CD4⁺LAP⁺ Tregs, namely Th3, that suppress experimental autoimmune encephalitis¹⁵ and autoimmune diabetes in a TGF- β -dependent fashion¹⁶. Autoimmune diseases are suppressed even by low doses of oral anti-CD3 antibody, associated with an increase in LAP⁺ Tregs. However, there is no evidence of antigen specificity with oral anti-CD3 antibodies and the cellular and molecular mechanisms underlying induction of Tregs remain unclear¹⁵. In one study, we applied this method to the treatment of atherosclerosis in apolipoprotein E-deficient (apoE^{-/-}) mice. Here we demonstrated that oral anti-CD3 antibody treatment induced Th3 and Foxp3⁺ Tregs, which suppressed pathogenic immune processes crucial for atherogenesis through a TGF- β -dependent mechanism, consequently inhibiting atherosclerotic plaque formation¹¹. In another study, we examined the effect of oral anti-CD3 antibody treatment on the phenotypes of DCs in the mesenteric lymph nodes in mice, and confirmed that expression of CD80 and CD86 in DCs were reduced in anti-CD3 antibody-treated mice compared to controls (**Fig. 1**). We found that active vitamin D₃ (calcitriol) was induced immature DCs and Tregs. Further, we tried to examine the effects of orally administered calcitriol on atherosclerosis in animal models, and demonstrated that it decreases atherosclerosis in apoE^{-/-} mice, by promoting induction of tolerogenic DCs and Foxp3⁺ Tregs¹². Although further studies are needed to clarify the precise role of several types of vascular DCs in atherogenesis, effective methods to induce atheroprotective DCs could be novel therapies for prevention of atherosclerosis^{11, 12}. Taken together, modulation of the intestinal immunity, including the function and quantity of Tregs and tolerogenic DCs, could be a novel strategy for preventing atherosclerotic CVD.

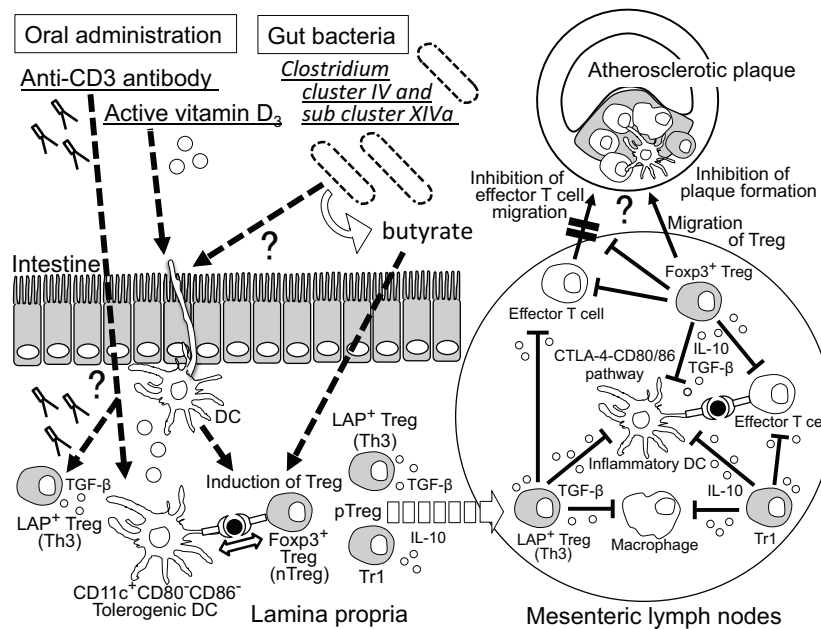


Fig. 1. Intestinal immunity as a possible therapeutic target for controlling inflammatory diseases including atherosclerosis

Oral administration of anti-CD3 antibody and active vitamin D₃ induces Tregs and tolerogenic DCs in mesenteric lymph nodes. *Clostridium* cluster IV and subcluster XIVa was shown to induce Foxp3⁺ Tregs in the colon of mice through butyrate-dependent manner. Some DCs in the intestine have a crucial role in determining tolerogenic immune responses by prompting the generation of Tregs. Peripherally inducible Treg (pTreg) is reported to differentiate mainly in the intestine. Foxp3⁺ Tregs inhibit antigen presentation through a cell-contact dependent manner or production of anti-inflammatory cytokines (IL-10 or TGF-β). These phenomena are similar to oral tolerance and may result in inhibiting atherosclerosis. Treg; regulatory T cell, LAP⁺ Treg; latency-associated peptide⁺ Tregs, pTreg; peripherally inducible Treg, DC; dendritic cell, IL; interleukin, IFN-γ; interferon-γ, TGF-β; transforming growth factor-β, CTLA-4; cytotoxic T-lymphocyte-associated protein 4

Gut Microbiota and their Regulatory Effects on the Intestinal Immunity

During birth, we are colonized with many microorganisms that will play a crucial role in defining our future metabolism and immunity. The profile of the predominant phylum in the gut changes during childhood and youth, and nearly stabilizes by adulthood¹⁷. Most bacterial species in the adult human and mouse gut belong to the phyla *Firmicutes* and *Bacteroidetes*, with less abundant bacterial phyla, such as *Actinobacteria*, *Proteobacteria*, *Fusobacteria*, and *Verrucomicrobia* also present (**Fig. 2**). A lot of gut bacteria cannot be cultivated *in vitro*, making categorization and identification of gut bacteria complicated. Although there is no clear definition of a “healthy” gut microbiome in humans, a recent metagenome study has allowed three major clusters, or “Enterotypes,” of gut bacteria to be distinguished in humans, based on the predominant bacterial genera in fecal specimens: type I is character-

ized by high levels of *Bacteroides*; type II has few *Bacteroides* but *Prevotella* are common; and type III has high levels of *Ruminococcus* (**Fig. 2**)¹⁸. The composition of our gut microbiota is remarkably diverse. Because dietary exposures significantly affect our microbial community, it is very dynamic and can change rapidly in a short period of time¹⁹. However, its composition appears to remain remarkably stable over longer periods, and to be conserved between individuals and their family members.

It has recently been demonstrated that specific bacterial species are associated with differentiation of specific subsets of T cells in the intestine¹⁹. Both human and mouse *Clostridium* cluster IV and subcluster XIVa, spore-forming components of indigenous intestinal microbiota, have been implicated in the induction of Foxp3⁺ Tregs in the colon of mice (**Fig. 1**)^{20, 21}. Furthermore, butyrate, a short chain fatty acid (SCFAs) produced by *Clostridium* species, promotes Foxp3⁺ Treg induction²². It can therefore be

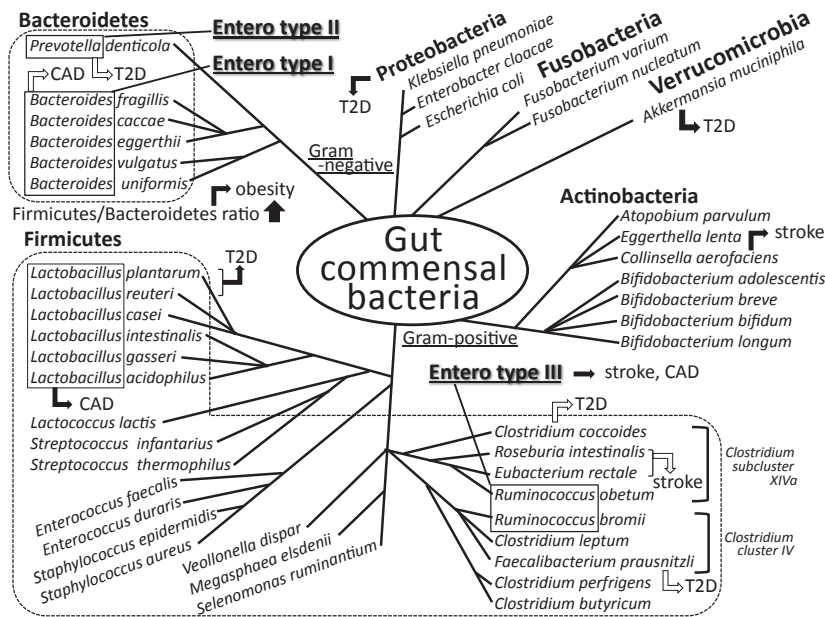


Fig. 2. Human gut commensal microbiota, their classification, and their relation to cardio-metabolic diseases

The phylum *Firmicutes* and *Bacteroidetes* is the most dominant gram-positive and gram-negative bacteria phylum in human gut microbiota, respectively. The major 4 phyla of *Firmicutes*, *Bacteroides*, *Actinobacteria*, and *Proteobacteria* occupy more than 98% of all human gut microbiota. The total number of bacteria in human intestine is more than one hundred trillions and are classified in several hundreds of species. The gut bacteria phenotype named “Enterotype” was reported in humans, based on the predominant bacterial genera in fecal specimens; type I is characterized by high levels of *Bacteroides*, type II predominates *Prevotella*, and type III has high levels of *Ruminococcus*. The *Firmicutes*/*Bacteroidetes* ratio was reported to be associated with obesity and lean. Black and white arrows show the positive and negative correlations with the incidence of the indicated diseases, respectively. T2D; type 2 diabetes mellitus, CAD; coronary artery disease

speculated that the propagation or sterilization of some specific bacterial species, resulting in augmented generation of Tregs or reduced differentiation of pathogenic T cells, may prevent inflammatory diseases, including atherosclerosis. Further studies are needed to prove this hypothesis and may contribute to the development of novel strategies for preventing atherosclerosis by modulating intestinal immunity.

Gut Microbial Alternations Associated with Obesity and Type 2 Diabetes

Recent studies in both mice and humans have suggested that gut microbiota may function as an environmental factor contributing to obesity and type 2 diabetes (T2D). This was first demonstrated by showing the increase of the phylum *Firmicutes* and the decrease of the phylum *Bacteroidetes* (increase of the *Firmicutes* /*Bacteroidetes* (F/B) ratio) in obese patients (Fig. 2), attracting attention from researchers in all over the world^{23, 24}. Although these findings suggest

that gut microbiota are altered in obesity, it is unclear whether the change of microbial composition is a cause or a result of obesity. If the altered microbiota contribute to the pathogenesis of obesity, modulating microbial composition could be a new therapeutic option for extreme obesity. In this regard, it was demonstrated that the obesity phenotype, an increase in body fat and weight, was transmissible by fecal microbiota transplantation into lean GF mice, resulting in increased capacity for energy harvest²⁵. Another paper demonstrated that metabolic disorders, such as insulin resistance, were also transmissible by fecal transplantation²⁶. Accordingly, the obese phenotype can be transplanted between individuals through the microbiota.

One potential mechanism by which gut microbiota contribute to the pathophysiology of obesity has been intensively examined. The microbiome of the obesity subjects was found to have increased fermentation capabilities, resulting in increased levels of fecal SCFAs that could be used as energy by the host. It was recently revealed that SCFAs such as acetate and pro-

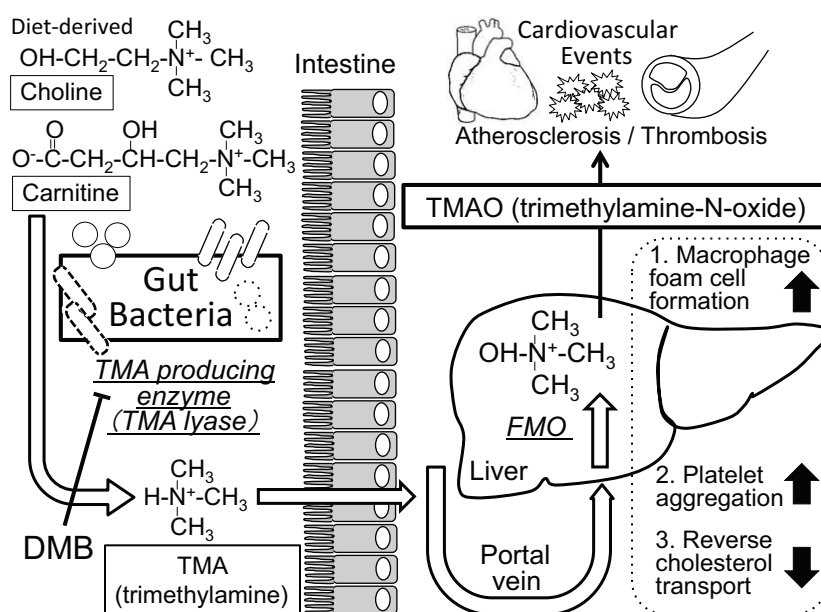


Fig. 3. Gut microbiota metabolic pathways related with the incident adverse cardiovascular events

Gut microbial-derived metabolites, trimethylamine (TMA) and trimethylamine N-oxide (TMAO), are shown to be associated with the incidence of cardiovascular events in humans and increase atherosclerosis in mice. TMAO is shown to increase the foam cell formation in macrophages, activate platelet aggregation, and decrease the reverse cholesterol transport from the atherosclerotic lesions to the liver. DMB; 3,3-dimethyl-1-butanol, FMO; flavin-containing monooxygenase

pionate regulate appetite via gut endocrine hormone and central homeostatic mechanisms²⁷). Taken together, these findings suggest that gut microbiota directly contribute to obesity via increasing appetite and energy harvest from the diet.

Two recent papers demonstrated the diagnostic and clinical value of fecal microbiota composition in T2D^{28, 29}. In both studies, diabetic subjects were characterized by a reduction of *Clostridiales* including butyrate-producing *Roseburia* species and *Feacalibacterium prausnitzii*. Whereas Karlsson *et al.* reported an enrichment of *Lactobacillus gasseri* and *Streptococcus mutans* in diabetic patients²⁹, Qin *et al.* found that an increased number of *Proteobacteria* could be a predictor of T2D²⁸. Using a sensitive quantitative reverse transcription PCR method, in fecal samples of Japanese patients with T2D, it became obvious that the *Clostridium coccoides* group, *Atopobium* cluster, and *Prevotella* were significantly lower, while the counts of total *Lactobacillus* were higher than in those of control subjects³⁰. Specifically, the counts of *Lactobacillus reuteri* and *Lactobacillus plantarum* subgroups were significantly higher in Japanese T2D patients (Fig. 2). Other papers demonstrated that *Akkermansia muciniphila* (*A. muciniphila*), a mucin-degrading bacterium, was associated with glucose metabolism^{28, 31}.

In human studies, the relationship between the abundance of *A. muciniphila* and type 2 diabetes is controversial. Oral administration of *A. muciniphila* led to decreased metabolic endotoxemia, improved glucose tolerance, and reduced atherosclerosis in mice. These findings indicate the need for further investigation to ascertain the therapeutic applicability of *A. muciniphila*.

CVD and the Chemical TMAO Connection

Recently, Hazen SL *et al.* have splendidly reported that gut microbial-derived metabolites, trimethylamine (TMA) and trimethylamine N-oxide (TMAO), are pro-atherogenic in both mice and humans (Fig. 3)^{32, 33}. First, they used a metabolomics approach to generate small-molecule metabolomics profiles in human plasma that can predict for CVD³². Three metabolites of the dietary lipid phosphatidylcholine; choline, TMAO, and betaine, were identified and these metabolites were associated with atherosclerotic CV risks in humans and the promotion of atherosclerosis in mice. Oral feeding of choline, rather than parenteral delivery, was necessary to generate TMAO, suggesting that a crucial phase in this biochemical pathway was performed within the intestine.

Generation of TMA, a precursor of TMAO, was shown to be dependent on the gut microbiota in both humans and mice. That is, they found that dietary choline is metabolized by gut bacteria to TMA, which is subsequently absorbed into the host and metabolized to TMAO in the liver by the FMO (flavin-containing monooxygenase) family of enzymes (**Fig. 3**)³⁴. Oral administration of phosphatidylcholine promoted up-regulation of multiple macrophage receptors and enhanced atherosclerosis in apoE^{-/-} mice³². Deletion of gut microbiota using broad spectrum antibiotics canceled the pro-atherosclerotic effect of dietary choline, which was associated with the reduction of plasma TMAO levels in antibiotic-treated mice. Furthermore, inhibition of microbial TMA lyases, enzymes that produce TMA, by 3,3-dimethyl-1-butanol (DMB) could reduce atherosclerotic lesion formation in apoE^{-/-} mice³⁵.

They also investigated the relationship between fasting plasma levels of TMAO and incidents of major adverse cardiovascular events (death, myocardial infarction, or stroke) during three years of follow-up in 4007 patients undergoing elective diagnostic cardiac catheterization³³. Increased plasma TMAO levels were associated with an increased risk of a major adverse CV event. Even after adjustment for traditional risk factors, elevated TMAO levels could predict an increased risk of major adverse CV events. Collectively, their findings suggested that pathways dependent on gut microbiota may contribute to the pathophysiology of atherosclerotic CVD and are potential therapeutic targets.

Microbial processing L-carnitine, which is abundant in red meat and contains a trimethylamine structure similar to that of choline, was found to elevate plasma TMAO concentrations and enhance atherosclerosis in a microbial-dependent manner³⁶. It was also recently found that γ -butyrobetaine is the major proatherogenic intermediate in gut microbial metabolism of L-carnitine to TMA and TMAO³⁷. One of the proatherosclerotic mechanisms of TMAO might be that it increases macrophage foam cell formation, suppresses reverse cholesterol transport, and enhances platelet hyperreactivity (**Fig. 3**)³⁴. Bacterial taxa belonging to the families *Clostridiaceae* and *Peptostreptococcaceae* were positively associated with TMAO production in humans, suggesting that L-carnitine-metabolizing bacteria may belong to these families. Although the molecular mechanisms through which gut microbial formation of TMAO leads to increasing atherosclerosis and CV events are not entirely clear, this study may in part explain why excessive red meat consumption has been associated with increased CVD and mortality risks. In any case, these studies suggest

that targeting the gut microbial TMAO pathway as a treatment strategy, whether it is via dietary manipulation, alteration in microbial community with a probiotic, or pharmacological inhibition of microbial enzymes (e.g., DMB) involved in TMA production, has the potential to inhibit atherosclerotic CVD associated with elevated TMAO.

Gut Microbial Alternations Associated with Atherosclerosis

The studies using GF mice of how the intestinal bacteria make an impact on the development of atherosclerosis are limited^{38, 39}. One study showed that, although plasma cholesterol levels were significantly higher in GF/apoE^{-/-} mice than in conventional apoE^{-/-} mice, the effect of gut microbiota on atherosclerosis was ambiguous³⁸. Lipidomics analysis on GF and conventionally raised mice proposed that gut microbiota affect host lipid metabolism⁴⁰. A detailed mechanism for how the commensal bacteria may contribute to host lipid and cholesterol metabolism could potentially be explained by microbial regulation of bile acid synthesis and metabolism, but further studies are required.

A sequencing study comparing the gut microbiome from patients who had stenotic and symptomatic atherosclerotic plaques in the carotid artery and from healthy controls showed that the microbiome was more pro-inflammatory in the people with atherosclerotic plaques⁴¹. The shotgun sequencing of the gut metagenome demonstrated that the genus *Collinsella* was increased in patients with symptomatic stroke, whereas *Roseburia* and *Eubacterium* were enriched in healthy controls (**Fig. 2**). They also demonstrated that patients with symptomatic atherosclerosis were under-represented in enterotype I, and overrepresented in enterotype III¹⁸.

We tried to clarify the specific profile of gut microbiota in CAD patients to investigate a diagnostic and therapeutical potential of gut microbiota⁴². We used terminal restriction fragment length polymorphism (T-RFLP) analysis to detect the profile of gut microbiota in CAD and control patients who had T2D, hypertension, and/or dyslipidemia. T-RFLP analysis is one of the most well-established and reliable 16S ribosomal RNA-based methods, especially when considering its high throughput and reproducibility. T-RFLP using BslI could classify gut microbiota into ten groups: *Prevotella*, *Bacteroides*, *Lactobacillales*, *Bifidobacterium*, *Clostridium* cluster IV, *Clostridium* sub-cluster XIVa, *Clostridium* cluster IX, *Clostridium* cluster XI, *Clostridium* cluster XVIII, as well as others, combining the operational taxonomic units that belonged

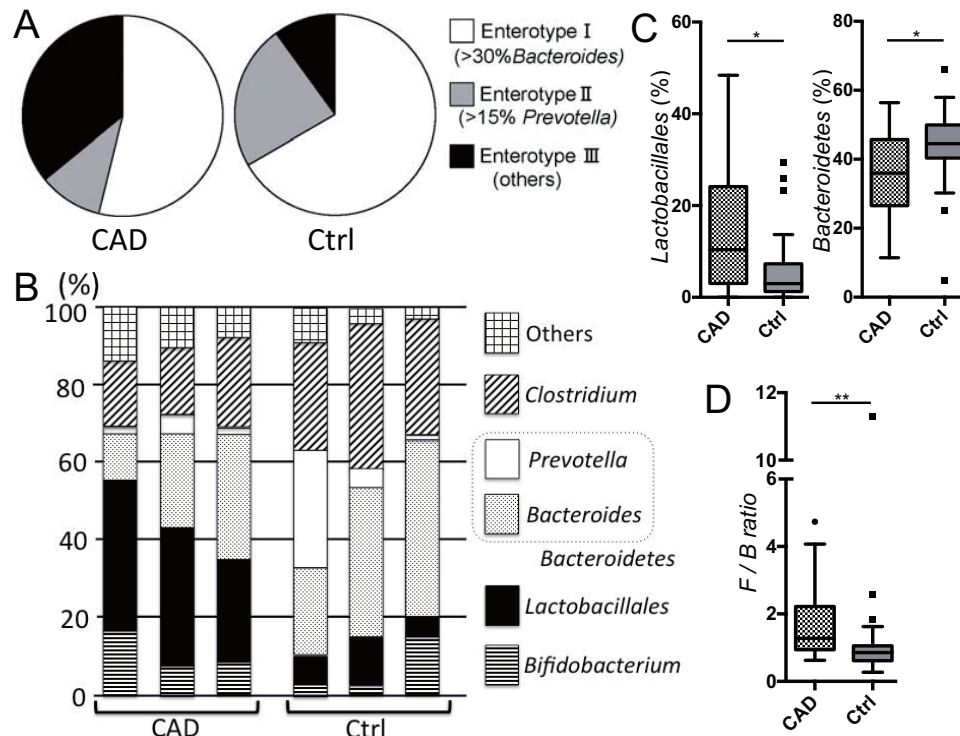


Fig. 4. Dysbiosis in coronary artery disease patients determined by terminal restriction fragment length polymorphism (T-RFLP)

(A) Enterotype III (high levels of *Ruminococcus* type) is predominant in coronary artery disease (CAD) patients. (B) Representative proportions of gut microbiota in CAD and control (Ctrl) patients were shown. (C) The percentage of the order *Lactobacillales* was increased; while the percentage of the phylum *Bacteroidetes* (*Prevotella* + *Bacteroides*) was decreased in CAD patients compared with Ctrl patients. (D) The F/B (*Firmicutes* / *Bacteroidetes*) ratio was increased in CAD patients. Kruskal-Wallis test followed by Dunn's post-hoc analysis was used to calculate *p*-values (**p* < 0.05 and ***p* < 0.01). Ctrl = age- and sex-matched controls with hypertension, type 2 diabetes and/or dyslipidemia who did not have coronary or other vascular diseases.

to the same group. Arumugam *et al.* suggested that the human gut microbiota could be stratified into three enterotypes¹⁸⁾. According to the report and our data, we defined enterotype I as *Bacteroides* >30%, enterotype II as *Prevotella* >15%, and the remaining as enterotype "others" (III), using this T-RFLP analysis of gut microbiota. Our data revealed that CAD patients were overrepresented in the enterotype "others" (III) compared with the control group (*p* < 0.001, chi-squared test; **Fig. 4A**)⁴²⁾.

We found that the order *Lactobacillales* was significantly increased and the phylum *Bacteroidetes* (*Bacteroides* + *Prevotella*) was significantly decreased in the CAD group compared with the control group (**Fig. 4B** and **C**). The F/B ratio, reported to increase in obesity²⁴⁾, was also increased in the CAD group compared with the control group (**Fig. 4D**). The order *Lactobacillales* is one of the main components of the human gut microbiome and belongs to the phylum *Firmicutes*. The order *Lactobacillales* is divided into sev-

eral genera, including *Lactobacillus*, *Streptococcus*, and *Enterococcus*. Because of the study protocol, we could not deny that medication could affect the composition of gut microbiota. The biological significance of the increase of the order of *Lactobacillales* and the decrease of the phylum of *Bacteroidetes* in CAD patients remains, as of yet, unknown. Although there are differences between carotid and coronary atherosclerosis, we found consistent results of low levels of the phylum *Bacteroidetes*. *Bacteroides fragilis*, which belongs to the phylum *Bacteroidetes*, affects mucosal T cell homeostasis by promoting regulatory T cell function. Other *Bacteroides* species can also establish mutualistic relationships with the host, by being able to flourish in the plant polysaccharide-enriched gut environment and by providing the biological byproducts necessary for the well-being of the host. Our work, and previous reports support the hypothesis that the phylum *Bacteroidetes* might help to prevent coronary atherosclerosis. Therefore, the role of bacteria in the

phylum *Bacteroidetes* in CAD should be assessed.

Conclusion and Perspectives

Intestinal immunity has been attracting much attention as a novel therapeutic target to treat atherosclerotic CVD. Since the discovery of Tregs and DCs, knowledge about the biology and pathophysiology of these regulatory immune cells has accumulated in the field of atherosclerosis as well as autoimmune diseases. It is now clear that several types of Tregs and tolerogenic DCs are essential for the regulation of pathogenic T cell immune responses in atherogenesis. Gut-associated immune tolerance induction by oral administration of drugs or therapeutic agents possessing immunoregulatory activities is a hopeful way to regulate inflammation. However, the details of mechanisms by which the intestinal immunity affect the systemic immunity remain to be clarified.

In association with intestinal immunity in atherogenesis, we have been interested in the gut bacteria that may be involved in the pathogenesis of atherosclerosis. We had already identified the types of gut microbiota susceptible to CAD. Further studies are needed to understand the functional level of some specific microbial pathways and their products, which contribute to maintaining our physiological homeostasis and drive disease progression. The next important step might be the application of this new knowledge to diagnostic and therapeutic approaches. The present status of the association between the gut microbiota and the incidence of T2D and atherosclerotic CVD was demonstrated in this review article. We hope that novel therapeutic strategies targeting the gut microbiome to prevent atherosclerotic CVD will be developed in the near future.

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