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Special Issue: Interaction between gut microbiota and host immune cells

Mini Review

Regulation of intestinal Th17 and Treg cells by gut microbiota

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Dysbiosis, an imbalance in the gut microbiota composition, is significantly associated with inflammatory bowel disease and other immune disorders. Dysbiosis can dysregulate immune system, compromise mucosal barrier integrity, and perpetuate chronic inflammation. Therefore, gut microbiota manipulation could be potentially used for treating various inflammatory diseases. Various intestinal bacteria differentially regulate the development and function of different immune cell populations. In particular, bacterial species falling within clusters VI and XIVa of the class Clostridia affect the generation and function of mucosal regulatory T cells, whereas segmented filamentous bacteria are the strong inducers for T helper 17 cells. This review discusses how the components of the gut microbiota affect the host immune system and disease susceptibility.

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Introduction

The gut microbiota is composed of diverse bacterial species and plays an important role in shaping intestinal immune responses[1, 2)]. Mucosal bacterial communities may not always play a quiescent role and may directly contribute to immune-mediated intestinal disorders such as inflammatory bowel disease (IBD). Recent studies have identified commensal bacterial species with immunomodulatory roles. For example, monocolonization of germ free mice with *Bacteroides fragilis* protected against colitis development by enhancing the production of anti-inflammatory cytokine (IL-10) production in regulatory T (Treg) cells, which play a significant role in suppressing intestinal inflammation[3)]. Another study showed that *Faecalibacterium prausnitzii* and its secreted products could attenuate chemically-induced colitis in mice by enhancing regulatory function of T cells[4)]. Furthermore, intestinal colonization with altered Schaedler flora (ASF), which

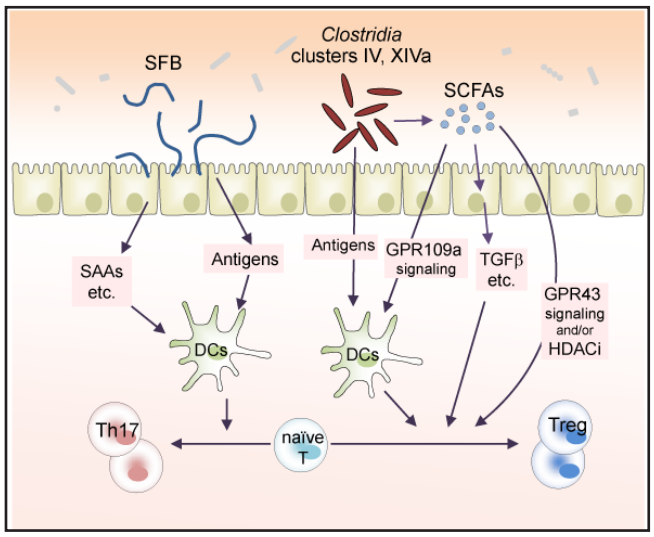

Fig.1 Effects of Clostridia and SFB on intestinal CD4$^+$ T cells
Segmented filamentous bacteria (SFB) activate intestinal epithelial cells (IECs) to produce serum amyloid A (SAA) and other molecules, which condition dendritic cells (DCs) to drive naïve CD4$^+$ T cells to differentiate into Th17 cells. Clostridia species produce short chain fatty acids (SCFAs) and other metabolites and stimulate IECs, DCs, or CD4$^+$ T cells to mediate Treg cell accumulation. Importantly, SFB and Clostridia both induce antigen-specific CD4$^+$ T cell responses. HDACi, histone deacetylase (HDAC) inhibition.

includes eight defined bacterial strains, resulted in Treg cell accumulation in the colon lamina propria (LP)[5]. Our previous studies, which were corroborated by other studies, have shown that segmented filamentous bacteria (SFB) can potently induce IL-17-producing CD4$^+$ T cells (Th17 cells) in the small intestine of mice[6, 7] (Fig. 1). We have also shown that a combination of 46 strains of Clostridia that are indigenous to conventionally reared mice can induce the accumulation of Foxp3$^+$ CD4$^+$ Treg cells in the mouse colonic LP and thereby protect mice against colitis and allergic response[8]. In our recent study, we identified 17 strains of human-derived Clostridia as potent inducers of Treg cells[9].

Since different bacterial species differentially affect host immune homeostasis, differences in microbial composition may contribute to inter-individual differences in immune responses and susceptibility against infection, autoimmunity, cancer, or other immunological conditions. Unveiling the underlying cellular and molecular mechanisms could lead to novel ways of regulating mucosal immunity.

Dysbiosis and inflammation

Dysbiosis, a loss of intestinal microbial diversity, has been documented in patients with diseases such as IBD, autoimmune disease, asthma, food allergy, diabetes, obesity, and liver cirrhosis. Animal studies have shown that dysbiosis can lead to immune dysregulation and compromised mucosal barrier integrity, and thereby perpetuate the cycle of disease-related chronic inflammation. For example, T-bet$^{-/-}$RAG2$^{-/-}$ mice develop spontaneous colitis, which is horizontally and vertically transmissible to wild-type mice that are co-housed with the T-bet$^{-/-}$RAG2$^{-/-}$ mice. Furthermore, 16S rRNA gene analysis revealed that T-bet$^{-/-}$RAG2$^{-/-}$ mice have increased numbers of *Klebsiella pneumoniae* and *Proteus mirabilis*[10]. Another study showed that mice deficient in NLRP6 have altered commensal microbiota and exhibit high sensitivity to dextran sodium sulfate (DSS)-induced colitis[11]. The study also revealed that colitogenic microbiota of NLRP6-deficient mice is transmissible to wild-type mice and that the NLRP6-deficient mice had increased numbers of members of the family Prevotellaceae and TM7 in their fecal microbiota.

Alteration of the gut microbiome via fecal microbiota transplantation (FMT), which involves transferring fecal bacteria from a healthy donor to the patient via duodenal tubing, colonoscopy, or enema, has been shown to be effective for treating colon inflammatory disorders. For example, FMT has been shown to be effective for treating patients with pseudomembranous colitis caused by *Clostridium difficile* infection[12]. Thus, there is an increasing research interest in assessing the potential applications of FMT for treating other diseases associated with inflammation, including multiple sclerosis and IBD. Although FMT treatment efficacy can serve as proof-of-principle for the feasibility of using human microbiome manipulation methods as a therapeutic strategy for inflammatory diseases, the commercial development of fecal transplant products encompasses several challenges associated with manufacturing, quality assurance, pathogen contamination risk, donor selection, and patient acceptance. Therefore, treatments that incorporate the use of a composite of well-characterized bacterial strains are more desirable.

Induction of Th17 cells by SFB

Th17 cells have been shown to infiltrate the inflamed intestine of patients with IBD and exacerbate inflammation by secretion of IL-17 and other inflammatory cytokines. Previous studies have also shown marked accumulation of Th17 cells in the intestinal LP of specific-pathogen free (SPF) mice during steady state conditions, particularly in

the small intestine. Notably, the percentage of LP Th17 cells is markedly reduced in germ-free (GF) or antibiotic-treated mice[13, 14]. Therefore, the development of Th17 cells in LP is dependent on the stimulation by intestinal microbiota. A previous study showed that mice deficient in toll-like receptor 9 (TLR9), a receptor for specific unmethylated CpG sequences of bacterial DNA, have decreased numbers of LP Th17 cells. Furthermore, *in vitro* differentiation of intestinal Th17 cells is enhanced by the addition of flagellin, a ligand for TLR5. Bacteria-infected apoptotic intestinal epithelial cells also provide TLR ligands that trigger dendritic cells (DCs) to produce IL-6 and TGF-β, resulting in the promotion of Th17 differentiation[15]. Taken together, these results indicate that intestinal bacteria mediate mucosal Th17 differentiation via TLRs and other innate immune receptors. However, mice deficient in mediators of TLR signaling pathways (double knockouts for MyD88 and TRIF) have normal numbers of LP Th17 cells in the small and large intestines. Thus, individual TLRs may convey divergent signals that differentially affect Th17 cell differentiation. Further studies are needed to clarify the role of each TLR in the induction of intestinal Th17 cells. In addition to pathogen-associated molecules that stimulate TLRs, extracellular adenosine 5'-triphosphate (ATP) derived from the microbiota can also induce Th17 cells[13]. Therefore, it is likely that several signaling events can contribute to the accumulation of Th17 cells in the intestine.

Mice housed in different SPF facilities were found to have marked differences in the number of LP Th17 cells[14]. This observation led to the identification of SFB as members of the indigenous microbiota responsible for gut Th17 cell accumulation. Monocolonization of mice with SFB, but not other assessed bacterial species, induced a marked accumulation of Th17 cells in the small intestine[6]. In accordance with this observation, mice expressing transgenic human defensin-alpha 5 (DEFA5) in Paneth cells exhibited loss of SFB, accompanied by a decrease in the number of Th17 cells in the small intestine[16]. SFB-mediated Th17 cell differentiation is likely mediated by a mechanism independent of TLR- or ATP-signaling[6]. A previous study showed that the attachment of SFB induces morphological changes in IECs such as the accumulation of actin around the attachment site. Furthermore, a recent *in vitro* co-culture study of SFB and IECs demonstrated that SFB adhere to IECs and induce the expression of inflammation-associated genes, such as those encoding the serum amyloid A (SAA), fucosyltransferase 2 (Fut2), and RegIIIγ[17]. SAA can stimulate DCs to produce IL-6 and IL-23, which have been implicated in chronic intestinal inflammation[6]. Taken together, the studies indicate that SFB attachment induces activation of IECs, which produce inflammatory molecules (such as SAAs) that then activate LP DCs that activate Th17 cells (Fig. 1). Further investigation is needed to establish the molecular basis underlying SFB-mediated Th17 cell differentiation.

Colonization of gut mucosa with SFB, and consequent induction of Th17 cells, has a protective function against pathogenic bacteria such as *Citrobacter rodentium*[6]. It is likely that Th17 cytokines stimulate IECs to produce antimicrobial peptides and other molecules, which limit the growth of *C. rodentium*. SFB colonization was also shown to prevent the colonization of enteropathogenic *Escherichia coli* O103 in rabbits[18]. Moreover, there is a strong correlation between the presence of SFB and a diabetes-free state in NOD mice[19]. On the basis of these findings, one may envisage that SFB-mediated induction of Th17 cells is beneficial to the host. However, this is not always the case. In the K/BxN mouse model of autoimmune arthritis, colonization with SFB enhances the production of autoantibodies and accelerates disease progression through induction of Th17 cells[20]. Mice harboring SFB are highly susceptible to experimental autoimmune encephalomyelitis (EAE) symptoms compared with GF mice[21]. These reports raise the possibility that the SFB-mediated Th17 induction may be harmful to the host. At present, the conditions that determine whether intestinal Th17 cells play a beneficial or harmful role in the host are not fully understood. IL-23 and IL-1β cytokine levels are important determinants of whether Th17 cells mediate harmful or protective immune responses. Further studies are required to elucidate the cellular and molecular mechanisms that determine the inflammatory or regulatory character of Th17 cells.

The finding that SFB-induced Th17 cells can contribute to autoimmune arthritis in K/BxN mice and host resistance to *C. rodentium* indicates that Th17 cells present in the intestine may have a broad repertoire of T-cell receptors (TCRs), and not be microbiota-specific. In contrast, recent studies indicate that most SFB-elicited Th17 cells have TCRs that are specific for SFB antigens[22, 23]. Indeed, two major protein antigens were identified to be responsible for SFB-mediated SI LP Th17 cell induction (SFBNYU_003340 and SFBNYU_004990)[23]. These findings may serve as a guide for future studies to determine the role of human commensal-specific pro-inflammatory T cells in the de-

velopment of autoimmune diseases such as rheumatoid arthritis and multiple sclerosis.

Induction of Treg cells by Clostridia

Foxp3$^+$ Treg cells are critical for maintaining immunological unresponsiveness to self-antigens and in suppressing excessive immune responses deleterious to the host. For example, Foxp3$^+$ Treg cells suppress the colitogenic activity of both effector T cells and innate lymphoid cells. Foxp3$^+$ Treg cells are derived from two sources: thymic Treg cells (tTreg), which arise and mature in the thymus, and peripheral Treg cells (pTreg), which develop extrathymically. pTreg cells can be distinguished from tTreg cells on the basis of lower expression levels of Helios and neuropilin-1[24, 25]. Helios- neuropilin-1$^-$ Foxp3$^+$ Treg cells (presumably pTreg cells) are most abundantly present in the intestinal LP[8]. In antibiotic-treated or GF mice, the frequency and absolute number of colonic Foxp3$^+$ Treg cells, particularly Helios$^-$ neuropilin-1$^-$ Foxp3$^+$ Treg cells, are considerably reduced. On the other hand, the number of Treg cells in the small intestine is unchanged or increased by the absence of microbiota[8]. Therefore, the intestinal microbiota has a profound effect on the number of colonic Treg cells; however, different mechanisms are involved in the induction of small intestinal Treg cells.

Several DC subsets and macrophages have been demonstrated to induce Treg cell accumulation in the intestine. In particular, a CD103$^+$ DC population in LP was shown to preferentially promote Treg cell generation and homing to the intestinal mucosa. Furthermore, CD103$^+$ DCs express retinal dehydrogenase (RALDH), an enzyme that converts retinal to retinoic acid[26]. Retinoic acid induces the expression of gut-homing receptors on T cells and also enhances pTreg cell differentiation in conjunction with TGF-β. In addition, granulocyte-macrophage colony-stimulating factor (GM-CSF) produced by innate lymphoid cells stimulates DCs and macrophages to produce retinoic acid, leading to the induction of pTreg cell accumulation in the large intestine[27].

As observed with Th17 cells, Treg cells are also activated by distinct constituents of the gut microbiota. Particularly, lactobacilli and bifidobacteria have been implicated in the induction of Treg cells. Treatment of mice with the probiotic mixture VSL#3 (a mixture of bifidobacteria, lactobacilli, and *Streptococcus salivarius*) or with probiotic strain *Lactobacillus reuteri* increases the percentage of Treg cells[28]. However, these probiotic strains are only transiently present and do not exist at high levels in the intestines of adult mice and humans. Furthermore, it is yet to be demonstrated whether monocolonization with any of these probiotic strains induces Treg cells. Therefore, the effects of probiotic strains on the induction of Treg cells are not well characterized. The probiotic strains may also mediate changes in the microbial ecology within the gut and, thereby, indirectly stimulate Treg cells. The human commensal *B. fragilis* has been shown to facilitate the functional maturation of Treg cells in mice[3]. Monocolonization with *B. fragilis* boosts IL-10 production in colonic Treg cells, although the effect on Foxp3$^+$ Treg numbers is marginal. The induction of IL-10 in Treg cells by *B. fragilis* was shown to be mediated by polysaccharide A (PSA). Furthermore, injecting or feeding mice with PSA was shown to be sufficient to replicate the *B. fragilis* immune effects. The study demonstrated that PSA binds to TLR2 on CD4$^+$ T cells to induce IL-10 production.

Previously, we have shown that bacteria belonging to clusters XIVa and IV Clostridia can induce an increase in the number of colonic Treg cells (Fig. 1). The colonization of GF mice with a defined mixture of 46 strains of Clostridia, which were originally isolated from chloroform-treated fecal material (spore-forming fraction) obtained from conventionally reared mice, induced Treg cells8). We also identified 17 strains of human-derived Clostridia as potent inducers of Treg cells[9]. The strains were isolated from human fecal samples collected from healthy donors by performing a sequence of selection steps and by using gnobiotic techniques for obtaining Treg cell-inducing human-derived bacterial strains. We demonstrated that Treg cells were induced in the colon of ex-GF mice orally inoculated with a chloroform-treated human fecal sample. Next, the cecal contents from these mice were treated with chloroform, diluted, and serially transplanted into other GF mice, while monitoring Treg induction capability. We succeeded in obtaining mice in which the complexity of the gut microbiota was greatly decreased without sacrificing Treg-inducing potency. From these mice, we cultured and selected 17 strains of Clostridia belonging to clusters IV and XIVa. When mixed together and orally administered to GF mice and rats, the 17 strains were able to induce a significant accumulation of CD4$^+$Foxp3$^+$ Treg cells in the colon. Importantly, mono-colonization of GF mice with one of the 17 strains was insufficient to induce Treg cells *in vivo*, indicating that synergistic effects are mediated in a microbial community-dependent manner[9]. Repeated oral ingestion

of the mixture of 17 strains rendered specific-pathogen-free mice resistant to experimental allergic diarrhea and trinitrobenzene sulfonic acid-induced colitis. Therefore, the 17 strains have a prophylactic effect in mouse colitis models. The proportion of Clostridia clusters XIVa and IV in the fecal microbial community is smaller in IBD patients than in healthy controls. These findings suggest that prophylactic administration of human-gut-resident Clostridia species could reduce the susceptibility to IBD.

The precise mechanism by which the 17 strains of Clostridia stimulate the colonic Treg cells remains to be elucidated. One suggested mechanism is the production of short chain fatty acids (SCFAs) (Fig. 1), which have multiple metabolic and immune functions[29]. In the context of Treg induction, SCFAs can elicit a TGF-β1 response in IECs, which can contribute to *de novo* induction of pTreg cells. SCFAs, particularly butyrate, can suppress DC activation by suppressing the expression of the NF-κB component RelB[30]. It has also been shown that butyrate activates signaling pathways through GPR109a to induce the expression of anti-inflammatory genes in DCs[31]. In addition, butyrate can directly stimulate tTreg proliferation through the activation of GPR43[32] as well as stimulate the differentiation of naïve $CD4^+$ T cells into pTreg cells through histone H3 acetylation of the *Foxp3* gene intronic enhancer by inhibiting histone deacetylase[30, 33].

The antigen specificity of colonic Treg cells has been previously studied and the data indicate that many Treg cells in the colon display TCRs specific for the gut microbiota[9, 34]. Thus, specific commensal bacteria induce naïve $CD4^+$ T cells to differentiate into antigen-specific colonic pTreg cells that, presumably, enforce immune system tolerance towards those bacteria. In contrast, a recent study described MHC class II-independent accumulation of colonic Treg cells[35]. Therefore, the gut microbiota may stimulate tTreg cell proliferation and/or recruitment. Further studies will be needed to determine how the microbiota influences T cell polarization.

Conclusion

In this review, we described the mutual interactions of the microbiota with the host mucosal immune system and the consequences of such interactions. It is likely that preserving the diversity of the microbiota and generating a variety of mucosal immune cell populations are mutually beneficial for the maintenance of bacterial and host homeostasis in the intestine. Loss of diversity can be a cause and a predisposing factor for various diseases. Several probiotics have been historically used in humans for medicinal purposes and have been shown to be safe for consumption. However, probiotics currently in use have generally been selected based on properties such as ease of culture and tolerance to acid and oxygen, and are not among the major colonizers of the human gut. In other words, they have not been isolated based on their ability to correct microbiome dysbiosis associated with human disease or to boost specific arms of the host immune system. Presumably as a result, the dysbiotic microbiota are refractory to treatment with currently available probiotic strains, and most probiotics tested to date have demonstrated, at best, mediocre effects in the clinic. Thus, there is a compelling need to identify more robust therapeutic organism compositions that are compatible and symbiotic to the host and, ideally, able to induce broader changes to the microbial ecosystem to correct dysbiosis and drive the immune system to normal homeostasis. Recent metagenomic and metabolomic studies together with gnotobiotic studies have offered new insights into the components and functions of the gut microbiota. However, further studies are required to elucidate the interplay between the host and the microbiota during the steady state and during disease development.

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Conflct of interests

None

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