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Review

Symbiotic and antibiotic interactions between gut commensal microbiota and host immune system

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ABSTRACT

The human gut commensal microbiota forms a complex population of microorganisms that survive by maintaining a symbiotic relationship with the host. Amongst the metabolic benefits it brings, formation of adaptive immune system and maintenance of its homeostasis are functions that play an important role. This review discusses the integral elements of commensal microbiota that stimulate responses of different parts of the immune system and lead to health or disease. It aims to establish conditions and factors that contribute to gut commensal microbiota's transformation from symbiotic to antibiotic relationship with human. We suggest that the host-microbiota relationship has been evolved to benefit both parties and any changes that may lead to disease, are not due to unfriendly properties of the gut microbiota but due to host genetics or environmental changes such as diet or infection.

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1. Introduction

It is now become evident that microbiota plays an essential role in the function, induction, and training of the host immune system. As a consequence, the immune system has evolved strategies to maintain this symbiotic relationship with a large number of diverse microbes. An average human gut contains approximately 10^{14} bacteria, most of which cannot be cultured. The vast majority of these commensals fall into one of two phyla: gram-negative *Bacteroides* and gram-positive *Firmicutes*. It has been approximated that these bacteria contain over 100 times more genes than a whole human genome [1]. Many of these genes directly influence host metabolic pathways and provide the host with nutrients that otherwise it would not receive [2]. Therefore, the gut commensals differ from pathogens in that they are allowed to co-exist due to the benefits they provide to the host; therefore the host does not try to eradicate them from the mucosa, but still maintains the ability to actively fight pathogens.

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Germ free (GF) mice are a tool often used to see the effects of ablated microbiota on host metabolic and immune function [3]. These mice also enable one to re-colonize the gut with a specific species of commensal bacteria to isolate its effects. This review will discuss the gut commensal microbiota interactions with the host immune system, focusing on the responses each part of innate and adaptive immune system elicits. These responses may be beneficial for the immune system, since they enhance immune system's ability to fight pathogens and maintaining the composition of gut microbiota. However, the responses can be unwanted since they may lead to local inflammatory disorders, such as inflammatory bowel disease (IBD) or autoimmune disease away from the gut [4]. This review will also attempt to juxtapose recent evidence on gut commensal interaction with the host to establish what contributes to switching from symbiosis to antibiosis.

2.   Commensal microbiota affects infection

Composition of gut commensal organisms is greatly affected when one is subjected to antibiotic treatment [5–7]. This change in composition has been associated with pathogenic infections of the gut [8,9]. Closely related species of pathogenic bacteria that are commensal in the gut seem to tolerate their related pathogenic bacteria colonization [10]. Therefore, it is safe to say that specific microbial species dictate pathogenic microbial colonization.

Even though, certain microbiota permits colonization of pathogens, some can limit pathogenic bacterial growth. *Salmonella typhimurium* induces inflammation which changes microbial composition and suppresses their growth [11]. Avirulent *S. typhimurium* does not cause colitis and fails to outcompete the microbiota unless inflammation is induced. IL-10 knockout mice, a model of IBD, allow the pathogen to overcome colonization resistance. However, transferring normal gut flora to *S. typhimurium* infected gut allows the mice to recover and eliminate the pathogen [12]. The process occurs in the absence of antibody response that clear the pathogen in the secondary infection, thus clearance results as a direct commensal microbiota pressure.

3.   Innate responses

Intestinal epithelial cells (IECs) form a barrier that protects the host from bacterial invasion. In addition to acting as a physical barrier, they perform a role in the immune cell regulation via expressing receptors for microbial-associated molecular pattern (MAMPs). Activation of these receptors leads to downstream cascades, which affect the inflammatory status of IECs. Apical expression of toll-like receptor 9 (TLR9) is involved in immune homeostasis [7]. Activation of TLR9 on the apical membrane of IECs leads to partial activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NFkB) without stimulating the release of pro-inflammatory cytokines. Apical introduction of CpG sites (where cytosine nucleotide occurs next to a guanine nucleotide) of gut commensals reduces the pro-inflammatory cytokine release when baso-lateral TLR9 receptors are activated in IECs [13]. Protective

effects of microbiota can also be evidenced by *Lactobacillus casei*, a common probiotic, inducing anti-inflammatory effects through inhibition of NFkB pathway via stabilization of IkBα during *Shigella flexneri* infection, which ameliorates the disease symptoms [14].

Short chain fatty acids (SCFA) are produced by fiber carbohydrate fermentation in the gut by *Bacteroides* and *Clostridium* species within the human gut [15]. Butyrate, one of the products of fiber fermentation, provides a signal for inhibition of pro-inflammatory cytokine expression in the IECs that involve inhibition of NFkB pathway [16]. Moreover, butyrate induces other protective mechanisms, such as production of mucin and antimicrobial peptides, as well as increases expression of tight junction proteins strengthening the epithelial barrier [17]. Lower butyrate levels have been associated with inflammatory bowel disease (IBD) such as Crohn's disease [16]. This shows that specific gut microbiota is important in keeping the unwanted organisms in check as well as preventing development of autoimmune disease, such as IBD.

It is important to point out that the host immune mediators play a significant role in controlling the microbiota. Changing part of the immune control system alters the gut flora composition. The effects of dysbiosis have been illustrated by the mice lacking Toll-like receptor 5 (TLR5) [18]. These mice are highly predisposed to type 2 diabetes and cardiovascular disease due to developed obesity. This metabolic syndrome is caused by the altered balance of *Firmicutes* and *Bacteroidetes*, which has been shown by transplanting the gut flora from the knockout mice into the wild-type, leading to development of the metabolic syndrome [18]. Altered host mechanisms of immune regulation have an impact on gut commensal composition and in this way cause the disease. However, under normal conditions the dysbiosis would be unlikely to occur and would not lead to disease.

A new population of innate cells that are of lymphoid origin has been identified recently. Not much is known about the interactions of these cells with the gut commensals. However, it seems that they can respond to direct and indirect actions of gut commensals to elicit inflammatory and barrier strengthening responses [19]. These cells produce a great variety of proinflammatory cytokines in response to activation through TLR and other receptors limited to innate lymphoid cells [20]. Therefore, it is possible to speculate that responses of these cells to gut microbiota affect immune homeostasis.

4.   Adaptive responses

4.1.   Th17 cells

T-cell responses have been shown to be dependent on the gut commensal composition. Germ-free mice tend to have diminished T helper 1 and 17 cells (Th1 and Th17) responses but maintained or increased CD4(+) T regulatory cells (T(regs)) frequency [21]. It has been found that segmented filamentous bacteria (SFB) play an important role in inducing intestinal Th17 cells. These bacteria adhere to the surface of the intestine, possibly contributing to their active sampling by the dendritic cells and the strong induction of Th17 cells [22]. This has been confirmed by increased expression of interleukin 17 and 22

(IL-17 and IL-22) cytokines upon colonization by SFB [23]. It has been elucidated by the same study that Th17 differentiation is mediated by the induced serum amyloid A in gnotobiotic mice colonized with SFB [23]. Induction of Th17 cells can be beneficial when fighting against pathogenic bacteria. SFB colonization protects the mice from *Citrobacter rodentium* infection by reducing their growth through stimulation of Th17 cells, which suggests that the commensals are involved in enhancing barrier function of the gut. Although experiments were done in mice, it is generally believed to be transferable to humans, since the presence of SFB 16sRNA has been detected not only in humans but also across a wide range of vertebrae species [24].

Although, SFB seem to confer a protective function, in the case of subjects with immune deficiencies, SFB induction of Th17 has been associated with autoimmune diseases, such autoimmune arthritis, where mice colonized with SFB had more auto reactive antibodies compared to GF mice [25], and experimental autoimmune encephalomyelitis (EAE) [26], a mouse model for human multiple sclerosis. In each model, mice have been bred to develop a genetic alteration which increases a susceptibility to a specific autoimmune disease. The disease progression and severity is then compared across each strain with different groups subjected to different microbiota conditions [27]. This allows for the recognition of the effects of specific commensals on progression of a specific disease. Whether induction of Th17 cells is beneficial or detrimental is not fully understood. Alterations in IL-1β and IL-23 molecules have been recognized as factors in developing autoimmune disease [28,29]. It seems that it is the combination of Th17 inducing microbiota and host factors that contribute to the disease state.

However, Th17 pro-inflammatory cells have been shown, using model of tolerance by CD-specific antibodies in the mouse model of sepsis, to be redirected and controlled in the small intestine [30]. These regulatory Th17 have an anti-inflammatory cytokine profile with IL-10 being the major player [31]. Conversion of Th17 could mean that their induction by microbiota may not be the main driving force for the disease and that Th17 cells could in fact greatly contribute to the regulation of immune responses.

4.2. T-regulatory cells

The proportion of CD4(+) T regulatory cells, which express the Foxp3 transcription factor, is much larger in the colonic mucosa than in other connective tissues or organs [32]. In the GF mice the proportion and the absolute numbers of T (regs) in the colonic mucosa of the colon are reduced. However, the number of T(regs) in the small intestine remains unchanged [32] and it can be induced by a mixture of Clostridia strains from human microbiota [33,34], leading to believe that microbiota play an important role in T(reg) cell induction.

A considerably important studies on adaptive immune system education has been conducted recently [35,36]. They showed that normal commensal microbiota in the mice intestine generated a specific group of T(reg) cells, limited to the gut. The T-cell antigen receptors (TCR) present in these T (reg) cell populations seemed to be reactive to specific commensal antigens. Creation of retroviral bone marrow

chimeras and a TCR transgenic line showed that normal gut flora is essential for the induction of colonic T(reg) cells from naive T-cells. Lastly, using adoptive transfer of microbiota reactive effector T-cells, into *Rag−/−* mice that have induced colitis illustrates the pathological consequences of T-cell recognition of gut commensals, under proinflammatory conditions [35].

It has been argued that certain commensal bacteria can induce CD4+ T-cell differentiation into T(reg) cells. One of the well-researched examples has been the spore-forming *Clostridium* species that belongs to clusters XIVa and IV [32]. Colonizing GF mice with specially isolated *Clostridium* species from the normally grown mice induced T(reg) cell differentiation. These T(reg) cells are helios-negative, thus are induced in the colon rather than matured in the thymus [37]. Furthermore, *Clostridium*-induced T(reg) cells express high levels of IL-10, which is an important cytokine for maintenance of immune homeostasis [32,38]. Mice were also subjected to dextran sodium sulphate (DSS)-mediated colitis, which made it possible to show that inoculations with *Clostridium* spp. reduced allergic and inflammatory diseases progression [32]. Therefore, specific gut commensal species can benefit the host by not just conferring tolerance to themselves but also creating a more generally tolerant environment by induction of T(reg) cells.

Other bacteria such as *Lactobacilli* and *Bifidobacteria* have been proposed to induce T(reg) cells. Treatment of mice with these probiotic bacteria or other strains of *Lactobacillus* increase T(reg) cell abundance [39]. *Bacteroides fragilis* is a human gut commensal that, through TLR2 ablation in CD4+ T cells study in mice, activates TLR2 receptors in naive T-cells to induce their differentiation toward the T(reg) cells [40]. The key factor in induction of T(regs) has been found to be the polysaccharide A (PSA) produced by *B. fragilis* that allows this tolerance to be induced. Furthermore, lack of PSA induces the Th17 axis that prevents successful colonization of these tolerance-inducing bacteria [40]. This evidence suggests that the immune system can recognize pathogens from the commensals and as a by-product receive regulation of immune homeostasis.

4.3. IgA of B-cells

Intestinal mucosa is a large site of immunoglobulin A (IgA) production where it plays an important role in maintenance of mucosal homeostasis, especially protecting from pathogenic bacteria invasion. It is believed that IgA contains bacteria from invading the lamina propria mucosa (LPM). Although, its role may be in fighting pathogens, it also appears to control gut commensal populations [41]. In the GF mice the IgA production is reduced due to lower levels of plasma cells. Colonizing mice with fecal flora containing commensal species such as *Alcaligenes* can restore the IgA production [42]. *Alcaligenes* tend to colonize the Peyer's patches where they can actively interact with the CD11+ dendritic cells which send signals to lymphocytes to induce IgA production. IgA production only increases at the sites of *Alcaligenes* colonization and no systemic spillover of IgA-producing plasma cell induction occurs [42]. As mentioned, IgA induction has an important role in maintaining immune homeostasis by controlling gut commensal populations as well as preventing pathogens from invading the LPM. GF mice

colonized by *B. thetaiotaomicron* commensal bacteria with no adaptive immune system (*Rag−/−*) tend to have elevated proinflammatory innate immune responses that are associated with gut inflammatory diseases [43]. However, introduction of hybridized plasma cells which express IgA binding *B. thetaiotaomicron* capsular polysaccharide, can balance the commensal populations so that proinflammatory responses are not too sufficiently strong to cause disease [43]. Abolishing IgA responses in mice have also shown expansion of segmented filamentous bacteria (SFB), which have been shown to induce Th17 mediated autoimmune disease [21,41]. Therefore, gut commensals have an important role in preventing development of dysbiosis by controlling their own and neighboring commensal populations through IgA induction as well as protecting the host from pathogenic colonization.

5. Diseases associated with changes in gut microbiota and deregulated immune response

In healthy organism, gut microbiota and host immune system acts symbiotically allowing the balanced induction of protective responses to antigens and pathogens. However, changes in diet, abuse of antibiotics, and other environmental factors contributed to alteration in composition of microbiota resulting in the lack of diversity required to keep balanced immune responses. This caused an antibiotic effect in gut microbiota-host immune system relationship contributing to the dramatic rise in autoimmune and inflammatory disorders [44,45]. Although there are many confirmed evidences that alterations in the gut microbiota composition affects immune responses, it is also becoming increasingly apparent that such changes can affect negatively the immunity in organs and tissues distal from the intestine [46]. Typical cases characterizing the gut microbiota changes associated with immune response-related diseases are summarized in Table.

A number of studies have shown that primary IBD, Crohn's disease (CD), and ulcerative colitis (UC) are associated with a decreased complexity of the gut microbiota and shift to the dysbiosis [44]. Both CD and UC were characterized by depletion of the phyla *Firmicutes* (predominantly *Costridia* class *Lachnospiraceae* family) and *Bacteroidetes* and by outgrowth of the phylum *Proteobacteria* (*Enterobacteriaceae* family) [47]. The CD has also been associated with such pathogens as *Clostridium difficile* and *Escherichia coli* [59,60], while colitis has been linked with commensals *Helicobacter hepaticus* and *Bacteroides* [61,62], which all have shown to be important contributors to IBD in mouse models. Notably, some commensal bacteria with enhanced inflammatory potential are closely related to pathogens allowing their survival under tough immune response conditions, and thus contribute to disease by promoting innate and adaptive immune responses to otherwise benign commensals or food antigens [63–65]. Role of gut microbiota has also been implicated in several other autoimmune diseases (coeliac disease, rheumatoid arthritis and encephalomyelitis), as well as allergies, autism, gastric cancer, obesity and type 2 diabetes (Table).

To date, the majority of research findings that focused on how the gut microbiota is contributing to host immune system related disease have mainly been tested on mouse models and it is not completely understood whether acquired knowledge can be directly applied to humans.

Table – Changes in the gut microbiota associated with immune response-related diseases and health conditions.

Disease and health condition	Implicated microbiota	Changes[a]	Relevant reference[b]	Potential preventative measures
IBD, Crohn's disease and ulcerative colitis	*Actinobacteria, Proteobacteria*	Increase	[47]	Clostridia and SCFA can induce directly T(reg)s opposing intestinal inflammation and colitis induction [34,48]
	Bacteroidetes, Lachnospiraceae	Decrease		
IBD, Crohn's disease	*Bacteroides ovatus, Bacteroides vulgatus*	Increase	[49]	
	Bacteroides uniformis	Decrease		
	Faecalibacterium prausnitzii		[50]	
Coeliac disease	*Bacteroides, Escherichia coli*	Increase	[51]	
Rheumatoid arthritis	*Bacteroides (Prevotella copri)*	Increase	[52]	
Encephalomyelitis	*Alteration of gut microflora after antibiotic treatment*	Decrease	[53]	Polysaccharide A produced by *Bacteroides fragilis* can protect against central nervous system demyelinating disease [53]
Allergy	*Bifidobacterium adolescentis*	Decrease	[54]	
	Lactobacillus spp.		[40]	
Autism	*Firmicutes (Clostridium)*	Increase	[55]	Human commensal *Bacteroides fragilis* corrects gut permeability, alters microbial composition and ameliorates autism spectrum disorder-related defects [55]
Gastric cancer	*Helicobacter pylori*	Increase	[56]	
Obesity	*Bacteroidetes*	Decrease	[57]	
	Lactobacilus	Increase		
Type 2 diabetes	*Firmicutes (Clostridia)*	Decrease	[58]	
	Escherichia coli	Increase		

[a] Changes relative to the healthy subject.
[b] One exemplary reference is provided.

6. Therapeutics

As discussed above, the host-commensal interactions are very complex and designing a right combination of these bacteria to tackle disease is very difficult. Gut commensals can directly and indirectly limit pathogen growth. Infection by *C. difficile* is a common type of hospital acquired infections due to their resistance to cleaning agents and antibiotics. Using the concept that commensals can limit pathogenic infections, a double blind, and placebo controlled study examined the role of probiotics containing *Lactobacillus* and *Bifidobacterium* [66]. The study showed that toxins produced by the pathogen were less frequently present among patients that were taking this probiotic than amongst those who did not. Furthermore, fecal transplants have become a viable option in treating Clostridium-associated diseases which attenuate their pro-inflammatory effects [67–69].

The protective effect of some bacterial species such as *Barnesiella* has been also confirmed in cases of broad antibiotic treatment [9], where intestinal microbiota was dominated with vancomycin-resistant enterococcus, a pathogen causing infections in immunocompromised patients [70].

Our knowledge that TLR9 pathway activation in epithelial cells leads to anti-inflammatory milieu [13], which can alleviate inflammation inducing infections [14]; this makes it possible to potentially exploit it for therapeutic purposes. TLR2−/− and TLR4−/− mouse model of colitis presented with *E. coli* CpG DNA fragments showed a decrease in disease progression. This positive effect of probiotics has been confirmed using the whole bacteria [71,72]. TLR9−/− mice were used to confirm that TLR9 receptors were crucial for anti-inflammatory actions of probiotics.

7. Concluding remarks

There are an increasing number of studies that attempt to describe gut commensal and host immune interactions. At the moment, it is clear that gut microbiota greatly contributes to the metabolic and immune homeostasis and influence health and disease. Gut commensals are essential for healthy metabolic function and their altered composition does correlate with various metabolic issues. A list of autoimmune diseases, many appearing outside of the gut, is also correlated to the dysbiosis [73]. Although, the evidence may suggest that the gut commensals can be beneficial and harmful, the complex host environment affects this friend or foe status to a large extent. Gut commensals, like any bacteria, whether pathogenic or not, are only concerned with their own wellbeing and do not differentiate between the host's health and its disease. Their survival depends on the symbiotic relationship. However, changes in the host environment (due to genetic or other environmental factors) can quickly reverse gut commensal role or it may disable them from surviving under new conditions. This may result in the host losing an important service, which in turn may lead to disease. It is difficult to determine whether the changes in commensal composition are a primary cause of disease. Therefore, further understanding of the mechanisms of microbial interactions with the host is crucial for future therapeutic uses of the gut commensal manipulation.

Conflict of interest

The authors state no conflicts of interest.

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