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Review

Relationship between gut microbiota and development of T cell associated disease

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ABSTRACT

The interplay between the immune response and the gut microbiota is complex. Although it is well-established that the gut microbiota is essential for the proper development of the immune system, recent evidence indicates that the cells of the immune system also influence the composition of the gut microbiota. This interaction can have important consequences for the development of inflammatory diseases, including autoimmune diseases and allergy, and the specific mechanisms by which the gut commensals drive the development of different types of immune responses are beginning to be understood. Furthermore, sex hormones are now thought to play a novel role in this complex relationship, and collaborate with both the gut microbiota and immune system to influence the development of autoimmune disease. In this review, we will focus on recent studies that have transformed our understanding of the importance of the gut microbiota in inflammatory responses.

1. Regulation of the immune response by the gut microbiota

The commensal bacteria, or microbiota, colonizing the gut performs several functions: it plays a critical role in the breakdown of indigestible complex plant polysaccharides, synthesizes important nutrients such as vitamin K, and provides an important layer of defense against invasion by pathogenic microorganisms. Interestingly, the intestinal microbiota affects the immune and/or inflammatory status of the host by modulating intestinal barrier function and by influencing the development of the immune response. Several gut microbial structures that play an important role in barrier functions have been identified. The secreted protein, p40, from Lactobacilli LGG ameliorates cytokine-mediated apoptosis and disruption of the gut epithelial barrier [1], and flagellin from *Escherichia coli* Nissle is associated with induction of β-defensin 2 in epithelial cells [2]. Gut microbiota has been shown to direct maturation of the host immune system [3], to play a key role in the induction of immunoglobulin (Ig) A [4,5] and germinal centers [6], and to drive Th1, Th17, and regulatory T cell (Treg) development in the gut [7–9]. In most individuals, the commensal-mediated induction of these different components of the immune response is beneficial for host health. However, the composition of the gut microbiota can differentially influence various immune cell populations and adversely affect autoimmune/inflammatory disease-susceptible hosts, e.g., the presence of segmented filamentous bacteria (SFB) has been associated with a strong Th17 response and development of Th17-mediated diseases [10–12].

Colonic commensal bacteria may "educate" both thymically and peripherally-derived regulatory T cells. One study using mice transgenic for T cells expressing a limited, but diverse [i.e., express identical T cell receptor (TCR) β], TCR repertoire showed that the TCR repertoire of colonic Tregs is unique (i.e., is not expressed by thymically-derived Tregs) and reacts to bacterial isolates, suggesting that encounter with gut commensal bacteria induces peripheral generation of colonic Tregs, and thereby, tolerance to the gut microbiota [13]. In contrast, a more recent study also using transgenic mice expressing a limited, but diverse, TCR repertoire (i.e., TCR^{mini} mice) showed that thymic and intestinal Tregs share a majority of dominant TCRs and many of these TCRs recognize microbial antigens. Moreover, treatment of the TCR^{mini} mice with a cocktail of antibiotics that alters the composition of the gut microbiota results in a concomitant change in the colonic thymic Treg TCR repertoire. These results suggest that the repertoire of thymus-derived Tregs is heavily influenced by the microbiota, and thymus-derived Tregs may play a dominant role in maintaining tolerance to gut commensal bacteria antigens [14]. The contradictory results of these two studies make it difficult to reach any

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definitive conclusions about the pre-dominant source of the Treg populations, i.e., thymic-derived versus peripherally-induced, that reside in the gut and mediate tolerance to components of the gut microbiota. Other populations of regulatory cells also appear to be activated/induced by commensal bacteria. A population of B regulatory cells (Bregs) was identified in B6 mice whose gut bacterial load was reduced by oral administration of a combination of broad spectrum antibiotics [15]. Importantly, this Breg population protected the animals from developing experimental autoimmune encephalomyelitis (EAE). EAE protection by reduction of the gut microbiota is associated with generally enhanced levels of CD19$^+$B220$^+$ cells in the mesenteric/cervical lymph nodes (LN) and spleen, increased percentages of a subpopulation of CD19$^+$CD5$^+$ B cells in the mesenteric/cervical LN, and higher frequencies of the CD1d^{hi}CD5$^+$ cell subset in the spleen/LN. Adoptive transfer of splenic and LN CD5$^+$ B cells isolated from antibiotic-treated mice conferred better protection against EAE than CD5$^+$ B cells from untreated mice by inducing a shift from a Th1/Th17 to a Th2-response [15] possibly through a mechanism involving IL-10. Interestingly, reduction of the gut bacterial load by broad spectrum antibiotics protects against EAE in different models via induction of different populations of regulatory cells, e.g., IL-10-producing CD1d^{hi}CD5$^+$ Bregs are induced in B6 mice [15] and IL-10-producing Tregs in SJL mice [16]. It is not clear why a reduction in gut bacterial load would favor regulatory T and B cells versus effector T cells. However, the antibiotics may not only reduce the bacterial load, but they may also alter the microbial composition, thereby, favoring commensal bacteria that induce regulatory cells and/or eliminating commensal bacteria that induce Th1/Th17 cells.

In the past several years, numerous reports have shown that an alteration in gut microbiota can favor induction of effector T cells over Tregs, and, consequently, trigger the development of autoimmune/inflammatory diseases (reviewed in [17]). Those studies identified specific gut commensals that appear to induce either Th17 or Treg responses that are associated with development or protection from disease, respectively. The presence of segmented filamentous bacteria (SFB) in the murine gut is associated with induction of Th17-mediated autoimmune/inflammatory diseases, such as colitis (induced by transfer of CD4$^+$CD45RB^{hi} cells into SCID mice), arthritis and experimental autoimmune encephalomyelitis (EAE) [10–12]. On the other hand, SFB protects from invasion by the pathogenic microorganism, *Citrobacter rodentium*, by inducing IL-22 production by Th17 cells that inhibits the growth of this microorganism [9]. Similarly, SFB protects in an IL-17-dependent manner against development of type 1 diabetes (T1D) in Non Obese Diabetes (NOD) mice [18], a spontaneous model of T1D. In this case, SFB-induced Th17 cells are not involved in disease pathogenesis, but appear to be protective through a mechanism that involves TGFβ [19]. These data indicate that a given gut commensal bacteria is not always beneficial or detrimental, but can have differential effects depending on the context. Furthermore, the presence of *Clostridium leptum* and *coccoides* bacteria, or colonization of mice with the human commensal, *Bacteroides fragilis* (*B. fragilis*) is associated with the induction of Tregs and prevention of dextran sodium sulfate (DSS)-induced or 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis, respectively [7,20]. The specific mechanisms by which gut commensals induce Tregs are beginning to be understood. As described below, molecules expressed or produced by commensal bacteria, such as polysaccharide A and short-chain fatty acids (SCFAs), modulate immunoregulation by acting directly on Tregs or on dendritic cells (DC). In contrast, very little is known about the mechanisms underlying the microbiota-mediated induction of CD5$^+$ Bregs that protect against EAE [15], and the specific commensal bacteria and molecules that trigger their induction remain to be identified. Moreover, although SFB colonization of germ-free mice triggers production of serum amyloid A (SAA) by cells of the terminal ileum, and SAA induces lamina propria DC to produce IL-23 and induce Th17 differentiation, the specific molecules produced by SFB that trigger this response are also unknown [9].

2. Specific interactions between commensal bacterial antigens and pattern recognition receptors of the immune system

Host–microbe interactions establish immunological homeostasis in the gut, and the molecular mechanisms underlying these interactions are currently under study. Commensals interact with the immune system via recognition of pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs), including toll-like receptor (TLR) and nucleotide-binding oligomerization domain-containing protein (NOD), expressed by a variety of host cells. The gut commensal bacteria are the source of peptidoglycan that prime the innate immune response through the two cytosolic PRRs, NOD1 and NOD2, expressed by intestinal epithelial cells, paneth cells, macrophages and DC. NOD plays an important role in controlling the immune response to commensal bacteria as indicated by the fact that *Nod1*- and *Nod2*-deficient mice exhibit a weakened intestinal barrier to gut microbes due to decreased production of the antimicrobial peptides, α- and β-defensins and RegIII-gamma [21,22], and are more susceptible to DSS-induced colitis [21]. Similarly, individuals with mutations in NOD2 exhibit an increased susceptibility to Crohn's disease [23,24], and NOD1 polymorphisms are also associated with inflammatory bowel disease (IBD) [25]. Interestingly, a mutation in NOD2 in Crohn's disease patients is associated with suppression of IL-10 transcription in monocytes [26]. It is, therefore, possible that the failure to control the bacterial burden in the gut in concert with a deficiency in anti-inflammatory cytokines contribute to the generation of immune responses against gut commensals, and the subsequent intestinal inflammation in IBD patients. The specific mechanisms by which commensals trigger the immune response, and subsequent disease are, however, unknown.

Bacteroides fragilis (*B. fragilis*), a human commensal, induces Tregs and prevents development of TNBS-induced colitis [7,20]. The precise mechanisms underlying the induction of Tregs by *B. fragilis* have begun to be understood, and involve polysaccharides (summarized in Fig. 1a). *B. fragilis* prevents TNBS-induced colitis in mice through polysaccharide A (PSA) which induces IL10-producing Tregs [20]. PSA can enhance Treg function via TLR2 signaling directly in Tregs [27]. *B. fragilis* can also release PSA in outer membrane vesicles (OMVs), that are sensed by dendritic cells (DC) via TLR2 resulting in upregulation of growth arrest and DNA-damage-inducible protein (Gadd45a) on DC and an increase in IL-10 production. These DC can subsequently induce Tregs *in vivo* and prevent TNBS-induced colitis [28]. Oral administration of PSA mediates protection against EAE in an IL-10-dependent manner, and is associated with accumulation of CD103-expressing DC and Tregs in the LN draining the central nervous system [29]. Altogether, these studies show that PSA from *B. fragilis* can activate Tregs directly and/or endows DC with the ability to induce Tregs.

On the other hand, SFB is known to induce IgA [4,5] and Th17 cell [8,9] differentiation, however, the specific SFB molecules triggering these effects remain to be determined. SFB may either activate B and T cells directly, or affect intestinal cells such as epithelial cells or DC, and trigger their ability to induce IgA and Th17 cell differentiation. Recent analysis of the whole genome of SFB shows that SFB encodes four types of flagellin, three of which are recognized by TLR5 [30]. TLR5 expression is limited in the mouse gut to intestinal epithelial cells (IEC) and CD11c^{hi}CD11b^{hi} lamina propria DC (LPDC). Interestingly, LPDC are critical for the generation of IgA and Th17 cells in the lamina propria, and produce

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