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Impact of gut microbiota on gut-distal autoimmunity: a focus on T cells

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Summary

The immune system is essential for maintaining a delicate balance between eliminating pathogens and maintaining tolerance to self-tissues to avoid autoimmunity. An enormous and complex community of gut microbiota provides essential health benefits to the host, particularly by regulating immune homeostasis. Many of the metabolites derived from commensals can impact host health by directly regulating the immune system. Many autoimmune diseases arise from an imbalance between pathogenic effector T cells and regulatory T (Treg) cells. Recent interest has emerged in understanding how cross-talk between gut microbiota and the host immune system promotes autoimmune development by controlling the differentiation and plasticity of T helper and Treg cells. At the molecular level, our recent study, along with others, demonstrates that asymptomatic colonization by commensal bacteria in the gut is capable of triggering autoimmune disease by molecular mimicking self-antigen and skewing the expression of dual T-cell receptors on T cells. Dysbiosis, an imbalance of the gut microbiota, is involved in autoimmune development in both mice and humans. Although it is well known that dysbiosis can impact diseases occurring within the gut, growing literature suggests that dysbiosis also causes the development of gut-distal/non-gut autoimmunity. In this review, we discuss recent advances in understanding the potential molecular mechanisms whereby gut microbiota induces autoimmunity, and the evidence that the gut microbiota triggers gut-distal autoimmune diseases.

Keywords: autoimmunity; microbiota; mucosal immunology; T cell.

Introduction

The mammalian gastrointestinal tract is home to an enormous and complex community of commensal bacteria.[1–3] This gut microbial community (microbiota) has co-evolved with its host over millennia, suggested by the fact that out of 52 bacterial phyla, two dominant phyla, Bacteroidetes and Firmicutes, represent 98% of the bacteria in the gut.[4,5] These gut microbes provide benefits to their host in many ways, including, but not limited to, digestion, production of nutrients, detoxification, protection against pathogens and regulation of the immune system.[1–3,6,7] As a result of its collective metabolic activity, and necessity for human health, the gut microbiota is often referred to as the 'forgotten' organ.[8] Interestingly, many of the metabolites derived from commensal bacteria have recently been found to directly impact the immune system. Commensal-mediated immunomodulation includes the promotion of T helper cell subset differentiation and T helper cell plasticity, the ability of a differentiated $CD4^+$ T cell to take on characteristics of other T-cell subsets simultaneously or at different times.[9] Plasticity is an especially important subject for mucosal immunity, as mucosal tissues are major sites where T-cell plasticity has been observed.[10–14] Therefore, the mucosa harbors the frontline immune response to commensal bacteria, and their interactions may subsequently control health versus disease status through T-cell differentiation and plasticity.

An autoimmune condition develops when the immune system mistakenly attacks our own self-tissues. A long-standing question in the field of microbe–host communication is how interaction of microbes with T cells

promotes autoimmune development. Molecular mimicry is one prominent hypothesis, theorizing that microbes trigger autoimmunity by a shared immunogenic epitope between microbes and a self-peptide, which leads to the activation of autoreactive T cells.[15,16] Another less well-known theory is that expression of dual T-cell receptors (TCRs) on T cells promotes autoimmunity by allowing autoreactive T cells to escape thymic clonal deletion.[17–19] Alterations in the composition of the gut microbiome, known as dysbiosis, have been implicated in many diseases and disease models, including those that show clear association with the gut such as inflammatory bowel disease, colorectal cancer, enteric infections, and obesity.[20–27] Dysbiosis has also been implicated in immune disorders that occur outside the gut, such as asthma, eczema, allergies, and gut-distal autoimmune diseases (autoimmune arthritis, type 1 diabetes, experimental autoimmune encephalomyelitis).[20,28–37] Although it is easy to see how the gut microbiota can influence immune responses within the gut, over the last decade an appreciation for the impact of the gut microbiome on gut-distal autoimmunity disease has developed. For example, a strong interest has emerged to characterize the impact of gut microbiota on health and disease status in the lung and brain, namely 'gut–lung axis' and 'gut–brain axis', respectively.[38,39]

This article aims to review the influence of microbiota on the immune system by producing key commensal-derived metabolites as well as controlling T-cell subsets and plasticity. Additionally, we discuss the recent findings on how gut commensals affect autoimmunity by molecular mimicry and skewing the expression of dual TCRs. Finally, we discuss the role of dysbiosis in the pathogenesis of common autoimmune diseases. As dysbiosis in inflammatory bowel disease has been extensively reviewed elsewhere (see ref. 40–42), this review will focus on the impact of gut microbiota dysbiosis in the development of gut-distal autoimmune diseases. Understanding the mechanisms of how gut commensals affect the immune system will be crucial for us to elucidate autoimmune pathogenesis and to generate novel therapies. This is an urgent subject, as dysbiosis-related diseases have emerged as new epidemics in the industrialized world.[43–45]

Commensal bacteria-mediated metabolites and immunity

Gut commensals are well-known for their function in digestion, a process involving extraction and synthesis of many metabolites, some of which are produced only by commensal bacteria and are crucial for host health.[46] Recently, several advancements have been made determining how the metabolites generated by commensal bacteria can directly influence the development and function of the immune system, directly impacting health and diseases (Table 1). Here, we review the most recent reports in this field.

Short-chain fatty acids

Short-chain fatty acids (SCFAs) including butyric acid, propionic acid, and acetic acid are the main metabolic products of undigested carbohydrates by gut commensal bacteria and have broad effects on the host immune system.[47] Among the fatty acid receptors, two orphan G protein-coupled receptors, GPR41 and GPR43, are activated by SCFAs.[48,49] SCFAs are significantly reduced in the colon of germ-free mice indicating that the gut microbiota is essential for their production.[50] Recently, one group reported that long-chain fatty acids enhanced

Table 1. The impact of commensal-derived metabolites on immunomodulation and disease development

Commensal	Metabolite	Disease phenotype	Immunomodulation	References
Clusters IV and XIVa of *Clostridium*	↑ IDO, enzyme involved in tryptophan catabolism, on IEC	↓ Colitis	↑ Colon Treg ↓ Th1	63,66
Lactobacillus reuteri	↑ Indole derivatives by metabolizing tryptophan		↑ Differentiation of DP IEL	69
Clostridium sporogenes	↑ IPA derivatives by metabolizing tryptophan, phenylalanine, and tyrosine	↓ Permeable intestines	↓ Neutrophils, monocytes, and memory T cells	72
General microbiota signal through MyD88	↑ RALDH, enzyme able to catalyze the synthesis of RA from Vitamin A	↓ EAE ↓ Autoimmune arthritis	↑ Treg ↓ Th17 ↑ Gut-homing integrin $\alpha_4\beta_7$ ↑ Maintenance of peripheral lymph nodes	78,79,92
Ruminococcaceae eubacterium	↑ SCFAs signal through GPR43	↓ EAE ↓ Type 1 diabetes	↑ Lamina propria Treg ↑ Antimicrobial peptides	57,53,55

Abbreviations: DP IEL, double-positive intraepithelial T lymphocytes; EAE, experimental autoimmune encephalomyelitis; IDO, indoleamine 2,3-dioxygenase; IEC, intestinal epithelial cells; IPA, indolepropionic acid; RA, rheumatoid arthritis; RALDH, retinal dehydrogenase; SCFA, short-chain fatty acids; Th1, T helper type 1; Treg, regulatory T.

the differentiation and proliferation of T helper type 1 (Th1) and/or Th17 cells, whereas SCFAs expanded gut regulatory T (Treg) cells.[51,52] Using experimental autoimmune encephalomyelitis (EAE) as a model for multiple sclerosis, Haghikia *et al.* showed that long-chain fatty acids decreased SCFAs in the gut, leading to exacerbated EAE by expanding pathogenic Th1 and/or Th17 cells in the small intestine.[51] Treatment with SCFAs ameliorated EAE by inducing lamina-propria-derived Treg cells. Consistent with the anti-inflammatory properties of SCFAs, treatment of germ-free mice with acetate, a type of SCFA, is sufficient to reduce intestinal inflammation in the dextran sulfate sodium model of colitis.[53] The protective effect of acetate was lost in GPR43$^{-/-}$ mice, indicating that signaling of acetate through GPR43 is necessary for the anti-inflammatory effect of SCFAs. Remarkably, administration of the SCFA butyrate ameliorates inflammation in patients with ulcerative colitis.[54] SCFAs also indirectly control type 1 diabetes through the production of antimicrobial peptides. Mechanistically, SCFAs produced by the gut microbiota have been shown to stimulate the production of antimicrobial peptides by pancreatic beta cells.[55] Furthermore, systemic administration of these antimicrobial peptides induce Treg cells in the pancreatic islets of pre-diabetic mice, reducing the incidence of autoimmune diabetes. Additionally, it has been recently demonstrated that administration of the SCFA propionate significantly attenuated HLA-B27-associated intestinal inflammation in Fischer 344 HLA-B27/β_2m transgenic rats.[56] Interestingly, this propionate-mediated inhibition of inflammation is independent of its role in regulating the Treg cell response.

Amino acids

An early study gives an indication that gut bacteria may play an important role in host amino acid homeostasis and health by showing that germ-free mice had an altered distribution of free amino acids along the gastrointestinal tract compared with conventionalized mice.[58] More recent reports suggest that the most abundant amino-acid-fermenting bacteria in the human small intestine are bacteria belonging to the *Clostridia*, the *Bacillus–Lactobacillus–Streptococcus* groups, *Proteobacteria*, and *Peptostreptococcus*.[59–61] These bacteria are therefore likely to be important for amino acid absorption in the gastrointestinal tract. Among the 20 amino acids, tryptophan belongs to one of the nine 'essential' amino acids that humans and higher vertebrates cannot synthesize, and that must be supplied in their diet.[62] Numerous studies suggest that tryptophan metabolites generated by gut commensal bacteria serve as important signaling molecules in host–microbe cross-talk. For example, the colonic intestinal epithelial cells from mice colonized with clusters IV, XIVa, and XVIII of *Clostridium* express high levels of indoleamine 2,3-dioxygenase,[63] an enzyme involved in the initial and rate-limiting step of tryptophan catabolism, which has been implicated in Treg cell induction.[64] Specifically, it has been demonstrated that kynurenine, the first product in the indoleamine 2,3-dioxygenase-dependent tryptophan degradation pathway, activates the aryl hydrocarbon receptor, leading to aryl hydrocarbon receptor-dependent Treg cell generation.[65] Indeed, an induction of colonic Treg cells was observed in mice colonized with *Clostridium* species.[63]

The small intestinal epithelium contains a unique population of CD4$^+$ CD8$\alpha\alpha^+$ double-positive intraepithelial T lymphocytes (DP IELs) which exhibit anti-inflammatory properties.[67,54] It has been shown that upon migration to the epithelium, Treg cells lose Foxp3 and convert to DP IELs in a microbiota-dependent manner, as microbiota depletion by treating mice with broad-spectrum antibiotics prevents Foxp3 loss.[68] Whereas all Treg cells express the CD4-lineage transcription factor T helper-inducing POZ/Krüppel-like factor (ThPOK), CD8$\alpha\alpha^+$ CD4$^+$ and $>$ 50% of Foxp3$^-$ CD8α^- CD4$^+$ cells in the small intestinal epithelium lack ThPOK expression. Later, it was found that loss of ThPOK corresponds to an IEL-like behavior in CD4$^+$ T cells. Interestingly, supplement of TCR stimulation can overcome the microbiota requirement for the DP IEL differentiation, as demonstrated by the relatively normal DP IEL differentiation in antibiotics-treated *Rag1*$^{-/-}$ OT-II (ova-specific) mice exposed to oral ovalbumin. By comparing the microbiota profile between mice housed at two vivaria, with mice at one vivarium displaying significant numbers and another displaying negligible or absent DP IELs, Cervantes-Barragan *et al.* discovered that the commensal *Lactobacillus reuteri* was responsible for the differentiation of DP IELs.[69] *Lactobacillus reuteri* generated indole derivatives by metabolizing tryptophan, which activated the aryl-hydrocarbon receptor in CD4$^+$ T cells, leading to their ThPOK down-regulation and differentiation into DP IELs. Hence, *L. reuteri* together with a tryptophan-rich diet can promote gut immune homeostasis by allowing the differentiation of intraepithelial CD4$^+$ T cells into anti-inflammatory DP IELs.

Only a few species of gut commensals, including *Clostridium sporogenes*, can break down tryptophan and produce the metabolite indolepropionic acid (IPA), a deamination product of tryptophan.[70] Bacterial tryptophanase catalyzes the conversion of dietary tryptophan to indole and subsequently to IPA.[71] A recent study identified a total of 12 compounds that can be produced in this process, nine of which can accumulate in the blood and three of which are produced exclusively by bacteria.[72] Specifically, the *C. sporogenes*-expressed gene *fldC* is necessary for the production of IPA. In fact, *fldC* is essential for the reductive metabolism of all three aromatic amino acids (tryptophan, phenylalanine, and tyrosine). Germ-

free mice receiving wild-type *C. sporogenes* exhibit high levels (~80 μM) of serum IPA whereas germ-free mice receiving the mutated version of *C. sporogenes* lacking *fldC*, had undetectable serum IPA. Importantly, mice with undetectable IPA had higher levels of immune cells, including neutrophils, classical monocytes, and memory T cells. In addition, the mice with the engineered version of *C. sporogenes* had increased intestinal permeability, a defect that is often seen in inflammatory bowel disease.

In addition to having a direct impact on the immune system, undigested amino acids have the potential to become supplemental precursors for SCFA generation by the gut microbiota, in addition to indigestible carbohydrates.[73] Therefore, amino acids could have indirect impact on the immune system through SCFAs as we discussed earlier. Numerous amino acids including glycine, threonine, glutamate, and ornithine can be metabolized by anaerobic bacteria to generate acetate, whereas threonine, lysine, and glutamate can be used for butyrate synthesis.[74] Moreover, at the molecular level, it has been shown that intracellular leucine concentrations can be sensed by the multiprotein complex leucyl-tRNA synthetase,[75,76] which activates the mechanistic target of rapamycin kinase, proving to be a vital link between immune function and metabolism.[77]

Retinoic acid

Retinoic acid, a metabolite of vitamin A, is one of the most active physiological retinoid metabolites and has a wide range of biological activity including regulating immune responses.[78] A major part of retinoic acid's anti-inflammatory effects depends on the inhibition of Th17 cells and promotion of Foxp3$^+$ Treg cell responses.[78,79] In addition, retinoic acid has been shown to be important for the expression of the gut homing receptor integrin $\alpha_4\beta_7$ on T cells.[80–82] The $\alpha_4\beta_7$ integrin receptors are imprinted on lymphocytes by dendritic cells (DCs) from Peyer's patches (PPs), and mesenteric lymph nodes.[82,83] In the absence of microbial toll-like receptor signaling in Myd88$^{-/-}$ mice, gut DCs express low levels of retinal dehydrogenase (RALDH) required for retinoic acid biosynthesis, so these Myd88$^{-/-}$ mice are unable to generate gut-homing lymphocytes.[84] AM80 is a synthetic retinoic acid that is characterized by higher stability and fewer potential adverse effects compared with retinoic acid.[85,86] It has been reported that both retinoic acid and AM80 ameliorate many autoimmune responses, including experimental autoimmune myositis, experimental autoimmune encephalitis, and collagen-induced arthritis.[87–90] We recently showed that oral administration of AM80 inhibits autoimmune disease in the joints as well as in the lung.[91] We elucidated a novel mechanism whereby AM80 suppresses the autoimmune pathology in both the lung and joints by inhibiting T follicular helper (Tfh) cells in addition to inhibiting Th17 responses. Specifically, AM80 increased the expression of the gut-homing integrin $\alpha_4\beta_7$ on Tfh cells, which diverted Tfh cells from systemic (non-gut) inflamed sites such as the lung draining lymph nodes into the gut (the non-immunopathological site) and so reduced systemic autoantibody production.

Moreover, the impact of retinoic acid can go beyond the cellular level and impact the development of whole lymphoid tissues.[92] It has been reported that cellular expansion in peripheral lymphoid tissues is controlled by gut microbiota in a retinoic acid-dependent manner.[93] The mucosal addressin MAdCAM-1 is the receptor for the gut-homing integrin $\alpha_4\beta_7$, and peripheral node addressin PNAd is the receptor for CD62L.[94] In neonatal mice, MAdCAM-1 expression in lymph nodes is elevated shortly after birth,[95] followed by a switch to a PNAd over a course of 2–3 weeks.[93] Zhang *et al.* demonstrated that commensal fungi drive a wave of RALDH$^+$ DCs to migrate to the peripheral lymph nodes after birth.[92] The arrival of these cells increases the amounts of retinoic acid *in situ*, mediates the neonatal MAdCAM-1 to adult PNAd addressin switch on endothelial cells, and directs the homing of lymphocytes to gut-associated lymphoid tissues. Finally, the authors found that a diet deficient in vitamin A causes reduced homing of RALDH$^+$ DCs into peripheral lymph nodes and a lack of maintenance of peripheral lymph node structures, suggesting a dependence on retinoic acid signaling for structural and functional maintenance of peripheral immune tissues.

Microbiota and T-cell subset determination

Understanding the role of microbiota in T-cell subset commitment and plasticity holds the key for unveiling the pathogenesis and therapeutic options for autoimmune diseases, as well as diseases associated with unbalanced immune responses such as cancers and infections. Not surprisingly, addressing this question has become a focused area of research in recent years. One study using transgenic mice expressing a limited but diverse TCR repertoire, through fixed TCR-β expression, showed that the TCR repertoire of colonic Treg cells is unique compared with thymically derived Treg cells.[96] Furthermore, these colonic Treg cells were shown to react with bacterial isolates, suggesting that encountering gut microbes in the intestines leads to peripheral Treg cell induction, and hence to tolerance to the gut microbiota.[96] A more recent study, also using transgenic mice expressing a constrained TCR repertoire, through fixed TCR-β expression and limited TCR-α rearrangement (TCR^{mini} mice[97]) showed that thymic and intestinal Treg cells expressed overlapping TCR repertoires, many of which recognize microbial antigens.[98] Alteration of the gut microbiota through treatment of TCR^{mini} mice with a cocktail of antibiotics concurrently altered the colonic and thymic Treg TCR

repertoire.[98] This suggests that the repertoire of thymically derived Treg cells is heavily influenced by the microbiota. Although these studies have differing results regarding the initial site of gut microbiota influence, in either case it is clear that direct recognition of microbial antigens by T cells can promote Treg cell lineage commitment. Beyond Treg cells, the gut microbiota can also influence T helper subsets. In the presence of a normal microbiota, CX_3CR1^+ intestinal antigen-presenting cells act to limit Th1 cell development and expansion.[99] However, antibiotic-induced dysbiosis leads to a shift in the function of CX_3CR1^+ antigen-presenting cells, allowing them to promote pathogenic Th1 cell development.[99]

Our laboratory and others have also demonstrated that a single type of commensal bacteria, segmented filamentous bacteria (SFB), can promote Th17 cell responses. In C57BL/6 mice, SFB specifically induce intestinal Th17 cells.[25] More recent studies, however, have demonstrated that SFB can also influence T helper subsets outside the gut. In 2011, Lee *et al.* discovered that SFB alone was able to promote Th17 cells specifically in the spinal cord of EAE mice.[33] Similarly, we have shown that in the K/BxN mouse model of autoimmune arthritis, SFB specifically promotes Th17 cell responses in the lung.[100] Additionally, SFB can also drive the differentiation of Tfh cells and promote their migration to systemic sites, leading to exacerbated autoimmune arthritis in K/BxN mice.[101] However, the exact molecule(s) employed by SFB to promote the differentiation of T helper cell subsets remain elusive. Although many microbiome studies focus on the microbes residing in the intestines, the entire digestive tract is colonized with bacteria. Hence, the oral microbiome has recently become an area of interest with regards to mucosal immunity. Data from Dutzan *et al.* showed that oral homeostatic Th17 responses, unlike gut Th17 responses, were unaffected by shifts in the oral or gut microbiomes.[25,102] This suggests that not all mucosal T cells are influenced by the microbiome, and that other environmental cues such as chewing activity in the oral cavity and chewing-associated damage can maintain the Th17 cell population. Interestingly, a more recent study from this same group found that inflammatory Th17 cells isolated directly from periodontal lesions were in fact heavily influenced by oral microbiome dysbiosis.[103] By focusing on Th17 isolated from periodontal lesions of both mice and humans, they found that increased abundance of Th17 cells and enhanced production of inflammatory cytokine IL-17 was associated with shifts in the oral microbiome. Specific outgrowth of *Enterococcus* and *Actinobacteria* along with loss of *Streptococcus* was associated with periodontitis and enhanced Th17 cell responses in the ligature-induced periodontitis mouse model. Together the studies suggest that only pathogenic but not homeostatic Th17 cell responses are influenced by dysbiosis. We recently addressed how both age and gut microbiota affect T-cell subsets in the context of autoimmunity.[104] Our results show an augmented autoimmune disease phenotype in both the joints and the lung of middle-aged compared with young mice. Mechanistically, we saw a soaring accumulation of Tfh, but surprisingly not Th17 cells with age. Our data suggest exposure to immunomodulatory commensals such as SFB may allow the young host to develop an overactive immune system similar to that found in middle-aged hosts. Our study also shows that age can independently increase the Tfh cell response without the help of immunomodulatory commensals.

CD_4 T helper cell plasticity and the microbiota

The development of specific transcription factor reporter mice has proven to be an invaluable tool in dissecting the plasticity of T-cell subsets. An early study using adoptive transfer of cells from $Foxp3^{eGFP}$ reporter mice showed that 80% of Treg cells lost GFP expression in the PPs compared with 50% in spleen.[12] Within the GFP-negative PP population, > 60% of the cells expressed CXCR5, consistent with a Tfh phenotype,[12] suggesting that PP, but not spleen, is the preferred site for a Treg to Tfh reprogramming. Two other studies found that retinoic-acid-receptor-related orphan receptor γ t (Rorγt), the hallmark Th17 transcription factor, was preferentially expressed by colonic Treg cells, and furthermore, that in the absence of gut bacteria, the Rorγt^+ Treg cell population was substantially diminished.[10,14] Interestingly, several bacterial species were able to induce Rorγt^+ Treg cells, which were indispensable for maintaining gut homeostasis as determined by enhanced IL-17, interferon-γ (IFN-γ), and colitis in their absence.[10] Another study showed that the pathogenic bacterium *Helicobacter hepaticus* promoted expansion of Rorγt^+ Treg cells in a c-MAF-dependent manner, which selectively suppressed pro-inflammatory Th17 cells in the intestines.[105] However, in the absence of Treg cells (in $Il10^{-/-}$ mice), *H. hepaticus* predominantly induced Th17 cells, suggesting that pathobionts can have an impact on T-cell plasticity by expanding potent suppressive T cells, which in turn, inhibit the inflammatory T helper cells necessary for pathogen elimination. Plasticity among T helper subsets has also been observed. Using cell fate reporter mice in which Th17 cells are permanently marked by YFP ($Il17^{cre}R26^{eYFP}$), Hirota *et al.* found that selectively in the PP, but not spleen, Th17 cells were reprogramed to a Tfh (PD-1^{hi} $CXCR5^{hi}$) phenotype.[13] Furthermore, these ex-Th17-Tfh cells localized in germinal centers where they assisted in T-cell-dependent IgA production. In a Th17 adoptive transfer model of colitis, it was demonstrated that Th17 cell conversion to a Th1 phenotype was necessary for colitis development as Th17 cells unable to produce IFN-γ ($Ifng^{-/-}$) were incapable of inducing colitis.[106] Interestingly, not only

did Th17 cells convert to Th1 and contribute to colitis development directly, but also Th17 were capable of providing help to naive T cells for Th1 lineage commitment. Together these studies suggest that gut microbiota is able to influence T-cell plasticity within the gut microenvironment and influence the development of autoimmunity and other inflammatory diseases.

Gut microbiota induces autoimmunity by TCR-mediated mechanisms

Central to T-cell function is the TCR, through which T cells recognize their cognate antigen and become activated. An important feature of the TCR is its ability to recognize multiple antigens, known as TCR cross-reactivity. Sequencing-based methods estimate that humans and mice express 10^7 and 10^6 productive unique $\alpha\beta$ TCRs, respectively, yet this is insufficient to cover the possible foreign antigen repertoire.[107,108] Therefore, TCRs possess the ability to recognize multiple antigens in order to recognize the plethora of possible foreign antigens (see ref. 109). One potential mechanism through which pathogenic autoreactive T cells may become activated is through weak recognition of a microbial antigen for which their TCR is cross-reactive. This is also known as molecular mimicry, a phenomenon in which a foreign antigen shares significant structural or sequence similarities with a self-antigen.[15,16] Later, this now activated autoreactive T cell can home to the tissue where its cognate self-antigen is expressed and elicit autoimmune pathology. In fact, cross-reactive TCR recognition of microbial peptides by autoreactive T cells has been seen in several autoimmune settings (Fig. 1). In 2015, Horai *et al.* showed that T cells restricted to expressing only the retina-specific TCR (R161H), became activated in the intestines in response to an unidentified microbial antigen.[110] T-cell activation was in response to TCR-signaling to a non-cognate microbial antigen, as T cells in the small intestines of R161H-Tcr$\alpha^{-/-}$ mice had clear co-localization of phosphorylated Zap-70 with CD4, known to be downstream of TCR signaling.[110–112] Later, it was shown that Islet-specific glucose-6-phosphate catalytic subunit-related protein (IGRP)-specific $CD8^+$ T cells in non-obese diabetic (NOD) mice were activated through recognition of a peptide from *Fusobacteria* and elicited autoimmune diabetes.[113] Interestingly, the *Fusobacteria* peptide shared significant homology with the IGRP peptide targeted by the IGRP-specific transgenic TCR,[113] suggesting that molecular mimicry in microbial peptides may pose a threat because they can activate pathogenic autoreactive T cells. Most recently, it was shown that gut commensal bacteria expressing an ortholog to human Ro60, a nuclear RNA-binding protein and primary targeted self-antigen in lupus, were able to activate human Ro60-specific $CD4^+$ T cells through cross-reactive TCR recognition.[114]

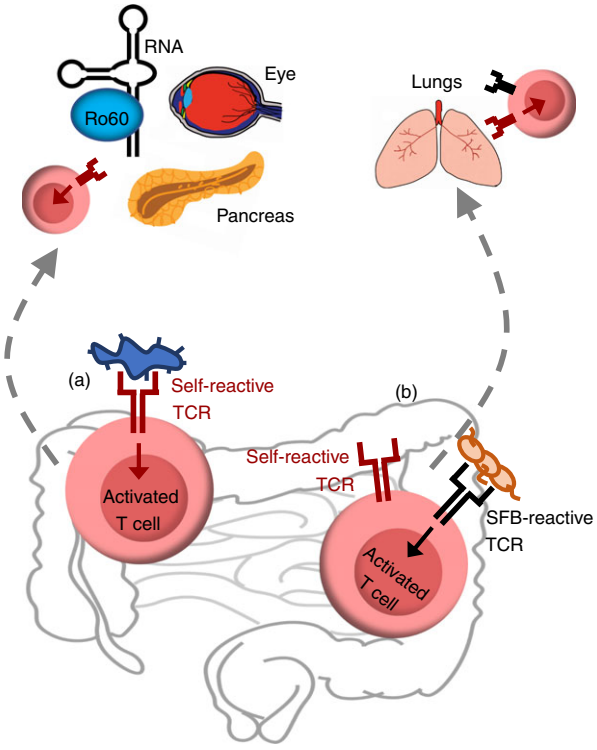

Figure 1. Mechanism leads to autoimmunity: recognition of commensal bacteria by T-cell receptor (TCR). (a) Molecular mimicry. Several studies have demonstrated, as a result of the cross-reactive nature of TCRs, that autoreactive T cells can recognize both a self-antigen and a gut commensal-antigen. Recognition of a microbial antigen is able to activate autoreactive T cells, which in turn migrate to the tissue where their cognate self-antigen is expressed and elicit autoimmune diabetes,[113] autoimmune uveitis,[110] and lupus.[114] (b) Dual TCRs. Another mechanism involves autoreactive T cells that are able to recognize gut commensal-antigens through expression of a secondary TCR in addition to self-antigen recognizing TCR and differentiate into T helper type 17 (Th17) cells.[100] These T cells differentiate into Th17 cells through TCR recognition of segmented filamentous bacteria (SFB), then traffic to the lung where they mediate lung pathology.

Furthermore, germ-free mice monocolonized with Ro60-ortholog-expressing commensal bacteria spontaneously developed lupus-like disease, as indicated by glomerular immune complex deposits. Together, these studies suggest that molecular mimicry is a mechanism through which the intestinal microbiota is able to propagate autoimmune responses leading to disease.

In addition to molecular mimicry, it has long been thought that pathogenic autoreactive T cells may arise from a subset of thymocytes expressing two unique TCRs, one of which allows them to evade negative selection along with one that has a high affinity for a self-antigen.[115–117] Approaching from a different angle, outside the central immune system, we found that in peripheral tissues, SFB selectively expand dual TCR-expressing T cells, leading to the augmentation of lung autoimmune pathology (Fig. 1).[100] This is the first

study to show that dual TCR T cells can be activated through the recognition of the cognate commensal-derived antigen and migrate to elicit autoimmune pathology in the lung, a gut-distal site. At the molecular level, we demonstrate that SFB selectively expand autoimmune T cells co-expressing SFB-specific TCRs in addition to their self-reactive TCRs. This additional SFB-specific TCR provides a proliferative advantage for autoreactive Th17 cells in SFB(+) hosts. Our data suggest that the induction of robust SFB-specific Th17 response by transferring SFB(+) recipients with SFB-specific 7B8 T cells could not rescue the autoimmune activity of $Rag^{-/-}$.KRN T cells through bystander activation. Instead, our data suggest that the same T cells need to specifically recognize self- as well as SFB-antigen to induce IL-17-expressing pathogenic T cells to cause autoimmunity. This is supported by previous data showing that in healthy mice, cognate TCR recognition of SFB-antigen is crucial for SFB-mediated Th17 induction.[118,119] Taken together, our study suggests that the same T cell needs to recognize both self-antigen and be activated by SFB to undergo preferential Th17 cell expansion and so enhance autoimmunity in an SFB-dependent manner. Beyond molecular mimicry, our study suggests an alternative, dual TCR-based mechanism for commensal-mediated autoimmunity.

Role of gut microbiota in human and murine gut-distal autoimmunity

Autoimmune disorders have been sharply increasing worldwide in recent decades.[26] The dramatic changes in the disease onset rate cannot be explained by genetic basis as it occurs in such a short period of time. On the contrary, these data suggest that environmental factors play a key role in the recent surge of autoimmune diseases. In recent years, we have begun to appreciate that the gut microbiota provides environmental cues controlling human health and disease, including its potential impact on autoimmune responses. Under healthy conditions, the human intestinal microbiota is composed primarily of bacteria of the phylum Firmicutes, with Bacteroidetes and Actinobacteria also represented.[120] On the family and species level, the gut microbiota varies widely among individuals, although it remains largely stable within an individual over the course of several years.[121] Studies using mouse models or samples from human subjects transplanted into mice have shown that age, gender, diet, smoking, autoimmunity, and other factors can alter the microbiota, and that the altered microbiota can be transferred and exert influence over the metabolism and immune system of the recipient.[122] These studies demonstrate that the host–microbiota relationship is not a one-way street, but rather a dynamic conversation that can have long-lasting impacts.

Many studies have investigated a correlation between gut microbiome composition and autoimmune disease in humans, but these studies are limited in their ability to determine causality. Exploring this field using animal models allows for perturbation of the gut microbiome to better distinguish cause from effect, permit mechanistic dissection and allow pre-clinical evaluation of suggested therapeutic strategies. Throughout this section, we will highlight the important observations made in both human patients and mouse models.

Multiple sclerosis (MS) is an autoimmune disease in which pathogenic $CD4^+$ T cells penetrate the blood–brain barrier and cause damage to the central nervous system.[123] Numerous studies have shown that patients with MS display intestinal microbiome dysbiosis.[124–126] Two independent studies comparing patients with relapsing–remitting MS and healthy controls found significant alterations in the composition of the microbiome from patients with MS.[124,127] Perhaps even more intriguing, when compared with treatment-naive patients, those receiving treatment displayed some restoration in their microbiome composition.[127] Untreated MS patients displayed increased relative abundance of *Methanobrevibacter* and *Akkermansia*, which were lower in healthy controls and treated MS patients. MS patients receiving treatment had increased *Sutterella*, which has been previously observed to be increased in healthy controls compared with treatment-naive patients with inflammatory bowel disease (Fig. 2).[128] This suggests that although dysbiosis may contribute to a predisposition for developing MS, perhaps treatment may act to normalize pro-inflammatory microbiota. A recent study by Cekanaviciute *et al.* showed that intestinal microbiome samples from MS patients displayed specific outgrowth of *Acinetobacter* and *Akkermansia.*[129] Importantly, they further demonstrated that in the presence of *Acinetobacter calcoaceticus*, human peripheral blood mononuclear cells preferentially shifted towards an inflammatory phenotype (IFN-γ) and away from a regulatory phenotype ($Foxp3^+$ $CD25^+$). This suggests that the specific dysbiosis observed in patients with MS favors an inflammatory T-cell response. More definitive studies using the EAE mouse model of MS have shown that in germ-free mice lacking gut microflora, EAE disease severity is reduced to an almost undetectable level, due to a more than twofold reduction in IFN-γ and IL-17 production from $CD4^+$ T cells.[33] Importantly, if $CD4^+$ T cells from germ-free mice were transferred to specific pathogen-free mice harboring a diverse intestinal microflora, these cells gained the ability to mediate EAE disease development, suggesting that the gut microbiota is necessary to elicit the pathogenic Th1 and Th17 responses seen in EAE and perhaps MS.[33] Importantly, it was recently demonstrated that colonization of germ-free Myelin oliodendrocyte glycoprotein (MOG)-specific TCR transgenic mice (RR mice) with gut microbiota from

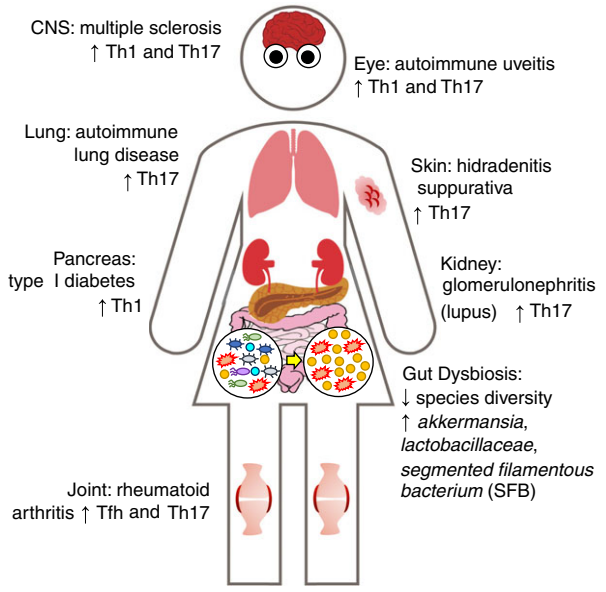

Figure 2. Dysbiosis leads to gut-distal autoimmunity. Several studies have demonstrated a link between alterations in the composition of the gut microbiota and the development of autoimmune disease. Multiple sclerosis (MS) and the C57BL/6 proteolipid protein-induced experimental autoimmune encephalomyelitis mouse model, which have autoimmune manifestations mediated by T helper type 1 (Th1) and Th17 cells in the central nervous system (brain and spinal cord).[33,123,124,127–130] Autoimmune uveitis and the R161H-TCR transgenic mouse model display autoimmune manifestations mediated by Th1 and Th17 cells in the retina of the eye.[110] Rheumatoid arthritis (RA) and the RA-associated lung pathology in the K/BxN mouse model have autoimmune manifestations mediated by follicular helper T (Tfh) and Th17 cells in the joints and by Th17 cells in the lung.[131,145–149] Hidradenitis suppurativa (HS) is an autoimmune skin condition associated with an increase of Th17 cells.[157] Currently, there is no good mouse model for HS, so the role of gut microbiota in this disease remains elusive. Systemic lupus erythematosus (SLE) and the NZB/W F_1 mouse model develop autoimmune manifestations mediated by Th17 cells and immune complex deposition specifically in the kidneys.[152,155,156] Type 1 diabetes and the various mouse models (C57BL/6 streptozotocin-induced model and IGRP-specific TCR transgenic model) develop autoreactive Th1 responses in the pancreatic islets.[113,160,161,163]

patients with MS resulted in increased incidence of spontaneous EAE development.[130] This suggests that the microbiota of patients is sufficient to precipitate autoimmune disease. Together these studies suggest a gut–brain axis of communication that allows gut microbes to elicit autoimmune responses in the brain.

Rheumatoid arthritis (RA) is an autoimmune disease that primarily affects joints but may also affect other parts of the body including the lungs and heart.[131–133] Several studies have suggested that many patients with RA display antigen–antibody complexes with complement fixation in joints that can cause tissue damage and tumor necrosis factor-α production.[134–139] Recent studies also suggest that anti-citrullinated protein antibodies are involved in RA pathogenesis.[140,141] Tfh cells are a T effector cell type specialized in helping B cells.[142–144] Overreactive Tfh responses pose a threat of triggering excessive autoantibody production and autoimmune disease.[145–149] In that regard, the presence of circulating $CXCR5^+$ Tfh (cTfh) cells in the blood has been observed in patients with different autoimmune diseases, including RA.[20] In a recent study assessing 77 treatment-naive patients with RA, 21 treated patients with RA, and 80 healthy controls, the gut microbiome was found to be altered in patients with RA.[150] In particular, *Haemophilus* spp. were depleted in individuals with RA and negatively correlated with serum autoantibodies titers, whereas *Lactobacillus salivarius* was over-represented in individuals with RA and abundance correlated with disease severity (Fig. 2). Interestingly, similar to what has been observed in patients with MS, patients with RA undergoing treatment displayed partial normalization of their intestinal microflora compared with treatment-naive patients.

By using a photo-labeling mouse model to track cell migration, our laboratory has demonstrated that a commensal bacterium, SFB, is able to promote the differentiation and migration Tfh cells from the PPs in the small intestine to systemic sites where they elicit autoimmune arthritis development.[101] At the molecular level, SFB induce PP Tfh cell differentiation by limiting the access of IL-2 to PP $CD4^+$ T cells and DCs are required for SFB-mediated IL-2Rα suppression and up-regulation of Bcl-6, a master regulator of Tfh cells, in PPs. Furthermore, we have recently shown that SFB can also promote RA-associated autoimmune lung disease through propagation and mobilization of gut-derived Th17 cells.[100] Importantly, this highlights a gut-lung axis of communication through which specific gut commensal bacteria are able to promote pathogenic T-cell responses leading to gut-distal autoimmune pathology. In the collagen-induced arthritis model of RA, dysbiosis characterized by outgrowth of *Lachnospiraceae* and *Lactobacillaceae* was detected before arthritis development.[151] Furthermore, antibiotic-induced depletion of the microbiota before induction of collagen-induced arthritis reduced disease severity by 40% and was accompanied by reduction in the inflammatory cytokines IL-17, IL-22, and IL-23 in the intestines along with reduced anti-collagen antibodies. This suggests that the gut microbiota is necessary for autoimmune disease development and that alterations in the composition of the gut microbiota precipitate autoimmune disease.

Systemic lupus erythematosus (SLE) is an autoimmune disease affecting almost all organs of the body, in which both autoreactive T cells and autoantibody-producing B cells contribute to the pathogenesis of SLE.[152] Like many gut-distal autoimmune diseases, recent studies have linked gut microbiota dysbiosis with SLE.[153] In

a 2016 study from Lopez *et al.*, it was shown that intestinal microbiota isolates from patients with SLE promoted naive T-cell differentiation into Th17 cells.[154] Furthermore, peripheral blood mononuclear cells from patients with SLE were enriched for both Th17 and Foxp3$^+$ IL-17$^+$ cells compared with healthy controls. As described earlier, due to T cell plasticity, Treg cells are able to shift between regulatory and potentially pathogenic phenotypes. Hence, the presence of Foxp3$^+$ IL-17$^+$ cells in the peripheral blood of patients with SLE suggests that the shift observed in the gut microbiome of these patients may promote inflammatory T-cell responses and may negatively impact Treg cell stability. More recently, in a study by Luo *et al.* which assessed the intestinal microbiota dynamics of both SLE patients and the NZB/W F_1 mouse model of SLE, it was shown that alteration in the gut microbiota resulted in decreased diversity with increased representation of Gram-negative bacteria (Fig. 2).[155] Interestingly, as NZB/W F_1 mice progressed to overt disease, the relative abundance of *Lactobacillaceae* increased from 0·1% to 10%. Furthermore, there was a positive correlation between *Lactobacillaceae* abundance and disease severity in female NZB/W F_1 mice. This suggests that reduction in α diversity, a measure of the number of different species present in a given site, and outgrowth of specific commensal microbes may promote autoreactive immune responses in susceptible individuals. Another report found a high frequency of Th17 cells in the kidneys of patients with glomerulonephritis, a form of SLE.[156] By using Kaede mice to track intestinal T-cell migration during glomerulonephritis induction, they observed that Th17 cells egress from the intestine and subsequently migrate to the kidney through CCR6 recognition of CCL20. Furthermore, depletion of gut Th17 cells in germ-free and antibiotics-treated mice ameliorated the autoimmune glomerulonephritis, suggesting that targeting the intestinal Th17 cells may offer a therapeutic strategy for autoimmune diseases.

Hidradenitis suppurativa (HS) is a chronic inflammatory skin condition of the hair follicles thought to be mediated in large part by Th17 cells (Fig. 2).[157] With respect to perturbations in the microbiota, most studies assess changes in the cutaneous microbiota as this is the site of the disease manifestation. Recently, HS has been identified to have a strong concurrence rate with inflammatory bowel disease, with over 17% of Crohn's patients also having HS.[158] Hence, while there are definite changes in the cutaneous microbiome of patients with HS,[159] taking a closer look at the gut microbiome may prove fruitful in the quest for identifying a causal mechanism.

Type 1 diabetes (T1D) is a T-cell-mediated autoimmune disease characterized by the selective destruction of insulin secreting β-cells in the pancreatic islets.[160] Many patients with T1D present with increased intestinal permeability or 'leaky gut', which has been shown to precede the onset of clinical disease.[161] In addition, patients with T1D also display an altered intestinal microbiome characterized by decreased α diversity and increased abundance of inflammatory species compared with healthy controls (Fig. 2).[162] Hence, it is tempting to assume that in combination with increased intestinal permeability, dysbiosis could promote an inflammatory environment in the gut–proximal pancreatic tissue leading to activation of an autoimmune response. In a 2016 study from Costa *et al.*, it was determined that autoimmune diabetes development in the streptozotocin-induced model of T1D is dependent on translocation of gut microbiota to the pancreatic lymph node.[163] Interestingly, islet-infiltrating T cells seem to be of gut origin as they often express $\alpha_4\beta_7$ integrin.[83,164,165]

We now know that the effect of commensal bacteria on autoimmune disease can be model dependent. For example, SFB is pathogenic in animal models of arthritis and multiple sclerosis[32,33] but protective in the NOD mouse model of type1 diabetes.[166,167] The beneficial and detrimental effects of SFB could depend on whether commensal-mediated immunomodulation is enhancing or inhibiting the pathogenesis of each disease. For example, autoantibodies are a key pathogenic factor and diseases can be induced by passive transfer of autoantibodies in the K/BxN and EAE models.[168–170] In contrast, type 1 diabetes in NOD mice is a T-cell-mediated autoimmune disease, and although B cells of NOD mice produce autoantibodies, these are not thought to play a diabetogenic role.[171,172] Hence, SFB, with their Tfh and autoantibody boosting effect, are more likely to play a pathogenic role in the K/BxN model and other antibody-mediated autoimmune diseases than in T-cell-mediated autoimmune diseases such as in NOD mice. One feature that many of the autoimmune diseases have in common is evidence of gut-derived T cells participating in the autoimmune response.[83,101,156,164,165] This would suggest that perhaps one mechanism through which the gut microbiota modulates autoimmune responses is through mobilization of T cells from the tolerogenic environment of the gut to systemic sites where alleviated tolerogenic pressure allows these cells to become active and initiate autoimmune disease development.[173–175] However, more studies are required to determine whether the altered microbiome drives changes in immunity or conversely the onset of immunological disease induces changes in the gut microflora in human patients. It is likely a combination, as changes in the gut microbiota precede onset of clinical autoimmune manifestations,[162] yet some evidence suggests that perhaps T cells, Foxp3$^+$ Treg cells in particular, regulate gut microbiota diversity.[176]

Conclusions and future directions

There is a surge of recent studies investigating the molecular mechanisms of dysbiosis-related autoimmune diseases. In both molecular mimicry and dual TCR theories, until recently, only infectious pathogens including viruses and bacteria have been implicated as the primary culprits,[15–17] and little is known about the molecular mechanism by which asymptomatic commensal colonization could trigger autoimmunity. Our recent study along with others demonstrates a previously unknown condition that asymptomatic colonization by commensal bacteria in the gut is capable of triggering systemic autoimmune disease by molecular mimicking self-antigen and skewing the dual TCR expression in the host. Notably, dual TCR expression is not limited to transgenic mouse models. In wild-type mice and in humans, up to 15% and 33%, respectively, of peripheral T cells have been reported to express dual TCRs, due to incomplete allelic exclusion at the *Tcra* locus.[177,178] Future investigation is required to determine whether dual TCR expression is involved in dysbiosis-related immune disorders in human patients. Another important, but poorly understood, aspect of microbiota–T-cell interaction is how different microbial signals impact T-cell stability and plasticity at the mucosal frontline. Understanding the plasticity and stability of T-cell subsets in mucosal tissues is highly relevant for future therapeutic strategies as we will not be able to manipulate T cells for therapeutic purposes without understanding the mechanisms by which T cells shift between alternative programs because of exposure to gut microbes.

As illustrated in this review, the use of animal models provides a powerful tool for mechanistic studies as it allows us to establish the causative effect of microbiota in disease development. By using germ-free and specific pathogen-free mouse models, numerous studies unravel the crucial roles of gut commensals in immune regulation in the context of health and disease. Although these studies provide promising clinical implications, various challenges need to be overcome by the field to harness these findings for future diagnostic and therapeutic approaches. One of the challenges is that the reductionist experiments in mice often do not reflect important features of the immune system in the adult human. In this regard, Beura *et al.* demonstrated that laboratory mice – like neonates, but not adult humans – lack effector-differentiated and mucosal memory T cells.[179] These cell populations are present in feral mice and pet store mice with constant and diverse microbial exposure. Furthermore, laboratory mice co-housed with pet store mice display profound changes in immune systems, resulting in an immune signature that is more closely reflected by adult humans than by neonates. Hence, the 'dirty' mouse model may provide a unique advantage over the current reductionist mouse models and should be considered for studies with translational purposes in human disease.

One of the biggest dilemmas facing the field of gut microbiota research is how to apply laboratory findings to clinical therapies. Currently, microbiota-based therapy relies on three major strategies: complete fecal microbiota transplants (FMTs), administering one or more species of bacteria orally (probiotics), or administering substrates to favor the expansion of certain kinds of bacteria or a shift in metabolite production, a strategy known as prebiotics.[180] Each of these approaches has limitations; for example, probiotics often colonize only transiently because of ineffective competition with the existing microbiota.[181] Additionally, despite the focus on taxonomic composition analyzed by 16S rRNA sequencing of microbial communities in many studies, the functional relevance of microbial communities is more likely to be revealed by their metagenomic gene expression and metabolomics profiles. This is further proved by the fact that bacterial isolates of the same strain can display very different immunoregulatory capabilities.[182] FMTs depend on severe depletion of the constituent microbiota through antibiotics and bowel flushing before administration of the transfer.[183,184] Other researchers are working to find alternative methods to circumvent the difficulties of FMTs, such as filtering donor material to the point of removing intact cells, leaving only bacterial byproducts such as metabolites.[185] An additional approach is to identify bacterial metabolites able to modulate human immune responses and artificially administer the identified metabolites as novel therapeutics.[186] This approach would be appealing from a pharmaceutical perspective, because it would allow the development of drugs that would need to be taken regularly. It would also minimize the factors that need to be controlled, since administering metabolites would not depend on the engraftment of a foreign bacterial species. However, a careful determination on how to achieve a comparable delivery of the metabolite that mimics the one produced by the intestinal microbiota will be crucial for the effectiveness of the treatment. In conclusion, the gut microbiota is a complex community that engages in significant cross-talk with the host. A better understanding of host–microbe interaction and the underlying microbiota-derived molecules that modulate the immune system and disease development may help to pave the way for better patient-tailored interventions and microbial molecule-based therapies for immune disorders.

Disclosures

The authors have no competing interests to declare.

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