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Host–microbiota interplay in mediating immune disorders

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Abstract

To maintain health, the immune system must keep a delicate balance: eliminate invading pathogens while avoiding immune disorders such as autoimmunity and allergies. The gut microbiota provide essential health benefits to the host, particularly by regulating immune homeostasis. Dysbiosis, an alteration and imbalance of the gut microbiota, is associated with the development of many autoimmune diseases in both mice and humans. In this review, we discuss recent advances in understanding how certain factors, such as age and gender, affect the gut microbiota, which in turn impact the development of autoimmune diseases. The age factor in microbiota-dependent immune disorders indicates a window of opportunity for future diagnostic and therapeutic approaches. We also discuss unique commensal bacteria with strong immunomodulatory activity. Finally, we provide an overview of the potential molecular mechanisms whereby gut microbiota induce autoimmunity, as well as the evidence that gut microbiota trigger extra-intestinal diseases by inducing the migration of gut-derived immune cells. Elucidating the interaction of gut microbiota and the host immune system will help us understand the pathogenesis of immune disorders, and provide us with new foundations to develop novel immuno- or microbe-targeted therapies.

Keywords

innate and adaptive immunity; microbiota; autoimmunity; age effect

Introduction

Microbes have traditionally been classified according to their relationship with their host: pathogens are harmful and invasive to the host, while commensals are normally harmless. Pathogens, including bacteria and viruses, have long been associated with autoimmune diseases.^{1,2} Recently, an expansion of pathogenic commensal bacteria—pathobionts—has been found in patients with autoimmune diseases and in animal autoimmune models. These types of commensals are often not pathogenic in and of themselves, but can trigger autoimmune symptoms in genetically susceptible hosts. Notably, the recent explosion of findings in the microbiota field is greatly facilitated by the advance of modern technology.

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Competing interests

The authors declare they have no competing interests.

The 16S ribosomal RNA (rRNA) subunit is an essential component in the ribosomal complex in prokaryotes.³ Owing to its conservation in evolution, the 16S rRNA gene is the most established marker for bacterial classification and taxonomic assignment. Because the majority of gut microbiota are unculturable, the 16S rRNA sequencing analysis, which is used by most of the studies discussed in this review, has replaced the traditional culture-based techniques for characterizing microbial communities in any given sample.⁴ Here, we review factors affecting the role of microbiota in autoimmunity, as well as the mechanisms whereby microbiota trigger an autoimmune response, with a focus on gut microbiota. This is an urgent subject, as dysbiosis-related diseases have emerged as new epidemics in the industrialized world.^{5–7} It is important to emphasize that, despite the focus of this review being the detrimental role of microbiota in autoimmunity, a healthy commensal community is beneficial to the host; for example, commensal bacteria can provide crucial nutrients, such as vitamins B and K, and also control immune homeostasis. This topic has been extensively discussed in a recent review.⁸

The role of microbiota and gender in autoimmune development

The gender dimorphism of susceptibility to autoimmune disease is a well-established phenomenon.⁹ A seminal discovery by Markle *et al.* provides a new perspective into the role of sex hormones in autoimmune disease development. Their study demonstrated that male puberty in non-obese diabetic (NOD) mice leads to changes in the gut microbiota that result in enhanced testosterone production, which acts as a protection against the development of type 1 diabetes (T1D).¹⁰ Importantly, the protective properties of the male-associated microbiota can be transferred to immature females. These protective effects correspond with an increase in male-like microbiota in recipient females and are dependent on androgen receptor activity. This study suggests a clear interaction between microbiota and sex hormones, raising the possibility that microbial transplantation might serve as a therapy for autoimmune diseases. Another research group also found different microbiota profiles between male and female NOD mice.¹¹ They further observed that the loss of interferon- γ (IFN- γ) signaling removes the gender bias from T1D, suggesting that IFN- γ signaling is required in sex- and microbiota-mediated disease protection.

Using a different disease model, Miller *et al.* demonstrated that the combined factors of gender and microbiota profile might predispose female myelin oligodendrocyte glycoprotein-specific 2D2 TCR transgenic mice with a TNF receptor 2 (TNFR2) deficiency to develop spontaneous autoimmune CNS demyelination.¹² Fecal 16S rRNA analysis identified a distinct microbiota profile in male *Tnfr2*^{-/-} 2D2 mice that is associated with disease protection, including *Akkermansia muciniphila*, *Sutterella* sp., *Oscillospira* sp., *Bacteroides acidifaciens*, and *Anaeroplasm* sp. Conversely, *Bacteroides* sp., *Bacteroides uniformis*, and *Parabacteroides* sp. were abundantly present in fecal samples from female *Tnfr2*^{-/-} 2D2 mice. Though it is not clear whether the increase in these bacteria is a cause or result of disease in these female *Tnfr2*^{-/-} 2D2 mice, this study suggests that anti-TNF therapy may disrupt commensal–host immune homeostasis in a gender-specific manner that leads to autoimmune demyelination.

The influence of age on microbiota and immune disorders

Age-dependent disease in mouse models

Airway exposure to bacteria can suppress allergic airway inflammation in adult mice.¹³ However, studies indicate that such treatments could be greatly optimized by administering the therapy at an optimal age window, because age is an important factor in immunoregulation of the lung. For example, an earlier report showed that exposure to endotoxin during the first 10 days of life, but not during adulthood, can cause the formation of tertiary lymphoid tissues in the lung.¹⁴ Later, it was shown that colonization by airway microbiota early in life induces T regulatory (T_{reg}) cells, a T cell type that plays a major role in maintaining immune tolerance and homeostasis, which contributes to reduced susceptibility to airway hyperresponsiveness in adulthood (Fig. 1).¹⁵ This effect is associated with an increase in the lung bacterial load and a shift in bacterial phyla from a predominance of Gammaproteobacteria and Firmicutes towards Bacteroidetes after the first 2 weeks of life. The increased accumulation of T_{reg} cells requires the interaction of programmed death 1 (PD-1) on T cells and programmed death-ligand 1 (PD-L1) on dendritic cells. Thus, the maturation of the lung microbiota during this 2-week window is a key factor for the host to develop protection against airway hyperresponsiveness.

Aside from the direct impact of lung microbiota on lung disease, recently a strong interest has emerged in characterizing the role of gut microbiota in lung disease. There is a clear gut–lung axis of communication, exemplified by gut microbiota's impact on immune disorders, such as asthma; lung diseases, such as chronic obstructive pulmonary disease (COPD); and respiratory infections.^{16,17} However, little is known mechanistically regarding how commensals in the gut modulate another mucosal site in the lung. Two reports point toward a tissue-specific effect of gut microbiota in regulating lung and intestinal inflammation. Invariant natural killer T (iNKT) cells recognize lipid antigens presented by CD1d.¹⁸ Initial findings showed that, compared with specific pathogen-free (SPF) mice, germ-free (GF) mice have an increase in iNKT cells in mucosal tissues, including colon and lung. They display an enhanced inflammatory response to chemical-induced colitis and exhibit airway hyperresponsiveness.^{19,20} The augmented inflammation in response to chemical assaults in the intestine can be dampened in GF mice by colonization with a type of gut commensal, *Bacteroides fragilis*, or oral gavage with *B. fragilis*-derived sphingolipid during the first 2 weeks of life, but not afterward (Fig. 1). Conversely, iNKT cell accumulation in the lung and susceptibility to airway hyperresponsiveness can be halted in GF mice by colonization with the general microbiota community—but not by monocolonization with *B. fragilis*—during the first 2 weeks of life. It is particularly interesting that *B. fragilis* monocolonization only protects against intestinal but not lung inflammation. This tissue-specificity may be due to the local immunoregulatory effect of *B. fragilis* and/or *B. fragilis*-derived sphingolipid.

Skin also exhibits a similar age- and microbiota-dependent T_{reg} regulation. Thus, *Staphylococcus epidermidis* colonization on neonatal (postnatal day 7) but not adult skin leads to immune tolerance characterized by an increase in commensal-specific T_{reg} cells in skin and skin-draining lymph nodes, which is accompanied by reduced commensal-specific

CD4⁺ T effector cells and diminished tissue inflammation (Fig. 1).²¹ An age-dependent effect is also present in another skin disease: psoriasis. A general antibiotic treatment in adult mice reduces the severity of psoriasis, with a decrease in IL-17- and IL-22-producing T cells. However, antibiotic treatment in neonatal mice induces long-term dysregulation of the microbial composition of the skin, an effect lasting until adulthood, which ultimately leads to an increased susceptibility to psoriasis development that is accompanied by an increase in IL-22-producing $\gamma\delta^+$ T cells (Fig. 1).²² In summary, age-dependent interactions of the host and microbiota in lung, intestine, and skin can cause beneficial and/or detrimental effects on the host. This knowledge presents a unique window of opportunity for future microbial-based therapies for immune disorder-related diseases.

Human diseases: early-life perturbations of microbiota lead to lifelong consequences

In humans, there is much evidence suggesting that a change in microbiota composition in early life may affect later susceptibility to disease as an adult. For example, children exposed to farm environments have a decreased risk for the development of allergic disease.²³ In an animal model of HDM-induced allergies, low-dose endotoxin or farm dust protected mice from developing allergies by suppressing the activation of epithelial cells and dendritic cells through induction of the ubiquitin-modifying enzyme A20 (encoded by *Tnfaip3*).²⁴ Combined with recent GWAS data that identified several SNPs in the TNFAIP3-interacting protein (TNIP-1) as being associated with asthma, the authors further discovered that *TNFAIP3* SNPs are linked to asthma in children growing up on farms. Most recently, an elegant study took advantage of two similar yet distinct U.S. agricultural populations, the Amish and the Hutterites, to elucidate the role of a farm environment in asthma risk.²⁵ Despite their similar genetic ancestries and lifestyle, one major difference between the two groups is that the Amish use traditional farming practices, whereas the Hutterites use industrialized farming practices. Children of Hutterites develop asthma 4–6 times more often than Amish children. The dust from Amish but not Hutterite homes protected mice from developing airway hyperreactivity and eosinophilia in a murine model of asthma. Mechanistically, the authors reveal that MYD88- and TRIF-dependent innate immune pathways are required for the protection mediated by Amish dust. Finally, the critical window for asthma control in early life, demonstrated first in animal models, has now been confirmed in human infants. Comparing the gut microbiota of 319 subjects, Arrieta *et al.* showed that infants at risk of asthma exhibited transient gut microbial dysbiosis during the first 100 days of life.²⁶ Thus, children at risk of asthma display reduced bacterial genera: *Lachnospira*, *Veillonella*, *Faecalibacterium*, and *Rothia*. Inoculation of GF mice with these four bacterial taxa ameliorated airway inflammation, demonstrating a causative effect of these bacteria on reducing asthma development.

With such strong evidence showing that a healthy/normal gut microbiota composition in early life is critical for lowering the incidence of immune disorders, it is not surprising to find that exposure to antibiotics can harm healthy microbiota and render the host more susceptible to many diseases. For example, antibiotic use within the first 6 months of life is associated with an increased susceptibility to allergy and asthma in later childhood.²⁷ Studies have also shown that antibiotic exposure during the first year of life is associated with the development of childhood wheezing and eczema, as well as inflammatory bowel

disease (IBD).^{28,29} In the IBD study, those receiving antibiotics were 2.9 times (95% CI: 1.2–7.0) more likely to develop IBD.²⁹ Another means of perturbation of the normal microbiota composition occurs in children born by cesarean section. Studies have shown that these children are more susceptible to type 1 diabetes mellitus, multiple sclerosis, allergy, and asthma later in life.^{30–32} The mechanistic basis for how these microbial perturbations affect disease susceptibility is unclear. However, it is clear that antibiotics can either transiently or permanently alter the composition of healthy microbiota, usually via depletion of one or several taxa.³³ Understanding this mechanism could help define the pathogenesis of dysbiosis-related immune disorders and offer major diagnostic and therapeutic opportunities.

Mucosa-associated microbiota and their immunomodulatory effects

Here, we focus on studies that show how changes in a single microbial species can alter the outcome of autoimmune diseases. Most commensals reside in the gut lumen, spatially separated from the host mucosal immune system.^{34,35} In contrast, mucosa-associated commensal species, though representing a minority within the commensal community, can powerfully modulate host immunity and disease status (Table 1).^{36–41} For example, segmented filamentous bacteria (SFB), which can breach the intestinal mucus layer and attach to intestinal epithelial cells, are potent inducers of lamina propria T helper 17 (T_H17) cells.^{28,38} Many studies focus on SFB in animal models,^{36,42–47} because these provide an efficient way to study the immune modulatory effect that may be induced by similar commensals in humans. Using the T_H17-inducing phenotype of SFB as a search criterion, one study discovered 20 human SFB-like commensal strains together displaying strong T_H17 induction capabilities and epithelial-adhesive features.⁴⁸ Notably, Mathis's and Benoist's group have identified a single species of human commensal, *Bifidobacterium adolescentis*, which was sufficient to induce T_H17 cells.⁴⁹ Similar to SFB, *B. adolescentis* is closely associated with the gut epithelium. Through collaboration with their group, we discovered a causative pathological effect of *B. adolescentis*, by showing that colonization by *B. adolescentis* can trigger autoimmune arthritis in genetically susceptible K/BxN mice.⁴⁹ Additionally, we have been studying human commensals isolated from spondyloarthritis (SpA) patients and their causative effect in inducing autoimmune arthritis. Roughly half of SpA patients show gut inflammation. By using a 16S rRNA compositional analysis that defined immune-relevant microbiota by IgA coating index, Viladomiu *et al.* identified an expansion of a pathobiont (pathogenic commensal) *Escherichia coli* isolate 2A (termed *E. coli* 2A) in patients with Crohn's disease-associated SpA (CD-SpA) compared with CD alone. We then used the K/BxN model to demonstrate a causative effect of *E. coli* 2A in enhancing T_H17 cell response, autoantibody (auto-Ab) titer, and disease progression.⁵⁰ Another commensal species, *Bacteroides fragilis*, which resides in the mucosal crypt, was shown to induce a T_H1 response as well as regulate the crucial balance between the T_H17 and T_{reg} response.³⁹ *Lactobacilli*, which makes up a high proportion of mucosal-associated bacteria (up to 13%), was shown to prevent IgE-mediated allergy.⁴⁰ Finally, two large-scale human studies have shown that there is an inverse association between *Helicobacter pylori*, a gastric mucosa pathobiont, and asthma and allergy.⁵¹ Some reports associate the protective effect of *H. pylori* with its ability to induce T_{reg} cells.^{52,53} However, the mechanisms by

which *H. pylori* colonization in the gastric mucosa reduces asthma risk remain to be determined.

Mechanisms by which commensals promote autoimmunity

A long-standing question in the field of host–microbe interaction is: how are microbes involved in the development of autoimmunity? Here, we discuss a few reported cellular and molecular mechanisms that contribute to gut microbiota–dependent autoimmunity. Although autoimmune diseases occur when the adaptive immune system fails to maintain self-tolerance, the innate immune system may be a culprit in facilitating the breakdown of self-tolerance. We will discuss how innate immunity is involved in microbiota-dependent autoimmunity, followed by the involvement of adaptive immunity, specifically molecular mimicry–mediated autoimmune diseases. Finally, while microbial recognition by the innate and adaptive immune system has been firmly established, recent studies have uncovered a crucial role for microbial metabolites in the orchestration of the host immune response.⁵⁴ We highlight examples of how microbiota-mediated metabolites and epigenetic modifications control the immune system and ultimately affect autoimmune development.

The innate immune system and the adjuvant effect

Infection has long been linked to the onset of autoimmune disease.^{2,55} Neutrophils are a key innate immune cell type at the front line of fighting infectious diseases.⁵⁶ Neutrophils are increasingly being recognized as integral players in the cross talk between microbiota and the host immune system, as they respond to signals from the microbiota and control the expansion of the microbiome. Oral microbiota affect neutrophil phenotype, as shown by the results of a report studying *Aggregatibacter actinomycetemcomitans* infection.⁵⁷ This oral pathogen releases a pore-forming toxin—leukotoxin A (LtxA)—which activates peptidylarginine deiminase enzymes in neutrophils and leads to hypercitrullination of self-proteins. Hypercitrullination is associated with rheumatoid arthritis (RA) development, and citrullination patterns induced by LtxA largely recapitulate those seen in RA, a finding further supported by the high concordance of anti-LtxA antibodies with RA development in human patients. These findings suggested a potential role for *A. actinomycetemcomitans* infection in triggering RA.

Another study compared the microbiome of three groups of infants in Northern Europe.⁵⁸ Early-onset autoimmune diseases are common in Finland and Estonia but are less prevalent in Russia. This study showed that Finnish and Estonian infants both have a greater proportion of *Bacteroides* species than Russia infants.⁵⁸ Therefore, the Finnish and Estonian infants are exposed to lipopolysaccharide (LPS) primarily derived from *Bacteroides*, which is structurally and functionally distinct from the LPS of *E. coli*. Unlike *E. coli* LPS, which is a potent innate immune activator, *Bacteroides* LPS does not inhibit innate immune signaling and endotoxin tolerance. The study further showed that, unlike LPS from *E. coli*, LPS from *B. dorei*, a species of *Bacteroides*, does not decrease the incidence of autoimmune diabetes in NOD mice. Interestingly, *B. dorei* has been shown to be associated with T1D pathogenesis.⁵⁹ Early colonization with immunologically silencing microbiota may thus preclude some aspects of immune education.

LPS is the ligand for Toll-like receptor 4 (TLR4), and another study along similar lines revealed the contribution of TLR4 in ameliorating autoimmune diabetes in NOD mice.⁶⁰ TRIF is a downstream signaling molecule of TLR4 and TLR3. TRIF gene deletion causes an increased incidence of autoimmune diabetes in NOD mice, suggesting a protective role for TRIF against autoimmune diabetes.⁶⁰ Mice with gene knockout of TLR4 but not TLR3 in an SPF facility have accelerated autoimmune diabetes,^{61,62} and TLR4 gene knockout mice in a GF facility have normal T1D incidence, suggesting that microbiota-dependent TLR4/TRIF signaling protects against T1D. Conversely, TLR2 gene deletion reduces the incidence of autoimmune diabetes, which is reversed when TLR2 gene knockout NOD mice are re-derived into the GF environment.⁶⁰ These findings suggest that microbiota deliver a prodiabetic signal through TLR2. Together, these results suggest that differences in microbiota-derived LPS among individuals may prevent their immune tolerance education, leading to autoimmune disease. These studies also indicate that microbiota can promote or inhibit autoimmunity by signaling through different receptors.

The IL-2 signaling pathway potently inhibits T follicular helper (T_{FH}) cell differentiation by decreasing Bcl-6 expression.^{63,64} We discovered that SFB drive the T_{FH} response via a DC-dependent adjuvant effect that is independent of TCR specificity.⁴⁷ Using a DC transfer model, we found that SFB-mediated Bcl-6 upregulation corresponds with SFB-mediated, DC-dependent IL-2R α ⁺ CD4⁺ suppression that occurs only in Peyer's patches (PPs). Consistent with other findings suggesting that IL-2 functions as a potent inhibitor of T_{FH} differentiation, anti-IL-2 treatment boosts the T_{FH} response, which leads to arthritis enhancement in SFB⁻ K/BxN mice, and SFB colonization does not further enhance these effects in the anti-IL-2-treated mice. These data suggest that the mechanism of SFB-triggered T_{FH} cell differentiation overlaps with anti-IL-2 treatment, which depends on suppression of the IL-2 signaling pathway. Our findings indicate that T cells can differentiate into T_{FH} cells upon receiving an SFB-mediated, DC-derived bystander signal that restrains IL-2 signaling, regardless of TCR Ag-specificity. Although molecular mimicry is a prominent mechanism whereby microbial infections trigger autoimmunity (see below), it is very unlikely that SFB induce differentiation of autoimmune T cells into T_{FH} cells by molecular mimicry of the self-antigen glucose-6-phosphate isomerase in the K/BxN model, because our data show that SFB also induce T_{FH} differentiation in NZB/NZW F1 mice, a non-transgenic murine SLE model.⁴⁷ Our finding that SFB mediate bystander activation of GPI-specific T_{FH} cells is supported by a recent report showing that SFB induced vigorous GC formation but a very low percentage of SFB-specific IgA in PPs compared with a commensal strain of *E. coli*.⁴⁴ Previous reports demonstrated an autoimmune-enhancing effect of SFB via the induction of T_H17 cells.^{42,65} Chappert *et al.* showed that, in a T_H1 cell-driven murine model of autoimmune arthritis, SFB modulate the activation threshold of self-reactive T cells and enhance autoimmune arthritis.⁶⁶ Interestingly, they found that SFB enhance disease by inducing T_H1 but not T_H17 cells. In the local microenvironment of gut-associated lymphoid tissues, inflammatory cytokines—particularly IL-12 elicited by the commensal flora—preferentially enhanced the T cell response to self-antigen that was otherwise tuned down.

Molecular mimicry

Molecular mimicry is a well-known mechanism for bacterial or viral infection–triggered autoimmunity.^{1,67} However, whether gut commensal bacteria can also trigger autoimmunity by molecular mimicry is poorly understood. Two studies have recently shed light on this topic (Fig. 2). The first report studied autoimmune uveitis, a major cause of blindness. Autoreactive retina-specific T cells were hypothesized to first become activated at non-eye sites and induce autoimmune uveitis by breaking through the blood–retinal barrier, given that their cognate antigens are sequestered within the immune-privileged eye.⁶⁸ To test this hypothesis, Horai *et al.* used a TCR transgenic mouse model that recognizes interphotoreceptor retinoid-binding protein (IRBP), a self-antigen, and demonstrated that gut commensals are required for the development of autoimmune uveitis. Retina-specific T cells are activated in a host that does not express self-antigen IRBP, and their activation is also independent of bacterial superantigens. Signaling through the retina-specific TCR by a protein extract derived from commensals is critical for the activation of retina-specific T cells, which can be blocked by anti-MHC class II antibodies, suggesting that the activation signals require TCR–MHC interactions. Although a molecular mimicry epitope was not identified in this study, it is conceivable that retina-specific TCRs can be activated by crossreactive products derived from commensals independent of the IRBP self-antigen.

The second study used islet-specific glucose-6-phosphatase catalytic subunit–related protein (IGRP)-specific CD8 TCR transgenic NOD mice to demonstrate that the gut microbiota strongly modulates CD8⁺ T cell–mediated T1D development (Fig. 2).⁶⁹ The authors further discovered that one type of commensal, fusobacteria, expresses peptides that share significant homology with IGRP and promote diabetes development. Thus, molecular mimicry between commensal antigens and an islet self-antigen provokes autoimmune diabetes. These studies raise a critical warning that activation of auto-reactive TCRs by commensal bacteria might be a more frequent trigger of autoimmune diseases than is currently appreciated.

Metabolites: 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 and have broad effects on the host immune system.⁷⁰ Recently, one group reported that long-chain fatty acids (LCFAs) enhanced the differentiation and proliferation of T_H1 and/or T_H17 cells, while SCFAs expanded gut T_{reg} cells.⁷¹ Using experimental autoimmune encephalomyelitis (EAE) as a model for multiple sclerosis, Haghikia *et al.* showed that LCFAs decreased SCFAs in the gut, leading to exacerbated EAE by expanding pathogenic T_H1 and/or T_H17 cells in the small intestine. Treatment with SCFAs ameliorated EAE by inducing lamina propria–derived T_{reg} cells. SCFAs can also indirectly regulate autoimmunity through other molecules, such as antimicrobial peptides (AMPs), expressed by epithelial and immune cells for their defense function against invading microbes.⁷² Interestingly, β cells have been shown to produce the cathelicidin-related antimicrobial peptide (CRAMP), and this production was defective in female but not male NOD mice.⁷³ Systemic CRAMP administration to female NOD mice induced T_{reg} cells in the pancreatic islets and reduced the incidence of autoimmune diabetes. Mechanistically, the production of CRAMP by β

cells was controlled by SCFAs produced by the gut microbiota. Fecal transplant of gut microbiota from male NOD mice to female NOD recipients increases CRAMP production and reduces the incidence of diabetes, indicating a causative effect of male gut microbiota in controlling autoimmune diabetes.

Metabolites: retinoic acid

Retinoic acid, a metabolite of vitamin A, has a wide range of biological activity, including regulating immune responses.⁷⁴ A major part of retinoic acid's anti-inflammatory effects depends on the inhibition of T_H17 and promotion of FOXP3⁺ Treg responses.^{74,75} AM80 is a synthetic retinoic acid that is characterized by higher stability and fewer potential adverse effects compared with all-*trans*-retinoic acid, one of the most active physiological retinoid metabolites.^{76,77} It has been reported that retinoic acid and AM80 ameliorate many autoimmune responses, including experimental autoimmune myositis, experimental autoimmune encephalitis, and collagen-induced arthritis.^{78–81} We recently showed that oral administration of AM80 inhibits autoimmune disease in joints as well as in lung.⁸² We elucidated a novel mechanism whereby AM80 suppresses the autoimmune pathology at both lung and joints by inhibiting T_{FH} and T_H17 responses. Specifically, AM80 increased the expression of the gut-homing integrin $\alpha_4\beta_7$ on T_{FH} cells, which diverted T_{FH} cells from systemic (non-gut) inflamed sites, such as lung, into the gut (the non-immunopathological site) and thus reduced the systemic auto-Abs.

Metabolites: uric acid

It is worth mentioning that, although most studies have focused on the bacterial microbiota, the gut microbial communities also include fungi and viruses. One study has reported that *Saccharomyces cerevisiae* exacerbated intestinal disease in a mouse model of colitis and increased gut barrier permeability by enhancing host purine metabolism, leading to an increase in uric acid production.⁸³ Thus, fungi in the gut may be able to potentiate metabolite production that worsens the development of IBD.

Microbiota-mediated epigenetic modifications

One of the mechanisms by which the microbiota affects the host is by triggering epigenetic changes in host cells. This often occurs through histone acetylation controlled by microbial metabolites, such as SCFAs.^{84–86} Administration of propionate rescued the T_{reg} population in GF mice, restoring percentage and numbers comparable to those in SPF mice, as well as increasing T_{reg} expression of FOXP3, TGF- β , and IL-10 *in vitro*. These effects were mediated by inhibition of histone deacetylases (HDACs) 9 and 6, which led to increased histone acetylation.⁸⁶ Similarly, butyrate was found by multiple groups to increase histone acetylation in order to promote peripheral or colonic T_{reg} differentiation.^{84,85} Naïve CD4⁺ T cells cultured under T_{reg}-inducing conditions in the presence of butyrate exhibited increased histone acetylation at the promoter and conserved noncoding sequence 3 (CNS3) compared with those cultured without butyrate, and *Rag1*^{−/−} mice fed butyrate were protected against T cell-induced colitis in a T_{reg}-dependent manner.⁸⁵ In another study, Arpaia *et al.* found that butyrate inhibited HDACs in order to increase histone acetylation in DCs, enhancing their ability to promote T_{reg} differentiation. This effect was dependent on peripheral T_{reg} differentiation, as mice lacking CNS1—an intronic enhancer required for extrathymic but

not thymic T_{reg} differentiation—in FOXP3-expressing cells failed to respond to butyrate.⁸⁴ These studies demonstrate that the commensal microbiota mediates protection against autoimmunity in part through histone modifications that promote T_{reg} proliferation and differentiation. It is interesting to note that these modifications occur in multiple cell populations, including T_{reg} cells themselves as well as DCs, demonstrating the wide target range of microbiota by-products.⁸⁴

The microbiota causes other epigenetic modifications, such as alterations in DNA or histone methylation. The previously mentioned iNKT-enhanced inflammatory response to chemical-induced colitis and airway hyperresponsiveness in GF mice were due to increased DNA methylation at five CpG sites in the gene for CXCL16, which results in a significant increase in iNKT cells numbers.²⁰ Another group found that NK cells were actually less reactive to stimuli than their counterparts from SPF mice.⁸⁷ Furthermore, TLR ligand-induced type I IFN production, as well as IL-6, TNF- α , IL-12 and IL-18, were systemically impaired in DCs of GF mice or mice treated with broad-spectrum antibiotics, which was connected to the absence of trimethylation on histone H3, lysine 4 (H3K4) in the regulatory regions of these cytokine genes in DCs, presumably impairing their ability to prime NK cells. Thus, the microbiota epigenetically regulates gene expression to dampen autoimmune inflammation but increase the ability to respond to outside challenges.

Linking gut microbiota to gut-distal autoimmune diseases

It is clear that dysbiosis of gut microbiota affects many extraintestinal autoimmune diseases and immune disorders, including rheumatoid arthritis, type 1 diabetes, multiple sclerosis, and asthma.^{7,88} How do microbiota residing in the gut affect disease outside the gut? To address this question, we took advantage of the KikGR transgenic mouse line, which ubiquitously expresses the green-to-red photoconvertible fluorescent protein in its cells.^{89,90} We first generated KikGR.K/BxN mice, which express the KikGR transgene on the K/BxN background. We then developed a surgical procedure to specifically photoconvert and hence mark PP cells by treating PPs with violet laser light and subsequently monitored the migration of these photoconverted PP cells to the spleen.⁴⁷ It has been reported that 3 days is the best time point to detect an arrival of photoconverted, colon-derived T conventional cells into the spleen, using Kaede mice, the transgenic photoconversion mouse model similar to the KikGR mouse model.⁹¹ Thus, we monitored the converted PP cell migration into the spleen 3 days after surgery. A significant number of photoconverted, migratory CD4⁺ T cells from PPs were detected in the spleens of the KikGR.K/BxN mice after PP violet light exposure compared with controls with sham surgery, regardless of their SFB status (Fig. 3). Interestingly, there is a cell type-specific contribution to the photoconverted CD4⁺ T cell pool in the spleen: a significantly higher portion of the newly arrived and PP-derived photoconverted CD4⁺ T cells in the spleen were T_{FH} cells, compared with the SFB⁻ group. Thus, these results provide direct evidence that SFB colonization preferentially boosts T_{FH} cell migration from PPs to the spleen and suggest a potential role for microbiota-driven and gut-derived immune cells in modulating systemic autoimmunity by entering systemic sites.

A later report provides further evidence for gut microbiota-dependent cell migration from the gut to another extraintestinal site, the kidney, contributing to autoimmune development

(Fig. 3).⁹² T_H17 cells are most abundant in the gut during homeostasis, and some reports have suggested that they are produced in a microbiota-dependent manner.³⁶ Krebs *et al.* found a high percentage of T_H17 cells in the kidneys of patients with anti-neutrophil cytoplasmic antibody-associated glomerulonephritis. They utilized Kaede mice to track intestinal T cell migration upon glomerulonephritis induction, and found that T_H17 cells egress from the intestine in an S1P receptor-1-dependent fashion. These T_H17 cells subsequently migrate to the kidney via the T_H17 chemoattractant CCL20 through interaction with CCR6 expressed by the T_H17 cells. Depletion of gut T_H17 cells in germ-free and vancomycin-treated mice ameliorated the autoimmune glomerulonephritis. Thus, targeting the intestinal TH17 cell reservoir may offer a therapeutic strategy for autoimmune diseases.

Conclusions and future directions

There is a surge of recent studies reporting the associations of host disease with dysbiosis, including reduction of microbial diversity and/or aberrant microbial communities.^{93,94} Although these studies provide promising clinical implications, various challenges need to be overcome by the field in order to harness these findings for future diagnostic and therapeutic approaches. One huge challenge lying ahead is to distinguish cause from effect (i.e., whether the gut microbiota is the cause of the disease or a result of the disease state). The use of animal models where the intestinal flora can be manipulated provides a powerful tool for such mechanistic studies. Second, 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 demonstrated by the fact that bacterial isolates of the same strain can display very different immunoregulatory capabilities.⁵⁰ Finally, the therapeutic effect of probiotics on dysbiosis-related disease may rely on a very specific composition of microbiota, with a few exceptions, such as in *Clostridium difficile* infection, as the antibiotic-induced dysbiosis that triggers *C. difficile* infection is so extreme that most fecal transplantations prove to be beneficial.⁹⁵ A stable engraftment of a defined commensal community can be challenging. A better understanding of host-microbe interaction and the underlying microbiota-derived molecules that modulate the immune system and disease development may help pave the way for better patient-tailored interventions and microbial molecule(s)-based therapies for immune disorders.

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Figure 1.

A critical age window for microbial-mediated immunomodulation that has long-term effects on health. The lung T_{reg} cell upsurge in early age is associated with an increase in the lung bacterial load and a shift in bacterial phyla from a predominance of Gammaproteobacteria and Firmicutes towards Bacteroidetes after the first 2 weeks of life. Another pioneer study showed that, in GF mice, iNKT cells accumulate in both colonic lamina propria and lung, resulting in increased disease in models of IBD and asthma compared with SPF mice. This increase of iNKT cells was associated with increased intestinal and pulmonary expression of the chemokine ligand CXCL16. Importantly, colonization of neonatal but not adult GF mice with normal microbiota protected the animals from mucosal iNKT cell accumulation disease development. An *et al.*¹⁹ further provided a mechanism whereby microbiota inhibit iNKT cell accumulation during the neonatal period. They found that the intestinal commensal *B. fragilis* reduces iNKT cells by producing sphingolipids, which inhibit iNKT cell proliferation during neonatal development. This effect lasts till adulthood, and early

exposure to these lipids protects the host from later iNKT cell–mediated colitis. Finally, early-life perturbations of microbiota also lead to long-lasting effects on immunomodulation in skin. Scharschmidt *et al.*²¹ demonstrated that skin colonization of *S. epidermidis* during the neonatal period was required for establishing immune tolerance to commensal microbes. This crucial window was characterized by an influx of T_{reg} cells into neonatal skin. Together, these studies suggest that the microbial community in neonatal life is crucial in shaping immune responses to commensals, and disrupting these interactions might have lifelong consequences.

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Figure 2.

Commensal-mediated molecular mimicry induces autoimmune diseases. A recent study demonstrated that microbial peptides from *Fusobacteria* share significant homology with islet self-antigen IGRP. Both the microbial mimic peptide and the *Fusobacteria* themselves directly activate IGRP-specific autoimmune CD8⁺ T cells and promote diabetes development. Another example of molecular mimicry was demonstrated in a novel mouse model of spontaneous uveitis. Activation of IRBP-specific CD4⁺ T cells is dependent on gut commensal microbiota. The activation of self-antigen-specific T cells occurs without the endogenous retinal autoantigen, which further suggests the non-self source of antigen for autoimmune T cell priming. Thus, microbial mimicry is involved in activation of both autoimmune CD4⁺ and CD8⁺ T cells, raising the possibility that activation of autoimmune T cells by commensal microbes might be more common than is currently appreciated.

Figure 3.

Gut microbiota-mediated immune cell migration triggers the systemic/non-gut autoimmune diseases. By photolabelling intestinal immune cells, two studies independently showed that microbiota-induced PP T_{FH} cells or intestinal T_H17 cells egress to systemic sites, and these gut-derived T_{FH} or T_H17 cells then cause systemic autoimmune diseases in joints or kidneys, respectively. In one case, PP depletion in autoimmune arthritic K/BxN mice reduced the splenic T_{FH} cell population, leading to a decrease in auto-Ab titers and arthritis development. Similarly, GF or antibiotic-treated B6 mice with deficient gut T_H17 cells display ameliorated renal diseases. Together, these studies suggest that targeting gut immune cells may serve as a novel therapeutic method to treat systemic diseases.

Table 1

The effects of mucosa-associated commensals on immunomodulation and disease development.

Commensal type	Location	Disease model phenotype	Immunomodulation	Reference
SFB	Mucosa (adhere to IEC)	RA ↑ EAE ↑	T _H 17 ↑ T _{FH} ↑ T _H 1 ↑	38, 43, 61, 62
<i>B. adolescentis</i>	Mucosa (adhere to IEC)	RA ↑	T _H 17 ↑	45
<i>E. coli</i> 2A	Mucosa (adhere to IEC)	RA ↑ Colitis ↑	T _H 17 ↑	46
<i>H. pylori</i>	Gastric mucosa	Asthma ↓ Allergy ↓	T _{reg} ↑	47–49
<i>B. fragilis</i>	Crypt	EAE ↓ Colitis ↓	T _{reg} ↑ T _H 1 ↑	35
Lactobacilli	Mucosa	Allergy ↓	IgE ↓	36