Type 1 diabetes and gut microbiota: friend or foe?

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Abstract

Type 1 diabetes is a T cell-mediated autoimmune disease. Environmental factors play an important role in the initiation of the disease in genetically predisposed individuals. With the improved control of infectious disease, the incidence of autoimmune diseases, particularly type 1 diabetes, has dramatically increased in developed countries. Increasing evidence suggests that gut microbiota are involved in the pathogenesis of type 1 diabetes. Here we focus on recent advances in this field and provide a rationale for novel therapeutic strategies targeting gut microbiota for the prevention of type 1 diabetes.

Type 1 diabetes (T1D) is an autoimmune disease characterized by the immune cell-mediated destruction of insulin-secreting pancreatic beta cells in genetically predisposed individuals upon environmental stimulation. The interaction between pancreatic β-cells and immune cells leads to the development of T1D (1). Strategies targeting cells or signaling pathways of immune system have been proven effective in preventing and reversal of T1D (2–7). Nevertheless, over the past few decades, there has been a steady 3–4% increase in the incidence of T1D, particularly in young children, in developed countries (8). Although genetic factors, especially genes in the HLA region, can predispose an individual to T1D, twin and family studies show that only a fraction of those genetically predisposed individuals will develop the disease (9–11). The cumulative incidence among monozygotic co-twins of persons with T1D is less than 50 % (11). Comparison of the frequency of HLA class II haplotypes in patients diagnosed more than 50 years ago with age and sex-matched patients between 1985 and 2002 suggests that the impact of environment on children with lower-risk HLA class II genes accounts for the rising incidence and decreasing age at diagnosis of T1D (12, 13). Thus, it is strongly believed that environmental factors are important for the development of T1D (14). Furthermore, environmental factors participate in the initiation, as well as various stages of the natural history of the T1D (15, 16).
There have been a variety of environmental factors, including viral infection and diet, suggested to promote T1D. A recent study provides evidence for the presence of enterovirus in pancreatic islets of newly diagnosed patients with type 1 diabetes (17), suggesting that a low-grade enteroviral infection in the pancreatic islets contributes to disease progression in humans. Consistent with the detection of virus in islets, virus-responsive interferon responsive factor 7 (IRF7) network genes and their regulatory locus are implicated in the pathogenesis of T1D by integrated genome-wide approaches (18). Coxsackie B virus (CVB) infected engrafted human islets in mice contain viral RNA, express viral protein, and show reduced insulin production compared to the grafts from uninfected mice (19). These observations imply that viral infection may trigger islet autoimmunity. In addition to virus, it has also been shown that various foods or food components such as cow’s milk and gluten affect the development of T1D (20–23). The insulin from cow’s milk may activate insulin-specific autoimmunity before the establishment of oral tolerance (22, 23). Other components from cow’s milk, such as casein, can alter gut permeability and potentially promote the incidence of T1D (22, 23). Food-derived gluten has also been shown to modify gut permeability and again potentially affect the development of T1D (20, 21). Interestingly, mice treated with a gluten-free diet showed modifications in gut microbiota and protection from T1D (20, 21). Consistently, accumulating studies showed in recent years the involvement of environment factors, particularly an association with gut microbiota, in the pathogenesis of T1D.

In this review, we will focus on the possible roles of gut microbiota in the development of T1D and provide the notion of using prebiotics, probiotics or even antibiotics as a potential strategy for the prevention of T1D.

**Understanding the Gut microbiota**

Gut microbiota and host have a symbiotic relationship due to their co-existence and co-evolution. On one hand, gut microbiota depend on the host for their growth and survival. On the other hand, most of the gut microbiota are non-pathogenic and can benefit the host in many ways: 1) extraction of nutrients and energy from diet intake (24–26), 2) protection from enteropathogen invasion (27), 3) contribution to the development of a normal immune system or function (28–30). In contrast, the imbalance between the gut microbiota and the host has been associated with many diseases including malnutrition (26), obesity (31–34), autoimmune disorders (35–40) and neurological diseases (41, 42). Thus, an understanding of how the gut flora may affect health and disease will provide an insight into how to promote health by the modification of gut microbiota.

The inability to culture the majority of the gut microbiota in the past had hindered our understanding of the microbial communities. With the advent of high-throughput sequencing technology, it has dramatically speeded up the dissection of the symbiotic relationship between gut microbes and their host. Several large-scale projects such as the US Human Microbiome Project and the European Metagenomics of the Human Intestinal Tract have substantially contributed to the understanding of healthy composition and functional states of gut microbiota (43–47). The analysis of 16S rRNA gene sequences of bacteria from human stool samples has identified that the majority of gut microbial populations comprise
bacteria from four main phyla: Bacteriodetes, Firmicutes, Actinobacteria and Proteobacteria (48). The results from metagenomics analysis also provided information about the functional properties of gut bacteria, particularly the important function of low-abundance gut microbes, involved in an array of physiological processes (47, 49). Furthermore, the metabolomics analysis revealed the effect of metabolites derived from microbiome on their host (50). Equipped with knowledge at different levels, we are gaining better recognition about the importance of gut microbiota in our health.

**Gut microbiota and type 1 diabetes**

During the last decade, there has been considerable research activity and a sharp increase in the amount of experimental data on the role of gut microbiota in health and disease. Of note, gut microbiota play an important role in the regulation of autoimmunity and tolerance (36, 39, 40, 51). However, the contribution of gut microbiota to the development of T1D remains limited, although involvement of the microbiota has been suggested in the development of T1D as early as 1987 (52). Following on from this, experiments using NOD mice that were transferred from specific pathogen-free (SPF) conditions to germ free (GF)-conditions showed a marked change in insulitis and the incidence confirmed the role of gut microbiota as a regulator of islet-specific autoimmunity (53, 54).

The first gut microbiota study in humans for T1D compared the microbiome between 4 Finnish children with T1D and 4 age- and HLA-DQ-matched healthy children (55, 56). By employing the16S rRNA pyrosequencing method, lower diversity and stability of the fecal microbiome was identified in the children in their first year of life who later went on to develop T1D when compared with the healthy control subjects (55). Recent data from the Human Microbiome Project also imply that higher diversity and stability of the gut microbiome is associated with health (46). Furthermore, in the follow-up study, those Finnish children who developed T1D had a decreased ratio of *Firmicutes* vs *Bacteroidetes*, supporting a cross-sectional study showing that *Bacteroidetes* were more abundant in islet-specific autoantibody-positive children than in autoantibody-negative children (57, 58). All the evidence from both animal and human studies thus far further supports the involvement of gut microbiota in the development of T1D. There are several mechanisms by which gut microbiota could affect the development of T1D as discussed below.

**Alteration of intestinal permeability**

Heightened gut permeability has been demonstrated to be one of the phenomena that precede the clinical onset of T1D in both animal models of autoimmune diabetes, as well as in patients with T1D and prediabetic individuals (59–63). Evidence from animal studies has been largely derived from two rodent models: NOD mice and the BioBreeding diabetes-prone (BBDP) rat. It has been suggested that the imbalance of bacteria, such as *Bacteroidetes*, which ferment short-chain fatty acid (SCFA), can affect the gut permeability. Indeed, in parallel to the changed gut permeability, BBDP rats, before clinic onset, have a different gut bacterial composition from that of diabetes-resistant (BBDR) rats, with relatively higher abundance of *Bacteroides* sp in diabetic rat (64, 65). At disease onset, the gut bacterial profile was also different between BBDP and BBDR rats (66). Specifically, the BBDP rats had a lower proportion of the probiotic-like bacteria, such as *Bifidobacterium*
and Lactobacillus, but had higher numbers of Bacteroides, Ruminococcus and Eubacterium (66). At the cellular level, there were also structural changes in the intestinal morphology, such as greater percentage of goblet cells and mucosal crypt depth, accompanying the increased permeability in BBDP rats (59, 62, 67). At the molecular level, the expression of multiple tight junction proteins was down- or up-regulated, in both BBDP rats and T1D patients, thus affecting the gut permeability, including occludin, members of claudin family and zonulin (61, 67, 68).

However, there is not much evidence, thus far, suggesting that the gut microbiota are actually responsible for the cellular and molecular changes in gut. Nevertheless, a study has shown that the metabolites of gut microbiota, such as butyrate, an anti-inflammatory factor, can affect gut permeability by enhancing the gut barrier function via tight junctions (69). In the children with beta cell autoimmunity, there was a low abundance of butyrate-producing bacteria including Clostridium clusters XIVa and IV (55–57). Butyrate can be metabolized from lactate. Children with T1D also have low numbers of lactate-producing bacteria, such as Bifidobacterium adolescentis (57). Those studies provided supporting evidence that gut microbiota could affect gut permeability through their metabolites. Nevertheless, gut permeability is only an index for the later possible beta cell autoimmunity. Although both BBDP and BBDR rats showed transient increases in gut permeability during early life, only BBDP rats that exhibited morphological changes and inflammation in intestine developed the disease (59), which implies that the enteropathy is fundamentally linked to the disease development. Thus, we have to be cautious in interpreting the gut permeability data in this setting.

Modification of intestinal immunity

Gut microbiota are essential for healthy development of the mammalian immune system (70). Direct evidence comes from germ-free mice, in which multiple defects in the gut immune system have been noted, including impaired development of gut-associated lymphoid tissue (GALT) (71), generation of colonic regulatory T cells (72) and production of IgA (73). Importantly, the profound effects that commensal microbiota have on immunity is not limited to the gut immune system, but extends to the systemic immune response (74). Germ-free mice have an elevated IgE level and overall are skewed toward Th2 immune responses, which can be normalized by exposure to a diverse microbiota during early life (75). Interestingly, administration of Bacteroides fragilis-derived polysaccharide A (PSA) to GF mice can correct the Th1/Th2 imbalance in the spleen (74).

Although the various mechanisms by which gut microbiota regulate host immunity are, as yet poorly elucidated, a couple of mechanisms have been proposed: 1) directly activating the innate immune response through Toll-like receptors (TLR) by molecular patterns from gut bacteria (76–79); 2) modulating immune responses via G-protein-coupled receptor (GPCR) by bacterially-derived metabolites (80, 81). The activation of innate immune responses by gut microbiota-derived molecular patterns that are mostly bacteria cell wall components such as flagellin (78, 82) and PSA (79), as well as commensal genomic DNA (77). For example, mice that are deficient in the flagellin receptor, TLR5, develop metabolic syndrome in a gut microbiota-dependent manner (82). The transfer of gut bacteria from
TLR5-deficient mice into germ-free mice leads to development of many features of the metabolic syndrome in the recipients (82). This suggests that malfunction of the innate immune system may promote the development of metabolic syndrome through modification of the gut bacterial profile. On the other hand, *Bacteroides fragilis*-derived PSA, a TLR2 ligand, can induce IL-10-producing CD4 T cells and reciprocally suppress Th17 responses, thus protecting against experimental colitis (79). Similarly, TLR9-deficient mice display an elevated frequency of Foxp3+ Treg at intestinal effector sites and suppressed constitutive IL-17- and IFN-γ-producing effector T cells (77). Further mechanistic studies indicate that DNA from gut flora plays a major role in intestinal homeostasis through TLR9 engagement (77). In the NOD mouse model of T1D, we also demonstrated that a deficiency of the master adaptor protein MyD88 led to resistance to the development of T1D through the modification of gut bacteria (54). All the observations imply that different innate immune-activating components of gut bacterial origin have a different role in the regulation of gut immune homeostasis and our results provide the first evidence of the “missing” link between gut microbiota and innate immunity with T1D development.

Similar to the diet-independent components, diet-dependent gut bacteria-derived metabolites, such as short-chain fatty acid (SCFA) and vitamins, have far-reaching effects on the immune responses (70, 83). SCFAs are produced through fermentation of dietary fiber by gut microbiota, which bind the G-protein-coupled receptor 43 (Gpr43), also called free fatty acid receptor 2 (FFA2/FFAR2). Studies have demonstrated the anti-inflammatory role of SCFAs on immune cells (80, 84). Several SCFAs, including acetate (27, 80, 84), butyrate (84), and propionate (84), have been well characterized as playing a role in gut immune homeostasis. Mice that take in acetate through drinking water display suppressed dextran sulfate sodium (DSS)-induced colitis, inflammatory arthritis and asthma in a Gpr43-dependent manner (80). The oral administration of acetate, butyrate or propionate not only augments population size but also enhances the suppressive function of colonic Treg in SPF mice (84). Among these three SCFAs, butyrate is the most potent inducer of the differentiation of naive T cells into Treg, while acetate and propionate are important for the migration of Treg to intestine (86, 87). On the other hand, certain *Bifidobacteria* produce B-group vitamins that can activate mucosa-associated invariant T cells and the Jurkat T cell line (88, 89). Although it has been shown that vitamin D plays a role in the development of T1D (90), studies identifying how SCFAs directly affect islet-specific autoimmunity are still lacking.

In humans, more males develop diabetes than females develop T1D after puberty. However, in NOD mice, females develop diabetes earlier and in a greater proportion than the male mice. In NOD mice, the gut microbiota were shown to be involved in the gender bias of T1D and there is sex hormones play a role in altering commensal gut microbiota which influence the development of diabetes through the IFN-γ signaling pathway (35).

It is clear that gut bacteria affect systemic immunity. Mounting evidence also suggests that gut microbiota have profound effects on autoimmunity. Several studies in animal models implied that alterations in the gut microbiota are associated with the development of T1D (54, 59, 91, 92). During early life, BBDDP rats have impaired intestinal barrier function that may be the underlying cause for the altered response to luminal antigens, but the intestinal
inflammation might be a trigger that leads ultimately to diabetes development (59, 62). Studies have confirmed the role of gut microbiota as a regulator/facilitator of inflammation in the pancreatic islets. Antibiotic treatment partially protects against T1D in BBDP rats (64, 65). Germ-free NOD mice display an imbalance between Th1, Th17 and Treg differentiation in the intestine (53). This imbalance is associated with accelerated insulitis, which can be interpreted by the shared immune cell homing receptors, such as α4β7-integrin, in the gut and inflamed pancreas (93). Compared with BBDP rats, BBDR rats carry more probiotic-like bacteria in stool, such as Lactobacillus johnsonii (66). Lactobacillus johnsonii can induce Th17 responses in mesenteric lymph nodes and spleen, thus, affecting the development of T1D (76, 94, 95), although the role of Th17 immunity in T1D has been controversial (96–102). The transfer of gut bacteria from diabetes-resistant MyD88 deficient NOD mice can reduce insulitis and significantly delay the onset of diabetes through the upregulation of IgA and TGF-β production in the intestine (92).

Modifying gut flora to prevent T1D

Existing evidence has suggested the role of dysbiosis in T1D development, including reduced bacterial and functional diversity, which are accompanied by impaired gut barrier function and elevated inflammation due to decreased induction of Treg (55–58). With the improvement in our understanding of the role of gut microbiota in autoimmunity, we can develop therapies targeting intestinal immunity by modification of gut microbiota to prevent T1D development. It has been shown that experimental manipulation of gut microbiota in young NOD mice can significantly protect them from T1D development, which provides proof of concept that therapy targeting gut microbiota is effective in genetically predisposed individuals (38, 92). During infancy, the intestinal environment undergoes major developmental changes and the gut microbiome is extensively remodeled; it later becomes relatively resistant to variation after puberty, due to the regulation by intestinal immunity (25, 103). Therefore, minor modifications at the early stages in life would have profound effects on normal intestinal immune homeostasis in adulthood (104). Previous studies also suggested that neonatal gut immunity plays an important role in controlling the development of diabetes (105, 106). Thus, early life treatment with the antimicrobial drug vancomycin can expand Akkermansia muciniphila and reduce diabetes incidence in the NOD mouse (107). Our recent study also demonstrated that the offspring from NOD mothers treated with antibiotics that target gram-negative bacteria had reduced and delayed T1D development (Hu, et al., unpublished data). Neonatal oral administration of DiaPep2, an analogue of HSP60 peptide p277, in combination with hydrolyzed casein diet can protect against T1D in BBBDP rats (105). These treatments during early life have a crucial effect on the intestinal barrier function, cytokine production and the development of diabetes (106). Long term administration of “friendly” gut bacteria or the probiotic compound VSL#3 to NOD mice starting from 4 weeks of age could also prevent the NOD mice from T1D development in regulatory cytokine IL-10 or TGF-β dependent mechanism (92, 108). Furthermore, administration of genetically modified gut bacteria can even reverse diabetes. Oral delivery of genetically-modified Lactococcus lactis alone or in combination with low dose of systemic anti-CD3 can reverse new-onset T1D in NOD mice (109, 110).
Although administration of gut microbiota can successfully prevent and reverse T1D in animal models, the application of this therapeutic strategy in humans has not yet been tested. One of the obstacles is the lack of reproducible development and manufacture of microbial mixtures with well-defined genetic content and metabolic output such as the one used for *Clostridium difficile* infection treatment (111, 112). Nevertheless, there are studies manipulating microbiota-induced immunoregulation by non-bacterial strategies including diet and/or bacterial metabolites. A gluten-free diet could affect gut microbiota and thus reduce the incidence of diabetes (20, 21). It has been shown that gut microbiota-derived metabolites, such as acetate, butyrate, propionate, can modulate intestinal immunity through induction or recruitment of Treg (80, 84–87). Regulatory T cells are essential for the controlling of islet autoimmunity. Thus, testing the effect of those SCFAs on the development of T1D would be important in future preclinical studies.

**Conclusion**

A better understanding of how gut bacteria-induced immunoregulation contributes to the pathogenesis of T1D is necessary. The existing evidence is exciting and encouraging in that modulation of gut microbiota can affect the progress of diabetes in preclinical studies. It is likely that the beneficial effects of gut microbes come from both the live bacteria and their metabolites (Figure 1). As an ecosystem, the gut microbiome is a community in which the components affect each other and a balance is important for the health of host. Although “omics” analyses can significant improve our understanding of the profile of gut flora and their metabolites, we have to be cautious in thinking that a single bacterial strain or molecule may be useful as therapeutics. Nevertheless, the advantages of microbial therapies are obvious: less expensive, less invasive and potentially long-lasting beneficial effects. Once we gain a better knowledge of specific host and gut microbial functional pathways involved in the development of T1D, direct or indirect microbiota-based therapies can be developed to prevent or cure T1D.

**Acknowledgments**

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**References**


Figure 1.
The role of gut microbiota in the development of T1D. Gut flora can affect islet autoimmunity through mechanisms: 1) expression of autoantigen mimicry to activate autoreactive T cells by antigen-presenting cells to destruct islet beta cells; 2) generating metabolites, such as acetate, butyrate etc, to induce the differentiation or migration of regulatory T cells to control autoreactivity through GPCR signaling pathway (such as Gpr43); 3) gut bacteria-derived pathogen-associated molecular patterns (PAMP) activate TLR signaling pathway to initiate the inflammation, which activates autoreactive T cells and/or directly cause injury to beta cells through inflammatory cytokines; 4) gut bacteria can penetrate the leaky gut and cause inflammation to destruct beta cells.
Table 1

Role of gut microbiota in T1D

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<td>Modified tight junction</td>
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<td>Modified mucosal immunity</td>
<td>Impaired GALT development Modified innate immunity:</td>
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<td>1) TLR2</td>
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GALT: Gut-associated lymphoid tissue