Published in final edited form as:

Clin Immunol. 2015 August ; 159(2): 143–153. doi:10.1016/j.clim.2015.05.013.

The Gut Microbiota and Type 1 Diabetes

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Abstract

Type 1 Diabetes (T1D) is a multifactorial, immune-mediated disease, which is characterized by the progressive destruction of autologous insulin-producing beta cells in the pancreas. The risk of developing T1D is determined by genetic, epigenetic and environmental factors. In the past few decades there has been a continuous rise in the incidence of T1D, which cannot be explained by genetic factors alone. Changes in our lifestyle that include diet, hygiene, and antibiotic usage have already been suggested to be causal factors for this rising T1D incidence. Only recently have microbiota, which are affected by all these factors, been recognized as key environmental factors affecting T1D development.

In this review we will summarize current knowledge on the impact of gut microbiota on T1D development and give an outlook on the potential to design new microbiota-based therapies in the prevention and treatment of T1D.

Keywords

Type 1 Diabetes; gut microbiota; gut permeability; Treg; Th17 cells

1. Introduction

Type 1 Diabetes (T1D) is one of the most common metabolic disorders in children and young adults. This autoimmune-mediated disease results in a progressive loss of insulin-producing beta cells in the islets of Langerhans in the pancreas.

Several diabetes-predisposing gene loci have been identified. The strongest association with T1D in humans is with the HLA-DR and -DQ genes, which account for approximately 40-50% of the disease risk [1]. However, despite intensive research, the triggers for T1D are still mostly unknown, whereas the incidence of T1D has sharply risen worldwide in the past few decades. Currently the overall increase in the incidence of T1D in Europe is about 3-4%

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per year. The fastest increase is observed in children less than 5 years of age [2]. Within this age group the incidence is anticipated to double by 2020 [3, 4]. These dramatic changes in the incidence rate cannot be explained merely by genetic changes. The importance of non-genetic, environmental factors contributing to the disease risk becomes obvious when considering that fewer than 10% of individuals who are genetically predisposed to T1D de facto develop the disease [5].

Environmental factors that may affect the risk of developing T1D include birth delivery mode [6, 7], diet in early life [8-11], and possibly usage of antibiotics [12, 13]. All of these potential environmental risk factors are related to the intestine and its microbiota. The gastrointestinal tract constitutes the largest surface area in the human body and is densely populated by 500-1000 different bacterial species [14]. In recent years, much research effort has been focused on elucidation of the interaction between the host and the gut microbiota in health and disease. The Hygiene Hypothesis, and a more recent refinement, the Old Friends Hypothesis postulates that the increase in the incidence of allergy and autoimmune diseases in Western Countries is associated with reduced or delayed exposure to microbes or parasites in our childhood [15]. The disappearing microbiota hypothesis in contrast focuses more on our ancestral microorganisms rather than cleanliness and postulates the lack of symbiotic microorganisms as being responsible [16]. Numerous studies have documented that coevolution with the microbiota has led to interdependence between the human host and the commensals, which are crucial for maintaining homeostasis. Disturbing the homeostasis, so-called dysbiosis, will have an important impact on immune responses that have been observed in different diseases. Recent studies also suggest that the gut microbiome contributes to the risk of developing T1D in genetically predisposed individuals [Tab. 1].

As various environmental factors that are known to influence the risk of developing T1D also modulate the composition of the gut microbiome [Fig.1], it is reasonable to consider microbiota to be a link between those factors and disease promotion, which will be discussed below.

2. Gut Microbiota and their Effect on T1D

Accumulating evidence from human studies emphasizes the crucial role of the composition of the gut microbiota in diabetes development. Patients with T1D exhibit a less diverse and less stable gut microbiome compared to healthy controls [17, 18] and changes of the ratio of Firmicutes to Bacteroidetes have been observed in the patients [17-20]. Prediabetic children harbor more *Bacteroidetes* compared to controls [19]. A decreased abundance of *Faecalibacterium prausnitzii* (butyrate-producing bacterium) in children who had more than two diabetes-related autoantibodies has also been observed [21]. However, these studies are limited at present and there is considerably more evidence in mouse models that had fuelled these studies in humans as discussed below.

The fact that the incidence of T1D in non-obese diabetic (NOD) mice is influenced by the microbial environment in different animal facilities worldwide [22] had suggested that microbiota play a crucial role in diabetes development.

Some early studies showed that NOD mice developed exacerbated diabetes under germ-free (GF) conditions, however, the recent reports did not confirm a disease aggravation in NOD mice housed in GF conditions [23, 24]. King, *et al.* demonstrated that an accidental contamination with *Bacillus cereus* in one cohort of otherwise GF NOD mice resulted in delayed diabetes onset and decreased incidence [23]. Hence, it seems more likely that the composition of the gut microbiome and the richness of certain bacteria are the key factors modulating diabetes development. Our recent study provided a strong evidence of another important player – innate immunity. Myeloid differentiation primary response 88 (MyD88) is a ‘master’ adaptive protein down stream of most innate immune molecules [25]. MyD88-deficient NOD mice were completely protected from diabetes development when housed in specific pathogen free (SPF) conditions; however, GF MyD88-deficient NOD mice developed full-blown diabetes. Colonization with defined gut bacteria restored diabetes protection in those mice although not 100% [26]. Our results reveal a novel influential pathway of innate immunity in T1D development through gut microbiota.

Individual strains of the gut bacteria may have different effects on diabetes. Using the Biobreeding (BB) rat model, Valladares and colleagues reported that *Lactobacillus johnsonii* isolated from diabetes-resistant BB (BB-DR) rats can attenuate diabetes development in diabetes-prone BB (BB-DP) rats whereas *Lactobacillus reuteri*, also from BB-DR rats, failed to affect diabetes development [27]. Contrary results were also obtained in terms of *segmented filamentous bacteria* (SFB) and their effect on diabetes development. Kriegel and coworkers reported an association between SFB colonization and diabetes protection in female SPF NOD mice [28], whereas using GF female NOD mice, Yurkovetskiy and coworkers found SFB did not confer protection in monocolonized gnotobiotic NOD females [29]. The authors showed that the protective effect of SFB could only be seen when other gut bacteria were present [29]. One explanation for this disparity is that SFB act in concert with other bacterial species to modulate the disease. Variations in SFB strains could also be responsible. Interestingly, male GF NOD mice were protected from diabetes development even monocolonized with SFB [29]. This suggests a complex association of sex hormones, gut microbiota and beta cell autoimmunity.

3. Gut Permeability and T1D Induction

The intestinal epithelial layer constitutes a barrier that separates the luminal antigens from the interior of the body. The adjacent epithelial cells exhibit lateral connections, namely tight junctions, which seal the paracellular space. Tight junctions consist of transmembrane barrier proteins such as occludin and claudins, which form fibrils that interact with the neighboring cell [30]. Intracellular scaffold proteins like ZO-1 – ZO-3 link the transmembrane barrier proteins to actin and microtubules, which play a decisive role in the architecture as well as the physiology of the tight junctions. The design of the intestinal epithelial barrier prevents food antigens, pathogenic as well as commensal bacteria from leaving the gut lumen and inducing a systemic immune response. Disruption of this crucial intestinal barrier is associated with intestinal autoimmune disorders including inflammatory bowel disease [31, 32], celiac disease [33], and irritable bowel syndrome [34]. Impaired integrity of the intestinal barrier, with increased permeability, has also been documented in both animal models and human T1D studies [Tab.2].

A study by Bosi and colleagues suggested that the increased gut permeability preceded the clinical onset of T1D [35]. Ultra-structural mucosal alterations and increased intestinal permeability in patients with T1D [36]. These studies support the notion that in genetically predisposed individuals impaired gut permeability may allow luminal antigens to escape the gut and promote islet-directed autoimmune responses. However, all the studies, thus far in humans, have not proven that the increased gut permeability is the cause and not a consequence of T1D onset. It is unlikely that increased gut permeability alone will be sufficient to induce T1D.

Increased intestinal permeability before the onset of diabetes was also seen in rodent models of T1D [37, 38]. In genetically modified NOD models, including MyD88-deficient mice, which lack the ability to signal through many of the innate immune Toll-like receptors (TLRs) and have altered diabetes development, for example, show increased gut permeability [39] but fail to accelerate diabetes development when maintained under SPF conditions [26]. This argument is reinforced by a study performed by Hadjiyanni and colleagues [40]. They showed that enhancement of the gut barrier function in NOD mice by applying a Glucagon-like peptide-2 did not result in delayed or decreased diabetes development [40].

Other rodent studies, however, suggest that increased gut permeability may be the cause rather than a consequence of T1D. Lee and colleagues demonstrated that gut barrier disruption induced by *C. rodentium* infection accelerated insulinitis in NOD mice [41]. Likewise, if the mice were infected with a strain unable to disrupt gut integrity, insulinitis was not affected. Visser and colleagues demonstrated that the gut permeability in BB-DP rats could be modulated by a hydrolysed casein diet [42]. Administration of this diet resulted in an improvement of the gut integrity and in prevention of T1D. Gut permeability can also be modulated through stimuli other than dietary products, which affect the function of the tight junctions in the epithelial layer [43]. Among these stimuli are inflammatory mediators, peptides produced by gut endocrine cells and microbial agents. Several of these agents induce the up-regulation and secretion of zonulin into the gut lumen by lamina propria cells [43, 44] (and our unpublished observation). Secreted zonulin is recognized by its receptor on intestinal epithelial cells. Signaling through the zonulin receptor causes remodeling of the cytoskeleton and down-regulation of ZO-1 and occludin [45]. This disassembly of the tight junctions results in enhanced gut permeability [43, 45]. The detrimental effect of zonulin on the gut barrier function was confirmed by Watts and coworkers. Using a zonulin antagonist in their study, the authors demonstrated that the improved gut integrity in BB-DP rats was accompanied by protection from developing T1D [46]. In accordance with this finding, a human study showed that zonulin upregulation in T1D patients and their high-risk-relatives was associated with increased gut permeability [47]. However, unlike the rodent study, clinical administration of a zonulin antagonist to patients with celiac disease failed to improve gut permeability [47]. To conclude from the aforementioned studies, gut permeability may be an important player in the development of T1D but, as yet, the findings in human studies have shown association but causation will be more difficult to prove.

4. Diet, Gut Microbiota and T1D

Early childhood (3 month) introduction to cereals [10, 11] and cow's milk [48] were shown to promote beta cell autoimmunity. It is interesting, in light of the gut involvement in T1D development, that different diets influence the incidence of diabetes in NOD mice [49, 50]. This dietary effect has also been shown in animal models where a gluten-free diet prevented diabetes in NOD mice [50]. In connecting these to alterations in the microbiota, a recent animal study revealed an association between low diabetes incidence in NOD mice raised on a gluten-free diet and alterations in the gut microbiota composition [51]. Similarly, in the BB-DP rat, a diet of hydrolyzed casein reduced the incidence of diabetes compared with a cereal-based diet [52]. Other dietary factors, including the acidity of water influences T1D risk in NOD mice through modulation of the microbiome [53].

The other dietary means by which gut bacteria could be altered includes the ingestion of probiotics – ‘live microorganisms that, when administered in adequate amounts, confer a health benefit on the host’ [54]. There is no data as yet available as to whether this might be a factor of influencing type 1 diabetes development. Many bacteria have been suggested to have probiotic effects through different mechanisms, including effects on barrier function of the gut. A study has documented a beneficial effect of *Lactobacillus plantarum* on barrier function [55].

A recent study by Patel and coworkers addressed the notion that probiotics could beneficially influence the gut permeability in animal models [39]. Their study documents the observation that the probiotic *Lactobacillus rhamnosus GG* promotes the maturation of the intestinal barrier by induction of claudin-3 expression. *Lactobacillus johnsonii* was also shown to induce claudin [27]. Prebiotics which are defined as ‘nonviable food components that confer a health benefit on the host associated with modulation of microbiota’ [56] are also known to improve the gut barrier function by improving tight junctions [57]. These findings suggest that prebiotics and probiotics could be potential therapeutic tools to improve gut integrity in various intestinal inflammatory and autoimmune diseases including T1D.

5. Mode of Birth, Early Life Feeding and T1D

The increased T1D incidence in babies who were delivered by Cesarean section has recently been found to be associated with altered composition of gut microbiota [6, 7]. The birth delivery mode determines which bacterial strains first colonize the newborn baby's gut. Infants who were born by Cesarean section had a gut microbiome that resembled the bacteria from the mother's skin [58] whereas infants who were born by vaginal delivery had gut flora predominantly resembling the bacteria in the mother's birth canal [58]. However, little is known about the effect of mode of birth on the immune system.

The newborn baby's intestine is first exposed to bacteria during the birth; their gut microbiome afterwards is mainly shaped by feeding behaviors, such as breast feeding or bottle feeding. Human breast milk is not sterile and many bacteria including *Lactobacillus gasseri*, *Lactobacillus rhamnosus*, *Lactobacillus plantarum*, *Lactobacillus fermentum* or *Enterococcus faecium* which are considered to be probiotic bacteria have been found in

human breast milk [59]. It is not surprising, therefore, that the breast-fed and bottle-fed infants differ in the composition of their gut microbiomes. The intestine of breast-fed infants is mainly colonized by *Bifidobacteria* and *Lactobacilli* [60] whereas bottle-fed infants show markedly decreased numbers of those bacteria but increased numbers of *Enterobacteriaceae* and *Bacteroides* [60, 61]. However, not all studies have found a clear distinction in gut bacteria between breast and bottle-fed babies [62] and it is controversial whether breast feeding is protective from T1D development. Some studies showed that the breast feeding reduced the risk and/or prolonged the time to T1D onset [8, 9], whereas other studies presented opposite findings [10, 11]. The beneficial effect of breast feeding suggested by some studies might be based on the promotion of gut maturation [63] and thus decreased gut permeability. In the mature gut, permeability is shaped by gut microbiota through degradation and fermentation of carbohydrates into short-chain fatty acids (SCFA) [17, 64]. Butyrate has been shown to enhance the intestinal barrier by regulating the assembly of tight junctions [65] and is also crucial for sufficient mucin synthesis [17]. The production of mucin, which is essential for a healthy gut, is counteracted by non-butyrate producers in autoimmune individuals. In line with this concept is a study showing a decreased abundance of *Faecalibacterium prausnitzii* (butyrate-producing bacterium) in children exhibiting more than two diabetes-related autoantibodies [21].

Some insight into immunological changes in the offspring has been shown in a recent study in NOD mice where fewer regulatory T cells were found in the mice born by Cesarean section, although in this study, no alteration in diabetes incidence was observed [66].

6. Antibiotics and T1D

Antibiotics have been used in modern societies to treat infections caused by pathogens. As a consequence, the intestinal microbiome is affected by antibiotic usage [67]. The use of the antibiotics can have long-term effects [7, 68]. However, to date, there is no direct evidence that antibiotic use in humans affects the development of T1D.

Animal studies demonstrate that antibiotic-induced modulation of the gut microbiome affects autoimmune diseases [12, 69]. A recent study showed that oral antibiotic treatment of pregnant mice could alter the gut microbiota composition in the offspring; but there was no significant change in diabetes development [70]. Other studies, however, demonstrate that the modulation of the gut microbiota composition by antibiotic treatment affects diabetes development in NOD mice and BB-DP rats [12, 13]. Long-term vancomycin treatment depleted many major bacteria genera, except for the species *Akkermansia muciniphila*, which became dominant and inhibited diabetes development in NOD mice [13]. However, it is noteworthy that diabetes development was critically affected by the type of antibiotic used as well as the timing of treatment. Our recent study suggests that targeting Gram-negative bacteria protects NOD mice from diabetes development whereas depleting Gram-positive bacteria accelerated diabetes in NOD mice (Hu *et al.*, unpublished). Our study also indicates that the most effective time of treatment is early in life (Hu *et al.*, unpublished) which is consistent with other studies of early-life microbiota disruption and other metabolic disorders, such as obesity [71].

7. Gut Immune Responses and T1D

As with many other aspects of interest in gut microbiota and the intestine that may play a role in development of T1D, the hypothesis that the gut microbiota may play a role in autoimmune diabetes development in humans is of considerable interest. However, studies are still at the early stages and mechanistic data is not easy to obtain in humans.

Much of the information has again come from animal models. In addition to affecting the gut integrity, gut microbiota also modulate the immune system. Colonization of the host's intestinal tract by microbiota is crucial for the development of the immune system as shown by the fact that GF mice exhibit underdeveloped intestinal and systemic lymphoid tissues [72]. Furthermore, they also have altered lymphocyte development and function [73-75], among which is the reduced Foxp3 expression in mucosal T cells and impaired regulatory function of Foxp3⁺ T cells (Treg) [24]. Monocolonization of GF mice with the commensal *Bacteroides fragilis*, however, promotes the differentiation of Treg and the production of the anti-inflammatory cytokine interleukin-10 [76]. The immunomodulatory effect of *Bacteroides fragilis* is mediated by the bacterial polysaccharide A (PSA) that induces the conversion of CD4⁺ T cells into CD4⁺ Foxp3⁺ Treg. Commensal bacteria also influence immune cells via their metabolites. The microbial metabolite butyrate, for example, can induce Treg cells [77, 78]. The immune-modulatory effect of gut microbiota was further evident in the study by Olszak and coworkers. They demonstrated that early life exposure to microbes had persistent effects on natural killer T cell function. Our own study showed that germ-transfer from diabetes-protected MyD88-deficient NOD mice to wild type mice resulted in an increase in CD8⁺ CD103⁺ and CD8αβ T cells in the lamina propria [68].

Both CD4⁺ (Th1) and CD8⁺ T cells contribute to the islet beta cell destruction in T1D. The role of Th17 T cells is also considered by some to be crucially involved in the pathogenesis of T1D [79, 80]. However, other investigators claim a diabetes-protective role of IL17-producing T cells [28, 81]. GF mice show impaired secretion of IL-17 in their lamina propria [24, 82], which is a thin layer in the mucosa beneath the intestinal epithelium. Lau and coworkers have recently reported that T1D protection in BB-DP rats after giving *Lactobacillus johnsonii* N6.2 was mediated by Th17 cells in mesenteric lymph nodes (MLN) [cf. section 'Gut Permeability and T1D Induction'] [81]. The fact that non-gut-draining lymph nodes lack a Th17 bias suggests that interactions between *Lactobacillus johnsonii* N6.2 and immune cells in the MLN are responsible for this bias that is most likely to contribute to the diabetes protection. The influence of distinct microbiota on the differentiation of Th17 cells in the small intestine was also shown by Ivanov and colleagues [82, 83]. They identified the commensal SFB as inducers of Th17 cells [83] and the lack of Th17-inducing commensals resulted in an increase of Treg in the lamina propria, which suggests that the microbiome regulates the balance of Th17 cells and Treg [82]. However, T cells featuring an IL-17A effector function are a heterogeneous population that has various effector functions. Therefore, further studies are needed to elucidate whether IL17A or another effector function exerts the observed effects. The heterogeneity of the IL17-producing T cell population might also be the reason for the controversial findings in terms of T1D promotion or protection.

As already mentioned above (cf. section ‘Gut Microbiota and their Effect on T1D’) our recent study in MyD88-deficient NOD mice suggests that in addition to the adaptive immunity including Th1 and Th17 responses, innate immunity plays an important role in gut immune responses in health and disease.

Pattern recognition receptors (PRR) like TLRs and nucleotide oligomerization domain-like receptor (NLRs) are highly expressed on intestinal epithelial cells and recognize conserved microbial components [84]. PRR play a crucial role in the initiation of immune responses. NOD mice that are deficient in a specific TLR exhibit an altered composition of gut microbiome and an altered diabetes development depending on which specific TLR deficiency (Gülden *et al*, unpublished data). Further studies are needed to elucidate the role of innate immunity in shaping the gut microbiome and altering diabetes development.

8. Potential Therapeutic Strategies

As described in section 4 (Diet, Gut Microbiota and T1D) pre- and probiotics can be considered as potential therapeutic tools to improve gut integrity in T1D. The beneficial effect of probiotics on the host's physiology is, however, not limited to the improvement of gut integrity.

As all microbes probiotics can shape host responses by direct interaction with cell wall bound molecules or via secreted products. Heat-killed probiotics cannot exert influence on the host through secreted products but are still able to interact with cell wall bound molecules. As early as 1997 a study by Matsuzaki and coworkers demonstrated that the application of heat-killed *Lactobacillus casei* prevented the onset of T1D in NOD mice [85]. The underlying mechanism, however, remained unclear. This study prompted Calcinaro and colleagues to administer a mixture of different strains of viable lyophilized probiotic bacteria to NOD mice which resulted in the induction of IL-10 and prevention from T1D [86]. Thus, probiotics are considered as potential tools for novel T1D therapies. These findings, however, have not been seen in all studies. Our own unpublished work (Peng et al, unpublished) had shown that probiotic treatment only has protective effects when started soon after weaning and continued for several months. The effects were no longer seen if the treatment was interrupted.

The challenge of developing new therapeutic intervention strategies for the treatment of T1D is to find an approach that is efficient without causing serious side effects. Current approaches aim at curing T1D by antigen-specific therapies and systemic immunomodulation and immunosuppression [87-89]. However, many strategies induce intense side effects due to suppression of immune responses. Antigen-specific therapies are attractive since they target only beta cell-reactive T cells without affecting general immune responses. Yet the common oral administration method results in reduced efficacy due to degradation during the passage of the gastrointestinal tract.

Novel therapeutic strategies have been designed to overcome this issue by using engineered *Lactococcus lactis* bacteria as a safe and effective method to transport autoantigens to the immune system to induce tolerance [90-92]. Robert and coworkers demonstrated that a combination of genetically modified *Lactococcus lactis* that express GAD65 and IL-10 and

short-term anti-CD3 treatment reversed diabetes in NOD mice [91]. This and other studies [90, 92] using engineered *Lactococcus lactis* suggest that genetically modified probiotics are safe and effective vectors for the treatment of T1D.

9. Conclusion

The research on gut microbiota and their interactions with the hosts has grown exponentially during the last 10 years or so, which has filled a huge gap in our knowledge. We have learned that many health problems including T1D can be influenced by the gut microbiome. GF mice provided a unique model system to conduct proof-of-concept studies. Although the overall composition rather than specific bacteria is likely to be more important for either pathogenic or protective effects on host health or disease, the knowledge of identified beneficial bacteria can be used to shape the gut microbiome in a positive way. It is clear that future research is required to test whether targeting the gut microbiome could be a basis for the establishment of potent new preventive or therapeutic T1D intervention strategies.

Acknowledgments

This work was supported by Deutsche Forschungsgemeinschaft fellowship GU 1122/3-1 (to EG), RC1 DK092882 and DK088181 (to LW), P30-DK045735 (to LW), EFSD/Novo Nordisk Programme (to FSW), MRC grant MR/K021141/1 (to FSW).

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Highlights

- Gut microbiota have been shown to play an important role in promoting or protecting from T1D development
- Gut microbiota modulate the host immune system
- Change of gut permeability may precede the clinical onset of T1D
- The route of birth delivery and early life environment including feeding method shape the composition of the gut microbiome

Fig. 1. Genetic and non-genetic factors determine the risk of developing diabetes in genetically predisposed individuals

T1D is a T cell-mediated autoimmune disease which occurs in genetically predisposed individuals when triggered by so far mostly unknown factors. In addition to genetics, environmental factors like diet, antibiotic usage, ‘hygiene’ and delivery mode may determine disease risk. Most of these environmental factors influence the gut microbiome composition, which is in close interaction with the immune system, and plays a role in shaping it. The gut microbiome composition also affects gut permeability. Increased gut permeability, thus a facilitated passage of luminal, potentially diabetogenic antigens, is suggested to play a role in initiating islet-directed autoimmunity.

Table 1
Gut microbiota related studies

Bacteria	Animal model/study group	Outcome	Ref.
<i>B. adolescentis</i> DSM 20083	children at risk to develop T1D	probably involved in predisposing children to β cell autoimmunity	[93]
Bacteroidetes	seroconverted subjects	positively associated with islet autoimmunity	[19]
<i>Bacteroides</i> , <i>Eubacterium</i> , <i>Ruminococcus</i>	BB-DP rats	more abundant	[98]
<i>Clostridium hylemonae</i>	BB-DR rats	increased abundance	[98]
Firmicutes, Bacteroidetes	NOD mice	decrease in Firmicutes and an increase in Bacteroidetes	[53]
Bifidobacteria, <i>Clostridium</i>	LEW1. WR1 rat	virus-induced T1D correlates with increased Bifidobacteria and Clostridia	[97]
<i>Bacteroides ovatus</i> , <i>Bacteroides fragilis</i>	T1D children	increase in <i>Bacteroides ovatus</i> and decrease in <i>Bacteroides fragilis</i>	[20]
Firmicutes, Bacteroidetes	T1D children	decreased abundances of Firmicutes; increase abundance of Bacteroidetes	[17, 18]
<i>Prevotella</i> and <i>Akkermansia</i>	T1D children	decreased abundance of <i>Prevotella</i> and <i>Akkermansia</i> in T1D children	[17]
<i>Prevotella</i>	T1D children	<i>Prevotella</i> genus was reduced in patients	[18, 95, 96]
<i>Bifidobacterium</i> , <i>Lactobacillus</i>	T1D children	decreased numbers of <i>Bifidobacterium</i> and <i>Lactobacillus</i>	[95]
<i>Clostridium</i> , <i>Bacteroides</i> , <i>Veillonella</i>	T1D children	increased amount of <i>Clostridium</i> , <i>Bacteroides</i> and <i>Veillonella</i>	[95]
Lactate and butyrate producers	seropositive subjects	lower levels of bacteria involved in the production of lactate and butyrate	[21]
<i>Bacteroides</i> , <i>Prevotella</i>	Mexican children with T1D	high levels of the genus <i>Bacteroides</i> ; control group exhibit high levels of <i>Prevotella</i>	[96]
Bifidobacteria	T1D children	reduced Bifidobacteria, increased Enterobacteriaceae in T1D	[94]
Firmicutes	seroconverted subjects	negatively associated with islet autoimmunity	[19]
<i>Faecalibacterium prausnitzii</i>	Children with diabetes-related autoantibodies	reduced abundance in children with two or more diabetes-related autoantibodies	[21]
Bifidobacteria	Children with diabetes-related autoantibodies	negatively associated with β cell autoimmunity	[21]
Bifidobacteria	T1D children	negatively associated with β cell autoimmunity	[94, 95]
Lactic acid bacteria	NOD mice; BB-DP rats	delay or prevent diabetes	[85, 86]
<i>Lactobacillus</i> , <i>Bifidobacterium</i>	BB-DR rats	negatively correlated with T1D onset	[98]
<i>Lactobacillus johnsonii</i> N6.2	BB-DP rats	delayed diabetes onset	[27]
SFB	NOD mice	diabetes protection	[28]
<i>Lactobacillus</i> species	BB rats	negatively correlated with T1D development	[98]
<i>A. muciniphila</i>	NOD mice	protective role in T1D development	[13]

Table 2
Human and animal studies that focus on assessing gut integrity in T1D

Animal model/study group	Finding	Intervention/treatment	Impact on diabetes development	Impact on gut integrity	Ref
BB-DP rats	diet modulates gut permeability	hydrolysed casein diet	prevention	improvement	[42]
BB-DP rats	increased gut permeability				[38]
BB-DP rats	increased permeability before T1D onset				[37]
NOD mice	Glucagon-like peptide-2 reduces intestinal permeability but does not modify T1D onset	Glucagon-like peptide-2	no impact	improvement	[40]
BB-DP rats	Enteropathy precedes T1D in BB rats				[104]
BB-DP rats	increased gut permeability in BB-DP rats	zonulin inhibitor (FZI/0)	protection	improvement	[46]
NOD mice	gut barrier disruption accelerates insulinitis	enteric bacterial pathogen	acceleration	disruption	[41]
T1D subjects and their relatives	Zonulin upregulation is associated with increased gut permeability				[99]
T1D subjects	Increased gut permeability precedes clinical onset of T1D				[35]
T1D subjects	Abnormal intestinal permeability to sugars in T1D.				[100]
T1D subjects	increased gut permeability is the cause of fluctuating postprandial blood glucose levels				[101]
T1D subjects	intestinal permeability to mannitol and lactulose in T1D subjects with HLA_DQB1*02 allele				[102]
T1D subjects	ultrastructural mucosal alterations and increased intestinal permeability				[36]
T1D subjects	altered intestinal permeability to mannitol				[103]