

# Microbiology of Type 1 diabetes: possible implications for management of the disease



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## Summary Points

- The environment plays a role in Type 1 diabetes in addition to a genetic predisposition.
- Antibiotics and probiotics prevent diabetes in murine models.
- Recent microbiome analysis suggests that bacteria in the gut may one day be used as early predictors of autoimmunity for Type 1 diabetes or as therapies to prevent Type 1 diabetes.
- No connection has been observed to date between antibiotic use in children and the incidence of Type 1 diabetes.
- The human microbiome changes in a variety of circumstances including during the development of other autoimmune diseases such as Crohn's and celiac disease.
- Continued analysis of the gut microbiome and its functions may one day lead to therapies for the prevention of autoimmunity.

**SUMMARY** The environmental factors that contribute to the onset of Type 1 diabetes are unknown but are of increasing interest. This article focuses on the possible role of the gut microbiome in the development of Type 1 diabetes. Administration of either antibiotics or probiotics prevents diabetes in murine models of the disease, which suggests a role for gut microbiota in insulin-dependent diabetes. The subsequent analysis of human gut samples led to the proposal that the gut microbiome may provide early diagnosis for autoimmunity for Type 1 diabetes and allow the identification of bacteria that may one day be useful in the prevention of autoimmunity. Future work should include microbiome analysis in more samples, functional analysis of the bacteria present and a search for small molecules of bacterial origin that may be responsible for a leaky mucosal layer.

### The environment & Type 1 diabetes

Characterized as a chronic autoimmune disease, Type 1 diabetes (T1D) is most often diagnosed in young children, but it can occur at any age. In terms of autoimmunity, the disorder is

caused by T-cell-mediated destruction of insulin-producing pancreatic  $\beta$  cells in the islets of the pancreas [1]. As yet unidentified environmental factors are thought to contribute to disease risk in a genetically susceptible individual [1–3]. T1D

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presents the highest incidence across countries in Northern Europe, especially in Scandinavia [4,5]. Scandinavia has the highest incidence of T1D in the world (ranging between 30 and 60 cases for each 100,000 individuals), with Finland having the highest incidence. Scandinavian countries share genetic, cultural and environmental characteristics, but no factors have yet been shown to be the cause of the high incidence of T1D [5,6].

In terms of genes influencing susceptibility to this disease, half can be associated with the human leukocyte antigen region [7]. Environmental factors likely contribute to this high-risk group, but seem to play a larger role in the increasing number of T1D cases in patients without the high-risk human leukocyte antigen genotypes [1]. Although genetic susceptibility plays a significant role [7], the incidence of T1D in children under 15 years of age has increased at a rate that is too rapid to be simply caused by changes in the population's gene pool [3,4]. During the 1990s, the incidence of T1D increased an average of 2.8% annually worldwide [8] and 4.7% every year in Finland [9].

Toxins, dietary factors, absence of breast-feeding, Cesarean section, ante- and peri-natal risk factors, stressful life events and even environmentally sensitive epigenetics have been suggested as factors that may trigger autoimmunity for T1D [5,6,10–13]. Biological factors such as bacteria and viruses have also been implicated as playing a role in T1D [10,14–17]. As others have reviewed the role of viruses in T1D [17–19], this article will focus on the role of bacteria.

Linking each of these facets together has been a long-term challenge for researchers. However, one model has posed a trio of factors to induce what was named 'the perfect storm' of events leading to autoimmunity in T1D [20]. Among these factors are an aberrant intestinal microbiota, a 'leaky' intestinal mucosal barrier and an altered intestinal immune responsiveness. The interplay of these factors seems to have a crucial role in the onset of several allergic and autoimmune diseases, including Crohn's disease, celiac disease, multiple sclerosis and, potentially, T1D [21–24]. The remainder of this article will focus on the aberrant intestinal microbiota identified recently in diabetic rodent models and in humans with or at increased risk for the disease.

#### Possible role of bacteria in murine models

Our understanding of the role of gut microbiome in the pathogenesis of common autoimmune disorders is limited, and in T1D is relatively

unexplored. Most of the evidence for an altered microbiome in diabetes comes from two rodent models, the nonobese diabetic (NOD) mouse model and bio-breeding diabetes-prone (BB-DP) rat model. When T lymphocytes are transferred from an adult diabetic mouse to a young NOD mouse weeks prior to the spontaneous development of diabetes, diabetes is induced 10 weeks after birth [25].

Recent evidence supports the role of microbes in the development of autoimmunity and subsequent T1D in rodent models. First, feeding antibiotics to BB-DP rats can prevent T1D [26], and this has also been demonstrated in NOD mice [27]. Second, the spontaneous development of diabetes in NOD mice increases in a germ-free environment [28–30]. Third, Freund's adjuvant, which contains desiccated *Mycobacterium*, protects NOD mice and BB-DP rats against diabetes [31–33]. And fourth, feeding probiotic bacterial strains, usually lactic acid bacteria, to NOD mice or BB-DP rats can delay or prevent diabetes [34–38]. In addition, pathogen-free NOD mice lacking an adaptor protein for multiple Toll-like receptors known to bind to bacterial ligands fail to develop diabetes, indicating that the interaction of the intestinal microbiota with the immune system is a critical factor to developing T1D [39]. The leaky gut described earlier was observed in prediabetic NOD mice when infected with the enteric bacterial pathogen, *Citrobacter rodentium* [40]. A protein called zonulin appears to be involved in the integrity of tight junctions in BB-DP rats [41]. However, the mechanism that disrupts tight junctions in the intestinal barrier remains unclear [40].

Roesch and coworkers conducted a culture-independent analysis of gut bacteria in BB-DP and BB diabetes-resistant (BB-DR) rats and showed that, at the time of diabetes onset, the bacterial communities in these two rat strains differed significantly [42]. Stool from BB-DR rats contained much higher populations of probiotic-like bacteria, such as *Lactobacillus* and *Bifidobacterium*, whereas BB-DP rats had higher numbers of *Bacteroides*, *Eubacterium* and *Ruminococcus*. Valladares and coworkers [38] isolated a strain of *Lactobacillus johnsonii* from the stool of the same set of BB-DR rats used by Roesch and coworkers [39] and showed that this strain prevents diabetes when fed to BB-DP rats [38].

The reduction of diabetes in animal models fed antibiotics or certain microbes suggests that bacteria are involved in inducing or reducing

disease. The reduction of diabetes through the feeding of probiotic strains or injection with Freund's adjuvant suggests that harmful bacteria may be involved in counteracting the action of those beneficial bacteria that reduce disease. The beneficial bacteria may trigger an immune response that reduces inflammation.

Diet has also been reported to prevent or reduce the incidence of diabetes in NOD mice by modifications in the composition of the intestinal microflora. A hydrolyzed casein diet prevented diabetes in BB-DP rats [43]. Several studies have shown reduced diabetes in NOD mice given a gluten-free diet. Funda and coworkers observed that 15% of NOD mice given a gluten-free diet became diabetic, while 64% of NOD mice on the standard diet became diabetic [44]. In addition, the mice that became diabetic on the gluten-free diet did so much later than those mice on the standard diet. In other studies, the incidence of diabetes in NOD mice decreased from 47% in standard-fed mice to 5% in the gluten-free-fed mice [45]. Based on culture-dependent techniques, the number of bacteria in the cecum was lower in gluten-free mice compared with the mice fed the standard diet. Fewer aerobic and microaerophilic bacteria were found in the gluten-free mice and in the nondiabetic mice. Gram-positive microorganisms such as *Lactobacillus* spp., *Lactococcus* spp. and *Enterococcus* spp. also appeared in smaller numbers in gluten-free mice. Dietary gluten was also linked to the development of autoimmune diabetes in an experiment in NOD mice [46]. The authors suggested that the nonexposure of NOD mice to dietary wheat and barley proteins is sufficient to delay the onset of T1D in mice.

Although rodent models support a role of gut microbes in T1D and indicate that diabetic rats have aberrant intestinal microbiota, the intestinal microbiota have not been characterized in humans at high-risk for T1D.

### Is there a role for bacteria in human T1D?

All these results in murine models are consistent with the notion that beneficial bacteria seem to provide a protective effect by delaying or preventing the onset of diabetes, as BB-DP rats have lower populations of species that contain suspected probiotic strains than BB-DR rats. Beneficial bacteria may be necessary for the maintenance of a healthy microbiome essential in preventing a leaky gut. However, it is unknown whether human intestinal microbes have a role in the development of autoimmunity that often leads to T1D.

Several groups around the world are engaged in the identification of infectious agents, dietary factors or other environmental exposures that are associated with T1D. The TEDDY consortium comprises six clinical centers located in the USA and Europe, and will screen 361,588 newborns for T1D [6].

High-throughput, culture-independent approaches identified bacteria that correlate with the development of T1D-associated autoimmunity in young children who are at high genetic risk for this disorder [47]. The level of bacterial diversity diminishes over time in these autoimmune subjects relative to that of age-matched, genotype-matched, non-autoimmune individuals. A single species, *Bacteroides ovatus*, comprised nearly 24% of the total increase in the phylum Bacteroidetes in cases compared with controls. Conversely, another species in the controls, represented by the human firmicute strain CO19, comprised nearly 20% of the increase in Firmicutes compared with cases over time.

In addition, bacteria that negatively correlated with the autoimmune state may prove to be useful in the prevention of autoimmunity development in high-risk children. These negatively correlated bacteria should be cultured from human subjects and tested for their ability to prevent diabetes in animal models. If efficacious in these models, the safety of these organisms when fed to human subjects should be tested.

These data also suggest bacterial markers for the early diagnosis of T1D [47]. The ratio of Bacteroidetes:Firmicutes is much higher in healthy children than in children who later become autoimmune 4 months after birth. However, at 2 years of age, this pattern reverses. The Bacteroidetes:Firmicutes ratio becomes much higher in autoimmune children compared with controls. As a high Bacteroidetes:Firmicutes ratio has been shown to be indicative of a disease state [21,23,24,48], this suggests that the autoimmune microbiome becomes increasingly unhealthy over time while the microbiome of healthy children becomes typical of healthy adults.

Three lines of evidence are presented that support the notion that, as healthy infants approach the toddler stage, their microbiomes become healthier and more stable, whereas children who are likely to develop autoimmunity develop a microbiome that is unstable and less diverse. Hence, the autoimmune microbiome for T1D may be distinctly different from that found in healthy children. For example,

the Shannon diversity index of the microbiome from healthy children increases over time while the diversity of the microbiome of autoimmune children is significantly lower [47]. Ecological theory tells us that a more diverse habitat is a healthier, more stable habitat [49,50]. There is also a significantly higher number of 16S rRNA sequences from healthy children that cannot be classified compared with autoimmune children. If an organism is a human pathogen, it is likely well characterized. If it is a benign organism, it is much more likely to be unclassified. Thus, a microbiome with a higher number of unclassified organisms is likely to be healthier than one where a larger proportion of organisms are known.

A third defining characteristic of the autoimmune microbiome may be instability of these communities [47]. That is, the phylogenetic distance between any two autoimmune microbiomes is far greater than the distance between the microbiomes of any two healthy children. Thus, although the microbiomes of healthy children are more diverse than those of autoimmune children, the healthy microbiomes share a more similar group of organisms than the autoimmune microbiomes.

### Human antibiotic use & T1D

Although antibiotics are correlated with T1D in murine models, several studies with humans have found no association between antibiotic use and T1D in children. A nationwide cohort study of all Danish children born between 1995 and 2003 was conducted. Among the 606,420 Danish children born during that period, 454 cases of T1D were identified. No association with antibiotic use and diabetes was found [51]. In a mother–child cohort with 437 children born in Finland between 1996 and 2000, no correlation was found between the use of antimicrobials by the mother before or during pregnancy and subsequent risk of becoming diabetic [52].

At first glance, this appears to contradict the observations that antibiotics prevent diabetes in animal models. However, the antibiotics used can differ greatly between human studies and animal experiments. Hence, their range of activity can vary. In addition, the bacteria that are correlated with disease can differ between humans and animals. Until we know the bacteria involved in disease in humans, it will be difficult to assess the role antibiotics could play in future prevention or treatment of T1D.

### Dysbiosis: the altered microbiome

The balance achieved in the gut mediated by healthy microbiota has to be seen as a group effort from the microbial community, not a single act of a sole species. The term dysbiosis was coined by Metchnikoff to describe an imbalance between healthy and pathogenic microbial species, which can lead to intestinal diseases [53]. A link between allergies and the gastrointestinal microbiome was first reported by Kuvaeva and coworkers [54]. At the time, the term ‘dysbacteriosis’ was coined to describe the food allergy caused by low Lactobacilli and Bifidobacteria compared with a high abundance of bacteria from the Enterobacteriaceae family. Cases of dysbiosis that may be caused by hygiene, gluten-rich and high-calorie diet, vaccinations and antibiotic use can result in inflammatory disorders [55,56]. Dysbiosis observed at the phylum level between Bacteroidetes and Firmicutes in the human gut has been described in several human disorders, especially in inflammatory bowel diseases [57]. The ratio between the phyla Firmicutes and Bacteroidetes declined when compared with controls in human Type 2 diabetes [48]. Wu and coworkers found that *Bifidobacterium* and *Bacteroides vulgatus* were in lower abundance in patients with Type 2 diabetes [58]. The authors suggested that obesity and diabetes might be associated with dysbiosis in the gut caused by a shift in the proportion of bacterial groups rather than being caused by a single strain.

Sechi and coworkers suggested that a single bacterium, *Mycobacterium avium* subsp. *paratuberculosis*, could be a trigger for the development of T1D in Sardinian patients [59]. This organism is known to cause chronic intestinal infections in ruminants and Crohn’s disease in humans. The microbial diversity in Crohn’s disease patients seems to be lower compared with healthy individuals [60,61]. Crohn’s disease patients also have an abnormal and reduced diversity of commensal bacteria, such as members of the phyla Firmicutes and Bacteroides, whereas Proteobacteria increases [21,23,60]. Conversely, Gophna and coworkers suggested that, although an imbalance in flora in Crohn’s disease exists, it is not sufficient to cause inflammation [62]. The reverse was observed in obesity where an imbalance is observed that is caused by a reduction in the proportion of Bacteroides in obese human subjects, with a corresponding increase in the Firmicutes:Bacteroidetes ratio. Among the Firmicutes, *Lactobacillus* strains can increase in obese patients [63–65].

Celiac disease and T1D co-occur in a high number of patients [44,66]. These two diseases share the same genetic background, with DR3 and DQ2 alleles thought to play a role [44]. Patients with both diseases develop T1D before celiac disease and not *vice versa*. Another important parameter to be considered when studying those two diseases is the geographic localization of T1D risk groups. Asian countries such as Japan and China have a low incidence of T1D (lower than five cases per 10,000 individuals) [4] and they are known for a low consumption of wheat flour in their diet [44]. A delayed exposure to gluten and cereal in children was described as playing a role in the prevention of T1D in relatives of patients with T1D [67–69].

### Future perspective

The microbial community changes that occur in the autoimmune microbiome over time, particularly when compared with healthy microbiomes, observed by Giongo and coworkers suggest that an early diagnosis for the development of autoimmunity can be achieved [47]. Furthermore, bacteria that are negatively correlated with the development of autoimmunity in humans might be useful as a therapy to prevent autoimmunity [47]. However, these ideas were based on the analysis of just 24 stool samples from eight Finnish children. The analysis of more samples is required from more locations in order to obtain a more generalized view of diagnostics and therapies. In addition, correlating the microbiome data with environmental factors such as diet, antibiotic use and fevers is an important goal over the next few years.

Functional analysis of the bacteria present in the autoimmune and healthy microbiomes is also required. Are there bacterial functions that can contribute to a leaky mucosal layer? Are there bacterial functions that limit the activity of those bacteria that can cause a leaky gut? To address these questions, metagenomics analysis

of samples is required. In addition, metabolomic analysis of stool samples may identify small molecules that are correlated with the presence or absence of autoimmunity, as has been shown for probiotic functions [70].

Continued work may one day lead to an early diagnosis for autoimmunity to T1D a few months after birth. Bacterial biomarkers may be identified that predict future autoimmunity for T1D. In addition, specific probiotic strains that are negatively correlated with autoimmunity may be useful in the future in the prevention of autoimmunity. Giongo and coworkers [47] suggested both of these aspects, but could probiotics be useful as a cure for autoimmunity? This could be investigated by treating diabetic animals with probiotic strains. However, once the integrity of the gut is disrupted, it is not known whether probiotic strains can play a role in its repair. Furthermore, the data to date suggest that the human and animal studies do not necessarily agree. For example, the bacteria that are negatively correlated with autoimmunity in humans may not be the same as those correlated with diabetes in rats [42,47].

Another broader question is the extent to which these results can apply to other autoimmune diseases such as Crohn's disease, celiac disease and multiple sclerosis [21,23,24]. Human trials that address the interactions of the microbiome and these diseases in a coordinated way are sorely needed.

### Financial & competing interests disclosure

*The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.*

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