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Modulation of Type 1 Diabetes Risk by the Intestinal Microbiome

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Abstract
Purpose of Review The purpose of this review is to summarize potential modulations of the intestinal microbiome aimed at preventing or delaying progression to overt type 1 diabetes in the light of recently identified perturbations of the gut microbiota associated with the development of type 1 diabetes.
Recent Findings Accumulated data suggest that the gut microbiota is involved at two different steps in the evolution of type 1 diabetes. At the first step, the intestinal tract is colonized by a microbial community unable to provide an adequate education of the immune system. As a consequence, the infant acquires susceptibility to immune-mediated diseases, type 1 diabetes included. At the other step, the young child seroconverts to positivity for diabetes-associated autoantibodies. This is preceded or accompanied by a decrease in the diversity of the intestinal microbiota and an increased abundance of *Bacteroides* species. These changes will affect the disease process promoting progression toward overt type 1 diabetes.
Summary By providing specific probiotics, one can affect the colonization of the intestinal tract in the newborn infant or strengthen the immune education in early life. Human milk oligosaccharides function as nutrients for "healthy" bacteria. Dietary interventions applying modified starches can influence the numbers and activities of both autoreactive and regulatory T cells and provide protection against autoimmune diabetes in non-obese diabetic mice. Modulation of the intestinal microbiome holds the promise of effective protection against human type 1 diabetes.

Keywords Type 1 diabetes · Microbiome · Microbiota · Prebiotics · Probiotics · Diet

Introduction

Type 1 diabetes (T1D) is one of the most common chronic immune-mediated diseases in children and adolescents, characterized by gradual destruction of the pancreatic insulin-producing β cells, eventually leading to total insulin deficiency in genetically susceptible individuals. Overt T1D is preceded by an asymptomatic preclinical period, during which autoantibodies against β cell structures appear into the peripheral circulation [1]. Seroconversion to autoantibody positivity may occur very early in life with a clear peak during the second year of life [2, 3]. The duration of the prediabetic period is highly individual ranging from a few months to more than 20 years [1]. If T1D is diagnosed in childhood, the average duration of the prediabetic period is 2.5–3 years. The genetic disease susceptibility allows the development of T1D, i.e., it is permissive, but non-genetic factors including both host-related and environmental determinants are considered to play

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a decisive role in the disease process leading to the manifestation of clinical T1D [4].

The incidence of T1D has increased in developed countries over the last 50–60 years [5]. For example, in Finland, the annual incidence was 12/100,000 children < 15 years of age in the early 1950s increasing to a rate of 65 in 2006 [6]. Such a steep increase in incidence cannot be due to genetic factors alone, as changes in the genetic composition in a population are likely to have an effect on the disease incidence over a long period of time; accordingly, fast changes in the environment and lifestyle must be the major contributors to the increasing T1D incidence [5, 7]. T1D is not the only disease for which a conspicuous increase in incidence has been observed over the past five to six decades. Other immune-mediated diseases, such as allergic disorders, celiac disease, and inflammatory bowel diseases, show a similar trend [7, 8], suggesting that exogenous factors are inducing an unbalanced development of the immune system resulting in increased predisposition to autoimmunity and allergy [9]. Altered living conditions, the increased use of processed water and food, the introduction of refrigerators, and extensive use of antibiotics have induced substantial changes in the human commensal microbiota over the past seven decades [10]. Such changes have been particularly dramatic for the intestinal microbiota [11]. The gut microbiota and the immune system interact closely [12], emphasizing the role of the intestinal microbiota in the maturation and education of immune functions.

The Intestinal Microbiome and Type 1 Diabetes

The adult human gut microbiota comprises 10^{13}–10^{14} microorganisms and the gut microbiome, defined as the aggregate genome of all intestinal microorganisms, is about 150 times larger than the human genome [13]. Bacteroidetes (gram-negative bacteria) and Firmicutes (gram-positive bacteria) represent the two major phyla in adults [14]. The outer cell membrane of gram-negative bacteria includes complex lipopolysaccharides (LPS) whose lipid portion acts as an endotoxin that induces a strong host immune response to protect against infections. Excessive inflammatory responses are in normal conditions inhibited by regulatory mechanisms to maintain tissue equilibrium [15–18]. The microbial colonization of the gut during the first few years of life is apparently critical for the development of a functional host immune regulation, and perturbations in either the microbiota composition or the host response may lead to chronic inflammation [16]. The gut microbial ecosystem may become imbalanced due to overgrowth of some microbes and loss of others. This condition is defined as intestinal dysbiosis.

Most studies based on sequencing data of the intestinal microbiota in subjects with preclinical T1D have been conducted so far in Finnish children. The first report published in 2011 [19] included eight children (four cases and four matched controls) from the DIPP (Diabetes Prediction and Prevention) birth cohort study [20]. The cases developed multiple (≥ 2) autoantibodies and presented with clinical T1D before the age of 5.5 years. The autoantibody-negative control children were matched with the cases for sex, HLA genotype, delivery hospital, and date of birth. Each case and control contributed to the analyses with three stool samples. The first sample was obtained before seroconversion to autoantibody positivity, the second close to the time of seroconversion to autoantibody positivity, and the third sample close to the clinical diagnosis of T1D in the cases. Samples were collected from the control participants at the corresponding ages as the case samples. In the cases, the 16S pyrosequencing data showed an increasing Bacteroidetes to Firmicutes ratio and a decreasing Shannon diversity index over time. These changes became more marked after seroconversion to autoantibody positivity. The same samples were later analyzed with shotgun metagenomics. Higher proportions of butyrate-producing and mucin-degrading bacteria were observed in the controls than in the cases, while the proportion of bacteria producing short-chain fatty acids other than butyrate was increased in the cases [21].

The gut microbiota in 18 children who tested positive for multiple autoantibodies was compared with the intestinal microbiota in 18 autoantibody-negative controls in a Finnish cross-sectional study. Half of the cases came from the TRIGR pilot study [22] and were 11-year-olds or older at the time of sampling, while the remaining cases were derived from the FINDIA study [23] and were 7-year-olds or younger when the stool samples were collected. A decreased abundance of bacteria-producing butyrate or lactate was associated with signs of β cell autoimmunity [24]. In addition, the cases were characterized by a dearth of the two most abundant *Bifidobacterium* species (*B. adolescentis* in the older children and *B. pseudocatenalatum* in the younger children) and a higher abundance of *Bacteroides* when compared with the controls. In contrast, an analysis of 22 autoantibody-positive BABYDIET children [25] and 22 autoantibody-negative control children matched for date of birth did not observe any differences in bacterial diversity, microbial composition, or abundance of single genera between cases and controls [26]. In that study, the collection of stool started at the age of 3 months and continued up to the age of 36 months at 3-month intervals. In subsequent analyses, substantial changes were seen in correlation-based bacterial interaction networks at the ages of 6 and 24 months. Whether these alterations play any role in the appearance of β cell autoimmunity has not been reported.

Seventy-six Finnish children with HLA-defined predisposition to T1D were included in a longitudinal study based on the DIPP Turku cohort and were observed from birth up to the

age of 2.2 years. Monthly stool samples were collected from the age of 4–6 months up to the age of 26 months. Twenty-nine case children developed multiple autoantibodies, out of whom seven progressed to clinical diabetes whereas 22 remained diabetes-free during the follow-up period. The remaining 47 who tested negative for autoantibodies served as controls. The cases were characterized by one highly abundant microbial group comprising two closely related species, *Bacteroides dorei* and *Bacteroides vulgatus*, which was more common in cases prior to seroconversion than in the control children [27]. The major constituent turned out to be *B. dorei* based on metagenomic sequencing. The abundance of this species reached a peak at the age of 7.6 months in the cases, which coincided with the introduction of solid food. On average, there was an interval of 8 months between that peak and the seroconversion to autoantibody positivity. The DNA methylation of *B. dorei* in stool samples (from one case and one control child) dominated by that species was analyzed later by the same researchers. The *B. dorei* genome from the control child showed no DNA methylation and lacked any DNA adenine methyltransferase genes, whereas the *B. dorei* genome from the case child contained more than 20,000 methylated sites [28]. This observation suggests that it might be important to look at DNA methylation patterns in the gut microbiome when analyzing functional diversity, as the degree of methylation may affect gene expression.

The DIABIMMUNE study was designed to test the hygiene hypothesis in T1D and other immune-mediated diseases by recruiting and monitoring newborn infants with HLA-conferred susceptibility to autoimmunity and young unselected children living in three adjacent countries (Estonia, Finland, and Russia [the region of Russian Karelia]) that have contrasting standards of living and hygiene [29]. The intestinal microbiome was analyzed in relation to the development of β cell autoimmunity and clinical T1D in Finnish and Estonian children participating in the birth cohort arm. The study population comprised 11 children who developed multiple (≥ 2) autoantibodies and 22 autoantibody-negative children. Four of the autoantibody-positive children were diagnosed with clinical T1D during the follow-up. The parents were asked to collect monthly stool samples starting from the age of 1 month up to the age of 36 months. In the time window between seroconversion to autoantibody positivity and the T1D diagnosis, we found a clear drop in α-diversity in children who were diagnosed with clinical disease [30••]. Compared with the controls, the children who presented with clinical T1D showed an increase in the relative abundance of *Blautia*, the *Rikennellaceae* and the *Ruminococcus* and *Streptococcus* genera, while a decrease was seen in the relative abundance of Lachnospiraceae and Veillonellaceae that are often depleted in inflammatory states. Shotgun metagenomics showed a complete absence of *Coprococcus eutactus* and *Dialister invius* in the children who were diagnosed with T1D. Those who progressed to clinical disease had also a reduced intestinal microbial gene content compared with the children who developed autoantibodies but not T1D and the control children. When analyzing the time period from the appearance of autoantibodies to clinical disease manifestation, the progressors were observed to experience an increase in the number of genes involved in the transport systems of multiple sugars and a decrease in the number of genes that facilitate the biosynthesis of a series of amino acids, such as tyrosine and phenylalanine.

A cross-sectional American study analyzed the intestinal microbiota in four groups of participants [31]. The first group included 35 patients with recently diagnosed T1D (within the previous 6 months); the second group comprised 21 first-degree relatives (FDRs) who had at least one diabetes-associated autoantibody; the third group consisted of 32 FDRs testing negative for autoantibodies; and the fourth group included 23 unrelated autoantibody-negative individuals. No differences were observed in bacterial diversity between autoantibody-positive and autoantibody-negative FDRs. In contrast, differences were seen in the abundance of the Firmicutes genera *Lactobacillus* and *Staphylococcus* between participants in the fourth group and participants in the three other groups. Autoantibody-positive FDRs had a decreased abundance of unclassified Bacteroidetes species and an increased abundance of the RC9 gut group, *Catenibacterium* and *Prevotellaceae*, compared with autoantibody-negative FDRs. The FDRs with multiple autoantibodies had an increased abundance of *Bacteroides* and *Akkermansia* and a reduced abundance of *Prevotella* compared with FDRs with a single autoantibody. The increased abundance of *Akkermansia* is surprising given that these bacteria were reported to be associated with partial protection from autoimmune diabetes in young non-obese diabetic (NOD) mice [32]. This finding underlines the need to confirm observations that suggest disease associations for specific microbes.

There are very few studies comparing the intestinal microbiota in patients with clinical T1D to that seen in healthy individuals. The first report, published in 2013, analyzed the gut microbiota in 16 children with T1D and an average disease duration of 4.8 years and 16 unaffected children using PCR-denaturating gradient electrophoresis and real-time quantitative PCR [33]. A notable reduction both in the bacterial number of Actinobacteria and Firmicutes and in the Firmicutes to Bacteroidetes ratio was observed, while the abundance of Bacteroidetes was increased in affected children compared with that in healthy controls. At the genus level, the authors found a marked increase in the levels of *Clostridium*, *Bacteroides*, and *Veillonella* and a decrease in *Lactobacillus*, *Bifidobacterium*, the *Blautia coccoides–Eubacterium rectale* group and *Prevotella* in the children affected by T1D. A Mexican study analyzed the intestinal microbiota in 21

children with T1D, of whom eight had newly diagnosed disease and the remaining 13 had a disease duration of at least 2 years. The intestinal microbiota of eight unaffected children was as well analyzed. A high abundance of *Bacteroides* was observed in the newly diagnosed patients, whereas *Prevotella* was the dominant genus among the participants without T1D [34]. A recent European study comprised 28 children with newly diagnosed T1D and 27 unaffected children. Of the 28 children with T1D, 18 were from Finland, four from France, three from Estonia, two from Lithuania, and one from Greece, whereas 26 control children were Finnish and only one Lithuanian. The analyses revealed that young patients with T1D (less than 3 years of age) had an increased abundance of Bacteroidetes and streptococci and a decreased abundance of species from *Clostridium* clusters IV and XIVa. Older patients (3 years old or older) had an increased microbial diversity and a reduced fraction of butyrate-producing species within *Clostridium* clusters IX and XIVa when compared to control children of similar age [35].

In a recent study, we have compared the development of the intestinal microbiome over the first 3 years of life in Finnish, Estonian, and Russian Karelian children [36••]. The incidence of T1D is about six times higher in Finnish children compared to that in Russian-Karelian children [37]. In addition, we have found earlier that the frequency of a series of immune-mediated diseases and phenomena, such as celiac disease, thyroid autoimmunity, and atopic sensitization, is six to ten times lower in Russian Karelia than that in Finland [38]. In the microbiome analysis, we noted in the Finnish subjects a dominance of the *Bacteroides* genus, which was substantially less abundant in the Russian-Karelian subjects over the first year of life. Different bacteria produce structurally different LPS molecules associated with alterations in their capacity to elicit immune responses. This prompted us to purify LPS from a number of bacterial species observed in the cohort. We found that LPS purified from *Bacteroides dorei*, the highest enriched species in Finland compared to Russia, failed to recognize and activate in vitro toll-like receptor 4 (TLR4) and to stimulate the secretion of a series of cytokines from human peripheral blood mononuclear cells. In addition, the *Bacteroides dorei*-derived LPS acted as a TLR4 antagonist, preventing TLR4-mediated activation by *E. coli*-derived LPS. Notably, *B. dorei* has recently also been associated with seroconversion to autoantibody positivity in T1D [27]. Our results suggest that early colonization by an immunoinhibitory microbiota in Finnish individuals may counter the immune stimulation by *E. coli* LPS, fostering a predisposition to autoimmune diseases, T1D included, and allergies in Finland. In addition, we observed that the Russian Karelian infants had an increased abundance of *Bifidobacteria* strains except for *Bifidobacterium breve*, which were more common in Finnish infants. Taken together, the gut microbiota in Finnish infants was characterized by dysbiosis including a decreased abundance of most *Bifidobacteria* species and an increased abundance of *Bacteroides* species.

A Concerted View on the Role of the Intestinal Microbiome in the Development of Type 1 Diabetes

Based on the current knowledge, one may hypothesize that the impact of the intestinal microbiome on the risk for progression to clinical T1D is a two-step process (Fig. 1). The disease process leading to clinical T1D has similarly two phases. The first phase covers the time from birth to seroconversion to autoantibody positivity, i.e., the appearance of the first autoantibody(ies), whereas the other phase stretches from initial seroconversion to

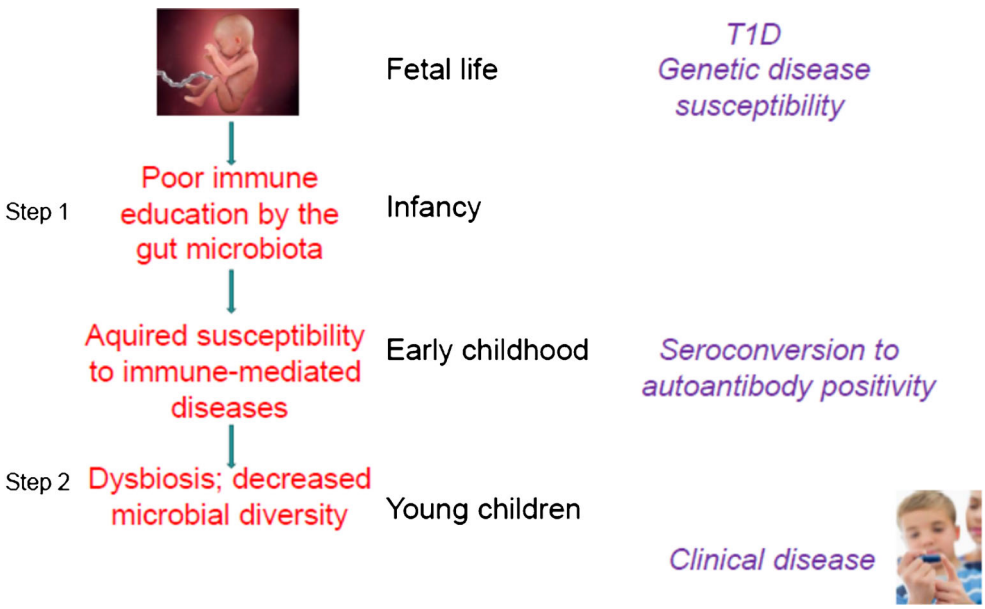

Fig. 1 The intestinal microbiome may affect the risk of type 1 diabetes (T1D) in two steps. An impaired immune education in infancy leads to an acquired susceptibility to immune-mediated diseases including type 1 diabetes resulting in the appearance of disease-associated autoantibodies as the first step. At the second step, a dysbiotic microbiome predisposes the autoantibody-positive child with genetic disease susceptibility to progression to clinical type 1 diabetes

overt disease. In the first phase, the infant and young child will need effective immune education to control inflammatory responses and to facilitate the induction of tolerance to autoantigens and allergens. An impaired immune education during the first year of life induces acquired susceptibility to immune-mediated diseases including T1D. In the second phase, a decreased intestinal diversity and the subsequent dysbiosis predispose the young child with genetic T1D susceptibility and a poorly trained and inadequately functioning immune system to develop progressive β cell autoimmunity reflecting a disease process that eventually will result in clinical T1D.

Based on this two-step process, we may consider how to intervene to prevent progression to overt T1D. At the first step, one has to consider how to educate the immune system adequately in early life. At the second step, one has to think of measures to increase the gut microbial diversity in a safe way. Modulatory treatment of the microbiome aimed at decreasing the risk of T1D is still in its infancy. In the following chapter, we will discuss possible means to modulate the T1D risk through measures affecting the gut microbiome.

Potential Measures for Reducing the Risk of Type 1 Diabetes Through the Intestinal Microbiome

So far, there are no evidence-based therapies modulating the gut microbiome for preventing or delaying progression to overt T1D. On the other hand, there are definitely changes in the gut microbiome associated with the disease process leading eventually to symptomatic disease, and the modulation of the intestinal microbiome provides novel strategies that may be safely and effectively applied for treatment of such aberrations. Table 1 lists perturbations of the gut microbiome reported to be associated with the development of T1D. Possible therapeutic modalities are as well presented including related comments and considerations.

When considering how to strengthen the immune education in infants born in a developed country, one option could be to administer orally the *Escherichia coli* Nissle 1917 (EcN) strain starting already on the first postnatal day. This probiotic was given to 54 newborn infants for the first 5 days of life in a randomized clinical trial. EcN was still detectable in stool samples taken at the age of 6 months in 90% of the actively treated participants [39]. Colonization with true and potentially pathogenic bacteria was significantly reduced in infants receiving EcN compared to the placebo group—both with respect to numbers of pathogens and to the spectrum of species. It has also been shown that EcN increases in vitro the interleukin 10 (IL-10) secretion but decreases the secretion of IL-2 and tumor necrosis alpha (TNF-alpha) from peripheral blood T cells implying that this strain has an anti-inflammatory effect [40]. Another potential alternative would be to perform a vaginal microbial transfer immediately after birth. This approach was assessed in a small pilot study including in total 18 infants, out of whom only four infants born by caesarean section (CS) were exposed to the maternal vaginal microbes [41•]. The aim was to restore the microbiota in the CS-born infants, since CS is known to be associated with differences in the diversity and colonization pattern of the gut microbiota when compared to the majority of vaginally born infants [42]. CS has been reported to be associated with an increased risk of T1D in the offspring [43]. The vaginal microbial transfer restored the oral and skin microbiota but less than the anal microbiota in the treated babies.

Probiotics are defined as live microorganisms which confer a health benefit to the host when given in adequate amounts

Table 1 Perturbations in the intestinal microbiome associated with the development of T1D, potential treatment strategies, and related comments and considerations

Perturbations	Potential treatment modalities	Comments and considerations
Poor education of the immune system in infancy	Probiotics, e.g., *E. coli* Nissle (EcN)	RCT in 54 healthy newborn infants [39]
	Vaginal microbial transfer	A small pilot study in four infants born by caesarean section [41]
Dysbiosis and decreased microbial diversity after the appearance of β cell autoimmunity	Probiotics	*Bifidobacterium longum infantis* colonizes effectively the intestine in breast-fed infants [47]
	Prebiotics	Human milk oligosaccharides (HMOs) are nutrients for commensal gut microbes, bifidobacteria in particular [51]
	Fecal microbiota transplantation (FMT)	FMT increases intestinal microbial diversity and introduces "healthy" microbes with altered metabolic profile [53]
Increased abundance of *Bacteroides*	Bacteriophages	Administration of lytic bacteriophages infecting specific *Bacteroides* species could be used to reduce the increased abundance of such species
		Selection of a donor microbiota with low abundance of *Bacteroides*
	Fecal microbiota transplantation (FMT)	Please see above
Reduced abundance of butyrate -producing bacteria	Dietary intervention, e.g., butyrylated starch	A butyrate- and/or acetate-containing diet protects against autoimmune diabetes in NOD mice [59••]

[44]. Currently, there are more than 100 different probiotic preparations on the market, some containing a single probiotic and others a combination of various probiotics. A combination preparation, VSL#3, comprising eight different strains has been shown to decrease the rate of autoimmune diabetes in NOD mice [45]. The international TEDDY study reported that early use of probiotics, i.e., during the first 4 postnatal weeks, was associated with a reduced risk of β cell autoimmunity in those carrying the HLA genotype conferring the highest risk for T1D [46]. This observation was largely driven by the Finnish participants with the highest frequency of probiotic use but on the other hand the highest T1D incidence. The *Bifidobacterium longum infantis* subspecies has been shown to colonize effectively the intestinal tract in breast-fed infants when given during the first 4 weeks after birth [47]. This bacteria is highly abundant as long as the mother is breast-feeding, which prevents colonization with pathogenic bacteria. Accordingly, this probiotic is a candidate to be tested whether it may protect from β cell autoimmunity and T1D in children with genetic T1D susceptibility. Recently, efforts have been made to increase the natural benefits of probiotics through a recombinant expression of therapeutic biomolecules [48••]. The potential benefits of such an approach remain to be defined.

Prebiotics are non-digestible food ingredients which selectively stimulate the growth or activity of anaerobic/microaerophilic microbes including *Bifidobacterium* and *Lactobacillus* in the colon [49]. Prebiotics include inulin, lactulose, and oligosaccharides. Human milk oligosaccharides (HMOs) are the most prevalent prebiotics, and they are present in breast milk. HMOs do not provide any energy to the infant but together with lactoferrin and lactoalbumin they promote specific growth of bifidobacteria, and these compounds are characterized as "bifidogenic factors" [50, 51]. Substantial differences have been observed in HMO utilization among infant bifidobacteria opening opportunities for fine-tuning of the intestinal colonization by using HMOs [52]. The previously mentioned *Bifidobacterium longum infantis* subspecies utilizes very effectively the HMOs in breast milk and as a consequence its abundance drops conspicuously after stopping breast-feeding [46].

Fecal microbiota transplantation (FMT) was initially introduced for the treatment of antibiotic refractory or recurrent *Clostridium difficile* infection with a success rate of up to 96% [53]. A recent review of the literature shows that FMT has also been applied with some success as an experimental treatment modality in a series of other diseases including gastrointestinal, hematologic, neurologic, metabolic, and infectious disorders [54•]. There are no reports on the use of FMT in any subjects with preclinical or clinical T1D. The unique feature of FMT is that it promptly restores a diverse composition of the recipient's gut microbiome. This is clearly different from probiotics that only provide a single or limited numbers of species into the intestinal tract [55]. A disadvantage to FMT is that it is almost impossible to standardize the composition of the fecal microbiota to be transplanted, as the microbiota varies both between individuals and within individuals over time. It may, however, be that standardized partially purified and frozen fecal microbiota might be as effective as the currently used fresh-harvest approach [56].

The intestinal virome being an essential part of the gut microbiome harbors a high number of bacteriophages, i.e., bacterial viruses [57••]. Lytic bacteriophages survive via infection and lysis of their bacterial target cells. Accordingly, bacteriophages infecting *Bacteroides* species could at least theoretically be given to reduce the increased abundance of *Bacteroides* observed in individuals with diabetes-associated autoantibodies [23, 26]. Speed and specificity of lytic action, a self-titrating dose, and activity against biofilms are definite advantages of phage therapy. The limited understanding of phage-bacterial interaction and of ecological interactions within the intestinal microbiota in the human host has, however, so far hampered the clinical application of phage therapy.

Observational studies have shown an association between the high-fat/low-fiber Westernized diet and the prevalence of many inflammatory and immune-mediated diseases [58]. Dietary fibers pass through the upper intestine and are fermented by large bowel anaerobic microbiota to produce short-chain fatty acids (SCFAs), including acetate and butyrate. Such acids promote gut epithelial integrity and exert immune effects, including stimulation of G protein-coupled receptors, promotion of innate (toll-like receptor 2) immune responses, and induction of regulatory T cells. In a recent paper, Morino et al. reported that feeding NOD mice an acetate-yielding diet reduced substantially the frequency of autoreactive T cells in lymphoid tissues through effects on B cells and their ability to expand populations of autoreactive T cells [59••]. A diet containing butyrate boosted the number and function of regulatory T cells. Acetate- and butyrate-yielding diets improved gut integrity and reduced serum concentrations of diabetogenic cytokines such as IL-21. Both diets decreased clearly the frequency of autoimmune diabetes and their combination provided full protection against autoimmune diabetes. These observations imply that butyrylated starches should be tested in subjects with preclinical T1D to assess whether such a dietary intervention may prevent progression to clinical disease. Butyrylated starch has already been shown to reduce increased levels of DNA adducts induced by a high intake of red meat [60].

The realization that microbial metabolic activity is involved in host physiology and pathophysiology opens up a totally new branch of pharmacology [61]. Instead of targeting host enzymes, the alternative is to focus on modifying biochemical processes in the microbiome. The development of small molecules targeted against bacterial enzymatic cascades will provide tools for modulating the metabolic activity of specific microbes.

Conclusions

Direct or indirect manipulations of the intestinal microbiome may provide effective measures for preventing or delaying the disease process leading to the manifestation of clinical T1D. We do not yet have any tools with proven track record in this field, but as discussed in this review there are a series of strategies the efficacy of which should be tested in the near future. The first challenge is to create optimal and safe conditions for the colonization of the intestinal tract in the newborn infant with microbes providing appropriate immune education in early life. If this opportunity is missed, the individual will acquire an increased susceptibility to immune-mediated diseases. The next challenge is to modify the microbiome in subjects who have already developed the first signs of β cell autoimmunity to provide protection against progression to clinical disease. The hope is to see a series of well-controlled randomized clinical trials exploring possible solutions to the challenges identified in this area.

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Compliance with Ethical Standards

Conflict of Interest Mikael Knip and Jarno Honkanen declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent The studies included in this review involving human participants and performed by any of the authors have been approved by the appropriate institutional research ethics committee and have been performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments. Informed consent was obtained from all individual participants included in the studies or from their guardians if the participant was a minor.

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- • Of importance
- •• Of major importance

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