Review

Microbiome and type 1 diabetes

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ARTICLE INFO

ABSTRACT

The steep increase in the incidence of type 1 diabetes (T1D), in the Western world after World War II, cannot be explained solely by genetic factors but implies that this rise must be due to crucial interactions between predisposing genes and environmental changes. Three parallel phenomena in early childhood – the dynamic development of the immune system, maturation of the gut microbiome, and the appearance of the first T1D-associated autoantibodies – raise the question whether these phenomena might reflect causative relationships. Plenty of novel data on the role of the microbiome in the development of T1D has been published over recent years and this review summarizes recent findings regarding the associations between islet autoimmunity, T1D, and the intestinal microbiota.

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1. Introduction

Type 1 diabetes (T1D) is a chronic immune-mediated disease in which the insulin-producing beta cells of the pancreatic islets are destroyed. The disease process starts often at a young age, during the first years of life. In T1D-affected patients, the lack of endogenous insulin causes a life-long need of exogenous insulin therapy [1,2]. The rate of T1D has increased conspicuously after World War II in most developed countries. For example, in Finland the incidence among children under the age of 15 years has increased from 12 to 65 new cases/100,000/year in five decades [3]. The increasing disease rate cannot be explained by genetic factors but implies that these changes are an outcome of interactions between the modern westernized environment and predisposing genes.

The human intestinal microbiome matures during the first years of life, after which its composition resembles the composition observed in adults [4,5]. The maturation of the gut microbiome is closely linked to the development of the immune system [6]. Three contemporary phenomena occurring in early childhood – the dynamic development of the immune system, th the maturation of the gut microbiome, and the appearance of the first T1D-associated autoantibodies – raise the question whether these phenomena are only temporarily associated, or do they reflect causative relationships. Even if the field of microbiome studies has expanded remarkably during recent years, causal relationships have not been established. Since our last review in which we explored the potential role of the gut microbiota in the development of T1D [7], ample new data has been generated. In this review, we set out to summarize recent findings from the microbiome studies.

2. Intestinal bacterial microbiota

2.1. Lessons learned from experimental studies

The idea that intestinal microbiota might play an important role in the development of immune-mediated diseases, such as T1D, was presented already in the 1980's [8]. To test this hypothesis, genetically modified rodents have been raised in germ-free conditions; colonized with various microbial communities; treated with probiotics, prebiotics, and antibiotics; fed with distinct diets, and given intestinal microbiota from other animals (Supplementary Table 1). Considering the interplay between the ntestinal microbiota and the developing immunity, several key findings have been observed (Table 1). Comparisons between these findings and those observed in man (Table 2) will be discussed in the next paragraphs [9–31]. While interpreting the results, one should bear in mind that autoimmune diabetes observed in NOD mice is not identical with T1D in humans [32]. Considering these two species, two major differences associate with the timing of the disease development: (i) in man, seroconversions peak during the first three years of life, while NOD mice typically seroconvert close to their sexual maturity, and (ii) approximately half of the cases appear before adulthood in humans, whereas in NOD mice, nearly all cases occur in adult animals, the typical age of onset being 12–30 weeks. Importantly, by 30 weeks of age, >80% of the NOD females and >50 of the NOD males are typically overtly diabetic, while in human populations the prevalence of T1D is under 1% [33].

2.2. The interactions between the genetic setup, nutrition, microbial composition, and susceptibility to islet autoimmunity

The genetic setup of the host may affect the host's microbial composition and function, the activation of innate and adaptive immunity and susceptibility to various diseases; both in animal models and in man [34–36]. In mice, the host's inherited disease susceptibility can be modified by colonizing with selected microbes, providing mice with specific diets, or treating them with antibiotics. Results from these interventions range from accelerated autoimmunity to total prevention of the disease (Supplementary Table 1). In humans, dietary modifications, such as weaning infants to highly hydrolysed formula after exclusive breast-feeding [37] or delaying the introduction of gluten-containing foods in infancy [38], have not provided any disease protection. The same holds true with oral antigens [39] and nicotinamide, a form of vitamin B3 suggested to protect beta-cells from destruction [40]. However, one observation from children with HLA-conferred disease susceptibility implies that early oral exposure to probiotics may decrease the risk of islet autoimmunity [41]. The protective effect was restricted to children carrying the high-risk HLA DR3/4 genotype. Recently, two intervention studies aimed at preservation of endogenous insulin secretion have been launched: one with L. *rhamnosus* GG and *B. lactis* Bb12 in children with newly diagnosed T1D, and one pilot study with prebiotic oligofructose-enriched inulin in children with a T1D duration of at least one year [42,43].

2.3. Intestinal microbiota matures early in life and affects the incidence of immune-mediated diseases

The human microbiota matures early in life. After being born from nearly sterile conditions, children encounter a lot of microbes and environmental antigens before reaching their third year of life, by which the intestinal microbiota has reached its adult-like composition and relative stability. From early on, the main modifiers of the microbial composition are the route of delivery, early nutrition (especially breast-feeding and introduction of supplementary feeding), use of antibiotics, and the microbial exposure and the hygiene level of the immediate environment [44,45]. Considering T1D, significant changes occur in the gut microbiota before any systemic signs of islet autoimmunity. These changes occur early in life and have a far-reaching effect on the development of the immune system [12,30,46,47]. In rodents, progression to autoimmune diabetes associates with altered composition and diversity of the intestinal microbial colonies, reduced abundance of Firmicutes in relation to Bacteroidetes, and decrease in butyrate-producing bacteria subgroups. This dysbiosis coincide with increased intestinal permeability, translocation of microbial materials through the epithelium, and increased and aberrant presentation of foreign and self-antigens. These alterations activate proinflammatory pathways in the gut, in the regional lymph nodes, and in the pancreas (Supplementary Table 1). In humans developing T1D, two comparable findings have been reported regardless of geographical location: the proinflammatory environment in the gut and the combination of an increased relative abundance of Bacteroidetes together with decreased levels of Firmicutes (Table 2). More specifically, in a recent study where young children with HLA-conferred disease susceptibility and signs of islet autoimmunity were compared to matched controls, the controls had higher levels of

Table 1
Summary on findings from experimental studies on the interplay between the intestinal microbiota and exogenous and host-related factors.

- Genetic setup of the host is associated with a tendency towards a specific microbial composition
- Microbial communities mature early in life
- Intestinal microbiota affects the incidence of immune-mediated diseases
- Prolonged or repetitive deviation from the optimal microbial homeostasis (dysbiosis) may lead to loss of self-tolerance and spreading of proinflammatory signals and effector cells
- In spite of a plethora of microbes, the functional net effects of the co-occurring microbial colonies resemble each other
- Considering the whole superorganism (host with its microbiota), crosstalk networks are extensive, ranging from molecular interactions to communication between microbiota and various organs of the host
- Sum vectors of these crosstalk networks may point towards proinflammatory or anti-inflammatory reactivity
- Modulation of the intestinal microbiota may lead to prevention or enhancement of the immune-mediated disease process, depending on the resulting compositional and functional changes
- Nutritional factors drive rodents towards tolerance or disease

Table 2

Recent studies investigating the associations between gastrointestinal microbiota and islet autoimmunity leading to type 1 diabetes (T1D) in humans.

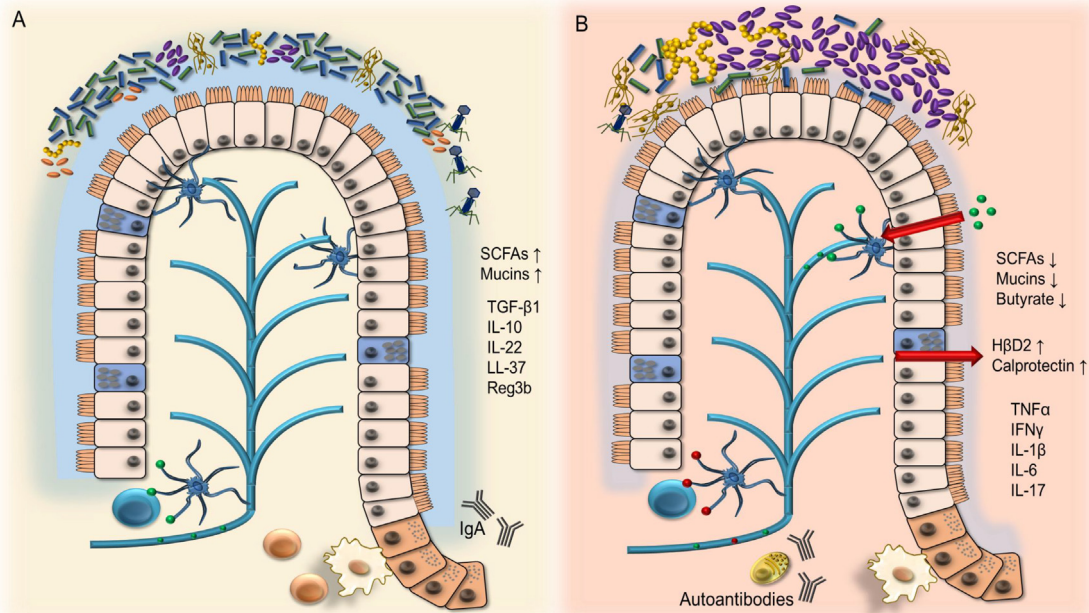| Ref. #	Author	Age (years)	Study groups; Geographical locations	Findings related to T1D and/or islet autoimmunity
[9]	Mejía-León ME et al. 2014	7–18	Children with T1D at diagnosis ($n = 8$) and after 2 years of insulin treatment ($n = 13$) vs. healthy controls (n = 8); Mexico	At diagnosis, T1D patients had high levels of *Bacteroides*, whereas controls had *Prevotella* dominance. After 2 years of insulin treatment, cases resembled the controls.
[10]	Kemppainen KM et al. 2015	0–2	Seronegative young children with high risk HLA genotype ($n = 15$ from each site); Finland, Sweden, Germany, and USA	Bacterial diversity differed by geographical location. Children from Finland and Colorado had lower bacterial diversity, and children from Sweden and Washington state had *Bifidobacterium* dominance.
[11]	Soyucen E et al. 2014	6–15	Children with newly diagnosed T1D (n = 35) and healthy controls (n = 35); Turkey	Compared to controls, *Bifidobacterium* colonization was lower in patients with T1D. *Candida albicans* and *Enterobacteriaceae* (other than *Echerichia coli*) were increased in controls.
[12]	Kostic AD et al. 2015	0–3	Young children with islet autoimmunity (n = 11) and healthy controls ($n = 22$). All have HLA-conferred risk for T1D; Finland and Estonia	Drop in alpha-diversity between seroconversion and T1D diagnosis, accompanied with an increase in proinflammatory organisms, changes in gene functions, and serum and stool metabolites. Microbial relationships were shared across most subjects. Strain compositions were highly variable between individuals, but stable within individuals throughout infancy. Metabolic composition and metabolic pathway abundances were constant across time.
[13]	Endesfelder D et al. 2016	0–3	Young children with persistent islet autoimmunity (n = 22) and healthy controls (n = 22); Germany	*Bacteroides*-dominated subgroup associated with early introduction of non-milk diet, increased risk for early autoantibody development, and lower abundances of genes for butyrate production. Alterations in the composition of mucin-degrading bacteria associated with early development of islet autoimmunity. Functional associations between diet, gut microbiome and islet autoimmunity existed in microbial co-occurrence networks.
[14]	Heintz-Buschart A et al. 2016	5–62	Four families with ≥2 generations of ≥2 cases of T1D per family (n = 20); Netherlands	Family membership has a pronounced effect on the structural and functional composition of the gastrointestinal microbiome. No differences in taxonomic or functional diversity and richness between T1D cases and their family members. Protein expression findings implicated that T1D cases may have slightly dysfunctional exocrine pancreas.
[15]	Maffeis C et al. 2016	6–16	Children with islet autoimmunity (10) and healthy controls (n = 10); Italy	Group-specific differentiation between cases and controls was challenging. Intestinal permeability was higher in children with islet autoimmunity. *Dialister invisus, Gemella sanguinis* and *Bifidobacterium longum* were associated with prediabetes.
[16]	Qi CJ et al. 2016	10–15	Children with T1D ($n = 15$) and healthy controls (n = 15); China	T1D cases had decreased bacterial richness, decreased *Haemophilus, Lachnospira, Dialister*, and *Acidaminococcus*, and increased *Blautia* levels. Percentage of *Blautia* correlated with HbA1c, number of T1D-associated autoantibodies, and IA-2A levels.
[17]	Vatanen T et al. 2016	0–3	Young children with HLA-associated risk for T1D ($n = 74$ from each country); Finland, Estonia, and Russia	*Bacteroides* strains were more abundant in Finnish infants compared to Russian Karelian infants, while *E. coli* was more frequent on Finnish infants *Bacteroides* LPS had immunoinhibitory properties that may preclude early immune education and contribute to development of T1D, while *E. coli* derived LPS was strongly immunostimulatory. Metabolism of human milk oligosaccharides (HMOs) maintained *Bifidobacterium*-dominant versus *Bacteroides*-dominant gut microbiota in the first year of life.
[18]	Cinek O et al. 2017	0–3	Young children with islet autoimmunity progressing to T1D ($n = 18$) and healthy controls (18); Finland	Four operational taxonomic units were less abundant in children developing islet autoimmunity, most markedly *Bacteroides vulgatus* and *Bifidobacterium bifidum*. Potential relation existed between *CrAssphage* and *Bacteroides dorei*. No differences were observed between cases and controls in alpha or beta diversity, or the taxonomic levels of bacterial phyla, classes or genera.
[19]	de Groot PF et al. 2017	25–45	Adults with T1D ($n = 53$) and healthy controls ($n = 50$); Netherlands	Both oral and gut microbiota differed between cases and controls. Oral microbiota had higher abundance of streptococci in cases, while gut microbiota showed decreased butyrate-producing species and less butyryl-CoA transferase genes. Plasma levels of acetate and propionate were lower in T1D. *Christensenella* and *Subdoligranulum* strains correlated with glycaemic control, inflammatory parameters, and level of short-chain fatty acids (SCFAs).
[20]	Pellegrini S et al. 2017	1–65	Children and adults with T1D ($n = 19$) or celiac disease (CD, $n = 19$) vs. controls ($n = 16$); Italy	T1D-specific increase in monocyte/macrophage infiltration in the intestinal biopsies. Cases had increase in Firmicutes and Firmicutes/Bacteroidetes ratio, and a reduction in Proteobacteria and Bacteroidetes in the duodenal mucosa. The expression levels of genes specific for T1D inflammation were associated with the abundance of specific bacteria in the duodenum.
[21]	Pinto E et al. 2017	8–11	Children with T1D ($n = 3$) and healthy controls ($n = 3$); Portugal	T1D cases had a microbial intestinal proteome enriched with proteins of clostridial cluster XVa and cluster IV and *Bacteroides*, while controls had more often bifidobacterial proteins. T1D children had lower levels of exocrine pancreatic enzymes.
[22]	Stewart CJ et al. 2017	25–30	Young adults with T1D ($n = 10$) and healthy controls (n = 10); UK	*Faecalibacterium sp., Roseburia* sp. and *Bacteroides* sp. were the most abundant microbes in both groups. Each bacterial profile was individual and no significant differences in the profiles or in the diversity indices were observed between the groups.
[23]	Cinek O et al.	7–15	Children at T1D onset ($n = 73$; 14–20 per country) and healthy	Genus *Escherichia* spp. correlated with T1D. T1D cases showed an

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Table 2 (continued)

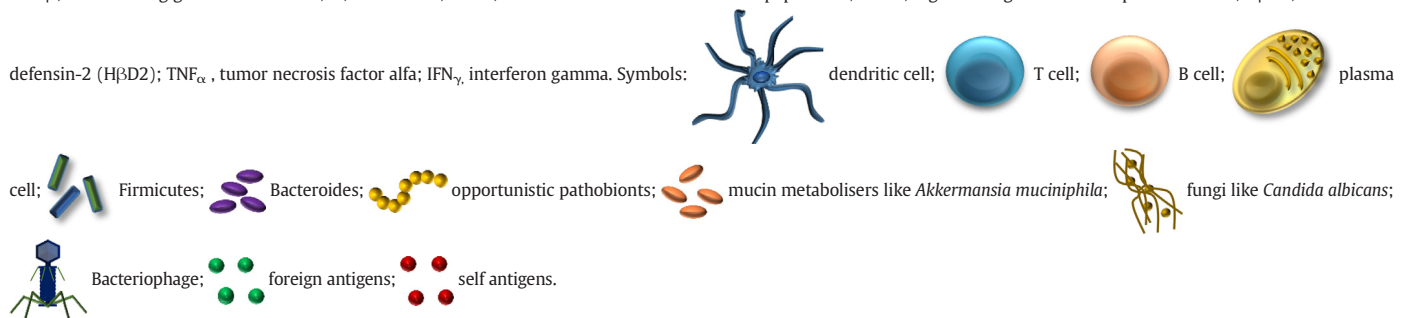| Ref. #	Author	Age (years)	Study groups; Geographical locations	Findings related to T1D and/or islet autoimmunity
	2018		controls ($n = 104$); Azerbaijan, Jordan, Nigeria, and Sudan	inverse association with *Eubacterium* and *Roseburia* (Firmicutes), and clostridial clusters IV and XIVa. No T1D-associations for microbial richness or enterotypes were observed.
[24]	Gao X et al. 2018	0–3	Young children with islet autoimmunity ($n = 11$) and healthy controls ($n = 22$). All have HLA-conferred risk for T1D; Finland and Estonia	More microbial interactions and the inhibition of *Clostridia* by *Gammaproteobacteria* were maintained throughout the first 3 years of life in healthy children. Gammaproteobacteria inhibited *Bacteroidia* in the cases, but not in controls. The inhibitory effects from *Actinobacteria* and *Bacilli* on *Bacteroidia*, from *Bacteroidia* on *Clostridia*, and the beneficial effect from *Clostridiao on Bacteroidia* were shared between cases and controls.
[25]	Gavin PG et al. 2018	2–45	Children and adults with recent-onset T1D (33) or islet autoimmunity ($n = 17$), and low-risk autoantibody-negative subjects ($n = 29$) and healthy controls (n = 22); USA	T1D patients had increased intestinal inflammation and decreased barrier function. Microbial taxa capable of promoting the host's mucous barrier, microvilli adhesion, and functions of the exocrine pancreas were depleted in T1D. Pancreatic exocrine dysfunction appeared in high-risk individuals before the disease onset. Both host-derived and microbial-derived proteins were able to differentiate the new-onset and the islet autoantibody-positive subjects from the low-risk subjects.
[26]	Higuchi BS et al. 2018	15–35	Teenagers and young adults with T1D ($n = 20$) and healthy controls ($n = 28$); Brazil	T1D patients had intestinal dysbiosis with prevalent Gram-negative bacteria like *Bacteroides vulgatus*, *Bacteroides rodentium, Prevotella copri*, and *Bacteroides xylanisolvens*. Cases had increased plasma levels of the proinflammatory interleukin-6. Poor glycaemic control associated with the relative abundance of Bacteroidetes, Lactobacillales, and *Bacteroides dorei*.
[27]	Huang Y et al. 2018	18–25	Young adults with T1D ($n = 12$) and healthy controls (n = 10); China	Various bacterial taxonomic clades differed between cases and controls. Bacteroidetes and Firmicutes were the dominant phyla in cases and controls, respectively. Abundance of *Faecalibacterium* correlated inversely with HbA1c levels. Numbers of islet autoantibodies correlated positively with *Bacteriodes* and *Bilophila* abundances and inversely with Streptococcus and Ruminococcaceae abundances.
[28]	Leiva-Gea I et al. 2018	9–16	Children with T1D ($n = 15$) or MODY2 (n = 15), and healthy controls ($n = 13$); Spain	T1D associated with (i) lower microbial diversity, (II) higher relative abundance of *Bacteroides, Ruminococcus, Veillonella, Blautia*, and *Streptococcus* genera, and (III) lower relative abundance of *Bifidobacterium, Roseburia, Faecalibacterium*, and *Lachnospira*. Proinflammatory cytokines and lipopolysaccharides were increased in T1D cases, as were the expression of genes related to lipid and amino acid metabolism, ATP-binding cassette transport, lipopolysaccharide biosynthesis, arachidonic acid metabolism, antigen processing and presentation, and chemokine signalling pathways. MODY2 associated with higher *Prevotella* abundance and a lower *Ruminococus* and *Bacteroides* abundance. Intestinal permeability was increased both in T1D and in MODY2 cases.
[29]	Mejía-León ME et al. 2018	9–14	Children with newly diagnosed T1D (n = 10)	The increasing *Bacteroides* proportion (during the first months after diagnosis) correlated with the consumption of saturated fat and carbohydrates at 3 months. After adjusting for HbA1C, consumption of carbohydrates associated with decreased *Bacteroides* abundance over time.
[30]	Stewart CJ et al. 2018	0–4	Young children ($n = 903$) with high risk HLA genotype; Finland, Sweden, Germany, and USA	Feeding with breast milk was the most significant factor associated with the microbiome structure. It associated with higher levels of *Bifidobacterium* sp. (*B. breve* and *B. bifidum*). *Bacteroides* associated with increased microbial diversity and faster maturation of the gut. Only subtle associations were observed between microbial taxonomy and islet autoimmunity: i) higher relative abundance of an unclassified Erysipelotrichaceae in children with autoimmunity; ii) five bacterial genera associated with T1D onset (*Parabacteroides* the most significant); iii) eleven bacterial genera were lower in T1D cases, including four unclassified Ruminococcaceae, *Lactococcus*, *Streptococcus*, and *Akkermansia*.
[31]	Vatanen T et al. 2018	0–4	Young children ($n = 783$) with high risk HLA genotype developing persistent islet autoimmunity or T1D vs. controls; Finland, Sweden, Germany, and USA	Control children had more genes associated with fermentation and biosynthesis of SCFAs. T1D-associated microbial compositions were taxonomically diffuse but functionally more coherent. Compared to children with autoimmunity, controls had higher levels of *Lactobacillus rhamnosus* and *Bifidobacterium dentium*, while children with autoimmunity had higher abundance of *Streptococcus* group *mitis/oralis/pneumoniae* species. Compared to children developing T1D, controls had higher levels of *Streptococcus thermophiles* and *Lactococcus lactis* species. T1D cases had higher levels of *Bifidobacterium pseudocatenulatum, Roseburia hominis*, and *Alistipes shahii*.

Lactobacillus rhamnosus and *Bifidobacterium dentium*, while children with autoimmunity had higher abundance of the *Streptococcus* group *mitis/oralis/pneumoniae* species. When children progressing to T1D were compared to non-progressors, the latter had higher levels of *Streptococcus thermophilus* and *Lactococcus lactis*, whereas the progressors had increased levels of *Bifidobacterium pseudocatenulatum*, *Roseburia hominis*, and *Alistipes shahii* species. The intestinal microbiomes of the control children had also more genes related to fermentation and to biosynthesis of short-chain fatty acids [31]. Other recent analyses on the host-microbiome interactions have also revealed that factors promoting the host's mucosal barrier, adhesion of the microvilli, and exocrine function of the pancreas are depleted in T1D patients [21,25]. Some seemingly contradictory findings observed in man (Table 2), may be at least partly explained by non-optimal selection of controls, or by the differences in sample collection and handling as well as in sequencing methods.

It appears that in man, a prolonged or repetitive deviation from the optimal microbial homeostasis (dysbiosis) may lead to loss of self-tolerance and systemic spreading of the proinflammatory signals and effector cells. Thus far it has been suggested that a health-promoting intestinal microbiota (i) has a certain level of microbial abundance, the optimal quantity of which depends on the functional net effects of the co-occurring microbial networks, (ii) has a composition that is resilient to transient perturbations, such as antibiotic treatment or gastrointestinal infections, (iii) provides the host with protection against pathogen invasions, and (iv) provides the host and the co-occurring commensal microbes with nutritional metabolites they are otherwise unable to obtain, e.g. certain vitamins, degraded complex polysaccharides, butyrate, and mucin metabolites (Fig. 1) [13,48].

	A) HEALTHY INTESTINAL ENVIRONMENT	B) DYSBIOSIS LEADING TO INTESTINAL INFLAMMATION AND AUTOIMMUNITY
MICROBIOTA	• Stabile and resilient microbial colonization maintaining symbiosis and homeostasis • Firmicutes >> Bacteroides • Beneficial synergistic relations between fungi, bacteria, and bacteriophages • Constrained niches for pathobionts (compete for nutrients and space) • Producers of SCFAs and mucin metabolites promote intestinal integrity and well-being	• Microbial colonization unstable and susceptible to disturbances • Bacteroides >> Firmicutes • Reduced microbial diversity and/or aberrant microbial composition • Decreased richness and beta diversity of the intestinal virome • Overgrowth of opportunistic pathobionts • Mucin-degraders disappear and number of butyrate-producers decreases
HOST	• Intact epithelium and firm tight junctions • Appropriate regeneration in the intestinal epithelium • Controlled and restricted transportation of foreign antigens and other molecules • Regulation of the inflammatory stimuli • Normal presentation of foreign antigens • Maintenance of self-tolerance and optimal development of the immunity	• Integrity of the intestinal barrier disturbed and permeability increased • Calprotectin and human beta defensin 2 into the intestinal lumen • Translocation of microbial material to lamina propria and adjacent organs • Pro-inflammatory environment both in the intestinal lumen and in the mucosa • Maturation of the immunity disturbed • Presentation of self-antigens, loss of tolerance and development of immune-mediated diseases

Fig. 1. Healthy intestinal environment (A) compared to dysbiosis (B) that leads to intestinal inflammation and emerging of autoimmunity. Abbreviations: SCFA, short-chain fatty acids; TGF-β, transforming growth factor beta; IL, interleukin; LL-37, cathelicidin-related antimicrobial peptide 37; Ref3b, regenerating islet-derived protein 3-beta; HβD2, human beta defensin-2 (HβD2); TNFα, tumor necrosis factor alfa; IFNγ, interferon gamma. Symbols: dendritic cell; T cell; B cell; plasma cell; Firmicutes; Bacteroides; opportunistic pathobionts; mucin metabolisers like *Akkermansia muciniphila*; fungi like *Candida albicans*; Bacteriophage; foreign antigens; self antigens.

3. Intestinal virome

3.1. Virus and T1D: From infections to virome

Viral infections have been associated with diabetes since 1864, when mumps was for the first time implicated as a cause of diabetes [49]. In the 1920's, the observed seasonal variation in the acute diabetes onset created the hypothesis that diabetes might have an infectious origin [50,51]. The technical developments in the next three decades rendered further findings possible: (i) viral infections can induce diabetes in mice [52–54], (ii) some patients with newly-diagnosed insulin-dependent diabetes have neutralizing Coxsackievirus B4 antibodies [55], and (iii) Coxsackievirus B4 isolated from a patients who died at the diagnosis of diabetes caused pancreatic islet inflammation and beta cell death when inoculated into mice [56].

Thereafter, the pathomechanisms behind the virus-induced insulitis and beta-cell autoimmunity leading to T1D have been intensively studied and discussed [e.g. 57,58]. In addition to Coxsackie B4, other enteroviruses and related viruses have been shown to induce diabetes in animals. In humans, enterovirus PCR-positivity has been correlated with subsequent development of T1D-associated autioantibodies in at-risk individuals, and post-mortem examinations have shown that unlike controls, patients with newly diagnosed T1D have beta-cell tropic destructive Coxsackie viruses in their pancreas [59–62]. The latest advancement in the field is the endeavour to develop a vaccine for prevention of T1D by inducing Coxsackievirus B immunity [63].

Studies on associations between viral infections and T1D have underlined the highly variable nature of the viruses, e.g. in some mice models inoculation with assumedly diabetogenic variants of encephalomyocarditis virus or Coxsackie B caused paradoxical protection against insulitis and diabetes [64,65]. Subsequently, it has been suggested that certain latent infections might bring immune benefits for the host and thus represent a part of the host's beneficial virome [66,67].

3.2. Early life dynamics of the intestinal virome in man

Lim et al. have described the dynamics of the early human intestinal virome in eight twins followed from birth until the age of two years [68]. For all virus groups, interpersonal variations were significantly lower within the twin pairs than between different pairs of twins. The main findings were that in man, populations of eukaryotic viruses have their nadir at the birth of the child, after which the abundance and the diversity of both RNA and DNA viruses increase by age. Entero-, parecho-, tombamo- and sapoviruses were the most common eukaryotic RNA viruses, while anelloviruses were the most common eukaryotic DNA viruses. Dynamics of the anelloviruses seemed to reflect the host's immunocompetence: they were rarely seen in breastfed infants before the age of 3 months but peaked at the age of 6–12 months, when the immune protection provided by the mother faded, and the infant's own immunity gradually prevailed.

Bacteriophages, viruses that infect bacteria, were observed in all samples from these twins and comprised the dominant viral component of the normal intestinal virome. Contrary to eukaryotic RNA and DNA viruses, richness and diversity of the bacteriophages peaked at birth and decreased therafter. Among the bacteriophages, *Caudovirales* (*Siphoviridae*, *Inoviridae*, *Myoviridae* and *Podoviridae* families) and *Microviridae* families were the most abundant ones. The abundance of the two groups of bacteriophages showed an inverse correlation, and the balance of the bacteriophage populations shifted towards *Microviridae* dominance by the age of 2 years. The otherwise commonly observed human intestinal bacteriophage, CrAssphage, appeared only in one sample taken at the age of 2 years, indicating that these bacteriophages peak at a later age. Predator-prey relationships between bacteriophages and their targets showed clear age-dependent trajectories: after high abundance of bacteriophages and low number of intestinal bacteria at birth, a decrease in *Caudovirales* and a shift towards *Microviridae* dominance allowed a physiological increase in the abundance and diversity of the intestinal bacterial colonies by the age of 2 years [68].

3.3. Human intestinal virome and islet autoimmunity

Thus far, there are few clinical studies exploring the role of the intestinal virome in the development of T1D in man [69–71]. In the first report, stool samples from 19 case children with early advanced islet autoimmunity and subsequent progression to clinical T1D, and their tightly matched controls, were analysed with next-generation sequencing. The detected human viruses were verified by using real-time PCR [69]. Samples in focus were gathered 3, 6, and 9 months before the first signs of islet autoimmunity. No dramatic changes in the intestinal virome preceded the appearance of the T1D-associated autoantibodies and no associations between islet autoimmunity and any of the detected human viruses were observed. In the second report from the same DIPP Study cohort, associations between islet autoimmunity and the intestinal bacteriome (16S rDNA profiles) and virome (both DNA and RNA viruses) were assessed [70]. Compared to controls, four bacterial operational taxonomic units were less abundant in children who progressed to islet autoimmunity. The concurrent quantitative relation between bacteriophage CrAssphage and prevalent species of the *Bacteroides* genus suggested that CrAssphage might possibly modify the intestinal bacteriome towards dysbiosis characteristic of islet autoimmunity [70].

In another small longitudinal study on young Finns and Estonians, comprising 11 children with T1D-associated autoantibodies and 22 controls, intestinal viromes of the controls had higher diversity and higher frequency of *Circoviridae*-related sequences, when compared to cases [71]. Human enterovirus, kobuvirus, parechovirus, parvovirus, and rotavirus sequences were frequently detected in the samples, but were not associated with islet autoimmunity. After adjusting for age, specific T1D-associated viral bacteriophage contigs could be identified. T1D-associated contigs were statistically linked to specific components of the bacterial microbiome, thus indicating an interplay between the two kingdoms. Results from this study confirmed that certain viral signatures, remarkably consistent across individuals, reflect normal intestinal development, and that the observed age-discriminative contigs are predictive of the developmental changes occurring in the gut microbiome [71]. The partly contradictory findings in these three early studies may, again, be explained by differences in sampling and handling methods, sequencing techniques, or in the identification of the viral sequences. Future larger studies on the role of the intestinal virome in the development of islet autoimmunity will show whether T1D-associated alterations in the intestinal bacterial microbiome are independent phenomena, or a result from earlier changes in the intestinal virome. As bacteriophages can modulate bacterial abundance and colonization, changes in their abundance and diversity might well be able to disturb the intestinal homeostasis and lead to bacterial dysbiosis. On the other hand, there are already indications that certain eukaryotic viruses modify mucosal immunity similarly to commensal bacteria, thus supporting the intestinal homeostasis [72].

4. Intestinal fungal community (mycobiota)

4.1. Mycobiota is an elementary part of the human microbiota

Recent studies of the intestinal microbiome in relation to T1D have largely focused on the role of alterations in the bacterial community. The human intestine, however, harbours a diverse range of bacteria, fungi, viruses, and protista [73,74]. Mycobiota is an important part of the human microbiota, but its role in various diseases is scarcely known. As all human barrier surfaces, including mucosa, are colonized with fungi, alterations in fungal communities may have profound effects on human health. The intestinal mycobiome may modulate the

composition and functions of the microbiota either directly by interacting with microbial organisms or indirectly by modulating the immune system of the host [75]. The development of culture-independent methods for the identification of the fungi has increased the knowledge of fungal colonization in humans and revealed associations between organ-specific diseases and the mycobiome. The modern methods for identifying fungal communities is based on sequencing the 18 s and 28 s sub-units of ribosomal DNA or so called internal transcribed spacer regions (ITS) [76].

4.2. Intestinal mycobiota in healthy humans

Several fungal species have been identified in the gastrointestinal tract of healthy humans, representing approximately 0.1% of the intestinal microbiota [77,78]. Stability of the intestinal mycobiota appears limited, as few fungal species have constantly been observed across different studies. Within the 36 studies analysed for a recent review, only 15 of the identified 267 fungal species were detected in more than five studies [77]. Fungal species most commonly detected belonged to the following genera: *Candida, Saccharomyces, Penicillium, Aspergillus, Cryptococcus, Malassezia, Cladosporium, Galactomyces, Debaryomyces,* and *Trichosporon.* The majority (~75%) of the fungal species were detected only in one study [77].

4.3. Disease associations of intestinal fungi

Even though it seems plausible that mycobiota plays an active role in the maintenance of the intestinal integrity, data on associations between mycobiota and human diseases is scarce. The main findings thus far relate to *Saccharomyces cerevisiae* and Candida species. Overgrowth of Candida spp. has been observed in humans with T1D or inflammatory bowel diseases. Poor glycaemic control may promote fungal overgrowth, and patients with established diabetes are more susceptible to have frequent and prolonged fungal infections [79]. The same holds true for immune compromised individuals [80,81]. In Crohn's disease, increased colonization with Candida species, elevated levels of antifungal-antibodies against *Saccharomyces cerevisiae* (ASCA Ig), and decreased abundance of *Saccharomyces cerevisiae* in the intestinal mycobiome have been observed [82]. Increased Candida colonization in patients with inflammatory bowel disease (IBD) may arise from alterations in the patients' immune system and from the frequent use of anti-inflammatory medications and antibiotics [83]. In addition to patients with IBD or autism spectrum disorders, overgrowth of Candida spp., especially that of *C. albicans*, has been observed in children with clinical T1D [11,83–85]. The idea that intestinal mycobiota has a modifying effect on extra-intestinal immunity has been supported by findings from a mouse model of allergy in which antibiotic-induced intestinal overgrowth of Candida promoted airway inflammation [86,87]. Mycobiota may also play its own important role in promoting the well-being of the mucosal barrier, especially when the bacterial microbiome is disturbed [88,89].

4.4. Mycobiome-bacteriome interactions

Inter-kingdom crosstalk between bacteria and fungi has been observed in patients with Crohn's disease, among whom fungal genera correlated with several bacterial taxa [90,91]. The potency of the mycobiota to regulate the bacterial microbiota is emphasised by findings showing that after antibiotic treatment, the restoration of the bacterial compartment is considerably influenced by colonization with *C. albicans* [91]. Animals treated with cefoperazone and subsequently fed with *C. albicans* had increased level of yeasts accompanied with other alterations in their intestinal bacterial microbiota [92]. In a mouse model of liver injury, administration of *Saccharomyces cerevisiae* var. boulardii changed the composition of the intestinal bacterial compartment by increasing the relative abundance of Bacteroidetes and

decreasing the Firmicutes [93]. The observation that fungal and bacterial communities interact with each other has been established, but the detailed crosstalk networks and their relevance regarding the health of the human host are yet to be more thoroughly characterized.

5. Microbiota in other sites

In addition to the gut, microbes colonize a series of other mucosal and epithelial barriers, such as the oral cavity, airways, skin, and genitalia. Data on the immunomodulatory role of the microbiota residing in other compartments than the intestine is limited. Hu et al. reported that in the NOD mice, specific islet autoimmunity related changes in the intestinal microbiota were predictive for future diabetes, and that changes in the microbiota were more obvious in the intestine than other sites [27]. A Dutch study compared the oral and the intestinal microbiota between adult patients with established T1D and healthy controls [94]. Marked differences were observed in the oral microbiota between patients and control subjects. In the oral microbiota, the abundance of the genera *Streptococcus, Rothia,* and *Actinomyces* were higher in cases, among whom there was also a decreased abundance of butyrate producing intestinal bacteria.

Microbial communities residing in other niches than the intestine may have a significant role in other immune-mediated diseases, such as anelloviruses in the development of asthma [95] or skin microbiota in the management of atopic dermatitis [96], but regarding the development of islet autoimmunity, gut with its microbiota seems to be the major early modifier of the human immune system.

6. Crosstalk between microbiota and the host

Data on microbiota and its modulatory effects on the host has expanded rapidly during the last five years and will be discussed elsewhere. However, to summarize the big picture, current discussions circle around three main categories: the known known, the known unknown, and the unknown unknown. The first category comprise data on the crosstalk between microbiota and various organs and tissues, for example brain [97], adipose tissue and gut [98], muscles [99], and liver [100]. Until future studies provide further knowledge, the unknown can only be deduced by exclusion. For example, we are still lacking data on the importance of the unidentified reads commonly detected in the microbiota studies; the interactions between all molecules in a particular cell (interactomes) and in wider systems of interacting molecules and cells (meta-interactomes); the crosstalk between various kingdoms of the microbiota, and details on communication networks between various organs and their microbiota.

7. Dysbiosis and diabetes –contemporary or causal phenomena?

Even though reports on the T1D-related changes in the gut microbiota are accumulating, proving the causality has remained challenging. Causality requires that when all possible confounders have been controlled for, *ceteris paribus* (other things being equal), change in one variable should lead to change in another variable in a repeatable and generalizable way [101]. As long as no randomized, controlled, placebo-controlled intervention studies showing long-term changes in the intestinal microbial colonization and in the immunological maturation that lead repeatedly to comparable changes in the T1D-associated outcomes have been performed in man, causality between dysbiosis and T1D remains open.

8. Hypothesis and future directions

Based on current knowledge, we hypothesize that intestinal microbiota may contribute to the development of T1D via a two-phased process. The first phase of the process starts at birth and ends with the appearance of the first T1D-associated autoantibodies. During that

phase, a successful training of the developing immune system is required in order to establish self-tolerance and to control inflammatory responses. If during that phase the gut microbiome tilts towards disproportion between the abundance of *Bacteroides, Bifidobacteria,* and *Esotericia coli*, the maturation of the immune system becomes distorted and susceptibility to immune-mediated diseases increases. The second phase from seroconversion to overt T1D seems to be characterized by a reduced microbial diversity and a proinflammatory intestinal dysbiosis. The mechanisms behind the spreading of the relatively local intestinal inflammation towards extra-intestinal autoimmunity remains to be explored in the future.

The challenge for the near future is to identify the causal relationships between intestinal microbiota and T1D in man. This will require extensive longitudinal studies with optimal methodology for collecting, processing and storage of the stool samples. In addition, standardized and reproducible methods for DNA and RNA extraction and sequencing, for stool transcriptomics, metabolomics and metaproteomics, and for bioinformatics are needed. We are only at the beginning of learning about the role of the intestinal microbiome in the development of T1D and other immune-mediated diseases. Further efforts should be directed to studies on the role of the intestinal mycobiota and virome, their interplay, and their interaction with the host and other microbes.

9. Search strategy and selection criteria

Data for this review were identified through PubMed and references from relevant articles using the search terms "microbiome", "microbiota", "mycobiota", "virome"," type 1 diabetes", and" dysbiosis". Articles between 2014 and 2018 were included in the initial analysis, with the exceptions of mycobiota and other topics for which recent data were scarcely available.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Acknowledgements

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ebiom.2019.06.031.

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