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Review

Beyond genetics. Influence of dietary factors and gut microbiota on type 1 diabetes

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ABSTRACT

Type 1 diabetes (T1D) is an autoimmune disease ultimately leading to destruction of insulin secreting β-cells in the pancreas. Genetic susceptibility plays an important role in T1D etiology, but even mono-zygotic twins only have a concordance rate of around 50%, underlining that other factors than purely genetic are involved in disease development. Here we review the influence of dietary and environmental factors on T1D development in humans as well as animal models. Even though data are still inconclusive, there are strong indications that gut microbiota dysbiosis plays an important role in T1D development and evidence from animal models suggests that gut microbiota manipulation might prove valuable in future prevention of T1D in genetically susceptible individuals.

1. Introduction

Type 1 diabetes (T1D) or insulin-dependent diabetes is an autoimmune disease ultimately leading to destruction of insulin secreting β-cells in the Langerhans islets within the pancreas [1]. With destruction of the β-cells the body losses control of blood glucose levels leading to hypoglycaemia, ketoacidosis and with time blindness, renal failure, and cardiovascular disease [1,2]. If left untreated T1D is deadly and even with well-managed insulin replacement T1D will still shorten life-expectancy with as much as ≈10 years [1,3]. Millions are diagnosed with T1D worldwide, and in general incidence rates are increasing exerting pressure on health and welfare systems [4], though this increase seems to level of in some countries [5,6].

The exact cause(s) of T1D development are not completely understood, but appears to be a combination of genetic predisposition and one or several environmental events [1]. A long list of genetic loci predisposing for T1D development have been identified, with specific HLA (Human Leukocyte Antigen) genotypes being the strongest identified genetic factor [1,7–9]. However, genetic predisposition is not the only factor leading to development of T1D. Twin studies have shown that for di-zygotic twins the pairwise T1D concordance rate is *ca.* 10%, and even for mono-zygotic twins the concordance rate is only around 50%, while the incidence for individuals without first degree relatives affected by T1D has been estimated to be around 0.4% [9,10], though with very large regional differences [11–13]. Further, the T1D incidence in Finland is 6 times higher than on the other side of the border in Russian Karelia, even though the predisposing HLA genotypes are equally frequent between the two populations [14] and immigrant studies have shown that the offspring of immigrants tend to approach the "risk profile" of the country they moved to [15–20], underlining that more than purely genetic factors drive T1D etiology (Fig. 1).

2. Dietary factors influencing T1D incidence and development

2.1. Infant feeding

The possible protective role of breastfeeding on T1D remains an unsettled issue, as some studies have shown a protective effect while others show no effect [21–25]. However, a recent meta-analysis of 43 studies found that breast-feeding tends to offer some, although limited protection against T1D development [26]. Similarly, early exposure to cow's milk protein (i.e. through infant formula) has in some studies been found to increase the risk of developing β-cell immunity and later T1D [15,22,23,27], but the results remain somewhat contradictory, as other studies found

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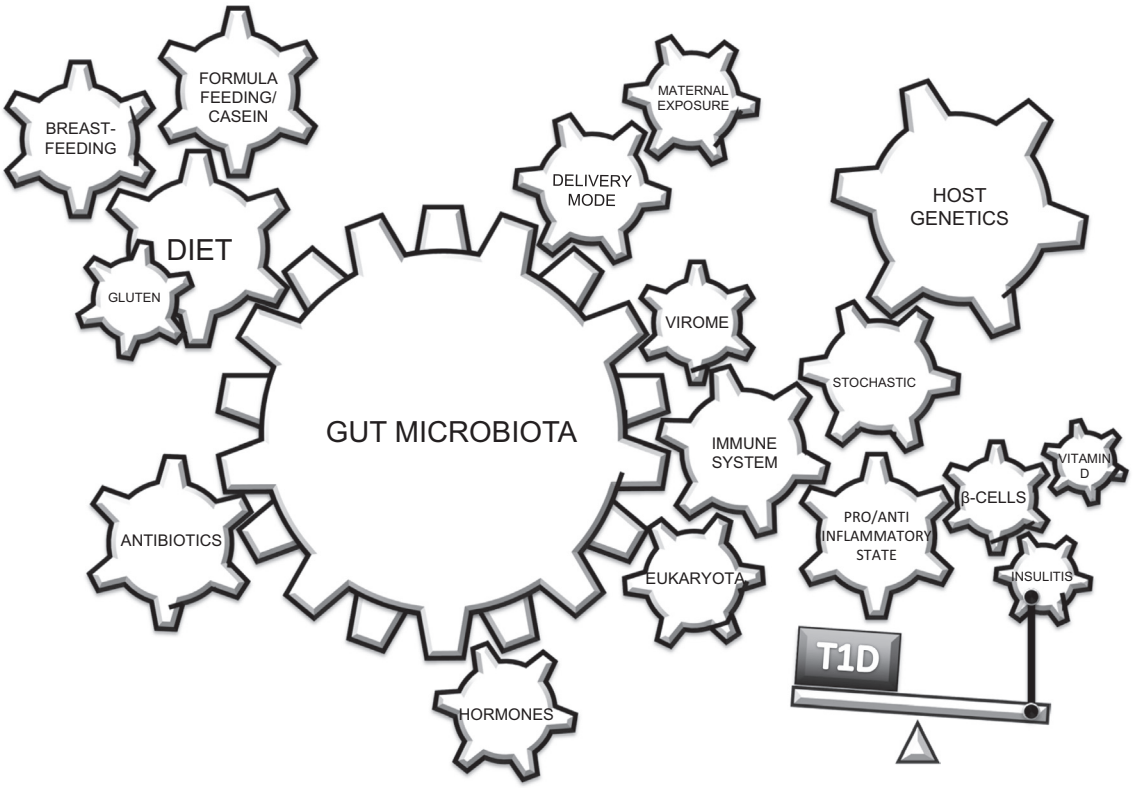

Fig. 1. The exact etiology of type 1 diabetes is still unresolved, but involves genetic as well as environmental factors. A range of possible disease drivers, many of which seem to be mediated via the gut microbiota, have been identified. See text for further details.

no effect [15,25,28,29]. However, recent studies using highly hydrolysed casein formula have showed promising results lowering T1D incidence in non-obese diabetic (NOD) mice, a mouse strain that spontaneously develop leukocytic infiltrations of pancreatic islets that subsequently develops into T1D with varying onset time and incidence depending on environmental factors [30,31]. Further, in humans, highly hydrolysed casein reduced the cumulative incidence of one of more T1D-associated antibodies with more than 50% in young (*ca.* 5 years) genetically predisposed children [32]. Knip et al. [32] speculate that the protective effect of the extensively hydrolysed casein formula could be due to (1) elimination of intact bovine insulin (that is present in cow's milk) by the protein hydrolysis carried out during the preparation of the formula; (2) decreased gut permeability, possibly mediated by the many short peptides present in the extensively hydrolysed formula, leading to less foreign peptides and proteins migrating into the gut associated lymphoid tissue (GALT) in the lamina propria, leading to better regulation of the gut associated immune system; (3) induced maturation of regulatory T cells, through an yet unknown mechanism; and/or (4) changed gut microbiota (GM) composition, that again influence immune system development [32]. A recent Finnish study seems to confirm the protective effect of removing bovine insulin from cow's milk based infant formula, as children fed bovine insulin free formula had significantly lower cumulative incidence of β-cell autoimmunity at the age of 3 years compared to children fed traditional whey based infant formula [33].

After weaning, excessive intake of cow's milk (more than 540 g/day) has been linked with a significantly increased risk of developing T1D, especially in children with HLA-DQB1 genotypes conferring an increased risk of T1D development [34]. The exact cause is not known, but large intake of casein has been associated with high T1D incidence in NOD mice [35] and the per capita consumption of casein variants A^1 and B has been linked with T1D incidence [36]. Possibly because casein variants A^1 and B yield β-casomorphin-7 when enzymatically cleaved, while casein variant A^2 does not, due to a slightly different amino acid composition. β-Casomorphin-7 has *in vitro* been shown to inhibit intestinal lymphocyte proliferation and is speculated to have immunosuppressive effects [36]. However, the actual risk associated with milk intake in terms of T1D development is far from elucidated as conflicting results have been reported [37]. Several studies report that milk intake correlates positively with β-cell autoimmunity and T1D incidence rates in children [36,38,39], but also studies showing no effect has been published [37,40].

2.2. Cereals

Early weaning and introduction of solid foods [41] and especially gluten containing cereals [42,43] has been associated with development of T1D associated autoimmunity, though other studies did not find the same relationship [28]. According to Norris et al. both early (before 3 months of age) and late (after 7 months) introduction to cereals were associated with an increased hazard ratio for developing islet autoimmunity [43], indicating that timing is important possibly due to interference with the developing immune system in the infant. In a more recent study, delaying gluten exposure from 6 months, as officially recommended in Germany, to 12 months did not have any influence on the risk of developing islet autoimmunity at the age of 3 years [44,45]. However, in animal models exposure to gluten has been found to have profound impact on T1D incidence. NOD mice fed a gluten-free diet, where cereal based protein have been replaced with meat-based protein, have a 4-fold lower diabetes incidence (15% vs. 64%) compared to mice fed a standard (gluten-containing) diet [46]. Further, compared to a gluten-free diet (with soya replacing wheat as

protein source), a wheat-based diet was found to induce a Th1-type, proinflammatory cytokine-bias in the gut of NOD mice [47]. Also in fully immune-competent BALB/c-mice a gluten-containing diet (same diet as in [46]) has been found to alter the cytokine-profile of lymphoid Foxp3$^-$ and Foxp3$^+$ regulatory T cells in a pro-inflammatory direction and in general result in a decreased proportion of γδT cells in lymphoid tissue compared to a gluten-free diet [48,49]. Similarly, in diabetes-prone BioBreeding rats (BB-DP) a cereal containing diet induces Th1 cytokine-bias with up-regulation of the proinflammatory cytokine *Ifng* [50]. Gluten (and cereal) free diets strongly influences GM composition compared to a standard, cereal-based chow diet [51–53] and it can been speculated that the protective effect on T1D development is due to diet-mediated changes of the GM which in turn influence immune system maturation and function, though dietary components themselves probably also play an important role, as a cereal free diet has been found to protect germ free mice against T1D development [50,52]. In an interesting case-study a 6 years old boy newly diagnosed with T1D was administered to a strict gluten-free diet which stabilized HbAc1 and fasting glucose levels within the non-diabetic range without the need for insulin treatment [54]. After having had T1D for 3 years he still does not need insulin regularly.

However, whether it is gluten *per se* that initiates the process leading to T1D in the animal models (as well as humans?) or whether other cereal (/wheat) dietary components might influence the picture is not fully elucidated. Surprisingly, it has for instance been found, that not only a gluten-free diet but also a diet enriched with purified gluten protects NOD mice against T1D development [55] compared to the standard Altromin diet used in [46,48,49]. A recent study on the other hand reported that when purified gluten was supplemented to a gluten-free diet, the T1D protective effect was abolished [52]. The finding that up to 10% of individuals diagnosed with T1D also have celiac disorders [56] does however indicate that gluten is likely to play a role T1D development, at least in some individuals [54].

2.3. Other major dietary groups

The average per capita intake of meat [39,40] and fruit and berry juices [38] has been found to correlate positively with β-cell autoimmunity. However, a later study could not confirm the association between meat consumption and β-cell autoimmunity [38] and possibly the associations are confounded by other factors.

2.4. Vitamin D

In Europe there is a North-South gradient in T1D incidence, with Finland in the north having the highest incidence worldwide, and more southern regions generally having lower incidence, though with exceptions such as Sardina, where incidence is very high, despite its geographical position in the southern part of Europe [11,13,57]. This has been ascribed to many factors including genetics, but also vitamin D deficiency has been speculated to play a role. The human body is able to synthesize vitamin D, but this requires the exposure of the skin to UV-light from the sun [58], putting the population in countries with only little daylight during winter time at risk of vitamin D deficiency [58]. *In vitro* vitamin D has been found to protect rat pancreatic islet β-cell function against interleukin-1β-induced inhibition [59] and *in vivo* administration of high doses of vitamin D has also been found to protect NOD mice against T1D development, but only if administered throughout life, while administration of vitamin D during pregnancy/lactation and early in life (3–14 weeks of age) had no protective effect [60]. Vitamin D supplementation in early childhood has been associated with significantly lowered T1D incidence [61] with e.g. Hyppönen et al. reporting that both regular and irregular intake of vitamin D supplementation reduced the T1D incidence with more than a factor 6 among Finnish children. However, it should be noted, that the population living in Russian Karalia neighboring Finland and with the same genetic T1D susceptibility has as mentioned previously approximately one-sixth the risk of developing T1D as Finns, despite the fact that their circulating vitamin D concentrations are almost similar [14,62]. Further, a recent study found no correlation between vitamin D status and increased risk of β-cell autoimmunity [63] leaving the influence of vitamin D on T1D incidence open for debate.

2.5. Other food related factors

Nitrite and nitrate has also been linked to T1D development in several studies [64,65]. Nitrate is found in many vegetables and depending on local conditions also in drinking water, while both nitrite and nitrate is found in meat products, such as sausages, where they are used to stabilize color and add an extra barrier towards the growth of *Clostridium botulinum* [66]. In case-control studies intake of nitrite and nitrate from food and drinking water has been linked to T1D incidence [64,65]. It is not the two compounds themselves that constitute a risk in terms of T1D development, but in the gut they may react with amines and amides in a process mediated by gut microbes and form toxic nitrosamines and nitrosamides [67,68]. Also the pH of drinking water has been directly linked with T1D development in NOD mice, with mice receiving acidified drinking water developing T1D faster and with a significantly higher incidence compared to mice receiving drinking water with a neutral pH [69].

Food and beverages might also indirectly become sources of pollutants such as the endocrine disruptor bisphenol A, used in food and beverage containers made of polycarbonate plastic. Through leaking from the packaging material food and beverages are the major sources of human exposure to bisphenol A. Though no direct link between bisphenol A exposure and T1D development has been established in humans, bisphenol A exposure has been linked to allergic asthma in children, modulate the immune system in mice models, and both perinatal and postnatal exposure has been shown to accelerate T1D development in NOD mice [70–72].

3. Exposure to microbial agents

The intriguing observation that there is an inverse relationship between the incidence of a wide range of infectious diseases (measles, mumps, rheumatic fever etc., incidence all decreasing) and diseases related to immune disorders (asthma, multiple sclerosis, T1D etc., incidence all increasing) led to the development of the so-called hygiene hypothesis [13,73,74], basically stating that due to better hygiene and health care systems we are less exposed to infectious agents, symbiotic microorganisms, parasites and allergens during childhood which influences immune system development leading to among other things an altered Th1/Th2 balance, which again might render us more susceptible to autoimmune diseases [75,76].

The above mentioned 6-fold difference in T1D incidence between Russian Karelia and Finland is one of several examples speaking in favor of environmental factors possibly linked to different hygiene levels and risk of infections playing an important role in T1D development [13,75].

3.1. Helminths

Decreasing incidences of helminth infections as a result of better hygiene conditions have been speculated to play a role in the increasing T1D incidence observed worldwide. Gale (2002) argue

that the decreasing rate of children infected with the pinworm *Enterobius vermicularis* correlates well with the simultaneously increasing T1D incidence in industrialized countries [77]. In addition to pinworms, also the helminth parasites *Heligmosomoides polygyrus*, *Trichinella spiralis*, *Litomosoides sigmodontis* and *Schistosoma mansoni* either completely inhibit or significantly slow T1D development and lower incidence in NOD mice [77–80]. In the case of *H. polygyrus* inoculation at 5 weeks of age protected entirely against T1D development, but even inoculation as late as 12 weeks of age significantly delayed onset time and reduced T1D incidence [79]. The protective effect is generally believed to be mediated through a shift in the Th1/Th2-balance, towards a more pronounced Th2-type response and with increased numbers of splenic regulatory T-cells [78–83]. However, recent findings from studies carried out in IL-4 deficient NOD-mice suggest that protection against T1D is independent of a Th2 shift, but requires secretion of the regulatory cytokines IL-10 and TGF-β [83,84]. No human studies investigating the potential of using helminths as "vaccines" against T1D development have been published to date, but for inflammatory bowel disease and multiple sclerosis pilot clinical trials have shown promising results [81,85,86]. The use of helminths in profylatic treatment of T1D is potentially promising, but not without concerns in terms of possible negative side effects [83]. Helminths cause chronic IgE-mediated activation of basophils and mast cells. In a proof of concept study it has been shown that instead of infecting with live helminths, protection against T1D development in NOD mice can also, at least to some extent be achieved by mimicking helminth infection through injection with anti-FcεR1 antibodies binding to the FcεR1 IgE receptor on basophils and mast cells thus releasing IL-4 and delaying T1D onset [87] pointing at a strategy for obtaining the protective effect of helminth infections, without the need for infection with actual helminths.

3.2. Viruses

Onset of T1D follows a seasonal pattern, with higher onset incidence during Autumn and Winter which already in 1926 led Adams to suspect a viral cause of T1D [88,89]. A range of viruses have been implicated in T1D etiology, but in most cases the associations were found to be weak or irreproducible in follow-up studies as recently reviewed [88]. However, in the case of single-stranded RNA enterovirus of the *Picornaviridae* family, the association between viral infection and T1D incidence seems rather strong. In a recent meta-analysis enterovirus infection was associated with T1D related autoimmunity at an odds ratio of 3.7 and with clinical T1D at an odds ratio of 9.8 [90]. Similarly, a Finnish study has also shown that enteroviral RNA is found in diabetics significantly more frequent than in both celiacs and healthy controls [91]. Further, it was found that the infection was prolonged or persistent in many of the T1D patients and that the infection was associated with gut mucosa inflammation, though a later Italian study was not able to confirm this finding, as they were unable to detect enterovirus in small intestinal biopsy samples from neither T1D patients, nor healthy controls [92]. Also perinatal exposure to enterovirus is possibly a risk factor, as a Swedish study found increased prevalence of enteroviral RNA in the blood of newborns who later developed T1D, while the prevalence of cytomegalovirus (CMV) and parvovirus B19 was similar between newborns who later developed T1D and healthy controls [93]. The presence of enteroviral RNA in blood has been linked with a particular, T1D-associated genotype of the *IFIH1* gene encoding interferon-induced helicase C that senses double-stranded RNA of the *Picornavirales* virus family including enterovirus [94]. During the first couple of years after birth there is also a high risk of enterovirus exposure with for instance 29% of a cohort of genetically T1D susceptible Finnish children aged 2 years or younger being positive [95], but the implications in terms of T1D development (if any) does probably not manifest until later in life, as a German study found no correlation between enterovirus infections during the first year of life and development of islet antibodies [91,96]. Another recent German study showed that also respiratory infections during the first year of life is associated with increased risk of islet antibody sero-conversion [97]. Furthermore, Encephalomyocarditis virus (EMCV), which is a picornavirus, induces fulminant type 1 diabetes in specific inbred strains of mice [98], and milder diabetes in Syrian hamsters [99] and Mongolian gerbils [100].

3.3. Gut microbiota

Recent years development within mainly high throughput sequencing technologies have enabled hitherto unseen detailed characterization of the human GM establishing links between GM and a range of disease conditions such as type 2 diabetes [101,102] and autoimmune diseases like atopic dermatitis [103]. The possible involvement of the GM in T1D development is receiving increasing attention and several lines of evidence not only suggest that GM is an important factor in the progression towards T1D, but also that GM manipulation offers possibilities for delaying and perhaps even preventing T1D development [104–106].

The human gastrointestinal (GI) system harbours a complex and dynamic consortium of 10–100 trillion microorganisms encoding as much as 100-fold more unique genes than the human genome itself. The genetic potential of the microbial inhabitants of our GI-tract is consequently massive and strongly influences human health and disease [107]. GM composition and development is determined in a delicate interplay between genetic and environmental factors [108–110] that we are only beginning to understand. *In utero* the GI tract is sterile, but during and after birth it is rapidly colonized. Mode of birth (vaginal birth vs. caesarean section (CS)) and feeding (breast feeding vs. bottle feeding) are both important drivers of GM development [108,109,111–114]. Initially the GM is dominated by oxygen tolerant species (e.g. staphylococci and Enterobacteriaceae), before obligate anaerobes (bifidobacteria, clostridia, eubacteria) take over and after a couple of years the child GM tend to approach the composition of the adult gut [108,109,115,116].

Several studies report that the GM differs between children with β-cell autoimmunity [117] or T1D and healthy, age and genotypically matched controls [118–121]. A recent German study comparing GM development in genetically at risk children up to the age of 3 years rather than identifying a particular GM compositional fingerprint associated with anti-islet cell autoimmunity, instead identified substantial alterations in microbial interaction networks between anti-islet antibody positive children and genetically and age-matched healthy controls [122]. In all cases the investigated cohorts are rather small (between 3 and 22 diabetics/individuals with β-cell autoimmunity) and even though they all provide valuable findings larger cohort studies investigating the link between GM and T1D development are highly needed. Several studies [117,120,121] indicate that β-cell autoimmunity and T1D is associated with a lower overall GM diversity, though in [122] no significant difference in GM diversity was found between children who developed anti-islet cell autoimmunity and healthy controls. Low GM diversity has also been linked to atopic dermatitis [103], another autoimmune condition, and low overall GM genetic diversity ("low gene count") has been linked to metabolic syndrome [123]. Birth by CS is associated with an increased risk of T1D development [124]. Recently, birth by CS has been linked with reduced GM diversity, and especially within the Bacteroidetes phylum infants born by CS had significantly lower diversity compared to infants born vaginally. Further, the

infants born by CS had significantly lower blood concentrations of the Th1 associated chemokines CXCL10 and CXCL11, while no significant differences where observed for the Th2 associated chemokines CCL17 and CCL22. Differences in GM composition between children born vaginally and by CS has been shown to persist up 7 years of age [125].

In the investigated Finnish and Spanish co-horts the abundance of *Bacteroides* is in general higher in cases compared to controls, with for instance *Bacteroides ovatus* and *Bacteroides uniformis* being associated with autoimmunity, while *Bacteroides fragilis* on the other hand seems to play a protective role [117,118,120,121]. However, no differences in *Bacteroides* abundance between children who developed anti-islet cell autoimmunity and healthy controls were observed in a recent German study [122]. Bifidobacteria, butyrate producers such as *Faecalibacterium* and *Roseburia* and mucin degraders like *Prevotella* and *Akkermansia* all constitute a larger proportion of the GM in healthy controls compared to cases indicating that they might play a protective role [117,118,120,121,126,127], though [120] could not confirm the tendency for *Prevotella* and [122] as mentioned above rather than identifying particular taxonomic units differing between children who developed anti-islet cell autoimmunity and healthy controls, instead identified substantial microbial interaction network disturbances in the anti-islet antibody positive children. The reason behind the possibly protective role of bifidobacteria is not clear, but de Goffau et al. [117] suggest that they reduce growth of e.g. *Bacteroides* members and/or reduce their translocation over the epithelium reducing inflammation. Butyrate is an inducer of mucin production [128,129]. In line with this, Brown et al. [118] suggests, that a GM rich in butyrate producers leads to increased mucin production, more tight junctions and increased gut integrity. The increased mucin production then creates a favorable niche for mucin degraders (*Prevotella*, *Akkermansia*), and [118] suggest that mucin degraders possibly can be used as indicators of gut integrity. In this context it is interesting to note, that even though not reported as significantly different, phylum Verrucomicrobia (of which *Akkermansia muciniphila* is the only known gut microbiota associated member [130]) has been found to be more abundant in children that did not develop anti-islet autoimmunity, compared to those that did, at all time points from 6 months of age to 3 years [122]. Metabolome studies support, that GM differs between T1D patients and healthy controls, as a range of metabolites of gut microbial metabolism differs between the 2 groups [131,132].

Evidence from animal models supports that GM plays an important role in T1D development. Bio-breeding diabetes-prone (BB-DR) rats have a GM significantly different from the GM of bio-breeding diabetes-resistant (BB-DR) rats, with the BB-DP rats having lower GM diversity, especially later in life (70 days), more *Bacteroides* and less *Bifidobacterium* compared to BB-DR rats [133], which by large corresponds well with the picture seen in humans [117,118,120]. Similarly, NOD mice that develop diabetes have a different GM already at weaning compared to NOD mice that do not develop T1D. This difference in GM composition persists into adulthood, where NOD mice that has developed T1D has a GM different from NOD mice that had not developed T1D up to 30 weeks of age [51 and own data, submitted for publication].

In some cases germ-free rearing has been found to exacerbate T1D development in NOD mice, but newer studies report divergent results. According to King and Sarvetnick [134] do germ-free mice not have higher T1D incidence compared to conventional mice, but a restricted gut microbiota offers some protection. Alam et al. [135] report that diabetes develops with equal incidence under both germ-free and specific pathogen free (SPF) conditions, but with significantly higher insulitis scores under germ-free conditions. The lacking GM results in an altered immune regulation in the colon, the mesenteric and the pancreatic lymph nodes with increased levels of IL17 and less FoxP3 cells which may explain the higher insulitis scores [135].

Wen et al. [136] showed that a specific protein, MyD88, involved in recognising microbial stimuli in the gut is essential for T1D progression in NOD mice. MyD88-knockout NOD mice were almost completely protected from developing T1D, whereas heterozygous MyD88$^{KO/+}$ NOD mice developed T1D. Interestingly, when reared under germ-free conditions, the MyD88-knockout NOD mice develops robust insulitis, while colonization of the germ-free MyD88-knockout mice attenuated T1D development. In a recent study it was found that not only MyD88, but also TLR3 is critical for T1D development in the RIP-B7.1 diabetes mouse model, confirming the important role of receptors recognising microbial stimuli in the gut [137].

T1D incidence in NOD mice has a strong gender bias, with female mice having higher incidence compared to male mice [138,139]. At weaning the male and female GM does not differ, but when the mice reach puberty, the GM and metabolome differs between sexes [138,139]. SPF male mice had significantly higher testosterone levels compared to SPF female mice, but when reared under germ-free conditions, male mice had comparable lower and female mice higher testosterone levels, indicating that a "sex specific" GM influences testosterone levels [139]. Transfer of adult male GM to young females altered the GM of the recipients, elevated testosterone levels, changed the metabolome and conferred protection against T1D [139]. Further, if castrated, the male GM does not differ from the female mice and their insulitis score is comparable to female mice [138]. Yurkovetskiy et al. [138] suggests a positive feedback mechanism, where hormones influence GM that again influence the hormone balance; a hypothesis overall in agreement with the finding in [139]. Also in adult humans, gender specific differences in blood metabolome has been identified [140] and a range of autoimmune diseases has a gender bias towards woman having higher incidence, but not in the case of T1D, where men and woman have approximately the same risk (with males even having a slightly higher risk in adulthood) [141]. Nevertheless, the findings in [138,139] underline that GM strongly influences host and T1D development and that disease development can be prevented or at least postponed by GM manipulation.

The influence of GM on T1D is further augmented by the finding that the presence or absence of segmented filamentous bacteria (SFB) in the gut has profound impact in T1D incidence in NOD mice [142]. SFB strongly influence host immune system development influencing T helper cell maturation and inducing intestinal Th17 cells [143–145] and has been found to be associated with protection against T1D development in female NOD mice (male NOD mice had low incidence regardless of SFB status) [142]. The exact mechanism behind the possible protective effect of SFB on T1D development in NOD mice has not been elucidated but Kriegel et al. [142] hypothesize that possibly the SFB induces a robust population of intestinal Th17 cells protecting against islet destruction by inhibiting the Th1-response, though this notion remains debateable [146]. However, in another study female NOD mice mono-colonized with SFB had a similar diabetes incidence as the germ-free mice [138]. No matter the exact role of SFB in protecting female NOD mice against T1D it should be noted that different vendors and experimental animal facilities differs with respect to gut microbiota composition, including SFB status [142,145], which probably explain the large differences in NOD mice T1D incidence occasionally reported.

3.4. Gut microbiota manipulation

Antibiotic treatment reduce diabetes incidence in both BB-DP rats and NOD mice [104,147,148]. In BB-DP rats, treatment with

a mixture of sulfamethoxazole, trimethoprim and colistine sulfate from weaning significantly reduced T1D incidence [147]. Exchanging the conventional plant-based diet, with a diet where hydrolysed casein was the sole protein source also offered some protection and if the hydrolysed casein diet was combined with antibiotic treatment no rats developed T1D [147]. A later study in NOD mice underlines that timing is important, as treatment with vancomycin from birth to weaning significantly reduced T1D incidence, while treatment only during adulthood did not offer the same protection [104]. Vancomycin treatment resulted in a switch from a GM dominated by Firmicutes and Bacteroidetes to a GM dominated by *A. muciniphila* (Verrucomicrobia) and to a lesser extent Proteobacteria [104]. The mechanism behind the protective effect of vancomycin treatment is not known, but it can be speculated, that the abundance of the mucin degrader *A. muciniphila* leads to an increased metabolisation of mucin, which might increase the possibility for other Gram-negative bacteria (like Proteobacteria) or ligands therefrom (e.g. lipopolysaccharides, LPS) to get in contact with intestinal immune cells at a critical stage during immune system maturation stimulating e.g. TLR-4, known to play a role in protection against T1D development [104], though other mechanisms are also possible. Humans diagnosed with T1D have a slightly decreased natural killer cell expression of NKG2D and also NOD mice have an altered natural killer cell NKG2D expression profile [149,150]. Intestinal epithelial cell expression of NKG2D has to date not been implicated in T1D etiology, but given that a receptor like My88D involved in recognition of intestinal microbial stimuli has a profound impact on T1D development in NOD mice, it is of interest, that vancomycin treatment has been found to decrease the expression of NKG2D ligands on intestinal epithelial cells [151]. Interestingly, propagation of *A. muciniphila* and decreased NKG2D ligand expression could also be achieved through feeding with dietary xylosaccharides as well, showing that also through a dietary intervention is it possible to influence gut epithelial ligand expression and increase *A. muciniphila* in the GI tract [151].

Recently, Sofi et al. [69] showed that female NOD mice receiving acidified water (pH 3.0–3.2) compared to neutral water (pH 7.0–7.2) markedly change GM composition and increase T1D incidence. Switching from acidified water to neutral water lowered abundance of *Bacteroides* but where in general associated with an increase in GM diversity, which corresponds well with previous findings indicating that low GM diversity is associated with autoimmunity and T1D [117,120,121]. Colonisation of mice receiving acidified drinking water with SFB lowered T1D incidence, while SFB colonisation did not influence T1D incidence in mice receiving neutral pH water [69], indicating that SFB influences T1D incidence in NOD mice, but only in collaboration with specific gut microbiotas.

Switching from a conventional chow-based diet to a gluten-free diet significantly reduces T1D-incidence in NOD-mice and is associated with pronounced GM differences between the 2 feeding regimes [46,51,52], with the gluten-free diet promoting *A. muciniphila*, while the gluten-containing chows were associated with increased *Tannerella*, *Barnesiella* and perhaps more surprisingly bifidobacteria [52], which also contradicts findings from our laboratory, showing that a gluten-free diet is associated with higher GM prevalence of bifidobacteria (own results, submitted for publication). Similar results have been obtained in BB-DP rats, where feeding a diet with hydrolysed casein as protein source resulted in significantly lower T1D incidence in both germ-free and SPF animals, compared to being fed a cereal-based, gluten-containing diet [50]. In a recent study, we show that maternal feeding with a gluten-free diet significantly reduces T1D incidence in the offspring (NOD mice). Pregnant NOD mice were fed either a gluten-free or a standard chow diet, until the pups were weaned to the standard diet – meaning that the pups were exposed to the effect of a gluten-free diet via cohousing with the mother. This resulted in pronounced GM differences between the 2 feeding regimes (also in the pups at weaning), with the gluten-free GM being characterized by increased *Akkermansia*, Proteobacteria, and TM7. Pancreatic FoxP3 regulatory T cells were increased in gluten-free fed offspring, while intestinal gene expression of proinflammatory cytokines was reduced. An increased proportion of pancreatic T cells expressing the mucosal integrin $\alpha4\beta7$ indicates the T1D protective mechanism involve increased transport of gut-primed immune cells to the pancreas [53]. Whether the protective effect of a gluten-free diet is transferable to humans is far from given, as the importance of gluten in human T1D etiology is still up for debate [43–45]. But a case report indicating that switching to a gluten-free diet as diabetes is diagnosed at least delays and possibly even prevents disease progression [see 54 and discussion above] warrants further studies. Furthermore, the findings that probiotic administration [152] and GM manipulation through diet [50,52], pH of drinking water [69], and antibiotics [104] prevents or delays T1D onset in experimental animals point towards the gut as a promising target for T1D prevention in individuals genetically at risk.

4. Conclusion

The drivers of T1D development are far from identified, but a range of candidates have been identified, including dietary habits (breast feeding vs. infant formula, highly hydrolysed infant formula vs. conventional infant formula, early/late exposure to gluten, vitamin D deficiency etc.), exposure to certain viruses and helminths. What unifies these candidates (with the exception of vitamin D) is that their effect one way or the other is mediated via the gut. The importance of our GI system in T1D etiology is further augmented by the differences in GM composition and gut microbial networks observed between individuals diagnosed with T1D/β-cell autoimmunity and healthy controls and perhaps even more importantly the increasing number of animal studies providing proof of concept of how GM manipulation can be used to prevent or reduce T1D incidence. Time is up for carefully designed, longitudinal studies with adequate power first establishing in greater detail how GM, metabolome, immune system development, and autoimmunity/T1D are connected and whether "early warning GM patterns" can be identified. In individuals at risk, it then becomes highly relevant to develop tools for directing the GM in a desired direction away from the "T1D risk profile" using appropriate means, be it dietary intervention, targeted use of antibiotics or possibly by using bacteriocin-producing bacteria [153] or phage cocktails [154] targeting unwanted GM members.

Conflict of interest

The authors declare no conflict of interest.

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