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Guts, Germs, and Meals: The Origin of Type 1 Diabetes

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Abstract Type 1 diabetes mellitus (T1DM) is due, in part, to non-genetically determined factors including environmental factors. The nature of these environmental effects remains unclear but they are important to identify since they may be amenable to therapy. Recently, the gut microbiota, the trillions of microorganisms inhabiting the gut, as well as diet, have been implicated in T1DM pathogenesis. Since dietary changes can reshape this complex gut community, its co-evolution could have been altered by changes to our diet, agriculture, personal hygiene, and antibiotic usage, which coincide with the increased incidence of T1DM. Recent studies demonstrate an association between altered gut microbiota and T1DM in both T1DM patients and animal models of the disease. Further studies should provide new insight into those critical host-microbial interactions, potentially suggesting new diagnostic or therapeutic strategies for disease prevention.

Keywords Microbiome · Virome · Enterotypes · Type 1 diabetes · Autoimmunity · Autoimmune disease · NOD mice · Toll-like receptors · Innate immune response · Guts · Germs · Meals

Introduction

Type 1 diabetes mellitus (T1DM) is due to the interaction of genetic and non-genetically or epigenetically determined factors leading, through an altered immune effector response, to destruction of insulin secreting beta-cells. Increasing evidence suggests the important role of those non-genetic factors including environmental factors. To prevent or cure this autoimmune disease, we must understand these factors since these are most likely amenable to therapy. Genetic associations [1•, 2] are reflected in the high risk of T1DM in monozygotic co-twins and non-diabetic siblings, with both HLA-DR3/4 and autoantibodies [3–5]. However, these genetic associations, including HLA alleles (the most important genetic risk) and up to 20 other genes, may account for no more than 50 % of the disease risk though that genetic risk declines with increasing age at diagnosis [3, 4, 6]. The importance of non-genetic factors is well recognized but the precise character of these factors has not been identified, though viruses and gut-related factors have been implicated. The purpose of this review is to discuss the potential role of the gut microbiota in predisposing to T1DM, where the microbiota refers to microorganisms in the gut, and their microbiome refers to their genomes or the activity of those genomes.

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Non-Genetic Factors Impact T1DM Pathogenesis

T1DM cannot be explained by genetic factors alone. Twin, migration, population, and birth-cohort studies emphasize the importance of environmental factors operating in early childhood when disease-predictive autoantibodies appear [1•, 2–6]. About 40 % of identical twin pairs are discordant for the disease, when the index twin is diagnosed under the age of 10 years, that is, 1 twin has T1DM and the other does not. That twin discordance becomes even more striking the older the age of diagnosis of the index twin, so that when the index twin is diagnosed after 25 years of age the discordance rate is 80 % or more [6]. There is substantial variation in the prevalence of T1DM world-wide (eg, T1DM is 18 times more frequent in the United States than Japan. Children in

industrialized countries are at much higher risk than those in developing countries; even within industrialized countries such as in Europe, the disease incidence is correlated with the wealth of the country, when expressed as gross domestic product. Migration of children from a low-risk zone is associated with the disease-incidence increasing in them to that of the children to the high-risk zone [7]. Moreover, disproportionate maternal and birth-related events influence the disease-risk (Table 1) [6]. Birth-cohort studies illustrate the development of early changes in metabolomics and the appearance of diabetes-associated autoantibodies, consistent with early exposure to diabetes-associated non-genetic factors [5]. Other putative T1DM-risk factors include: temperate climate [1•], increased hygiene [1•], overcrowding in childhood [2], virus infections [2, 5], early diet including exposure to cow's milk [5], reduced rates or duration of breast feeding [5], vitamin D [3], increased birth weight and weight gain [8], and high levels of a glycotoxin [9]. Paradoxically, powerful evidence characterizing such factors comes from genetic studies as such studies which unequivocally invoke a disease predisposition and exclude the effect being an epiphenomenon (eg, 2 genes, *DHCR7* and *CYP2R1*, each directly linked to vitamin D levels, are among 3 key genes associated with 25(OH) vitamin D metabolism and diabetes-susceptibility [3, 10]. Moreover, a macrophage gene cluster containing the interferon regulatory factor 7 (IRF7)-driven inflammatory network is enriched for diabetes-associated genes and includes rare genetic variants of IFIH1, a cytoplasmic helicase involved in protection from viruses [11, 12]. It is difficult to attribute relative risk to any one of these factors, but it is notable that many of them are related to diet (eg, early diet, breast feeding, vitamin D, glycotoxin, weight gain) and to viruses, of which the main group implicated are enteroviruses ie gut viruses. For these reasons, attention has recently focused on the gut and specifically, the gut microbiome.

It is likely that the number of non-genetic factors that lead to T1DM are limited. Having identified that shared familial environmental effects explain the increase in T1DM in a blood glycotoxin, the advanced glycation end-product carboxymethyllysine (CML), we assessed whether an increase in CML or diabetes autoantibodies could predict T1DM in children [9]. Of 7287 unselected school children screened for islet cell autoantibodies (ICA), we found 115 were ICA positive and they were followed for median 7 years, during which time 33 developed T1DM. We found that elevated serum CML in this cohort was a persistent, independent predictor of diabetes progression in addition to autoantibodies and HLA risk. As a twin study showed that familial, that is shared, environmental factors explained 75 % of CML variance, while non-shared environment explained all autoantibody variance, by implication 2 distinct environmental events account for 2 distinct biomarkers, CML and autoantibodies, which together can predict about 75 % of childhood cases developing T1DM over a 7 year period [9]. How might CML operate to cause T1DM? CML is an advanced glycation end-product and high levels are likely to have proinflammatory effects. Moreover, absorption by the gut of advanced glycation end-products from the diet probably determines the level of such glycation end-products in the blood [13]. It follows that the gut microbiota could play a key role in determining the levels of CML in the blood and, by implication, the gut microbiota could play a key role in the origins of T1DM.

Recent evidence points to factors operating even before the development of diabetes-associated autoantibodies. A study of 56 children who progressed to T1DM found that cord blood phosphatidylcholine, which is metabolized by the gut microbiome, was reduced at birth, independent of HLA-risk, with increased blood levels of proinflammatory lysoPC several months before seroconversion to autoantibody positivity, but not thereafter [14]. Thus, early lipid dysregulation with increased oxidative stress may influence disease pathogenesis. The same metabolomic method was applied to the Munich birth-cohort. After seroconversion to autoantibody positivity, children showed increased odd-chain triglycerides and polyunsaturated fatty acid-containing phospholipids compared with autoantibody-negative children [15], while children who developed autoantibodies by age 2 years, but not later, had persistent 2-fold lower concentrations of methionine compared to children who either developed autoantibodies later or were autoantibody-negative [15]. This result implies that pathways using methionine could influence the appearance of autoantibodies, and even clinical disease. Methionine, like choline, is an epigenetic regulator as a methyl-donor, important in transmethylation and one-carbon moiety pathways. Methionine is, therefore, involved in DNA methylation, an epigenetic effect putatively involved in autoimmunity [15]. A recent twin and population study of whole genome-scale DNA methylation

Table 1 List of potential birth-related risk factors for type 1 diabetes

Likely genetic effect
- Born to diabetic fathers rather than diabetic mothers
- Having a diabetic father aged less than 9 years at diagnosis compared with a father diagnosed later

Likely non-genetic effect
- Increasing maternal age at delivery
- First born
- Season of delivery
- More maternal enterovirus infections
- Early cessation of breast feeding
- Caesarean section
- Birth interval greater than 3years

has also implicated an epigenetic effect in the development of T1DM, with DNA methylation variable positions evident both before and after diagnosis, consistent with changes operating before the onset of clinical disease [16•]. In summary, there are changes in blood metabolomics, in serum autoantibodies, and in DNA methylation that antedate the development of clinical T1DM, can be identified in early childhood, and are, most likely, determined by environmental/non-genetically determined factors. Among these changes, reduced phosphatidylcholine levels, a metabolite metabolized by the gut microbiome, is a very early marker of T1DM-risk. Since the gut microbiome can also be considered to be part of the internal environment, as well as being epigenetic because it is substantially transmitted at birth from mother to infant, it is possible that gut microbiota may impact T1DM-risk. It is also likely that the many known risk factors alone do not sufficiently account for the non-genetically determined contribution to T1DM-risk. Many of these factors could be dependent on the gut microbiota; of which choline is a prime example, but other dietary factors, including breast feeding, early environmental exposures, weight gain, and exposure to glycotoxins such as advanced glycation end-products, could depend on the microbiota to mediate their adverse impact.

The Gut Microbiome and Immune Tolerance

A critical theater for both dietary factors and microorganisms to impact physiology is provided by the gut. It follows that environmental events associated with T1DM-risk, which include both dietary factors and microorganisms, might also include altered gut microorganisms (the gut microbiome). These 2 factors, the gut microbiome and diet, can be conflated since the food we eat is processed, in part, by these microorganisms, and the composition of the diet shapes the structure and function of the gut microbiome [17, 18]. There is, moreover, an exquisite balance between our gut immune system and material in the lumen of the gut, which includes dietary factors, ingested items (which are not always dietary), and the gut microbiome, none of which are quite ‘self’ as we understand it and should elicit an adverse immune response. That they do not, in general, implies some process of immune education or tolerance, whereby the gut contents do not evoke a massive immune-mediated rejection.

Microbiome and Immunity

The human immune system is intimately linked to the gut microbiome, which influences the development of immune system, susceptibility to infection from pathogens, and inflammation [19–21]. It is also likely that our immune system, whilst rejecting or destroying many ingested bacteria and viruses, is tolerant of those that derive from this symbiont community. It follows that either the microbiota adapts to the gut immune response, vice versa, or both. Certainly, there is evidence that immune development is dependent on the gut microbiome [22••, 23]. An important step in this interaction is that between bacterial zwitterionic polysaccharides and the host immune system. Such polysaccharides are found in numerous commensal organisms, and can elicit both proinflammatory and immunoregulatory responses. This exquisite effect is achieved by modifying the balance between T-helper 17 cells and interleukin-10-producing regulatory T cells. This process protects the host against various diseases and could account at a molecular level for the 'hygiene hypothesis' of allergic immune disorders such as asthma [22••].

Several immune mechanisms could be involved in our tolerance of the gut microbiome. They include the innate and adaptive immune response that would normally be activated in the face of a microbial invasion. At the interface of the environment and the innate immune response are Toll-like receptors (TLRs), one of the receptor families for innate immunity. These TLRs recognize molecular patterns of a variety of microbial products (pathogenic or non-pathogenic), as well as endogenous stress signals, with consequent activation of antigen-presenting cells, production of inflammatory cytokines thus inducing inflammation and also affecting immune regulatory cells [23]. As a result, the immune response is directed to deal with the immediate invasion of pathogens, or endogenous stress, leading to more specific immune responses through the adaptive immune system. To establish or deter immune tolerance, immune cells are educated at an early age through contact with commensal microbes. For example, invariant natural killer T (iNKT) cells in the gut mucosa can acquire protection from immune-mediated diseases such as inflammatory bowel disease and asthma [22••]. These authors investigated the age-dependent regulation of invariant natural killer (iNKT) cells in mice models of inflammatory bowel disease and allergic asthma. They found that germ-free mice were more susceptible to chemically induced colitis with increased expression of CD1d, chemokine ligand CXCL16 and accumulation of invariant natural killer T (iNKT) cells in the mucosal tissue. Blocking CD1d by monoclonal antibody or generating mice deficient in iNKT cells ameliorated the disease. Interestingly, recolonization of gut microbiota in germ-free mice also attenuated the disease. More importantly, microbial exposure during early life attenuated the disease whereas microbial exposure in adulthood did not. Using an experimental asthma model, the authors showed a similar protective effect in asthma. The immune protection was mediated by iNKT cells, a subset of innate immune cells [22••]. The results are in keeping with the hygiene hypothesis which proposes that immune education is through exposure in childhood to microbes is important in limiting adverse immune responses

later in life. Regulatory T cells (Tregs) controls a threshold for peripheral tolerance including downregulation of dendritic cell co-stimulation [23]. Central tolerance is controlled by thymus. However, it is not complete, self-reactive T cells can escape negative selection in the thymus and expand in the periphery when the opportunity arises, thus peripheral Treg cells become important in control harmful immune responses in the periphery such as at the interface with the environment.

Different gut bacterial species can have different effects on the immune system. An imbalance in the composition of the bacterial microbiota, dysbiosis, could be a major factor in human diseases such as inflammatory bowel disease. For example, *Bacteroides fragilis* can alleviate colitis in animal models, and certain *Clostridium* strains can promote expansion of Treg-cells [24]. The human symbiont *B. fragilis* can protect animals from experimental colitis induced by *Helicobacter hepaticus*, a normal commensal bacterium which has pathogenic potential [25•]. It is known that $CD45RB^{hi}$ CD4+ T cells can induce colitis in laboratory mice whereas $CD45RB^{low}$ CD4+ T cells can protect from colitis induction by $CD45RB^{hi}$ CD4+ T cells in mice. Therefore, $CD45B^{low}$ CD4+ T cells could be regulatory T cells, especially for experimental colitis. Mazmanian and colleagues reported that germ-free mice expressed low levels of $CD45RB^{low}$ CD4+ T cells than conventionally-reared mice [25•]. Mono-colonization of by the human commensal bacterium *Bacteroides fragilis* in germ-free mice restored the profile of $CD45RB^{low}$ CD4+ compared with $CD45RB^{hi}$ CD4+ T cells to the levels found in conventionally-reared mice. However, if germ-free mice were colonized by mutant *B. fragilis* that cannot produce polysaccharide A (PSA), the CD45RB profile was unchanged. This study suggested that PSA could affect $CD45RB^{low}$ protective CD4 T cells. The authors further tested this hypothesis using purified PSA in an experimental colitis mouse model and found that PSA could suppress the pro-inflammatory cytokine IL-17 but promoted the anti-inflammatory cytokine IL-10 producing regulatory CD4 T cells [25•]. By implication, gut microbiota and their products play an important role in health and disease.

Viral infections including influenza and adenovirus may also be important in molding this regulatory immune balance, perhaps by directly modifying viral particles, recruiting immune effectors that promote viral replication, or modulating host immunity [26, 27].

Microbiome and Metabolism

The gut microbiome is associated with the trillions of microorganisms that inhabit the distal gastrointestinal tract. The aggregate genomes of these microbes encode a variety of metabolic functions including vitamin and amino acid biosynthesis, degradation of otherwise inaccessible host -and diet-derived polysaccharides, production of short chain fatty acids, and choline metabolism [28, 29••, 30, 31]. As a result, a comprehensive view of human metabolism requires understanding the gut microbiome.

Metagenomic analysis of the human gut microbiome, largely driven through decreases in sequencing cost coupled to increasing throughput have provided an unprecedented view of the spatial, temporal, and inter-individual variation in the composition of the human microbiota [29••, 30, 32, 33••]. Studies of adult female monozygotic and dizygotic twin pairs concordant for leanness or obesity, and their mothers, revealed that the human gut microbiome is more similar among family members [28]. There was a comparable degree of co-variation between adult monozygotic and dizygotic twin pairs but with an extensive, identifiable 'core microbiome' at the gene, rather than at the organismal lineage, level.

Interestingly, studies have recently linked the human gut microbiome to obesity and other aspects of metabolic syndrome. The gut microbiome is certainly thought to influence both sides of the energy balance equation; enabling the digestion and absorption of substrates inaccessible to human enzymes and promoting the accumulation of body fat. Various studies have associated obesity with phylum-level changes in the microbiota [34], reduced bacterial diversity [29••], altered representation of bacterial genes and metabolic pathways and network-wide differences in the representation of bacterial genes [35]. Interactions between the gut microbiome and both our metabolic capability and our immune system could contribute to multiple diseases involving altered energy balance or aberrant inflammation but can also be important in disease protection as set out by the hygiene hypothesis. These effects may even be conflated as illustrated when germ-free mice colonized with a microbiota harvested from obese donors gain significantly more fat than those colonized with samples from lean donors [29••, 34]. Mice deficient for TLR5, the receptor for bacteria flagellin, showed different gut microbiota from the TLR5 sufficient mice under the same environment and expressed the phenotype of metabolic syndrome, in which the altered metabolic state is associated with obesity, dyslipidemia and hyperglycemia [36]. These observations, whilst directly relevant to the origins of type 2 diabetes, could also be relevant to the origins of human T1DM. In fact, a study using non-obese diabetic (NOD) mouse, an animal model of human T1DM, provided the direct evidence that the development of T1DM was associated with gut microbiota [37••]. Metabolic changes associated with progression to T1DM in humans have already been discussed and include: reduced levels in early life of cord blood phosphatidylcholine [14]; raised levels of the advanced glycation end-product, serum CML [9], and accelerated weight gain in prediabetic children [8]. Taken together, these observations relate the gut

microbiota to both immunity and metabolism, which could be relevant to the origin of T1DM or rates of progression towards clinical presentation.

The Gut Microbiome and T1DM

An altered gut microbiome is widely believed to be critical to the development of inflammatory bowel disease [38]. The gut has also been implicated in the development of the quintessential gut autoimmune disease, namely celiac disease, which shares much of the same genetic susceptibility (eg, HLA, to that of T1DM [39]. For celiac disease, the critical non-genetic effect precipitating the disease is an early exposure to dietary wheat, specifically gluten. For T1DM, the nature of these environmental effects is less clear [2, 6].

Diet is only one of the potential intestinal factors causing disease and gut changes could extend to an increase in gut permeability, in intestinal inflammation with impaired regulatory mechanisms and in dysregulated oral tolerance, all of which have been observed in T1DM [40]. Preliminary results imply that is likely that the gut microbiome is involved, at one or more stages, in the pathogenesis of the disease [41••]. Moreover, studies showed that early diet modulates the development of diabetes-associated serum autoantibodies and weaning to hydrolyzed casein formula decreases the risk of diabetes-associated autoimmunity [5].

The hygiene hypothesis has been supported by many epidemiological studies for T1DM. However, it was not clear how the hypothesis worked. Checkpoints for the development of autoimmune diabetes identified in animal and human studies include intestinal factors noted earlier and encompassing dietary factors and the intestinal microbiota [40]. Wen and colleagues studied the relationship of the intestinal microbiota and T1DM using an innate immune deficient diabetic mouse model, namely MyD88 deficient NOD mice [37••]. MyD88 deficient NOD mice were completely protected from diabetes in specific pathogen free housing conditions. However, these mice developed a high incidence of diabetes when they were raised in a germ-free environment. Interestingly, when a cocktail of the bacterial "flora" normally found in the mammalian gut was given to the germ-free MyD88 deficient NOD mice, the incidence of diabetes was significantly reduced [37••]. This study provided evidence that commensal microbiota could prevent T1DM in individuals who are predisposed to the disease. This study also demonstrated the role played by the innate immune system in T1DM. Most importantly, this study provided evidence to support the hygiene hypothesis.

By implication, it might be possible to mediate protection from autoimmune diabetes by modulation of the gut microbiota [42]. Preliminary studies in human T1DM found that 8 Finnish children at T1DM-risk (based on presence of serum diabetes-associated autoantibodies) had a lower total bacterial diversity and less stable microbiota than normal [43]. Such functional changes are important to consider when identifying disease-related changes and might extend to lower total bacterial diversity, less stable microbiota or the association with non-butyrate-producing lactate-utilizing bacteria as found in a preliminary study of 4 T1DM patients [43, 44]. If confirmed, it may be that progression to T1DM in genetically susceptible subjects may be associated with colonization by a distinctive set of bacterial or viral species [45].

Enterovirus and T1DM

Viruses have been implicated in T1DM pathogenesis, most notably enteroviruses [2]. Indeed, a recent study found that the load of enteroviruses in the gastrointestinal tract of patients with T1DM exceeded that of controls [45]. However, among the many viruses in the gut, bacteriophages are particularly prevalent, yet we know very little about them in the context of T1DM, so the true character of the gut virome in T1DM remains to be resolved.

Conclusions

Type 1 diabetes is determined to a substantial degree by environmental factors. Predominant factors in the disease pathogenesis include diet and enteroviruses. These observations have focused attention on the gut and on the gut microbiome in the pathogenesis of T1DM. The gut is a critical theater to impact both health and disease since the gut microbiome processes our food and has lived as a symbiont community within us for thousands of years. Given the close relationship of this substantial gut microbiota with our immune system, it has been argued that the microbiota drives immune tolerance and maturation. Mammalian species carry a core microbiome at the gene level though in man, gut microbial communities are seemingly not determined by the host-genome. Setting aside the issue as to whether the gut microbiota is a part of our self or of our non-self and by implication part of either our acquired self or our environment, it is likely that the microbiome has an important role in our predisposition to disease including T1DM. Preliminary studies point to an altered core microbiota in T1DM, while animal studies implicate the gut immune response to the microbiome as a potential critical factor in leading to the disease. Such observations, if confirmed in man, have the potential to guide a therapeutic approach towards the prevention of auto-inflammatory diseases.

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- •• Of major importance

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