Complex interactions in T1D

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Type 1 diabetes (T1D) is an auto-immune disorder characterized by a complex interaction between the host immune system and various environmental factors in genetically susceptible individuals. Genome-wide association studies (GWAS) identified different T1D risk and protection alleles, however, little is known about the environmental factors that can be linked to these alleles. Recent evidence indicated that, among those environmental factors, dysbiosis (imbalance) in the gut microbiota may play a role in the pathogenesis of T1D, affecting the integrity of the gut and leading to systemic inflammation and auto-destruction of the pancreatic β cells.

Keywords: microbial dysbiosis ; intestinal permeability ; HLA ; virome

1. Introduction

Type 1 diabetes (T1D) is an auto-immune disorder caused by a complex interaction between the host immune system and different environmental factors in genetically predisposed individuals [1][2][3][4]. Furthermore, it is well known that T1D exhibit gender-related differences in which males are more predisposed to T1D in populations with the highest incidence, whereas a female bias was observed in the lowest risk populations (non-European origin), due to various factors [5][6][7][8].

According to the recent report from the International Diabetes Federation (IDF), a total of 600,900 children and adolescents up to 14 years old have T1D [9]. The incidence of T1D in children is increasing worldwide, with strong indications of a geographical-specific increase, with the highest rates of T1D (>5 per 100,000) found in North Africa and America [9].

Recently, a substantial increase in T1D incidence was observed [10], suggesting that multiple contributing factors must be involved in this higher incidence. Those factors include genetic and epigenetics contributors, autoimmunity, viral infections, antibiotics-mediated dysbiosis, gut microbiome composition, and lifestyle factors such as nutrition and modern diet [11][12][13][14][15]. Although certain *HLA* risk alleles are known to increase the susceptibility to T1D in children at risk, only 5% or fewer of them actually develop T1D [16], highlighting the importance of the non-genetic modifiers, in addition to other environmental factors in T1D pathogenesis [17][18]. While the genetic predisposition is considered to have a direct effect on the disease initiation, recent evidence indicated that different T1D risk alleles are also affecting the gut microbiome composition, however, it remains unclear how these risk alleles are interacting with the host gut microbiome and how these gut microbiomes affect the host epigenome leading to destructive autoimmunity [17][19]. The increasing incidence of T1D in western countries could be explained by the hygiene hypothesis, in which a lack of exposure to infectious agents can affect the maturation of the immune system [20].

While a great deal of progress has been made in our understanding of T1D pathogenesis, translating this knowledge into a clinical decision is still far from being achieved. This review aims to discuss the triple interaction between the host genome, the epigenome, and the gut microbiome in T1D.

2. Genetic Predisposition to T1D

The rapid evolution in genome-wide association studies (GWAS) along with the availability of large genomic consortia have transformed our ability to link between specific gene loci and their association with auto-immune diseases including T1D [21][22][23][24][25][26]. Recent GWAS studies highlighted that the risk for developing T1D is explained by the presence of certain Human leukocyte antigen class-II (*HLA* class II) risk alleles in addition to 60 plus non-*HLA* single nucleotide polymorphisms that have been recently identified 26. These key genetic factors include the *HLA* alleles (mainly *HLA DR* and *DQ* genes) at position 6p21, which represent 30–50% of the T1D risk genes, in addition to *HLA I* and *HLA III* [27][28]. Around ~50% of the children carrying the *HLA-risk* genotypes *DR3/4-DQ8* or *DR4/DR4* develop T1D at a very young age (up to five years old), this risk increases when the child has a family history of T1D [29][30]. Interestingly, studies on familial T1D showed a higher incidence of T1D in offspring of fathers withT1D compared to mothers having T1D, in which the

DR4-DQ8 haplotype was the most frequent haplotype in these children [31][32]. In addition to the *HLA* genes, 60 plus non-*HLA* risk alleles were shown to be involved in T1D pathogenesis, including several genetic variants in key immune genes such as the insulin gene (*INS*), the protein tyrosine phosphatase non-receptor type 22 gene (*PTPN-22*), the cytotoxic T-lymphocyte-associated protein 4 gene (*CTLA4*), the interleukin 2 receptor alpha (*IL-2RA*), and interferon-induced with helicase C domain 1 (*IFIH1*) in addition to *PXK/PDHB* and *PPIL2* [33][34][35][36]. Furthermore, growing evidence from the T1D twins studies showed concordance rates range between ~23–47% in monozygotic twins compared to ~3.8–16.4% in dizygotic twins which depends on the age at diagnosis [37][38][39][40][41]. It is also worth noting that these genetic factors do not provide a 100% positive predictive value, indicating that the progression into T1D is a complex interaction of both genetic determinants and other environmental factors such as the gut microbiome [42].

3. Gut Microbiome, Immunity, and T1D

Gut microbiota is the collection of microorganisms living in the gastrointestinal tract including bacteria, viruses, fungi, protozoa, archaea, and accounting for 500–1000 different species, which in healthy individuals, is predominated by two major bacterial phyla *Bacteroidetes* and *Firmicutes* [43][44][45]. The benefits of these microbes range from vitamin synthesis, energy homeostasis, maturation of the immune system among others [46][47]. Besides, the byproducts of the gut microbiota can modulate the host physiology and metabolism by facilitating digestion and extraction of energy from indigestible substrates such as extraction of short-chain fatty acids (SCFAs) from indigestible fibers [46]. SCFAs are used as an energy source by the intestinal mucosa, which can in turn maintain the intestinal homeostasis by regulation of the immune response and tumorigenesis in the gut [47].

Different factors affect the composition of the gut microbiota including the mode of delivery, diet, lifestyle, sex hormones, genetic background, pharmaceutical agents, use of antibiotics, and even the pH of the drinking water [48][49]. The alteration in the gut microbial composition has been involved in the pathogenesis of a wide array of diseases such as cardiovascular disease, gastrointestinal disease, and metabolic disorders including T1D, T2D, and obesity among others [50][51][52][53]. Interestingly, many studies have compared the microbiome composition between individuals with T1D (or those having a genetic risk of T1D) and healthy controls, in which they found differences in the gut microbiome composition, suggesting a role of the dysbiotic gut microbiome in disease pathogenesis [54][55][56][57][58][59].

4. Microbial Metabolites, Probiotics, and T1D

Intestinal microbes are well known to produce a range of molecules or metabolites, such as SCFAs including acetate (C2), propionate (C3), and butyrate (C4) that can modulate various host functions [60]. These molecules are byproducts for the bacterial fermentation of the dietary fibers in the colon known to maintain the gut epithelial integrity, enhance the colonic T-reg cells function, and to provide strong anti-inflammatory functions by modulating immune response [60][61]. Butyrate mediates anti-inflammatory functions in the intestinal mucosa through inhibition of the NF-κB transcription factor and activation of the *CX3CR1* of macrophages (Mfs) [61][62][63]. Intestinal butyrate is also involved in the regulation of TLR 4 gene expression, by reducing the LPS translocation and blocking of LPS stimulated dendritic cells (DC), in addition to enhancing the activity of Treg cells, as well as inhibiting immune response against the gut microbiota [61][62][63]. Moreover, butyrate is also reported to maintain the intestinal integrity by modulating the intestinal forkhead box protein P3 *FOXP3* (transcription factor responsible for activation of regulatory T-cell) [64]. Similar findings were documented in the mice model, for example, feeding NOD.Myd88$^{-/-}$ with a diet rich in starch, fibers, or SCFA resulted in enhanced production of acetate and butyrate in their stool, hepatic, and peripheral blood, and protecting them against T1D [65][66][67]. This indicates the beneficial role of the microbial metabolites in providing immune- regulatory functions [65][66][67]. In addition, feeding of NOD mice with SCFA even at the onset of T1D gave them protection against T1D, by lowering the number of islets auto-reactive T cells and enhancing the proliferation of *FoxP3*$^{+}$ Treg cells in the gut mucosa, spleen, and pancreatic lymph nodes, thus enhancing immune tolerance [68].

On the other hand, the progression into T1D in TLR4-deficient (TLR4$^{-/-}$) NOD mice showed to be associated with the gradual decrease of SCFA concentration in their portal blood vein [69]. This indicates the beneficial effects of microbial metabolites in maintaining a healthy gut and regulating immune response which shed the light on the potential therapeutic role of probiotics[70] many probiotic bacterial strains showed an immune regulatory function leading to protection against the autoantibody destruction of the pancreatic β cells [70]. Indeed, many microbial-based probiotics have been identified with potential benefit against T1D, for example, *VSL3#* which consists of eight beneficial *Lactobacillus* strains shown to prevent or delay T1D in NOD mice, this is mediated by releasing a number of molecules with anti-inflammatory activities, modulating the number of splenic CD8 T cells, enhancing the immune tolerance and the growth of beneficial bacteria in the gut [71][72] (Figure 2). Furthermore, probiotic species such as *Lactobacillus* spp. (*Lactobacillus. fermentum*) and

Clostridium cluster IV and XIV spp., can promote the integrity of the gut epithelial barrier and protect against leaky gut through enhancing the expression of tight junction proteins including *ZO*-1, *Claudin*-1, *Occludin*, and *Cingulin*, protecting against T1D associated auto-immune response in the gut [73][74].

Due to the beneficial effects of probiotics and SCFA in maintaining the immuno-hemostasis, many clinical trials have been conducted in human subjects with T1D such as the TEDDY study, linking the early life consumption of probiotics and islet autoimmunity in genetically susceptible T1D children [75]. This study found a positive correlation between early life (the first 27 days of life), administration of probiotics (mainly *Lactobacillus* and *Bifidobacterium),* and the reduced risk of islet autoimmunity, especially in children with the highest risk [75]. Furthermore, a recent probiotic study showed that the administration of *Lactobacillus rhamnosus* GG can increase the serum tryptophan levels in kids with T1D, which in turn lowers the production of the inflammatory cytokines (IFN-γ, IL-17F) that is associated with the auto-immune response in PBMCs [76]. This suggests that prospective clinical studies are vital for the identification of potential novel microbiome-based therapeutic strategies for T1D.

5. Role of Genetic Predisposition on the Gut Microbiome Composition in Individuals with T1D

Although GWAS studies have identified T1D associated genes including both *HLA* and non-*HLA* alleles, the predicted genetic contribution does not give us a 100% positive predictive value indicating the possible role of other environmental factors such as gut bacteriome and virome [77][78]. For example, the association between various SNPs in *PTPN22*, *PTPN2*, *IL10*, *IL2*, *IFIH1*, *INS*, *HLA-DRA*, and *CTLA4* genes and their impact on the gut microbiota composition has recently been revealed in individuals with autoimmunity and T1D [79]. Furthermore, rs2476601 and rs1893217 SNPs on *PTPN22* and *PTPN2* genes, respectively, were associated with a lower abundance of beneficial bacteria such as *Faecalibacterium*, *Bilophila*, and *Coprococcus*, in addition to a higher abundance of *Bacteroides* in many auto-immune diseases such as Crohn's disease, and since these SNPs are common also in T1D, it may indicate a possible correlation with gut microbiome [79][80]. These genes are involved in the regulation of innate and adaptive immune responses against different viral and bacterial infections that are associated with the susceptibility to T1D [78]. Interestingly, several cohort studies have found an association between the *DR3/4* risk genotype (haplo-genotype A) and the higher abundance of *CV-B4* viral antibody levels, *Parabacteroides*, *Bacteroides*, *Clostridium*, *Ruminococcus Saccharimonadaceae*, *Klebisella*, *Veillonella*, *Akkermansia*, and *Erysipelotrichaceae*, in T1D children as shown in Figure 1 [78]. This could be explained by the fact that *DR3/DR4* risk alleles can increase the susceptibility to infection by making the immune system hyper-reactive [78]. Furthermore, non-*HLA* T1D associated SNPs can affect the immune system by lowering the activation and the signaling functions of T cells [81]. For example, *PTPN22* gene is involved in both T and B cell receptor signaling pathways in which SNPs in this gene can disturb the ability of T and B cells to recognize self from non-self-antigens [81]. In addition, SNPs on *PTPN2* are associated with an increased expression of pro-inflammatory cytokines in the intestinal epithelium, stimulating the activated Th1 and Th17 cells and impairing the function of regulatory T cells [78][81]. The stimulated Th1 and Th17 cells can mediate an inflammatory response in the pancreatic tissues, this was shown to be associated with molecular mimicry between the pancreatic cells and microbiome antigens mainly in *Bacteroides* and *Parabacteroides*. however, Further studies are required in order to identify the specific correlation between each SNP and its effect on the gut microbiome composition [79][81].

Interestingly, rs1990760 SNP on the *IFIH1* gene showed to be associated with variant levels of *Enterovirus* RNA in peripheral blood of children at risk for T1D [82][83]. It is well known that The *IFIH1* gene codes for the pattern recognition receptor MDA5 which is an innate immune receptor able to detect and interact against viral infection via activation of a cascade of antiviral responses including the stimulation of type I interferons and proinflammatory cytokines that showed to be associated with T1D [84].

As most of T1D related genes have been identified using advanced linkage studies and GWAS studies, genetic risk scores can be used for early prognosis of T1D combing early life factors such as diet, exposure to infections, and the measuring of early life auto-antibodies that appeared before the initiation of the disease, in which the combination of these factors with the associated gut microbial dysbiosis can expand our knowledge regarding the gut microbiome interaction network, by which this disease is initiated and the possible therapeutic targets that can be applied [85].

6. Role of Gut Microbiome in Gene Expression and Epigenetic Regulations of T1D

As discussed, the interactions between genetic predisposition and environmental factors are significantly associated with the initiation and development of T1D. One of the most important interactions is epigenetic regulation which includes histone modifications, DNA methylation, and non-coding RNA binding [86][87]. Those interactions can be triggered by the dysbiosis in the gut microbiome and their metabolites, these metabolites act as cofactors for the key epigenetic enzymes, affecting the methylation and acetylation, in addition to mediating variations in mi-RNA expression in different T1D related genes including *NF-KB P65*, *CTLA4*, *IL2*, and *FOXP3* [86][87].

7. Conclusions

This review summarizes the association between different genetic, epigenetic, and gut microbiome factors that together, can enhance the pathogenesis and progression of T1D. Although some complex interactions between the gut microbiome, the host genome, and epigenome in T1D have been revealed, still little is known about the effects of the host genome and different T1D variants on the gut microbiome, and whether these dysbiotic microbiomes are genetically determined. Further studies are required to elucidate the molecular mechanisms by which the microbial composition can contribute to protection from T1D, by understanding which bacterial species provide a specific beneficial protective role in T1D, and which type of metabolites can mediate this protective mechanism. Implementing multi-omics approaches will help to move towards identifying novel T1D initiating mechanisms and thus enable us to develop new therapies.

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