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Heterogeneity of autoimmunity in type 1 diabetes

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Abstract

Type 1 diabetes (T1D) develops in distinct stages; however, there is heterogeneity among patients in the rate at which this occurs, and different clinical endotypes have been defined. T1D is an autoimmune disease, but target β cells also play a role in its development. It is possible that the observed heterogeneity is a result of variable contributions of immune regulation and β-cell vulnerability to T1D pathogenesis. Possible mechanisms for increased β-cell vulnerability include: the secretome from accumulating senescent β cells altering adjacent β cells and activating additional immune cells; human leukocyte antigen class I hyperexpression resulting in a more diversified antigen repertoire, including 'non-self' neo-antigens, thus increasing β-cell visibility; and the ability of β cells to sensitize T cells from a distance by releasing antigens in the bloodstream. Conversely, stressed β cells can also mount protective mechanisms, and these present possible therapeutic opportunities to develop β-cell–protective agents. In addition to cross-talk between the immune system and β cells, evidence suggests a pathogenic role of the exocrine pancreas in T1D. An increased understanding of the different mechanisms involved in the progression of T1D is crucial for developing predictive biomarkers and identifying novel therapeutic targets.

Key words: autoimmunity, β cells, heterogeneity, pathogenesis, type 1 diabetes

Introduction

The development of type 1 diabetes (T1D) in the genetically at-risk population occurs in distinct stages, related to the decline of β-cell function over time.[1] Prior to stage 1, numerous known and unknown risk factors can cause β-cell autoimmunity to emerge. Stage 1 disease is characterized by asymptomatic β-cell autoimmunity with normoglycemia, stage 2 is also asymptomatic but with dysglycemia, and stage 3 is symptomatic clinical T1D, which occurs when the destruction of β cells has reached a critical threshold. Although T1D always develops in this predictable manner, there is considerable heterogeneity among patients in the rate at which this occurs, depending on the slope of β-cell decline.[1] This observed heterogeneity has led to the definition of clinical endotypes of the disease.[2]

Clinical endotypes of T1D

The different clinical endotypes of T1D are generally related to the age of disease onset. For patients with stage 3 clinical disease, it is commonly observed that children progress to complete insulin deficiency more quickly than adults. In preclinical disease (stage 0–1), two endotypes have been defined: in children who seroconvert within the first 2 years of life, the first autoantibodies are more often against insulin (IAA), and their genotype is mostly HLA-DR4/DQ8; whereas, in those who seroconvert later, the first autoantibodies are more commonly against GAD65, and the genotype is HLA-DR3/DQ2.[3] Children who seroconvert earlier generally progress to clinical disease more quickly and are less likely to revert to an autoantibody-negative status.[4] Two endotypes can also be defined by histopathologic means. Endotype 1 (T1DE1), in patients who are diagnosed at <13 years old, is characterized by very few remaining insulin-containing islets (ICIs), with the remaining ICIs showing significant immune infiltration dominated by $CD20^+$ B cells and $CD8^+$ T cells.[3] In contrast, endotype 2 (T1DE2, adult-onset) is characterized by more remaining ICIs and more modest immune filtrates. Thus, it appears that the autoimmune mechanisms underlying these two endotypes are dissimilar.

The role of $CD8^+$ T cells in T1D

In TD1, $CD8^+$ T cells are the main components of immune infiltrates, and human leukocyte antigen class I (HLA-I) hyperexpression in ICIs is a histopathologic hallmark.[5,6] Both are key players in the cross-talk between β cells and the immune system in T1D; HLA-I molecules present antigens (in the form of peptides) recognized by $CD8^+$ T cells, which ultimately leads to cytotoxic activity and the destruction of β cells. However, circulating autoimmune islet-reactive $CD8^+$ T cells are found at similar frequencies in patients with T1D and in healthy subjects.[7,8] Furthermore, these $CD8^+$ T cells have a largely naïve phenotype, which suggests they are not directly involved in the autoimmune process.[7] If these naïve $CD8^+$ T cells are differentiated in vitro, they are similarly capable of killing β cells, regardless of whether the T cells originate from T1D patients or healthy donors.[8] Therefore, healthy individuals are in a state of 'benign' autoimmunity, which leads to the question: what causes only some people to progress to T1D while others remain in this benign state?

Possible mechanisms of progression from benign islet autoimmunity to T1D

There are different possibilities for mechanisms of progression from benign islet autoimmunity to T1D; these include different efficiencies of immune regulation and different degrees of β-cell vulnerability between individuals.

Different degrees of immune regulation

It is possible that defective immune regulation leads to progression to T1D in some individuals. This possibility is supported by findings from older studies, in which functional interferon (IFN)-γ ELISpot readouts were able to accurately differentiate between samples from patients with T1D and samples from healthy volunteers or patients with T2D.[9-12] This assay, performed using unfractionated peripheral blood mononuclear cells (PBMCs), leaves $CD8^+$ T cells under the influence of regulatory networks that may be exerted by other circulating immune cells.

Although defective immune regulation may contribute to the progression of T1D, effective immune regulation can also occasionally slow T1D progression in some patients. Our team has described the case of a 56-year-old woman with T1D who underwent a partial pancreatectomy.[13] In spite of showing the hallmarks of classical T1D (i.e., anti-GAD autoantibody positive, significant β-cell loss, and insulitis on pancreas histology), this patient remained on low insulin for several years after diagnosis. Her insulin requirements eventually increased after seroconversion to a second autoantibody, anti-zinc transporter 8 (ZnT8). Pancreatic histopathology showed an immune filtrate with the classical $CD8^+$ T cells, but also a high number of forkhead box protein 3 (FOXP3)-positive T regulatory cells. PBMC analysis showed a dominance of a regulatory,

interleukin-10-secreting phenotype of islet-reactive T cells, compared with the IFN-γ-secreting phenotype that is more typical of T1D; IFN-γ responses increased after seroconversion to anti-ZnT8. These results suggest a spontaneously regulated islet autoimmune process.

Absolving T cells: not always the sole culprits for T1D

Although $CD8^+$ T cells dominate immune infiltrates and play a key role in β-cell loss, it is becoming clear that they are not always the sole, or lead, culprits for the development of T1D, but rather that autoimmunity is a result of cross-talk between immune cells and target β cells.[3] For numerous other autoimmune disorders, it is possible to create a mouse model in which autoimmunity is induced by immunization with organ extracts or purified self-antigens. However, it has not been possible to develop a T1D model by immunizing non-transgenic mice with islet extracts. Furthermore, adoptive transfer of human islet reactive T cells into immunodeficient mice is not diabetogenic, unless β-cell damage is also induced in the mice.[3,14]

Although T cells are not the only culprits for T1D, T1D can still be defined as an autoimmune disease. In non-obese diabetic (NOD) mice, early and transient β-cell dedifferentiation (and consequent reduction in β-cell autoantigen expression) can prevent T1D.[15,16] These results suggest that there is an early critical time period during which T cells are primed by β-cell antigens, and therefore, if the β cells are not visible to the T cells, there is no autoimmunity. However, these results cannot be taken as evidence for an active role of β cells in initiating autoimmunity.

There is also genetic evidence for synergy between autoimmunity and β cells in T1D.[3] While β-cells express T1D-predisposing alleles, these play a minor role in T1D predisposition compared with HLA Class II. The function of HLA molecules in antigen presentation lends support to the role of T cells in T1D. Moreover, the major effect of HLA Class II haplotypes is on the rate of autoantibody seroconversion rather than on clinical progression, suggesting that other genes (including β-cell–related genes) may be synergistically involved in later stages of clinical progression.

Heightened β-cell vulnerability in T1D

Another potential mechanism for progression from benign islet autoimmunity relates to increased vulnerability of β cells in people who subsequently develop T1D; this vulnerability may occur in a variety of ways, some of which are outlined below.

Senescence

A study in NOD mice has shown that there is a critical point in disease progression, at the time of transition from peri-insulitis to destructive insulitis, during which senescent β cells start to accumulate.[17] This accumulation suggests ineffective immune-mediated clearance. The senescent β cells acquire a senescence-associated secretory phenotype (SASP), characterized by factors that can cause adjacent β cells to become senescent, and also secrete chemokines and cytokines that may further amplify the autoimmune attack. When the senescent β cells were eliminated, by treating the mice with an anti-Bcl-2 pro-apoptotic molecule, the remaining β cells were preserved and the mice were protected from T1D. This suggests a counterintuitive strategy of eliminating senescent β cells in order to protect the remaining non-senescent β cells.

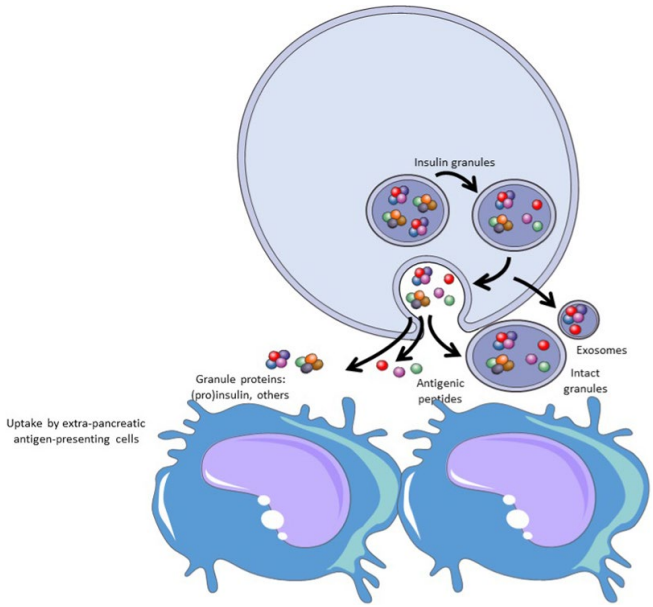
Increased HLA/antigen visibility

Another possible mechanism for increased β-cell vulnerability relates to HLA-I hyperexpression. This hyperexpression translates into a larger and more diversified panel of peptide antigens, which probably makes the β cells more visible to the immune system. In a study designed to elute the peptides present in the HLA-I molecules, unsurprisingly, many of the eluted peptides were from the insulin granule.[7] In addition to the known peptides, we identified several previously undescribed peptides derived from insulin secretory granule proteins, including secretogranin-5 (SCG5), proconvertase-2 (PCSK2), and urocortin-3 (UCN3). These peptides were selectively recognized by pancreas-infiltrating $CD8^+$ T cells from T1D donors, as well as by circulating naive $CD8^+$ T cells from T1D and healthy donors.[7] Furthermore, we have demonstrated that these novel antigens are diabetogenic: when their corresponding T cells are transferred into immunodeficient mice, T1D is induced quite efficiently (similar to what is observed with the transfer of insulin-reactive T cells).[18] It is likely that the reason these novel antigens are diabetogenic is because they share several features with insulin, in addition to originating from granules.[6] Both are produced as soluble pro-proteins which are converted to their bioactive products via proconvertases. Furthermore, several studies have shown that the proconvertase pathway is impaired in β cells of patients with T1D.[6] This impaired processing of proinsulin and other peptides may deviate the degradation of the antigens towards the HLA-I presentation pathway and thus increase the visibility of β cells.

Priming role of secreted granule antigens

A feature of β cells important to their physiology is that their metabolic activity can be exerted from a distance by releasing insulin into the circulation.[19] This ability of β cells to communicate from a distance can also increase their vulnerability, as they can sensitize remote T cells by sending antigens to extra-pancreatic antigen-presenting cells.[19,20] In addition to being antigens themselves, the secretion of the above-mentioned secretory granule antigens, along with insulin, during granule exocytosis causes other antigenic peptides that are ready for presentation to T cells to be released into the circulation (**Figure 1**).

Figure 1: *Some possible mechanisms of heightened β-cell vulnerability in T1D. β cells secrete insulin and other granule antigens into the bloodstream, which leads to T cells being primed from a distance. Reprinted by permission from Springer Nature: Diabetologia. Presumption of innocence for beta cells: why are they vulnerable autoimmune targets in type 1 diabetes? Mallone R, Eizirik DL. 2020;63(10):1999-2006. © 2020.*



Neo-antigen generation

Among the antigens displayed by β cells, there are a number of neo-antigens, so-called because they are not templated in the genome and are regarded as 'non-self' by the immune system. There are several mechanisms through which these antigens may be generated. For example, SCG5-009 is an isoform of SCG5, generated by mRNA splicing, which has a completely different sequence at the C-terminus of the protein.[7] Another mechanism is when non-contiguous fragments from the same protein, or even two different proteins, fuse during degradation in the proteasome (and possibly in the granules), resulting in fusion peptides which may be particularly immunogenic; an example is insulinoma antigen-2 (IA-2, PTPRN)$_{576\text{-}580/708\text{-}711}$ (formed by the fusion of the 576-580 and 708-711 non-contiguous IA-2 fragments).[18]

β cells also mount protective mechanisms

While it is becoming clear that β cells have some role to play in the pathogenesis of T1D, protective mechanisms are also mounted by stressed β cells. In one study in NOD mice, investigators identified a subpopulation of β cells that survived the immune attack during the progression of T1D.[21] In response to immune cell activity, these β cells showed a dedifferentiated phenotype with stemness features, a reduction in expression of target antigens, and an upregulation of immune inhibitory markers, including the programmed death-ligand 1 (PD-L1) molecule. Other groups have also shown an upregulation of PD-L1, in response to inflammation, in islets from NOD mice[22] and human T1D samples.[22,23]

Several immunomodulatory agents have been trialed, but clinical benefits were mostly transient and observed early during treatment.[19] Pharmaceutical agents that may therapeutically boost the protective mechanisms mounted by β cells are therefore being investigated as a complementary strategy. Agents being investigated for the possibility of relieving endoplasmic reticulum stress include verapamil, a thioredoxin-interacting protein (TXNIP) inhibitor, and tauroursodeoxycholic acid (TUDCA). A trial of the tyrosine kinase inhibitor imatinib[24] demonstrated a reduction in the rate of β-cell decline at a later stage of T1D progression than the immunomodulators just mentioned. Though there was room for improvement in the benefit observed with imatinib, this later stage effect may suggest the possibility of a synergistic effect with combination treatment.

Beyond β cells: an additional pathogenic role for the exocrine pancreas?

In addition to the cross-talk between the immune system and β cells, the exocrine pancreas may contribute to T1D pathology. Alterations of the exocrine pancreas can be observed during the progression of T1D, including at pre-clinical stages.[25] These alterations include reductions in pancreas weight[26,27] and serum trypsinogen and lipase levels.[25,28] There are also immune cell infiltrates in the exocrine pancreas of patients with T1D.[29] Furthermore, a recent landmark study used single-cell epigenomics to show that there is increased expression of T1D risk gene variants in acinar and ductal cells in the exocrine pancreas; the authors concluded that these results support the possibility that gene regulation in the exocrine pancreas plays a role in the pathogenesis of T1D.[30]

Conclusion

While T1D can still be regarded as an autoimmune disease, target β cells also have a role to play in the pathogenesis of the disease. It is possible that the observed heterogeneity of T1D relates to a variable contribution of the immune system (different degrees of immune regulation) and β cells (different degrees of β-cell vulnerability) to clinical progression. Stressed β cells also mount protective mechanisms, which presents a therapeutic opportunity to develop β-cell–protective agents. The picture of this cross-talk between the immune system and β cells may be even more complex, with an additional putative role of the exocrine pancreas. It is critical that we understand the mechanisms through which different endotypes progress from 'benign' autoimmunity to clinical T1D, as this understanding is the key to developing biomarkers capable of predicting the risk of progression along this continuum, as well as identifying novel therapeutic targets.

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