

The beta cell-immune cell interface in type 1 diabetes (T1D)



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ABSTRACT

Background: T1D is an autoimmune disease in which pancreatic islets of Langerhans are infiltrated by immune cells resulting in the specific destruction of insulin-producing islet beta cells. Our understanding of the factors leading to islet infiltration and the interplay of the immune cells with target beta cells is incomplete, especially in human disease. While murine models of T1D have provided crucial information for both beta cell and autoimmune cell function, the translation of successful therapies in the murine model to human disease has been a challenge.

Scope of review: Here, we discuss current state of the art and consider knowledge gaps concerning the interface of the islet beta cell with immune infiltrates, with a focus on T cells. We discuss pancreatic and immune cell phenotypes and their impact on cell function in health and disease, which we deem important to investigate further to attain a more comprehensive understanding of human T1D disease etiology.

Major conclusions: The last years have seen accelerated development of approaches that allow comprehensive study of human T1D. Critically, recent studies have contributed to our revised understanding that the pancreatic beta cell assumes an active role, rather than a passive position, during autoimmune disease progression. The T cell-beta cell interface is a critical axis that dictates beta cell fate and shapes autoimmune responses. This includes the state of the beta cell after processing internal and external cues (e.g., stress, inflammation, genetic risk) that that contributes to the breaking of tolerance by hyperexpression of human leukocyte antigen (HLA) class I with presentation of native and neoepitopes and secretion of chemotactic factors to attract immune cells. We anticipate that emerging insights about the molecular and cellular aspects of disease initiation and progression processes will catalyze the development of novel and innovative intervention points to provide additional therapies to individuals affected by T1D.

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Keywords Type 1 diabetes; Tolerance; T cell infiltrates; Islets; Neoepitopes; Beta cells

1. INTRODUCTION

T1D pathogenesis involves genetic susceptibility, beta cell stressors and the outcome of that stress, the innate and adaptive immune systems, and undefined environmental and other factors which yield an incompletely understood and complex interplay of events that proceed from normal functions of both the beta cell and the immune system to pancreatic beta cell specific autoimmunity and clinical manifestation of the disease. The current consensus is that progression to clinically overt T1D is characterized by four stages [1]. T1D risk varies according to age, metabolic status, genetic susceptibility, and islet autoantibody (AAb) type and number. Having ≥ 2 AAbs is one of the most important determinants for progression to clinical T1D [2,3], however not all at risk subjects develop the disease, adding complexity. To date, in our understanding of human T1D, beta cell destruction is primarily caused by immune attack driven by CD4⁺ and CD8⁺ T cells of the adaptive arm of the immune response [4], immune cells of the innate immune response playing a less well-defined role,

and with emerging evidence for a direct contribution of the beta cells in this disease process.

The nonobese diabetic mouse (NOD) [5,6] model of autoimmune diabetes has contributed significant understanding of T1D pathogenesis. While some therapies are in use in trials [7–10], translation of successful therapies from the NOD model to human disease has proven difficult [11] and it is widely accepted that the mouse model doesn't fully recapitulate human T1D. Thus, the need to fully understand the beta cell-immune cell interface in human T1D pathology studying human tissues is critical.

Currently, *in vitro* cell population experiments, *in vivo* humanized mouse experiments [12], and use of human tissues are becoming more accessible and commonly employed. These approaches will likely allow us to close existing knowledge gaps in human T1D pathogenesis. Thus, we anticipate that resulting advances will enable effective prevention strategies and novel therapeutic treatment of human T1D. Here, we consider current knowledge and knowledge gaps concerning the interface of the islet beta cell and the immune cell populations in T1D.

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Received July 1, 2023 • Revision received September 1, 2023 • Accepted September 15, 2023 • Available online 20 September 2023

<https://doi.org/10.1016/j.molmet.2023.101809>

Abbreviations

<i>AIRE</i>	autoimmune regulator gene	<i>INS1</i>	gene or transcript for murine insulin 1
AAb	autoantibody	<i>G6PC2</i>	gene or transcript for glucose-6-phosphatase catalytic subunit 2
APCs	antigen presenting cells	<i>G6PC2</i>	glucose-6-phosphatase catalytic subunit 2. Glucose-6-phosphatase, catalytic subunit 2-formerly islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP)
APECED	autoimmune polyendocrinopathy candidiasis dystrophy	IPEX	immunodysregulation polyendocrinopathy enteropathy X-linked syndrome
ChgA	chromogranin A	MDA5	a dsRNA helicase enzyme encoded by the <i>IFIH1</i> gene
<i>CHGA</i>	gene or transcript for ChgA	MHC	major histocompatibility complex
CXCL10	C-X-C motif chemokine ligand 10	NOD	nonobese diabetic mouse
CXCR3	C-X-C Motif Chemokine Receptor 3	PAD	peptidyl arginine deaminase
DR3	HLA DRA*01:01-DRB1*03:01	PD-1	programmed cell death protein 1
DR4	HLA DRA*01:01-DRB1*04:01	PD-L1	programmed death ligand 1
DQ2	HLA DQA1*05:01-DQB1*02:01	PD-L2	programmed death ligand 2
DQ8	DQA1*03:01-DQB1*03:02	PTM	post translationally modified
DRiPs	defective Ribosomal Products	<i>PTPN2</i>	gene or transcript for tyrosine-protein phosphatase non-receptor type 2
ER	endoplasmic reticulum	<i>PTPN22</i>	Protein Tyrosine Phosphatase Non-Receptor Type 22
FoxP3	forkhead box protein 3	<i>PTPRN</i>	gene or transcript for IA-2
<i>GAD2</i>	gene or transcript for GAD65	TCR	T cell receptor
GAD65	glutamic acid decarboxylase 65	TECs	thymic epithelial cells
HLA	human leukocyte antigen	TG2	transglutaminase 2
HIPs	hybrid insulin peptides	Tregs	T regulatory cells
Ins-ChgA	insulin-chromogranin A HIP	TRA	intrathymic expression of tissue-restricted antigen
IA-2	islet tyrosine phosphatase 2	<i>TYK2</i>	gene or transcript for tyrosine kinase 2
IAPP	islet amyloid polypeptide	T1D	type 1 diabetes
IFN	interferon	TNF- α	tumor necrosis factor- α
IFN- α	interferon- α	UPR	unfolded protein response
IFN- γ	interferon- γ	VNTR	variable number of tandem repeats
<i>IFIH1</i>	gene encoding interferon induced with helicase C domain 1 (MDA5)	ZnT8	zinc transporter 8
IL-1 β	interleukin-1 β		

2. BETA CELLS IN HEALTH AND T1D

The concept that beta cells may contribute to their own demise during T1D pathogenesis was first postulated many years ago [13], and this notion is becoming more widely accepted in the field [14,15]. T1D pathogenesis is a slow process, often taking years to develop clinically. It has been hypothesized that progression to T1D is due, at least in part, to features of the beta cells such as a highly secretory phenotype and a well vascularized niche that render them more vulnerable to cellular stress that can tip the delicate cellular balance and promote dyshomeostasis [16]. It is well established that beta cells are specifically targeted by the immune system, however, the roles that beta cells play in enabling or resisting this targeting are not completely understood. Multiple pathways likely contribute to beta cell dysfunction, an altered beta cell-immune cell dialogue, and immunogenicity. These pathways may be potentiated by intrinsic stressors such as endoplasmic reticulum (ER) stress, or to extrinsic stressors such as viral infections and inflammatory cytokines. In the following section, we describe current modes of examining beta cells, beta cell heterogeneity, the current state of knowledge on these pathways and identify the key beta cell processes that contribute to the autoimmune response.

2.1. Modes of examination of beta cells

Given the nature of the pancreas as a relatively inaccessible and fragile internal organ, many studies have focused on the use of human islets *ex vivo* [17], mouse models of autoimmune diabetes [5,6], and cultured glucose-responsive beta cell lines [18–20] to provide insight into disease pathogenesis. These traditional approaches to study the

mechanisms that might contribute to immune attack have provided valuable insight into T1D pathogenesis, but limitations with respect to ineffective recapitulation of the human islet niche have left many questions unanswered. The key caveats for traditional approaches are multifold: rodent models do not fully recapitulate human disease and species-specific differences exist; isolated human islets are intrinsically stressed by being from deceased donors and from the isolation process; isolated islets lack functional vasculature, innervation, and systemic communication; and cultured beta cell lines do not retain full beta cell identity, and lack other islet and immune cell types, vasculature, and microenvironment.

More recent models have attempted to mitigate these caveats as much as possible allow for more appropriate modeling of the human beta cell-immune cell interface. These include novel humanized mouse models [21–24], islet-on-a-chip approaches [25], generation of relevant cell and tissue types from stem cells [26–31], intravital imaging [32–35], and analysis of live pancreas slices [36,37]. Live pancreas slices in particular appear to retain some immune cell components [38], and thus may prove especially beneficial in particular for studying endogenous beta cell-immune cell interactions; however, they do lack access to the circulation.

Advances in stem cell technology allow modeling of discrete aspects of interactions between autoimmune immune and beta cells in an HLA matched manner. For example, immune cells and beta cells can be obtained from the same individual via direct differentiation of patient-derived induced pluripotent stem cells and/or using genome engineering technologies to manipulate either cell compartment. Such models can provide complementary insights into previously inaccessible aspects of human T1D [39]. However, these models are continuously

being refined, and thus our understanding of the changing dialogue between the islet cells and immune cells remains a work in progress. Importantly, due to these difficulties in analysis of the islet-immune interface in a physiologic context, current established models fail to effectively recapitulate both the potential role that beta cells play in pathological processes (and susceptibility or resistance to them) and the inherent or induced heterogeneity of beta cells. Thus, continued development of physiologic models to study beta cells will undoubtedly be necessary to advance our understanding of the complex interplay between beta cells and immune cells leading to beta cell destruction.

2.2. Beta cell heterogeneity and links with T1D

As the primary target of autoimmune attack in T1D, the beta cells have long been of interest for studies of disease pathophysiology. Although it was once thought that all beta cells were lost in the context of T1D, several recent studies have clearly demonstrated a clear residual population of surviving (though largely dysfunctional) beta cells even in longstanding T1D [40–43]. A similar subpopulation of persistent beta cells that are capable of prolonged resistance to autoimmune attack has been identified in the pancreata of the NOD mouse model of autoimmune diabetes [44]. This suggests that not all beta cells are equally susceptible to autoimmunity, and that a subpopulation of beta cells may be resistant to immune-mediated death. This is perhaps mediated by intrinsic beta cell protective factor(s) (Section 3.3) that contribute to cell survival to varying degrees in the context of genetic risk, a hypothesis supported by the observations that not all “at risk” individuals go on to develop diabetes [45].

Further support for this hypothesis comes from observations of beta cell heterogeneity under normal conditions from the level of transcription and phenotype [46–49] to function and communication across individual islet cells [50–54]. Together these data suggest that differences among beta cells themselves may contribute to their propensity for resistance to immune attack in T1D. Indeed, beta cell subpopulation distribution in individuals with type 2 diabetes has been shown to be skewed towards a less functional phenotype, indicating a potential role in disease development [46]. It remains unclear whether this same phenomenon occurs in the context of T1D, though the independent observations from NOD mice that 1) a subset of cells become senescent [55] and 2) cells that escape autoimmunity have reduced expression of beta cell identity genes [44] suggests that this may also be the case in this setting. Collectively, these data support that loss or inactivation of a critical subpopulation of beta cells may therefore exert a disproportionate effect on the process of autoimmunity, potentially resulting in disease progression if the beta cells no longer function as normal beta cells and therefore are no longer recognized as “self”. Again, however, this hypothesis is somewhat speculative as it is currently unclear how or when this subpopulation of normal beta cells loses its function/identity and how this phenomenon might contribute to altered recognition by the immune cells.

Remarkably, despite extensive research efforts, detailed knowledge on causative events that trigger a break in tolerance (section 3.1) and contribute to autoimmunity is still largely missing. An attractive working hypothesis that has received increased attention recently is the idea that a discrete stress event (or events) on beta cells results in a temporarily increased diabetogenic phenotype that leads to immune cell activation and disease progression in genetically susceptible individuals (Figure 1). However, testing this idea in the human context is challenging due to genetic heterogeneity at both the population level and at the individual islet/beta cell level, and thus in samples arising from human tissue donors. An additional layer of complexity that is also largely neglected in stress modeling approaches is the normal beta cell

heterogeneity described above, a critical component affecting stress response outcomes that likely impacts T1D pathogenesis [56,57]. Despite these limitations, a wealth of data indicates involvement of a number of stress pathways at different stages of T1D development. These include primarily inflammatory stress originating from both innate and adaptive immune responses, and ER stress that is always present due to the inherent demands of insulin production but intensifies as beta cell function is compromised. Stressed beta cells may become susceptible to autoimmune attack by three complementary mechanisms: beta cells offer themselves as targets through display of class I and class II immune receptors and epitopes/(neo)epitopes; beta cells exhibit compromised function through senescence, impaired autophagy, and/or stress-related pathways; and in predisposed subjects beta cells exhibit increased fragility in response to translational burden and immunologic and metabolic stress.

2.3. Induction of beta cell stress by viruses

Viral infection can lead to stress on the beta cells via both direct and indirect mechanisms. Multiple studies have demonstrated some indications of virus in the pancreatic islets of organ donors with T1D, but the exact underlying mechanism are still poorly understood. A notable example is coxsackie B4 which has been shown to directly infect beta cells and is associated with inflammation and beta cell functional impairment [58]. Additional viruses, including Epstein-Barr Virus, rotavirus, rubella, and cytomegalovirus have been linked to disease either through epidemiologic associations or specific cases [59–62]. Further, animal studies suggest that virus can be directly taken up into the beta cell [63]. Viral infection can directly impact the beta cell through cytolytic effects and through the energetic demands of translating the viral genome [64]. Indirect effects are often the result of increased cytokine production (either systemically or locally) that leads to inflammatory stress and the resulting cascade of deleterious events.

2.4. Induction of beta cell stress by inflammation

Production of inflammatory cytokines, including interferon- γ (IFN- γ), interferon- α (IFN- α), interleukin-1 β (IL-1 β) and tumor necrosis factor- α (TNF- α) [65,66], is a key cellular defense mechanism in the response to infection. These cytokines are produced and secreted as a beacon to warn cells of pathogen presence and to activate an immune response. In the pathogenesis of T1D, viral infection may play a role in the induction of inflammation that may exceed the capacity of the body to mount a productive response. In this scenario, excess cytokines can cause cellular damage, referred to as inflammatory stress. In addition to damage to proteins and organelles caused by the excess accumulation of reactive oxygen species [67,68], inflammatory stress also causes a reduction in beta cell function [69] and in some cases dedifferentiation [70], stimulation of a senescent phenotype [71], or even cell death [72,73].

There is a significant amount of evidence supporting a role for type I interferon (IFN) signaling in T1D pathogenesis, both systemically and at the level of the pancreatic beta cell. Several T1D susceptibility loci can modify IFN- α -mediated signaling [74–76], and IFN- α signaling may induce downstream effects that play a role in the beta cell-immune cell interface in early T1D [77,78]. IFN- α can dramatically alter the phenotype and behavior of beta cells, editing HLA class I overexpression, ER stress and apoptosis [79]. The downstream effects of inflammatory cytokines on beta cells include broad transcriptomic and proteomic alterations, which can alter their interactions with and perception by immune cells. Notably, susceptible variants of genes such as in the gene encoding for Tyrosine Kinase 2 (TYK2) and Protein Tyrosine Phosphatase Non-Receptor Type 2 protein (PTPN2) can

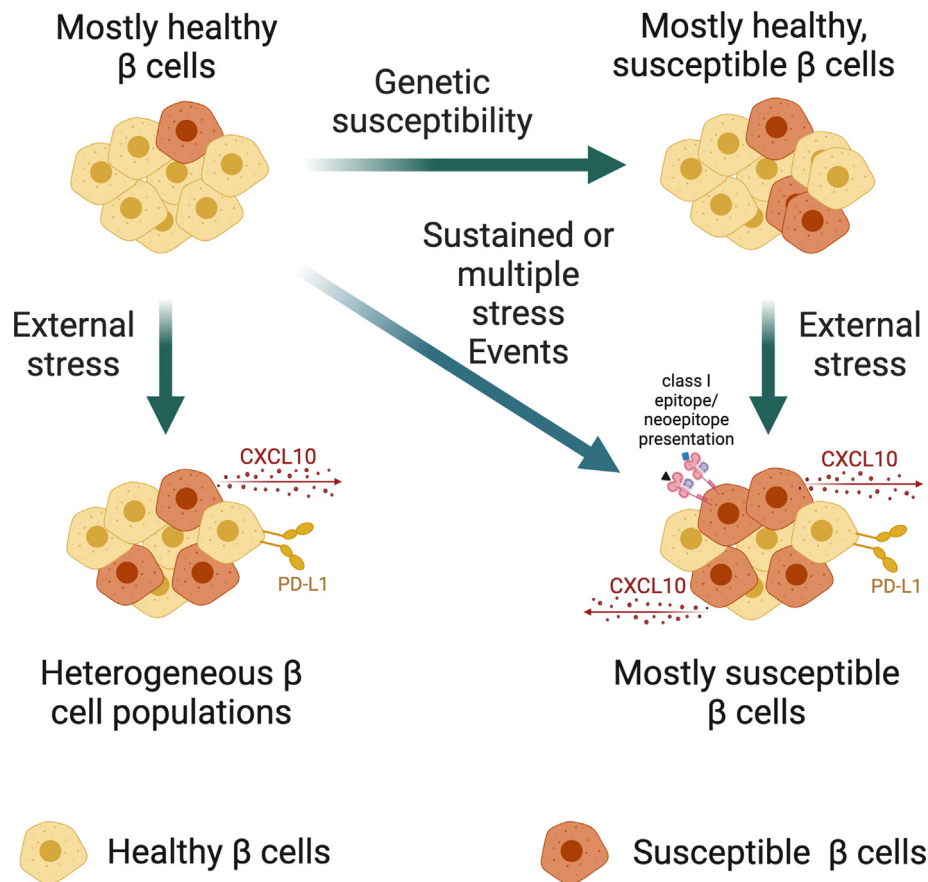


Figure 1: Schematic showing how beta cell phenotype, susceptibility or resistance to autoimmune attack, and external impact could influence the propensity to attract an autoimmune attack. This schematic depicts a hypothetical view of beta cell susceptibility to an autoimmune attack. Beta cells from a healthy, non-risk gene expressing individual are mostly of a healthy phenotype, with only few, if any, beta cells that have a disease susceptible phenotype. Presence of genetic risk increases the probability for autoimmunity. Individual or combined internal and/or external stresses on beta cells subtypes induce (temporary?) resistance to attack with PD-L1 expression or the propensity for disease development with chemoattractant factor secretion and factors such as hyperexpression of HLA class I and epitope/neoepitope presentation.

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amplify these effects [80,81]. Further, children genetically at risk for T1D display a type I IFN-inducible transcriptional signature that precedes appearance of autoantibodies [82,83]. Type I IFN is also expressed in pancreatic islets from people with T1D [58,75,84–88] and laser-captured islets obtained from donors with recent onset T1D show an increase in IFN-stimulated genes [89,90]. Excess IFN- α can even trigger human beta cell apoptosis synergistically with IL-1 β [79]. Interestingly, heterogeneity in IL-1 β -induced toxicity appears to be more severe in the most glucose-responsive rat beta cells [91], suggesting that functional properties such as hyperactivity may render certain beta cells more susceptible to cytokine-induced apoptosis.

2.5. T1D susceptibility gene variants modulate beta cell stress responses

Genome wide association studies have identified more than 60 susceptible loci that are associated with T1D [92]. The majority of these are traditionally classified as immune related genes, but it is estimated that at least 40% of the genes in these risk loci are expressed in pancreatic beta cells [93]. Consequently, it is probable that specific risk genes deleteriously impact the phenotype and/or stress response of pancreatic beta cells — including those elicited by immune cell populations or by direct insults. As an example, the T1D risk gene encoding *PTPN2*, classically important in immune cell function, has

also been shown to also alter apoptosis of pancreatic beta cells when exposed to viral infection mimicking conditions [94]. Deletion of *PTPN2* in stem cell derived beta cells results in increased HLA class I expression at steady state and under proinflammatory conditions leading to increase stimulus of autoreactive T cell receptor (TCR) T cell avatars in co-culture assays [95]. Additional variants in the gene encoding for Interferon Induced With Helicase C Domain 1 (*IFIH1* that encodes for MDA5) and in *TYK2* have been shown to modulate the sensitivity of beta cells to inflammation and environmental stresses [16,74–76]. Therefore, it is plausible that pancreatic beta cells in individuals who carry specific combinations of risk variants have an increased level of sensitivity to sources of stress and inflammation, conferring them more susceptible to beta cell loss, dysfunction and consequently T1D disease development.

2.6. ER stress impacts beta cell survival and prohormone processing

As professional secretory cells, pancreatic beta cells exhibit high levels of ER stress even under normal conditions [96]. Although they have a reserve capacity, under high demand the adaptive unfolded protein response (UPR) is triggered and seeks to attenuate stress and resolve the increased demands of glucose stimulated insulin synthesis [97,98]. If ER stress persists or is further compounded through inflammation and

cytokine exposure, the terminal UPR is triggered, leading to beta cell loss through apoptosis and an increased burden on the remaining beta cells [73]. These effects have been shown to trigger beta cell dysfunction [99]. In particular, dysfunction suggestive of ER stress has been postulated to contribute to the secretion of proinsulin in both pre-diabetic NOD mice [100,101] and humans [102,103]. Likewise, impaired processing of pro-islet amyloid polypeptide (IAPP) has also been identified in the context of T1D [104]. Impaired proinsulin and IAPP processing have been shown to occur in at risk subjects and remain a persistent feature in established disease [103,105,106]. These studies have led to suggestions that levels of circulating unprocessed pro-hormone relative to mature, bioactive insulin could be used as a biomarker for T1D risk [105]. Given the importance of the secretory granule degradative process of crinophagy in modulation of aged or damaged secretory granules in the beta cell [107], and the recent observation that this process is defective in human T1D [108], it is likely that ineffective turnover of granules contributes to their continued presence. Global cellular deficiencies in degradation of granules containing unprocessed hormones may thus play a role in their elevation during disease pathogenesis. In support of this, it has been demonstrated that beta cells in NOD mice deliver insulin-containing vesicles to phagocytes for presentation to T cells [109], and that blood leukocytes can present beta cell derived peptide epitopes bound to major histocompatibility complex (MHC) class II to autoreactive CD4 T cells [110]. These data suggest that alterations in the degradation of vesicles containing improperly processed insulin could lead to the presentation of altered peptides, for which no central tolerance exists, that activate autoreactive T-cells recruited to the islets.

In addition to having a direct impact on beta cell function, the accumulation of intact and incorrectly processed prohormone is likely to play an indirect role in the accelerated formation of neo-epitopes by increasing ER stress (promoting previously discussed mechanisms). Furthermore, the accumulation of incorrectly processed protein has the potential to offer an increased amount insulin fragments for hybrid insulin peptide (HIP) formation (Section 2.8). IAPP deposition has been shown to be a prominent feature in type 2 diabetes and a recent study reported islet amyloidosis in a child with T1D suggesting a possible role in autoimmune development as well [111]. Therefore, impaired proinsulin and IAPP processing may play a fundamental role both in undermining function and in amplifying immune recognition of beta cells.

2.7. Upregulation of HLA class I and HLA class II expression on beta cells

HLA class I hyper-expression on insulin⁺ beta cells is now an accepted phenotype of T1D [88] and pre-diabetes [112]; a stressed inflammatory microenvironment is a key culprit in initiating this beta cell phenotype with *in vitro* evidence [113–115]. The outcome of class I hyperexpression on insulin⁺ beta cells is thought to increase their recognition by islet-infiltrating CD8⁺ T cells which can recognize and kill beta cells expressing surface class I molecules presenting auto-antigenic epitopes [116–118].

Expression of HLA class II on beta cells has been a controversial topic [13,84,119–122]. As beta cells are not professional antigen presenting cells, some suggested that the co-localization of insulin and class II staining was a result of infiltrated macrophages engulfing damaged beta cells [121,123]. However, *in vitro* experiments indicate that class II expression in human islets is induced by pro-inflammatory cytokines [124–126]. More recent data indicates that beta cells from islets donors with T1D had upregulated expression of mRNA species for HLA class II and its antigen presentation pathway components as compared to those from control donors via transcriptome analyses. It

was also shown by immunohistochemical analyses that class II upregulation could be seen in insulin⁺CD68⁺ (macrophage marker) cells in donors with T1D. In addition, in one study, class II was detected by flow cytometry on a subpopulation of CD45^{neg}insulin⁺ islet cells (15.64 ± 8.01%), albeit not as abundantly expressed as on macrophages or B cells while others demonstrated the presence of class II molecules only intracellularly [125,127]. The function of beta cell expression of class II, regarding its expressed peptidome or as targets for islet autoreactive CD4⁺ T cells is not well known at this time. Naïve CD4 and CD8 T cells require costimulation, which most likely occurs in the draining lymph nodes with professional antigen presenting cells (APCs), and with primed T cells then drawn to the islets via a chemokine gradient. Beta cells from donors with T1D were found to lack CD80 (B7-1) and CD86 (B7-2) expression [128]; thus, it is unlikely that epitope presentation by class II on beta cells is a priming event for CD4⁺ autoreactive T cells. However, at the time of the aforementioned study, many costimulation proteins were unknown and therefore not examined; it remains an unanswered question as to whether beta cells *in situ* express any of the currently known costimulatory proteins, possibly expressed in a tissue-specific manner. Potential mechanisms of *in situ* activation of memory islet-infiltrating CD4⁺ T cells may include islet-derived epitopes in the context of class II expression by beta cells and by islet-infiltrating professional APCs.

2.8. Beta cell stress leads to new autoantigenic targets

In addition to native epitopes, it is now appreciated that the presentation of islet antigens includes the formation of multiple classes of neo-epitopes when homeostasis is destabilized by inflammatory or ER stress. The resulting landscape of self-epitopes (native epitopes and neopeptides) is more effectively presented to CD8⁺ T cells through increased HLA class I expression on beta cells [88] and also presented to CD4⁺ T cells through beta cell death and subsequent uptake by islet-resident or islet-infiltrating APCs [129]. The net effect is a broad increase in the immunogenicity of stressed beta cells, a phenomenon that has been directly documented using the NOD mouse model [130]. The generation of neopeptides through multiple mechanisms appears to play an important role in the loss of tolerance to beta cells [131,132]. Autoreactive T cells have been shown to react to neoantigens formed through various processes, including transpeptidation, disulfide bond formation, deamidation, and citrullination [133–135], formation of epitopes such as HIPs [136–140], alternative splicing [141], splice variant peptides [113,114], and defective ribosomal insulin products (DRIPs) [142] and there is growing evidence that these neopeptides are relevant to disease [143]. Immune recognition of neopeptides can be enhanced as compared to their native counterparts due to altered HLA binding or increased TCR recognition. For example, protein deamidation by tissue transglutaminase 2 (TG2) has been shown to generate negatively charged peptides with enhanced presentation by disease-associated HLA-DQB1*02:01 and HLA-DQB1*03:02 (DQ8) proteins as heterodimers with their associated HLA-DRA chain proteins and increased recognition by self-reactive T cell receptors [144]. These processes were shown to generate high affinity neopeptides derived from proinsulin and islet tyrosine phosphatase 2 (IA-2) [135,145]. Analogously, conversion of arginine into citrulline by peptidyl arginine deaminases (PADs) [144] enzymes at key residues on self-peptides can enhance their presentation by the disease-associated DRB1*04:01 protein as a heterodimer with the associated HLA-DRA chain protein.

HIPs in particular have gained traction as potentially key antigens in T1D pathology [146]. Several TCRs that were widely used to study development of T1D in transgenic NOD mice were identified to

recognize HIPs with much stronger affinity as compared to their cognate antigen, providing an attractive explanation for a break in tolerance upon increased generation and/or presentation of HIPs by pancreatic beta cells [139,147–150]. For instance, one of the most well-studied TCRs isolated from NOD mice, the BDC2.5 transductant is known to recognize preproinsulin_{15–24}, but was also shown to recognize an insulin-chromogranin A (Ins-ChgA) HIP with orders of magnitude increased affinity; the first half of this peptide (amino acid sequence LQTLAL) is derived from insulin, whereas the second half (amino acid sequence WSRMD) is derived from ChgA [151]. Importantly, HIP-reactive CD4⁺ T cells have been observed in patients with T1D [152]. Whether neoantigen-specific T cells play an essential and/or inciting role in human disease is yet to be determined. However, the finding that HIP-specific TCRs such as BDC2.5 are sufficient to initiate T1D in mice suggests that HIPs in particular may be inciting antigens. Whether HIP formation increases under conditions of beta cell stress is not clear, in fact, early evidence for HIP formation stemmed from studies of beta cells in absence of external stressors. Thus, much more work is required to provide comprehensive understanding on the specificity and hierarchy of islet infiltrating T cells.

It has been shown in experiments with both, beta cell lines and primary islets that the expression level and activity of modifying enzymes such as TG2 and PADs are increased in response to ER stress and inflammatory cytokines [133,145]. TG2 and PADs are calcium dependent enzymes [153,154]; therefore, their level of activity is dependent both on their expression level and calcium availability. Notably, inflammatory stresses have been shown to increase calcium flux in beta cells [145,155]. As such, inflammatory and ER stress conditions can increase the proportion of modified self-proteins within the proteome of beta cells if ER-associated degradation mechanisms are not appropriately responsive, potentially leading to increased immune recognition.

In parallel, inflammatory stresses have been shown to substantially alter the transcriptional profile of pancreatic beta cells [79] and to increase the frequency of defective ribosomal initiation [142]. These changes are directly reflected in the HLA class I peptidome of beta cells, leading to the presentation of unique epitopes that are recognized by cytotoxic CD8⁺ T cells [113,114]. The relative immune-dominance or immune-prevalence of T cells that recognize mRNA splice variants and alternatively translated RNA species remains unclear, but T cells with these specificities have been documented within human islets [114].

Collectively, these data demonstrate that alterations in beta cell functional response to stress likely contribute to the generation and perhaps presentation of peptides that are previously unknown to the immune system. However, there are still many unknowns associated with these pathophysiologic processes. Further, a dysfunctional beta cell is unlikely to be the only culprit leading to disease and as detailed in section 3, an overactive immune response to this dysfunction is also a significant contributing factor to T1D pathogenesis.

3. AUTOREACTIVE T CELLS IN T1D

Recruitment of lymphocytes into islets in humans with T1D has been appreciated for decades [156,157], however, the driving forces behind this infiltration are poorly understood. The role of self-reactive T cells in T1D is well supported through genetic associations, histology, and evidence from rodent models. As will be elaborated below, T1D has a strong association with specific susceptible HLA class II alleles more modest association with susceptible HLA class I alleles. The role of T cells is also supported by the association of a number of other genes

that modulate immune signaling and function [158,159] along with with the control of autoreactive T cells including central and peripheral T regulatory cell (Treg) mechanisms.

3.1. Autoreactive T cell control mechanisms

A critical component of autoreactive T cell responses in any autoimmune disease is their selection in the thymus and the regulation by mechanisms of tolerance induction in the periphery. The natural mechanisms of central and peripheral tolerance are geared towards deleting or suppressing the activity of autoantigen-reactive effector T cells. However, in T1D, these mechanisms may be bypassed or broken in different ways. Breakage of central tolerance can lead to the release of autoreactive T cells into the periphery. Failure of peripheral tolerance can lead to ineffective suppression of autoimmunity. Finally, neoepitopes-specific T cells may bypass central tolerance altogether. In the subsequent sections, we discuss how these mechanisms are broken or bypassed in T1D.

3.1.1. Induction of central immune tolerance

The glandular thymus organ, located in the chest cavity ventrally from the heart, is critical in facilitating the tolerance mechanism of the adaptive immune system [160,161]. T cell progenitors entering the thymus undergo the critical processes of positive and negative selection. Only T cell progenitors able to generate a functional TCR that can interact strongly with peptides mounted on HLA on the surface of cortical thymic epithelial cells (TECs) receive a survival signal before proceeding to negative selection in the medullary region of the thymus [162,163]. Interaction with HLA class I or class II molecules during positive selection determines the lineage of distinct cytotoxic CD8 or helper CD4 T cells, respectively. Negative selection is critical for establishing a self-tolerant adaptive immune system by removing positively selected T cells that bind too strongly with self-peptides presented on HLAs of medullary TECs.

Medullary TECs, in addition to ubiquitous self-peptides, also express tissue restricted antigens (TRAs) such as insulin, whose expression is otherwise restricted to pancreatic beta cells. TRA expression is reliant on the autoimmune regulator gene (*AIRE*) [164,165]. Human *AIRE* deficiency results in autoimmune polyendocrinopathy candidiasis dystrophy (APECED), a rare and life-threatening disease caused by a T cell mediated autoimmune attack of multiple organs, highlighting the critical need for functional negative selections. Experiments using animal models have demonstrated a critical need for functional expression of specific TRAs in the thymus. Deletion of thymic insulin expression results in autoimmune diabetes by autoreactive T cells that did not undergo negative selection for insulin [166]. Animal studies further demonstrate that reduced levels of thymic insulin expression correlate with increased susceptibility to destructive autoimmunity. Indeed, a variable number of tandem repeats (VNTR) minisatellite upstream of the insulin gene has been identified in humans. Shorter class I VNTRs exhibit 2–3 times less insulin expression in fetal thymic tissues compared to longer class III VNTRs elements thus providing a potential mechanism for increased risk of developing T1D in humans [167,168]. Thus, altering presentation of self-peptides in the thymus could serve as an intervention point to prevent or treat autoimmunity in the future. In concert with the effects of insulin expression, susceptible variants of PTPN2 and PTPN22 (Protein Tyrosine Phosphatase Non-Receptor Type 22) alter T cell signaling, with potential impacts on T cell selection and the stability of regulatory T cell subsets in the periphery [169].

Despite a detailed understanding of the critical stages of T cell development in animal models, many knowledge gaps in the human

setting still exist. Increased numbers of human based model systems have recently been described, including artificial thymic organoid technology and direct differentiation of human stem cells into thymic cells [170–179]. Recent single cell sequencing studies have also emphasized the extreme cellular heterogeneity within the thymus and raised questions as to their respective functions [180,181]. Continuous development of new study settings will be necessary to enable a better understanding of the governing principles of negative selection in a human context and may in fact point to novel approaches that counteract autoreactivity and/or re-establish tolerance [39,182] for this crucial mechanism of the adaptive immune system.

3.1.2. Peripheral tolerance in T1D

A fraction of T cells that escape thymic selection and exit to the periphery can adopt a regulatory phenotype identified by the expression of forkhead box P3 protein (FoxP3) and other markers such as CD3, CD4, CD25, and low or absent expression of CD127 [183,184]. CD4⁺ Tregs exhibit immune suppressive functions in the periphery and provide localized tolerance mechanisms for effector T cells [185]. Due to their immune modulatory abilities, *ex vivo* expanded or modified Treg technology has been extensively explored as a potential therapy in transplantation, autoimmune and cancer settings [186,187].

Tregs have been shown to be crucial for suppressing autoimmunity in mouse models and humans. Absence of Tregs due to Foxp3 deficiency in mice causes systemic autoimmunity [188] and when introduced in the NOD background, elimination of Tregs caused hyper-accelerated autoimmune diabetes [189]. In humans, patients with a genetic Foxp3 deficiency suffer from immunodysregulation polyendocrinopathy enteropathy X-linked syndrome (IPEX), which manifests in patients with T1D as its most common symptom [190]. In addition, several genetic variants associated with T1D risk have also been shown to affect Treg function [191]. Taken together, these lines of evidence point to a crucial role of Tregs in controlling autoimmunity. However, several key aspects of exactly how and where Tregs exert their suppressive effect in pancreatic autoimmunity are poorly understood. Tregs have shown to infiltrate islets in NOD mice [192], and in pancreatic draining lymph nodes from donors with T1D [193], albeit at very low frequencies [194]. But whether they exert their suppressive function only locally within islets, or in pancreatic lymph nodes, or both is not clear. In addition, there is evidence indicating that Tregs are dysfunctional in T1D [195,196] and/or T effector cells in T1D may be resistant to the function of Tregs [197,198].

The antigen specificities of Tregs have not been elucidated to the same extent as for conventional effector CD4⁺ and CD8⁺ T cells in T1D though a number of islet-antigen specific Tregs have been identified from the peripheral blood of donors with T1D [199]. There is very little overlap between the TCR repertoires of effector and regulatory T cells in mice, suggesting a distinct non-overlapping antigenic landscape recognized by Tregs. Several studies have shed light on Treg antigens in the NOD, demonstrating a high propensity for Tregs to recognize insulin-derived epitopes and thus critical to regulate disease [192]. Expression of a protective mutated insulin residue led to development of dysfunctional Tregs also suggests the importance of insulin-reactive Tregs for suppression of diabetes in NOD [200]. Moreover, as it would be expected, absence of HIPs or other post translationally modified (PTM) neopeptides in the thymus leads to a lack of Tregs recognizing them [201]. While this presents as a hole in the Treg repertoire, leading to failure of peripheral tolerance, it also presents an opportunity to engineer Tregs. Indeed, engineered Tregs targeting insulin or HIPs have recently been reported as a way to suppress T1D [202–204]. One of the key elements missing from the

development of engineered antigen-specific Tregs is the knowledge of which antigens will afford dominant protection, as determined by their importance for disease.

3.2. Genetic associations of HLA with T1D

T1D has a strong association with specific HLA class II A and B chain alleles: HLA-DQ8 (DQA1*03:01-DQB1*03:02), HLA-DQ2 (DQA1*05:01-DQB1*02:01), HLA-DR3 (DRA1*01:01-DRB1*03:01), and HLA-DR4 (DRA*01:01-DRB1*04:01) [159], and more modest association with susceptible HLA class I alleles (HLA-B*39:06 and HLA-A*24:02) [205]. T1D susceptible HLA alleles or alleles in combination called haplotypes (referred to as DR3-DQ2 and/or DR4-DQ8) confer risk for T1D development. Interestingly, the MHC class II allele in NOD mice, I-Ag7, which is similar to the HLA-DQ8 in structure and preferences for peptide presentation, is also sufficient to drive T1D [206–208]. Consequently, it is believed that susceptible HLA types generate a repertoire that includes T cells that are poised to be primed to islet-epitope expressing APCs coupled with co-stimulation in the pancreatic draining lymph nodes, and after chemoattraction to the islets, recognize and execute their effector functions to beta cells.

3.3. Islet autoreactive T cells and the islet environment in T1D

In the study of human T1D, the vast majority of information regarding autoreactive T cells has been derived, by necessity, from peripheral blood. T cell reactivity to known tissue-specific epitopes in subjects without T1D and comparison to those with T1D has formed the basis of numerous studies over the past two decades; however, the number of studies of autoreactive T cells in the peripheral blood of individuals without risk of T1D, first degree relatives, and those at-risk for T1D (with >1 AAb) are in the minority and additional studies are required to increase our knowledge of the autoreactive T cell status before serum AAb appearance and clinical diagnosis. Indeed, it has been proposed that healthy individuals have a state of ‘benign autoimmunity’ observable both in the periphery, in pancreatic acinar tissue, and in islets [114,209,210].

In the periphery, increased proportions of recently activated islet-specific CD4⁺ T cells have been observed in T1D versus healthy subjects [211,212]. Despite their low numbers in peripheral blood, there has been some evidence that self-reactive T cells are present at higher numbers (suggesting expansion) in subjects with T1D, whereas other studies have observed similar numbers in this population [210,211]. In a recent study, interrogation of self-reactive CD8⁺ T cells observed similar overall numbers in subjects with T1D and HLA matched controls in peripheral blood but demonstrated increased frequencies within pancreatic islets [213]. Indeed, in order to monitor immune-based therapies for T1D, we will have to reconcile what we have learned about autoreactive T cells in the periphery of individuals with T1D or at-risk for T1D with what we are learning about the composition and functions of islet-infiltrating T cells. However, despite the importance of this task, it is certainly not trivial, and studies must be carefully designed to provide relevant insight into therapeutic efficacy.

Functional examination of autoantigenic T cells has demonstrated that T cells from individuals without disease were found to be dependent on co-stimulation for a functional response in contrast to autoreactive T cells from the periphery of individuals with multiple sclerosis or T1D [214,215], suggesting that in disease, autoreactive T cells are in a more memory-like state. Autoreactive CD8⁺ T cells with an antigen experienced (memory) phenotype were observed both in the periphery in children with T1D and at-risk subjects [205] with both CD4⁺ and CD8⁺ T recognizing a broad array of epitopes and antigens [143].

Furthermore, they have been shown to take on a wide range of phenotypic states, encompassing the complete spectrum from naïve through terminal exhaustion [216]. Some phenotypes are implicated as playing a pathogenic role in disease, including stem cell memory [217], CD57⁺ effector memory phenotype [218], and an activated T cell subset [216,219]. Others are associated with non-progression or protection, including exhausted T cells [220,221], and regulatory T cells [212,222].

As previously discussed (Section 2.4), an inflammatory islet micro-environment is strongly tied to the development of T1D. Pro-inflammatory cytokines control the transcription from the promoter for C-X-C motif chemokine ligand 10 (CXCL10) [223] which is a potent chemoattractant for immune cells [224,225]. Islet-infiltrating T cells, in murine models of virus-induced T1D and from tissue from human donors with T1D, have been found to express C-X-C motif chemokine receptor 3 (CXCR3), the receptor for CXCL10 [226–228]. Thus, the inflammation-controlled CXCL10:CXCR3 axis is critical for immune cell recruitment into islets. In addition, exposure of islets to inflammatory cytokines upregulates programmed death-ligand 1 (PD-L1 or CD274) but not programmed death ligand 2 (PD-L2 or CD273) expression on beta cells islets and insulin⁺ beta cells from donors with T1D express PD-L1 [229,230]. The interaction with programmed cell death protein 1 (PD-1) expressing T cells mediates inhibition of T cell function, suggesting a mechanism for beta cells resisting T cell attack through the PD-1:PD-L1 axis, though the dynamics and efficacy of PD-L1 expression by beta cells from donors with T1D is not understood. These data indicate that an inflammatory islet environment has pleiotropic effects on islets which cause interactions with the immune system.

3.4. Direct evidence of and composition of T cell islet infiltrates

As mentioned, immune infiltration of islets in those with T1D has been recognized for decades [156,157]. T1D, in humans and in NOD mice, is marked by insulinitis or peri-insulinitis, which are inflammatory lesions comprised of CD45⁺ cells within or on the periphery of islets, which are comprised of multiple immune cell types. The current histopathological definition of insulinitis is the presence of ≥ 15 CD45⁺ leukocytes/islet (alternatively ≥ 6 CD3⁺ lymphocytes) in three islets with the presence of pseudotrophic (insulin negative) islets [231]. Interestingly, in human T1D, detection of insulinitis or peri-insulinitis is rare, even in newly diagnosed donors or autoantibody positive donors, and insulinitis is heterogeneous or ‘patchy’, within lobes or even in islets in close proximity to each other, infiltrated or without infiltration [232]. CD8⁺ T cells, CD4⁺ T cells, B cells, macrophages/dendritic cells, and NK cells have been shown to infiltrate islets in humans and in NOD mice [78,233–235] and CD4⁺Foxp3⁺ Treg cells (section 3.1.2) from the islets of NOD mice [192]. B cells are also found within islets of donors with T1D, and their presence and frequency has been correlated with age of T1D onset [236,237]. While immune cells of the innate arm of the immune response (e.g., macrophages, dendritic cells, NK cells) have been described in the islets of human donors with T1D, their critical phenotypes, functions, and interactions with cells of the adaptive immune response (antigen presentation, regulation of the microenvironment), their phenotypes and functions are under current investigation [78,85,87,233,235–238]. Dendritic cells are important players in connecting innate and adaptive immunity and, along with macrophages, are components of human insulinitis [85,87,233,235,238]. In NOD mice, macrophages that are defined as islet resident cells are necessary for disease [239] with an alteration to a pro-inflammatory phenotype [240]. However,

the roles these immune cells play, specifically, the cellular interactions in infiltrating islets and their interactions with infiltrating CD4⁺ and CD8⁺ T cells in human T1D leading to signaling cascades and effector functions are poorly understood at present. CD4⁺ T cells are thought to be the orchestrator of the immune attack on the beta cell with CD8⁺ T cells playing a crucial role in direct beta cell destruction and representing a significant constituent in insulinitis [114,213,241].

3.5. Antigen specificity of islet-infiltrating T cells

Among the immune cells that infiltrate the pancreas [4], islet epitope-reactive CD8⁺ T cells can be found in the islets and in the acinar tissue of donors with T1D [114,241,242]. In addition to an inflammatory/chemoattractant islet environment, T cells require specific antigen epitope recognition to perform their effector functions. A substantial array of beta cell proteins has been implicated as T cell antigens, including characterized native epitopes and neopeptides [143] (Section 2.8). As a key protein expressed in beta cells, prepro-insulin (also a known susceptibility locus in T1D) has the most obvious relevance, and indeed, insulin specific CD8⁺ T cells have been directly visualized within human islets [241]. Accumulating evidence shows that insulin reactive CD4⁺ T cells are also well represented within human insulinitis [243–246]. Whether reactivity to islet antigens is a requirement for T cells to enter islets is an ongoing conundrum with two schools of thought. One set of studies have reported that islet entry in NOD mice is tightly regulated by reactivity to islet autoantigens [247–251]. In contrast, several studies have reported the presence of naïve, non-diabetogenic, or highly polyclonal T cells in islets of NOD mice [240,252–254]. One of the complicating factors in addressing this question is limited knowledge about the antigenic specificity of T cells. Despite being one of the most well-studied diseases from an antigen discovery viewpoint, the majority of antigenic specificities of islet-infiltrating T cells are unknown [4]. To date, 16 endogenous proteins have been reported as autoantigens recognized by CD8⁺ T cells [143], whereas 10 proteins have been reported to be sources of epitopes for CD4⁺ T cells. Autoantigens such as ChgA, proinsulin, glutamic decarboxylase 65 (GAD65), IA-2, glucose-6-phosphatase, catalytic subunit 2 (G6PC2); formerly islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP), and zinc transporter 8 (ZnT8) are shared among multiple T1D patients and NOD mice. In addition, as discussed (Section 2.8), generation of islet neopeptides and their targeting by T cells is a major focus of interest in T1D.

The class I and II peptidomes from human beta cell lines and NOD mice is under examination [114,255] and, in parallel, mass spectrometry and RNA sequencing are being used to verify suspected epitopes and to reveal new candidate antigens including HIPs [256]. The unbiased data sets generated through these efforts verify the formation of known epitopes and support the relevance of several different classes of neopeptides. Developing strategies to identify potentially formed neopeptides from large proteomic data sets will allow discovery in an unbiased manner and further accelerate this field of investigation. Indeed, as of now, only a small subset of these specificities has been shown to be present within islet infiltrates [243]. The common thread between all different classes of neoantigens is a theorized short-circuiting of central and peripheral tolerance mechanisms. The idea is that neopeptides are underrepresented or absent from the thymus and healthy tissue but readily generated in islets under conditions of stress, thus triggering an autoimmune attack [257]. For example, it should be noted that insulin hybrid peptides have been detected in the islets of donors without risk of T1D [256].

3.6. Islet epitope hierarchy

An important feature of the antigenic landscape of T1D in NOD mice is an apparent epitope hierarchy. NOD mice lacking the gene for Chga expression (*Chga*) or the gene for insulin 1 in mice (*Ins1*) [258,259] fail to develop T1D, but for mice lacking the gene for protein tyrosine phosphatase receptor type N (*Ptprn*, the gene for IA-2), GAD65 (*Gad2*), or G6PC2/IGRP (*G6pc2*) expression, T1D still developed [260–263]. The mechanism of such hierarchy is suggested to be epitope spreading [264,265], where the pro-inflammatory cytokine secretion by CD8⁺ and CD4⁺ T cells may lead to enhanced antigen presentation and alterations in the presented landscape of beta cells [266]. The known features of the antigenic landscape of islet-infiltrating T cells in the NOD model implies two waves of T cell recruitment to the islets. The first wave consists of a focused, highly restricted repertoire, followed by a second wave consisting of a broader repertoire. In human disease, this is hard to capture given limited availability of islet samples, heterogeneity of islet infiltration, variation in HLA alleles, and relatively long timeframe of disease development. Because of technical limitations and under-sampling, it is currently not possible to draw firm conclusions about epitope hierarchy within islets of human donors with T1D. In addition, the structure and temporal landscape of the epitope specificities is complicated by the recent research findings focused on neopeptides within the context of T1D (Section 2.8).

3.7. Autoreactive TCR diversity

TCR repertoire studies conducted in NOD mice [267–269] have reported a bias towards certain TCR variable beta chain families, despite minimal inter-animal overlap. TCRs reactive to common beta-cell autoantigens can be detected in peripheral blood of T1D patients and healthy controls [270]. Early studies comparing clonal expansion of T cells in T1D patients have suggested overlap of clones between islets and peripheral blood [271]. Recent reports have presented conflicting evidence of whether CD4⁺ T cell repertoires were found to be tissue specific, whereas CD8⁺ T cell repertoires were shared amongst multiple tissues [213,272] suggesting that analyzing peripheral blood may not be optimal to study the development of autoreactive islet-infiltrating T cells. Another complicating factor is high overall diversity of clones reactive to a given antigen [273], which is coupled with overall low levels of clonal overlap between two individuals [272]. However, oligoclonal T cells can be found within islets of donors with T1D and their epitope targets, here epitopes in proinsulin, can be identified [244].

Finally, as the TCR sequences themselves have almost no predictive power with respect to their cognate epitope, there is a disconnect between the TCR repertoire and the antigen specificity landscape. Several landmark studies have identified antigenic specificities of islet-derived T cells and TCRs *de novo* [114,243–246,274,275], but the antigenic specificity of most islet-derived T cells remains unknown. Current single cell RNA sequencing techniques, which can inform the TCR clonotype, along with newer TCR-directed epitope discovery techniques [276] will be instrumental in providing a comprehensive picture of the antigenic landscape seen by islet-infiltrating T cells in NOD mice and patients with T1D [194].

4. THE BETA CELL-IMMUNE CELL INTERFACE

Mounting evidence suggests that at its most fundamental level, the pathology of T1D is an escalating disruption of normal, non-pathogenic interactions between immune cells and beta cells. In health, beta cells take on a spectrum of phenotypes and synergize with one another and other endocrine cell populations within the islets to maintain

homeostasis and glucose control. Tissue resident immune cells (including T cells) are present within the healthy pancreas, but significant insulinitis is not generally seen in healthy organ donors. We do not fully understand the phenotypic attributes that predispose individual beta cells and/or specific beta cell subpopulations to immune attack or how their function/dysfunction and stress responses contribute to susceptibility or resistance to immune attack. However, it seems clear that critical changes in beta cell phenotype and the islet microenvironment do happen, attracting insulinitis and inviting beta cell destruction. Indeed, several pieces of evidence suggest key influences that may disrupt the interface between beta cells and the immune system. We understand that intrinsic stress in the beta cell (e.g., ER stress) and extrinsic stressors (cytokine exposure and potentially, virus exposure) can be amplified through genetic predisposition, leading to altered RNA and protein processing, HLA class I hyperexpression and epitope/neopeptide presentation by beta cells. This and other factors (e.g., secretion of CXCL10 for immune cell chemotaxis) can influence the propensity for the beta cell to become recognizable to immune cells (Figure 2, points A–C). In some cases, transient protective effects (e.g., Treg activity or PD-L1 expression on a subset of beta cells) may oppose immune attack, but in disease, these are insufficient to preserve functional beta cell mass. In tandem, it is clear that loss of tolerance and recognition of functional beta cells by immune cells contributes to both temporal and spatial changes in the pancreas and islet-infiltrating T cell and immune cell repertoire (Figure 2, points D–F) with possible alterations in T cell phenotype that sustain autoimmunity. The breadth of autoreactive T cell targets, the islet specific TCR repertoire, and the mechanism(s) driving the switch of islet-autoreactive T cells from a more naïve ('benign') phenotype to a more memory, potentially pathogenic phenotype are under active investigation. We anticipate that emerging insights from those studies will help answer these pressing questions.

Importantly, each of these mechanisms can happen independently, but compound one another to foment deleterious interactions at the beta cell-immune cell interface. It remains to be discovered how these two elements of dysfunction, the beta cells, and the immune cells, change and interact at different stages of disease development. There is significant opportunity for new contributions in this arena, and we are confident that novel models and approaches to studying the disease will accelerate the pace of discovery.

5. CONCLUSIONS

The last years have seen accelerated development of approaches that allow researchers now to study T1D in a human context comprehensively. Animal models and system have laid a crucial foundation of knowledge to facilitate research in a focused and informed manner using human cells. Taken together, recent studies have contributed considerably to our revised understanding that the pancreatic beta cell assumes an active role instead of a passive position during autoimmune disease progression. However, many knowledge gaps remain (Table 1). Addressing our knowledge gaps are certainly complicated by the uncertainty if T1D progression is a highly personalized process dependent on individuals (genetics, cell-cell interactions, inflammation, and lifestyle choices) or if key processes are shared among large patient populations. Taking advantage of recent model systems that allow to study these questions in a consistent genetic background reducible will be critical. Similarly (as outlined above), despite our rapidly expanding knowledge on T cell biology in T1D the exact nature and subsequence of immune infiltration and its interaction with human beta cells is still poorly understood. Defining epitope reactivity of T1D islet associated T

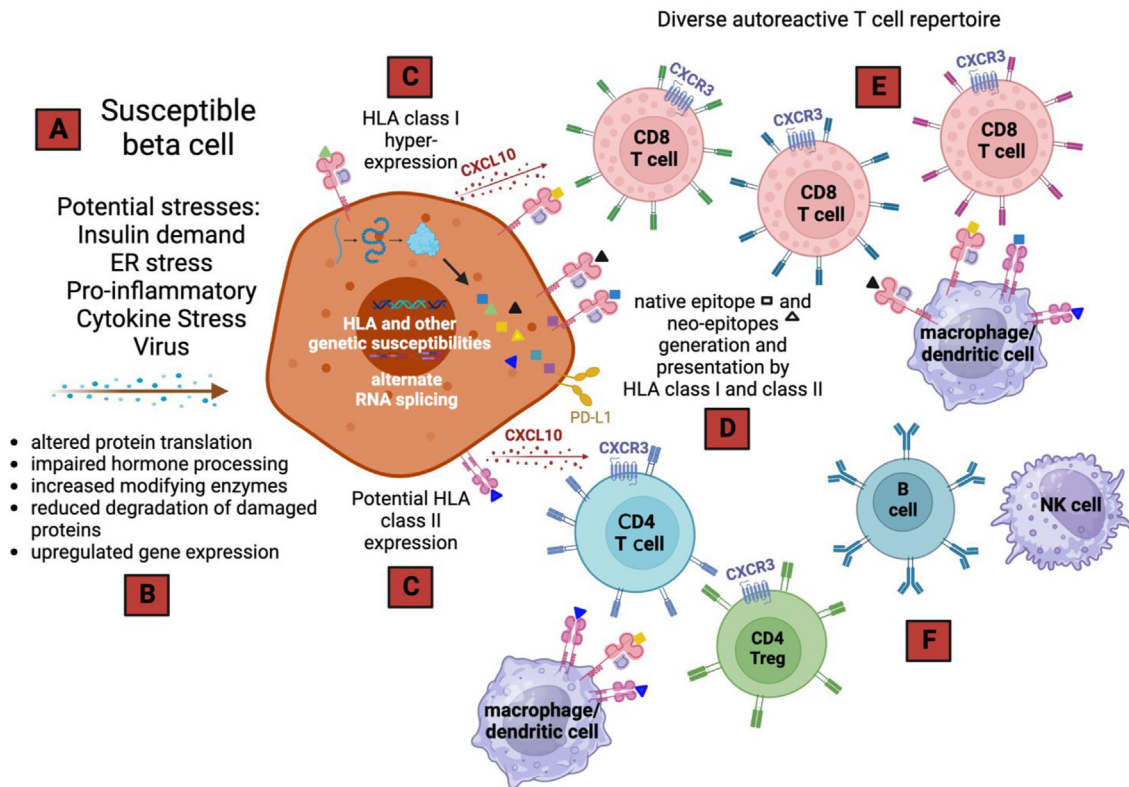


Figure 2: The beta cell-immune cell interface in T1D. This schematic depicts some of the known components of the beta cell-immune cell interface, though not every aspect of each and how each aspect contributes to pathology in T1D is fully known. **Point A:** Susceptible beta cells experience additional stress, including increased insulin demand, multiple sources of ER stress, proinflammatory cytokine exposure, or potential viral infection, the effects of which may be further augmented by expression of susceptible HLA and other genetic susceptibilities. **Point B:** These processes can result in alterations in gene expression, RNA splicing, dysregulation of protein processing and expression, and generation of modified neo-epitopes. **Point C:** Class I hyperexpression is a hallmark of beta cells in T1D while beta cell expression of Class II is controversial. Islets secreting CXCL10 attract activated, CXCR3 expressing T cells. However, PD-L1 surface expression can counteract activating immune mechanisms. **Point D:** Both beta cell class I hyperexpression and class II expression could lead to direct increased native and neoepitope presentation to both CD4⁺ and CD8⁺ T cells. **Point E:** The full breadth of the autoreactive T cell repertoire is unknown and under investigation as is how central tolerance may be broken in T1D. **Point F:** The full function of other immune cell populations (macrophages, CD4 Tregs, NK cells, B cells, other immune cells?) in islet infiltration and pathology are similarly under investigation.

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Table 1 — Knowledge gaps in the beta cell biology, immune cell biology, and in the beta cell-immune cell interface in T1D.

Knowledge gaps in beta cell-immune cell interface in T1D

- Why are beta cells specifically targeted in T1D?
- Why are some beta cells susceptible to immune attack while others are resistant?
- Do 'resistant' beta cells respond to stress differently than susceptible beta cells?
- How do genetics influence beta cell susceptibility or resistance to attack?
- What are the environmental triggers that enhance susceptibility of beta cells to immune attack?
- What initially attracts activated immune infiltrates into islets?
- What is the full complement of islet-infiltrating immune cell types, how do they interact, and how are they involved in pathogenesis?
- What causes the spatial heterogeneity of immune infiltration of islets?
- Are there unknown mechanisms of neo-epitope generation?
- How do individual HLA haplotypes influence the autoreactive T cell repertoire infiltrating the islets in human T1D?
- What is the breadth of the islet-infiltrating autoreactive T cell repertoire and TCR repertoire in human T1D?
- What mechanism(s) drive(s) naive autoreactive T cells to a memory, more pathogenic, phenotype?
- What is the 'tipping point' at the beta cell-immune cells interface that promotes beta cell damage?
- Will key reactivities differ for different stages of disease?
- What are the key T cell reactivities to target in therapies?
- What is the upper limit of the efficacy of Treg therapies?
- Can specific elements of beta cell function be therapeutically reversed?

cells will likely provide new insights into differential pathways for disease progression. These efforts should be pursued in conjugation with better defining the array of translated proteins and peptides and the immunopeptidome presented on the surface of beta cells (and/or beta cells subpopulations). The advances in our understanding of the underlying molecular and cellular mechanisms leading to human T1D development have been truly remarkable and revealed novel pathways. We predict with the future refinement of human model systems and analysis approaches we can further advance to field towards the discovery and implementation of novel intervention and therapy strategies that will benefit T1D patients.

AUTHOR CONTRIBUTIONS

AKL, AVJ, EAJ, HAR and SCK all contributed to the content of this review article.

All authors are members of the Human Islet Research Network (HIRN).

FUNDING

AKL is supported by a new investigator award from NIDDK/HIRN RRID: SCR_014393, a NIH/NIDDK New Investigator Gateway Award R03 DK127766, and NIH/NIDDK grant R01DK124380. AVJ is supported by NIH/NIDDK New Investigator Gateway Award R03 DK127447-01, NIH/NIDDK/dKNET New Investigator Pilot Award in Bioinformatics, Pittsburgh Autoimmunity Center of Excellence in Rheumatology (PACER) Innovative Discovery Award, and JDRF grant 3-SRA-2023-1354-S-B. EAJ was supported by U24 DK104162-07 (HIRN, Niland PI), 2 R01 DK081166-09 (Haskins PI), and JDRF grant # 1-SRA-2020-978-S-B. HAR is supported by NIH/NIDDK grant R01DK12044 and R01DK132387, a new investigator award from NIDDK/HIRN RRID: SCR_014393; UC24DK1041162, the Juvenile Diabetes Research Foundation (JDRF 2-SRA-2019-781-S-B, 2-SRA-2023-1313-S-B, 3-SRA-2023-1367-S-B). SCK was funded by UC4 DK104218-02 and UC4 DK116284 and is the The George F. and Sybil H. Fuller Term Chair in Diabetes.

DECLARATION OF COMPETING INTEREST

AKL has been on a scientific advisory panel for Janssen Research & Development, LLC. AVJ has been a consultant for Pfizer Inc. and has received research funding from Mitsubishi-Tanabe Pharma. EAJ has been a consultant for ProventionBio and has research projects sponsored by Bristol-Myers Squibb and Novartis. HAR has been a SAB member for Prellis Biologics and Sigilon Therapeutics and consultant to Eli Lilly, Minutia, Guidepoint, and Axon. SCK is an SAB member for 4Immune Therapeutics.

DATA AVAILABILITY

No data was used for the research described in the article.

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