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From immunobiology to β-cell biology

The changing perspective on type 1 diabetes

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Abbreviations: ATF6, activating transcription factor 6; ER, endoplasmic reticulum; IRE1α, inositol requiring 1α; NOD, non-obese diabetic; PERK, protein kinase R-like endoplasmic reticulum kinase; RIP-LCMV-GP, rat insulin promoter-lymphocytic choriomeningitis virus–glycoprotein; sXBP1, spliced X-box binding protein 1; T1D, type 1 diabetes; TUDCA, taurine-conjugated ursodeoxycholic acid; UPR, unfolded protein response

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Type 1 Diabetes (T1D) is characterized by the immune mediated destruction of β cells. Clinical studies have focused on drug therapies to modulate autoimmunity, yet none of these interventions has resulted in durable preservation of β-cell function. These findings raise the possibility that initiating or propagating events outside of the immune system should be considered in future efforts to prevent or reverse T1D. An emerging concept suggests that defects inherent to the β cell may trigger autoimmunity. A study by Engin et al. in type 1 diabetic NOD mice suggests that excessive β-cell endoplasmic reticulum stress arising from environmental insults results in abnormal protein synthesis, folding, and/or processing. Administration of the chemical protein folding chaperone TUDCA resulted in recovery of β-cell endoplasmic reticulum function and a diminished incidence of diabetes in NOD mice. We propose here that these data and others support a model whereby an inadequate or defective β-cell endoplasmic reticulum response results in the release of β-cell antigens and neoantigens that initiate autoimmunity. Pharmacologic therapies that either mitigate these early β-cell stressors or enhance the ability of β cells to cope with such stressors may prove to be effective in the prevention or treatment of T1D.

Type 1 diabetes (T1D) arises when loss of islet β-cell function or mass results in the absolute deficiency of insulin. T1D is thought to occur from a breakdown in immune tolerance, resulting in the infiltration into the islet of auto-reactive T cells that target β cells.[1] The immunobiology of T1D has been studied most extensively in the non-obese diabetic (NOD) mouse, which exhibits early and gradual infiltration of immune cell types into the pancreas ("insulitis") with accompanying loss of β-cell mass.[2] In the setting of a dysregulated immune system, it has been proposed that externalization of β-cell antigens activates autoreactive T cells and give rise to invasive insulitis.[3]

Although insulitis of the extent observed in NOD mice has not been as uniformly documented in human autopsy studies, a similar failure of immune tolerance is thought to occur in humans.[4] Several immune modulatory drugs with well characterized responses in other autoimmune diseases have been attempted in clinical trials of new-onset T1D subjects. Whereas administration of some of these drugs (such as anti-CD3, anti-CD20, CTLA4-Ig) have led to the preservation of β-cell function (as assessed by C-peptide secretion) for periods of months, subsequent declines in β-cell function paralleled those of placebo.[5-8] In no case has true remission, as defined by insulin independence, been observed. These outcomes could be explained by the low replicative capacity of human β cells,[9,10] coupled with the possibility that interventions were initiated too late in the disease—at a time in which molecular stress pathways in β cells were so aggressively activated that even a respite from autoimmunity could

not prevent β-cell decline. If true, what remains unanswered is the cause and nature of these intrinsic β cell stress pathways, and whether therapies that target these pathways might substantially alter outcomes. It should be noted that these outcomes in humans differ substantially from those seen in NOD mice, in which a number of interventions have been shown to prevent or even lead to remission of T1D.[2] Collectively, these considerations have sparked a new perspective in the field, in which greater emphasis has been placed on elucidating the molecular mechanisms within β cells that might initiate or perpetuate autoimmunity and eventual β cell demise.

By all accounts, β cells are a vulnerable cell type with little reserve. Notably, β cell mass is relatively small—less than 1 g in most humans,[11] and possibly even lower in individuals susceptible to T1D.[12,13] To compound the low mass reserve, β cells have a low proliferative rate. In humans, β cell replication falls exponentially in the immediate postnatal period, to virtually zero by adulthood.[9,10] In addition to limited mass and proliferation, β cells also have limited functional reserve. As professional producers and secretors of insulin, β cells rely heavily upon the endoplasmic reticulum (ER) to ensure that proteins are produced robustly and folded efficiently. As such, even minor perturbations in calcium homeostasis, oxidative stress, and peripheral insulin demand can impose stresses that can cause the β-cell ER to decompensate ("ER stress") and fail to efficiently produce, fold, and process relevant proteins.[14] Unfolded or improperly processed proteins that exit the β cell have the potential to trigger autoimmunity.[15] Dysfunction of the β cell—as judged by an impaired insulin secretory response to glucose—appears to precede the development of frank T1D in both mice and humans.[16-19] Only recently have studies correlated the decline in β-cell function with the appearance of ER stress,[16,20] thus raising the question of whether ER stress is a cause or consequence of β-cell dysfunction in T1D.

In a recent study, Engin, et al.[21] engaged 2 mouse models of autoimmune diabetes and a chemical chaperone of protein folding to address if ER stress is causative of β-cell dysfunction and subsequent T1D. Both models, the NOD mouse and the RIP-LCMV-GP mouse (rat insulin promoter-lymphocytic choriomeningitis virus–glycoprotein, in which diabetes is induced by viral infection), exhibited invasive insulitis and β-cell destruction. The authors first studied the 3 major arms of the unfolded protein response (UPR) cascade—PERK (protein kinase R-like endoplasmic reticulum kinase), ATF6 (activating transcription factor 6), and IRE1α (inositol requiring 1α)—as they became activated in an attempt to resolve ER stress. These arms collectively result in global inhibition of mRNA translation initiation, increased production of protein folding chaperones, and ER biogenesis. Specifically, PERK phosphorylates the translation initiation factor eIF2α, resulting in inhibition of CAP-dependent mRNA translation. ATF6, upon cleavage by S1P and S2P proteases, is translocated to the nucleus to activate gene transcription. IRE1α is an endoribonuclease that splices *Xbp1* mRNA to produce sXBP1 protein. Both ATF6 and sXBP1 enhance the transcription of genes encoding ER chaperones and other UPR intermediates (for reviews, see refs. 22 and 23). By pancreas tissue immunofluorescence, Engin, et al. showed that both NOD and RIP-LCMV-GP mice exhibit age-dependent loss of ATF6 and sXBP1 in the weeks preceding the development of T1D, a finding suggestive of a failure of the "proresolution" functions of the UPR. To correlate these findings to human T1D, the authors then studied tissue sections from human subjects. Interestingly, when compared with controls, T1D subjects exhibited reduced ATF6 and sXBP1 staining intensity in residual β cells, a finding more striking in females with diabetes for 8–20 y duration (for unclear reasons).

To address more directly whether failure of the proresolution function of the UPR contributes to β-cell dysfunction and frank T1D, the authors next administered the chemical protein folding chaperone taurine-conjugated ursodeoxycholic acid (TUDCA).[24] In both mouse models, TUDCA treatment reduced β cell death, restored insulin secretion, and reduced substantially the incidence of T1D. The molecular mechanism linking TUDCA to its effect appeared to be via stimulation of ATF6 and sXBP1 expressions in β cells. Consistent with this possibility, TUDCA had no effect in preventing T1D in a mouse model of RIP-LCMV-GP lacking ATF6 protein in β cells. Taken together, these data support the notion that ER stress is an important contributor to β-cell dysfunction and eventual T1D in mice, and that β cells in these mouse strains appear to have defective adaptive UPRs.

Whereas prior studies suggested a role for ER stress in the pathogenesis of T1D,[16,20] the study by Engin, et al.[21] is the first to provide a direct link between ER stress, β-cell dysfunction, and T1D development. Nevertheless, important questions still remain. First, does this study rule out a role for immune cells in triggering β-cell destruction? Because TUDCA was administered systemically, some uncertainty still exists as to whether the drug might have affected immune cells directly. In this regard, immune cells undergo rapid protein production and ER expansion upon antigenic stimulation,[25] and therefore it remains possible that systemic TUDCA administration has combined and synergistic effects on both β cells and immune cells. Although the ineffectiveness of TUDCA in the β-cell-specific ATF6-deficient mouse model seemingly discounts this possibility, it is unclear whether the RIP-LCMV-GP mouse model can be used interchangeably with the NOD model, as the viral etiology of T1D remains controversial.[26] Therefore, it would seem important to study NOD mice in which ATF6 is either specifically deleted or overexpressed in islet β cells to directly test a role for protein folding in diabetes pathogenesis. Nonetheless, from a β-cell perspective, it is tempting to speculate that the reduced insulitis in TUDCA-treated animals arose from reduced β-cell auto-antigen exposure.

Second, if not autoimmunity, what are the factors that initiate β-cell ER stress in the setting of T1D, and third, are these findings relevant to human Type 1 diabetes? Considerable work has been performed to address triggers of ER stress, which has been shown to be induced by hyperglycemia and resultant oxidative stress, saturated free fatty acids, pro-inflammatory cytokines, proinsulin

mutations, and double-stranded RNA, among others.[14] Using a bioassay with peripheral blood mononuclear cells in vitro, Wang, et al.[27] noted that serum of pre- and recent-onset T1D individuals induce a strong transcriptional signature of innate immunity. Similar findings were noted in the biobreeding rat model of T1D.[28-30] These findings suggest that systemic inflammation is present in individuals destined to develop T1D, and that sustained inflammation could lead to ER stress in β cells. The source of this systemic inflammation remains vague, but environmental factors such as viruses or other infections, components of the gut microbiome, prenatal factors, insulin resistance, and diet remain possible candidates.[31-33] Observational studies such as TEDDY (The Environmental Determinants of Diabetes in the Young) are focusing on correlation of T1D with such factors.

The observation by Engin, et al.[21] that human β cells exhibit similar expression profiles to the mouse models, is reassuring, but by no means definitive that these findings can be extended to human disease. A number of monogenic disorders have been described that lead to β-cell ER stress and death, resulting in early onset of diabetes without apparent autoimmunity. For example, mutations in the *WFS1* gene lead to Wolfram syndrome, which is characterized by childhood-onset diabetes, hypoinsulinemia, diabetes insipidus, optic atrophy, and deafness.[34,35] Mutations in the gene encoding PERK results in Wolcott-Rallison syndrome, which includes neonatal or early onset diabetes, skeletal dysplasia, and growth retardation.[36] At present, genome-wide association studies have not uncovered polymorphisms or T1D risk alleles associated with the UPR. However, studies are beginning to identify UPR genes associated with a number of neurodegenerative disorders including progressive supranuclear palsy and Alzheimer disease.[37] Interestingly, while GWAS studies have primarily identified polymorphisms in genes thought to solely impact immune function, over 60% of T1D candidate genes are also expressed in human islets exposed to proinflammatory cytokines.[38] Similar to prevailing theories regarding the development of type 2 diabetes, the idea of T1D emerging in the context of a β cell that is inherently susceptible to UPR activation and/or ER stress is intriguing and one that bears further testing. In support of this notion, Donath and colleagues identified a family with T1D stemming from a point mutation in the histone deacetylase SIRT-1 that resulted in increased β-cell nitric oxide synthesis and cytokine production in the context of inflammation.[39]

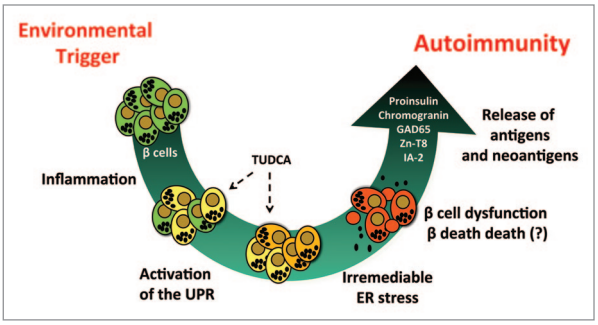

Figure 1. The β cell-centric model of T1D pathogenesis. The model proposes that sustained systemic inflammation arising from environmental factors (e.g., viral infections, gut microbiome, diet) leads to development of ER stress in β cells. Irremediable ER stress, as a result of failed compensatory responses by ATF6 and sXBP1, leads to the dysfunction and death of β cells. The subsequent release of β-cell antigens and endogenous neoantigens triggers secondary autoimmunity. A vicious cycle then ensues, leading to destruction of β-cell mass and development of T1D. The figure shows the potential stages in pathogenesis where intervention with TUDCA might allow for ER stress remediation.

Taken together, the study of Engin, et al.[21] and an emerging body of work from others suggests a model of T1D pathogenesis that shifts the focus from immunobiology to β-cell biology. The accompanying figure (**Fig. 1**) depicts a process in which systemic inflammation arising from the environment leads to inflammatory signaling in islet β cells. When inflammation is sustained or in the context of a susceptible islet β cell, there is activation of the UPR and triggering of ER stress that can result in either β-cell death or dysfunction, with the release of β-cell antigens and endogenous "neoantigens" (i.e., in this case an antigenic determinant emanating from misfolded or misprocessed β-cell proteins), which secondarily induce autoimmunity. As noted previously, many of the autoantigens described in T1D, including proinsulin, ZnT8, chromogranin, GAD65, and IA-2, are channeled through the ER.[15,40,41] In this hypothetical model, several factors can increase the susceptibility to β-cell dysfunction, such as reductions in starting β-cell mass and genetic factors that may diminish and/or enhance β cell to ER stress-responsiveness.

We acknowledge that many aspects of this model remain controversial. Most importantly, several reports suggest a disconnect between β-cell apoptosis and/or death and activation of ER stress. A study by Satoh, et al.[42] demonstrated that CHOP is dispensable in the development of type 1 diabetes. There is also disagreement with regard to how cytokines induce β cell death. At least 2 reports suggest that death is independent of ER stress in cell lines[43] and the NOD mouse.[44] By contrast, a study in INS-1 cells suggests that pro-inflammatory cytokines and classical ER stress inducers like thapsigargin are both capable of inducing death and ER stress, but differ significantly in how they activate specific components of UPR signaling.[45]

Whereas β-cell death may not be sufficient or necessary for induction of an autoimmune response,[46] these studies do not rule out the important possibility that secretion or liberation of neo-antigens in the context of ER stress leads to the loss of immune tolerance. Based on our understanding of rare disorders such as Wolfram and Wolcott-Rallison syndromes, hyperactivation of ER stress alone is unlikely as well to completely drive this process, as individuals with these disorders lack detectable β-cell autoantibodies. Notwithstanding these controversies, this model should serve as a framework

for further refinement using humans and human model systems. Recent application requests from the Juvenile Diabetes Research Foundation (JDRF) and the National Institutes of Health (NIH) increasingly emphasize the need to translate studies from rodent diabetes models to humans and, in this respect, utilization of clinically-approved drugs in rodent models is an important first step in that translation process. As noted by the Engin, et al.,[21] TUDCA has been approved in certain cases of liver disease,[47,48] and therefore has potential to move into clinical trials of T1D. These strategies will need to be tested in individuals in whom T1D risk is high enough to justify initiation of the drug for prevention studies. Assuming that appropriate biomarkers can be identified that predict—with reasonable certainty—the development of T1D, the opportunity may exist to prevent diabetes using drugs that target β-cell ER function and protein folding. At the very least, this study informs the growing dialog suggesting that intrinsic β-cell stress pathways play important role in T1D progression. This dialog is increasingly important as results from recent large clinical trials in subjects with new onset T1D using drugs that solely impact immune function have met with limited success.[7,8,49,50] As such, rational combinations of drugs that address aspects of both T1D immunobiology and β-cell biology are urgently needed.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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