From Pancreatic β-Cell Gene Networks to Novel Therapies for Type 1 Diabetes

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Completion of the Human Genome Project enabled a novel systems- and network-level understanding of biology, but this remains to be applied for understanding the pathogenesis of type 1 diabetes (T1D). We propose that defining the key gene regulatory networks that drive β-cell dysfunction and death in T1D might enable the design of therapies that target the core disease mechanism, namely, the progressive loss of pancreatic β-cells. Indeed, many successful drugs do not directly target individual disease genes but, rather, modulate the consequences of defective steps, targeting proteins located one or two steps downstream. If we transpose this to the T1D situation, it makes sense to target the pathways that modulate the β-cell responses to the immune assault—in relation to signals that may stimulate the immune response (e.g., HLA class I and chemokine overexpression and/or neoantigen expression) or inhibit the invading immune cells (e.g., PDL1 and HLA-E expression)—instead of targeting only the immune system, as it is usually proposed. Here we discuss the importance of a focus on β-cells in T1D, lessons learned from other autoimmune diseases, the “alternative splicing connection,” data mining, and drug repurposing to protect β-cells in T1D and then some of the initial candidates under testing for β-cell protection.

The Human Genome Project opened the door for a systems- and network-level understanding of biology (1). This remains, however, to be fully applied for understanding the pathogenesis of type 1 diabetes (T1D) and, based on this knowledge, identify new therapies for the disease. We will discuss the hypothesis that defining the key gene regulatory networks that drive β-cell dysfunction and death in T1D might enable the design of therapies that target the ultimate causative factor of T1D, namely, the progressive loss of functional β-cell mass, instead of only managing hyperglycemia or attempting to inhibit the immune system to delay disease. This approach has already been attempted for other human diseases, such as heart valve diseases (2). Importantly, early (“stage 1”) T1D may now be detected years ahead of clinical outbreak (3), indicating the potential for effective β-cell-protective therapies that may modify the natural history of diabetes.

Many successful drugs do not directly target individual disease genes; instead, they modulate the consequences of defective steps, targeting proteins located one or two steps downstream (4). For instance, during the ongoing coronavirus disease 2019 (COVID-19) pandemic the use of artificial intelligence to search for drugs to be repurposed for the treatment of the disease found that only 1 of 77 candidates directly targeted the virus, while the 76 other drugs that reduced viral infections relied on network-based cellular responses to the virus (5). If we transpose this to the T1D situation, instead of only targeting the immune system as is usually proposed (6), it would make sense to also target the pathways that modulate the β-cell responses to the immune assault—intrinsic β-cell signals that may stimulate the immune response (e.g., HLA class I and chemokine overexpression or neoantigen generation (7,8)) or inhibit the invading immune cells (e.g., PDL1 and HLA-E expression) (9,10).

Why Focus on the β-Cells in T1D?

We can learn from the Buddha’s advice as outlined in the Kalama Sutta: “Do not go upon what has been acquired by repeated hearing; nor upon tradition . . . nor upon an axiom . . . nor upon a bias towards a notion that has been pondered over. . . .” In other words, let’s leave aside for a moment the “axiomatic and many times pondered” view that prevention of T1D equals inhibiting or modulating...
the immune system in T1D (an approach that—even when implemented in the prediabetes state—at most delays but has not yet been shown to completely prevent disease [11]) and focus instead on the key role for β-cells in the disease. This has been the subject of recent reviews by us and others (7,12,13), and we will here only emphasize key points suggesting the relevance of the β-cells in T1D. Priority is on data obtained with human islets/β-cells and histology of the pancreas from individuals affected by T1D. We are aware that the role for β-cells as a key agent in T1D pathogenesis is coming into increasing focus (7,12,13) and that the notion that new interventions in T1D should also target β-cell protection is gaining traction; we hope that this article will further support this welcome trend.

Some individuals at increased risk for T1D, due to strong family history, show a decrease in their first-phase glucose-stimulated C-peptide release and an increase in proinsulin-to-insulin ratios in the circulation even in the absence of autoantibodies against β-cells, indicating that disturbed β-cell function may precede in some cases the autoimmune assault (14).

There is histological evidence that many patients—including those who died of ketoacidosis close to diagnosis—retain up to 40% of their normal β-cell mass during the first year of T1D (15), and islets isolated from T1D patients are dysfunctional but regain at least part of their ability to release insulin in response to glucose after 5–6 days in culture at 5.6 mmol/L glucose (16,17). These observations suggest that there remains a population of “sleeping” β-cells after clinical disease onset of T1D, still alive but unable to release insulin. Some patients with long-term T1D and with undetectable circulating C-peptide still release proinsulin (18), suggesting that a fraction of β-cells may survive for >3 years but are not able to convert proinsulin into mature insulin. HLA class I hyperexpression (7), markers of endoplasmic reticulum (ER) stress (19), chemokines, and an interferon (IFN) signature (10,20) are present in these remaining insulin-containing islets from T1D patients, suggesting that they are engaged in a cross talk with the invading immune cells. This cross talk may have negative or potentially positive consequences. Thus, triggering of ER stress (which by itself may lead to apoptosis) and changes in alternative splicing (AS) by proinflammatory mediators, together with upregulation of HLA class I and production of chemokines by β-cells, can lead to increased presentation of neoantigens to the immune cells homing into the islets (7,8,21), amplifying the immune assault. This suggests that acting at the β-cell level may be relevant not only to prevention of immune-mediated β-cell death but also to modulation of the components of β-cell dysfunction that contribute to autoimmunity in the first place—an area where much remains to be learned.

On the other hand, cytokines such as IFNα and IFNγ induce the expression of potentially protective molecules in human islets, namely, PDL1 and HLA-E (7,10), a finding confirmed by histology of insulin-containing islets from patients affected by T1D (9,10). PDL1 is a ligand of PD1, a protein expressed in T cells, and PDL1-PD1 binding inhibits T cell function: for instance, overexpression of PDL1 in β-cells protects mice against immune-mediated diabetes, while inhibiting PD1-PDL1 in the context of immune checkpoint therapy for metastatic tumors triggers immune-mediated endocrine diseases, including T1D (22). HLA-E is a potential inhibitor of both natural killer and T cells (7). A challenge that remains to be solved is how to dissociate the cytokine-induced harmful effects (i.e., induction of HLA class I, ER stress, and chemokines) from the potentially beneficial ones (i.e., induction of PDL1 and HLA-E).

Learning From the Target Tissues in Other Autoimmune Diseases

There is conclusive evidence of increased incidence of other autoimmune diseases in patients affected by T1D, and T1D and other autoimmune diseases have many candidate genes in common (23). We recently compared the gene networks expressed in β-cells isolated from patients affected by T1D against the target tissues of three other autoimmune diseases, namely, kidney cells in systemic lupus erythematosus, optic chiasma in multiple sclerosis (MS), and joint tissue in rheumatoid arthritis (RA) (24). While the target tissues of these diseases are different, they share genetic risk loci and have in common local inflammation (with a role for innate immunity), ER stress (24), and the generation of reactive oxygen species and proinflammatory cytokines as causes of tissue damage. Available RNA sequencing (RNA-seq) data for these different tissues (25–28) were downloaded and processed by a common pipeline (24), and comparisons between diseases were done with the rank-rank hypergeometric overlap analysis (RRHO) (29), a genome-wide approach that enables comparisons between two equally ranked data sets. There were confluent signatures mostly among the upregulated genes, many of them related to types I and II interferon signaling. In agreement with these observations, there was an enrichment of binding motifs for IFN-induced transcription factors in expressed genes at the target tissue level in all four diseases. On the other hand, downregulated pathways were mostly disease specific, indicating dysfunction at the level of the target tissues. For example, β-cells obtained from patients affected by T1D had downregulation of pathways involved in energy metabolism (required for insulin release) and in key transcription factors for the maintenance of the differentiated β-cell phenotype, including PDX1 and MAFA (24).

Of particular relevance, in all four diseases >80% of known candidate genes were expressed at the target tissue level (24), which is in line with previous observations that candidate genes for T1D regulate β-cell responses to potential “danger signals,” such as viral infections or endogenous nuclear acid fragments and type 1 IFN
signaling (30,31). T1D-associated variants have been described as "enriched for immune cell types" but not or only marginally present in pancreatic islets regulatory elements under basal conditions (32). This apparent discrepancy was clarified by recent findings showing that human islet regulatory elements responsive to the cytokines IL1β and IFNγ are enriched for T1D risk variants (33), indicating that T1D risk variants indeed impact β-cells but this only becomes detectable upon exposure of these cells to relevant immune stimuli, such as proinflammatory cytokines (7,33).

Interestingly, one of the few candidate genes expressed in the target tissues of the four autoimmune diseases studied is TYK2, a key kinase activated during the early steps of the signal transduction of type I IFNs. Genetic variants that partially decrease TYK2 activity are protective against T1D diabetes (31), and a 50% inhibition of TYK2 by specific siRNAs or chemical inhibitors protects human β-cells against death caused by exposure to the viral by-product double-stranded RNA or the proinflammatory cytokine IFNα (34,35) without sensitizing β-cells to infections by the potential diabetogenic enteroviruses Coxsackie B virus types 1 and 5 (35). TYK inhibitors are being tested as therapeutic agents for psoriasis (phase 2 trial) (36) and RA and inflammatory bowel disease (preclinical studies) (37).

The studies described above involved comparisons between an autoimmune endocrine disease, i.e., T1D, against three other autoimmune diseases that do not target primarily endocrine glands. We recently gained access to RNA-seq of thyroid tissue obtained from patients affected by Hashimoto’s thyroiditis (HT) as compared with normal thyroid tissue (38). These data sets were analyzed with our pipeline (24) and compared by RRHO to RNA-seq data from β-cells from T1D patients as compared with respective control subjects (25) (Fig. 1). As an external comparison, we also included the RRHO analysis of β-cells from T1D patients versus optic chiasma from patients affected by MS (27) (Fig. 1B), the nonendocrine autoimmune disease with the closest similarity to T1D regarding changes in global gene expression (24). There is clearly a more marked similarity between the upregulated genes in the target tissues of the two endocrine diseases as compared with optic chiasma in MS (Fig. 1B). Enrichment analysis of the upregulated pathways in common between target tissues of T1D and HT identified neutrophil degranulation, signaling downstream of types I and II IFNs, cytokine-cytokine receptor interactions, and cell adhesion molecules, among others (Fig. 1C and D). These novel observations reinforce the role for IFNs in autoimmune endocrine diseases and point to novel therapeutic possibilities (see below).

The AS Connection
AS is a major generator of transcriptome and proteome diversity. Pre-mRNA splicing is a highly regulated process that involves the interaction between specific cis-acting pre-mRNA elements and different trans-acting factors (RNA-binding proteins [RBPs]), which together form the so-called "splicing code" and shape splicing decisions. AS outcome is influenced by modifications at the level of cis-regulatory regions (e.g., mutations, methylation) or the expression of trans-acting factors (39,40). Proinflammatory cytokines such as IFNα or IL1β plus IFNγ and diabetes candidate genes such as GLIS3 modulate the AS repertoire of human pancreatic β-cells (10,41–43). Proinflammatory cytokines modify the expression of >30 RBPs in β-cells (40,43). The effects of cytokines in AS seem to be specific, as they induce particular AS patterns that are different from those induced by the metabolic stressor palmitate (39). Inhibition of the candidate gene GLIS3 has a proapoptotic effect on human β-cells, which is mediated at least in part via downregulation of the splicing factor SRSF6 (also known as Srp55). SRSF6 inhibition impairs directly or indirectly the splicing of pre-mRNAs encoding key proteins for β-cell function (e.g., INS, SNAP25), and survival (e.g., BIM-Small, BAX–B) (41,44).

Many of the cytokine-induced changes in gene expression in human islets are similar to the ones observed in human β-cells isolated from patients affected by T1D (7,10) and are also present in the target tissues of other autoimmune diseases (24) (Fig. 1), suggesting that the putative “dialog” between β-cells and the immune system may be valid for other autoimmune diseases. This may also extend to changes in the regulation of AS. As such, human islets exposed to IL1β plus IFNγ share a large number of modified RBPs in common with joint tissues of patients affected by RA (Fig. 2).

Most T1D risk loci are located in intronic regions, suggesting that they are involved in β-cell genomic regulation rather than protein function (7). T1D-associated risk single nucleotides polymorphisms may disrupt splicing, altering the transcription of T1D candidate genes. This, and the impact of inflammatory mediators on β-cell splicing, suggests that antisense oligonucleotide (ASO)-mediated splicing modulation could be a tractable therapeutic approach in T1D. This approach is already being tested in the clinic for other diseases caused by AS defects, such as in Duchenne muscular dystrophy and spinal muscle atrophy (45).

Data Mining and Drug Repurposing to Protect β-Cells in T1D—An Unmet Need
In silico correlation between disease-related pathways and drugs that induce an opposite gene signature (“perturbagens”) may reveal new therapies and/or agents to be repurposed between autoimmune diseases (46). We compared a panel of drug-modified data sets (46) against the gene pathways induced by exposure of human β-cells to relevant proinflammatory cytokines (7,10) or against the pathways present in β-cells obtained from patients affected by T1D (24,25). This identified interesting candidates, including bile acid–derived molecules (that may modulate ER stress),
Figure 1—RRHO analysis comparing the gene expression signatures of FACS-purified β-cells in T1D, thyroid tissue samples in HT, and optic chiasm samples in MS reveals similarities in upregulated genes and enriched pathways. A and B: After a differential expression analysis between the target tissues in T1D, MS, HT, and their healthy controls (CTL) tissues as previously described (24), genes were ranked by their fold change from the most down- to the most upregulated ones and submitted to the RRHO algorithm. The level map colors indicate the adjusted log $P$ values of the overlap (Benjamini and Yekutieli correction) between genes upregulated in both data sets (bottom-
bromodomain inhibitors, and TYK2 inhibitors (10,24) (see below). This approach is, however, limited by two main factors: 1) the use of surrogate cell lines that are not directly related to the disease under study and 2) the fact that surrogate cells are exposed to the tested agents under basal conditions, which is different from the disease situation. For instance, in our previous search for perturbagens, we correlated human β-cells exposed to cytokines or isolated from the pancreas of patients affected by T1D (10,24) against a database containing thousands of standard cell lines—none of them insulin producing—exposed to a panoply of test agents under basal conditions (46). Disease biology is in most cases cell type specific, and pancreatic β-cells have particular characteristics; for instance, to sense nutrients and release insulin, β-cells are unique by increasing, instead of decreasing, mitochondrial ATP production in face of rising glucose concentrations. Furthermore, adult human β-cells have very low proliferative capability (47), which is not the case for the cell lines used in the global screening for drug-modifying signatures (46). Thus, comparing stressed β-cells with cell lines derived from other tissues and exposed to putative therapeutic agents under nonstressed conditions has clear limitations.

A better approach would be to map the dysregulated networks in the relevant cell type, i.e., human β-cells in the case of T1D. This is difficult due to the limitations in obtaining sufficient amounts of human islets and their resistance to genetic modifications. An alternative is to use induced pluripotent stem cell (iPSC)-derived β-cells from patients with type 1 diabetes with relevant disease-predisposing polymorphisms or to introduce these polymorphisms by CRISPR/Cas9 and then expose the cells to relevant stimuli, such as proinflammatory cytokines (7,48) or CD8+ T cells (49), ahead of RNA-seq and proteomics to define the key hubs that drive β-cell dysfunction and death. The next step would be interfering with these disease-related hubs to restore physiology; for instance, the kinase JAK1 is a key hub for the deleterious effects of IFNα on human β-cells, and decreasing JAK1 activity using specific inhibitors preserves β-cell function and survival in the presence of IFNα (10) (see below). Such a global approach has been used with success to identify therapeutic candidates for heart valve disease (2).

**Initial Candidates for β-Cell Protection**

Most β-cell–targeting therapies are still at the preclinical stage or undergoing clinical trials at phase I (Table 1). To exemplify this approach, we list below potential therapies to increase β-cell resistance to IFNα (Fig. 3). This cytokine and its footprints are present in islets from T1D patients, and it may play a crucial role in the early phases of insulitis and β-cell destruction (reviewed in 7). Some of the downstream consequences of IFNα exposure (Fig. 3), such as ER stress, are probably a common end point for diverse β-cell stresses (7,50,51), while some of the proposed approaches, such as exercise, may have benefits that go well beyond protection against β-cell ER stress (52).

The first step of type 1 IFN signaling is binding to its receptors (IFNαR1 and IFNαR2) and activation of the kinases JAK1 and TYK2. This is followed by phosphorylation and activation of the transcription factors STAT1/STAT2, which then dimerize and move into the cell nucleus, leading to a series of effects that, in pancreatic β-cells, may trigger ER stress and upregulation of HLA class I and chemokines (consequently increasing antigen presentation and the attraction of immune cells) but also the upregulation of the potentially protective proteins PDL1 and HLA-E (7,34) (Fig. 3). Potential therapies targeting TYK2, a candidate gene for T1D, discussed above, provide a promising strategy for an early therapy for T1D (35). Currently approved JAK inhibitors usually block the activity of JAK1 and JAK2, affecting both IFNα/β and IFNγ. These inhibitors, including baricitinib, have been approved as a therapy for RA (53) and are being tested in phase 2 trials for systemic lupus erythematosus (54). JAK inhibitors prevent the deleterious effects of IFNα on human β-cells, decreasing HLA class I and CXCL10 expression and IFNα plus IL1β–induced apoptosis (10), and reverse diabetes in newly diagnosed diabetic NOD mice (55). A clinical trial based on the use of baricitinib to prevent progressive β-cell death in T1D is starting now (Table 1) (clinical trial reg. no. NCT04774224, ClinicalTrials.gov).

The fact that both TYK2 (36) and JAK inhibitors (53,54) are already in use, or in advanced stages of clinical tests, increases their potential as a T1D therapy. Indeed, repurposing drugs whose toxicology, pharmacodynamics, and pharmacokinetic profiles are already characterized facilitates the bench-to-bedside transition (56). While systemic inhibition of this family of kinases has been linked to some rare safety concerns, newer generations of these inhibitors with increased specificity against single JAK family members are poised to address these.

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left quadrant), downregulated in both (top-right quadrant), upregulated in the left-hand pathology and downregulated in the bottom part (top-left quadrant), and downregulated in the left-hand pathology and upregulated in the bottom part (bottom-right quadrant). The color palette was scaled up to the highest —log(P) value adjusted—to allow the comparison between the two results. Pairwise analysis between FACS-purified β-cells in T1D and thyroid tissue samples in HT (4) and optic chiasm samples in MS (8), C and D: Overlapping genes between T1D and HT in the upregulated quadrant were submitted to a hypergeometric test using clusterProfiler to highlight common enriched pathways between the target tissues of the two diseases and then analyzed using the Reactome (C), and Kyoto Encyclopedia of Genes and Genomes (KEGG) (D) pathway databases. We downloaded data for T1D (from 25), HT (27), and MS (38), and they were reanalyzed as previously described (24). Adj., adjusted. IMMUNOREGULATORY INTERACT LYMPH NON LYMPH, immunoregulatory interactions between a lymphoid and a non-lymphoid cell.
**Table 1**

| RNA binding proteins (gene name) | A | B |
|----------------------------------|---|---|
| PPARGC1A                        |   |   |
| RBFOX3                          |   |   |
| THOC3                           |   |   |
| PCBP4                           |   |   |
| FAM172A                         |   |   |
| SNRNP48                         |   |   |
| TSEN2                           |   |   |
| HABP4                           |   |   |
| PPIL3                           |   |   |
| PRMT7                           |   |   |
| CPSF1                           |   |   |
| NONO                            |   |   |
| RP9                             |   |   |
| SNRNP70                         |   |   |
| LUC7L                           |   |   |
| TSEN54                          |   |   |
| SRSF8                           |   |   |
| GEMIN6                          |   |   |
| CIRBP                           |   |   |
| PCBP2                           |   |   |
| LSM2                            |   |   |
| SNU13                           |   |   |
| PPIE                            |   |   |
| LUC7L3                          |   |   |
| FXR1                            |   |   |
| PPP2R1A                         |   |   |
| HNRNPC                          |   |   |
| GTF2F1                          |   |   |
| DBR1                            |   |   |
| SRSF4                           |   |   |
| CDC40                           |   |   |
| IK                              |   |   |
| PRPF38B                         |   |   |
| CPSF3                           |   |   |
| THOC2                           |   |   |
| ESS2                            |   |   |
| HNRNPH3                         |   |   |
| YTHDC1                          |   |   |
| PPP2CA                          |   |   |
| COIL                            |   |   |
| PRPF40A                         |   |   |
| BCAS2                           |   |   |
| PAPOLA                          |   |   |
| POLR2D                          |   |   |
| RBM12                           |   |   |
| MTREX                           |   |   |
| HTATSF1                         |   |   |
| RBM4                            |   |   |
| RRP1B                           |   |   |
| RBM28                           |   |   |
| CCAR1                           |   |   |
| CLP1                            |   |   |
| TCERG1                          |   |   |
| SCNM1                           |   |   |
| DHX16                           |   |   |
| PRCC                            |   |   |
| SFPQ                            |   |   |
| RBM10                           |   |   |
| YJU2                            |   |   |
| SRSF10                          |   |   |
| SCAF1                           |   |   |
| DHX15                           |   |   |
| USB1                            |   |   |
| POLR2B                          |   |   |
| NCBP1                           |   |   |
| RBM42                           |   |   |
| HNRNPH2                         |   |   |
| SON                             |   |   |
| SNRPF                           |   |   |
| NSRP1                           |   |   |
| PHF5A                           |   |   |
| PRPF4B                          |   |   |
| MAGOH                           |   |   |
| RBM41                           |   |   |
| DDX5                            |   |   |
| SART3                           |   |   |
| SNRPG                           |   |   |
| U2SURP                          |   |   |
| RBM23                           |   |   |
| RBM39                           |   |   |
| SNRPD1                          |   |   |
| SNRNP35                         |   |   |
| LSM1                            |   |   |
| SNRPB                           |   |   |
| BUD31                           |   |   |
| HNRNPK                          |   |   |
| ZBTB8OS                         |   |   |
| SCAF11                          |   |   |
| CWC25                           |   |   |
| CELF4                           |   |   |
| PSPC1                           |   |   |
| LSM8                            |   |   |
| GEMIN7                          |   |   |
| IWS1                            |   |   |
| CSTF2T                          |   |   |
| PLRG1                           |   |   |
| TSEN15                          |   |   |
| PPP4R2                          |   |   |
| SRPK1                           |   |   |
| DAZAP1                          |   |   |
| RBM15                           |   |   |
| PCBP1                           |   |   |
| SRSF2                           |   |   |
| DYRK1A                          |   |   |
| SRSF1                           |   |   |
| AL YREF                         |   |   |
| PTBP1                           |   |   |
| SNRPB2                          |   |   |
| HNRNPR                          |   |   |
| SNIP1                           |   |   |
| ZBTB7A                          |   |   |
| FMR1                            |   |   |
| EIF4A3                          |   |   |
| SRSF3                           |   |   |
| HSPA8                           |   |   |
| TGS1                            |   |   |
| TRA2A                           |   |   |
| SYMPK                           |   |   |
| AFF2                            |   |   |
| NUP98                           |   |   |
| TXNL4B                          |   |   |
| HNRNPM                          |   |   |
| PTBP3                           |   |   |
| TRA2B                           |   |   |
| MAGOHB                          |   |   |
| SRSF7                           |   |   |
| ZPR1                            |   |   |
| HNRNPF                          |   |   |
| RRAGC                           |   |   |
| SLC38A2                         |   |   |
| PPIL1                           |   |   |
| ERN1                            |   |   |
| LSM6                            |   |   |
| RBP7                             |   |   |
| SETX                            |   |   |
| METTL4                           |   |   |
| WTAP                            |   |   |

**Figure 2**—Similar modifications on the mRNA expression of RBPs in human islets exposed to proinflammatory cytokines (IL1β plus IFNγ) and in the target tissue of RA. A and B: Bar charts depict log2-transformed fold change of the 137 differentially expressed RBP genes (adjusted P value < 0.05) in human islets (A) exposed to IL1β plus IFNγ and in the target tissue of RA (B) as compared with their respective controls. We downloaded data for human islets (from 33) and RA (28), and they were reanalyzed as previously described (24). Adj., adjusted.
Table 1 — Registered clinical trials aiming, at least in part, to directly protect β-cells in T1D

| Clinical trial reg. no. (ClinicalTrials.gov) | Title                                                                 | Target population          | Location | Main outcomes                                      | Current phase of clinical trial |
|---------------------------------------------|----------------------------------------------------------------------|----------------------------|----------|----------------------------------------------------|---------------------------------|
| NCT04233034                                 | Hybrid Closed Loop Therapy and Verapamil for Beta Cell Preservation in New Onset Type 1 Diabetes (CLVer) | T1D (age 7–17 years)       | U.S.     | C-peptide, CGM, etc.                               | 3                               |
| NCT04545151                                 | Verapamil SR in Adults with Type 1 Diabetes (Ver-A-T1D)               | T1D (age 18–45 years)      | Europe   | C-peptide, proinsulin/insulin, CGM, etc.           | 2                               |
| NCT02218619                                 | Tauroursodeoxycholic Acid (TUDCA) in New-Onset Type 1 Diabetes        | T1D (age 18–45 years)      | U.S.     | C-peptide                                         | 2                               |
| NCT02407899                                 | Protective Effects of Saxagliptin (And Vitamin D3) on β Cell Function in Adult-onset Latent Autoimmune Diabetes | LADA (age 18–70 years)     | China    | C-peptide                                         | 4                               |
| NCT02820558                                 | Neuropeptide Therapy of Recent Onset Type 1 Diabetes                  | T1D (age 10–18 years)      | Canada   | C-peptide                                         | 1                               |
| NCT04774224                                 | Baricitinib in New-onset Type 1 Diabetes (BANDIT)                     | T1D (age 12–30 years)      | Australia| C-peptide                                         | 2                               |
| NCT02384889                                 | DFMO in Children with Type 1 Diabetes                                | T1D (age 12–40 years)      | U.S.     | C-peptide, proinsulin/insulin, etc.                | 1                               |
| NCT03635437                                 | Evaluation of Safety and Diabetes Status Upon Oral Treatment With GABA in Patients With Longstanding Type-1 Diabetes | T1D (age 18–50 years)     | Sweden   | C-peptide, safety of oral GABA                    | 1/2                             |
| NCT04729296                                 | Anti-TNFα to Delay or Prevent Progression to Stage 3 T1D              | T1D (age 3–46 years)       | U.S.     | Not provided                                      | 2                               |

All trials listed above are intervention trials, aiming to preserve/restore β-cell function in patients with early or, in one case (gamma-aminobutyric acid [GABA]) long-standing, clinical T1D or, in one trial, adult-onset latent diabetes mellitus (LADA). CGM, continuous glucose monitoring.
A key issue in interfering with IFN signal transduction is dissociation of the beneficial from the deleterious effects of the cytokine at the β-cell level. Knocking down the transcription factor STAT2 induces these effects, preventing HLA class I expression while upregulating PDL1 (9). Furthermore, repeated short-term exposures to IFNα also dissociates these two pathways in iPSC-induced islet cells, likely via epigenetic mechanisms, and restricts activation of T cells cocultured with iPSC-derived human islets (57). IFNs have, however, multiple stimulatory effects on the immune system, and it is difficult to envisage translation of this approach to the early stages of T1D.

Many of the approaches discussed here will not act at the β-cells only. For example, targeting JAKs and TYK2 will affect many pathways in the immune system, while imatinib may interfere with T cell function. Furthermore, many of the gene networks addressed here are not unique to β-cells, although the way human β-cells respond to their activation may indeed be specific due to unique characteristics of the β-cells, including high insulin synthesis, low proliferative capacity, and a particular susceptibility to viral- and metabolic-induced stress (12,31).

Localized drug delivery of inhibitors with improved specificity to key nodes in this signaling cascade could obviate this issue and address potential safety concerns. For example, blocking of STAT2, either by small molecules or via in vivo use of cell-targeted siRNAs, is an interesting approach, particularly if targeted to the β-cells. RNA-based therapies are being developed for multiple oncology...
indications, rare/orphan diseases, and severe hypercholesterolemia (58). In the case of T1D, siRNA-mediated STAT2 blockage could be achieved by targeting the siRNA to proteins specifically expressed at the β-cell surface, as has recently been done with another cargo with use of a GLP1 analog coupled to an ASO (59). Targeting moieties for β-cell delivery of therapeutic siRNA can be readily developed from the ligands validated in human pancreatic imaging studies such as exendin 4, nanobodies against dipeptidyl peptidase like 6 (DPP6), and others (60). Another interesting approach in this context is the use of sulfated semisynthetic polysaccharide dextran sulfate, which upregulates PDL1 expression in mouse islets (among other anti-inflammatory effects) and reverses diabetes in NOD mice (61).

Once the dimer STAT1-STAT2 moves to the nucleus, often after forming a trimer with members of the IRF1 family, it binds to interferon-stimulated responsive elements and triggers expression of several pathways that will lead to ER stress (which may also be caused by other cytokines such as IL1β plus IFNa and metabolic stress), upregulation of HLA class I, production of chemokines, and, on the positive side, upregulation of PDL1 and HLA-E (7). Several of these pathways can be targeted for β-cell protection (Table 1 and Fig. 3).

Exposure of β-cells to the proinflammatory environment prevailing during insulitis, including the exposure to the cytokines IFNα, IL1β, TNFα, and IFNγ, triggers ER stress (7,19). As discussed above, this may contribute to both the presentation of neoantigens and, if unresolved, to β-cell death. The key pathway for cytokine-induced human β-cell ER stress and consequent apoptosis is activation of IRE1α and, downstream of it, degradation of relevant mRNAs (including insulin, a target of IRE1α RNase activity) and JNK phosphorylation (7). The facts that β-cells have endogenous protection mechanisms against excessive induction of IRE1α, namely, cytokine induction of N-MYC interactor and ubiquitin D (proteins that provide a specific negative feedback against JNK activation) (7), and that a blation of IRE1α in β-cells of NOD mice decreases diabetes incidence (62) indicate the importance of this pathway. Two drugs have been shown to prevent excessive IRE1α activation and revert diabetes in NOD mice, namely, ATP-competitive IRE1α kinase–inhibiting RNase attenuator 6 (KIRA6) and imatinib (63–65) (KIRA6 also prevents diabetes in the Akita mouse, a monogenic model of diabetes where ER stress is caused by insulin misfolding (64)). Imatinib has been approved by the U.S. Food and Drug Administration as an antican- cer inhibitor of tyrosine kinase and may prevent destruction of β-cells in people when administered early after T1D diagnosis. Another broader inhibitor of the deleterious effects of ER stress is the chemical chaperone tauroursodeoxycholic acid (TUDCA); it partially protects human β-cells from cytokine-induced apoptosis (66) and delays (but apparently does not revert) diabetes in mouse models of T1D (67). TUDCA is used for the therapy of liver diseases, and there is an ongoing clinical trial with use of TUDCA to delay β-cell loss after the onset of T1D (Table 1) (clinical trial reg. no. NCT02218619, ClinicalTrials.gov). Other interesting strategies include verapamil, an antihypertensive agent that acts as a TNXIP inhibitor and has shown promising results in a small-scale clinical trial (68); blockers of TNF, which were validated by a recent clinical trial in patients with early T1D and may both directly protect β-cells and modulate the immune assault (69); and exercise, which protects β-cells against ER stress probably via the release of protective myokines (70).

One of the key chemokines released by β-cells is CXCL10. While CXCL10 expression is increased in the islets affected by insulitis, infiltrating lymphocytes express the corresponding receptor CXCR3, illustrating the "dialogue" between β-cells and the invading immune cells (7). Small molecules are being developed to block CXCL10 or CXCR3 in autoimmune thyroiditis and other diseases (71), and they may eventually be considered as an adjuvant therapy in T1D.

In conclusion, therapies for β-cell protection in T1D seem to face a situation similar to that of green energy, usually criticized for not being economically viable, which is probably due to the fact that, during decennia, very limited public investments were done to make it economically viable. In the case of therapies for β-cell protection, since most interest is focused on the immune system, there are very few clinical trials aiming to protect β-cells. Worse, these rare trials usually disregard the fundamental issue that once the immune system starts recognizing β-cells as "foreign" and attacks them, β-cell protection alone will not suffice. Indeed, the immune system is resourceful and persistent, and β-cell–protective therapies must be combined with therapies aiming to reduce the immune assault. By not taking this into consideration, we may be wasting useful agents that could be used either very early in the disease process or as supportive/combi- nation therapies. There is an increasing interest in other autoimmune diseases, such as MS and inflammatory bowel disease, to develop adjunctive therapies to protect target tissues from inflammation-mediated damage (72), and it is our hope that this trend will also gain further traction for T1D.

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