A parallax view of type 1 diabetes

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The year 2021 marks the 100th anniversary of the discovery of insulin, a therapy that transformed type 1 diabetes (T1D) from a once fatal diagnosis into a chronic condition that can be successfully managed with exogenous insulin administration. In this article, we celebrate the remarkable journey that led to the discovery of insulin and our subsequent understanding of T1D as a disease of immune-mediated β-cell destruction. Further, we discuss an alternative and parallax view of disease development, highlighting maladaptive β-cell responses that act to amplify immune responses and have been implicated in diabetes development.

In 1921, headlines around the world read 'Diabetes is no longer a fatal disease'

Type 1 diabetes (T1D) is a chronic autoimmune disease caused by destruction of the insulin-producing β-cells of the pancreas, resulting in insulin deficiency and severe hyperglycaemia. T1D is estimated to cause 5%–10% of all cases of diabetes and affects nearly 46 million individuals worldwide. In 2021, we are celebrating the 100th anniversary of the discovery of insulin by Dr Frederick Banting, medical student Charles Best, Dr James Collip, and Dr J.J.R Macleod, a therapy that transformed T1D from a once fatal diagnosis to a chronic condition that can now be managed medically.

While the discovery of insulin dramatically changed the treatment of diabetes, it would take another 50 years to more clearly understand the origins of T1D. In 1974, Dr Gian Franco Bottazzo demonstrated the autoimmune basis of T1D by observing the presence of antibodies that reacted to islet β-cells in the serum of individuals with autoimmunity against other endocrine organs and diabetes. Subsequent molecular studies would show that defects in the immune system central and peripheral tolerance permit the propagation of a β-cell-specific autoimmune attack, which is is likely triggered by one or more environmental insults. Multitudes of studies followed, detailing the specifics of autoimmune-mediated β-cell destruction showing that naïve, autoreactive T cells encounter molecular fragments of damaged β-cells, called islet autoantigens, in the pancreatic lymph nodes. There, these islet autoantigens are presented to T cells and B cells by antigen-presenting cells. Recognition of an islet autoantigen by a T or B cell leads to activation of the T or B cell. Some activated B cells develop into antibody-secreting cells (plasma cells) and secrete islet antibodies into circulation. Other activated B cells and T cells travel from the pancreatic lymph node to the islet. Infiltration of the islet by these islet-reactive T and B cells, termed insulitis, generates inflammatory cytokines that induce the expression of MHC class I and MHC class II molecules on the surface of β-cells and antigen-presenting cells, respectively. In addition to the original islet autoantigens that were recognized by T and B cells in the pancreatic lymph nodes, new autoantigens, called neoantigens, are generated at the site of autoimmune attack and presented via MHC molecules, further exacerbating the immune response, amplifying the repertoire of autoantigens, and creating a feed forward cycle of inflammation, β-cell death, and dysfunction.

A decade after Dr Bottazzo's seminal discovery, Dr George Eisenbarth proposed a unified and longitudinal model of T1D disease development (Figure 1). This model highlighted (1) the presence of baseline genetic risk; (2) a precipitating environmental event leading to islet-specific autoimmunity, now characterized by autoantibodies against several different islet autoantigens, including insulin, glutamatic acid decarboxylase (GAD65), islet antigen 2 (IA-2), and zinc transporter 8 (ZnT8); and (3) progressive loss of β-cell insulin secretion and rising blood glucose levels. Observation of newborns with high genetic risk has demonstrated that the presence of two or more islet autoantibodies is associated with a nearly 80% risk of developing T1D over 15 years of observation. These findings have led to the development of a new staging system for T1D, where stage 1 T1D is defined as the presence of two or more autoantibodies and normal blood glucose levels, while stages 2 and 3 are defined as the presence of two or more antibodies plus impaired glucose tolerance (stage 2) or overt diabetes (stage 3) (Figure 1).

A step towards T1D prevention: one century later

Even today with improved insulins, continuous glucose monitors and insulin pumps, including those with automated insulin dosing algorithms, only ~21% of

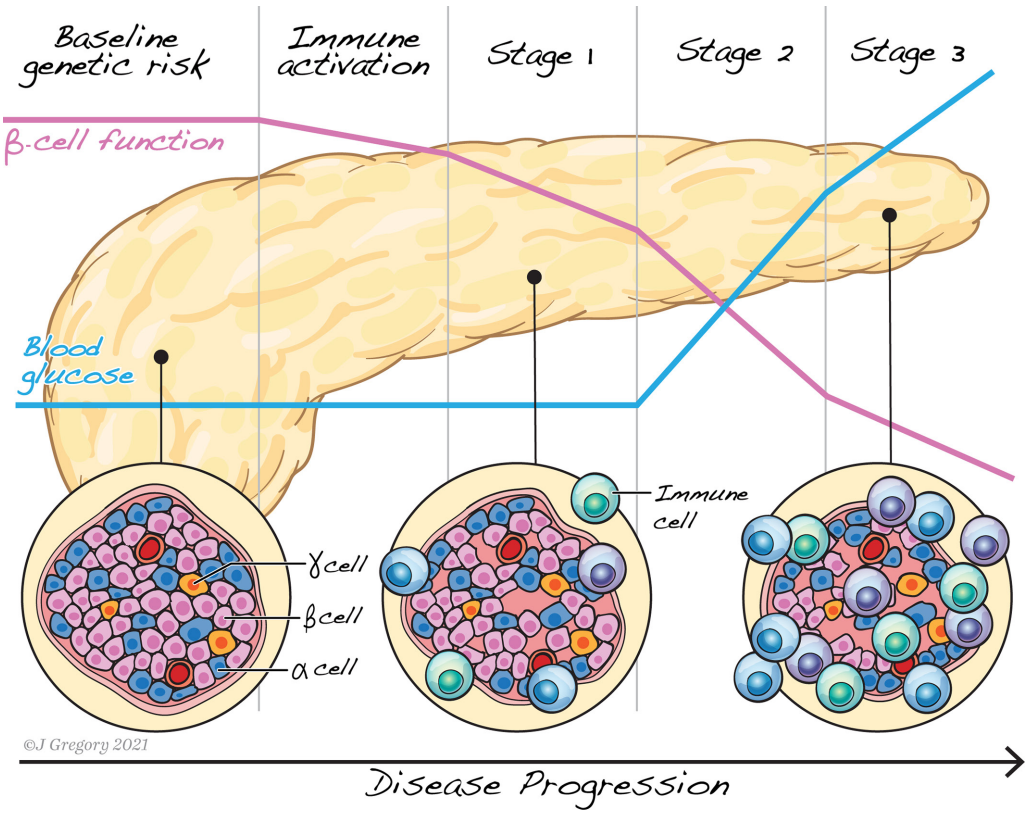

Figure 1. The stages of type 1 diabetes (T1D). T1D is initiated by an event that triggers islet autoimmunity, and risk is higher in those with a family history of T1D, which indicates baseline genetic risk. Islet autoimmunity is detected by the identification of serum autoantibodies against β-cell antigens. Stage 1 is defined as the presence of two or more autoantibodies and normal blood glucose levels or 'euglycaemia', while stages 2 and 3 are defined as the presence of two or more antibodies plus impaired glucose tolerance (stage 2) or overt diabetes (stage 3).

people with T1D are able to achieve near-normal blood glucose levels. According to the landmark Diabetes Control and Complications Trial (1982–1993), uncontrolled blood glucose directly leads to diabetic complications such as retinopathy, nephropathy, neuropathy and cardiovascular disease. For over three decades, the research community and those affected by T1D have sought a cure for this disease. To date, clinical trial efforts have largely focused on targeting different aspects of the immune response with several attempts to tolerize the immune system against islet antigens. In this approach, low amounts of islet autoantigens have been administered either orally or intravenously in an attempt to train the immune system to recognize these proteins as self. Other approaches have utilized drugs to block the activation of T cells or B cells and to block specific pro-inflammatory cytokines; however, all showed limited success.

The majority of these efforts have been initiated at the time of stage 3 disease onset, and while a handful of drugs improved insulin secretion, none resulted in insulin independence. Realizing stage 3 may be too far advanced in disease progression to produce a durable remission, the field has shifted its focus to earlier interventions. In 2019, results from the clinical trial of teplizumab, an anti-CD3 (T-cell) antibody aimed to reduce autoimmune destruction of β-cells, showed for the first time that T1D can be delayed in high-risk individuals, with teplizumab-treated individuals in stage 2 experiencing a nearly three year delay in, diabetes onset. The results of this trial have rightly been heralded as a ground-breaking milestone in T1D clinical trial efforts. However, in the teplizumab trial as well as every trial preceding it, responses to disease-modifying therapies continue to be heterogeneous, suggesting there is continued room for improvement in how we approach T1D intervention and prevention therapy.

Analogous to cancer therapy, there is growing interest in testing combinations of drugs that target distinct aspects of T1D development, including drugs that directly target the β-cell. As early as 1985, Dr Bottazzo first proposed the question of whether T1D is a disease of 'β-cell suicide or homicide', a notion that continues to be revisited 36 years later. Indeed, there is accumulating evidence to suggest that β-cells are not simply innocent bystanders nor the victims of a unidirectional immune assault during T1D progression. Rather, a variety of studies suggest they may

actually 'amplify' immune responses through activation of numerous cell stress pathways. These learnings provoke us to re-examine our view of T1D pathogenesis and to consider a parallax view of T1D development from the perspective of the β-cell. Here, we highlight key molecular pathways in the β-cells that have been linked with T1D pathology and highlight research efforts focused on improving β-cell health in T1D.

β-cells uncloak themselves to the immune system

In response to cellular stress, including exposure to proinflammatory cytokines, reactive oxygen species (ROS) and other toxic molecules, normal pathways of protein production may be altered resulting in the generation of erroneous proteins. The cell degrades these erroneously synthesized proteins into recyclable peptides, amino acids and potential antigens. Specific transporter proteins recognize peptides for antigen generation and route them to the endoplasmic reticulum (ER) for selective pairing with MHC class I molecules for presentation. The immune cells then 'read' these antigens on the cell surface to determine cellular health and respond accordingly.

During the evolution of T1D, a number of pathways in the β-cell can lead to modification of antigens, resulting in additional new antigens, or neoantigens. In particular, cytokines and ROS can impact the function of key β-cell organelles such as the ER and mitochondria, which may be involved in the processing and generation of neoantigens (Figure 2). Dysfunction of the ER and the development of ER stress have been shown to alter folding patterns and post-translational modifications of islet proteins such as insulin, GAD65, GRP78, and chromogranin A, creating neoantigens that are perceived by the immune system as foreign. Similarly, mitochondrial stress in the β-cell generates excessive ROS, which can alter already synthesized β-cell proteins. Consistent with this, sera from recently diagnosed individuals with T1D showed enhanced reactivity to a hydroxyl radical modified form of GAD65, which supports the idea that ROS can modify islet proteins to create neoantigens that more strongly

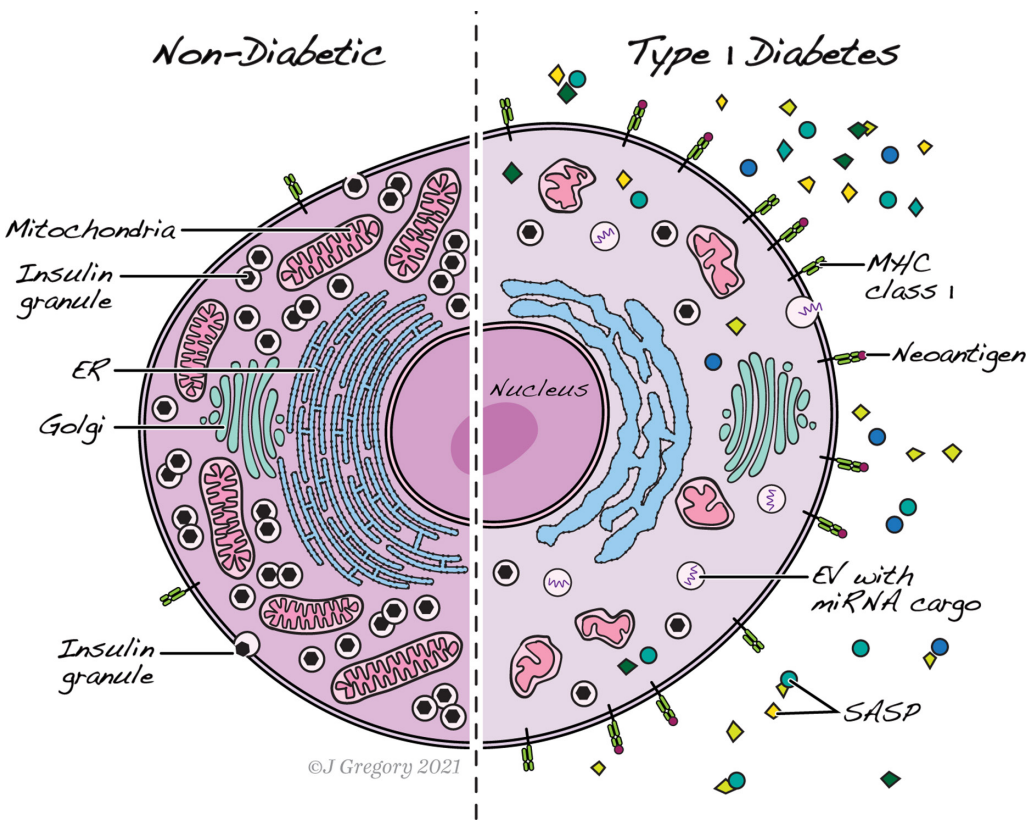

Figure 2. Profound alterations in β-cell homeostasis are observed in type 1 diabetes. Endoplasmic reticulum (ER) and mitochondrial stress alter organelle function and morphology and are linked with the generation and presentation of neoantigens on up-regulated MHC class I surface molecules on the β-cell. Additionally, β-cells heighten communication with neighbouring β-cells and immune cells via secretion of pro-inflammatory cytokines and chemokines known as SASPs. Extracellular vesicles are differentially loaded with cargo material under stress conditions, displaying a 'snapshot' of cellular activity and modulating cell-to-cell communication. In aggregate, these changes in the β-cell lead to increased visibility and communication with the immune system, leading to a feed forward cycle of inflammation and β-cell destruction.

activate the immune system. Finally, organelle stress and inflammation in the β-cell has been linked with the up-regulation of the surface marker, MHC class I, leading to increased surface presentation of these neoantigens (Figure 2). In fact, MHC class I hyper-expression in the β-cell is considered a defining feature of T1D. Thus, newly generated autoantigens and neoantigens are heavily 'advertised' to the immune system, promoting destruction.

Factors released from the β-cell amplify immune responses and β-cell dysfunction

Whereas the β-cell is a professional secretory cell that bears sole responsibility for the synthesis and release of the hormone insulin, there is evidence to suggest that not all the factors released from the β-cell are beneficial. In fact, the β-cell uses its enhanced secretory pathways to release multiple factors used to communicate to other β-cells as well as the immune system during T1D development. β-cells have been shown to up-regulate expression of proteins that attract immune cells during T1D. A recent study suggests this up-regulated protein expression could be part of a senescence-associated secretory phenotype (SASP), where β-cells exit the cell cycle and begin to secrete proinflammatory cytokines and chemokines (Figure 2). Secreted SASP proteins allow for significant means of communication during progression of T1D by acting in an autocrine fashion to directly damage nearby β-cells or in a paracrine fashion to influence the behaviour of nearby immune cells.

β-cells may also release factors that contribute to disease development via differential loading and release of extracellular vesicles (EVs; Figure 2). EVs are nanometre-sized membrane-bound spherical structures that are released from the cell and carry proteins, lipids, DNA, RNA, microRNA (miRNA) and active metabolites as cargo. This cargo material serves as a 'snapshot' of cellular activity and may be selectively packaged into EVs in response to intracellular stress. Accordingly, there is considerable interest in identifying the signature of cargo within EVs that can be leveraged to identify β-cell stress and serve as a biomarker of disease risk. EVs have been linked with a bi-directional communication between the β-cell and the immune system across multiple studies. For example, a recent study found an increase in EV-derived miRNA-122-5 p and miRNA-192-5 p in individuals with newly diagnosed T1D. While the cell source of the miRNAs was not clear, the two miRNAs were found to activate both CD4+ and CD8+ T cells. Additionally, human islet-derived EVs have been shown to package well-known islet autoantigens, such as GAD65 and ZnT8, that can be taken up by antigen-presenting cells in peripheral blood. Furthermore, recent evidence suggests that human islet-derived EVs mediate the activation of B and T cells in the peripheral blood of individuals with T1D.

Forthcoming steps towards T1D prevention

Multiple lines of evidence suggest that T1D should no longer be considered only a disease of the immune system, but also a disease of the β-cell. Increased understanding of the response pathways activated from the perspective of both the immune system and the β-cell provides opportunities for novel approaches to disease modification. For example, two β-cell-targeted therapies, verapamil (a calcium chain blocker) and TUDCA (a taurine conjugated bile acid), are currently in clinical trials to slow or halt T1D progression in newly diagnosed individuals by alleviating β-cell ER stress. More recently, GLP-1 receptor agonists, commonly used to treat type 2 diabetes, are being investigated in T1D based on data suggesting they may modulate β-cell stress pathways. Lastly, variations in cytokine blockers continue to be tested in clinical trials, with efforts aimed at inhibiting proinflammatory cytokine generation and secretion from both immune and β-cell viewpoints. Going forward, β-cell-focused interventions have the potential to be used in conjunction with immunomodulatory agents that have been shown already to have some clinical benefit. It is likely that combination therapies that simultaneously slow the immune response and preserve β-cell function will be required to achieve disease prevention and insulin independence. These strategies, combined with on-going efforts to identify biomarkers that are able to detect early stage T1D, illustrate how we can overcome this parallax view of T1D development. As we celebrate the 100th year anniversary of the discovery of insulin and the ensuing research following this remarkable accomplishment, we are filled with optimism for what the next 100 years will bring.

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