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Type 1 diabetes mellitus as a disease of the β-cell (do not blame the immune system?)

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Abstract | Type 1 diabetes mellitus is believed to result from destruction of the insulin-producing β-cells in pancreatic islets that is mediated by autoimmune mechanisms. The classic view is that autoreactive T cells mistakenly destroy healthy ('innocent') β-cells. We propose an alternative view in which the β-cell is the key contributor to the disease. By their nature and function, β-cells are prone to biosynthetic stress with limited measures for self-defence. β-Cell stress provokes an immune attack that has considerable negative effects on the source of a vital hormone. This view would explain why immunotherapy at best delays progression of type 1 diabetes mellitus and points to opportunities to use therapies that revitalize β-cells, in combination with immune intervention strategies, to reverse the disease. We present the case that dysfunction occurs in both the immune system and β-cells, which provokes further dysfunction, and present the evidence leading to the consensus that islet autoimmunity is an essential component in the pathogenesis of type 1 diabetes mellitus. Next, we build the case for the β-cell as the trigger of an autoimmune response, supported by analogies in cancer and antitumour immunity. Finally, we synthesize a model ('connecting the dots') in which both β-cell stress and islet autoimmunity can be harnessed as targets for intervention strategies.

For several decades, type 1 diabetes mellitus (T1DM) was believed to be a T cell-mediated autoimmune disease[1–3]. This notion still holds, but several observations in the past few years point to a role of β-cells that goes beyond being a non-provoking victim of an autoimmune attack[4–6]. The lack of durable effects of immune-suppressive intervention therapies, islet autoimmunity occurring without the development of T1DM, a remarkably low rate of insulitis at diagnosis and the unexpectedly high proportion of β-cells that persist (although they do not always function) after the diagnosis of T1DM prompted a revision of our take on the pathogenesis of T1DM[7–9]. In this Review, we build the case for β-cells as active participants in the dialogue with the immune system. We propose that therapies targeting β-cell health, vitality and function might prove essential, in combination with immunotherapy, in changing the course of events leading to β-cell destruction.

T1DM as an autoimmune disease
A connection between the immune system and T1DM was first suggested in 1973, when HLA antigens were found to be associated with insulin-dependent diabetes mellitus but not with insulin-independent diabetes mellitus[10]. Since then, genome-wide association studies have confirmed that HLA genes account for up to 50% of the genetic risk of T1DM (in particular HLA class II loci), which suggests that the selective presentation of specific autoantigen peptides is involved in the pathogenesis of T1DM[11–13]. Meta-analyses have also linked non-HLA high-risk polymorphisms within *INS*-VNTR (variable number of tandem repeats), *PTPN22*, *CTLA4* and *IL2RA* with a reduction in central and peripheral immune tolerance to self and increased T cell activation and proliferation[14–17], which emphasizes the participation of the immune system in the development of T1DM[18].

During the development of T1DM, seroconversion of islet autoantibodies to insulin, glutamate decarboxylase, insulinoma antigen 2 or zinc transporter 8 represents the first notable sign of autoimmunity and their combined presence in serum remains the best predictor for both loss of immune tolerance (that is, induction of autoimmunity) and clinical manifestation of T1DM, albeit that their role in β-cell destruction remains unclear[19,20]. During disease progression, immune cells that infiltrate the pancreas and target insulin-producing cells create an inflammatory environment characteristic of insulitis that triggers and accelerates T1DM development by increasing exposure of islet antigens presented by HLA class I molecules to the immune system[7,21–23] (BOX 1).

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Key points

- Autoreactive T cells are part of the normal T cell repertoire.
- β-Cells are poorly equipped to survive an inflammatory milieu and participate in their own destruction.
- Metabolic activity drives β-cell dysfunction and destruction.
- Inflammation triggers profound metabolic, epigenetic and autoantigenic changes, which expose β-cells to the immune system.
- The immune response to distressed β-cells might be one with 'good intentions', as infected tissues or tumours provoke the immune system in similar ways.
- Immunotherapy might be insufficient to cure type 1 diabetes mellitus; β-cell therapy might contribute to reducing β-cell immunogenicity and islet autoimmunity.

Thymic education
The process that takes place in the thymus leading to the establishment of central immune tolerance to self-proteins and the potential for immune response against foreign proteins (such as viruses, bacteria, donor tissue and allergens).

Self-proteins
Proteins normally produced by a particular organism.

Thymic selection
The processes of positive and negative selection in the thymus through which T cells acquire the capacity to distinguish self-proteins from foreign proteins.

The presence of islet-specific autoreactive CD4^{+} and CD8^{+} T cells in peripheral blood, pancreatic draining lymph nodes and insulitic lesions[7,24–28] provided evidence for T1DM as an autoimmune disease, where an impaired thymic education was responsible for the immune attack directed against self-proteins of insulin-producing cells[29–31]. Yet, despite their importance in T1DM pathology, the frequency of these autoreactive cells in peripheral blood is low and quite similar between patients with T1DM and healthy individuals[32]. Although the presence of naive autoreactive cells in healthy individuals indicates that these cells are part of the normal T cell repertoire and that 'we are all autoimmune', the increased frequency of CD8^{+} T cells (in particular resident memory cells) in the pancreata of patients with T1DM compared with those of control individuals implies a differential peripheral activation and/or regulation in patients with T1DM[7,32,33]. Indeed, regulatory T (T_{reg}) cells, which have an important role in repressing these autoreactive T cells in healthy individuals, show a similar frequency in control individuals and in patients with T1DM but with a reduced suppressive capacity in patients with T1DM[34–36].

Intriguingly, islet autoreactive T cells have unusual characteristics compared with T cells that protect us from cancer and infection, such as a fairly low epitope binding affinity for HLA, low T cell receptor (TCR) avidity for HLA–epitope complexes, tilted or even reversed docking of the TCR on the HLA–peptide complex, suboptimal synapse formation in the interphase between T cells and antigen-presenting cells or target cells and abnormal expression of signalling molecules that might have contributed to incomplete thymic education and thymic selection[37–45]. Consistently, patients with cancer who are treated with immune checkpoint inhibitors (that is, anti-PD1, anti-PDL1 or anti-CTLA4 therapies) aimed at reducing immune regulation and initiating an immune response against the tumour tissue (FIG. 1) are at risk of developing adverse effects, including acute T1DM, presumably due to loss of immune regulation combined with activation of naive autoreactive T cells[46,47].

In the past couple of years, it has been argued that autoimmune diabetes mellitus induced by immune checkpoint blockade and T1DM are different diseases[48], but this contention is perhaps premature. Indeed, T1DM is not one disease, as can easily be appreciated by comparing T1DM diagnosed in children versus that diagnosed in adolescents or adults[8,49–51]. Some of the major differences in presentation of autoimmune diabetes

mellitus induced by immune checkpoint inhibition and other types of T1DM relate to the acute manifestation and short prodromal phase of the former, leading to fairly frequent and severe ketoacidosis and paucity of autoantibodies at diagnosis[46,47]. After all, islet autoantibodies take time to be generated, following T cell activation. The demonstration that T1DM can be transferred with bone marrow from a donor with T1DM to an immune-suppressed recipient who did not have T1DM only when T cells are not depleted, underscores the relevance of T cells in the immunopathogenesis of T1DM[52]. Furthermore, pancreatitis rarely leads to T1DM, even in patients with an increased genetic risk of T1DM, which in turn emphasizes that loss of immune tolerance and induction of islet autoimmunity are a prerequisite for development of the disease[53]. This finding is supported by the rapid recurrence of islet autoimmunity, selective β-cell destruction and T1DM following partial pancreas transplantation from non-diabetic donors to their monozygotic twins with T1DM, as well as islet autoimmunity predicting failure or poor prognosis of allogenic islet transplantation and autologous bone marrow transplantation[54–58]. Finally, the fact that, until now, immunotherapeutic strategies have shown temporal efficacy in delaying disease progression implicates the immune system in T1DM pathology[59,60].

Inconsistencies in the role of T cells

A different stand on a role of T cells in the pathogenesis of T1DM can easily be defended[61] (BOX 2). Islet autoreactive T cells are common in the healthy population, and nine out of ten individuals with islet autoantibodies will never develop T1DM[32,62,63]. Most patients with T1DM have immune regulation that is indistinguishable from that of healthy individuals, and over 99% of patients with cancer who are treated with immune checkpoint inhibitors do not develop T1DM[46,47,64]. Furthermore, some patients with T1DM present with negligible T cell autoimmunity[51]. Moreover, induction of autoimmune diabetes mellitus in mice by vaccination with islet autoantigens is very difficult, if not impossible[65]. Even when transduction of human islet autoreactive TCRs in humanized mice leads to high frequencies of T cell autoimmunity to islets, no diabetes mellitus was induced[29]. In addition, thus far, progression of T1DM has not been found to accelerate after patients with T1DM are injected with islet autoantigens[59,66–68]. Of note, HLA upregulation as an early sign of islet distress frequently occurs without inflammation, even if β-cells are still present[7,69], while insulitis is a rare feature in individuals who have islet autoantibodies but not T1DM[70]. Furthermore, immunotherapies in T1DM have not yet shown a durable effect on disease progression[60]. These inconsistencies in our understanding of the critical role of islet autoimmunity, and T cells in particular, require reconciliation.

T1DM as a disease of β-cells

Given that autoreactive T cells are part of a normal T cell repertoire, it is implausible that the disease is entirely the result of dysfunctional immune cells; rather, peripheral activation of the immune system is required locally in the targeted tissue[32,71]. A role for β-cells in their own

demise was first proposed by Bottazzo[72]. Different triggers that might lead β-cells to provoke an immune response have been proposed, ranging from the size of the pancreas and β-cell mass to viral infection and metabolic stress[4,73,74]. Indeed, the pancreata of patients with T1DM are smaller than those from unrelated control individuals[73] (BOX 3). Yet, at-risk individuals and patients with T1DM have pancreata of similar sizes[73], and no data at this time suggest that the pancreas decreases in size with disease progression. Obviously, less β-cell mass might equal less β-cell functional capacity and increased pressure on β-cells to cope with glycaemic control. In addition to metabolic stress, viral infections or intestinal inflammatory agents 'leaking' into the pancreas might create a pro-inflammatory environment[61,74,75]. β-Cells are exposed to viral infection as they express specific receptors and adhesion molecules. Indeed, the presence of a coxsackievirus and adenovirus receptor (CAR) that is unique to β-cells, found in the insulin-containing granules, might leave β-cells vulnerable to viral infection during insulin secretion, as illustrated by studies correlating enteroviral infection by coxsackievirus B4 with islet autoimmunity (but not T1DM)[76,77]. Viral infection might be a risk factor in, at best, a small minority of patients with T1DM[78]. A viral contribution to the development of T1DM is certainly not limited to coxsackievirus;

for example, rotavirus and cytomegalovirus have also been implicated[78–81].

Role of diet and microbiota. Similarly, dysbiosis of the gastrointestinal tract (a 'gut storm') provoked by changes in intestinal microbiota and an increased Bacteroidetes to Firmicutes ratio has been correlated with seroconversion and onset of T1DM (the pancreas being an intestinal organ)[82]. Microbiota shape peripheral immune tolerance, modulating both migration and differentiation of immune cells to maintain intestinal homeostasis; furthermore, local inflammation is limited through short-chain fatty acids (SCFAs) generated by resident gut bacteria from fermentation of non-digestible carbohydrates[83]. SCFAs have a direct effect on T cell subsets via histone deacetylase inhibition and activation of mTOR and STAT3 signalling, leading to an increased proportion of regulatory T cells that produce IL-10 and express FOXP3. In addition, SCFAs can exert their anti-inflammatory effect on neutrophils, macrophages and plasmacytoid dendritic cells via antimicrobial peptides produced by innate lymphoid cells or by β-cells themselves[84–87]. Strong evidence from studies in mice demonstrates the protective role of these cationic antimicrobial peptides against autoimmune diabetes mellitus, and SCFAs have been used to prevent cytokine-induced cell death of human islet cells and to improve β-cell function[88]. Despite these positive findings, a first-in-human crossover clinical trial conducted in patients with longstanding T1DM (mean diabetes mellitus duration of 8 years) that aimed to restore epithelial integrity by short-term oral butyrate supplementation failed to show improvement in adaptive and innate immune system parameters[89].

Diet can also affect the microbiome favourably or unfavourably with regard to the predisposition for developing T1DM. A low gluten diet can induce favourable changes in the intestinal microbiome of healthy adults, while low maternal gluten intake during pregnancy shows a remarkable correlation with reduced development of T1DM in the offspring[90,91]. While it remains to be established whether patients at risk of T1DM or patients newly diagnosed with T1DM would benefit from a low gluten diet, these data might also suggest that once initiated, local inflammation in the pancreas is sufficient to drive disease progression, given the inherent molecular fragility of β-cells (the 'domino effect').

Genetic risk. Genetic risk, determined by certain genetic variants in the gene encoding insulin (*INS*), might affect β-cell function and glycaemic control[14,15,92,93]. Early studies suggest that protective variants of *INS* result in increased *INS* expression in the thymus, thereby increasing the probability that the immune system will be educated to avoid immune reactivity to insulin; however, differences in *INS* activity in pancreatic islets have also been linked to these genetic polymorphisms, as well as effects on β-cell function and resilience[14,15,92–97]. Other genetic variants associated with increased risk of T1DM might affect β-cell health, vitality and self-defence[98–100]. β-Cell mass and function might have been declining for more than 10 years before clinical manifestation of

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Box 1 | Evidence supporting a role for T cells in T1DM pathogenesis

- Insulitis[7,22]
- HLA association[10]
- HLA class I upregulation in inflamed islets[7,69]
- Autoreactive CD8+ T cells in insulitis[7,24]
- Autoreactive CD4+ T cells in insulitis[25,26]
- Recurrence of islet autoimmunity, insulitis and type 1 diabetes mellitus (T1DM) after twin pancreas graft into T1DM recipient[54]
- Adoptive transfer of T1DM after bone marrow transplantation not depleted for T cells from a donor with T1DM[52]
- T cell-dependent islet autoantibodies[19,20]
- Recurrent islet autoimmunity and chronic progressive loss of islet allografts transplanted into patients with T1DM[56–58,163]
- Recurrent islet autoimmunity in patients with T1DM who relapse after pancreas transplantation[54,164]
- No islet autoimmunity in pancreas or T1DM in chronic pancreatitis (even with high-risk HLA)[53]
- Higher islet autoreactivity of T cells in patients with T1DM than in healthy individuals[27,165]
- Therapeutic effect of anti-T cell immune-suppressive therapy[166,167]
- Complete and durable remission after autologous bone marrow transplantation in patients with new-onset T1DM[55,168]
- Therapeutic effect of co-stimulation blockade of progression of T1DM[169,170]
- Development of T1DM after co-stimulation blockade in cancer[46,47]
- Development of insulitis and selective loss of β-cells in humanized mice carrying insulin-specific TCR[29]
- Phenotypical and functional features in islet autoreactive T cells[37,38,43,45,171]
- Functional abnormalities in regulatory T cells in T1DM[35,41]
- Genetic risk associated with polymorphisms in genes involved in immune regulation (*PTPN22*, *IL2R* and *CTLA4*)[11]
- Genetic defects of immune regulation genes causing T1DM (*FOXP3* and *AIRE*)[172,173]
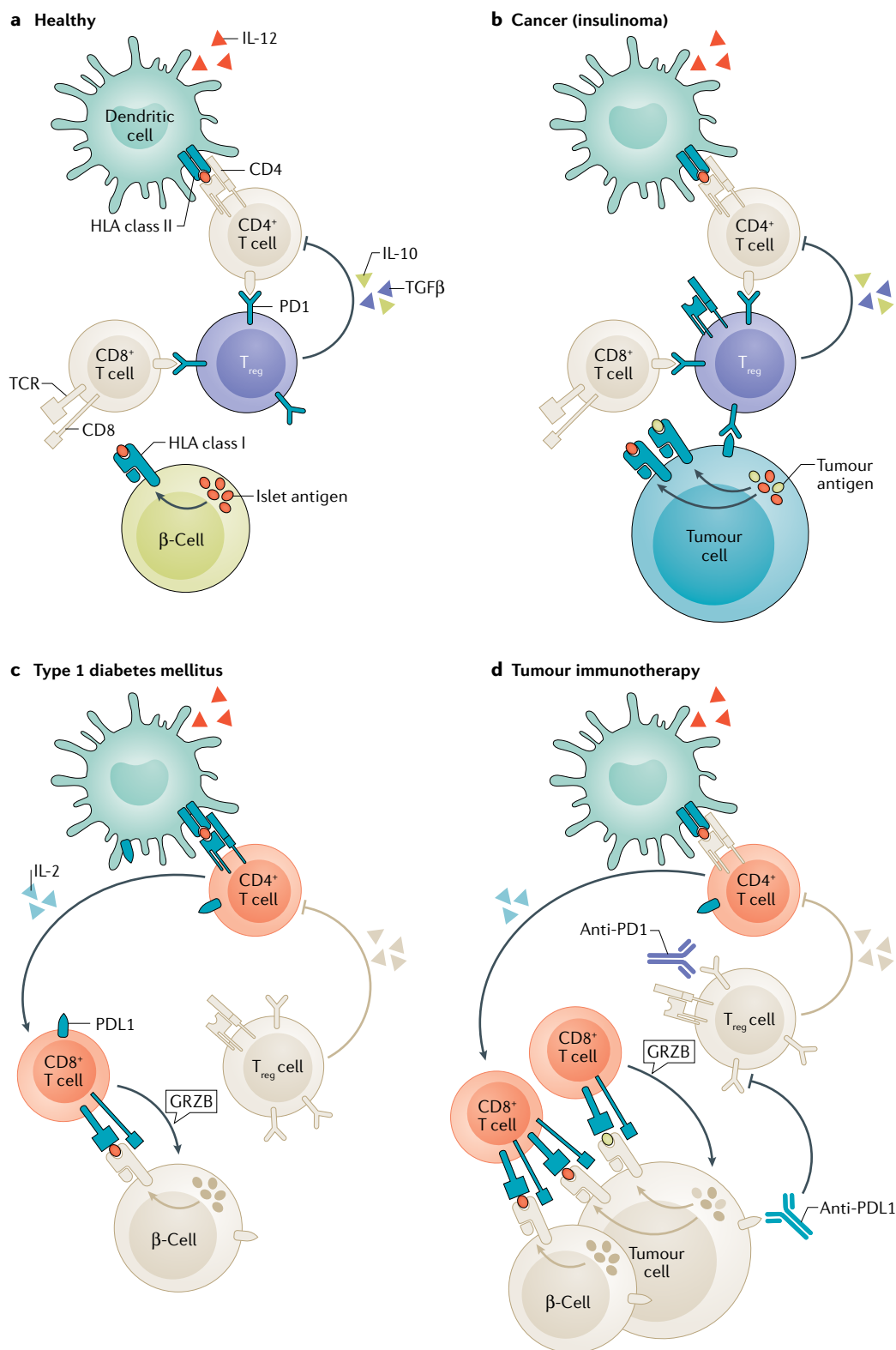- Genetic risk associated with *INS* gene associated with thymic education and central tolerance[14,15,94,95,97]

Fig. 1 | **Immunoregulation in health, and immune dysregulation in cancer, T1DM or immunotherapy. a** | In healthy individuals, β-cells are protected from autoimmune β-cell destruction by immune regulation exerted by regulatory T (T_reg) cells and PD1 ligation. **b** | While advantageous in preventing autoimmunity, T_reg cells impede antitumour immunity. **c** | In type 1 diabetes mellitus (T1DM), insufficient immune regulation can result in an autoimmune response by autoreactive T cells, particularly if these cells are provoked by β-cells. **d** | The response in T1DM resembles effective antitumour immunity as a result of immunotherapeutic blockade of PD1 or its ligand PDL1 that otherwise keep autoimmune responses in check. In addition to resulting in antitumour immunity, other immune and autoimmune responses might be triggered, including those against pancreatic islets. T1DM is a serious adverse effect of tumour immunotherapy. GRZB, granzyme B; TCR, T cell receptor.

T1DM, adding to increasing metabolic stress in β-cells and vulnerability to autoimmune insults[101,102].

Insights from human studies of insulitis. Our understanding of the effect of insulitis on β-cells has exploded with the increased access to pancreata from donors with diabetes mellitus (Network for Pancreatic Organ Donors with Diabetes), even though the condition of the donors (factors such as cause of death, presence of brain death, stay and treatment in an intensive care unit, cold ischaemia, ketoacidosis, injury and stress) might influence some of the observations made on the pancreata, in terms of the effects of stress[7,21,22]. An increased expression of markers specific for the unfolded protein response to stress in β-cells during insulitis suggests that adaptive mechanisms are engaged to help β-cells deal with the environmental pressure[103]. Stressed β-cells have a reduced overall translation rate, initiate degradation of proteins accumulated in the endoplasmic reticulum (ER), increase the translation rate of chaperones and promote autophagy to return to cellular homeostasis[104–106]. However, the extraordinary capacities of β-cells to produce up to 1 million insulin molecules per minute and to increase production in excess of 50-fold in response to glucose[106], combined with low expression of superoxide dismutase and anti-apoptotic factor BCL-2 make β-cells poorly equipped to survive the inflammatory milieu. β-Cells are more sensitive than α-cells to environmental stimuli, as illustrated by studies conducted on islets challenged by metabolic stress mimicking pathophysiological conditions in type 2 diabetes (T2DM)[107,108]. In addition to the cytoprotective function, activation of the ER stress sensors is known to lead to a cascade of events promoting direct apoptosis via activation of the IRF–STAT1 pathway[98,109], necroptosis via activation of TNFR1–RIP1 and necrosis by increased production of reactive oxygen species as well as induction of a form of β-cell senescence[108,110,111] (FIG. 2). These mechanisms might participate in the amplification of inflammation and destruction of β-cells by starting communication with other endocrine cells and resident immune cells. Stress-induced senescence drives β-cells to a senescence-associated secretory phenotype, which is correlated with intra-islet infiltration of CD45^{+} immune cells in patients with T1DM[112].

Studies of human insulitis have revealed that 'danger signals' from stressed β-cells might precede insulitis. Among these signals, hyper-expression of HLA class I (and possibly HLA class II) was noted across pancreata from patients with newly diagnosed T1DM[7]. In addition, islets secrete the chemokine CXCL10, attracting leukocytes expressing its receptor CXCR3 to the lesion[113]. This chemokine production by stressed β-cells might present a master switch of islet inflammation and has attracted interest from the pharmaceutical industry as an opportunity for intervention therapy[114]. Other strategies include efforts to reduce β-cell stress with verapamil, where early studies have shown promise for delaying T1DM disease progression[115]. Intriguingly, high levels of insulin-specific autoreactive human T cells only precipitated insulitis and selective β-cell destruction in humanized mice in vivo after the mice had been vaccinated with insulin peptide to prime an autoimmune response and subjected to low-dose streptozotocin to stress the β-cells. This finding underscores the need for β-cell perturbation and loss of autoimmune tolerance to β-cells to create a 'perfect storm' that causes their destruction[29].

Role of the exocrine pancreas. T1DM seems to affect both the endocrine and exocrine pancreas, as studies have shown inflammation and loss of exocrine parenchyma[7,22,73]. This finding is a potentially important missing link to be discussed and incorporated in any hypothesis aiming to clarify the mechanisms that lead to T1DM. Yet, in spite of efforts to prove an actual decline in total pancreas mass longitudinally, no evidence indicates that pancreas mass decreases with time in patients with T1DM. Indeed, although patients with T1DM often have a small pancreas, the pancreata of first-degree relatives of patients with T1DM, with or without islet autoimmunity, tend to be smaller than those of the general population as well, possibly pointing to inherent small pancreas sizes in individuals prone to developing T1DM[73]. It is tempting to speculate that a smaller pancreas and subsequent reduced endocrine mass would increase the burden on the reduced number of β-cells trying to cope with hyperglycaemia; that is, 'size matters'. In terms of exocrine inflammation, the argument about whether this effect is secondary to the fatal condition of the pancreas donor and organ procurement has not been settled yet; however, pancreas tissue obtained from biopsy samples of living patients with newly diagnosed T1DM tends to show less pronounced or no exocrine involvement compared with samples obtained at autopsy[7,22,116]. Importantly, insulitic lesions early after diagnosis of T1DM point to monoclonal or oligoclonal infiltration with islet autoreactive CD8^{+} T cells only, with little evidence of 'bystander' T cells or exocrine involvement, which underscores the central role of autoimmunity in pancreas immunopathology at that stage[7]. In addition, islets depleted of β-cells no longer show insulitis[7,22], which suggest that β-cells are the driving force of this inflammatory process characteristic of T1DM and underscores the central role of β-cells in the disease process.

Box 3 | **Evidence supporting a role for β-cells in T1DM pathogenesis**

- Smaller size of pancreas and islet mass in patients with type 1 diabetes mellitus (T1DM) and individuals at risk[73]
- Genetic risk associated with *INS* gene polymorphism associated with β-cell function[14,15,93–95,97]
- Genetic risk associated with polymorphisms in genes with protein products involved in β-cell protection, health and vitality[99]
- β-Cell stress[31,103,106]
- Abnormal β-cell function preceding diagnosis of T1DM (in spite of sufficient β-cell mass)[101,102]
- HLA class I upregulation on endocrine cells in inflamed islets[7,69]
- HLA class I upregulation preceding islet inflammation[7,69]
- Paucity of insulitis in individuals with islet autoantibodies[70]
- Development of post-translational modifications (such as deamidation, citrullination and transpeptidation)[26,133,134,138,177]
- Stress-induced ribosomal errors; post-transcriptional modification[31]
- Alternative splicing of islet autoantigens[99,131,136,178]
- No development of insulitis or selective loss of β-cells in humanized mice transduced with islet antigen-specific T cell receptors unless β-cells are distressed (with streptozotocin)[29]
- Histologically distinct lesion endotypes that correlate with age at diagnosis[8,49,50]
- Beneficial effects of verapamil on preservation of β-cell function in new-onset T1DM[115]

Islet-resident macrophages and inflammation. In the dialogue between β-cells and the immune cell compartment, islet-resident macrophages have a mediator role as they engulf, process and present catabolic products from insulin granules or products that are carried by exosome particles secreted by β-cells[117]. The localization of islet-resident macrophages near blood vessels and in close contact with β-cells, forming synapse-like structures, emphasizes their role in the effector phase of T1DM as they secrete pro-inflammatory cytokines and free radicals, triggering NF-κB and STAT1 downstream signalling pathways and FAS-mediated apoptosis in β-cells[118,119]. Conventionally, macrophages recognize pathogen-associated molecular patterns or damaged tissue-associated molecular patterns (DAMPs) via Toll-like receptors (TLRs). DAMPs derived from β-cell-specific antigens (such as insulin and islet amyloid polypeptide (IAPP, also known as amylin)) have been described. Interestingly, in mouse models susceptible to autoimmune diabetes mellitus, members of the TLR family in the presence of β-cell DAMPs trigger T1DM, while in the absence of the corresponding ligands, the same TLRs exert tolerance; this finding shows the importance of β-cells in the balance between tolerance and autoimmunity[120]. In mice, therapies affecting macrophages limit T1DM progression[121,122], while a study using pancreatic biopsy samples from patients with recent onset T1DM (3–9 weeks after diagnosis) showed that islet-resident macrophages (and infiltrating dendritic cells) are the main source of pro-inflammatory cytokines released during insulitis, positioning macrophages at the centre of the pathology of T1DM[123].

Exposure to cytokines can cause substantial metabolic and epigenetic changes. For instance, DNA methylome profiles, histone acetylation and deacetylation levels and chromatin structure are altered in β-cells, exposing promoters and enhancers for inflammatory response

Translation infidelity
The ribosomal errors during the translation of mRNA into proteins that lead to changes in amino acid sequences of mRNA-encoded proteins (such as, alternative start site, frameshifts, readthrough of stop codons or premature termination of translation).

Ligandome
The complete range of peptides presented by HLA molecules on the surface of β-cells.

factors and T1DM genetic predisposition loci, as shown in NOD mice and human islets after exposure to pro-inflammatory cytokines[124–127]. Combining these results with other 'omics' studies indicates that genes responsive to interferon, protein degradation and HLA loading machinery processes are the main factors that are disturbed during inflammation, which suggests that insulitis not only leads to β-cell dysfunction but also to increased β-cell visibility to immune surveillance[99,128–130]. We have also described how inflammation induced by ER stress can shape β-cell immunogenicity and control cytotoxic destruction by miRNA-mediated regulation of ERAP1 and its effect on preproinsulin processing[104].

Peptide presentation by β-cells. The effect of cytokines on β-cells is not limited to an increased peptide–HLA density at the cell surface but also affects the nature of the peptides presented. Currently, several autoantigens have been identified and while many peptides are derived from native proteins, a new range of neoantigens (protein products from mutations, frameshifts, alternative mRNA splicing and post-translational modifications) originating from alternative splicing[131], translational mistakes[31], post-translational modifications[5,107,132,133], peptide fusion[134] and possibly immunoproteasome activation[135] has emerged that strongly activate the immune system response (FIG. 2). In inflammatory conditions, the increased splicing events measured by RNA-seq in human islets combined with translation infidelity and increased activity of post-translational enzymes (such as protein arginine deiminases and tissue transglutaminase 2) contribute to the diversity of the islet proteome[99,136–138]. A β-cell ligandome landscape was presented by combining HLA class I peptidomic and transcriptomic analyses after cytokine stimulation[130]. While these results demonstrated that most of the presented (β-cell-specific) epitopes were derived from secretory granule components, which are hyper-immunogenic[27,28,117,130], most of the alternative epitopes were not detected, despite evidence that they were able to trigger a pro-inflammatory T cell response. The low expression rate of most of these neoantigens is probably close to the sensitivity limits of proteomic analyses, so they might not be detected. Alternatively, neoantigen synthesis might require chronic rather than acute exposure to cytokines, while immune cells producing pro-inflammatory cytokines might provide additional extra stress signals to the β-cells.

Interestingly, dendritic cells can convert native islet autoantigens into immunogenic neoantigens, revealing a role for islet-resident dendritic cells in the induction or expansion of islet autoimmunity[133]. Yet, these results have shed light on new mechanisms implying that hybrid peptides are generated by β-cells during proteolysis in the proteasome, where some protein fragments can be retained and bound to other N-terminal peptides in a process called peptide splicing (for example, *cis*-peptidation reaction within PTPRN and transpeptidation reactions between IAPP and PTPRN, SLC30A8 and PCSK2, and PIK3R3 and PIK3R1), before loading on HLA[134]. Even though progress has been made, our knowledge of neoantigens in T1DM is still at an early stage and is limited. Furthermore, other potential
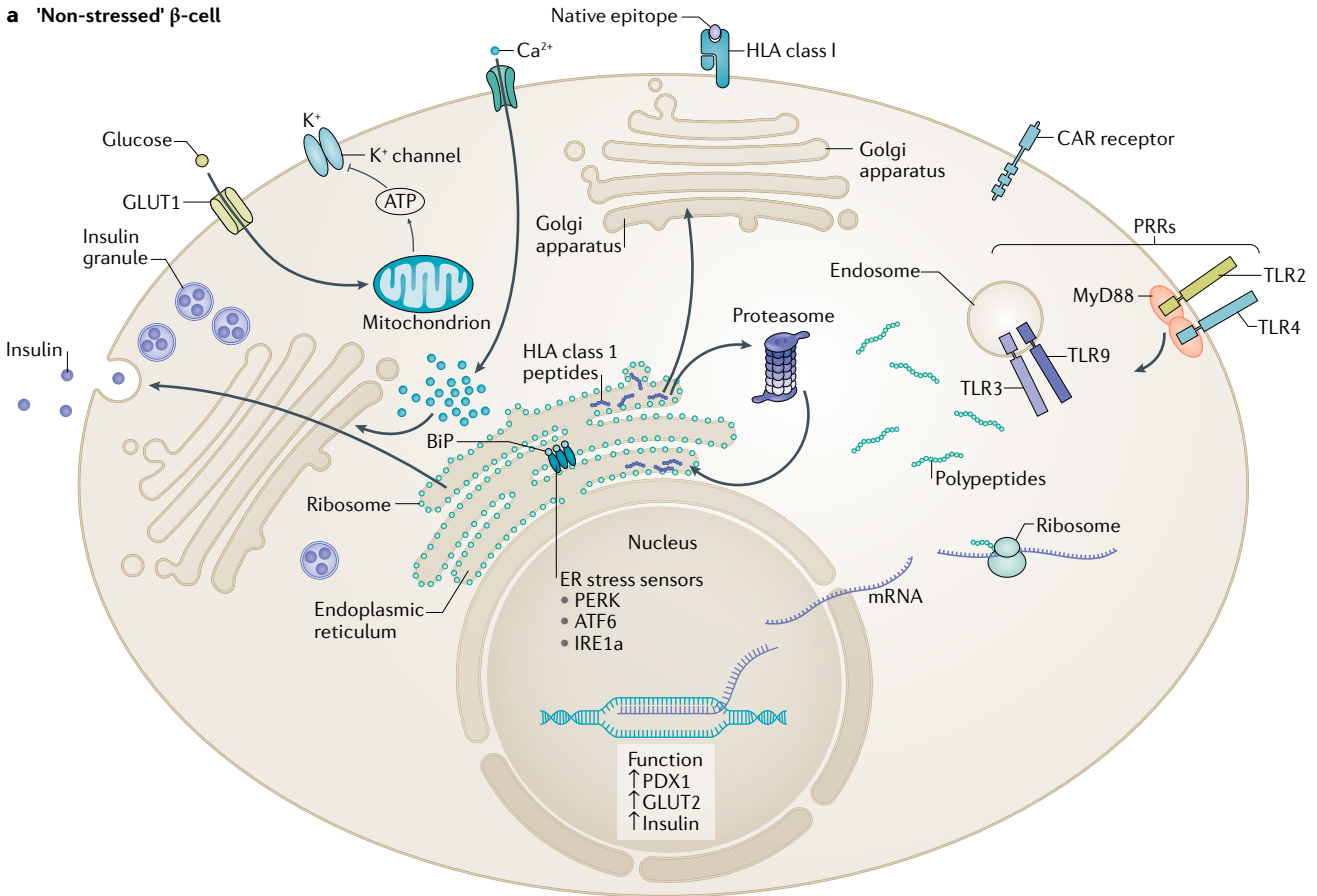
a 'Non-stressed' β-cell

b β-Cell under 'stress'

◀ Fig. 2 | **The effect of stress on β-cell function and immunogenicity. a** | In non-stressed ('happy') β-cells, glucose uptake via the glucose transporter GLUT1 leads to pyruvate formation through glycolysis and to increased ATP production by mitochondria. The resulting increased cytosolic level of ATP promotes the closure of the potassium channels, membrane depolarization and opening of the voltage-dependent calcium channels. The rise in intracellular levels of calcium triggers exocytosis of the insulin-containing granules. In these conditions, several genes and proteins are upregulated to restore the cellular stock of insulin. During this process, non-translocated preproinsulin, endoplasmic reticulum (ER)-resident insulin signal peptide and non-mature proinsulin molecules are degraded through the proteasome directly or after retro-translocation and are presented by HLA class I at the cell surface (normal β-cell ligandome). **b** | In type 1 diabetes mellitus pathophysiological conditions, cytokines lead to profound changes in gene and protein expression, mainly by activation of the STAT1, IRF1 and NF-κB downstream pathways, that ultimately drives hyper-expression of HLA class I, and also the surface expression of inhibitory receptors (that is, PDL1). In the stress condition, calcium uptake by the mitochondria is responsible for increased permeability of the mitochondria that precedes the release of pro-apoptotic factors (such as reactive oxygen species (ROS) and cytochrome *c* (cyt *c*)). Calcium depletion in the ER leads to activation of cytosolic calcium-dependent enzymes (such as transglutaminase 2 and peptidyl arginine deiminase) that are involved in the post-translational modification processes by inducing protein deamidation and citrullination, respectively. The recruitment of the ER chaperones (binding immunoglobulin protein (BiP)) in response to the accumulation of misfolded protein within the ER leads to the activation of the sensors (PERK, IRE1a and ATF6) expressed at the surface of the ER membrane. The PERK pathway attenuates mRNA translation by phosphorylation of the eIF2a translation initiation factor. Phosphorylation of IRE1a and translocation of ATF6 activate the ER accumulated protein degradation pathway and the transcription of chaperone encoding genes with protein products involved in degradation of misfolded proteins, and also restores ER homeostasis. Long-term exposure to inflammatory stress promotes additional coping mechanisms, including initiation of recycling programmes and selective secretion of proteins and small RNAs in microvesicles, and ultimately leads to the induction of an apoptosis programme mediated by the transcriptional activation of CHOP by the combined activity of PERK and ATF6. During stress, normal transcription, translation and degradation is affected, which generates alternative RNA splicing, defective ribosomal products and hybrid peptides, respectively (stressed β-cell ligandome). CAR, coxsackievirus and adenovirus receptor; DRiP, defective ribosomal product; PRR, pattern recognition receptor; SASP, senescence-associated secretory phenotype; TLR, Toll-like receptor.

mechanisms have just been presented (that is, hybrid peptides and defective ribosomal products) or have been overlooked (RNA editing). Mechanisms to create neoantigens in tumours should be investigated as additional potential sources of neoantigens in T1DM. For instance, the double-stranded RNA-specific adenosine deaminase ADAR1 switches adenine to inositol, thereby changing aspartic acid into arginine. Expression of this enzyme in breast cancer correlates with high infiltration of T cells into tumours and immune reactivity to edited antigens[139]. Increased expression of ADAR1 in patients with systemic lupus erythematous is associated with increased RNA editing events, indicating the possible involvement of RNA editing in the autoimmune reaction[140].

All the aforementioned data highlight the importance of endogenous characteristics of β-cells and their response to exogenous inflammatory stimuli for disease progression and exacerbation. These findings also demonstrate the need for intensification of efforts to fully unravel β-cell physiology in health and autoimmunity.

Lessons from cancer

The dogma describing T1DM as a disease characterized by total destruction of the insulin-producing β-cells has been shaken by immunohistochemistry studies performed on pancreatic specimens from patients with longstanding T1DM showing the presence of β-cells and

insulin microsecretion (C-peptide value of <30 pmol/l) in the majority of these patients, implying that some β-cells resist or escape the immune attack, or that new β-cells are formed[141,142]. The lobularity of this feature (where β-cells in certain pancreatic lobules seem unaffected, while β-cells in other lobules are depleted) might imply formation of new pancreatic lobules with unaffected islets, which increases the sense of urgency to protect β-cells after a diagnosis of T1DM. Confirming these observations, the latest single-cell analysis methods (that is, transcriptomics, mass cytometry and imaging mass spectrometry) have revealed wide heterogeneity in the β-cell population in healthy pancreata but also during disease progression, which might contribute to different sensitivities of β-cells to immune responses[143–146]. Evidence of this concept is found in multiple sclerosis, where different oligodendrocyte phenotypes have different levels of autoimmune reactions, potentially driving self-destruction[147]. Importantly, while the presence of insulin-positive cells and lack of insulitis in longstanding T1DM might suggest that 'normal' islets are present, the lack of detectable C-peptide and differential clustering from islets of non-diabetic donors in multidimensional mass cytometry analyses points to intrinsic differences in patient-derived islets that might reflect prodromal islet distress and prediabetic lesions[145]. Intriguingly, studies of insulin and proinsulin in pancreata from patients with T1DM support the existence of aetiopathological endotypes of T1DM that are associated with age at diagnosis, and point to age-related intrinsic differences in distressed β-cells during insulitis that might lead to different autoimmune reactions[8,50,51].

A concept is emerging that the immune response seen in T1DM might be one with 'good intentions', where the immune response to distressed tissue resembles the immune response that has evolved to detect infected tissue or tumours. Indeed, people carrying T1DM risk gene variants have a hyper-inflammatory immune system[148]. It can be argued that patients with T1DM have an immune system that might be beneficial in patients with cancer. A clear analogy in support of this provocative contention is presented by Lambert–Eaton myasthenic syndrome, which has two different aetiologies: one associated with immune hypersensitivity and autoimmunity (a phenotype shared with T1DM) and one where an antitumour immune response against the voltage-gated calcium channels expressed by small cell lung carcinoma cells and nerve endings causes cross reactivity in the neuromuscular synapse. Patients with small cell lung carcinoma who develop Lambert–Eaton myasthenic syndrome have a better prognosis for cancer survival than patients who do not develop this syndrome[149–151]. In addition, in patients with cancer, immune responses that are initiated after antitumour immunotherapy tend to be directed to neoantigens rather than native autoantigens[152].

In a similar manner to tumour cells that evade immune responses to become more invasive, β-cells have developed active self-protective mechanisms to limit further autoimmune destruction; the upregulated expression of inhibitory receptors (such as PDL1) at their cell surface and the increased expression of IDO1

after cytokine challenge illustrate these changes[153,154]. A correlation between loss of IDO1 expression and β-cell destruction extends proof for the participation of these protective mechanisms in the maintenance of the β-cell integrity[155]. In addition, several studies have suggested that increased degranulation and/or a loss of β-cell identity occurs under environmental pressure, which is supported by the defect in insulin production and the presence of polyhormonal cells in the pancreata of patients with T1DM. From these findings, a concept of a 'β-cell identity crisis' has emerged where β-cells dedifferentiate into other endocrine cells (α-cells or δ-cells) as a defence mechanism[156–158]. Along with this β-cell identity crisis, levels of 'semi' β-cells that only express chromogranin A (chromogranin-positive, hormone-negative (CPHN) cells) are increased in the pancreata from patients with T1DM and T2DM and they are scattered throughout the pancreas regardless of inflammation level[158,159]. The origin of these cells is still unknown; however, the mere fact that CPHN cells express the autoantigen chromogranin A without this leading to their destruction might suggest that insulin production and the inherent negative molecular effects are needed to drive autoimmunity. Similarly, not all T1DM autoantigens are β-cell-specific; chromogranin A and receptor-type tyrosine-protein phosphatase N2 are also expressed in other tissues not affected by an immune attack in patients with T1DM[160].

By comparing islet and tumour microenvironments, increasing evidence supports the notion that in autoimmune diseases, as in effective tumour immunity or following antitumour immunotherapy, the immune system is acting on dysfunctional cells or tissues that have accumulated aberrant or modified proteins[147].

Conclusions

The appreciation of a role for β-cells in their own demise, the importance of ER stress in T1DM pathology, the identification of residual β-cells in patients who have had T1DM for several decades and the presence of dormant ('hibernating') β-cells that evade immune attack suggest that β-cell dysfunction and destruction are driven by their metabolic activity, and might lie at the heart of the aetiologies of both T1DM and T2DM. Both types of diabetes mellitus are chronic inflammatory diseases, and both are β-cell diseases. Thus far, immunotherapy alone has proven insufficient to achieve lasting preservation of β-cell function, pointing to the need to combine this strategy with β-cell therapy. In T2DM, inflammatory cytokines (secreted by stressed adipocytes or stressed β-cells) and recruitment of macrophages, B cells and T cells have been found to participate in β-cell failure and pathology. Accordingly, several intervention strategies for T2DM aimed at alleviating pressure exerted on β-cells and improving glycaemic control have been evaluated in the context of T1DM: metformin, GLP1 analogues (liraglutide, exendin 4 or sitagliptin) and verapamil have shown some benefit when combined with insulin therapy in the treatment of patients with T1DM[115]. We favour the engagement of the immune system, rather than suppression of the immune system, to reverse the immunopathogenesis of T1DM, in combination with β-cell therapy to improve β-cell stamina and vitality and to protect these cells from metabolic and inflammatory assaults. At a time when the coronavirus disease 2019 (COVID-19) pandemic reminds us of the need for a fully functional immune system, we cannot afford to suppress it and put patients with inflammatory disorders in danger of infection or cancer[60]. Novel therapies are already being assessed in the clinic that 'negotiate' with the immune system, rather than suppress it, including 'inverse' vaccination strategies that aim to induce selective immune tolerance to islet autoantigens, similar to desensitization when treating allergies[59,66,161,162]. This strategy, in combination with β-cell therapy, is an attractive strategy to achieve durable remission in T1DM.

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