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Pancreatic β-cells in type 1 and type 2 diabetes mellitus: different pathways to failure

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Abstract | Loss of functional β-cell mass is the key mechanism leading to the two main forms of diabetes mellitus — type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM). Understanding the mechanisms behind β-cell failure is critical to prevent or revert disease. Basic pathogenic differences exist in the two forms of diabetes mellitus; T1DM is immune mediated and T2DM is mediated by metabolic mechanisms. These mechanisms differentially affect early β-cell dysfunction and eventual fate. Over the past decade, major advances have been made in the field, mostly delivered by studies on β-cells in human disease. These advances include studies of islet morphology and human β-cell gene expression in T1DM and T2DM, the identification and characterization of the role of T1DM and T2DM candidate genes at the β-cell level and the endoplasmic reticulum stress signalling that contributes to β-cell failure in T1DM (mostly IRE1 driven) and T2DM (mostly PERK–eIF2α dependent). Here, we review these new findings, focusing on studies performed on human β-cells or on samples obtained from patients with diabetes mellitus.

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Loss of functional β-cell mass is the key mechanism leading to diabetes mellitus — as long as β-cells are able to compensate, for instance, for insulin resistance, normoglycaemia is preserved[1]. The American Diabetes Association (ADA) defines type 1 diabetes mellitus (T1DM) as autoimmune β-cell destruction, usually leading to absolute insulin deficiency, and type 2 diabetes mellitus (T2DM) as progressive loss of β-cell insulin secretion frequently occurring on the background of insulin resistance[2]. T1DM and T2DM are complex and heterogeneous diseases with a common outcome — hyperglycaemia. Novel ways of clustering patients with diabetes mellitus into subgroups that predict disease progression and risk of complications are being investigated[3,4]. These new classifications remain to be fully validated and, for the purpose of this review, we follow the ADA's definitions of T1DM and T2DM.

T1DM is caused by autoimmune-mediated β-cell dysfunction and apoptosis, leading to the lifelong need for exogenous insulin therapy. The disease is the consequence of a complex dialogue between invading or resident macrophages and T cells, which release chemokines and cytokines in the islet microenvironment and deliver cell–cell pro-apoptotic signals, and β-cells via signals generated physiologically (for instance, degradation products of insulin or other components of the β-cell dense core granules) or by stressed, injured or dying β-cells that attract and activate immune cells to the islets[5–7]. This dialogue is determined by the host genetic background and age as well as by environmental factors such as viral infections and diet, among others[8–10]. Pathogenic crosstalk between immune cells and β-cells can trigger local inflammation (insulitis) and progressive β-cell dysfunction and death, mainly via apoptosis[5,8,11], or might be arrested by local mechanisms that dampen the immune response and restore physiology[12,13]. Of note, some individuals from families affected by T1DM show evidence of β-cell dysfunction, such as decreased first phase glucose-stimulated C-peptide release or increased circulating proinsulin–insulin ratios, in the absence of β-cell autoantibodies[14]. This observation suggests that β-cell dysfunction could actually precede the autoimmune assault in T1DM or might reflect 'scars' of a previous, resolved autoimmune episode.

The prevalence of T1DM in children is doubling every 25 years[15,16], and currently causes an average loss of 11–12 years of life expectancy[17,18]. Loss of life expectancy is slightly higher when disease starts earlier in life — for instance, patients diagnosed before age 15 years live 2.5 fewer years than those diagnosed after age 30 years[18]. A staging classification system has been proposed for T1DM, defining stage 1 as the presence of β-cell autoimmunity (that is, two or more autoantibodies) in normoglycaemic individuals, stage 2 as dysglycaemia (but no overt diabetes mellitus) in the presence of β-cell autoimmunity, and stage 3 as clinical T1DM[19]. Presently, no therapeutic approaches exist that prevent or cure T1DM[8,20], although a recent trial in stage 2 patients, using

a monoclonal antibody against CD3 (a surface molecule present on CD8^{+} T cells), delayed — but did not prevent — disease onset by ~2 years[21].

In T2DM, relative insulin deficiency owing to β-cell dysfunction is a key factor for the development of disease[22–24] that often coexists with insulin resistance. Although T2DM represents the bulk (80%) of all cases of diabetes mellitus, it remains an ill-defined form of disease and a diagnosis of exclusion: no specific diagnostic criteria exist for T2DM. Clustering approaches using age at diagnosis, BMI, HbA$_{1c}$, HOMA estimates of β-cell function and insulin resistance, and glutamic acid decarboxylase autoantibodies have subtyped patients into moderate or severe forms of T2DM, with predominance of insulin resistance or insulinopenia[3]. One subtype can evolve into another over time[25]. Obesity, energy-dense 'western' diets, older age and sedentary lifestyle are key risk factors for T2DM[26] that have led to a four-fold increase in the number of cases over the last 4 decades[27]. These risk factors can precipitate both β-cell failure and insulin resistance.

Although rates of chronic complications have improved over time, T2DM remains associated with considerable morbidity and mortality. Life span is shortened by 6 years on average, although the loss in life expectancy reaches 12 years in people who develop T2DM at a younger age[28]. A large number of drug classes exist to treat T2DM, none of which has been convincingly shown to modify the progressive decline in β-cell function over time. In the RISE study, patients with early T2DM (defined here as impaired glucose tolerance or recently diagnosed T2DM) randomized to treatment with metformin, metformin plus GLP-1 analogue or insulin plus metformin had improvements in β-cell function whilst on treatment for 1 year; however, these beneficial effects disappeared 3 months after treatment withdrawal[29]. In the ACT NOW study, the beneficial effects of pioglitazone on β-cell function also waned after treatment discontinuation[30].

As the different forms of diabetes mellitus progress, β-cells might become subjected to unbearable levels of stress and can undergo similar final steps of apoptosis

such as caspase 3 activation. As Saul Bellow writes in *The Adventures of Augie March*, " … there is no fineness or accuracy of suppression. If you hold down one thing, you hold down the adjoining." However, there are fundamental differences in the pathogenesis of T1DM and T2DM that affect the early stages of β-cell dysfunction and eventual fate. T1DM is clearly autoimmune, with CD8^{+} T cells recognizing and targeting specific antigens expressed on the β-cell surface in the context of HLA class I, whereas no specific autoimmune attack against β-cells exists in T2DM[31]. T2DM might have an inflammatory component that affects β-cells[32], but its pathogenic importance remains to be proven or translated into therapies for the disease (reviewed in REF.[33]). A global unbiased comparison of transcriptomes of β-cells obtained from donors with T1DM or T2DM compared with transcriptomes from human islets exposed to the pro-inflammatory cytokines IL-1β and IFNγ shows strong overlap between cytokines and T1DM but no or marginal overlap between cytokines and T2DM (FIG. 1). The anti-IL-1β antibody canakinumab did not reduce T2DM incidence in the CANTOS trial and only mildly and transiently improved glycaemic control in patients with established T2DM[34].

In 2005, we reviewed the mechanisms of pancreatic β-cell dysfunction and death in T1DM and T2DM, concluding that there were many differences and few similarities[1]. Major advances have occurred in the field since then. In particular, many studies have been conducted on islet morphology and human β-cell gene expression in T1DM and T2DM. The genetics of both diseases have been largely uncovered and the role of some candidate genes at the β-cell level has been characterized. Furthermore, the community now has a detailed understanding on the role of endoplasmic reticulum (ER) stress in β-cell dysfunction and death in T1DM and T2DM. The present Review focuses on these new findings from the past 15 years, with particular emphasis on studies performed in human β-cells or samples obtained from patients with T1DM or T2DM.

Islet histology and gene expression

Until 25–30 years ago, most of our knowledge on islet morphology and the mechanisms leading to diabetes mellitus were based on limited studies of human pancreata collected at necropsy and on animal models such as non-obese diabetic (NOD) mice, ob/ob and db/db mouse models of obesity and T2DM, and the GK rat model of T2DM, which reproduce the human disease to a rather limited extent. The development of the Network for Pancreatic Donors with Diabetes[35] as well as the systematic organization and expansion of the Exeter Archival Diabetes Biobank[36] have already provided access to ~500 well-preserved pancreata from individuals with T1DM and non-diabetic controls from both necropsy and organ donors, with different ages of disease onset and duration[36]. Numbers of pancreata in these tissue banks are increasing and include tissue from individuals who are autoantibody-positive but do not have overt T1DM (stages 1 and 2, see earlier text). Valuable material has also been obtained from pancreas tail biopsies taken from six patients with T1DM at disease
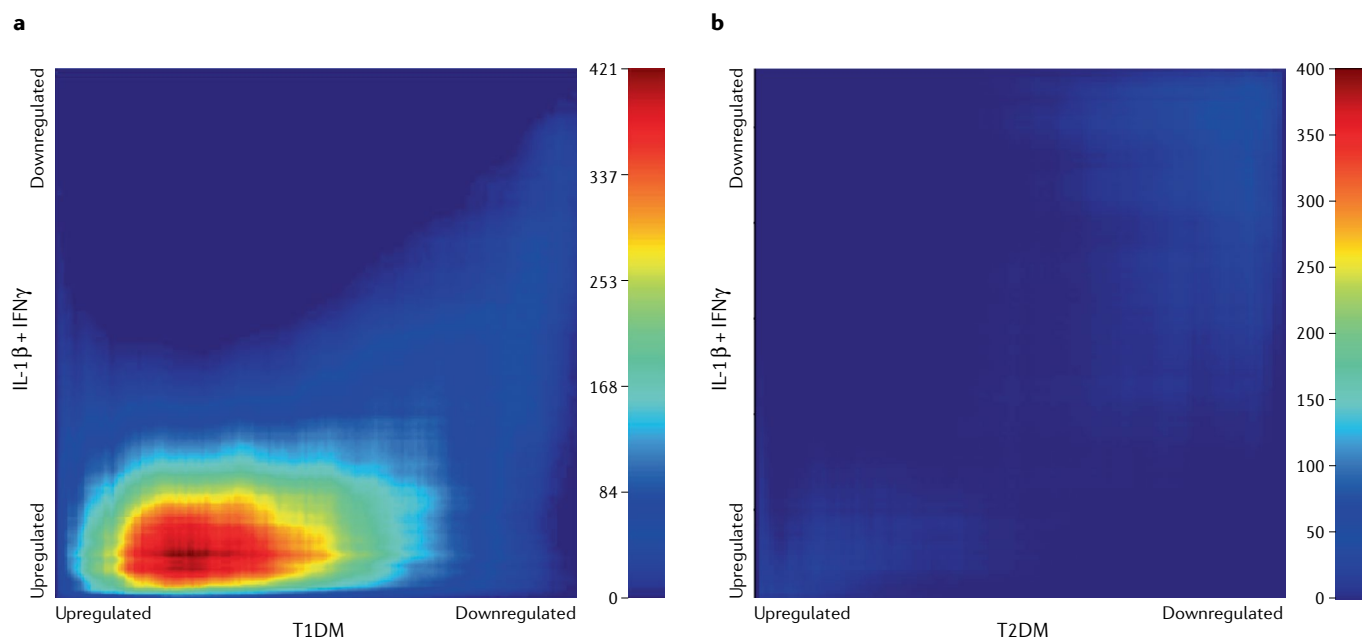
Fig. 1 | **Transcriptomes of human islets exposed to pro-inflammatory cytokines versus β-cells from donors with T1DM or T2DM.** The unbiased Rank–Rank Hypergeometric Overlap[186] map compares the transcriptome of human islets exposed to IL-1β and IFNγ to that of β-cells from donors with type 1 diabetes mellitus (T1DM) (part **a**) or type 2 diabetes mellitus (T2DM) (part **b**). For these comparisons, ranked lists of genes were generated using the median-$\log_2$-fold change for β-cells from patients with T1DM ($n=4$) versus non-diabetic control individuals ($n=12$)[69] or from patients with T2DM ($n=6$) versus non-diabetic controls ($n=12$)[70] and cytokine-treated versus non-treated human islets from non-diabetic donors ($n=5$)[6,68]. The scale bars show -log(P value), indicating the strength of the overlap between the gene expression signatures. Figure courtesy of Dr M. Colli, ULB Center for Diabetes Research.

onset[37]; however, this approach has been stopped due to risks associated with the procedure. Novel approaches, including the integrated analysis of histology, physiology, mass cytometry, genomics and immunology of T1DM organ donors[38], should enable even better information to be gleaned from these precious samples.

Characteristics of human β-cells in T1DM. Results obtained from the human T1DM histology collections available from the Network for Pancreatic Donors with Diabetes and the Exeter Archival Diabetes Biobank have been reviewed in detail elsewhere[36,39]. Several key findings have been described in these studies. For example, in human T1DM, considerable heterogeneity of inflammation and β-cell destruction is found; some lobes contain only islets devoid of β-cells, whereas other lobes have nearly normal looking islets and/or β-cell content, with few infiltrating immune cells. In addition, HLA class I hyperexpression was observed, particularly in insulin-containing islets[39], as well as the presence of markers of β-cell stress such as ER stress[40] (see later text). Amyloid has been described in islets from some patients with recent-onset T1DM and could contribute to elicit ER stress[41,42].

At disease onset, β-cell loss is far from complete, with >95% of patients with <1 year disease duration presenting with insulin-containing islets[36]. In some patients with recent-onset T1DM who died in ketoacidosis, the remaining β-cell mass approached 40–50% of normal, suggesting that severe immune-mediated β-cell dysfunction precedes actual β-cell death[43,44]. Compared to non-diabetic donors, in patients with recent-onset T1DM (disease diagnosed within 0–6 years), β-cells more often express markers of cellular senescence, such as CDKN1A and β-galactosidase, as well as IL-6 and serpine 1, which suggests an inflammatory senescence-associated secretory phenotype[45]. Interestingly, clearance of senescent β-cells in T1DM mouse models reduces diabetes mellitus incidence[45]. In imaging mass cytometry studies of human T1DM, loss of the β-cell markers insulin, proinsulin and amylin, precedes β-cell death[46].

Islets obtained from NOD mice affected by severe insulitis are dysfunctional upon isolation, however, they regain function once freed from the infiltrating immune cells after 7 days in culture[47]. Furthermore, similar findings were observed in islets isolated from patients with T1DM after 5–6 days in culture[48,49]. Of note, β-cells from four donors with T1DM (2–7 years of duration) had preserved insulin release in response to glucose, when expressed as insulin secretion per insulin content, but showed defective first-phase insulin release[50]. These islets were maintained in culture with 5.5 mM of glucose for 24–72 h prior to the assessments, which could have allowed them to partially recover from the in vivo T1DM-related stress. Curiously, α-cell gene expression and function are severely impaired in islets[50,51] or single cells[50] from donors with T1DM, as compared with islets from normoglycaemic individuals. In T1DM, α-cells are not targeted by the autoimmune assault and have increased resistance to metabolic-induced[50,52] or virally induced[53] stress compared to β-cells. The reasons for α-cell dysfunction remain to be determined but

could be related to loss of normal islet architecture and β-cell–α-cell contacts.

Insulin release, as evaluated by stimulated urinary C-peptide, is detectable in 80% of patients with T1DM after a mean disease duration of 21 years, most often at very low levels: 70% of patients have urinary C-peptide values below 1% of controls[54]. Serum or plasma C-peptide is also detectable, most commonly at very low concentrations, in 11–80% of long-standing patients with T1DM, depending on the method used (reviewed in REF.[44]). Interestingly, some C-peptide-negative patients with T1DM still produce and release proinsulin[55], suggesting that surviving insulin-producing cells have defective pro-insulin–insulin conversion. The cellular source of this (pro)insulin remains to be identified, but findings from 2019 suggest that other islet cells — particularly α-cells — start expressing low levels of insulin following severe β-cell loss[56]. This observation is in line with findings in mice pointing to transdifferentiation of α-cells into β-cells following β-cell ablation[57] or after blocking of the key α-cell transcription factor ARX[58]. These observations indicate that rodent and perhaps human islet cells have unexpected plasticity. Whether this plasticity is clinically relevant or can be therapeutically exploited remains to be determined[59].

The immune system has major effects on β-cell function, survival and the generation of signals that feedback to the immune system[5]. Thus, β-cell exposure to cytokines present in the islet environment at the different stages of insulitis, for example, type I interferons (mostly IFNα) during the early stages of inflammation, and then IFNγ plus IL-1β, tumour necrosis factor (TNF) and, potentially, IL-17 at the latest stages[5], will trigger ER stress, changes in alternative splicing[60,61] and upregulation of HLA class I (REFS[39,62]). These effects, together with increased β-cell production of chemokines and increased cell death, are predicted to lead to the augmented presentation of β-cell neoantigens to infiltrating immune cells, with the potential to aggravate and/or amplify the immune assault[5,6].

Not all responses induced by these cytokines are deleterious to β-cells. For example, both IFNα and IFNγ upregulate β-cell expression of PDL1 (REFS[12,63]) and HLA-E[64], proteins that respectively provide a negative feedback to T cells and natural killer cells and, at least in animal models, protect β-cells against immune-mediated cell death[13]. PDL1 and its ligand PD1 probably also have a protective role in human T1DM, as their blockade in the context of cancer therapy is associated with autoimmune endocrine diseases, including T1DM[65]. Although most of the pro-inflammatory and pro-apoptotic signals delivered by IFNs to β-cells are mediated by the transcription factors STAT1 and STAT2 (REFS[12,66]), the 'protective' signals, such as PDL1, are mostly regulated by IRF1, a downstream transcription factor in the IFN signal transduction pathway[12,66]. Interestingly, an IFN signature is observed in islets isolated from patients with T1DM in the first weeks to months of the disease[67], suggesting that this IFN–STAT–IRF1–PDL1 pathway could contribute to the honeymoon phase of T1DM. A feasible therapeutic approach might involve preserving protective signals downstream of IFNs (such as PDL1) whilst blocking deleterious signals (such as HLA class I overexpression, ER stress and chemokine production). Indeed, STAT2 blockade prevents HLA class I overexpression, ER stress and chemokine production in human β-cells whilst preserving and even increasing PDL1 expression[12].

When comparing global gene expression between human islets exposed to the pro-inflammatory cytokines IL-1β and IFNγ[6,68] and human β-cells from donors with T1DM (with disease duration 0.4–7 years)[69], a strong overlap is seen between genes upregulated in both conditions (FIG. 1a). By contrast, there is no similarity between transcriptomes of cytokine-exposed human islets and T2DM β-cells[70] (FIG. 1b), suggesting that these cytokines do not play a major role in islet dysfunction and/or death in T2DM.

Characteristics of human β-cells in T2DM. In T2DM, the histology of pancreatic islets comprises an ~40% reduced β-cell mass (range 25–60%), increased β-cell apoptosis, greater amyloid deposition and reduced pancreatic insulin content compared with that of non-diabetic pancreatic islets[71–76]. Amyloid deposits are formed of islet amyloid polypeptide (IAPP) and IAPP aggregates can trigger ER stress[77]. Macrophage numbers are mildly increased from 0.5 macrophages per islet in non-diabetic islets to 1.3 in T2DM[78] (inflammatory stress in islets in T2DM is comprehensively reviewed in REF.[33]). The absolute α-cell mass is overall unchanged[79]. The reduction in β-cell mass in T2DM is limited in recent-onset cases and becomes more marked with longer duration of disease[71]. Considering that β-cell function is substantially impaired at T2DM onset, reduced by ~80% according to some estimates[24,80], these data show that β-cell dysfunction is an early player in T2DM pathogenesis and occurs independently of β-cell loss. This situation resembles that seen in T1DM; however, loss of residual β-cell function and mass will occur over a much longer timespan in T2DM compared with the rapid evolution in T1DM.

β-cell apoptosis in T2DM is an infrequent event, which can be partly explained by the difficulty in detecting apoptosis in vivo as neighbouring cells rapidly clear apoptotic cells[81]. Human β-cells are very long-lived cells with limited potential for β-cell neogenesis or replication. Different approaches, such as mathematical modelling of β-cell lipofuscin accumulation[82,83], thymidine analogue incorporation and ^{14}C radiocarbon dating of β-cells[84], and histological assessment of β-cell proliferation[85], all provide evidence that the human β-cell mass is established in the first 2–3 decades of life and that, subsequently, β-cells age with the body. Cell death and/or turnover is therefore expected to be an uncommon phenomenon under conditions where β-cells are not rapidly depleted. It is plausible that the same causative factor(s) induce both β-cell dysfunction and β-cell death in each disease, potentially through similar downstream signal transduction pathways; however, the causative factors differ between T1DM and T2DM. For example, cytokines can lead to β-cell dysfunction and death in T1DM, whereas free fatty acids (FFAs) might elicit ER stress and thereby impair β-cell function and survival in T2DM.

Dedifferentiation and/or transdifferentiation have also been implicated in the β-cell pathology of T2DM by the loss of β-cell markers and acquisition of progenitor or non-β-cell genes, respectively. The prevalence of dedifferentiation, defined as hormone-negative endocrine islet cells, in T2DM varies considerably, from 0.1–0.4 cells/islet in some studies[86,87] to 10–17% of islet cells in others[88,89]; technical issues could underlie this variability. Glucagon and insulin co-expressing cells are increased in islets from patients with T2DM (3–4% versus 0.5–3% in non-diabetic islets)[89–91] and cells containing insulin and amylase-containing acinar granules were also observed in T2DM[92]. The impact of β-cell dedifferentiation and transdifferentiation on functional β-cell mass in T2DM remains uncertain.

Accumulating evidence suggests that accelerated β-cell ageing and senescence occurs in T2DM. In keeping with the long lifespan of β-cells, one study showed that β-cell and α-cell telomere shortening is most pronounced before age 20 years and flattens thereafter[93], pointing to replicative senescence of postmitotic adult β-cells. More marked telomere attrition was seen in β-cells in T2DM[94]. Cellular senescence in β-cells is characterized by acid β-galactosidase and p16[INK4A] expression and leads to loss of β-cell markers as well as to induction and secretion of pro-inflammatory cytokines (the so-called senescence-associated secretory phenotype). In T2DM islets, more β-galactosidase-positive cells were detected and the β-galactosidase-positive β-cell fraction had higher p16[INK4A], CCL4 and IL-6 expression[95]. Whether senescence actually contributes to β-cell dysfunction in T2DM is unclear.

Studies on β-cell gene expression in T2DM initially put forward one or few genes responsible for the phenotype based on only a few human islet samples[96]. These approaches resembled the initial candidate gene studies in small cohorts to dissect T2DM genetics that resulted in an array of false positive findings. Studies from different groups using qPCR, microarray and RNA sequencing (RNA-seq) analyses have included growing numbers of human islet preparations, now typically counting 10–80 non-diabetic and 10–20 T2DM samples[97–99]. Overall, the overlap of differentially expressed genes between different studies is very limited, questioning the validity of the findings. Considering the lack of specific diagnostic criteria and the heterogeneity of T2DM, very large collections of human islets are needed to map T2DM islet transcriptomes with a fair degree of confidence, as has been the case for genome-wide association studies (GWAS). Efforts are under way, through the European consortium T2DSystems' TIGER (the Translational Human Pancreatic Islet Genotype Tissue-Expression Resource), Rhapsody and others, to bring together >1,000 human islet transcriptomes and correlate gene expression with donor genotypes.

Another advance has been the introduction of single-cell RNA-seq and other single-cell omics approaches[100,101]. These new studies confirmed earlier findings in rodent β-cells that were based on other methods, suggesting that β-cells are functionally heterogeneous[102–104] and have variable susceptibility to stressful agents (such as pro-inflammatory cytokines) with the more active cells being preferentially affected[105]. It is of concern, however, that different single-cell transcriptome papers obtained discordant gene expression signatures for both physiological human β-cell 'sub-populations' and T2DM β-cells (reviewed in REFS[100,106]). This finding casts doubts on whether these signatures are biologically relevant or merely driven by different methods of cell separation and bioinformatics analysis, and by working at or below the limit of resolution of single-cell analysis[106]. Furthermore, only a limited number of genes can be detected by single-cell RNA-seq, from hundreds to a few thousand, as compared with >50,000 transcripts, including splice variants, in bulk analyses[6,61]. In addition, the fact that islet cells function as micro-organs, and not as single cells, suggests that these single-cell analyses should be still taken with a 'grain of salt' and complemented by bulk RNA-seq of human islets or enriched β-cell preparations.

Insights from GWAS

During the last decades, the human genome of thousands of individuals was scanned in search of DNA sequence variants associated to common traits and diseases. GWAS revealed >400 distinct genetic signals associated with T2DM[107] and >50 influencing T1DM[108]. Excluding the HLA region[109], which has a major effect on T1DM risk[110], the most common associated variants with T1DM and T2DM have only modest effects on disease risk but, in the context of T1DM pathogenesis, could affect the speed of functional β-cell loss following the appearance of autoantibodies[111,112]. Knowledge of such association signals is precious for gaining mechanistic insight into the development of the disease and for capturing genes implicated in the process; such information has utility for the development of novel therapies. Nevertheless, this process is challenging since most susceptibility loci harbour numerous genes and the majority of associated variants are located in non-coding genomic regions, suggesting that risk variants can act by affecting gene regulatory relationships, as opposed to exerting a direct effect through changes in gene coding sequences[113]. Moreover, GWAS do not inform on the tissue and/or cell type implicated in disease.

Candidate genes in T2DM. In the case of T2DM, the data emerging from GWAS indicate that a substantial fraction of the association signals are driven by dysregulation of β-cell development and insulin secretion, as opposed to influencing the tissues of insulin action such as fat, muscle and liver[114]. To date, the main candidate genes associated with T2DM that exert an adverse impact on β-cell function include *ABO*, *IGF2BP2*, *MTNR1B*, *TCF7L2*, *HNF1A*, *HNF1B*, *ADCY5*, *SLC30A8*, *CCND2* and *PAM*. Variants in *WFS1* are also associated with increased T2DM risk[115,116] through modulation of insulin secretion[117].

The human genome contains instructions to generate a vast number of cell-fate programmes and each cellular state (including disease states) utilizes distinct sets of non-coding genomic regulatory regions[118–120]. Studies profiling accessible chromatin, histone modifications and transcription factor binding sites in an

unbiased genome-wide manner are enabling research-ers to unravel the coordinates of non-coding regula-tory elements throughout the genome in an array of human tissues and cells, including pancreatic islets[121–124]. Contrasting maps of non-coding regulatory elements in islets with T2DM GWAS signals have implicated islet-specific non-coding function with genetic sus-ceptibility for T2DM[121]. Such studies prove the central role of islets in T2DM and suggest the disruption of islet-specific regulatory elements by common vari-ants as a major pathogenic mechanism. For instance, rs58692659 (*ZFAND3* locus)[121], rs1635852 (*JAZF1* locus)[125], rs11257655 (*CDC123* locus)[126], rs11603334 and rs1552224 (*ARAP1* locus)[127], rs231362 (*KCNQ1* locus)[128], rs7732130 (*ZBED3/PDE8B*)[129], and rs7903146 (*TCF7L2* locus)[123] are all examples of T2DM-associated variants disrupting islet regulatory functions. Yet, these studies lack information on the actual genes affected by T2DM susceptibility enhancer variants, a crucial piece of knowledge for the development of interventions targeting pathogenic mechanisms.

Attempts have been made to bridge these gaps in knowledge by studies applying state-of-the-art tech-niques to reconstruct regulatory relationships between distal regulatory elements and their target genes[129,130]. For instance, using chromatin capture and genome editing techniques one study showed that, for 72% of the susceptibility loci, the target gene could not be pre-dicted based on linear proximity. Examples of unexpec-ted distal target genes include *SOX4* (in the *CDKAL1* locus), *OPTN* (*CDC123/CAMK1D*), *TRPM5* (*MIR4686*), *PDE8B* (*ZBED3*), *SLC36A4* (*MTNR1B*), *POLR3A* and *RPS24* (*ZMIZ1*), and *PHF21A* (*CRY2*)[130]. Consistent with T2DM regulatory variants affecting islet-specific *cis*-regulatory networks, genetic variation in islet enhanc-ers affects the heritability of β-cell function. Remarkably, common variation in islet enhancers can explain ~10% of the heritability for T2DM, >20% for the acute insu-lin secretory response in intravenous glucose tolerance tests and ~30% for the insulinogenic index in oral glu-cose tolerance tests[130]. Such results confirm the promi-nent role for variants affecting islet regulatory elements in the heritability of T2DM and insulin secretion.

Importantly, refining the potential impact of T2DM variants enables the precise genetic risk to be defined for individuals to develop the disease. Polygenic risk scores integrating a large number of variants, including many that lack genome-wide significance, can be used to identify individuals at high risk for developing polygenic diseases, including T2DM. Furthermore, variants in islet enhancers can be used to generate distinct T2DM risk scores that confer risk at an earlier age and lower BMI.

Candidate genes in T1DM. T1DM has a strong heri-table component (twin concordance rate up to 70%[131] and sibling risk of approximately 8%[15]), which enables disease prediction based on individual genetic back-ground. Disease prediction can be used for surveillance or inclusion in trials of early immunologic intervention. Although initial diagnostic discrimination was based exclusively on HLA alleles[132], genetic risk scores later evolved to incorporate non-HLA variants[133–135]. In 2019,

a genetic risk score was developed based on 67 single nucleotide polymorphisms to include HLA DR-DQ hap-lotype interactions as well as non-HLA single nucleotide polymorphisms. The resulting score allowed discrimina-tion of T1DM in the UK Biobank dataset with an out-standing accuracy (receiver operating characteristic area under the curve of 0.92)[136].

Numerous GWAS have been performed for T1DM, identifying >50 non-HLA regions associated with incr-eased risk, including loci such as *INS* variable number of tandem repeat, which is also associated with T2DM and predisposition to other metabolic syndromes[137,138]. The most extensive fine mapping studies[108,139] were designed to make genetic comparisons across auto-immune disorders. These studies confirmed a pri-mary enrichment of T1DM association signals in immune-cell enhancers affecting mainly T cells and B cells. These observations are in line with the autoim-mune pathogenesis of T1DM and the central role of the immune system. Nevertheless, the lack of enrichment in islet regulatory elements is in contrast with the observa-tion that >60% of T1DM candidate genes are expressed in human β-cells[61]. These candidate genes regulate key steps related to 'danger signal recognition' and innate immunity[9]. Furthermore, substantial evidence points to an active role of β-cells in their own demise in T1DM[5]. Such apparent contradiction might be reconciled by experimentally exposing β-cells to external stimuli that can have a role in T1DM such as pro-inflammatory cytokines[5,68].

Fig. 2 | **T1DM and T2DM risk variants affect pancreatic islet *cis*-regulatory elements. a** | Results of permutation tests assessing the significance of the overlap between different classes of islet *cis*-regulatory elements (stable regulatory elements (SREs) or induced regulatory elements (IREs)) by the pro-inflammatory cytokines IFNγ and IL-1β (*y* axis) and trait-associated loci (*x* axis). Non-shared loci associated with type 2 diabetes mellitus (T2DM) and type 1 diabetes mellitus (T1DM) were tested. T2DM but not T1DM risk loci overlap with human islet non-cytokine-responsive regulatory elements (that is, SREs) more than expected by chance (SREs in T2DM risk loci $P = 2 \times 10^{-4}$, $Z = 6.6$). By contrast, T1DM but not T2DM risk loci are enriched for human islet IREs (IREs in T1DM risk loci $P = 4 \times 10^{-3}$, $Z = 4.8$). The size of the circle is proportional to $-\log_{10}P$; fill represents Z-score of the observed versus expected value. Significance was assessed by permutation tests; **$P < 0.01$, ***$P < 0.001$. The raw data was processed from Ramos-Rodriguez et al.[68]. **b** | Single nucleotide polymorphisms associated with T2DM and fasting glycaemia are enriched in pancreatic islet active regulatory elements. This confirms, from a genetic perspective, that altered β-cell insulin secretory function is relevant to T2DM pathogenesis. T1DM risk variants are primarily enriched in regulatory elements active in immune cells but not in islets. Nevertheless, exposure of human islets to pro-inflammatory cytokines results in pervasive activation of islet IREs. T1DM but not T2DM risk loci are enriched for human islet IREs. This observation implicates the islet response to pro-inflammatory cytokines in T1DM genetic susceptibility. Genes linked to islet IREs are enriched in immune response, β-cell stress and apoptosis pathways. GWAS, genome-wide association studies. Part **a**, image courtesy of the M. Ramos-Rodriguez, IGTP.

A 2019 study of islet exposure to pro-inflammatory cytokines demonstrated profound chromatin remodelling and pervasive activation of distal regulatory elements[68]. Of note, analysis of islet chromatin immunoprecipitation sequencing data and sequence composition of the activated regulatory sites showed that more than one-third of the islet cytokine-responsive regulatory elements are pre-bound by islet-specific transcription factors such as HNF1A, HNF1B, NEUROD1, PDX1, MAFB and NKX6.1, among others. These regulatory elements are further induced by inflammatory response-activated transcription factors such as interferon regulatory factors (IRFs), signal transducers and activators of transcription (STATs) and nuclear factor-κB (NF-κB). Of particular relevance, this work revealed that islet cytokine-responsive regulatory elements are enriched in T1DM risk variants (FIG. 2a). Taken together, these findings led to the hypothesis that enhancers pre-bound by tissue-specific transcription factors could facilitate cell type-specific responses to ubiquitous pro-inflammatory signals[68]. These observations,
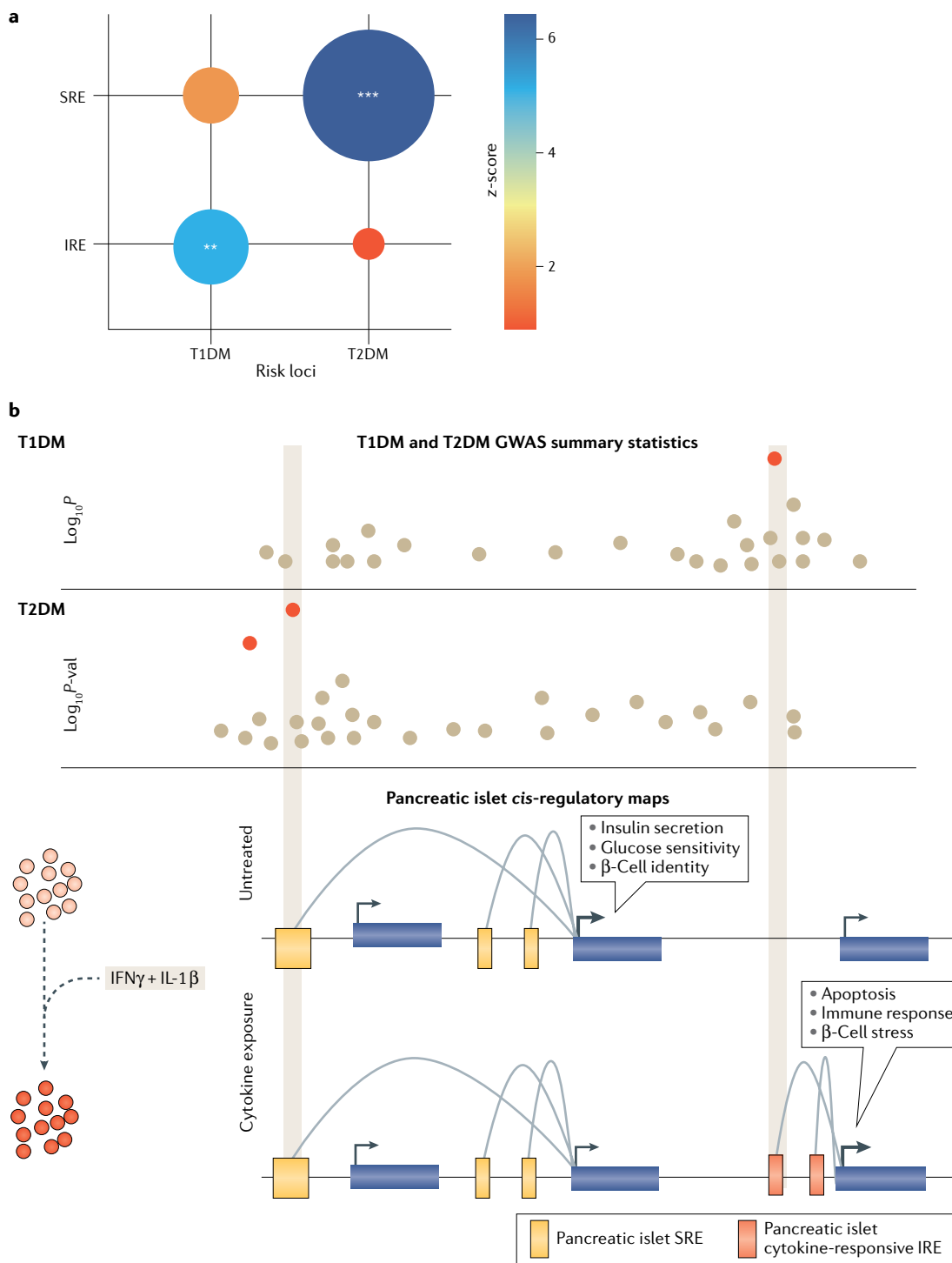
together with results obtained from other non-islet studies[140,141], suggest that enhancer priming could result in tissue-specific genetic susceptibility in autoimmune diseases.

Overall, these observations suggest a new mechanism for the disease, linking T1DM genetic susceptibility with β-cell responses and external stimuli. In other words, T1DM variants might act at the β-cell level but only become manifest upon islet cell perturbation. Hence, T1DM islet functional variants do not map to islet regulatory elements in an unperturbed state but can be captured by stimulus-specific islet *cis*-regulatory maps (FIG. 2b). By contrast, T2DM variants are enriched in pancreatic islet active regulatory elements under basal conditions, and these variants can thus be detected as determining expression quantitative trait loci (eQTL) without the need of islet exposure to disease-relevant perturbations (FIG. 2b).

Summary. Taken together, these findings suggest that a subset of T1DM functional variants could interfere with the regulatory responses to external stress stimuli; this effect is less so regarding T2DM variants. In line with this hypothesis, genes linked to islet cytokine responses are enriched for β-cell stress, immune response and apoptosis pathways[68].

In light of accumulating evidence implicating a role for β-cells in the risk of developing T1DM and T2DM, it might be timely to develop genetic risk scores designed to capture β-cell fragility. Such scores would aim to identify individuals with insulin-secreting cells particularly sensitive to immune or metabolic stresses and thus at high risk of developing diabetes mellitus. The Innovative Medicines Initiative-supported project INNODIA is presently developing combined therapies to prevent or revert T1DM with a focus on both modulation of the immune system and β-cell protection. Novel β-cell-targeted genetic risk scores, if validated, could help in identifying patients that would particularly benefit from β-cell-protective approaches such as the use of ER chaperones.

ER stress in T1DM and T2DM

Over the past 15 years, evidence has accumulated indicating that ER stress contributes to β-cell failure in T1DM and T2DM. Here, we define ER stress and its downstream signalling pathways, and then review the salient features of β-cell ER stress in both types of diabetes mellitus.

The ER stress response. ER stress develops when the demand to synthesize and process proteins in the ER exceeds capacity. This situation can arise as a result of a variety of perturbations such as, for example, expression of a mutant protein, translational misreading, increased demand for protein synthesis, ATP shortage, ER Ca^{2+} depletion, impaired N-linked glycosylation, alterations in the oxidizing environment of the ER, shortage of ER folding enzymes or chaperones, and accumulation of cholesterol or saturated lipids in the ER membrane, among others[142]. The resulting perturbation in organelle homeostasis results in the accumulation of unfolded or misfolded proteins in the ER and triggers an adaptive response, termed the unfolded protein response or ER stress response[143].

The overall aim of the ER stress response is to restore organelle homeostasis by transiently decreasing protein translation, transcriptionally expanding ER size, upregulating folding enzymes and chaperones, and promoting ER-associated degradation (ERAD) of terminally misfolded proteins. The process is essential for the cellular adaptation to changes in protein synthesis demand and is particularly critical for the development, function and survival of pancreatic β-cells (reviewed in REF.[144]). ER stress is sensed by ER transmembrane proteins, called ER stress transducers. Upon activation, the canonical ER stress transducers PERK and IRE1 will homodimerize or homo-oligomerize and trans-autophosphorylate. IRE1 has an endoribonuclease activity that results in intron splicing of XBP1 mRNA, generating the transcription factor XBP1s[143]. This transcription factor activates a lipogenic programme to expand the ER and induces components of the ERAD machinery as well as folding enzymes and chaperones. In addition, IRE1 RNase activity mediates regulated IRE1-dependent decay, that is, the cleavage of ER-localized mRNAs[145], thereby reducing the protein synthesis and folding demand placed upon the ER (FIG. 3).

Activated PERK phosphorylates the α-subunit of the eukaryotic translation initiation factor 2 (eIF2)[143]. Phosphorylated eIF2α inhibits eIF2B, the guanine nucleotide exchange factor that exchanges eIF2γ-bound GDP for GTP to enable the ternary complex to initiate translation. eIF2α phosphorylation globally attenuates protein translation but, in parallel, it facilitates the translation of specific mRNAs by ribosomal skipping of short inhibitory upstream open reading frames in the promoter (for example, ATF4 and CHOP). The transcription factors ATF4 and CHOP upregulate transcriptional programmes of oxidative stress response, amino acid transport and apoptosis. Signalling in the PERK branch is terminated by the upregulation of GADD34, a non-enzymatic co-factor of protein phosphatase 1 that dephosphorylates eIF2α. Other proteins attenuate PERK signalling, namely p58[IPK] and CReP, which respectively decrease PERK and eIF2α phosphorylation.

A third ubiquitously expressed ER stress transducer is ATF6, which, upon activation, traffics to the Golgi to be proteolytically cleaved, thereby generating an active transcription factor[143]. ATF6 induces ER chaperones such as BiP, XBP1 and ERAD proteins.

Evidence of β-cell ER stress in diabetes mellitus. ER stress markers such as CHOP and BiP are detected in islets from pre-diabetic NOD mice[146,147] and patients with T1DM[40]. In vitro studies in both rodent and human β-cells exposed to pro-inflammatory cytokines, which trigger ER stress and β-cell apoptosis[148,149], suggest that IRE1α and downstream signalling are crucial for the transition between a compensatory unfolded protein response and the final steps of pro-apoptotic pathways[148] (FIG. 3). Activation of IRE1α is fine-tuned by the formation of molecular complexes both at its ER luminal and cytosolic regions. During severe ER stress, cytosolic ABL tyrosine kinases move to the ER membrane, where they bind and hyperactivate the RNAse activity of IRE1α in NOD mouse islets[150]. IRE1α-induced RNase[150,151] and
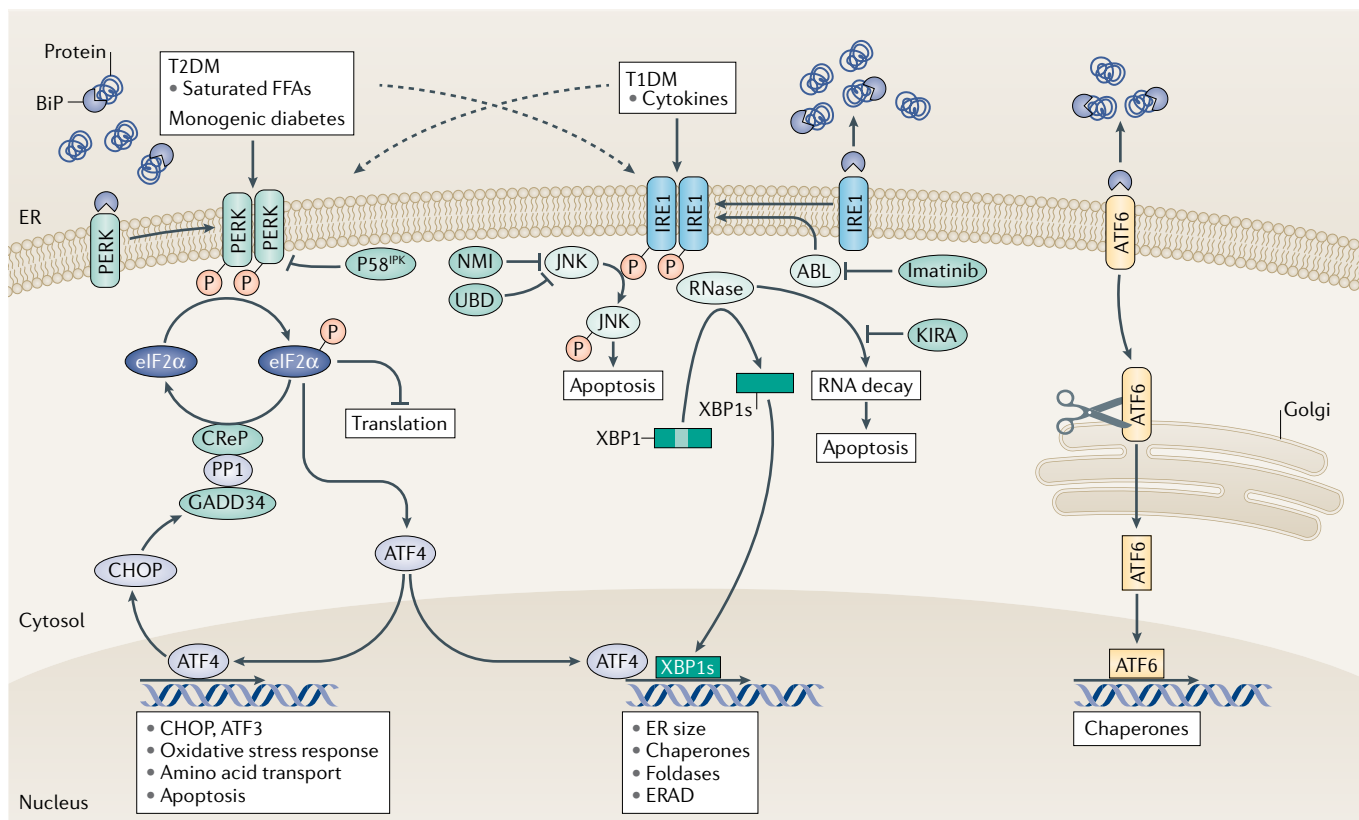
Fig. 3 | **ER stress signalling in β-cells in T1DM and T2DM.** IRE1 activation leads to XBP1 splicing (XBP1s), which induces endoplasmic reticulum (ER) expansion, expression of ER chaperones and foldases, and ER-associated degradation (ERAD), a favourable, adaptive response to ER stress. Cytokines and the immune assault in type 1 diabetes mellitus (T1DM) elicit particularly strong β-cell IRE1 activation that, through JUN N-terminal kinase (JNK) phosphorylation and endoribonucleo-lytic decay of ER-localized mRNAs, will culminate in secretory dysfunction and apoptosis. JNK activation is tonically inhibited by N-MYC interactor (NMI) and ubiquitin D (UBD). Kinase-inhibiting RNase attenuators (KIRAs) inhibit mRNA decay but not splicing of XBP1, a highly specific IRE1 substrate, and preserve β-cell function and survival in T1DM models. By sequestering c-Abl in the cytoplasm, the tyrosine kinase inhibitor imatinib prevents c-Abl translocation to the ER and IRE1 binding and hyperactivation. Non-toxic unsaturated free fatty acids (FFAs) elicit adaptive signalling in the three branches of the ER stress response. Saturated FFAs, such as palmitate, strongly activate PERK and induce eukaryotic translation initiation factor 2α (eIF2α) phosphorylation, and thereby trigger β-cell dysfunction and apoptosis. In the context of lipid overload in β-cells in type 2 diabetes mellitus (T2DM), excessive or prolonged eIF2α phosphorylation is detrimental. Similarly, exaggerated or persistent signalling downstream of PERK occurs in monogenic forms of diabetes mellitus caused by loss-of-function mutations in p58[IPK], CReP or the γ-subunit of eIF2.

JUN N-terminal kinase (JNK) activation[148] contribute to cytokine-induced rodent and human β-cell apoptosis.

Interestingly, β-cells express endogenous defence mechanisms against excessive cytokine-induced IRE1α activation, namely N-MYC interactor[152] and ubiquitin D[153]. These proteins do not modify cytokine-induced IRE1α RNAse activity as evaluated by XBP1 splicing and Ins-2 mRNA degradation; however, they do provide a negative feedback signal on IRE1α-induced JNK activation[152,153]. Although this effect could prevent excessive β-cell apoptosis during a limited innate immune response, with low or transitory cytokine exposure and ER stress, it will not prevent cell death in the course of a protracted autoimmune assault and the resulting long-term ER stress activation.

New tools have been developed that aim to prevent excessive IRE1α-induced RNase activity and thus promote β-cell survival during severe ER stress, for example, KIRA6 as well as imatinib (an FDA-approved anticancer tyrosine kinase inhibitor); both drugs reverted diabetes in NOD mice[150,151,154]. Imatinib is currently being inves-tigated as a therapy in patients with T1DM[155]. The ability to modulate β-cell ER stress could be an interesting adju-vant treatment to new approaches aiming to decrease immune-mediated β-cell death in early T1DM. This approach is particularly interesting considering that ER stress can augment local inflammation[149] and con-tribute to the triggering of neoantigen generation[156,157], which, together with the increased transfer of antigens to islet resident macrophages[158], could augment β-cell vulnerability to the immune system.

In patients with T2DM, several reports documented β-cell ER stress, with increased protein expression of p58[IPK], ATF3 and CHOP (markers of PERK signalling) as well as BiP[159–162]. Conversely, ATF6, XBP1s and phos-phorylated eIF2α proteins were reduced in islets from patients with T2DM[163]. Electron microscopy showed ER expansion, which is an ultrastructural hallmark of ER stress signalling, in β-cells and α-cells from individuals

with T2DM[52,164]. In db/db and ob/ob mice, islet p58[IPK], ATF3, CHOP, BiP and XBP1s mRNA levels were overall increased, albeit to a lesser extent in diabetic db/db versus prediabetic db/db mice[165]. Furthermore, db/db mouse islets had higher p58[IPK], phosphorylated eIF2α and XBP1s protein than wild-type islets[159]. At the protein level, islet ATF6 and XBP1s proteins were reduced in diabetic ob/ob and high fat diet-fed mice, whereas phosphorylated eIF2α was increased, the latter reportedly only in α-cells[163].

In vitro studies have identified potential triggers of β-cell ER stress in T2DM, notably high glucose or saturated FFA levels and IAPP (reviewed in REFS[77,142,166]). High glucose exposure elicits mild ER stress, with signalling in the IRE1 and ATF6 branches and, to a lesser extent, downstream of PERK[167,168]. By contrast, saturated FFAs produce substantial signalling downstream of PERK and this effect, together with IRE1-induced JNK activation, is pro-apoptotic in β-cells[169,170] (FIG. 3). Non-toxic unsaturated FFAs, which elicit modest signalling in the three branches of the ER stress response, can become pro-apoptotic when eIF2α phosphorylation is pharmacologically[171–173] or genetically[174] induced. Thus, hyperactivation of the PERK branch of the ER stress response in the context of lipid overload is detrimental to β-cells.

In addition to the compelling role for ER stress in β-cells in these polygenic forms of diabetes mellitus, several types of monogenic diabetes mellitus are caused by loss-of-function mutations in ER stress response components, particularly in proteins pertaining to the PERK branch (reviewed in REF.[144]). For example, mutations in *EIF2AK3* (REF.[175]) (encoding PERK) lead to the inability to elicit PERK signalling, which causes β-cell demise and diabetes mellitus in Wolcott–Rallison syndrome. Similarly, mutations in *EIF2B1* (encoding the α-subunit of eIF2B), which affect its interaction with P-eIF2α and also impair downstream signalling, cause very young-onset diabetes mellitus[176]. Conversely, excessive signalling downstream of PERK causes diabetes mellitus in patients with mutations in *DNAJC3* (REF.[177]) (encoding p58[IPK]), *PPP1R15B*[174] (encoding CReP) and *EIF2S3* (REF.[178]) (encoding eIF2γ). Collectively, these diseases highlight the β-cell sensitivity to aberrant eIF2α phosphorylation and perturbed mRNA translation[144]. Recessive[179] or dominant[180,181] mutations in *WFS1* (a candidate gene for T2DM) cause Wolfram syndrome, another monogenic form of diabetes mellitus related to β-cell ER stress.

β-cell ER stress signalling thus differs in T1DM and T2DM, with more IRE1-mediated damage in the former, and a predominant role for PERK–eIF2α in monogenic forms of disease and T2DM (FIG. 3). ER stress can result in β-cell dysfunction by preventing adequate insulin synthesis and secretion as well as expression of components of the secretory machinery, that is, membrane-expressed proteins that are synthesized in the ER. ER stress will also trigger protein degradation by ERAD and autophagy. If protracted, ER stress can lead to dedifferentiation or β-cell death by apoptosis[144,182].

Targeting ER stress. ER stress could represent a common target to protect β-cells in both polygenic forms of diabetes mellitus. In the context of T1DM, the chemical chaperone TUDCA (already in clinical use for liver diseases) partially protects human β-cells in vitro against cytokine-induced apoptosis[183] and delays diabetes onset in two mouse models of immune-mediated disease[147]. A clinical trial is presently ongoing to test whether TUDCA improves β-cell survival in patients with new-onset T1DM[184]. Of some concern, however, is that neither is TUDCA specific to ER stress nor is ER stress the sole mechanism of β-cell dysfunction and death in T1DM. Furthermore, TUDCA has been shown to protect NOD mice when given ahead of disease outbreak[147], but no data indicates that it can revert established disease. Independently of the outcome of this trial, TUDCA or other similar agents under development could still be considered for the treatment of multiple autoantibody-positive individuals (stage 1 T1DM) or be used in combination with agents aiming to decrease the autoimmune assault in patients with new-onset T1DM.

The effect of TUDCA on β-cell function in people with or at risk for T2DM has not been assessed, but sodium phenylbutyrate, another chemical chaperone, has been shown to prevent lipid-induced β-cell dysfunction[185]. For a comprehensive review on the therapeutic modulation of β-cell ER stress and ER stress signalling, the reader is referred to REF.[144].

Conclusions

β-cell failure is the central event in both T1DM and T2DM but the pathways that lead to this failure are different. β-cell dysfunction and death in T1DM is mostly immune mediated, whereas metabolic stress has a clear role in the progressive loss of functional β-cell mass in patients with T2DM. Over the past decade, research focus on the pathogenesis of diabetes mellitus has shifted from animal models to morphological and functional genomics studies on human islet cells. Furthermore, focus has been on the clarification of the functional role for genetic variants associated with T1DM or T2DM acting at the human β-cell level. Since human islet material is rare, this approach has — similarly to what happened in human genetic studies — stimulated large collaborative efforts in the field, which are starting to bear fruits. These findings have greatly augmented our understanding of disease, but the knowledge remains to be translated into novel therapies to prevent β-cell death or to restore lost β-cell mass in advanced disease.

Several key questions remain to be answered in order to allow translation of these novel findings into better therapies. First, how do β-cells from individuals with obesity who remain normoglycaemic in the face of insulin resistance differ from β-cells that fail early in other individuals with obesity who develop T2DM? Future work could aim to ask if genetically determined β-cell mass, functional plasticity and/or β-cell 'endurance capacity' have a role. Second, it remains to be assessed whether the drivers of β-cell failure are distinct in moderate and severe insulin-deficient and insulin-resistant subtypes of T2DM. That is, do the clustering approaches identify subtypes with different aetiologies? Third, is the unexpected plasticity of pancreatic islet cells clinically relevant to any extent in people with T1DM? If yes, can newly transdifferentiated β-cells be killed? If not, does the negative

effect of the immune attack on α-cells decrease their plasticity? Fourth, most candidate loci that potentially act at the β-cell level in T1DM and T2DM are located in non-coding regions; future research should aim to address the mechanisms linking genetic variation to disease risk at these loci — which are their proximal and/or distant targets and how can this knowledge be exploited therapeutically? Fifth, the numbers of human islets genotyped and RNA sequenced should reach >1,000 in coming months, allowing eQTL estimation (TIGER). However, these analyses will be done on islets under basal conditions. It would be of interest to investigate if *cis*-eQTL and *trans*-eQTL change when these human islets are exposed to relevant immune or metabolic stresses. Sixth, ER stress affects β-cells in T1DM and T2DM, but the signalling differs. Can novel approaches be developed to improve ER function in both forms of diabetes mellitus? Seventh, can the addition of novel therapies aiming to protect β-cells in T1DM improve the limited benefits of ongoing attempts to revert disease based on targeting the immune system only? Last, how can we translate the growing understanding of β-cell fate in diabetes mellitus into novel biomarkers that allow us to predict disease or to follow β-cell loss and response to therapy?

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Review criteria
Relevant publications were identified by searching the PubMed database (Jan 1, 2005 to October 30, 2019) using combinations of the following terms: "pancreatic beta cells", "pancreatic islets", "insulin release", "insulin secretion", "diabetes", "type 1 diabetes", "type 2 diabetes", "pathogenesis", "histology", "transcriptome", "genetics", "candidate genes", "islet gene regulation", "islet epigenomics", "endoplasmic reticulum stress" and "apoptosis". We preferentially selected publications in the past 5 years, plus earlier key publications for citation. A manual search of some references cited in these papers or in relevant articles related to the role of pancreatic β-cells in the pathogenesis of diabetes was also done. All selected papers were English-language, full-text articles. Review articles are often cited to provide the readers with additional references. Many of the references identified could not be included owing to space restrictions.

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