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Perspective

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Why does the immune system destroy pancreatic β-cells but not α-cells in type 1 diabetes?

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Abstract

A perplexing feature of type 1 diabetes (T1D) is that the immune system destroys pancreatic β-cells but not neighbouring α-cells, even though both β-cells and α-cells are dysfunctional. Dysfunction, however, progresses to death only for β-cells. Recent findings indicate important differences between these two cell types. First, expression of *BCL2L1*, a key antiapoptotic gene, is higher in α-cells than in β-cells. Second, endoplasmic reticulum (ER) stress-related genes are differentially expressed, with higher expression levels of pro-apoptotic *CHOP* in β-cells than in α-cells and higher expression levels of *HSPA5* (which encodes the protective chaperone BiP) in α-cells than in β-cells. Third, expression of viral recognition and innate immune response genes is higher in α-cells than in β-cells, contributing to the enhanced resistance of α-cells to coxsackievirus infection. Fourth, expression of the immune-inhibitory HLA-E molecule is higher in α-cells than in β-cells. Of note, α-cells are less immunogenic than β-cells, and the $CD8^+$ T cells invading the islets in T1D are reactive to pre-proinsulin but not to glucagon. We suggest that this finding is a result of the enhanced capacity of the α-cell to endure viral infections and ER stress, which enables them to better survive early stressors that can cause cell death and consequently amplify antigen presentation to the immune system. Moreover, the processing of the pre-proglucagon precursor in enteroendocrine cells might favour immune tolerance towards this potential self-antigen compared to pre-proinsulin.

Sections

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Key points

- Pancreatic β-cells and α-cells are both dysfunctional in type 1 diabetes (T1D) but, while β-cells are killed, α-cells survive.
- Exposure of islet cells to interferon-α (IFNα), a cytokine that is induced early in T1D pathogenesis, induces expression of both similar genes (such as HLA-related genes) and different genes (such as *BCL2L1*, endoplasmic reticulum (ER) stress-related genes, innate immune response genes and antiviral response genes) in β-cells and α-cells.
- Expression of candidate genes for T1D shows major differences between β-cells and α-cells.
- The antigen presentation capacity seems similar in β-cells and α-cells, but either α-cells are less antigenic than β-cells (perhaps owing to higher HLA-E expression) or their capacity to better endure viral infections and ER stress increases their survival when facing diabetogenic stressors and thus decreases antigen presentation.
- Pre-proglucagon processing in enteroendocrine cells might favour immune tolerance towards glucagon and further limit α-cell immunogenicity.

Introduction

A popular tenet states that type 1 diabetes (T1D) is characterized by the selective autoimmune destruction of insulin-producing pancreatic β-cells[1]. This destruction is ultimately mediated by cytotoxic $CD8^+$ T cells recognizing antigenic peptides (epitopes), which are largely, but not exclusively, derived from insulin and its precursor pre-proinsulin[2] and are presented in the context of surface HLA class I molecules on β-cells[3,4]. The hyper-expression of surface HLA class I in β-cells along with a variable degree of immune infiltration (insulitis) dominated by $CD8^+$ T cells is a histopathological hallmark of T1D[5,6]. Mirroring the heterogeneity of immune infiltration, not only between patients but also across the islets of the same pancreas[7], the persistence of both insulin-containing and insulin-deficient islets is another hallmark of the pancreas of a patient with T1D[8]. In both types of islets, α-cells and δ-cells are spared[9,10]. The question as to why such destruction is so selective for β-cells has been largely overlooked. This omission is even more striking considering that α-cell dysfunction and inadequate glucagon production is another feature of T1D[9,10] that contributes to hyperglycaemia and metabolic dysregulation[11–13], and can even precede β-cell dysfunction and death[14].

Of note, α-cell dysfunction is present in both T1D and type 2 diabetes (T2D) and can contribute to insulin-induced hypoglycaemia in T1D and, at least in the initial phases of the disease, to hyperglycaemia in T2D[9,15,16]. α-Cells are located in close proximity to β-cells in human islets[17], and these cells engage in a crosstalk that contributes to the regulation of their function[18]. The reduced functional β-cell mass in T1D and T2D, together with the consequent hyperglycaemia, have been hypothesized to indirectly affect α-cells and contribute to their dysfunction in both forms of diabetes[9]. Recent single-cell RNA sequencing (scRNA-seq) studies, however, indicate that α-cells in T1D and T2D have limited common changes in gene expression: <5% of differentially expressed genes detected were similarly upregulated or downregulated in both T1D and T2D (compared to α-cells from age-matched and sex-matched normoglycaemic controls)[19]. The gene signatures of α-cells[19] and β-cells[20] in T1D indicate exposure to similar immune mediators, suggesting that α-cell dysfunction might not be secondary to β-cell pathology but rather directly immune-induced. Thus, despite the fact that both β-cells and α-cells are exposed to deleterious immune signals, β-cells die while α-cells survive even in long-term T1D (Fig. 1).

The fact that α-cells do not die in the course of T1D is reminiscent of the dialogue between Inspector Gregory and Sherlock Holmes in the short story 'The Adventure of Silver Blaze' by Conan Doyle: Gregory: "Is there any other point to which you would wish to draw my attention?"; Holmes: "To the curious incident of the dog in the night-time"; Gregory: "The dog did nothing in the night-time"; Holmes: "That was the curious incident"[21]. In other words, the fact that α-cells are also exposed to immune mediators and show early dysfunction — probably even preceding β-cell dysfunction[14] — but somehow remain alive even in long-standing T1D is 'the curious incident' that is addressed in this Perspective. Understanding the mechanisms of autoimmune resistance of α-cells is important not only from a pathophysiological standpoint, but also because it could reveal underlying pathways amenable to therapeutic interventions aiming to increase the resistance of the β-cells themselves to the immune attack. Indeed, human β-cells that switch to a dual α/β-cell phenotype under immune attack are more resistant than pure β-cells to apoptosis[22]. We provide an overview of the available evidence for key differences between β-cells and α-cells that could account for their differential autoimmune vulnerability, and how these differences may translate into mechanisms of the preferential endurance and survival of α-cells over β-cells.

Single-cell studies of islets in T1D

Technical aspects

A major challenge in the study of individual cell types is the ability to effectively isolate them via in vitro or in silico approaches in order to capture a phenotype as unbiased and as representative as possible[23]. Physical cell sorting methods such as flow cytometry require identifying cell surface markers that are uniquely expressed at a sufficient level to allow isolation of >90–95% pure populations to enable accurate downstream interpretation. Physical cell sorting might also influence the observed phenotype through disruption of the microanatomy of islets by disrupting gap junctions[24] and the communication between β-cells and α-cells[25]. scRNA-seq technologies offer an alternative to physical cell sorting methods by leaving most of the 'dissecting' part to in silico tools (although the processing of islets for scRNA-seq can also disrupt important islet cell interactions) and by providing another layer of information on genomics dynamics[26] through studies of cell trajectory[27] and RNA velocity[28]. Public sharing of data sets in database depositories such as the European Molecular Biology Laboratory Nucleotide Sequence Database of the European Bioinformatics Institute and the Gene Expression Omnibus enables re-analysis and/or integration of the data as new algorithms and methods are developed.

In recent years, collaborative initiatives such as the Human Pancreas Analysis Program[29] have generated large data sets based on scRNA-seq of islets from normoglycaemic individuals and patients with T1D or T2D, enabling for the first time a global view of the impact of diabetes on the different islet cell populations and islet-invading immune cells[29,30]. The availability of these data sets from actual human disease enables comparison and validation of the gene expression observed by studies in in vitro models, where human islets or human β-cell lines[31,32]

or stem cell (SC)/induced pluripotent stem cell (iPSC)-generated islet-like cells[33] are exposed to potential mediators of islet cell dysfunction and death in T1D, such as pro-inflammatory cytokines[31–34]. The use of SC/iPSC-induced islet-like cells enables detailed studies on the role of candidate genes acting at the islet cell level[35] and of the development of novel approaches to protect β-cells from the immune system[36–38].

Differences between α-cells and β-cells following IFNα exposure and antiviral responses

A limitation of the use of SC/iPSC-derived islet cells is that, by the end of their in vitro differentiation, they are not fully functional, with a phenotype that is closer to that of the β-cells of a neonate than that of an adult human islet; also, they present a higher proportion of multi-hormonal cells than primary islets and often retain a population of poorly differentiated 'enterochromaffin cells'[34,39]. On the other hand, the fact that autoimmunity in T1D can be triggered at an early age, possibly even in utero[40–42], makes the use of those relatively 'immature' β-cells an interesting tool to obtain novel insights into the early stages of T1D pathogenesis[33]. A recent study used a scRNA-seq approach to characterize the impact of interferon-α (IFNα; a pro-inflammatory cytokine produced in the vicinity of islets in early T1D)[20] on iPSC-derived islet cells, enabling detailed studies of the impact of IFNα on the gene expression of α-like and β-like cells[33] (Fig. 2). Although an overlap was observed between the top three pathways detected as being enriched by gene set enrichment analysis in α-cells and β-cells[33], the leading edges of those pathways were not identical (Fig. 2a). Around 20% of the genes in the leading edges of 'interferon signalling' pathways in α-cells and β-cells were different, including seven genes (*GBP1*, *GBP3*, *OAS2*, *TRIM22*, *UBA7*, *UBC* and *XAF1*) uniquely present in the α-cell leading edge and two genes (*IRF1* and *IFIT2*) present only in the β-cell leading edge. Guanylate-binding proteins (GBPs) are IFN-induced GTPases that play a role in the immune response by cooperating with the core inflammasome machinery[43,44] and, together with the antiviral factors TRIM22 (ref. 45) and XAF1 (ref. 46), might contribute to α-cell resistance to viral infection. Likewise, *OAS2* is a member of the leading edge of the IFN-stimulated genes[33] involved in the antiviral mechanism[47]. This upgrade of the antiviral responses in α-cells is an important point, as environmental factors interact with predisposing genes and contribute to the initiation of the autoimmune assault against β-cells that eventually culminates in T1D. Among these environmental factors, coxsackievirus B (CVB), which belongs to the *Enterovirus* genus, probably plays a role in triggering insulitis and T1D[48–50]. Several T1D candidate genes regulate antiviral responses in islet cells and the immune system[48], and their observed differences of expression in α-like versus β-like cells (Fig. 2c) might play a role in these responses. In line with this hypothesis, gene expression of the pattern recognition receptor *IFH1* — a T1D candidate gene encoding MDA5, a helicase involved in the recognition of viral double-stranded RNA[51,52] — is much higher in human α-cells than in β-cells[33], supporting the observation that α-cells clear viruses more effectively than do β-cells[53]. This difference in MDA5 expression was confirmed by histological studies showing that human α-cells from both normoglycaemic donors and T1D donors have a higher expression of MDA5 than β-cells from these donors[54].

After IFNα exposure, both α-cells and β-cells show increased expression of HLA class I as well as increased expression of the peptide loaders TAP1 and TAP2 (ref. 33) (Fig. 2b), suggesting a similar capacity of both cell types to present antigens to $CD8^+$ T cells. This likely similar antigen-presenting capacity is, however, counterweighted by a higher α-cell expression of HLA-E — an atypical HLA protein with a protective role against the autoimmune assault, potentially making α-cells 'less visible' to invading immune cells[31]. α-Cells also show increased expression of the protective endoplasmic reticulum chaperone BiP (encoded by *HSPA5*), of the antiapoptotic protein BCL2L1 (also known as Bcl-XL), of the free radical scavengers catalase (CAT) and superoxide dismutase (SOD2) (Fig. 2b). All of these genes and their encoded proteins have been previously shown to protect islet cells against immune or metabolic stressors[55,56].

Importantly, candidate genes for T1D risk[57,58] have different levels of expression in β-cells and α-cells (Fig. 2c). We previously reported that >80% of the T1D candidate genes are detected in human β-cells purified by fluorescence-activated cell sorting[59], and the expression of several of these genes is modified in human islets by exposure to pro-inflammatory cytokines[32,60]. This phenomenon seems to be a broad one, as candidate genes for three other autoimmune diseases (lupus erythematosus, multiple sclerosis and rheumatoid arthritis) are also highly expressed in their respective target tissues (namely kidney cells, optic chiasma and joint tissue)[59]. Owing to limitations of scRNA-seq[61], which usually detects only the 20–25% the most highly expressed genes, only a subset of these genes were retrieved (Fig. 2c). Nonetheless, the difference in expression levels offers an additional layer of information: for example, *BACH2* — which has an anti-apoptotic role in islet cells[62] — is more expressed in α-cells than in β-cells[33]. *BACH2* participates in crosstalk with *PTPN2* (ref. 62), a gene that is heavily downregulated in α-cells, but slightly upregulated in β-cells[33]. Similarly, the kinase TYK2, implicated in type I IFN signalling, has higher basal expression in α-cells than in β-cells[33]. Increasing knowledge about the function of these candidate genes will enable clearer hypotheses to be made on the impact of their different expression levels in β-cells and α-cells.

The observations described above suggest that both α-cell and β-cell types use a common repertoire of genes in response to IFNα, but that α-cells have higher basal expression of key genes and/or deploy a more effective response against viral infections and stress and/or apoptosis mitigation, as discussed below.

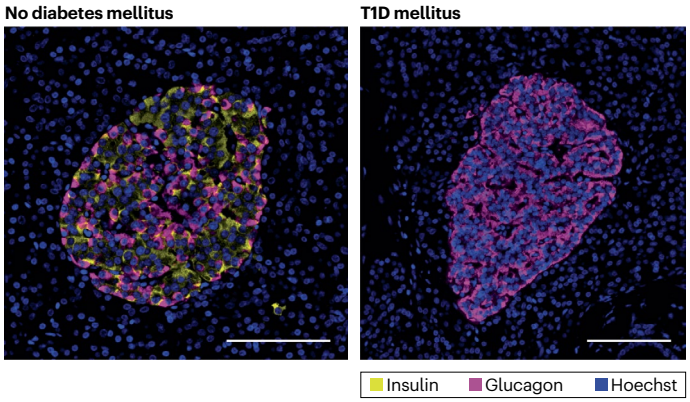

Fig. 1 | Immunofluorescence imaging of islets in individuals with T1D and with normoglycaemia. Representative images show insulin (yellow), glucagon (magenta) and nuclear staining (Hoechst) in islets from a non-diabetic donor (left) and a donor with type 1 diabetes (T1D) with 7 years of disease duration (right). Note the near complete absence of β-cells in the islet from the donor with T1D, while the α-cell population remains similar to that present in the islet from the non-diabetic donor. Images are courtesy of T. Rodriguez-Calvo, Institute of Diabetes Research, Munich, Germany.

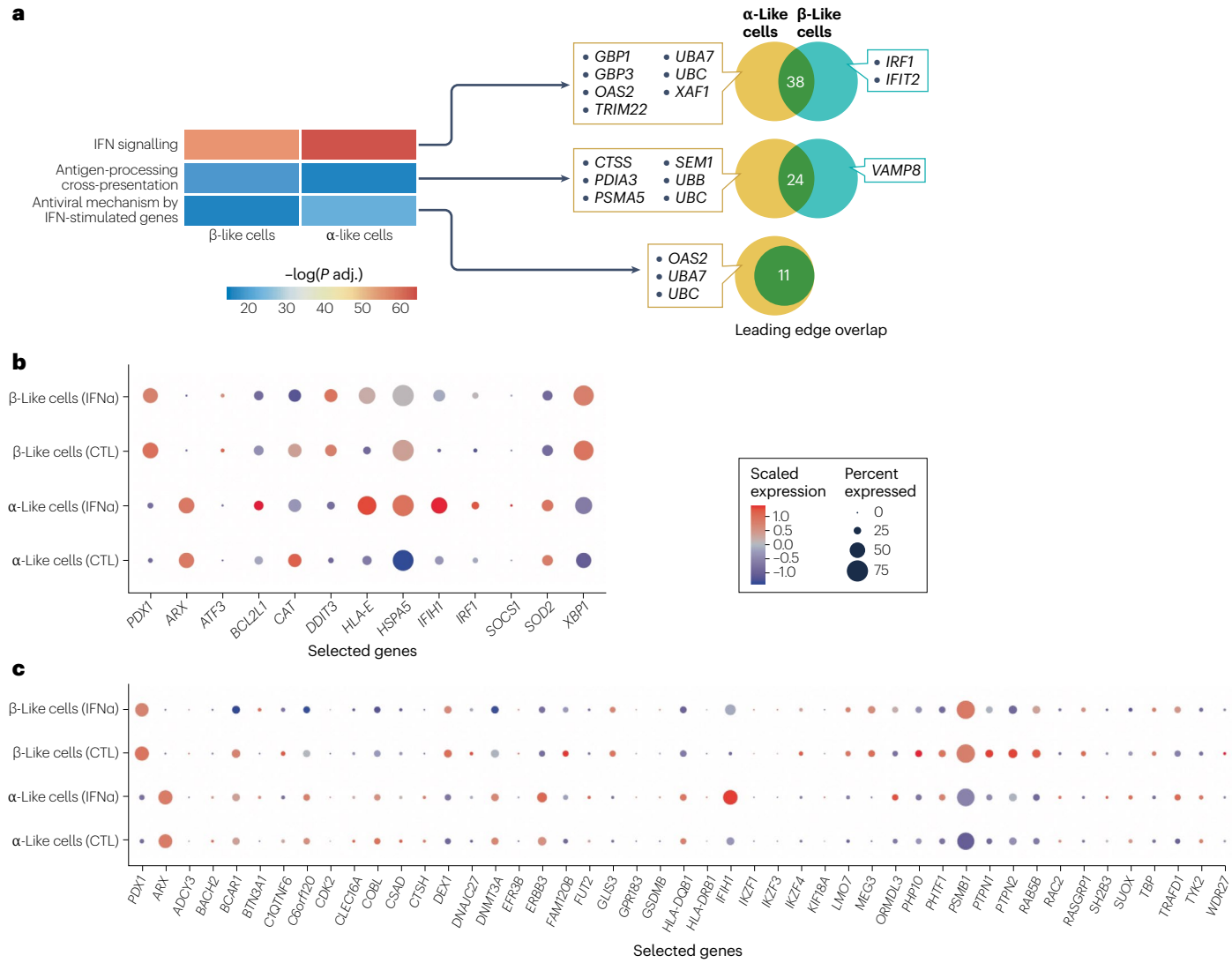

Fig. 2 | Different basal and IFNα-induced gene expression in α-like and β-like cells. a, Gene set enrichment analysis of α-like and β-like cells after exposure to interferon-α (IFNα) shows three common upregulated REACTOME pathways, as represented by their $-\log_{10}$(*P*-adjusted) values. Leading edge components are represented as Venn diagrams with the amount of overlap indicated in number of genes. **b**, Dot plot representing the expression of selected key IFNα-induced genes in β-like and α-like cells. The dot sizes indicate the percentage of cells expressing the gene of interest, while the colour scale indicates the level of expression. **c**, Expression of detected candidate genes in β-like and α-like cells. The dot sizes indicate the percentage of cells expressing the gene of interest, while the colour scale indicates the level of expression. The figure was created by re-analysis of the data presented in ref. 33.

Innate immune responses

Recognition of viral double-stranded RNA by MDA5 or other pattern recognition receptors (for example, RIG-I and TLR3) in islet cells[63–65] is followed by local release of type I IFNs which contribute to the generation of antiviral responses in both the infected and neighbouring cells. Type I IFNs are expressed in pancreatic islets from individuals with T1D[20,66], and an IFN signature is detected in peripheral blood in early disease stages (for example, in children genetically at risk of T1D (stage 1))[67,68], supporting the concept that type I IFNs are key cytokines in human T1D. In line with this idea, IFN-stimulated genes are present in the islets of patients with recent-onset T1D[69,70], and exposure of human islets to IFNα induces expression of HLA class I and markers of endoplasmic reticulum (ER) stress and, together with IL-1β, leads to apoptosis, three hallmarks of human islets in T1D[31,71]. Interestingly, rat α-cells trigger a more efficient antiviral response against CVB infection than do rat β-cells owing to higher expression of the transcription factor STAT1 (and probably STAT2) and of downstream antiviral proteins such as MX1, PKR and viperin[53]. β-Cells allow viral proliferation and undergo progressive apoptosis, whereas α-cells show high expression of *STAT1*/*STAT2* and downstream genes that enables them to eradicate CVB infection without undergoing apoptosis[53]. This finding is reminiscent of the fact that different neuronal subtypes have unique innate immune response programmes, which explains why cerebellum granule neurons survive while cortical neurons are killed during infection by

positive-strand RNA viruses[72]. A systematic comparison of genes that are upregulated in α-cells versus β-cells as compared to genes upregulated in granule neurons versus cortical neurons identified a close similarity, with 70% of the upregulated mRNAs shared between α-cells and granule neurons, many of them downstream of STATs[53], indicating that α-cells and granule neurons are better poised to clear viral infections than are β-cells and cortical neurons[48,53,72,73]. This finding might help explain why putative chronically infected β-cells, but not α-cells, are recognized by the immune system during the course of T1D.

CD8$^+$ T cell responses

Virus-infected cells expose viral peptide antigens in the context of HLA class I, leading to their recognition and lysis by CD8$^+$ T cells[74]. Interestingly, and as discussed above, α-cells show higher expression of the co-inhibitory molecule HLA-E than do β-cells, as detected by histology and scRNA-seq[33,75]. HLA-E is a non-classic HLA molecule that inhibits both CD8$^+$ and NK cells, and is being considered as a target for cancer checkpoint immunotherapy[76,77]. It is thus conceivable that the increased HLA-E expression in α-cells represents an additional level of resistance against cell death induced by virus-reactive CD8$^+$ T cells.

The stress of synthesizing hormones

Pancreatic β-cells and α-cells must devote most of their energy to hormone secretion and readily increase protein synthesis during stimulation. Following activation by high glucose, for example, β-cells augment the synthesis of (pro)insulin by more than tenfold, reaching ~50% of the total protein production[78]. This process places a burden on the ER, where (pro)insulin and (pro)glucagon synthesis and folding takes place, forcing islet endocrine cells to deploy adaptive mechanisms to match the functional capacity of the ER to the demand, a process known as the unfolded protein response (UPR)[79]. If the ER stress is severe and prolonged, and cannot be compensated for by the UPR, the cells will eventually undergo apoptosis, at least in part mediated by the pro-apoptotic transcription factor CHOP (also known as DDIT3)[20,79–81]. Importantly, markers of ER stress are present in β-cells in both T1D and T2D[20,82–85].

One adaptation driven by the UPR is an increase in ER volume density. In islets from patients with T2D, both α-cells and β-cells show an increase of more than threefold in ER volume density, indicating that these cell types are functionally overloaded and are triggering a compensatory UPR[86]. A similar observation was made in human islets exposed in vitro to palmitate, a saturated free fatty acid that contributes to metabolic stress in T2D: both α-cells and β-cells showed a more than threefold increase in ER volume density[86]. Curiously, however, in the case of islets from patients with T2D, or islets from normoglycaemic donors exposed to palmitate, an increase of more than fourfold in β-cell apoptosis occurred while α-cells remained fully viable[86]. In short, both β-cells and α-cells experience ER stress in diabetes, but only β-cells die. Previous findings, obtained in rat α-cells and β-cells, indicate that α-cells have higher expression of the chaperone BiP (which helps to fold or re-fold proteins in the ER) and of the antiapoptotic proteins BCL2L1 and BiP[86]. BCL2L1 is a key protein here, as knocking down its expression sensitizes rat α-cells to palmitate-induced apoptosis, akin to β-cells exposed to the same stressor[86]. Some of these findings have recently been confirmed by scRNA-seq in human islet cells exposed to IFNα: as discussed above, human α-like cells (differentiated from iPSC) have much higher expression of BiP and BCL2L1 than do β-like cells[33]. Increased BiP expression in α-cells has also been observed in another scRNA study of islet cells from adult normoglycaemic individuals and individuals with T1D[85]. On the other hand, human β-cells exposed to IFNα have higher expression of the ER stress-induced and pro-apoptotic transcription factor CHOP compared with human α-cells exposed to IFNα, which might contribute to the observed higher rate of cell death[33].

Collectively, these findings suggest that while both α-cells and β-cells must trigger the UPR to adapt to the challenging conditions present in both T1D and T2D, this event leads to β-cell but not α-cell death.

Immunogenicity of β-cells

Although still incomplete, the catalogue of self-antigens displayed by surface HLA class I molecules on β-cells and/or recognized as epitopes by CD8$^+$ T cells has been significantly expanded in recent years[3,87]. Several lines of evidence point to pre-proinsulin-derived peptides as central targets in the autoimmune pathogenesis. In non-obese diabetic (NOD) mice, the knocking out of *Ins1* (which in β-cells is more expressed than the second murine isoform *Ins2*) protects against diabetes[88]. Even a single point mutation in the key insulin B9-23 epitope completely protects NOD mice from development of diabetes[89]. This finding also suggests an initiating role for pre-proinsulin-reactive T cells, with subsequent spreading of the autoimmune response towards additional β-cell antigens[90]. In humans, the identification of the epitope targets of islet-infiltrating CD8$^+$ T cells in patients with T1D has highlighted that a large proportion of them also recognize pre-proinsulin-derived peptides[2], including an epitope derived from an insulin-defective ribosomal product following mis-initiated mRNA translation[91]. Moreover, hybrid insulin peptides are generated in secretory granules by the fusion of peptide stretches derived from insulin C peptide and other granule proteins such as chromogranin A, islet amyloid polypeptide, neuropeptide Y and the non-contiguous sequence of the insulin C peptide itself, and are recognized by CD4$^+$ T cells in both patients with T1D and NOD mice[92–95]. Insulin is thus also capable of generating so-called neoantigens, which are peptide sequences not templated in the genome and that can therefore be preferentially recognized as non-self and trigger autoimmunity.

Insulin as an autoantigen in T1D

The role of insulin as a central autoantigen of T1D is not unexpected when considering its high and constantly modulated rate of biosynthesis in β-cells, which favours errors in mRNA transcription and translation, thus generating unstable by-products readily degraded by the proteasome and potentially diverted towards the HLA class I presentation pathway[1]. The accumulation of proinsulin in secretory granules and its intermediate processing by proconvertases to generate mature insulin is another feature that may favour its antigenicity[1]. The same principle may apply to pre-proinsulin processing in the ER, which leads to the cleavage of its signal peptide by-product, which is a major source of autoimmune epitopes[96,97]. Moreover, these insulin by-products can be released by β-cells and taken up by antigen-presenting cells at distance. This antigen uptake and presentation might favour the initial priming of autoimmune T cells before their migration into the pancreas[98]. Such immune sensitization might, however, require additional inflammatory cues, and the physiological outcome of this constant antigenic release can instead be immune tolerance under steady-state conditions[99].

Other potential β-cell autoantigens

Other β-cell granule proteins besides insulin are increasingly being recognized as antigenic targets in T1D, and secretory granules are

known to be a rich source of target antigens in both humans and NOD mice[4]. These granule proteins include zinc transporter 8 (ZnT8)[100,101], islet antigen 2 (IA-2)[101,102], chromogranin A[3], islet-specific glucose-6-phosphatase catalytic subunit-related protein[6], and the more recently described secretogranin 5 (SCG5), urocortin 3 (UCN3) and proconvertase-2 (refs. 3,4). Several of these proteins, like proinsulin, undergo intermediate processing inside secretory granules, and their degradation products can also be released, and favour priming of distant cognate T cells[1]. The degree of evidence for their antigenic role is fairly solid, including the detection of autoantibodies against ZnT8 and IA-2 and diabetogenicity of cognate T cells for SCG5, UCN3 and proconvertase-2 (ref. 4). Of note, none of these proteins is selectively expressed by β-cells, and many are expressed at similar levels in α-cells[103]. This observation is open to several interpretations. First, insulin may be the key initiating antigen triggering the first autoimmune attack against β-cells, with subsequent spreading to other accessory antigen specificities that are not sufficient on their own to initiate autoimmunity against other endocrine cells. Second, generation of hybrid neoantigens (with several granule proteins involved together with insulin in these transpeptidation events) may be another feature favouring β-cell vulnerability. Whether neoantigens can also be generated by α-cells is currently unknown. Third, these antigens might simply provide the molecular targets of autoimmunity, which do not contribute to cell damage in the absence of an intrinsic vulnerability of other islet cells, as is probably the case for α-cells (see above).

Glucagon immunogenicity

The other question stemming from these observations is why glucagon, the signature protein of α-cells, does not provide a favourable autoimmune target as does insulin. Indeed, a systematic screening of islet-infiltrating $CD8^+$ T cells from patients with T1D for responses against pre-proglucagon (overlapping peptides spanning the whole protein sequence), elicited no responses[2]. Yet, similar to insulin, glucagon production accounts for most of the burden of protein synthesis in α-cells, with its synthesis and degradation rates constantly modulated according to metabolic demand. Like insulin, glucagon is first synthesized as a pre-prohormone, with a signal peptide cleaved and released in equimolar amounts in the ER, with the resulting proglucagon subsequently processed by proconvertases in secretory granules to yield the bioactive hormone(s)[104]. However, α-cell and β-cell secretory granules also have some structural differences, the most notable being the high Zn^{2+} content (in the millimolar range) of insulin granules[105]. Zn^{2+} modulates several features of innate and adaptive immunity[106,107] (for example, by inducing dendritic cell maturation[108] and T cell activation[109]). The release of Zn^{2+} by β-cells together with insulin might thus provide an adjuvant-like signal favouring immunogenic $CD4^+$ T helper 1 and $CD8^+$ cytotoxic responses[110,111]. Secretion of unprocessed proinsulin is also a well-established marker of β-cell stress[112–114] at different T1D stages, including the preclinical normoglycaemic phase[115,116]. The accumulation of proinsulin in secretory granules may be another feature favouring its immunogenicity. Whether proglucagon similarly behaves as a marker of α-cell stress is currently unknown.

Another key feature that may impede glucagon immunogenicity is that, although insulin expression is exquisitely β-cell-specific, glucagon-related peptides are also produced by other cells, notably enteroendocrine L cells. Here, the preferential processing of proglucagon by proconvertase-1 and proconvertase-3 (without the intervention of proconvertase-2, as in α-cells) yields the intestinal hormones glicentin and oxyntomodulin, which encompass the glucagon sequence. The local release of these hormones in a microenvironment that is inherently tolerogenic under physiological conditions[117–119] may drive an immune tolerance state towards glucagon-derived peptides. Moreover, the glucagon-like peptide 1 produced by L cells has been shown to attenuate T cell proliferation and favour maintenance of peripheral regulatory T cells[120]. Thus, glucagon tolerance might be hardwired in the immune system and difficult to overcome even in the immunogenic microenvironment of insulitis.

Conclusions

A number of key differences between α-cells and β-cells may contribute to their different fates in T1D (Fig. 3). In T1D, both α-cells and β-cells are affected and become dysfunctional but only β-cells die. Multiple layers of evidence suggest that α-cells and β-cells do not handle the initial inflammation and immune assault in the same fashion, with α-cells mounting a better response to viral infections, ER stress and deleterious immune mediators, even though both cell types are hormone-producing and have a similar embryonic origin. Furthermore, although β-cells are essential to life (neither humans nor animal models can survive without them), mice with 98% α-cell ablation retain near-normal glucose

Glossary

Antigens
Molecular structures (proteins, peptides, polysaccharides, lipids or nucleic acids) that can bind to an antigen receptor (for example, antibodies for B cells and T cell receptors for T cells) and trigger an immune response. Antigens can originate from within the body (self-antigens or autoantigens) or from the external environment (foreign antigens).

Candidate genes
Genes related to particular traits that either increase or decrease the risk of disease, either as a result of their protein product or their position on a chromosome.

Epitopes
The specific parts of the antigen (most commonly peptides) to which antigen receptors bind.

Gene set enrichment analysis
Computational method to determine whether an a priori defined set of genes shows statistically significant differences between two biological states (for example, phenotypes).

Immune tolerance
The state of unresponsiveness of the immune system to antigens that have the potential to induce an immune response. Immune tolerance to self-antigens is achieved through both central tolerance and peripheral tolerance mechanisms in the thymus and in the periphery, respectively.

Insulitis
Inflammation of the islets of Langerhans, characterized by infiltration of immune cells within and at the periphery of islets.

Leading edges
Subsets of genes in a gene set that contribute the most to the enrichment or depletion in a gene set enrichment analysis.

Neoantigens
Peptide sequences not templated in the genome that can be preferentially recognized as non-self and trigger autoimmunity. Neoantigens can be generated by mis-initiated mRNA transcription, alternative mRNA splicing and post-translational modifications (that is, the addition of chemical groups to amino acid residues or the fusion of non-contiguous fragments from the same protein (*cis*-splicing) or of two fragments from different proteins (*trans*-splicing, generating so-called hybrid peptides)).

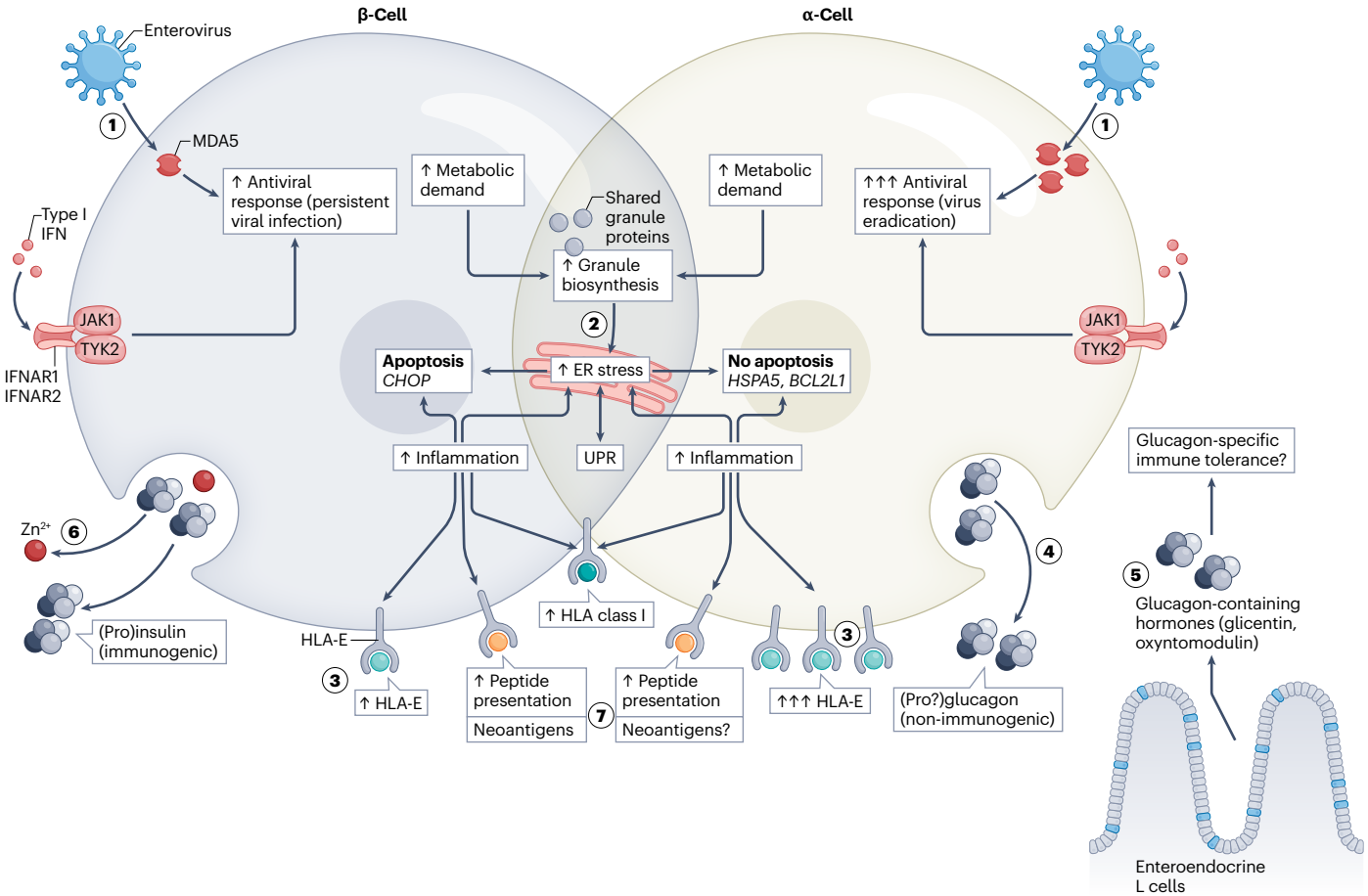

Fig. 3 | Points of increased resistance of α-cells to different stressors and to autoimmunity as compared to β-cells. During the course of type 1 diabetes, both α-cells and β-cells are affected and become dysfunctional, but only β-cells die. α-Cells and β-cells do not react to the initial inflammation and immune assault in the same way, with α-cells showing more efficient protective responses to the following stressors: viral infections (step 1) (for example, enteroviruses), with higher expression of viral sensors such as MDA5 and key transcription factors such as STAT1 (not shown) in α-cells; endoplasmic reticulum (ER) stress (step 2), exemplified by less expression of the pro-apoptotic gene *CHOP* and higher expression of the protective genes *HSPA5* (which encodes BiP) and *BCL2L1* (which encodes Bcl-XL) in α-cells; and deleterious immune mediators (step 3), exemplified by higher expression of the immunomodulatory molecule HLA-E mounted by α-cells. Furthermore, glucagon (step 4), the main hormone produced by α-cells, does not seem to be immunogenic, and this immune-tolerant state may be favoured by the production of glucagon-containing hormones by enteroendocrine L cells (step 5). Conversely, it is possible that the high amounts of Zn^{2+} secreted by β-cells together with insulin (step 6) may exert an adjuvant effect favouring insulin immunogenicity. Whether neoantigen generation and display on the surface HLA class I molecules by β-cells (step 7) also take place in α-cells is currently unknown. UPR, unfolded protein response.

homeostasis[121]. It is thus difficult to understand, from an evolutionary perspective, why β-cells are more fragile than α-cells.

A number of key questions remain to be answered. For example, what exactly human α-cells do better than β-cells to handle ER stress and inflammation, and can we find ways to boost β-cell resistance to stress by mobilizing comparable mechanisms? Another question is why are α-cells able to interrupt CVB infections while β-cells allow the viruses to replicate and lead to apoptosis, particularly considering the essential nature of β-cells for the maintenance of life? Also, what is the role for the differential expression of candidate genes for T1D in α-cells and β-cells, and can these genes contribute to their differential survival during T1D? And finally, can enteroendocrine L cells produce glucagon-related peptides that drive an immune tolerance state towards glucagon and α-cells?

Answers to these questions will require detailed bioinformatic analyses of diverse data sets obtained from scRNA-seq of human α-cells and β-cells, both obtained from individuals with T1D and from human islets or iPSC-derived islet-like cells exposed to pro-inflammatory cytokines or T cells. These analyses should be followed by detailed confirmation in isolated human α-cells and β-cells, both unmanipulated and after inhibition or overexpression of the key genes identified in α-cells and β-cells, followed by exposure of these cells to pro-inflammatory cytokines, viral infections or T cells.

Data availability

The data used to create Fig. 2 are available in ref. 33.

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Author contributions

All authors researched data for the article, contributed substantially to discussion of the content, wrote sections of the article and reviewed and/or edited the manuscript before submission.

Competing interests

D.L.E. received grant support from Eli Lilly, Indianapolis, IN, for research on new approaches to protect pancreatic β-cells in T1D (not directly related to the present study). The other authors declare no competing interests.

Additional information

Peer review information *Nature Reviews Endocrinology* thanks Maureen Gannon, Jason Collier and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

Review criteria

Relevant publications were identified by searching the PubMed database (1 January 2005 to September 2022) using combinations of the following terms: 'pancreatic beta cells', 'pancreatic alpha cells', 'pancreatic β-cells', 'pancreatic α-cells', 'pancreatic islets', 'insulin release', 'insulin secretion', 'glucagon release', 'glucagon secretion', 'diabetes', 'type 1 diabetes', 'type 2 diabetes', 'pathogenesis', 'histology', 'transcriptome', 'genetics', 'candidate genes', 'islet gene regulation', 'islet epigenomics', 'viral infection', 'endoplasmic reticulum stress' and 'apoptosis'. We preferentially selected publications from the past 5 years, plus earlier key publications for citation (of note, the literature on α-cells in T1D is rather limited). Some references cited in these papers or in relevant articles related to the fate of pancreatic β-cells and α-cells in diabetes were also searched manually. All selected papers were full-text articles in English. Review articles are often cited to provide the readers with additional references.