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REVIEW

Presumption of innocence for beta cells: why are they vulnerable autoimmune targets in type 1 diabetes?

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Abstract

It is increasingly appreciated that the pathogenic mechanisms of type 1 diabetes involve both the autoimmune aggressors and their beta cell targets, which engage in a conflicting dialogue within and possibly outside the pancreas. Indeed, autoimmune $CD8^+$ T cells, which are the final mediators of beta cell destruction, circulate at similar frequencies in type 1 diabetic and healthy individuals. Hence a universal state of 'benign' islet autoimmunity exists, and we hypothesise that its progression to type 1 diabetes may at least partially rely on a higher vulnerability of beta cells, which play a key, active role in disease development and/or amplification. We posit that this autoimmune vulnerability is rooted in some features of beta cell biology: the stress imposed by the high rate of production of insulin and other granule proteins, their dense vascularisation and the secretion of their products directly into the bloodstream. Gene variants that may predispose individuals to this vulnerability have been identified, e.g. *MDA5*, *TYK2*, *PTPN2*. They interact with environmental cues, such as viral infections, that may drive this genetic potential towards exacerbated local inflammation and progressive beta cell loss. On top of this, beta cells set up compensatory responses, such as the unfolded protein response, that become deleterious in the long term. The relative contribution of immune and beta cell drivers may vary and phenotypic subtypes (endotypes) are likely to exist. This dual view argues for the use of circulating biomarkers of both autoimmunity and beta cell stress for disease staging, and for the implementation of both immunomodulatory and beta cell-protective therapeutic strategies.

Keywords Antigen · Autoimmunity · Benign · Coxsackievirus · Endotype · Epitope · Islet · Proinsulin · Review · T cell

Abbreviations

APC	Antigen-presenting cell
CVB	Coxsackievirus B
DRiP	Defective ribosomal products
ER	Endoplasmic reticulum
HLA-I	HLA Class I
PD-1	Programmed cell death protein 1
PD-L1	Programmed cell death ligand 1
pLN	Pancreatic lymph node
UPR	Unfolded protein response

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Introduction

There is mounting evidence that type 1 diabetes is a disease not only of autoimmunity, but also of the target beta cell itself. The active pathogenic role of the latter is suggested by several observations: (1) the heterogeneity of immune infiltrates across islets within a given pancreas [1]; (2) the expression by beta cells of several type 1 diabetes-susceptible gene variants [2, 3], which modulate islet inflammation [4, 5]; (3) the increased endoplasmic reticulum (ER) stress [6] and metabolic biomarkers that precede the first signs of autoimmunity [7]; and (4) the impaired proinsulin processing that affects stressed

beta cells [8, 9], which may promote their immunogenicity by further favouring ER stress and HLA Class I (HLA-I) presentation of proinsulin peptides. Notwithstanding possible differences in $CD8^+$ T cell phenotype between type 1 diabetic and healthy individuals [10, 11], our group [12, 13] and others [10, 11] have reported that islet-reactive $CD8^+$ T cells circulate at similar frequencies in type 1 diabetic and healthy individuals. This may suggest that the progression from a physiological state of 'benign' islet autoimmunity to type 1 diabetes may be favoured by increased beta cell vulnerability to this universal autoimmune T cell repertoire. Indeed, at variance with those in the blood, islet-reactive $CD8^+$ T cells are enriched in type 1 diabetic pancreases [12, 13], pointing to local factors that promote their homing to the target organ.

A conflicting dialogue between beta cells and the immune system is increasingly recognised as a key player in type 1 diabetes pathogenesis. Although this dialogue is bidirectional, we will focus on the effects that the beta cell itself may have on nurturing islet inflammation and autoimmune activation, setting up compensatory mechanisms that become deleterious in the long term. To do so, we suggest that we start with this question: why are beta cells such privileged targets of autoimmunity? Endocrine cells are defined as hormone-secreting cells organised in glands devoid of a ductal system that secrete their products directly into the bloodstream through their rich vascularisation. Several features of this definition may explain beta cell vulnerability–a paradigm that may also apply to other endocrine cells.

Weakness 1: making insulin and other granule proteins is a stressful job

Following nutrient stimulation, (pro)insulin synthesis by beta cells is increased by more than ten-fold, representing nearly 50% of total beta cell protein production. This functional adaptation imposes a constant burden on beta cells, particularly considering that they are long-lived cells with limited replicative capacity. Beta cells fulfil this task by adapting ER function to these changing demands by triggering the unfolded protein response (UPR) [14]. The UPR decreases the ingress of newly synthesised proteins into the ER and increases the extrusion of misfolded proteins and the synthesis of ER chaperones, thus upgrading the folding capacity of the organelle. If this fails to restore ER homeostasis, beta cells eventually activate the apoptotic pathway [14]. This dysregulated ER stress also augments local inflammation via activation of the NF-κB pathway, increasing the local release of chemokines that attract immune cells [14] and the generation of neo-antigens [15].

It remains to be determined what triggers the transition from 'physiological' to 'pathological' UPR, and why this occurs in relatively few individuals [14]. For example, morbidly obese individuals with severe insulin resistance and consequent beta cell functional overload do not usually develop islet autoimmunity. Both genetic and environmental factors are probably at play. Predisposing HLA Class II alleles account for most of the genetic risk, which points to the role of antigen-presenting cells (APCs) in driving disease progression via the uptake of beta cell material and the priming of $CD4^+$ T cells. Other type 1 diabetes-associated gene variants can synergise with ER stress to potentiate local inflammation [16]. These include *MDA5* (also known as *IFIH1*), which encodes a cytoplasmic receptor for viral double-stranded RNA and is upregulated in human islets upon Coxsackievirus B (CVB) infection and potentiates chemokine release, and *TYK2* and *PTPN2*, which encode key interferon signalling transducers in beta cells. Similarly, environmental cues, such as islet-tropic CVB infections, which are candidate environmental triggers for type 1 diabetes, may synergise with ER stress when stochastically coming together in time and space. Of note, recent findings suggest that CVBs use the UPR pathway IRE1α/XBP1s/JNK1 to foster their own replication in beta cells [17]. Further indirect support for the role of ER stress and environmental factors such as CVBs come from the observation that neighbouring alpha cells are spared by islet autoimmunity. This may reflect their higher resistance to ER stress-induced apoptosis, partly as a result of their higher expression of the anti-apoptotic protein Bcl2l1/Bcl-xL [18]. Moreover, alpha cells mount more efficient anti-CVB responses, resulting in faster viral clearance that may limit cell death and antigen release [16].

As described in other cell types [19], the increased rate of (pro)insulin translation upon increased metabolic demands and the resulting ER stress may also lead to an increased frequency of misfolding events and accumulation of defective ribosomal products (DRiPs), which are subsequently degraded by the proteasome. DRiPs constitute a major source of peptides for the HLA-I processing and presentation pathway [19]. Indeed, T cell epitopes derived from an insulin DRiP have been described [20]. Several other epitopes originate from the pre-proinsulin signal sequence [21], which becomes an abundant by-product of proinsulin biosynthesis upon its translocation into the ER. Moreover, ER stress upregulates the ER aminopeptidase 1 that trims the pre-proinsulin signal sequence [22]. The burden imposed on the ER by the secretory activity involves the biosynthesis not only of insulin, but also of other granule proteins, e.g. chromogranin-A, secretogranin-5, urocortin-3 and proconvertases. These proteins are major contributors to the HLA-I peptidome presented by beta cells and recognised by T cells [13] and share several features with insulin: they are soluble proteins translated as precursors, whose maturation into their bioactive products requires cleavage of their signal sequence (with several T cell epitopes mapping to this region), followed by further cleavage. It is thus possible that the impaired proinsulin processing described in beta cells from type 1 diabetic patients [8, 9] may also apply to these proteins and divert them

towards the HLA-I presentation pathway, thus increasing the immunogenicity of beta cells.

A similar shunting towards the HLA-I pathway may take place under inflammatory conditions, in which glucose-stimulated insulin secretion is downregulated and unused granules are degraded through crinophagy. Islet inflammation may also lead to the increased generation of neoepitopes that are not encoded in the genome. They are therefore regarded as 'non-self', possibly escaping thymic deletion, although such deletion may be marginal overall [12, 23]. Neo-epitopes can be generated by modulation of mRNA splicing [13] and by post-translational modifications [15] via the upregulation of modifying enzymes, either expressed by beta cells or released by immune cells (e.g. neutrophils). 'Hybrid' peptides generated by the fusion of non-contiguous sequences from the same proteins or from distinct partners have also been described [24]. This so-called transpeptidation process was originally reported to generate $CD8^+$ T cell epitopes through the proteasome pathway [25], but in beta cells it may also generate $CD4^+$ T cell epitopes and take place in the insulin granules and, more likely, in the crinosomes (formed from the fusion of granules and lysosomes). The peculiar biology of granule degradation through crinosomes, which contain high amounts of proteins constantly catabolised within a restrained space, may indeed favour transpeptidation events and beta cell immunogenicity [26]. It is currently uncertain whether this is a physiological process upregulated by inflammation and whether neo-epitope generation may play an initiating or amplifying role in type 1 diabetes pathogenesis.

Weakness 2: islets are highly vascularised, which favours face-to-face encounters between immune cells and beta cells

At the local level, the encounter between immune cells and beta cells is favoured by the rich vascularisation of islets, which provides easy access for immune cells from distant sites. While only activated immune cells, likely primed in pancreatic lymph nodes (pLNs), can readily cross the vascular endothelium, the ensuing inflammatory microenvironment increases vascular permeability and further facilitates access, even for naive and non-islet-reactive T cells [27, 28].

This access is also facilitated by pLNs, which drain the contents not only of the pancreas itself, but also of adjacent regions of the gut, thus providing an ideal crossroad for immune cells transiting between these compartments. This is relevant in light of the importance of the immune environment of the gut in shaping peripheral tolerance, and its modulation by nutrients, the endogenous microbiome [29, 30] and the intercurrent infections occurring through the gastrointestinal tract, e.g. by CVBs [31].

The 'words' of the resulting local dialogue between beta cells and immune cells are (1) cytokines (e.g. type I IFNs, IL-1β and TNF-α) released by immune cells; (2) chemokines (e.g. CCL2 and CXCL10) released by beta cells, which further attract and activate immune cells; and (3) putative 'danger' signals (e.g. nucleic acid fragments and modified proteins/peptides released by damaged or dying beta cells) [32]. Indeed, observations in the NOD mouse suggest that beta cell death releases self-DNA and recruits neutrophils to the pancreas [33]. The concerted action of self-DNA, anti-DNA antibodies and DNA-binding anti-microbial peptides secreted by neutrophils activates plasmacytoid dendritic cells, leading to IFN-α production and amplification of the autoimmune response through T cell recruitment. In this scenario, IFN-α plays a key role in early insulitis by linking the activation of innate and adaptive immunity, as documented in other autoimmune diseases such as lupus. This process may be further ramped up by the genetic background and by putative environmental cues that induce beta cell death, such as CVB infections [16, 32].

The other key amplifying role of inflammatory cytokines is to make beta cells more 'visible', and hence vulnerable, to T cells. Indeed, inflammatory cytokines upregulate HLA-I expression on beta cells and increase the number of peptides presented for scanning and recognition by $CD8^+$ T cells [13]. IFN-α also displays some peculiar features in this scenario. First, an IFN-α signature precedes the appearance of autoantibodies in the peripheral blood [34, 35] and may be associated with neutrophils [36]. This is likely to reflect an induction phase of islet autoimmunity, orchestrated by innate immune cells and followed by an amplification stage triggered by other cytokines, e.g. IFN-γ, TNF-α and IL-1β. Second, IFN-α induces greater ER stress in beta cells compared with IFN-γ, which translates into apoptosis only upon concomitant exposure to IL-1β [37]. Third, IFN-α induces long-lasting HLA-I upregulation [38], which likely translates into sustained antigen presentation.

New findings suggest that the components of this dialogue are not always deleterious to the beta cells. Indeed, IFN-α and IFN-γ upregulate beta cell expression not only of chemokines and HLA-I [37], but also of programmed cell death ligand 1 (PD-L1), which is hyper-expressed in the beta cells of type 1 diabetic patients [39, 40], possibly reflecting an active yet insufficient peripheral tolerance mechanism through programmed cell death protein 1 (PD-1) binding and T cell inhibition. Therapeutic anti-PD-1/PD-L1 antibodies are increasingly used for treating cancers that evade immune surveillance by overexpressing PD-L1. While highly effective at inducing tumour remission, 8–10% of treated patients develop endocrine autoimmune disorders, e.g. hypophysitis, thyroiditis, adrenal disease and, in 0.4–0.9% of patients, a

fulminant form of diabetes [41]. The mechanisms underlying this form of diabetes remain unclear, but they may involve the neutralisation of the key PD-1 'brake' in the autoimmune T cells that we all harbour [42]. In line with this possibility, PD-1/PD-L1 inhibition accelerates diabetes, while PD-L1 overexpression in beta cells prevents or reverts diabetes in NOD mice [42].

Relevant to this theme of immune defence mechanisms in human islets is the paucity of information on tissue-resident T cells, which are non-recirculating memory T cells that localise stably in the organs, expand upon local inflammation and may provide a first response against infection re-encounters [43]. In islets from non-diabetic donors, the group of Cilio found that most (80%) immune cells are $CD3^+$ T cells, 80% of which had a central/effector memory phenotype expressing the CD69 and CD103 markers of tissue residency [44]. One feature that contrasts with the high vascular accessibility of the islets is that, in non-diabetic donors, relatively few immune ($CD45^+$) cells are found here (~1 per islet equivalent) [45], while a larger proportion can be found scattered in the exocrine and peri-islet tissue [44]. Another recurrently reported striking observation is the near absence of regulatory T cells [44] compared with other organs, irrespective of disease status. This may represent another feature making islets more vulnerable to autoimmunity.

Weakness 3: beta cells secrete their products into the bloodstream—The 'Wi-fi' dialogue with immune cells?

The other key feature that may render beta cells susceptible autoimmune targets is their ability to communicate at distance. This is an integral part of their physiology, as insulin and other granule contents are released directly into the bloodstream to exert their metabolic effects at a distance. Since insulin and other granule proteins [13] are also target antigens of islet autoimmunity, not all APCs may need to reach the pancreas for antigen uptake, as such antigens may also become available at distant sites. Furthermore, stressed beta cells also release high amounts of proinsulin, which has a longer half-life than insulin and binds poorly to the insulin receptor. It can thus remain systemically available for a longer time, favouring uptake by APCs outside the pancreas. This 'Wi-Fi' system of extra-pancreatic antigen delivery may also involve other mechanisms. For example, secretory granules also contain antigenic insulin peptides, which, together with intact granule proteins, may provide another source of T cell sensitisation at a distance [46]. According to the model proposed by Unanue, this represents a physiological process. Indeed, such a release is found in both pLNs and non-draining LNs in the NOD mouse and other murine strains and is triggered by glucose challenge [46]. Interestingly, the most immunogenic

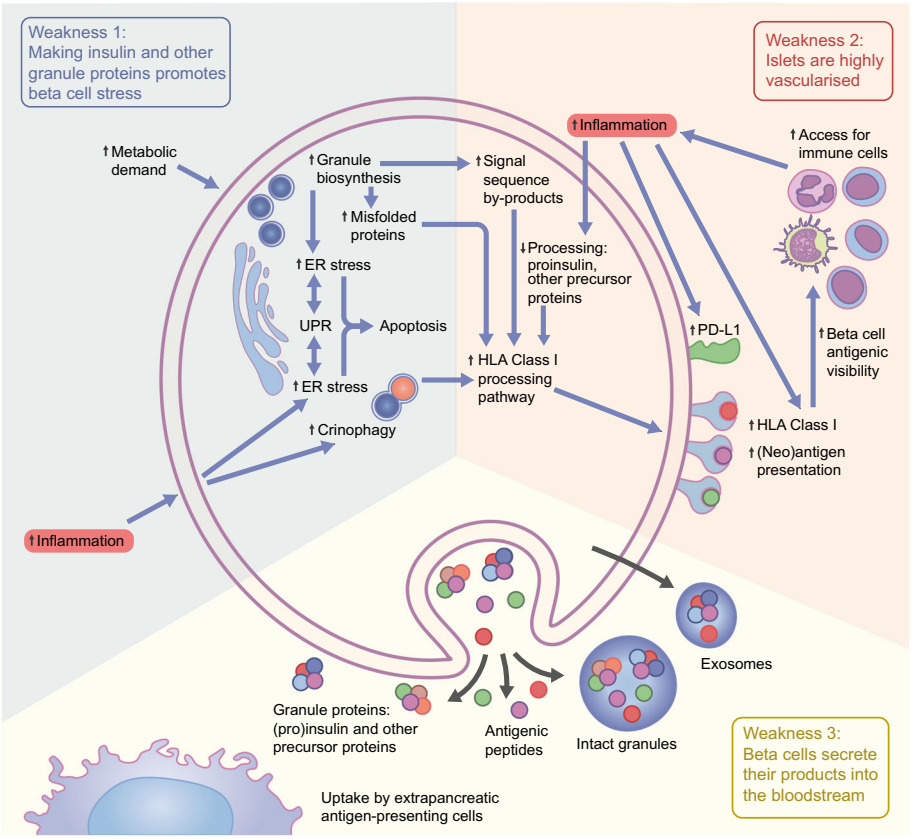

Fig. 1 Weaknesses in the biology of beta cells that may promote their autoimmune vulnerability. This figure is available as part of a downloadable slideset

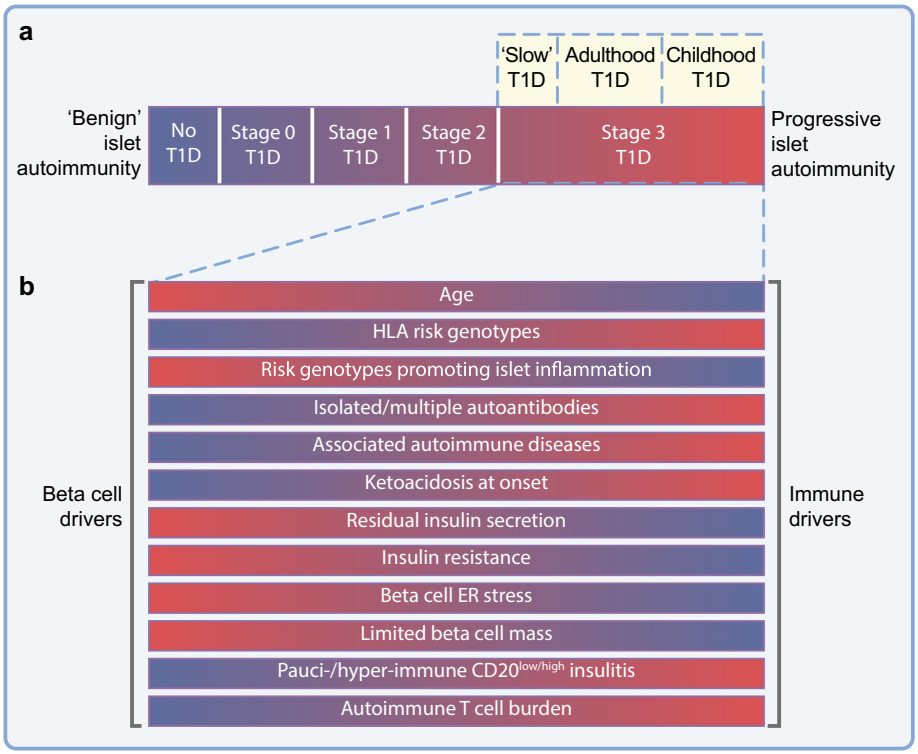

Fig. 2 Autoimmune aggressiveness in health and type 1 diabetes and disease endotypes according to beta cell and immune drivers. (**a**) The continuum between health and the sequential stages of type 1 diabetes (T1D), which could reflect the transition between a 'benign' state and an increasingly aggressive state of islet autoimmunity. At stage 3 of type 1 diabetes, progressive autoimmunity may also be graded according to clinical phenotype ('slow' vs classical type 1 diabetes) and age of onset (adulthood vs childhood). (**b**) Illustration of how the relative weight of beta cell vs immune drivers may differ according to type 1 diabetes endotypes. These drivers are however dynamic and interconnected. For example, islet autoimmunity induces ER stress in beta cells, which may enhance inflammation and antigen presentation, thus aggravating autoimmunity and creating a vicious cycle. This figure is available as part of a downloadable slideset

$CD4^+$ T cell epitopes in the NOD mouse are derived from insulin B-chain peptides that are found proximally in beta cell crinosome and secretome fractions, and distally bound to MHC Class II molecules in islets, pLNs and spleen [26], lending strong support to this model of systemic antigen seeding. In most cases, the outcome of this process may be systemic tolerance. The switch to autoimmunity may depend on the prior history of T cells and on the presence of danger signals, which may come from stressed beta cells themselves or from exogenous inflammatory triggers (e.g. viral infections) and microbiome composition [47]. Beta cells also release intact granules [48] and exosomes carrying antigens such as proinsulin, GAD65 and islet antigen 2 (IA-2) [49], which are endocytosed more efficiently than soluble proteins/peptides and may further feed distant APCs. Beta cell-derived microRNAs released into the bloodstream, either in soluble form or associated with exosomes [50], may exert additional immunomodulatory effects.

Translational relevance

Figure 1 summarises the pathways that may make beta cells active players in their own destruction and favour their vulnerability. These emerging concepts are of major importance not only for our understanding of type 1 diabetes pathophysiology, but also for improving disease management.

The first implication is that circulating biomarkers of beta cell stress may be complementary to immune biomarkers such as autoantibodies [51]. Besides the established value of following residual insulin secretion by measuring C-peptide release after a glucose challenge, circulating proinsulin is now widely accepted as one such biomarker. Other products released by beta cells (e.g. GAD65, IAPP, microRNAs) are under scrutiny, while the detection of DNA species carrying beta cell-specific methylation marks and released upon cell death may not be suitable outside the 'acute' islet transplantation setting.

The second implication is that agents aimed at limiting the autoimmune vulnerability of beta cells should find their place in the search for disease-modifying treatments, either alone or in combination with immunotherapies. Indeed, a recurrent observation from the immunotherapies that achieved some impact on the decline of insulin secretion after the clinical onset of type 1 diabetes is that their efficacy is typically confined to the first months of treatment [52]. The beta cell insulin secretion subsequently resumes its usual slope of

decline, possibly suggesting residual pathogenic mechanisms that remain untargeted. Such mechanisms may well be intrinsic to beta cells and their inappropriate, self-amplifying response to the immune assault, which is reminiscent of the beta cell 'suicide' concept coined by G. F. Bottazzo, who noted 'The aim is noble but the act smacks of danger!' [53].

This wish to integrate beta cell-protective agents into the therapeutic arsenal of type 1 diabetes remains, however, unfulfilled to date. Which pathways should be targeted? Reducing ER stress and oxidative damage are obvious candidates, and some agents already licensed for other indications have been proposed. These include ursodeoxycholic acid, a bile acid used to reduce cholestasis which can also function as a chaperone to quell ER stress [6]; and verapamil, an antihypertensive, anti-arrhythmic drug that inhibits thioredoxin-interacting protein (TXNIP), a pro-oxidative factor that activates the inflammasome [54]. Glucagon-like peptide 1 (GLP-1) agonists have also been shown to have beneficial effects on beta cells in rodent models, but whether the benefits observed in some trials on type 1 diabetic patients reflect an improvement in residual insulin secretion is currently debated [55]. Other agents under study target IFN-α signalling pathways in beta cells, e.g. JAK/TYK2/STAT inhibitors [38, 40, 56].

Autoimmune vs beta cell disease: chicken or egg?

Is the initiation of islet autoimmunity triggered by immune cells or by the beta cells themselves? In support of the latter possibility, altering the identity of beta cells by boosting their proliferation protects NOD mice from autoimmune diabetes [57]. However, it is noteworthy that islets from these protected mice are less vulnerable but not resistant to autoimmunity when transferred into unprotected recipients and that, conversely, bone marrow transfer from unprotected mice into protected recipients triggers diabetes. Indeed, proliferating beta cells exerted an indirect protective effect by modulating T cell self-reactivity and boosting regulatory T cells [57], an observation that underlines the far-reaching effects of this immune–beta cell crosstalk. Mirroring these mouse studies, two human case reports lend strong arguments for an initiating role of immune cells: (1) twin-to-twin transplantation of a non-diabetic pancreas into a type 1 diabetic recipient led to rapid autoimmune relapse [58]; and (2) conversely, bone marrow transplantation from a HLA-identical type 1 diabetic sibling into a non-diabetic recipient led to the development of type 1 diabetes [59]. Thus, the role of the beta cell may be to amplify rather than to initiate the pathogenic process of type 1 diabetes.

Conclusions and perspectives

A unifying picture is emerging that prompts us to consider type 1 diabetes as a disease of both the immune system and beta cells, resulting from a conflicting dialogue between these two players. Given the phenotypic heterogeneity of type 1 diabetes, different phenotypic subtypes (endotypes) of islet autoimmunity are increasingly recognised based on putative pathogenic mechanisms and, likely, different responsiveness to immunotherapy ('theratypes') [60, 61]. It is conceivable that distinct endotypes may also exist according to the aggressiveness of islet autoimmunity (benign vs progressive) and to the relative contribution of immune and beta cell drivers to such aggressiveness (Fig. 2). The recent description of an overlap between the hyper-immune ($CD20^{high}$) immunohistological endotype of insulitis and altered proinsulin processing in beta cells exemplifies the intertwining of the two participants in the dialogue [62].

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