Trends box

In T1D, β cells are highly sensitive to selective damage and recruit immune cells by chemokine production. These immune cells directly damage β cells as well as induce enzymes and cytokines that cause free radical and cytokine-induced apoptosis.

Damaged islets express innate immune receptors, engagement of which may amplify β cell destruction, contributing to their own destruction. Interestingly, damaged and functional islets co-exist. Immune regulatory cells and regulatory mechanisms induced by islet cells counterbalance inflammation. Communication between immune cells and resident islet β cells during inflammation is dependent on the pancreatic microenvironment.

Therapeutically targeting the direct and indirect mediators of islet β cell damage to prevent further destruction, combined with boosting islet β cell number and function are important joint targets in developing therapy in T1D.

Immune and pancreatic β cell interactions in type 1 diabetes

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Abstract

The autoimmune destruction of the pancreatic islet β-cells is due to a targeted lymphocyte attack. Different T-cellular subsets communicate with each other and with the insulin-producing β-cells in this process, with evidence not only of damage to the tissue cells but also lymphocyte regulation. Here we explore the various components of the immune response as well as the cellular interactions that are involved in causing or reducing immune damage to the β-cells. We consider these in the light of the possibility that understanding may help to identify therapeutic targets to reduce the damage and destruction leading to type 1 diabetes.

Type 1 diabetes and Islet Inflammation

Type 1 diabetes (T1D) is an organ-specific autoimmune disease that results in a loss of insulin-producing **β-cells** (see glossary) in the pancreatic **islets of Langerhans**, leading to an inability to maintain glucose regulation (1). Insulin administration is essential in patients with type 1 diabetes. However, maintaining optimal blood glucose is difficult, and many patients present with complications including blindness, kidney failure and vascular complications (1). Although T1D is T-cell-mediated, other immune cells are crucial for this multifunctional process with a dynamic progression of inflammation. **Antigen presenting cells** (APCs) are critical for antigen presentation of peptides to **T-cells** (both CD4 and CD8 T-cells), resulting in immunological and molecular events leading to apoptosis of β-cells (2). Understanding these cellular interactions and subsequent events will provide important information to enable us to identify new targets and deliver potential therapies.

The autoimmune response in human T1D progresses slowly, and intact islets can coexist with islets undergoing immune infiltration and β-cell destruction. Historically, it was thought only when 70-90% of β-cells are destroyed that clinical symptoms in patients appear, although recent reports suggest this is overestimated (3, 4). Furthermore, experimental mouse models have demonstrated that leukocyte infiltration into islets does not always lead to disease (5). We suggest that different islets, within the same tissue, may have a different cellular profile with heterogeneous cell types, which may evolve depending on the microenvironment they encounter. It is essential to understand why some insulin-producing β-cells are destroyed and some escape destruction. Emerging literature on the dynamics of the pancreatic infiltrate in the non-obese diabetic (NOD) mouse (Box 1) during the course of diabetes has shown how diverse and constant cellular flux can be, although not an 'open house' to all cell types (6). Furthermore, work on the antigen-specific T-cell:APC interactions in the islets throughout disease progression has demonstrated that the tissue microenvironment can control T-cell functions and motility (7, 8). During the early stages of inflammation in the NOD mouse, development of T1D is dependent on crosstalk in the pancreas between islet β-

cells and **B-1a cells**, neutrophils and interferon alpha (IFNα) secreting **plasmacytoid dendritic cells** (pDCs) (9). With these studies in mind, we aim to discuss cellular interactions in the islets, focusing on islet-resident cells and the interplay with infiltrating immune cells (Figure 1, Key Figure). This will take into account different heterogeneous populations and what will be required to improve our understanding of these complex interactions. We will discuss the autoimmune processes in the pancreas of mice developing **insulitis**, but cross-reference to human pancreas, fully acknowledging that processes may not be identical.

Islet Organization and Immune Control

The pancreas consists of exocrine and endocrine tissues that are functionally distinct. The exocrine cells produce digestive enzymes and will not be discussed here. The endocrine cells maintain glucose homeostasis. Pancreatic islets comprise multiple hormone-secreting cells that are highly organized; in rodents, insulin-producing β-cells are at the core of the islet. Other endocrine cells include the glucagon-secreting α□cells, somatostatin-secreting δ cells, pancreatic polypeptide (PP)-secreting cells, and ghrelin-producing ε cells, and reside in the islet periphery. In human islets, the organization of these endocrine cells is more intertwined and compact (10). They are highly vascularized to enable the efficient secretion of insulin into the bloodstream. For a full review on in-depth control of insulin release, see (11).

Immunofluorescence studies demonstrate that pancreatic islets are surrounded by a peri-islet basement membrane (BM) and an interstitial matrix, that together create a capsule. The BM acts as a barrier for the insulin-secreting β-cells, and leukocytes must pass through this membrane during islet infiltration. A loss of this capsule, during the progression of diabetes in the NOD mouse model and human T1D, specifically correlates with leukocyte infiltration (12). This suggests that the peri-islet BM is a key regulator of islet infiltration and progression of T1D. Here, it should be noted that mouse and human BM differs in organization, with the human BM having a second BM layer (13).

The islets of the pancreas are highly vascularized, and communication between β-cells and surrounding cell types is particularly important. β-cells are in close contact and communicate with intra-islet endothelial cells, and normally this communication maintains homeostasis (14, 15); however this is also important in the context of autoimmune disease (Figure 1A). It is well documented, in mice and humans, that cell adhesion molecules on the endothelium in the pancreas can enable T-cell binding and interactions (16) resulting in T-cell activation and movement into the islets (17). Additionally, patients with T1D have increased expression of Major Histocompatibility Complex (MHC) I and induced expression of MHC II on islet endothelial cells (18, 19), allowing endothelial cells to present autoantigens to T-cells (18). This is a potential mechanism in humans and mice by which T-cells may enter the islets. Finally, isolated human endothelial cells can express the co-stimulatory molecule CD86 that is involved in CD4 T-cell adhesion through interaction with cytotoxic T lymphocyte associated protein 4 (CTLA4), a key negative regulator on T-cells (20). Thus, the highly specialized islet structure and microvascular cells of the pancreas can facilitate T-cell infiltration and contribute to the progression of disease.

Influence of β-cells in the islet microenvironment

The β-cell is highly specialized for insulin release but also intra-islet communication and immune regulation of the islet (Figure 1). β-cells are targeted by diabetogenic T-cells, and upon lysis, β-cells release islet-specific antigens that trigger or perpetuate inflammation. MHC I expression is upregulated on both mouse and human β-cells during inflammation (19, 21). Furthermore, this MHC I expression can influence β-cell destruction by CD8 cytotoxic T lymphocytes (CTL); mice lacking MHC I expression on β-cells have reduced diabetes incidence (17, 22). Historically, presentation of murine islet-specific antigens to CD4 T-cells by β-cells requires APC help (23, 24). However, more recent evidence suggests that murine islet β-cells can upregulate MHC II during inflammation and present antigen to diabetogenic CD4 T-cells *in vitro* (25). Interestingly, co-stimulatory molecule expression has not been found on β-cells (25), and therefore it is possible that β-cells present antigen *in vivo* to

induce anergic CD4 T-cells to control inflammation. However, the function of CD4 T-cells stimulated by β-cells has yet to be established. MHC II expression (HLA) can be found on human β-cells in insulin-containing islets, but appears to be limited to a small number of cells (26, 27). This expression is observed without insulitis present, suggesting upregulation just prior to infiltration (27).

β-cells are also a source of chemokine production, which may have inflammatory or protective roles in both the NOD mouse and human islets (28, 29). Multiple chemokines including C-X-C motif chemokine 10 (CXCL10) are up-regulated on β-cells in islets undergoing infiltration (28, 29), which drives immune cell recruitment to the pancreas via the chemokine receptor CXCR3 (28). Human islet β-cells express the Interleukin-22 receptor (IL-22R) (30), which can be engaged by IL-22 produced from T-cells, in patients with T1D (31). IL-22 up-regulates genes that protect β-cells from apoptosis and can enhance β-cell regeneration (32). During inflammation, cytokines such as IFNα, in the presence of IL-22, can induce nitric oxide synthase (iNOS) and resulting oxygen free radicals contribute to tissue destruction (33). This is pertinent in T1D, as IFNα production in the pancreatic islets is essential for disease initiation (9). Conversely, however, in NOD mice deficient in IFNα receptor 1 (IFNAR1) diabetes is not delayed or prevented (34). Synergistic islet interactions, under pro-inflammatory conditions, have also been demonstrated in both rat and human islets (35, 36). In the presence of interleukin 1 (IL-1) β-cells undergo destruction in an iNOS dependent manner (36). Production of IL-1 can be induced in islet macrophages by tumor necrosis factor alpha (TNFα) and lipopolysaccharide (LPS) stimulation, and depletion of islet macrophages inhibits expression of IL-1 (36).

In common with other tissue cells, both endocrine as well as infiltrating immune cells may express innate immune receptors (reviewed in (37)). Whilst these receptors are normally involved with the detection of pathogen-induced molecular patterns (PAMPs), they may also sense damage-induced molecular

patterns (DAMPs) (37). Thus, inflammation within islets may be amplified by recognition of molecules released by islet damage (37).

Regulatory costimulatory molecules may also be important. β-cells express **programmed death-ligand 1** (PD-L1) during insulitis and may play a key role in regulating inflammation, demonstrated by the NOD-*Pdcd1-/-* mice that have PD-L1 deficiency and develop accelerated diabetes (38, 39). Patients receiving anti-**programmed death 1** (**PD-1)** treatment for cancer can present with autoimmune T1D (40). It is clear that although β-cells are key in tissue regulation and homeostasis, they can self-destruct when exposed to certain environmental cues. However, we have yet to gain full understanding of β-cell communication with immune cells through this pathway, during different stages of inflammation. Critical interactions in the evolving microenvironment, involving both soluble factors as well as cell-to-cell contact need to be uncovered. It is also interesting to speculate that E-cadherin, a key protein involved in adhesion on epithelial tissues, in the pancreatic islets may interact with its ligand CD103, expressed on effector memory CD8 T-cells, which can reside in non lymphoid tissues (41). In a model of pancreatic islet transplant rejection, $CD8^+CD103^+$ T-cells are found at the graft site and are essential during the immune response (42). Together, islet β-cells are integral in regulating the local environment, but during inflammation, multiple mechanisms can contribute to their own cell death.

Islet Antigen Presenting Cells (APCs)

Myeloid tissue resident cells in the pancreas play a key role in tissue homeostasis as well as in inflammatory conditions. Islet APCs are needed for normal islet function (43). In the murine islet, two main APC subtypes have been identified. The islet mostly contains APCs that are $CD11c^+CD11b^+$ $F4/80^+$ $CD80^+$ and express MHC II (44), consistent with a macrophage phenotype (45). A smaller population lacking CD11b but expressing CD103 ($CD11c^+$ MHC II^+) (46), consistent with a dendritic cell (DC) population has been identified (45, 47). These APC subsets are in close contact with islet blood vessels, as shown by two-photon microscopy (46, 48), suggesting these APCs are in-

volved in surveillance of surrounding areas. Further to this role, islet APCs are essential for islet antigen presentation, and express MHC-peptide complexes derived from β-cell proteins (44). They are efficient at presenting these peptide complexes to CD4 T-cells. β-cells transfer secretory granules to resident islet APCs and it is this interaction that allows the recognition of β-cell antigens by CD4 autoreactive T-cells (49).

Tissue resident $CD103^+$ DCs, which are of the same lineage as CD8α DCs found in lymphoid tissues (50), are under the control of the ***Batf3*** transcription factor (51) and are a key DC subset presenting MHC I-bound peptides to CD8 T-cells. Interestingly, this DC population was increased in the islets of pre-diabetic NOD mice (12 weeks of age), which synchronized with CD4 T-cell infiltration (47). Furthermore, *Batf3-/-* mice do not develop spontaneous diabetes as a result of lack of antigen presentation to antigen-specific CD4 T-cells in the islet and diminished CD8 T-cell priming in the pancreatic lymph node (47). This suggests that $CD103^+$ DCs are essential for autoimmune diabetes. More recently, the importance of the pancreatic milieu on macrophage phenotype has been shown in the C57BL/6 mouse model (52) but is yet to be tested in NOD mice. Furthermore, the islet microenvironment influences macrophages, demonstrated in mice lacking NADPH oxidase (NOX)-derived superoxide (an enzyme complex involved in free radical production), which display an alternatively activated **M2 macrophage** phenotype and have delayed onset diabetes (53) (reviewed in (54)).

Heterogeneous populations of myeloid cells are present in the murine islet during inflammation, including recruited populations of macrophages and DCs (6, 7). pDCs producing IFNα, essential for disease initiation, are present in the islets during early stages of insulitis (9). In line with this, pDCs are increased in peripheral blood of newly-diagnosed, but not long-standing patients (55). In contrast, in the NOD mouse model, pDCs can negatively regulate diabetes as their loss leads to accelerated insulitis (56). This disparity suggests that pDCs may have dual roles in T1D, dependent on the environment and cell milieu present in the islet. APC populations alter with the severity of islet inflamma-

tion and mediate T-cell:APC interactions, and in turn change T-cell effector function (7). This further demonstrates the importance of the evolving micro-environment in the pancreas. Importantly, new imaging techniques will enable the visualization of multiple islets, allowing further understanding of the varying infiltration in each islet (7, 8).

Islet infiltrating T-cells

T-cells are essential in the development of diabetes, although other populations are required for full clinical manifestation. Evidence accumulated over many years has shown that CD4 and CD8 T-cells are directly involved in the destruction of β-cells, particularly in mouse models (57). In recent years, the topic of antigen specificity has been widely discussed, with conflicting hypotheses. Whether islet entry is dependent on antigen specificity, or whether T-cell infiltration is controlled by chemokine and cytokine cues, is a much-researched question. Additionally, whether islet infiltration is largely autoreactive, or whether bystander T-cells are present during this inflammation, has also been an important focus and conclusions have differed, dependent on the methodology used. Sophisticated **retrogenic mouse** models, whereby hematopoietic stem cells are transduced with a retroviral construct containing the TCR α and β chains encoding T-cell receptors from diabetogenic T-cell clones and then infused into recipient mice, have demonstrated that bystander T-cells do not infiltrate pancreatic islets (58). Furthermore, work on the initiation of events revealed that antigen-specific CD4 T-cells are the first T-cells to infiltrate the pancreas and interact with local intra- islet APCs (48). This first interaction induces changes in the local microenvironment, including vascular cell adhesion molecule 1 (VCAM-1) upregulation on the endothelium of islet blood vessels and intercellular adhesion molecule 1 (ICAM-1) upregulation on islet β-cells. These events allow subsequent infiltration of non-antigen-specific CD4 T-cells (59). The initial entry of CD4 T-cells is supported by further studies in the NOD model demonstrating that only CD4 T-cells were detected in 4 week-old mice; CD8 T-cells and B-cells are found at 6 weeks old within the islet; by 8 weeks all major leukocyte subsets have infiltrated (60). However, antigen specificity was not probed in this study. A new investigation using the

Kaede/NOD mouse model, which allows non-invasive labeling and tracking of cells, reported that not all CD4 T-cells that infiltrate the islets are antigen specific; in fact activated effector memory CD4 T-cells were a minority of the population found in the islets (6). Detection of antigen specific CD4 T-cells in humans with T1D is challenging given the low frequency of antigen specific T-cells. Recently, however, recently islets isolated from a patient with T1D revealed CD4 T-cells responsive to proinsulin peptides (61). The degree of heterogeneity of CD4 T-cells that recognize β-cell epitopes within individuals with T1D remains unknown.

In respect of CD8 T-cell antigen specificity, studies in mice show MHC class I expression and local antigen recognition is required for the homing of CD8 T-cells to the pancreas (17, 62). However, this does not exclude the possibility that chemokine and cytokine cues are also important (17, 63). Earlier murine studies of antigen-specific CD8 T-cells indicated that whilst less numerous than CD4 T-cells, CD8 T-cells can be found within the islet in the early infiltrate (64, 65). Recently, CD8 T-cells specific for islet autoantigens have been found in human islet tissue from both newly diagnosed and long standing patients (66). Of note, CD8 T-cells were found to recognize multiple islet autoantigens only in long standing patients, building a case for persistent antigen release (66). In mice, CD8 T-cells acquire an effector memory phenotype in the islets that have increased expression of IFNγ and granzyme B, indicating encounters with cognate antigen (67, 68). Further to this, tetramer-stained antigen-specific CD8 T-cells upregulate KLRG1 and CD127, markers associated with antigen exposure (69). Another marker associated with chronic antigen stimulation is PD-1, a key negative regulator and expressed on exhausted CD8 T-cells, particularly in viral infections. An exciting study recently revealed that exhausted CD8 T-cell transcriptional profiling could predict the prognosis of autoimmune disease, including T1D (70). However, whether CD8 T-cell exhaustion occurs in the pancreatic islets during inflammation has yet to be determined (outstanding questions), but it is an interesting possibility, as destruction of β-cells releases further antigen, perpetuating chronic exposure. Additionally, IL-7 receptor blockade in the NOD mouse model can prevent and reverse diabetes by the induction of PD-1 upregulation and reduced IFNγ

production in CD4 T-cells (71). PD-1 expression on both CD8 and CD4 T-cells may provide a regulatory mechanism by interaction with its ligand on β-cells in the islets during autoimmune diabetes (See Figure 1).

Regulatory T-cells (Tregs) are a well-studied population in T1D, as dysregulation leads to spontaneous autoimmunity in humans. There is also evidence that Treg function is impaired in human T1D (72). In mouse models, Treg adoptive transfers can protect mice from T1D and manipulation of Treg mechanisms can accelerate disease progression (73). The transcription factor required for Treg function, forkhead box P3 (FoxP3), is a hallmark of natural Treg cells. Ablation of FoxP3 T-cells in a CD4 double transgenic model using an inducible system demonstrated accelerated immune attack on islets, characterized by increased effector T-cells and activated natural killer cells (NK cells) (74). This has led to therapeutics aiming to increase Tregs, such as IL-2 injections, which increases Tregs in the pancreatic islets, resulting in reversal of established disease (75). Recently, the full extent of Treg interaction with other cells in the pancreatic islets has been more thoroughly investigated. Treg treatment in a mouse model of accelerated diabetes revealed that Tregs not only inhibit CD4 T-cell effector function in the islets, but also have a profound effect on CD8 T-cells (76). Here, CD8 T-cells were fewer in Treg-treated mice, though this was not a result of apoptosis or inhibition of proliferation but reduced chemokine recruitment. Furthermore, Tregs had the ability to inhibit IFNγ production from CD8 T-cells through mTOR signaling directly in the target tissue.

Islet Infiltrating B-cells

B-cells play an important role in the pathogenesis of T1D, including insulin-specific antigen presentation to CD4 T-cells, and the production of pro-inflammatory cytokines, both contributing to the destruction of islet β-cells. B-cell depletion therapy using an anti-human CD20 (hCD20) monoclonal antibody can reverse diabetes in the hCD20 transgenic NOD mouse model (77), as could targeting CD22 (78). For a full review of B-cell depletion therapy in type 1 diabetes in both humans and mice see (79). During inflammation, infiltrating B-cells can be organized into tertiary lymphoid structures (TLS) (80),

including germinal centers. Molecular analysis of the B-cell receptors (BCR) in TLS shows a different light chain usage, compared to the pancreatic lymph node (80). This suggests that islet-infiltrating B-cells are specific to the pancreas and do not reflect the repertoire of B-cells found in the secondary lymphoid organs. Supporting this, B1a-like B-cells (found mostly in the peritoneum), that are $CD5^{+}$ $B220^{low}$, infiltrate islets in NOD mice (81). Furthermore, B-cells in the pancreatic islets are mostly antigen-specific and show markers of antigen experience (80, 82, 83). Taken together, the evidence suggests that there is specific cross-talk between B-cells and T-cells in the islets during inflammation. This interplay between B- and T-cells has been dissected by the use of B-cell depletion therapy. B-cell depletion can improve the outcome of diabetes in part by the induction of Treg subsets (77, 78), although this occurs only during the reconstitution of B-cells. In fact, before B-cell reconstitution, antigen-specific CD4 T-cells respond more robustly to antigen *in vitro,* and a reduction of CD4 Tregs was observed in the islets, compared to non-depleted control mice (84). However, once B-cell populations are restored, they acquire enhanced regulatory function that depends on cell-to-cell contact rather than IL-10 (84). It is important to note that B-cells infiltrating islets can become plasma cells and lack CD20 expression (85). In humans, $CD20^{lo}$ and $CD20^{high}$ B-cells are found in the islets of patients, which represent different profiles of insulitis correlating with diagnosis (4). Together this may impact on the efficacy of anti-CD20 B-cell therapy.

Other studies using B-cell depletion show an impact on other cell subsets. B-cell absence during TNFα-mediated inflammation impacts CD8 T-cell accumulation in the islet, with increased apoptosis, resulting in fewer intra-islet CD8 T-cells (86). B-cell depletion also expands $CD11b^{+}Gr1^{+}$ myeloid population that can suppress T-cell function through IL-10, NO and cell contact (87). It is clear that B-cells significantly alter the progress of inflammation, and in their absence, key interactions in the islet can be altered to favor tissue regulation (See Figure 2).

Conversely, B-cells have also been reported to regulate autoimmunity, including T1D. Different **regulatory B-cell (Bregs)** populations have been de-

scribed that regulate through anti-inflammatory cytokines such as IL-10, TGFβ and IL-35. The participation of Bregs in the islets during insulitis is relatively undefined; possible interactions are shown in Figure 3 for the NOD mouse and we speculate that this is also possible in humans. B-cells activated with LPS can produce TGFβ and express Fas ligand and leads to the apoptosis of diabetogenic T-cells (88). In addition, Bregs can induce Tregs, possibly through a TGFβ mechanism and promote graft survival (89). Interestingly, increased IL-10 levels from B-cells in the islets of long-term normoglycemic mice have been reported (90). Moreover, increased populations of $CD40^+$ and anergic B-cells are also found in islets of mice that are 'protected' from diabetes (90). Whether this altered B-cell repertoire is functionally impaired or negatively regulating the microenvironment is yet to be determined. Of note is that IL-35 can induce IL-10 producing B-cells (91) and ectopic expression of IL-35 on β-cells can protect from diabetes and β-cell destruction (92). Although this IL-35 ectopic expression resulted in decreased CD8 and CD4 T-cells, the effect on B-cells was not addressed in the study. Emerging literature also reveals new populations of Breg cells, including Tim-1-expressing Bregs that are able to alter T-cell responses and reduce the severity of EAE (experimental autoimmune encephalomyelitis) through an IL-10-dependent mechanism (93). Recent evidence describes pDC production of IFNα, along with CD40 stimulation, induces IL-10-producing immature Bregs, and this pathway is altered in patients with systemic lupus erythematosus (SLE) (94). It is interesting to speculate whether the conflicting roles of IFNα and pDCs seen in T1D contrasting with this recent data in SLE may be an unexplored mechanism providing insight into new regulatory pathways. Whether these populations of Bregs are present in the pancreatic infiltrate or important in T1D is a question that is yet to be answered.

Other regulatory cell populations include invariant NK T-cells that may interact with potential Breg populations may also be present in 'protected' NOD mice (See Figure 3).

Concluding remarks and future perspectives

T1D is a multifactorial autoimmune disorder that leads to β-cell destruction. It is clear that more than one immune cell population governs local inflammation. Resident islet cells play a role in tissue regulation but also contribute to tissue destruction. The protection of β cells within the islet requires a dynamic balance of a heterogeneous population of cells, all of which provide specific signals. It would be useful to understand the factors that upset this balance (outstanding questions).

More examination is required into the heterogeneity of islets within the pancreas with regards to their immune infiltrate and the status of islet resident cells. Understanding the contribution of these resident cells may allow differentiation between pathogenic and potentially protective mechanisms (outstanding questions). The triggers of β-cell destruction are important, but we should be particularly interested in the immune phenotype of islets that have immune cell infiltrates but do not undergo β-cell destruction (see outstanding questions). This would allow consideration of how best to target the multiple cell types that contribute to β-cell destruction and their interaction with the β-cells (Text box 2).

Understanding both the pathogenic pathways as well as the regulatory components that reduce islet β-cell damage is important, as we seek to develop immunotherapy to reduce loss of insulin production in diabetes. Ultimately, boosting natural regulation or protective pathways may be equally important in protection of β-cell regeneration or replacement in immunotherapy and prevention of type 1 diabetes.

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Glossary

Antigen Presenting Cell (APC): A cell that can present antigens via either MHC I or MHC II.

B1-a cells: A subset of B lymphocytes mainly localized in the peritoneal cavity that are involved in humoral immunity.

Basic leucine zipper transcriptional factor ATF-like 3 (Batf3): A transcription factor that controls the maturation of CD8α classical dendritic cells.

Beta-(β) cells: Resident islet β-cells responsible for secreting insulin into the bloodstream. These cells are the primary target during autoimmune type 1 diabetes.

CD4 T-cell: A T lymphocyte, which is part of the adaptive immune system, that expresses a CD4 molecule (part of the T-cell receptor) on the surface that upon recognition of antigen presented by MHC II produces cytokines.

CD8 T-cell: A T lymphocyte, which is part of the adaptive immune system, that expresses a CD8 molecule (part of the T-cell receptor) on the surface that is involved in targeted killing upon recognition of antigen presented by MHC I.

Insulitis: Infiltration of immune cells into the Islets of Langerhans.

Islets of Langerhans: Islands of endocrine tissue within the large body of pancreatic acinar tissue making up 1-2% of the total cell mass.

M2 macrophage: A group of heterogenous macrophages that can be alternatively activated by cytokines such as IL-4 and IL-13. These cells are involved in tissue repair and wound healing.

Plasmacytoid DCs (pDCs): A unique subset of dendritic cells which play a role in antiviral immune responses and specializes in the production of interferons, and importantly interferon alpha (IFNα) in the context of T1D.

Programmed death-ligand 1 (PD-L1): A ligand for PD-1 which is expressed on multiple cell types including lymphocytes and myeloid cells.

Programmed death-receptor (PD-1): A cell surface receptor expressed on lymphocytes that can deliver essential negative signals to T and B cells upon ligation with its ligands

Regulatory B-cells (Breg): A heterogeneous group of B lymphocytes defined by immunoregulatory function.

Regulatory T-cells (Treg): A specific population of T lymphocytes that have the ability to suppress effector T-cell responses.

Retrogenic mouse: A mouse developed using retrovirus technology which allows the study of multiple T-cell receptors simultaneously.

Box 1. Islet inflammation and kinetics of the NOD mouse

The use of the NOD mouse model has provided many advances and insights into human T1D, because it has allowed study of the kinetics and initiation of diabetes. The importance of the NOD mouse model has been extensively discussed in reviews (57, 95). This animal model develops diabetes spontaneously with genetics and pathological outcomes that parallel those of human T1D (95). Genetically, the NOD mouse expresses MHC II molecules I-A^{g7}, which is the primary contributor to disease susceptibility (95). Correspondingly, the genetic region strongly associated with T1D in humans is the HLA locus; however, in both mice and humans, environmental factors are also a contributing factor (1). Disease incidence in NOD mice is higher in females than in males at about 70-80% compared to 20-30%, respectively, and varies between different laboratory colonies (2). Inflammatory cells can be observed in the islets as early as 2-4 weeks of age in the NOD mouse. Infiltration of the islets continues to amplify, inducing β-cell death, causing the full clinical manifestations of diabetes (2). In comparison, human T1D is very slow and insulitis seems to be less extensive with regards to the amount of cellular infiltrate and proportion of islets compromised (4). In both human and mouse, this infiltration comprises a variety of leukocytes, including lymphocyte populations such as CD4 and CD8 T-cells, B-cells, in addition to myeloid populations and NK cells (96). In the NOD mouse, innate immune cells can be detected in the pancreas, including neutrophils, plasmacytoid DCs and B-cells, as early as 2 weeks of age (9). Myeloid cell populations comprise the majority of the immune cells during early stage insulitis, then lymphocyte populations become more prominent as disease is established (6). In mice, CD4 T-cells are more numerous in the islet lymphocyte population, although in humans CD8 T-cells are more prominent (6, 96, 97). It is important to note that some NOD mice are ‘protected’ from diabetes (aged 30 weeks onwards), as the incidence is

rarely 100%, and even more noteworthy is that these mice still have islet infiltration although do not succumb to full clinical disease (90). Moreover, there are well known transgenic mouse models that develop pancreatic infiltrate yet do not present with spontaneous disease (5, 98). This interesting observation, along with the parallel existence of intact and destroyed islets in the same pancreas, raise many important questions that need future clarification.

Box 2. Therapies and therapeutic targets

There are a number of potential therapeutic targets for T1D. 1) The pathogenic T-cells could be directly disabled or destroying indirectly controlled by boosting regulatory cell activity, reviewed in (99). 2) The APCs that present antigens to pathogenic T-cells, both in the islet (resident APCs) and in secondary lymphoid organs could be tolerized such that they fail to activate/tolerize T-cells. Tolerizing B-cells as antigen-specific APCs, may be part of this strategy. 3. The β-cells that are the target of the specific damage need to be protected and allowed to replicate/regenerate (100). Disabling the interactions within the islet may be more difficult, but reducing damage by oxygen free radicals within the islet and boosting β-cell repair and regeneration would be of considerable benefit. Currently there is a gulf between experimental therapeutic strategies that have been successful in animal models and those that can be practically targeted in heterogeneous human individuals with T1D. Many successful strategies have targeted the pre-diabetic period in the NOD mouse, and if safety and accurate identification of individuals could be made, then some of the currently successful strategies in mouse models could be tested in humans. However, once diabetes occurs, relatively few strategies targeting immune cells have been successful in mice, and this underlines the difficulty that has also been experienced in immunotherapy in humans. Ideally, therapy would target pathogenic lymphocytes, as well as deal in combination with β cell regeneration (100).

Figure legends

Figure 1. Pancreatic islet cells are involved in immune cell regulation during inflammation in T1D.

A pancreatic islet (bottom right) is depicted with an immune infiltrate and undergoing β-cell destruction. 1. Immune cells entering the pancreas are influenced by islet endothelial cells. Endothelial cells enable T-cell adhesion and binding along with induced expression of MHC I and MHC II allowing antigen presentation to CD4 and CD8 T-cells. 2. β-cell interactions with islet infiltrate influence the microenvironment. (A) Antigen presentation by β-cells to CD8 T-cells via MHC Class I can initiate cytotoxic CD8 T-cells to target β-cells, allowing antigen release and perpetuation of β-cell apoptosis. β-cell antigens are processed by DCs and presented via MHC Class II by DCs to CD4 T-cells; direct presentation through upregulation of MHC Class II may also induce anergic CD4 T-cells. (B) β-cells express the IL-22 receptor (IL-22R), engaged by IL-22 produced by CD4 T-cells, allowing protection from apoptosis and encouraging β-cell regeneration (left). In the presence of IFNα and inflammation, engagement of IL-22R may lead to tissue destruction (right). 3. β-cells may modulate the islet environment via PD-L1. PD-L1: PD-1 interaction on CD4 T-cells can lead to a down-regulation of the inflammatory cytokine IFNγ. Antigen release from apoptotic β-cells may induce PD-1 up-regulation on CD8 T-cells upon which engagement with PD-L1 on β-cells and macrophages can, in turn, down-regulate CD8 T-cell activation. Together these will have an indirect effect on β-cell destruction.

Figure 2. B cell depletion reveals key immune cell interplay within the pancreas.

Evidence from B-cell depletion studies suggest that removal of B-cells from the pancreas can (A) induce a population of IL-10 producing myeloid population (B) increase apoptosis in intra-islet CD8 T-cells and (C) allow expansion of CD4 Tregs during B-cell reconstitution which influences antigen presenting cells (APCs) to down-regulate CD4 and CD8 T-cell activation. This has the potential to regulate the immune cell environment within the islet in addition to the systemic effect.

Figure 3. Proposed interactions between regulatory B-cell populations local islet inflammatory milieu in the NOD mouse.

Bregs can be induced with a variety of stimuli including CD40 ligation, TLR ligands and cytokines IL-21, IL-35, IL-1β and IL-6. Plasmacytoid DCs (pDC) producing IFNα along with CD40 ligation also promote Bregs. Bregs can control inflammation through the production of IL-10, IL-35 and TGFβ and the induction of Tregs. IL-10, IL-35 and TGFβ cytokines can all inhibit CD4 T effector cells (Teff), cytotoxic CD8 T-cells and pro-inflammatory cytokines from dendritic cells (DCs), which in turn down-regulate T-cells. In protected mice, increased surface expression of CD40 and IL-10 production has been shown (red boxes) in the pancreatic islets, although we speculate as to the type of Breg present and how they are induced/maintained. Interactions in the islets between Bregs and invariant NKT (iNKT) cells may provide further regulation of diabetogenic T-cells. We also consider that IL-10-expressing Breg can influence macrophage populations that are involved in immunoregulation and tissue remodeling.

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Outstanding questions box

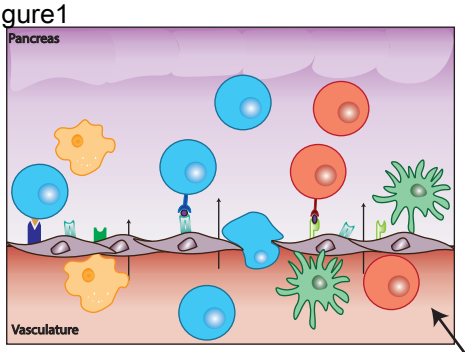
What are the triggers that lead to immune β cell destruction? Do the islets play an active role in this process? Can the process be halted before diabetes manifests?

What is the difference between islets with insulitis but no β cell destruction compared to islets undergoing destruction? Are the islet endocrine cells themselves contributing to regulation of the infiltrating immune cells or is this protection carried out by islet-resident immune cells? Do protected NOD mice have a different immune cell phenotype in the islets compared to NOD mice that develop diabetes? Is this protection governed by local Breg interactions?

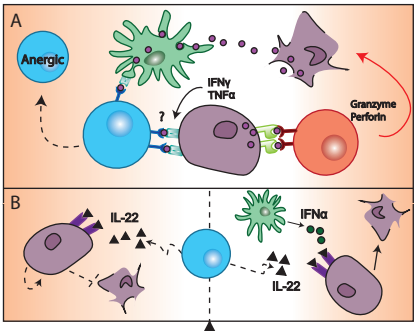
CD8 T cells are key effectors in β cell destruction. Is there any evidence of CD8 T cell exhaustion, indicated by upregulation of PD-1, and could this be a potential regulatory mechanism within the target tissue?

Tissue resident CD8 T cells (Trm) have recently been characterized; could Trm cells take up residency in islets of the pancreas over time and contribute to the CD8 T cell population seen in the infiltration?

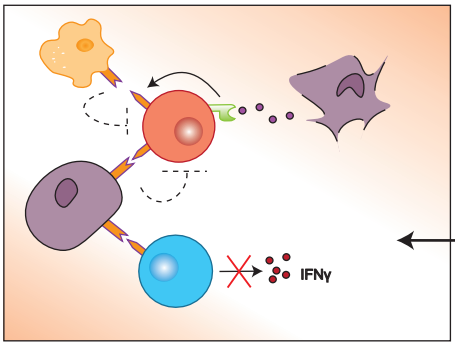
Figure1

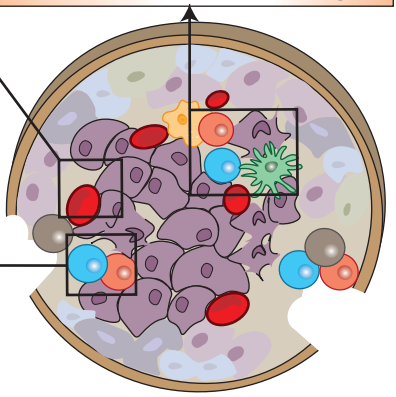

Pancreas
Vasculature

2.


A
Anergic
IFNγ
TNFα
?
Granzyme
Perforin
B
IL-22
IFNα
IL-22

3.


IFNγ



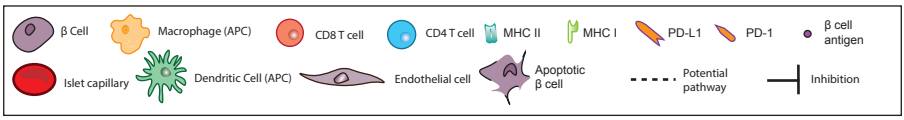
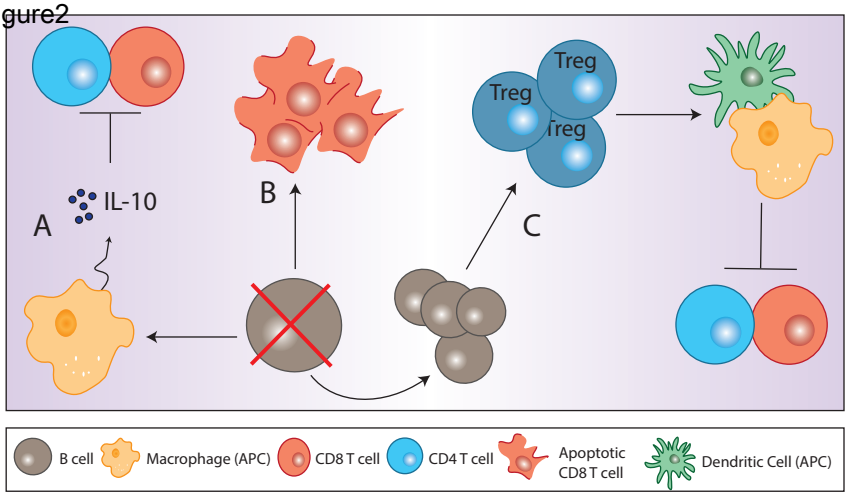
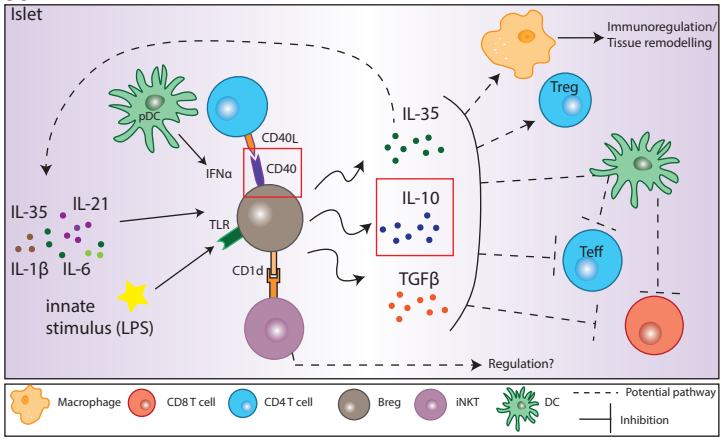

β Cell
Macrophage (APC)
CD8 T cell
CD4 T cell
MHC II
MHC I
PD-L1
PD-1
β cell antigen
Islet capillary
Dendritic Cell (APC)
Endothelial cell
Apoptotic β cell
Potential pathway
Inhibition

Figure2


A
IL-10
B
C
Treg
Treg
Treg

B cell
Macrophage (APC)
CD8 T cell
CD4 T cell
Apoptotic CD8 T cell
Dendritic Cell (APC)

Figure3


Islet
Immunoregulation/
Tissue remodelling
pDC
IFNα
CD40L
CD40
IL-35
IL-21
IL-1β
IL-6
TLR
CD1d
innate
stimulus (LPS)
IL-35
IL-10
TGFβ
Treg
Teff
Regulation?
Macrophage
CD8 T cell
CD4 T cell
Breg
iNKT
DC
Potential pathway
Inhibition