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### Trends box

In T1D,  $\beta$  cells are highly sensitive to selective damage and recruit immune cells by chemokine production. These immune cells directly damage  $\beta$  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  $\beta$  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  $\beta$  cells during inflammation is dependent on the pancreatic microenvironment.

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

1 Immune and pancreatic  $\beta$  cell interactions in type 1 diabetes

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3

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7

8 **Keywords**

9 Immune crosstalk, Pancreatic islets, Inflammation, Type 1 diabetes, Autoim-  
10 mune disease

11 **Abstract**

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

## 21 **Type 1 diabetes and Islet Inflammation**

22 Type 1 diabetes (T1D) is an organ-specific autoimmune disease that results in  
23 a loss of insulin-producing **β-cells** (see glossary) in the pancreatic **islets of**  
24 **Langerhans**, leading to an inability to maintain glucose regulation (1). Insulin  
25 administration is essential in patients with type 1 diabetes. However, maintain-  
26 ing optimal blood glucose is difficult, and many patients present with compli-  
27 cations including blindness, kidney failure and vascular complications (1). Alt-  
28 though T1D is T-cell-mediated, other immune cells are crucial for this multi-  
29 functional process with a dynamic progression of inflammation. **Antigen pre-**  
30 **senting cells** (APCs) are critical for antigen presentation of peptides to **T-**  
31 **cells** (both CD4 and CD8 T-cells), resulting in immunological and molecular  
32 events leading to apoptosis of β-cells (2). Understanding these cellular inter-  
33 actions and subsequent events will provide important information to enable us  
34 to identify new targets and deliver potential therapies.

35

36 The autoimmune response in human T1D progresses slowly, and intact islets  
37 can coexist with islets undergoing immune infiltration and β-cell destruction.  
38 Historically, it was thought only when 70-90% of β-cells are destroyed that  
39 clinical symptoms in patients appear, although recent reports suggest this is  
40 overestimated (3, 4). Furthermore, experimental mouse models have demon-  
41 strated that leukocyte infiltration into islets does not always lead to disease  
42 (5). We suggest that different islets, within the same tissue, may have a dif-  
43 ferent cellular profile with heterogeneous cell types, which may evolve de-  
44 pending on the microenvironment they encounter. It is essential to understand  
45 why some insulin-producing β-cells are destroyed and some escape destruc-  
46 tion. Emerging literature on the dynamics of the pancreatic infiltrate in the  
47 non-obese diabetic (NOD) mouse (Box 1) during the course of diabetes has  
48 shown how diverse and constant cellular flux can be, although not an ‘open  
49 house’ to all cell types (6). Furthermore, work on the antigen-specific T-  
50 cell:APC interactions in the islets throughout disease progression has demon-  
51 strated that the tissue microenvironment can control T-cell functions and mo-  
52 tility (7, 8). During the early stages of inflammation in the NOD mouse, devel-  
53 opment of T1D is dependent on crosstalk in the pancreas between islet β-

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

63

### 64 **Islet Organization and Immune Control**

65 The pancreas consists of exocrine and endocrine tissues that are functionally  
66 distinct. The exocrine cells produce digestive enzymes and will not be dis-  
67 cussed here. The endocrine cells maintain glucose homeostasis. Pancreatic  
68 islets comprise multiple hormone-secreting cells that are highly organized; in  
69 rodents, insulin-producing  $\beta$ -cells are at the core of the islet. Other endocrine  
70 cells include the glucagon-secreting  $\alpha$ -cells, somatostatin-secreting  $\delta$  cells,  
71 pancreatic polypeptide (PP)-secreting cells, and ghrelin-producing  $\epsilon$  cells, and  
72 reside in the islet periphery. In human islets, the organization of these endo-  
73 crine cells is more intertwined and compact (10). They are highly vascularized  
74 to enable the efficient secretion of insulin into the bloodstream. For a full re-  
75 view on in-depth control of insulin release, see (11).

76

77 Immunofluorescence studies demonstrate that pancreatic islets are surround-  
78 ed by a peri-islet basement membrane (BM) and an interstitial matrix, that to-  
79 gether create a capsule. The BM acts as a barrier for the insulin-secreting  $\beta$ -  
80 cells, and leukocytes must pass through this membrane during islet infiltration.  
81 A loss of this capsule, during the progression of diabetes in the NOD mouse  
82 model and human T1D, specifically correlates with leukocyte infiltration (12).  
83 This suggests that the peri-islet BM is a key regulator of islet infiltration and  
84 progression of T1D. Here, it should be noted that mouse and human BM dif-  
85 fers in organization, with the human BM having a second BM layer (13).

86

87 The islets of the pancreas are highly vascularized, and communication be-  
88 tween  $\beta$ -cells and surrounding cell types is particularly important.  $\beta$ -cells are in  
89 close contact and communicate with intra-islet endothelial cells, and normally  
90 this communication maintains homeostasis (14, 15); however this is also im-  
91 portant in the context of autoimmune disease (Figure 1A). It is well document-  
92 ed, in mice and humans, that cell adhesion molecules on the endothelium in  
93 the pancreas can enable T-cell binding and interactions (16) resulting in T-cell  
94 activation and movement into the islets (17). Additionally, patients with T1D  
95 have increased expression of Major Histocompatibility Complex (MHC) I and  
96 induced expression of MHC II on islet endothelial cells (18, 19), allowing en-  
97 dothelial cells to present autoantigens to T-cells (18). This is a potential  
98 mechanism in humans and mice by which T-cells may enter the islets. Finally,  
99 isolated human endothelial cells can express the co-stimulatory molecule  
100 CD86 that is involved in CD4 T-cell adhesion through interaction with cytotox-  
101 ic T lymphocyte associated protein 4 (CTLA4), a key negative regulator on T-  
102 cells (20). Thus, the highly specialized islet structure and microvascular cells  
103 of the pancreas can facilitate T-cell infiltration and contribute to the progres-  
104 sion of disease.

105

### 106 **Influence of $\beta$ -cells in the islet microenvironment**

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

120 induce anergic CD4 T-cells to control inflammation. However, the function of  
121 CD4 T-cells stimulated by  $\beta$ -cells has yet to be established. MHC II expres-  
122 sion (HLA) can be found on human  $\beta$ -cells in insulin-containing islets, but ap-  
123 pears to be limited to a small number of cells (26, 27). This expression is ob-  
124 served without insulinitis present, suggesting upregulation just prior to infiltration  
125 (27).

126

127  $\beta$ -cells are also a source of chemokine production, which may have inflamma-  
128 tory or protective roles in both the NOD mouse and human islets (28, 29).  
129 Multiple chemokines including C-X-C motif chemokine 10 (CXCL10) are up-  
130 regulated on  $\beta$ -cells in islets undergoing infiltration (28, 29), which drives im-  
131 mune cell recruitment to the pancreas via the chemokine receptor CXCR3  
132 (28). Human islet  $\beta$ -cells express the Interleukin-22 receptor (IL-22R) (30),  
133 which can be engaged by IL-22 produced from T-cells, in patients with T1D  
134 (31). IL-22 up-regulates genes that protect  $\beta$ -cells from apoptosis and can en-  
135 hance  $\beta$ -cell regeneration (32). During inflammation, cytokines such as  $\text{IFN}\alpha$ ,  
136 in the presence of IL-22, can induce nitric oxide synthase (iNOS) and resulting  
137 oxygen free radicals contribute to tissue destruction (33). This is pertinent in  
138 T1D, as  $\text{IFN}\alpha$  production in the pancreatic islets is essential for disease initia-  
139 tion (9). Conversely, however, in NOD mice deficient in  $\text{IFN}\alpha$  receptor 1 (IF-  
140 NAR1) diabetes is not delayed or prevented (34). Synergistic islet interac-  
141 tions, under pro-inflammatory conditions, have also been demonstrated in  
142 both rat and human islets (35, 36). In the presence of interleukin 1 (IL-1)  $\beta$ -  
143 cells undergo destruction in an iNOS dependent manner (36). Production of  
144 IL-1 can be induced in islet macrophages by tumor necrosis factor alpha  
145 ( $\text{TNF}\alpha$ ) and lipopolysaccharide (LPS) stimulation, and depletion of islet mac-  
146 rophages inhibits expression of IL-1 (36).

147

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



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

154

155 Regulatory costimulatory molecules may also be important.  $\beta$ -cells express  
156 **programmed death-ligand 1** (PD-L1) during insulinitis and may play a key role  
157 in regulating inflammation, demonstrated by the NOD-*Pdcd1*<sup>-/-</sup> mice that have  
158 PD-L1 deficiency and develop accelerated diabetes (38, 39). Patients receiv-  
159 ing anti-**programmed death 1 (PD-1)** treatment for cancer can present with  
160 autoimmune T1D (40). It is clear that although  $\beta$ -cells are key in tissue regula-  
161 tion and homeostasis, they can self-destruct when exposed to certain envi-  
162 ronmental cues. However, we have yet to gain full understanding of  $\beta$ -cell  
163 communication with immune cells through this pathway, during different stag-  
164 es of inflammation. Critical interactions in the evolving microenvironment, in-  
165 volving both soluble factors as well as cell-to-cell contact need to be uncov-  
166 ered. It is also interesting to speculate that E-cadherin, a key protein involved  
167 in adhesion on epithelial tissues, in the pancreatic islets may interact with its  
168 ligand CD103, expressed on effector memory CD8 T-cells, which can reside  
169 in non lymphoid tissues (41). In a model of pancreatic islet transplant rejec-  
170 tion, CD8<sup>+</sup>CD103<sup>+</sup> T-cells are found at the graft site and are essential during  
171 the immune response (42). Together, islet  $\beta$ -cells are integral in regulating the  
172 local environment, but during inflammation, multiple mechanisms can contrib-  
173 ute to their own cell death.

174

### 175 **Islet Antigen Presenting Cells (APCs)**

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

185 volved in surveillance of surrounding areas. Further to this role, islet APCs are  
186 essential for islet antigen presentation, and express MHC-peptide complexes  
187 derived from  $\beta$ -cell proteins (44). They are efficient at presenting these pep-  
188 tide complexes to CD4 T-cells.  $\beta$ -cells transfer secretory granules to resident  
189 islet APCs and it is this interaction that allows the recognition of  $\beta$ -cell anti-  
190 gens by CD4 autoreactive T-cells (49).

191

192 Tissue resident CD103<sup>+</sup> DCs, which are of the same lineage as CD8 $\alpha$  DCs  
193 found in lymphoid tissues (50), are under the control of the **Batf3** transcription  
194 factor (51) and are a key DC subset presenting MHC I-bound peptides to CD8  
195 T-cells. Interestingly, this DC population was increased in the islets of pre-  
196 diabetic NOD mice (12 weeks of age), which synchronized with CD4 T-cell  
197 infiltration (47). Furthermore, *Batf3*<sup>-/-</sup> mice do not develop spontaneous diabe-  
198 tes as a result of lack of antigen presentation to antigen-specific CD4 T-cells  
199 in the islet and diminished CD8 T-cell priming in the pancreatic lymph node  
200 (47). This suggests that CD103<sup>+</sup> DCs are essential for autoimmune diabetes.  
201 More recently, the importance of the pancreatic milieu on macrophage pheno-  
202 type has been shown in the C57BL/6 mouse model (52) but is yet to be tested  
203 in NOD mice. Furthermore, the islet microenvironment influences macrophag-  
204 es, demonstrated in mice lacking NADPH oxidase (NOX)-derived superoxide  
205 (an enzyme complex involved in free radical production), which display an al-  
206 ternatively activated **M2 macrophage** phenotype and have delayed onset di-  
207 abetes (53) (reviewed in (54)).

208

209 Heterogeneous populations of myeloid cells are present in the murine islet  
210 during inflammation, including recruited populations of macrophages and DCs  
211 (6, 7). pDCs producing IFN $\alpha$ , essential for disease initiation, are present in the  
212 islets during early stages of insulinitis (9). In line with this, pDCs are increased  
213 in peripheral blood of newly-diagnosed, but not long-standing patients (55). In  
214 contrast, in the NOD mouse model, pDCs can negatively regulate diabetes as  
215 their loss leads to accelerated insulinitis (56). This disparity suggests that pDCs  
216 may have dual roles in T1D, dependent on the environment and cell milieu  
217 present in the islet. APC populations alter with the severity of islet inflamma-

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

223

### 224 **Islet infiltrating T-cells**

225 T-cells are essential in the development of diabetes, although other popula-  
226 tions are required for full clinical manifestation. Evidence accumulated over  
227 many years has shown that CD4 and CD8 T-cells are directly involved in the  
228 destruction of  $\beta$ -cells, particularly in mouse models (57). In recent years, the  
229 topic of antigen specificity has been widely discussed, with conflicting hypoth-  
230 eses. Whether islet entry is dependent on antigen specificity, or whether T-cell  
231 infiltration is controlled by chemokine and cytokine cues, is a much-  
232 researched question. Additionally, whether islet infiltration is largely autoreac-  
233 tive, or whether bystander T-cells are present during this inflammation, has  
234 also been an important focus and conclusions have differed, dependent on  
235 the methodology used. Sophisticated **retrogenic mouse** models, whereby  
236 hematopoietic stem cells are transduced with a retroviral construct containing  
237 the TCR  $\alpha$  and  $\beta$  chains encoding T-cell receptors from diabetogenic T-cell  
238 clones and then infused into recipient mice, have demonstrated that bystand-  
239 er T-cells do not infiltrate pancreatic islets (58). Furthermore, work on the initi-  
240 ation of events revealed that antigen-specific CD4 T-cells are the first T-cells  
241 to infiltrate the pancreas and interact with local intra- islet APCs (48). This first  
242 interaction induces changes in the local microenvironment, including vascular  
243 cell adhesion molecule 1 (VCAM-1) upregulation on the endothelium of islet  
244 blood vessels and intercellular adhesion molecule 1 (ICAM-1) upregulation on  
245 islet  $\beta$ -cells. These events allow subsequent infiltration of non-antigen-specific  
246 CD4 T-cells (59). The initial entry of CD4 T-cells is supported by further stud-  
247 ies in the NOD model demonstrating that only CD4 T-cells were detected in 4  
248 week-old mice; CD8 T-cells and B-cells are found at 6 weeks old within the  
249 islet; by 8 weeks all major leukocyte subsets have infiltrated (60). However,  
250 antigen specificity was not probed in this study. A new investigation using the

251 Kaede/NOD mouse model, which allows non-invasive labeling and tracking of  
252 cells, reported that not all CD4 T-cells that infiltrate the islets are antigen spe-  
253 cific; in fact activated effector memory CD4 T-cells were a minority of the pop-  
254 ulation found in the islets (6). Detection of antigen specific CD4 T-cells in hu-  
255 mans with T1D is challenging given the low frequency of antigen specific T-  
256 cells. Recently, however, recently islets isolated from a patient with T1D re-  
257 vealed CD4 T-cells responsive to proinsulin peptides (61). The degree of het-  
258 erogeneity of CD4 T-cells that recognize  $\beta$ -cell epitopes within individuals with  
259 T1D remains unknown.

260

261 In respect of CD8 T-cell antigen specificity, studies in mice show MHC class I  
262 expression and local antigen recognition is required for the homing of CD8 T-  
263 cells to the pancreas (17, 62). However, this does not exclude the possibility  
264 that chemokine and cytokine cues are also important (17, 63). Earlier murine  
265 studies of antigen-specific CD8 T-cells indicated that whilst less numerous  
266 than CD4 T-cells, CD8 T-cells can be found within the islet in the early infil-  
267 trate (64, 65). Recently, CD8 T-cells specific for islet autoantigens have been  
268 found in human islet tissue from both newly diagnosed and long standing pa-  
269 tients (66). Of note, CD8 T-cells were found to recognize multiple islet autoan-  
270 tigen only in long standing patients, building a case for persistent antigen re-  
271 lease (66). In mice, CD8 T-cells acquire an effector memory phenotype in the  
272 islets that have increased expression of IFN $\gamma$  and granzyme B, indicating en-  
273 counters with cognate antigen (67, 68). Further to this, tetramer-stained anti-  
274 gen-specific CD8 T-cells upregulate KLRG1 and CD127, markers associated  
275 with antigen exposure (69). Another marker associated with chronic antigen  
276 stimulation is PD-1, a key negative regulator and expressed on exhausted  
277 CD8 T-cells, particularly in viral infections. An exciting study recently revealed  
278 that exhausted CD8 T-cell transcriptional profiling could predict the prognosis  
279 of autoimmune disease, including T1D (70). However, whether CD8 T-cell ex-  
280 haustion occurs in the pancreatic islets during inflammation has yet to be de-  
281 termined (outstanding questions), but it is an interesting possibility, as de-  
282 struction of  $\beta$ -cells releases further antigen, perpetuating chronic exposure.  
283 Additionally, IL-7 receptor blockade in the NOD mouse model can prevent and  
284 reverse diabetes by the induction of PD-1 upregulation and reduced IFN $\gamma$

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

288

289 **Regulatory T-cells (Tregs)** are a well-studied population in T1D, as dysregu-  
290 lation leads to spontaneous autoimmunity in humans. There is also evidence  
291 that Treg function is impaired in human T1D (72). In mouse models, Treg  
292 adoptive transfers can protect mice from T1D and manipulation of Treg  
293 mechanisms can accelerate disease progression (73). The transcription factor  
294 required for Treg function, forkhead box P3 (FoxP3), is a hallmark of natural  
295 Treg cells. Ablation of FoxP3 T-cells in a CD4 double transgenic model using  
296 an inducible system demonstrated accelerated immune attack on islets, char-  
297 acterized by increased effector T-cells and activated natural killer cells (NK  
298 cells) (74). This has led to therapeutics aiming to increase Tregs, such as IL-2  
299 injections, which increases Tregs in the pancreatic islets, resulting in reversal  
300 of established disease (75). Recently, the full extent of Treg interaction with  
301 other cells in the pancreatic islets has been more thoroughly investigated.  
302 Treg treatment in a mouse model of accelerated diabetes revealed that Tregs  
303 not only inhibit CD4 T-cell effector function in the islets, but also have a pro-  
304 found effect on CD8 T-cells (76). Here, CD8 T-cells were fewer in Treg-  
305 treated mice, though this was not a result of apoptosis or inhibition of prolif-  
306 eration but reduced chemokine recruitment. Furthermore, Tregs had the abil-  
307 ity to inhibit IFN $\gamma$  production from CD8 T-cells through mTOR signaling direct-  
308 ly in the target tissue.

309

### 310 **Islet Infiltrating B-cells**

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

319 including germinal centers. Molecular analysis of the B-cell receptors (BCR) in  
320 TLS shows a different light chain usage, compared to the pancreatic lymph  
321 node (80). This suggests that islet-infiltrating B-cells are specific to the pan-  
322 creas and do not reflect the repertoire of B-cells found in the secondary lym-  
323 phoid organs. Supporting this, B1a-like B-cells (found mostly in the peritone-  
324 um), that are CD5<sup>+</sup> B220<sup>low</sup>, infiltrate islets in NOD mice (81). Furthermore, B-  
325 cells in the pancreatic islets are mostly antigen-specific and show markers of  
326 antigen experience (80, 82, 83). Taken together, the evidence suggests that  
327 there is specific cross-talk between B-cells and T-cells in the islets during in-  
328 flammation. This interplay between B- and T-cells has been dissected by the  
329 use of B-cell depletion therapy. B-cell depletion can improve the outcome of  
330 diabetes in part by the induction of Treg subsets (77, 78), although this occurs  
331 only during the reconstitution of B-cells. In fact, before B-cell reconstitution,  
332 antigen-specific CD4 T-cells respond more robustly to antigen *in vitro*, and a  
333 reduction of CD4 Tregs was observed in the islets, compared to non-depleted  
334 control mice (84). However, once B-cell populations are restored, they acquire  
335 enhanced regulatory function that depends on cell-to-cell contact rather than  
336 IL-10 (84). It is important to note that B-cells infiltrating islets can become  
337 plasma cells and lack CD20 expression (85). In humans, CD20<sup>lo</sup> and CD20<sup>high</sup>  
338 B-cells are found in the islets of patients, which represent different profiles of  
339 insulinitis correlating with diagnosis (4). Together this may impact on the effica-  
340 cy of anti-CD20 B-cell therapy.

341

342 Other studies using B-cell depletion show an impact on other cell subsets. B-  
343 cell absence during TNF $\alpha$ -mediated inflammation impacts CD8 T-cell accu-  
344 mulation in the islet, with increased apoptosis, resulting in fewer intra-islet  
345 CD8 T-cells (86). B-cell depletion also expands CD11b<sup>+</sup>Gr1<sup>+</sup> myeloid popula-  
346 tion that can suppress T-cell function through IL-10, NO and cell contact (87).  
347 It is clear that B-cells significantly alter the progress of inflammation, and in  
348 their absence, key interactions in the islet can be altered to favor tissue regu-  
349 lation (See Figure 2).

350

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

353 scribed that regulate through anti-inflammatory cytokines such as IL-10, TGF $\beta$   
354 and IL-35. The participation of Bregs in the islets during insulinitis is relatively  
355 undefined; possible interactions are shown in Figure 3 for the NOD mouse  
356 and we speculate that this is also possible in humans. B-cells activated with  
357 LPS can produce TGF $\beta$  and express Fas ligand and leads to the apoptosis of  
358 diabetogenic T-cells (88). In addition, Bregs can induce Tregs, possibly  
359 through a TGF $\beta$  mechanism and promote graft survival (89). Interestingly, in-  
360 creased IL-10 levels from B-cells in the islets of long-term normoglycemic  
361 mice have been reported (90). Moreover, increased populations of CD40<sup>+</sup> and  
362 anergic B-cells are also found in islets of mice that are 'protected' from diabe-  
363 tes (90). Whether this altered B-cell repertoire is functionally impaired or  
364 negatively regulating the microenvironment is yet to be determined. Of note is  
365 that IL-35 can induce IL-10 producing B-cells (91) and ectopic expression of  
366 IL-35 on  $\beta$ -cells can protect from diabetes and  $\beta$ -cell destruction (92). Alt-  
367 hough this IL-35 ectopic expression resulted in decreased CD8 and CD4 T-  
368 cells, the effect on B-cells was not addressed in the study. Emerging literature  
369 also reveals new populations of Breg cells, including Tim-1-expressing Bregs  
370 that are able to alter T-cell responses and reduce the severity of EAE (exper-  
371 imental autoimmune encephalomyelitis) through an IL-10-dependent mecha-  
372 nism (93). Recent evidence describes pDC production of IFN $\alpha$ , along with  
373 CD40 stimulation, induces IL-10-producing immature Bregs, and this pathway  
374 is altered in patients with systemic lupus erythematosus (SLE) (94). It is inter-  
375 esting to speculate whether the conflicting roles of IFN $\alpha$  and pDCs seen in  
376 T1D contrasting with this recent data in SLE may be an unexplored mecha-  
377 nism providing insight into new regulatory pathways. Whether these popula-  
378 tions of Bregs are present in the pancreatic infiltrate or important in T1D is a  
379 question that is yet to be answered.

380

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

384

385 **Concluding remarks and future perspectives**

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

393 More examination is required into the heterogeneity of islets within the pan-  
394 creas with regards to their immune infiltrate and the status of islet resident  
395 cells. Understanding the contribution of these resident cells may allow differ-  
396 entiation between pathogenic and potentially protective mechanisms (out-  
397 standing questions). The triggers of  $\beta$ -cell destruction are important, but we  
398 should be particularly interested in the immune phenotype of islets that have  
399 immune cell infiltrates but do not undergo  $\beta$ -cell destruction (see outstanding  
400 questions). This would allow consideration of how best to target the multiple  
401 cell types that contribute to  $\beta$ -cell destruction and their interaction with the  $\beta$ -  
402 cells (Text box 2).

403

404 Understanding both the pathogenic pathways as well as the regulatory com-  
405 ponents that reduce islet  $\beta$ -cell damage is important, as we seek to develop  
406 immunotherapy to reduce loss of insulin production in diabetes. Ultimately,  
407 boosting natural regulation or protective pathways may be equally important in  
408 protection of  $\beta$ -cell regeneration or replacement in immunotherapy and pre-  
409 vention of type 1 diabetes.

410

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414 studies that have contributed to current understanding of this topic but these  
415 investigations could not be discussed here because of space limitations.

416

417

418



419 **Glossary**

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

422 **B1-a cells:** A subset of B lymphocytes mainly localized in the peritoneal cavi-  
423 ty that are involved in humoral immunity.

424 **Basic leucine zipper transcriptional factor ATF-like 3 (Batf3):** A transcrip-  
425 tion factor that controls the maturation of CD8 $\alpha$  classical dendritic cells.

426 **Beta-( $\beta$ ) cells:** Resident islet  $\beta$ -cells responsible for secreting insulin into the  
427 bloodstream. These cells are the primary target during autoimmune type 1 di-  
428 abetes.

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

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

435 **Insulinitis:** Infiltration of immune cells into the Islets of Langerhans.

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

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

441 **Plasmacytoid DCs (pDCs):** A unique subset of dendritic cells which play a  
442 role in antiviral immune responses and specializes in the production of inter-  
443 ferons, and importantly interferon alpha (IFN $\alpha$ ) in the context of T1D.

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

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

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

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

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

455

#### 456 **Box 1. Islet inflammation and kinetics of the NOD mouse**

457 The use of the NOD mouse model has provided many advances and insights  
458 into human T1D, because it has allowed study of the kinetics and initiation of  
459 diabetes. The importance of the NOD mouse model has been extensively dis-  
460 cussed in reviews (57, 95). This animal model develops diabetes spontane-  
461 ously with genetics and pathological outcomes that parallel those of human  
462 T1D (95). Genetically, the NOD mouse expresses MHC II molecules I-A<sup>g7</sup>,  
463 which is the primary contributor to disease susceptibility (95). Corresponding-  
464 ly, the genetic region strongly associated with T1D in humans is the HLA lo-  
465 cus; however, in both mice and humans, environmental factors are also a  
466 contributing factor (1). Disease incidence in NOD mice is higher in females  
467 than in males at about 70-80% compared to 20-30%, respectively, and varies  
468 between different laboratory colonies (2). Inflammatory cells can be observed  
469 in the islets as early as 2-4 weeks of age in the NOD mouse. Infiltration of the  
470 islets continues to amplify, inducing  $\beta$ -cell death, causing the full clinical mani-  
471 festations of diabetes (2). In comparison, human T1D is very slow and insulinitis  
472 seems to be less extensive with regards to the amount of cellular infiltrate and  
473 proportion of islets compromised (4). In both human and mouse, this infiltra-  
474 tion comprises a variety of leukocytes, including lymphocyte populations such  
475 as CD4 and CD8 T-cells, B-cells, in addition to myeloid populations and NK  
476 cells (96). In the NOD mouse, innate immune cells can be detected in the  
477 pancreas, including neutrophils, plasmacytoid DCs and B-cells, as early as 2  
478 weeks of age (9). Myeloid cell populations comprise the majority of the im-  
479 mune cells during early stage insulinitis, then lymphocyte populations become  
480 more prominent as disease is established (6). In mice, CD4 T-cells are more  
481 numerous in the islet lymphocyte population, although in humans CD8 T-cells  
482 are more prominent (6, 96, 97). It is important to note that some NOD mice  
483 are 'protected' from diabetes (aged 30 weeks onwards), as the incidence is

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

490

## 491 **Box 2. Therapies and therapeutic targets**

492

493 There are a number of potential therapeutic targets for T1D. 1) The pathogen-  
494 ic T-cells could be directly disabled or destroying indirectly controlled by  
495 boosting regulatory cell activity, reviewed in (99). 2) The APCs that present  
496 antigens to pathogenic T-cells, both in the islet (resident APCs) and in sec-  
497 ondary lymphoid organs could be tolerized such that they fail to acti-  
498 vate/tolerize T-cells. Tolerizing B-cells as antigen-specific APCs, may be part  
499 of this strategy. 3. The  $\beta$ -cells that are the target of the specific damage need  
500 to be protected and allowed to replicate/regenerate (100). Disabling the inter-  
501 actions within the islet may be more difficult, but reducing damage by oxygen  
502 free radicals within the islet and boosting  $\beta$ -cell repair and regeneration would  
503 be of considerable benefit. Currently there is a gulf between experimental  
504 therapeutic strategies that have been successful in animal models and those  
505 that can be practically targeted in heterogeneous human individuals with T1D.  
506 Many successful strategies have targeted the pre-diabetic period in the NOD  
507 mouse, and if safety and accurate identification of individuals could be made,  
508 then some of the currently successful strategies in mouse models could be  
509 tested in humans. However, once diabetes occurs, relatively few strategies  
510 targeting immune cells have been successful in mice, and this underlines the  
511 difficulty that has also been experienced in immunotherapy in humans. Ideal-  
512 ly, therapy would target pathogenic lymphocytes, as well as deal in combina-  
513 tion with  $\beta$  cell regeneration (100).

514

## 515 **Figure legends**

516

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

519 A pancreatic islet (bottom right) is depicted with an immune infiltrate and un-  
520 dergoing  $\beta$ -cell destruction. 1. Immune cells entering the pancreas are influ-  
521 enced by islet endothelial cells. Endothelial cells enable T-cell adhesion and  
522 binding along with induced expression of MHC I and MHC II allowing antigen  
523 presentation to CD4 and CD8 T-cells. 2.  $\beta$ -cell interactions with islet infiltrate  
524 influence the microenvironment. (A) Antigen presentation by  $\beta$ -cells to CD8 T-  
525 cells via MHC Class I can initiate cytotoxic CD8 T-cells to target  $\beta$ -cells, allow-  
526 ing antigen release and perpetuation of  $\beta$ -cell apoptosis.  $\beta$ -cell antigens are  
527 processed by DCs and presented via MHC Class II by DCs to CD4 T-cells;  
528 direct presentation through upregulation of MHC Class II may also induce  
529 anergic CD4 T-cells. (B)  $\beta$ -cells express the IL-22 receptor (IL-22R), engaged  
530 by IL-22 produced by CD4 T-cells, allowing protection from apoptosis and en-  
531 couraging  $\beta$ -cell regeneration (left). In the presence of IFN $\alpha$  and inflammation,  
532 engagement of IL-22R may lead to tissue destruction (right). 3.  $\beta$ -cells may  
533 modulate the islet environment via PD-L1. PD-L1: PD-1 interaction on CD4 T-  
534 cells can lead to a down-regulation of the inflammatory cytokine IFN $\gamma$ . Antigen  
535 release from apoptotic  $\beta$ -cells may induce PD-1 up-regulation on CD8 T-cells  
536 upon which engagement with PD-L1 on  $\beta$ -cells and macrophages can, in turn,  
537 down-regulate CD8 T-cell activation. Together these will have an indirect ef-  
538 fect on  $\beta$ -cell destruction.

539

540 **Figure 2.** B cell depletion reveals key immune cell interplay within the pan-  
541 creas.

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

549

550 **Figure 3.** Proposed interactions between regulatory B-cell populations local  
551 islet inflammatory milieu in the NOD mouse.  
552 Bregs can be induced with a variety of stimuli including CD40 ligation, TLR  
553 ligands and cytokines IL-21, IL-35, IL-1 $\beta$  and IL-6. Plasmacytoid DCs (pDC)  
554 producing IFN $\alpha$  along with CD40 ligation also promote Bregs. Bregs can con-  
555 trol inflammation through the production of IL-10, IL-35 and TGF $\beta$  and the in-  
556 duction of Tregs. IL-10, IL-35 and TGF $\beta$  cytokines can all inhibit CD4 T effec-  
557 tor cells (Teff), cytotoxic CD8 T-cells and pro-inflammatory cytokines from  
558 dendritic cells (DCs), which in turn down-regulate T-cells. In protected mice,  
559 increased surface expression of CD40 and IL-10 production has been shown  
560 (red boxes) in the pancreatic islets, although we speculate as to the type of  
561 Breg present and how they are induced/maintained. Interactions in the islets  
562 between Bregs and invariant NKT (iNKT) cells may provide further regulation  
563 of diabetogenic T-cells. We also consider that IL-10-expressing Breg can in-  
564 fluence macrophage populations that are involved in immunoregulation and  
565 tissue remodeling.

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### Outstanding questions box

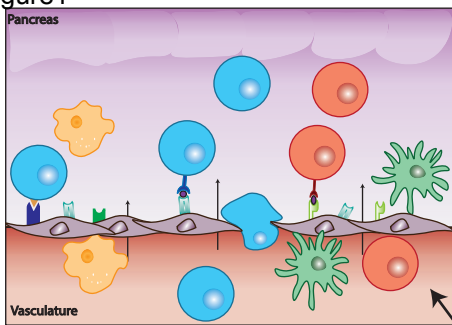
What are the triggers that lead to immune  $\beta$  cell destruction? Do the islets play an active role in this process? Can the process be halted before diabetes manifests?

What is the difference between islets with insulinitis but no  $\beta$  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?

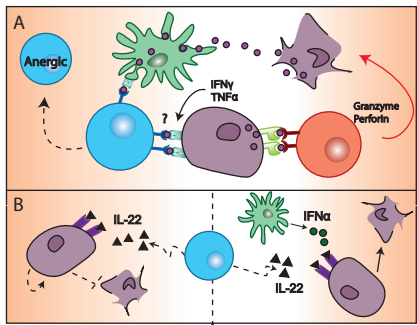
CD8 T cells are key effectors in  $\beta$  cell destruction. Is there any evidence of CD8 T cell exhaustion, indicated by upregulation of PD-1, and could this be a potential regulatory mechanism within the target tissue?

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?

Figure 1



2.



3.

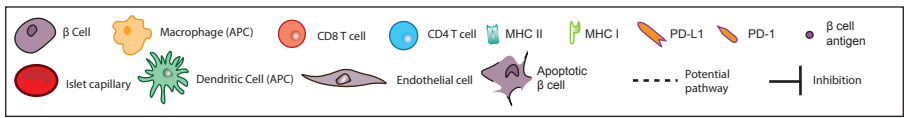
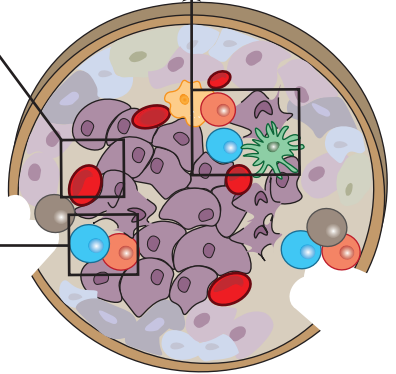
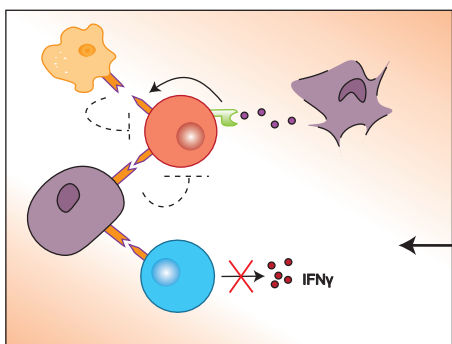
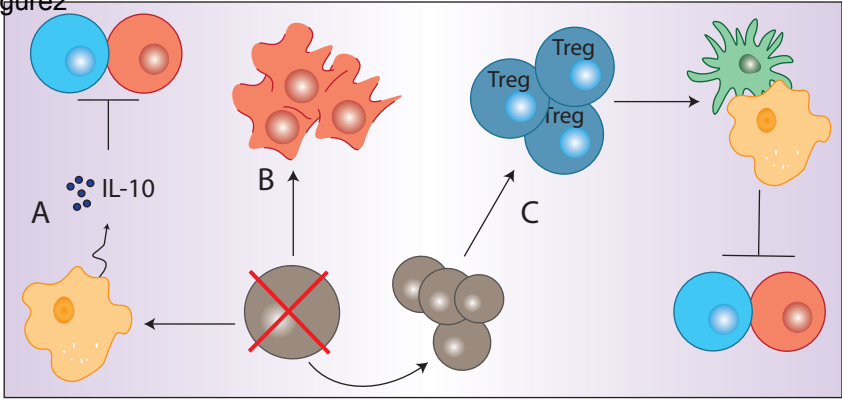


Figure 2



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



Figure3

