Is there a role for the adaptive immune system in pancreatic beta cell failure in type 2 diabetes?

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Abstract Pancreatic beta cell failure dictates the clinical onset of type 2 diabetes, with insulin secretion insufficient to overcome peripheral tissue insulin resistance. Over the past 5–10 years, a convincing case has emerged supporting the contribution of islet inflammation to this beta cell failure. IL-1 is central to this insult, impairing insulin secretion in preclinical and clinical studies. Further, islet-infiltrating macrophages are a major source of IL-1 and other cytokines in response to elevated levels of nutrients (glucose, saturated fatty acids), endocannabinoids and islet amyloid polypeptide (IAPP). In this issue of *Diabetologia*, Butcher et al have further characterised immune cell subsets present in islets from individuals with type 2 diabetes (DOI: [10.1007/s00125-013-3116-5](https://doi.org/10.1007/s00125-013-3116-5)). Increased numbers of CD45⁺ leucocytes were found in these islets compared with islets from healthy controls, with an elevated proportion of CD20⁺ B cells within the CD45⁺ population. Their data also suggest that absolute numbers of CD3⁺ T cells and CD11b⁺CD11c⁺ myeloid cells may be increased in islets from individuals with type 2 diabetes. While many aspects of islet inflammation await further exploration, the study from Butcher and colleagues suggests a role for immune cell-mediated inflammation early in disease pathogenesis, and supports the concept that targeting the immune system may slow continued beta cell demise in type 2 diabetes.

Keywords B cells · Inflammasome · Inflammation · Insulin · Interleukin-1 · Macrophage · Pancreatic beta cell · Pancreatic islet · T cells · Type 2 diabetes

Abbreviations

HFD	High-fat diet
hIAPP	Human islet amyloid polypeptide
IAPP	Islet amyloid polypeptide
IL-1Ra	IL-1 receptor antagonist
IEQs	Islet equivalents

World Diabetes Day on November 14, 2013 once again highlighted the global health and economic impact of this disease. Current estimates indicate that 382 million people are living with diabetes, and 592 million are predicted to be diagnosed with diabetes by 2035 [1]. These statistics are frightening, considering that—only 10 years ago—the International Diabetes Federation predicted that 333 million people would be diagnosed with the condition by 2025 [2], a figure that has now been surpassed.

Ninety per cent of diabetic individuals have type 2 diabetes, which is a complex disease resulting from the interaction between predisposing genes and an unhealthy environment. Clinical onset occurs when pancreatic beta cells fail in the face of insulin resistance, which is likely to be due to a combination of impaired insulin secretion and reduced beta cell mass [3]. Several mechanisms have been proposed to explain this failure, with recent attention falling on a chronic inflammatory process deleterious to beta cell insulin secretion [4].

It has long been appreciated that chronic activation of the innate immune system is associated with type 2 diabetes [5, 6]. Preclinical evidence now suggests that many organs

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critical to the regulation of glucose homeostasis, including the pancreatic islets of Langerhans, show signs of a chronic inflammatory process. Analysis of human tissue has indicated the presence of infiltrating CD68⁺ macrophages in islets in individuals with type 2 diabetes [7, 8], together with increased expression of macrophage chemoattractants (CCL2, CCL13) in laser-captured beta cells [9]. Increased expression of IL-1 β has also been found in autopsy pancreases from type 2 diabetic individuals [10]. Indeed, recent clinical trials targeting IL-1 have confirmed that inflammation contributes to beta cell failure in humans with this disease. These include our original study of the IL-1 receptor antagonist (IL-1Ra) in type 2 diabetes [11], industry sponsored studies using IL-1 β -specific antibodies [12] and a recent clinical study in non-diabetic volunteers with the metabolic syndrome [13]. The latter study showed that IL-1Ra improved measures of beta cell function with no effect on insulin resistance as assessed by hyperinsulinaemic–euglycaemic clamping [13], supporting our observations [11]. Because beta cell dysfunction can precede the clinical onset of type 2 diabetes, this suggests a very early role for IL-1-driven beta cell dysfunction in type 2 diabetes. It also confirms the islet-specific role of IL-1 in this disease, perhaps due to inflammasome activation by islet-localised amyloid deposits [14, 15] and/or high sensitivity of the beta cell to IL-1 due to high IL-1 receptor expression [16].

Despite these advances, there remain a number of unanswered questions with respect to the contribution of immune cells to beta cell function in type 2 diabetes. What are the phenotypes of resident and infiltrating islet macrophages in type 2 diabetes? Are other innate or adaptive immune cells involved in the regulation of beta cell function? Some of these questions have started to be addressed in rodent models of type 2 diabetes. An elegant study by Eguchi and colleagues recently demonstrated that infusion of ethyl palmitate in mice impaired beta cell function via toll-like receptor 4 (TLR4) signalling [17]. This study also showed a direct contribution of macrophages to beta cell dysfunction. Proinflammatory M1 macrophages were recruited to islets in mice infused with ethyl palmitate, in the *db/db* mouse, and in the KK Δ y mouse. Macrophage depletion in vivo in all these models increased *Ins* and *Pdx1* mRNA expression in islets and increased glucose-stimulated insulin secretion in vivo and in isolated islets ex vivo [17].

In another recent study, Jourdan and colleagues found higher levels of endocannabinoids in islets of the Zucker diabetic fatty rat compared with islets from lean rats [18]. Peripheral CB1 cannabinoid receptor blockade reduced islet inflammation and improved beta cell insulin secretion with no effects on insulin resistance, as confirmed by hyperinsulinaemic–euglycaemic clamping. Macrophage depletion in vivo in Zucker diabetic fatty rats paralleled these effects, and macrophage-specific CB1 receptor deletion confirmed the role of this receptor in the deleterious effects of proinflammatory macrophages on beta cells [18].

Finally, recent work led by us confirmed that transgenic mice expressing human islet amyloid polypeptide (hIAPP) in beta cells have increased IL-1 β expression in their islets, localised to macrophages [15]. hIAPP, which aggregates to form islet amyloid deposits in type 2 diabetes (unlike the non-amyloidogenic rodent form), was recently identified as a novel activator of the NOD-, LRR- and pyrin domain-containing protein (NLRP3) inflammasome (a multi-protein complex that generates active IL-1 β from pro-IL-1 β) in macrophages and dendritic cells [14]. However, only when transgenic mice expressing hIAPP in beta cells were fed a high-fat diet (HFD) did beta cell dysfunction become evident. This was associated with increased islet macrophage IL-1 β expression in the face of reduced IL-1Ra expression, the latter due to HFD feeding [15]. Depletion of resident islet macrophages in vivo reduced islet IL-1 β expression, improved beta cell insulin secretion as measured in isolated islets, and improved glucose tolerance in these mice [15]. Macrophage depletion also improved glycaemia and reduced islet IL-1 β expression in hIAPP transgenic mice with insulin resistance and obesity caused by expression of the agouti viable yellow allele [15]. Thus, IAPP-activated islet macrophages are directly linked to beta cell dysfunction in mice with features of type 2 diabetes. Overall, these publications strongly implicate islet macrophages as critical mediators of beta cell dysfunction in type 2 diabetes.

However, rodent models of disease are no substitute for human tissue, and in this issue of *Diabetologia*, Butcher and colleagues performed the most definitive characterisation to date of the types of immune cell that are present in the islets of humans with type 2 diabetes [19]. The availability of human islet preparations from individuals with type 2 diabetes is very limited, making it difficult to understand the pathophysiology underlying beta cell failure in this disease. In this study, human islets isolated from 21 individuals without diabetes were compared with those from 18 individuals with type 2 diabetes, an impressive sample size well matched for islet purity and clinical characteristics other than BMI (age, sex, race, cause of death). Impaired glucose-stimulated insulin secretion in islets from individuals with type 2 diabetes was confirmed by islet perfusion, and increased *TNF* and *CCL2* mRNA expression was found at the whole islet level, correlating with severely impaired beta cell function. Interestingly, expression of *ALOX12* mRNA, encoding 12-lipoxygenase (an enzyme that catalyses oxygenation of arachidonic acid in the eicosanoid synthesis pathway), was only increased in islets demonstrating mild impairments in insulin secretion.

Investigation of total leucocyte numbers indicated that approximately twofold more CD45⁺ leucocytes were present per 1,000 islet equivalents (IEQs) in islets from individuals with type 2 diabetes. CD45⁺ cell number positively correlated with beta cell function and *ALOX12* expression, and not with

BMI, *CCL2*, or *TNF* expression. Further analysis of immune cell subtypes showed that islets have resident myeloid ($CD11b^+CD11c^+$) cells, T cells ($CD3^+$), and B cells ($CD20^+$). Most islet leucocytes were T cells (~25–30% of $CD45^+$ cells), followed by $CD11b^+$ myeloid cells (~15% of $CD45^+$ cells), followed by B cells (~2.5% of $CD45^+$ cells). The percentages (of $CD45^+$ cells) of $CD3^+$ T cells, $CD11b^+$ cells, and $CD11b^+CD11c^+$ cells were unchanged between groups. $CD68^+$ macrophages were not assessed, so it is unclear what proportion of $CD11b^+$ cells were $CD68^+$, or if $CD11b^+CD11c^+$ cells indeed represent a dendritic cell population that is $CD68^-$. However, a 2.2-fold increase in the proportion of $CD20^+$ B cells relative to $CD45^+$ cells was found in islets from individuals with type 2 diabetes.

A number of interesting observations are worth highlighting from this study. First, $CD45^+$ leucocytes and *ALOX12* expression were increased in islets showing mild rather than severe beta cell impairment. This suggests a role for immune cell-mediated inflammation early in disease pathogenesis, and supports the clinical findings of van Asseldonk and colleagues [13] and our initial observations in the HFD-fed mouse [7]. It also highlights the dynamic nature of tissue inflammation. The nature and extent of the inflammatory response depend on numerous factors including time of disease onset, age, genetics, sex and the state of beta cell function. With respect to this, longitudinal studies on islet inflammation in rodent models of this disease will be informative. Indeed, if greater numbers of human islet samples become available, together with associated clinical history, it will be helpful to correlate islet function and inflammation (including immune cell subtypes, activation state and function) with duration of disease, medication and autoantibody status.

Second, because total $CD45^+$ cells/1,000 IEQs were increased (with a change in proportion noted only for B cells, which comprise ~2.5% of $CD45^+$ cells and do not account for the total increase in this population), absolute numbers of T cells, macrophages/dendritic cells and B cells all appear to be increased in islets from donors with type 2 diabetes, with possible contributions from other uncharacterised haematopoietic cells such as granulocytes, natural killer (NK) cells and other innate lymphoid cells. The greatest increase was seen in B cells. Thus, in addition to the well-supported role of macrophages in beta cell failure in type 2 diabetes, the study by Butcher and colleagues suggests a role for B cells (and potentially T cells and other myeloid cells) in the pathophysiology of this disease. B cells were found to induce insulin resistance during diet-induced obesity in mice [20]. Recent human data showed that circulating B cells are hyperactivated in individuals with type 2 diabetes and elicit T cell-derived proinflammatory IL-17 production in T cell/B cell co-cultures via contact-dependent mechanisms [21]. Monocytes are also required to maintain the proinflammatory Th17 signature in type 2 diabetes [22]. Interestingly, increased numbers of circulating T cells

reactive to islet proteins are detectable in some individuals with type 2 diabetes [23]. Whether islet B cells (or macrophages) also regulate proinflammatory cytokine production by islet T cells, or affect other features of islet T cell activation in type 2 diabetes, awaits investigation. Indeed, further characterisation of the activation state of macrophages (M1 vs M2), and T and B cells, is warranted in addition to further classification and functional analyses of immune cell subsets (e.g. naive vs memory $CD4^+/CD8^+$ T cells, regulatory T cells [Tregs], B1 vs B2 cells, plasma cells and regulatory B cells). Finally, information regarding the antigen specificity of T and B cell receptors within the islets will be important. Such characterisation will be critical to determining whether increases in specific immune cell subsets represent an adaptive response to islet damage rather than an instigation of an inflammatory response.

The study by Butcher and colleagues has highlighted some intriguing changes in innate and adaptive immune cell types in islets in type 2 diabetes; however, we are at the tip of the iceberg with respect to understanding the inflammatory process ongoing in islets in this disease. It is hoped that further characterisation of islet inflammation will lead to novel targeted therapies to help prevent the decline of beta cell mass and function in type 2 diabetes.

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