Macrophages, cytokines and β-cell death in Type 2 diabetes

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Abstract
The pathology of islets from patients with Type 2 diabetes displays an inflammatory process characterized by the presence of immune cell infiltration, cytokines, apoptotic cells, amyloid deposits and, eventually, fibrosis. Indeed, analysis of β-cells from patients with Type 2 diabetes displays increased IL-1β (interleukin 1β) expression. Furthermore, increased islet-associated macrophages are observed in human Type 2 diabetic patients and in most animal models of diabetes. Importantly, increased numbers of macrophages are detectable very early in high-fat-fed mice islets, before the onset of diabetes. These immune cells are probably attracted by islet-derived chemokines, produced in response to metabolic stress, and under the control of IL-1β. It follows that modulation of intra-islet inflammatory mediators, particularly interleukin-1β, may prevent islet inflammation in Type 2 diabetes and therefore presents itself as a promising therapeutic approach.

Introduction
In this review, we present evidence for the presence of islet inflammation in patients with Type 2 diabetes. Indeed, the pathology of the islet in this condition is characterized by the presence of immune cell infiltration, cytokines, Fas up-regulation, nuclear factor-κB (NF-κB) activation, apoptotic cells, amyloid deposits and, eventually, fibrosis. Although some of these changes have already been discussed extensively elsewhere (for reviews see [1,2]), in the present article we focus on the role of IL (interleukin)-1β, immune cell infiltration and clinical consequences.

Locally produced IL-1β plays an integral role in the deleterious effects of high glucose concentration and a Type 2 diabetic milieu on human pancreatic islets
Most of the knowledge of the properties of the IL-1 family is derived from the field of immunology where IL-1β was reported to have some unique features not shared by other chemokines and cytokines [3,4]. Most notably, the signal transduction pathway via the IL-1 receptor (type I) is unusually effective. Indeed, fewer than ten molecules of IL-1β bound per cell can induce biological responses that could consequently be observed at concentrations 10–100-fold less than that of the dissociation constant. Because of its potent cytotoxic effect on cells [5], several ‘road guards’ tightly control the processing, release and receptor binding of IL-1β to target cells. First, unlike other secreted cytokines, IL-1β does not have a leader sequence and has to be processed from pro-IL-1β to IL-1β by intracellular inflammasomes before secretion [6]. Secondly, once secreted, IL-1β associates with binding proteins [7]. These IL-1β-binding proteins are also the reason conventional ELISA detection of IL-1β is problematic and leads to underestimation of IL-1β concentrations [8]. Thirdly, IL-1β-producing cells also synthesize their own antagonist IL-1RA (IL-1 receptor antagonist), which binds to the IL-1 receptor without having agonistic properties and thereby modulates inflammatory responses [9]. The low level of expression and the aforementioned ‘road guards’ render IL-1β protein difficult to detect [3].

The spectrum of IL-1β-producing cells is very wide: classically, IL-1β production was observed by activated immune cells, but it was also shown to be expressed constitutively and at very low levels by many different cell types such as skin keratinocytes and mucosal, hypothalamic, glial and vascular smooth muscle cells [9]. In islets, IL-1β expression has been demonstrated in resident islet lymphoid cells [10,11], ductal cells and islet vascular endothelial cells [12], as well as in insulin-producing β-cells [11,13,14]. It has been proposed that intra-islet expression of inflammatory cytokines, particularly IL-1β, contributes to β-cell glucotoxicity in the pathogenesis of Type 2 diabetes [13]. This hypothesis was based on observations of increased IL-1β expression in pancreatic sections of patients with Type 2 diabetes by immunofluorescence and by in situ hybridization, as well as in islets from the hyperglycaemic Psammomys obesus [13]. Furthermore, high glucose concentrations in vitro induced IL-1β release in most, but not all, human islet cell preparations, followed by impaired insulin secretion and apoptosis. In an attempt to elucidate the reason for this variable result, this study was repeated recently and expanded upon under defined conditions [15]. Cultured human islets exposed for 4 days to elevated glucose concentrations exhibited increased IL-1β mRNA levels in six out of 12 islet batches. Islet preparations where glucose did not increase

Key words: apoptosis, β-cell death, cytokine; islet inflammation, macrophage, Type 2 diabetes.

Abbreviations used: G-CSF, granulocyte colony-stimulating factor; UC, Urotensin-C; IL, interleukin; K-1RA, K-1 receptor antagonist; KC, keratinocyte chemoattractant; MIP-1α, macrophage inflammatory protein 1α; NF-κB, nuclear factor-κB.

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IL-1β mRNA displayed higher basal IL-1β mRNA levels than responsive preparations, and there was a significant negative correlation between basal IL-1β mRNA levels and glucose-stimulated IL-1β mRNA. Furthermore, the ability of elevated glucose to increase islet IL-1β mRNA was masked in the presence of increased concentrations of IL-1β. IL-1β expression was increased by IL-1β auto-stimulation in whole islet cultures and FACs-purified human β-cells and decreased by the addition of IL-1RA. Auto-stimulation of IL-1β mRNA was transient and NF-κB-dependent. High glucose concentrations regulated IL-1β dually, by decreasing endogenous IL-1RA expression and by increasing IL-1β mRNA via promotion of IL-1β auto-stimulation. Importantly, islet-derived IL-1β was shown to be biologically active, as reflected by the ability to induce the production of the inflammatory factors IL-8 and IL-6. Finally, a gene array analysis of near pure populations of β-cells obtained by laser-capture microdissection revealed increased IL-1β mRNA in patients with Type 2 diabetes compared with controls. The gene array screening was validated by real-time PCR, confirming IL-1β up-regulation in β-cells of patients with Type 2 diabetes.

Macrophages in islets of patients with Type 2 diabetes

Pancreatic islets from Type 2 diabetes patients are known to present with amyloid deposits, fibrosis and increased cell death [16–19]. Furthermore, and as mentioned above, cytokines and chemokines are released from islets exposed to metabolic stress, partly under the control of IL-1β. These events are typically associated with an inflammatory response, and it was therefore expected that the pancreatic islet in Type 2 diabetes is associated with immune cell infiltration. Surprisingly, it is only recently that the first study demonstrating increased islet macrophage infiltration in patients with Type 2 diabetes was published [20]. Increased islet-associated immune cells were also observed in a variety of animal models of this disease including the GK (Goto-Kakizaki) rat [21], the high-fat-diet fed and db/db mouse [20], and the Cohen diabetic rat [22]. Whether the presence of macrophages is causative of Type 2 diabetes islet pathology requires further investigation. Of note is the observation that increased numbers of macrophages were detectable very early in high-fat-fed mouse islets, before the onset of diabetes. Possibly, early infiltration of macrophages may be beneficial to islet function [20] and plasticity [23]. However, as the disease progresses, macrophages may become activated [22] and play a role in accelerating pancreatic islet cell dysfunction and death. The presence of macrophages may also be a consequence of β-cell death, acting to phagocytose dead islet tissue. To this end, macrophages were not detected in the vicinity of apoptotic cells [20].

The concept that macrophages are an integral part of the islet pathology in Type 2 diabetes and not merely scavenger cells is supported by their possible mechanism of attraction [20]. Indeed, increased islet-derived inflammatory factors are produced and released by islets exposed to a Type 2 diabetic milieu (high glucose and/or non-esterified fatty acids) and by islets isolated from high-fat-fed mice, including IL-6, IL-8, chemokine KC (keratinocyte chemoattractant), G-CSF (granulocyte colony-stimulating factor) and MIP-1α (macrophage inflammatory protein 1α). The specificity of this response was demonstrated by direct comparison with non-islet pancreatic tissue, which fails to display such a response to metabolic stress. Also, induction of islet cell death by other means (streptozotocin and staurosporine) does not increase chemokine release. Finally, IL-8 has emerged as a possible key mediator of immune cell attraction to human Type 2 diabetic islets, although this will need in vivo confirmation.

The role of IL-1β with respect to this Type 2 diabetic islet inflammatory response has recently been tested in vivo in mouse and GK rat islets and in vivo in the GK rat [24]. Indeed, increased islet-derived inflammatory factors [IL-6, chemokine KC, G-CSF, MIP-1α and MCP-1 (monocyte chemoattractant protein 1)] due to a diabetic milieu in vivo or in vitro (GK rat) could be reversed by IL-1RA treatment in vivo. Furthermore, using IL-1β-knockout mice, the effect of IL-1RA was confirmed to be specific to IL-1β [24]. Finally, treatment of the GK rat, a spontaneous model of Type 2 diabetes, with IL-1RA reversed this islet inflammatory phenotype in vivo and reduced hyperglycaemia (J.A. Ehses, M.-H. Giroix, G. Lacraz, F. Schmidlin, J. Coulaud, S. Akira, F. Homo-Dalarche and M.Y. Donath, unpublished work). Thus IL-1β appears to be a master regulator of islet inflammation in Type 2 diabetes.

Clinical consequences

Understanding that a key role of IL-1β in the pathogenesis of diabetes is to regulate the pancreatic β-cell, and given the availability of the human recombinant IL-1RA, we conducted a clinical trial of IL-1 receptor antagonism in Type 2 diabetes [25]. A total of 70 patients were randomized to subcutaneous injection of anakinra once daily or placebo. At 13 weeks, glycated haemoglobin was significantly lower in the anakinra than in the placebo group. β-Cell secretory function was enhanced, and there was a reduction in the proinsulin to insulin ratio, an indicator of β-cell stress. As highlighted recently [26], this might be a disease-modifying treatment for Type 2 diabetes and the next generation of mechanistically targeted therapeutic agents. Ongoing and planned studies will evaluate the benefit of IL-1 antagonists with prolonged half-lives, allowing weekly or monthly injections, and therefore greater improvement of glucose metabolism.

Summary and working hypothesis

On the basis of the above, we propose a hypothesis for the development of islet inflammation in Type 2 diabetes, and consecutive failure to produce sufficient insulin (Figure 1). According to this hypothesis, metabolic stress (high glucose and non-esterified fatty acid concentrations) and leptin [27] induce β-cell production of IL-1β. Locally produced IL-1β will enhance its own production via auto-induction in addition to inducing production of inflammatory factors,
including IL-1β. This will attract macrophages which may become activated in a paracrine manner by islet-derived cytokines, such as IL-1β. In turn, activated macrophages will produce even more IL-1β, which will contribute to β-cell secretory failure and death. This may be precipitated by unprocessed amyloid [16,17], which will accumulate into post-inflammatory fibrotic deposits.

References
9 Mandrup-Poulsen, T (2007) Cytokines and β-cell function by unprocessed amyloid [16,17], which will accumulate into post-inflammatory fibrotic deposits.

Figure 1 | Islet inflammation in Type 2 diabetes
For explanation, see the Summary and working hypothesis section in the text.

Metabolic stress
(hyperglycaemia, dyslipidaemia, adipokines)

β-cell
Secretory failure / Apoptosis
Amyloid
Macrophage
Aβ

Il-1β


15 Böni-Schnetzler, M., Marselli, L., Ehses, J.A., Marchetti, P., Weir, G.C. and Donath, M.Y. (2007) IL-1β expression is induced by glucose and IL-1β auto-stimulation, and increased in β-cells of type 2 diabetes. Diabetes 56 (Suppl.), 413A

Received 2 January 2008
doi:10.1042/BST0360340