Islet Inflammation Impairs the Pancreatic β-Cell in Type 2 Diabetes

Onset of Type 2 diabetes occurs when the pancreatic β-cell fails to adapt to the increased insulin demand caused by insulin resistance. Morphological and therapeutic intervention studies have uncovered an inflammatory process in islets of patients with Type 2 diabetes characterized by the presence of cytokines, immune cells, β-cell apoptosis, amyloid deposits, and fibrosis. This insulin is due to a pathological activation of the innate immune system by metabolic stress and governed by IL-1 signaling. We propose that this insulitis contributes to the decrease in β-cell mass and the impaired insulin secretion observed in patients with Type 2 diabetes.

Major progress has been achieved in our understanding of the pathogenesis of Type 2 diabetes (3, 13, 34, 52). Over-nutrition and inactivity promote insulin resistance. To cope with this increased demand of insulin secretion, the pancreatic islet needs to enhance its secretory activity. In most individuals, such an adaptation does occur during early stages of increased metabolic stress (characterized by over-nutrition). Although successful adaptation of the β-cell permits maintenance of normal metabolism throughout life in most subjects, this adaptation eventually fails in some individuals, depending on the genetically determined ability of the β-cell to adapt and the severity of the resistance to insulin. The reasons for this failure to maintain sufficient insulin secretion are a combined decrease in β-cell mass and defective insulin secretion. Several mechanisms have been proposed to explain this failure, including ER stress, oxidative stress, amyloid deposition, lipotoxicity, and glucotoxicity (28, 33, 51, 54, 65). Interestingly, all these factors may induce an inflammatory response, whereas some may be the result of the inflammation (16, 17, 19, 31). Initially, an inflammatory response is probably deployed to promote β-cell repair and regeneration. However, as it becomes chronic, the situation gets complicated by, e.g., the activation of auto-inflammatory processes that may then become deleterious. Interestingly, similar auto-inflammatory processes occur in insulin-sensitive tissues. Therefore, Type 2 diabetes can be considered an auto-inflammatory disease. Consequently, it can be treated by agents blocking this vicious cycle such as IL-1 antagonists, or more downstream by NF-κB modulation via salsalate or similar compounds (15, 59).

In this article, we will review the evidence for insulitis in Type 2 diabetes, the mechanisms inducing this inflammatory process, its physiological and pathological role, and the therapeutic consequences.

Evidence for an Islet Inflammatory Process in β-Cell Failure During Type 2 Diabetes

In 1967 it was first proposed that Type 2 diabetes is not solely due to insulin resistance but also to a failure of the insulin producing β-cell to secrete an adequate amount of insulin (5). Based on numerous investigations, it is now evident that impaired insulin secretion is not only an important etiological factor in the pathogenesis of the disease but also the driving force dictating the dynamics of the disease. Indeed, although insulin resistance is present in all stages from pre-diabetes to overt diabetes, it remains constant in a single individual as long as his or her body weight remains unchanged. In contrast, the onset of diabetes and its progression is largely determined by the progressive failure of the pancreatic islet. At a pre-diabetic stage, insulin production will increase to adapt to the enhanced demand. When this adaptation fails, diabetes occurs. This failure then continues to progress, and affected individuals will require increasing anti-diabetic treatment, until eventually all Type 2 diabetics require exogenous insulin to control their hyperglycemia. In an attempt to understand the underlying cause of this progressive failure, several mechanisms have been described. It appears that in all instances they constitute various components of an inflammatory process. The first evidence for an inflammatory process in the pancreatic islet arose from the observation that hyperglycemia induces β-cell apoptosis (14). By unravelling the underlying mechanism, it turned out that high glucose concentrations induce the Fas receptor, which in turn is upregulated via glucose-induced IL-1β production (41, 43, 44). Similarly, recent evidence shows that fatty acids also promote an inflammatory response (Refs. 1, 21, and see below). More downstream, ER stress and oxidative stress also appear linked to inflammatory events. In support of
insulins in Type 2 diabetes, elevated numbers of immune cells have been detected in islets of patients with Type 2 diabetes in conjunction with increased levels of cytokines and chemokines (2, 21, 53). Of note, every animal model of Type 2 diabetes investigated to date displays islet immune cell infiltration (19, 21). Furthermore, a strong argument for the occurrence of an inflammatory process in islets is the well described fibrosis observed in tissue sections of patients with Type 2 diabetes, characterized by amyloid deposits. Indeed, fibrosis is a hallmark of the end stage of a chronic inflammatory process. Although the concept of insulitis is recent in Type 2 diabetes, it is well established in Type 1 diabetes and was considered as pathogenicomic. Although the precise aetiology of the insulitis in both diabetes types remains to be fully understood, differences certainly exist; for example, Type 1 diabetes is a more autoimmune-mediated process. However, a common final effector pathway seems to be activated in both types of diabetes (17). Keeping in mind that metabolic stress is a more recent threat than viruses or other possible causes of Type 1 diabetes, it is not surprising that islets respond with similar mechanisms developed over their long period of evolution. Of interest is the predominate role of IL-1β, which is upregulated in islets of patients with both Type 1 and Type 2 diabetes (2, 43, 45). This master cytokine regulates numerous cytokines and also chemokines (Refs. 12, 20, and see below). Thereby, it contributes to the recruitment of immune cells, implo-

menting a broad inflammatory response. Of note is that IL-1β will also induce itself in β-cells, engendering a vicious cycle (Ref. 2, and see below). Thus insul

tis may be considered as an integral component of the pathology observed in Type 2 diabetes, and its dependence on IL-1 supports the concept of the auto-

inflammatory nature of the disease.

Regulation of IL-1β Expression by Nutrients in Human Islets

Elevation of circulating nutrients such as glucose and free fatty acids (FFAs) induce an inflammatory process within numerous tissues in the body (32, 68). The first demonstration that a high glucose concentration indeed induces IL-1β release was obtained with human islets (43), with similar observations following in rat islets (24), retinal cells (70), and human monocytes (8). Moderately elevated glucose concentrations (11 mM) were sufficient to induce transcriptional ac-
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sion rates to changes in local IL-1β expression (1) and the release of various cytok- and chemokines (21).

But how do these distinct and "nonspecific" nutri-
tons lead to elevated IL-1β expression? The well described IL-1β-positive feedback loop with IL-1β auto-stimulation via its receptor plays a pivotal role in the regulation of intra-islet IL-1β expression pro-

ved by nutrients. Noteworthy, we observed the highest levels of IL-1β receptor 1 expression in mouse islets and in an insulin-secreting β-cell line compared with 22 other rodent tissues (1). These data suggest that the pancreatic β-cell may be uniquely sensitive to changes in local IL-1β concentration due to its high

number of IL-1β receptors (1, 55).

Although these findings are compatible with a role for the IL-1 system as an amplifier of pro-inflammatory mediators, we leave it to the question of how glucose or FFAs trigger and maintain this process. This is currently an open question, particularly for glucose, whereas for FFAs several mechanisms were postulated. It has been hypothesized that FFAs stimulate pro-inflammatory factors via direct activation of the lipid-sensing TLR2 and TLR4 in various cell types (39). Results with islets from TLR KO mice indeed show that FFA-induced pro-inflammatory factors are partly TLR dependent. Human islets express functional TLR2 and TLR4 (1, 64), and their activation results in increased expression of IL-1β and various cytok- and chemokines. Again, the TLR2/4 response is further

amplified by IL-1Ra (9), thereby reducing the stimulatory effects of FFAs on proinflammatory mediators (49). In human islets, spe-
cific TLR2 and TLR4 agonists induce IL-1β; however, the strongest inducer of IL-1β expression is IL-1β itself, which increases its own expression by an auto-

stimulatory mechanism. IL-1β auto-stimulation was particularly pronounced in purified β-cells compared with whole islets, which consist of different cell types (2). Furthermore, it was NF-κB dependent and could be interrupted by blocking the IL-1 receptor with the specific IL-1 receptor antagonist (IL-1Ra) (9), neutralizing the receptor ligand or by deleting the intracellular receptor docking protein required for signal transduc-

ion (Myd88). Using these tools, we could demonstrate that both elevated glucose concentrations and FFAs increase IL-1β expression by promoting IL-1β auto-

stimulation via IL-1 receptor activation. Thus IL-1β auto-stimulation via its receptor plays a pivotal role in the upregulation of intra-islet IL-1β expression pro-

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IL-1 is a Master Regulator of Tissue and Islet Inflammation in Type 2 Diabetes

It has been known for a long time that obesity and Type 2 diabetes are associated with chronic activation of the innate immune system (50). Indeed, this activation appears to be systemic, given the increased levels of circulating cytokines and chemokines correlating with the disease and their potential in predicting Type 2 diabetes onset (30, 61). Local tissue inflammation is increasingly recognized in the pathology of both obesity-associated insulin resistance and increasing prevalence of Type 2 diabetes (16, 68). In all cases, this local tissue inflammation is characterized by increased tissue-infiltrating macrophages are an underlying feature. We recently extensively characterized the islet inflammatory process in the GK rat, showing increased islet expression of IL-1β and other pro-inflammatory cytokines (IL-6, TNFα), increased expression of a number of chemokines (CXCL1/ KC, MCP-1, MIP-1α, 1β), together with immune cell infiltration in the islet (20). Since an important property of IL-1β in a pro-inflammatory disease is to increase the local expression of chemokines and adhesion molecules (12), we investigated the role of IL-1 in islet inflammation in the GK rat, with a focus on the pancreatic islet. Elevated IL-1β mRNA levels were found in 2-month-old GK rat islets and liver, with no increases in skeletal muscle or adipose tissue. To assess the biological impact of this finding, we blocked IL-1β activity with IL-1Ra in vitro and in vivo. IL-1Ra was able to suppress GK islet IL-6 and chemokine secretion (CXCL1/KC, MCP-1, and MIP-1α) in vitro, indicating that IL-1 activity is partially responsible for driving GK islet cytokine and chemokine expression. Treatment of GK rats with IL-1Ra, administered via either mini-osmotic pumps or subcutaneous injections, reduced fed hyperglycemia over the 4 wk of treatment. This was due to both peripheral and islet anti-inflammatory effects impacting on both insulin sensitivity and β-cell function in a dose-dependent manner. Indeed, analysis of liver and islet inflammation in high-dose IL-1Ra-treated animals (100 mg/kg day IL-1Ra) indicated that IL-1Ra not only amplified by IL-1β auto-stimulation. Thus the IL-1 system in islets not only amplifies the response to circulating nutrients but also to specific TLR agonists. Interestingly, it was observed that obese subjects and rodents display elevated levels of circulating gut flora-derived endotoxins that could potentially trigger TLRs (4, 7), or, alternatively, inflammatory factors derived from other organs could stimulate TLRs in islets. To this end, it was recently shown that serum amyloid A binds to TLR2 (6), which is prominently expressed on purified human and rodent β-cells (1). Elsses, unpublished data, and our own observations, suggest that the role of IL-1β, however, is unknown until recently.

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FIGURE 1. Regulation of IL-1β in islets by metabolic stress

Elevated glucose and FFA initiate the expression and release of low levels of IL-1β (stimulation) required for the adaptive response of islet cells. Continuous or prolonged stimulation with nutrients (metabolic stress) will lead to the activation of the IL-1β leading to a further increase of IL-1β by an autostimulatory process and to the production of IL-1β-dependent cytokine and chemokines expression (amplification). Autocrine and paracrine activation will precipitate of a broad inflammatory response including elevated cytokine and chemokine production, attraction of macrophages, apoptosis and amyloidosis, fibrosis, and impaired insulin secretion (precipitation).
IL-6 Regulation of the Pancreatic α-Cell

Pancreatic islet pathology in Type 2 diabetes is characterised not only by reduced β-cell function and mass but also by increased proportion of α-cells relative to β-cells, together with relative hyperglucagonemia due to α-cell dysfunction (10, 63, 71). Having recently found elevated IL-6 levels in pancreatic islets of models of Type 2 diabetes (20, 21), we re-explored its role in the regulation of α- and β-cells (22).

We confirmed the previously published detrimental effects of IL-6 on insulin secretion in both human and rodent islets in vitro (35). Beyond this, however, we found that the glucagon-producing α-cell is a primary target of IL-6. Islet α-cells express the IL-6 receptor and functionally respond to IL-6 kinetically prior to β-cells. IL-6 was found to increase glucagon secretion under fasting conditions (low glycemia) in isolated islets and when systemically administered in vivo. Furthermore, IL-6 promoted α-cell proliferation and prevented α-cell apoptosis due to metabolic stress while exacerbating β-cell apoptosis. Thus, in contrast to the negative effects on the β-cell, IL-6 acts as a growth and survival factor and promotes glucagon secretion in the α-cell.

Further studies in vitro supported this hypothesis. For this, we studied the role of IL-6 in the setting of obesity and insulin resistance by feeding wild-type and IL-6 KO mice HFD. Although HFD increased systemic IL-6 levels in wild-type mice, this did not occur in IL-6 KO animals. Most striking, IL-6 KO mice did not show increased α-cell mass under these conditions, demonstrating that IL-6 signaling is necessary for the stimulating effect of HFD on α-cell mass. Consistent with these α-cell-specific findings, IL-6 KO animals displayed reduced fasting glucagon levels relative to wild-type animals on HFD. However, in contrast to the expected improvement in glucose tolerance in these animals, IL-6 KO mice on HFD displayed fed hyperglycemia, impaired glucose tolerance, and impaired glucose-stimulated insulin secretion compared with diet-matched wild-type animals. This effect has previously been published using IL-6 KO animals from the same source that we used (11). Since insulin sensitivity was similar among genotypes, the phenotype could be attributed to impaired β-cell function and suggests a role for the α-cell in maintaining β-cell function in vivo. Indeed, recent reports support this notion. Thus α-cell expression of prohormone convertase 1/3 (allowing the α-cell to produce GLP-1) or overexpression of the glucagon receptor on β-cells promotes improved insulin secretion (25, 69), thus supporting the hypothesis that the pancreatic α-cell can regulate β-cell function in vivo in a paracrine manner. That β-cell mass expansion is an important feature of the pathology of Type 2 diabetes was shown in Type 2 diabetic baboons, in which increased α-cell proliferation and mass together with hyperglucagonemia were recently described as part of the islet remodeling process (26).

Thus IL-6 regulation of the pancreatic α-cell represents a novel endocrine loop in the regulation of glucose homeostasis. Although it appears that this mechanism may exist to compensate for impaired β-cell function in the setting of obesity and/or Type 2 diabetes, it cannot be ignored that elevated IL-6 levels may also drive the relative hyperglucagonemia of diabetic patients. How this pathway contributes to the IL-6-mediated effects during physiology [e.g., during exercise, where systemic IL-6 levels are elevated (23)] or during the pathophysiology of obesity and diabetes in humans remains to be examined.

Role of Insulitis in Obesity and Type 2 Diabetes: From Islet Adaptation to Failure

Inflammation is not in itself a disease but a manifestation of a disease. Initially, it has beneficial effects such as preventing the deleterious effects of obesogenic exposure. However, if unchecked, inflammation may exacerbate diabetes, producing a vicious cycle. In the case of type 1 diabetes, islet α-cells may adapt to complex inflammatory processes by upregulating its functional hyperglycemia-promoting mechanisms (25, 69), thus supporting the hypothesis that the pancreatic α-cell is an important feature of islet remodeling. That IL-1β plays a central role in orchestrating this inflammatory process (25, 69), thus supporting the hypothesis that the pancreatic α-cell is an important feature of islet remodeling, has been evidenced by our recent reports that IL-1Ra treatment of the GK rat also reduced islet IL-1β expression itself, supporting that the auto-stimulatory role of IL-1β in the islet (as described above) plays an important role in the inflammatory process in vivo.

In summary, both our in vitro data on human and rodent islets and our in vivo studies on the GK rat support a central role for IL-1α in driving the expression of tissue proinflammatory cytokines and chemokines that subsequently attract immune cells to islets. This inflammatory process leads to impairment of β-cell function in vivo. In particular, we have demonstrated that markers of endothelial cell activation, such as adhesion molecule expression (Vcam1), were also suppressed by IL-1Ra treatment (36). Finally, reduction in islet inflammation correlated with improvements in proinsulin to insulin processing in IL-1α-treated animals. This effect has previously been published using IL-6 KO animals from the same source that we used (11). Since insulin sensitivity was similar among genotypes, the phenotype could be attributed to impaired β-cell function and suggests a role for the α-cell in maintaining β-cell function in vivo. Indeed, recent reports support this notion. Thus α-cell expression of prohormone convertase 1/3 (allowing the α-cell to produce GLP-1) or overexpression of the glucagon receptor on β-cells promotes improved insulin secretion (25, 69), thus supporting the hypothesis that the pancreatic α-cell can regulate β-cell function in vivo in a paracrine manner. That β-cell mass expansion is an important feature of the pathology of Type 2 diabetes was shown in Type 2 diabetic baboons, in which increased α-cell proliferation and mass together with hyperglucagonemia were recently described as part of the islet remodeling process (26).

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as preventing spread of infection or promoting regeneration. However, if prolonged or excessive, it may exacerbate disease by tissue destruction. It is likely that, in the case of overt inflammation in Type 2 diabetes, similar phenomena occur. Indeed, the endocrine pancreas has a remarkable capacity to adapt to conditions of increased insulin demand as encountered in obesity and pregnancy by increasing its functional mass. This may be triggered by limited hyperglycemic events that would provoke \( \beta \)-cell production of very low concentrations of IL-1\( \beta \) followed by a self-perpetuating state. At low concentrations of IL-1\( \beta \) and in the presence of FLIP, Fox engagement would lead to \( \beta \)-cell proliferation and enhanced function via NF-\( \kappa \)B and PDX1 (56). Indeed, NF-\( \kappa \)B has been reported to have beneficial effects on both insulin secretion and \( \beta \)-cell proliferation (27, 48). This response becomes excessive due to prolonged or repetitive exposure to nutrients, this initially adaptive process may become deleterious. IL-1\( \beta \) will then decrease FLIP and Fox engagement and switch to promote deleterious effects. In addition, IL-1\( \beta \) initiates the vicious cycle of inducing itself and promoting an enhanced release of chemokines, which leads to recruitment of macrophages (2). Possibly, these macrophages then produce a high amount of IL-1\( \beta \) (67) and other cytokine factors, which then impair the function of the \( \beta \)-cell. It is important to note that these mechanisms may be responsible for both decreased \( \beta \)-cell mass and impaired function. Depending on duration and magnitude of the effect as well as the individual regenerative capacity, the functional impairment may predominate over the more definitive decrease in \( \beta \)-cell mass. Interestingly, such an auto-inflammatory process occurs also in the insulin-sensitive tissues and in end organs such as kidney, eye, and the vasculature. Therefore, tissue inflammation may not only underlie beta-cell failure and insulin resistance but also participate directly in the complications of diabetes, such as nephropathy, retinopathy, and cardiovascular disease.

Clinical Validation and Therapeutic Consequences

Based on the above-described predominant role of IL-1\( \beta \), we initiated clinical trials of IL-1 antagonism in Type 2 diabetes. In a proof-of-concept study, the naturally occurring antagonist of IL-1\( \beta \), IL-1\( \beta \)-12, was tested in a placebo-controlled study of 70 patients (38). At 13 wk, glycated hemoglobin was significantly improved due to enhanced \( \beta \)-cell secretory function. Remarkably, the improvement promoted by IL-1\( \beta \) blockade lasted for at least 39 wk following treatment withdrawal (37), thus reflecting the disease-modifying potential of this therapy. Novel therapeutic approaches designed to modulate IL-1 are under development with agents lasting a month or longer (18). Rather than just palliating hyperglycemia, IL-1 antagonism may represent a novel treatment principle directed against the pathogenesis that underlies diabetes mellitus, whereby the progressive decline in functional \( \beta \)-cell mass could be prevented or even reversed. Due to the auto-inflammatory nature of the whole metabolic syndrome and based on preclinical studies, it is expected that IL-1 antagonism will also enhance insulin sensitivity and prevent complications like blindness, cardiovascular events, and nephropathy. Numerous ongoing clinical studies are based on this assumption.

References


