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Review Series

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The Journal of Clinical Investigation

Islet inflammation in type 2 diabetes and physiology

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The finding of islet inflammation in type 2 diabetes (T2D) and its involvement in β cell dysfunction has further highlighted the significance of inflammation in metabolic diseases. The number of intra-islet macrophages is increased in T2D, and these cells are the main source of proinflammatory cytokines within islets. Multiple human studies of T2D have shown that targeting islet inflammation has the potential to be an effective therapeutic strategy. In this Review we provide an overview of the cellular and molecular mechanisms by which islet inflammation develops and causes β cell dysfunction. We also emphasize the regulation and roles of macrophage polarity shift within islets in the context of T2D pathology and β cell health, which may have broad translational implications for therapeutics aimed at improving islet function.

Introduction

Metabolic syndrome comprises a cluster of diseases associated with excess nutrition and insufficient physical activity. Studies over the last two decades have shown that chronic inflammation is a common and potentially unifying mechanistic cause of these diseases (1–4). Inflammation can be viewed as an evolutionarily selected protective response enabling the host organism to cope with stresses from external factors, and can be classified as acute or chronic (5). Acute inflammation is characterized by prominent local and systemic signs, as well as infiltration of the affected area by immune cells, mainly neutrophils. By contrast, chronic inflammation is characterized by less prominent local and systemic signs, enhanced tissue injury and fibrosis, and infiltration of the affected area mainly with monocytes/macrophages and lymphocytes (5).

Inflammation in the context of metabolic syndrome largely exhibits the characteristics of chronic inflammation and is thus often accompanied by tissue infiltration by monocytes/macrophages (6) and lymphocytes (7, 8). Type 2 diabetes (T2D) is a common and serious complication of metabolic syndrome, and although the disease can exist in isolation, many T2D patients meet the diagnostic criteria for metabolic syndrome (9). Given that chronic metabolic stress induced by excess nutrition was not a driving force during evolution, it is perhaps not surprising that inflammation in response to this stress eventually results in deleterious effects on tissue function and contributes to T2D pathology.

Insulin resistance and β cell dysfunction are the two major components of T2D pathology, and β cell function starts to decline even before the onset of impaired glucose tolerance (10, 11). Histologic changes characteristic of inflammation occur within the islets of T2D subjects, including immune cell infiltration (12–16), amyloid deposition (14, 17, 18), cell death, and fibrosis (18, 19). These reports suggest inflammation is involved in β cell dysfunction, though inflammatory pathologic changes have been observed in only a portion of T2D patients, suggesting that islet inflammation and its contribution to T2D pathology may vary among patients (12–14, 18).

Several rodent experimental models (20–23) as well as observations in humans (12–14) have made it clear that macrophages play a key role in the islet inflammation seen in T2D. The most well-studied mechanism by which islet macrophages cause β cell dysfunction is through secretion of IL-1β, and it has been demonstrated that interference with the IL-1 pathway relieves T2D and restores β cell function in both rodents (24, 25) and humans (26–28). Factors that stimulate islet macrophages to secrete IL-1β in vivo include human islet amyloid polypeptide (hIAPP) (20, 23), palmitate (21), and endocannabinoid (22). The possibility that other immune cell types are involved in islet inflammation in T2D remains to be confirmed; while one study reported an increase in the B cell number (15), other groups have not observed changes in the number of immune cell types including neutrophils, lymphocytes, and mast cells (refs. 12–16, 29, 30, and Table 1). It has also been suggested that some T2D patients develop islet autoimmunity during the course of disease and that contributes to β cell functional decline (31).

There is significant heterogeneity among macrophages in terms of both their function and origin in vivo, and the transition between functional states occurs along a continuum regulated by the microenvironment, especially in the context of sterile inflammation (32–34). Using the concept of M1-like and M2-like polarization of macrophages to describe an essentially heterogeneous population of tissue macrophages is a simplified operational framework (35). During islet inflammation, overall macrophage polarity shifts toward the proinflammatory classically activated M1-like phenotype, and this shift has been shown to contribute to β cell dysfunction in T2D mouse models (21–23). Additionally, alternatively activated M2-like macrophages, which include macrophages with antiinflammatory (36), pro-fibrosis (37), and pro-angiogenic (38) phenotypes, were recently found to be the key mediators of β cell proliferation during both development (39, 40) and adulthood (41–44) in mice.

In this Review we present an overview of the links between islet inflammation and β cell dysfunction in T2D, with a focus on the regulation of islet macrophage polarity through commu-

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Table 1. Analysis of immune cells in islets of T2D subjects

Study	Change in number of immune cells
Ehses et al. (12)	Increased $CD68^+$ macrophages
	No change in $CD3^+$ lymphocytes
	No change in neutrophils
Richardson et al. (13)	Increased $CD68^+$ macrophage
Kamata et al. (14)	Increased $CD68^+$ macrophages in subjects positive for islet amyloid deposits
Butcher et al. (15)	Increased $CD45^+$ leukocytes
	Increased $CD20^+$ B cells
	No change in $CD3^+$ T cells
Rodriguez-Calvo et al. (29)	No change in $CD8^+$ T cells
Martino et al. (16)	Increased macrophages (analyzed by electron microscopy)
	No change in lymphocytes
	No change in mast cells

nications among multiple cell types within the islet and its roles in T2D pathology and physiology. From this viewpoint we argue that immune cell function within islets is seamlessly regulated and thus plays an indispensable role in maintaining β cell health as well as serving as a primary source of proinflammatory cytokines that cause β cell dysfunction in T2D. Expanding our understanding of the cells that mediate islet inflammation may have broad translational implications for therapeutics to improve islet function.

Evidence for the involvement of islet inflammation and islet macrophage polarity shift in T2D

Initial evidence of immune cell infiltration of the pancreatic islets in T2D came from immunostaining analyses showing increased numbers of islet macrophages in rodent T2D models (the Goto-Kakizaki [GK] rat and the *db/db* [leptin-deficient] mouse; refs. 12, 45), as well as in human T2D patients (12, 13). Additionally, microarray profiling of human β cells obtained through laser-capture microdissection revealed a three-fold increase in the expression of chemokines *CCL2* and *CCL13* in T2D subjects compared with controls, providing molecular evidence of immune cell infiltration and islet inflammation in human T2D patients (46). These results suggest that islet inflammation in T2D is mainly driven by innate immunity, whereas inflammation in type 1 diabetes (T1D) is known to be mainly driven by adaptive immunity (47, 48). Several studies also showed that prolonged exposure to high glucose levels causes β cells to secrete IL-1β, which contributes to islet inflammation in human islets in vitro (49) and in mice (50).

Given the heterogeneity of macrophages (32) and the importance of macrophage polarization in the pathology of obese adipose tissue (AT) (6), we asked whether there are multiple subtypes of macrophages within the islets and analyzed the extent to which their characteristics are altered in T2D islets. Compared with islets from control *db/+* mice, islets from *db/db* mice expressed several-fold higher levels of such chemokines and cytokines as *Ccl2*, *Cxcl1*, *Il1b*, and *Tnf*. Flow cytometric (FCM) analysis demonstrated that islets from two murine models of T2D, obese *db/db* mice or KKAy mice (mouse model described in ref. 51), contain more $CD11b^+Ly\text{-}6C^+$ macrophages without a significant change in islet-resident $CD11b^+Ly\text{-}6C^-$ macrophages, compared with their respective controls (21). We observed that $CD11b^+Ly\text{-}6C^+$ cells from islets of obese mice do not express the cell surface proteins CD206 and CD301, which are characteristic of M2-like alternatively activated macrophages, and exhibit greater expression of *Il1b* and *Tnf* and lower expression of *Il10* than islet-resident $CD11b^+Ly\text{-}6C^-$ macrophages. These findings demonstrate that macrophages shift to an inflammatory M1-like phenotype within the islets of at least two different T2D mouse models (Figure 1). Another report found that M1-like $CD68^+F4/80^-$ macrophages are present only in the islets of *db/db* mice and islet-resident M2-like CD206-expressing $CD68^+F4/80^+$ macrophages in the islets of both *db/+* and *db/db* mice, confirming that islet macrophages in *db/db* mice undergo a shift in polarization to an M1-like phenotype (52). In contrast, one recent study described *Il1b* and *Tnf* expression in non-diabetic islet-resident macrophages, although this study did not compare these macrophages with the macrophages of inflamed islets (53).

Islet macrophages link β cell dysfunction and islet inflammation

The first in vivo evidence that islet inflammation plays a causative role in T2D was derived from a human clinical study on the effect of anakinra, a recombinant human IL-1 receptor antagonist (IL-1Ra) that blocks both IL-1α and IL-1β signaling, in 70 patients with T2D (26). Thirteen weeks of treatment with anakinra significantly reduced serum IL-6 and C-reactive protein levels, improved glycemia and C-peptide secretion, and reduced the serum proinsulin/insulin ratio without significantly affecting homeostatic model assessment of insulin resistance (HOMA-IR). These effects of anakinra on insulin secretion were also seen in prediabetic patients with impaired glucose tolerance (54). Further, studies utilizing multiple mAbs against IL-1β confirmed the contribution of IL-1β signaling to systemic inflammation, glycemic control (55), and β cell dysfunction (27, 28) in T2D (56, 57). A dose-escalation study of gevokizumab, a recombinant humanized mAb against IL-1β, demonstrated that an intermediate dose (0.03–0.1 mg/kg) but not a high dose (>0.3 mg/kg) of gevokizumab significantly improved glycated hemoglobin A1C (HbA1C), suggesting the effects of IL-1β in T2D are multifaceted (see below) (27). For greater mechanistic insight into the role of IL-1 signaling in β cell dysfunction in T2D, IL-1Ra was administered to mice fed a high-fat diet (HFD) (25) and to GK rats (24). Ehses and colleagues clearly showed that treating GK rats with IL-1Ra resulted in decreased hyperglycemia that was accompanied by increased insulin mRNA levels within islets, reduced serum proinsulin/insulin ratios, reduced macrophage infiltration into islets and mRNA levels of chemokine/proinflammatory cytokine within islets, and ameliorated insulin resistance (24). There was no change in islet mass with IL-1Ra treatment (24, 25), suggesting that a functional decline of β cells rather than an apoptotic effect is the primary mechanism by which islet inflammation decreases insulin secretion in T2D (Figure 1). This finding is consistent with the earlier observation that in T2D individuals, islets containing increased numbers of macrophages did not contain higher numbers of TUNEL-positive cells and macrophages were not observed in proximity to apoptotic

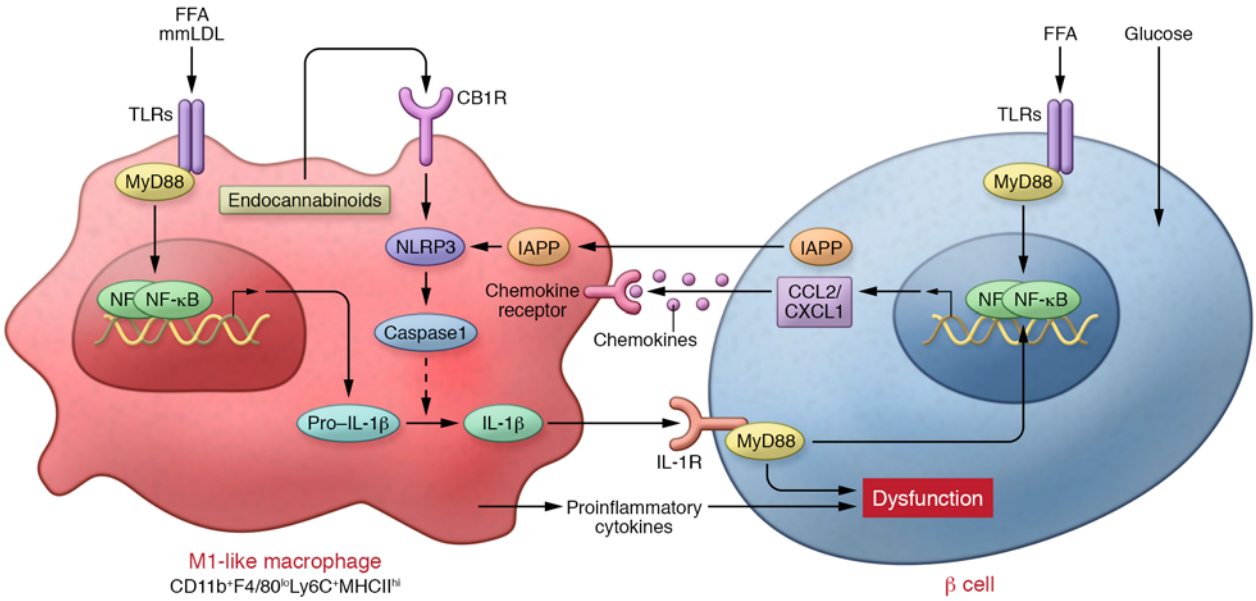

Figure 1. M1-like polarization of islet macrophages plays important roles in islet inflammation and β cell dysfunction in T2D. The communication between islet macrophages and β cells via hIAPP, chemokines (e.g., CCL2 and CXCL1), and proinflammatory cytokines (e.g., IL-1β) initiate and amplify the M1-like polarity shift of islet macrophages and islet inflammation. The inflammasome/IL-1β pathway in islet macrophages is a common pathway that causes β cell dysfunction within inflamed islets in T2D.

β cells (12). This may be an important distinction from the effect of islet inflammation in T1D, where the primary mechanism of disease is the autoimmune destruction of β cells (47).

The actions of macrophages leading to β cell dysfunction in obese mice were further interrogated using clodronate liposome–mediated suppression of macrophage recruitment to islets in *db/db* and KKAy mice. In both models, mice treated with clodronate liposomes showed improved glucose tolerance with improved insulin secretion in an oral glucose tolerance test (OGTT), and islets isolated from these mice exhibited increased glucose-stimulated insulin secretion (GSIS) as compared to the blunted GSIS seen in islets from mice treated with empty liposomes. Levels of pancreatic and duodenal homeobox-1 (*Pdx1*) and insulin mRNA were also increased in islets from clodronate liposome–treated mice (21). These data clearly demonstrate the causative involvement of islet inflammation and macrophage infiltration in β cell failure in T2D rodent models (Figure 1).

Mechanisms of islet inflammation and inflammation-induced β cell dysfunction

The aforementioned studies support the therapeutic strategy of targeting islet inflammation to ameliorate T2D. To gain mechanistic insight into the pathophysiology of islet inflammation, experiments using a specific factor that initiates inflammation have several advantages. These include the ability to set up in vitro preparations to dissect the complex communication between multiple cell types within islets, easy utilization of mutant mice, and the ability to set up short-term in vivo models to eliminate confounding effects from insulin sensitization due to systemic antiinflammatory manipulations. With these advantages in mind, in the next section we review reports analyzing islet inflammation induced by specific stimuli, including hIAPP, saturated free fatty acids (FFAs), and endocannabinoids.

Islet inflammation induced by TLR activation in T2D. In determining the mechanisms by which inflammation is induced in obese AT, the roles of pattern-recognition receptors, including TLRs (58, 59) and NLRs (60) have been extensively analyzed. Several factors, including saturated FFAs, minimally modified LDL (mmLDL) cholesterol, advanced glycation end products of LDL cholesterol, and damage-associated molecular patterns (61) such as HSP60, S100 calcium-binding protein A8 (62), and high-mobility group B (HMGB) proteins (63) all reportedly activate TLRs in obese AT (64). Indeed, saturated FFAs have been reported to induce chemokine (*Cxcl1* and *Ccl3*) expression within islets in vitro (12, 46). Additionally, HMGB1 has been shown to activate the NF-κB pathway in isolated murine islets via TLR2 and TLR4 pathways (65). Furthermore, *Tlr2*$^{-/-}$ mice fed a HFD for 20 weeks were protected from HFD-induced β cell dysfunction and insulin resistance (66), indicating a critical role for the TLR2 pathway in islet inflammation.

Treatment of islets isolated from healthy human control subjects with palmitate, but not oleate or high glucose, increased secretion of cytokines and chemokines, including IL-6 and CXCL1, mimicking the inflammation seen in islets from T2D patients. Palmitate-induced expression of inflammatory genes (*IL6* and *IL8*) in human islets was IL-1 dependent, as IL-1Ra abolished the effects of palmitate (46). Similarly, in both human and mouse islets, exposure to FFAs induced *Il1b* and *Cxcl1* expression and pro–IL-1β expression. This inflammatory response was partially inhibited by IL-1Ra (67). Consistently, exposure of MIN6 β cells to culture medium conditioned by palmitate-treated macrophages, with or without addition of IL-1β– and TNF-α–neutralizing antibodies, confirmed that those proinflammatory cytokines are produced by macrophages, not by β cells, and that they promote β cell dysfunction and enhance chemokine expression in β cells (21). These reports demonstrate the ability of saturated FFAs to initiate an

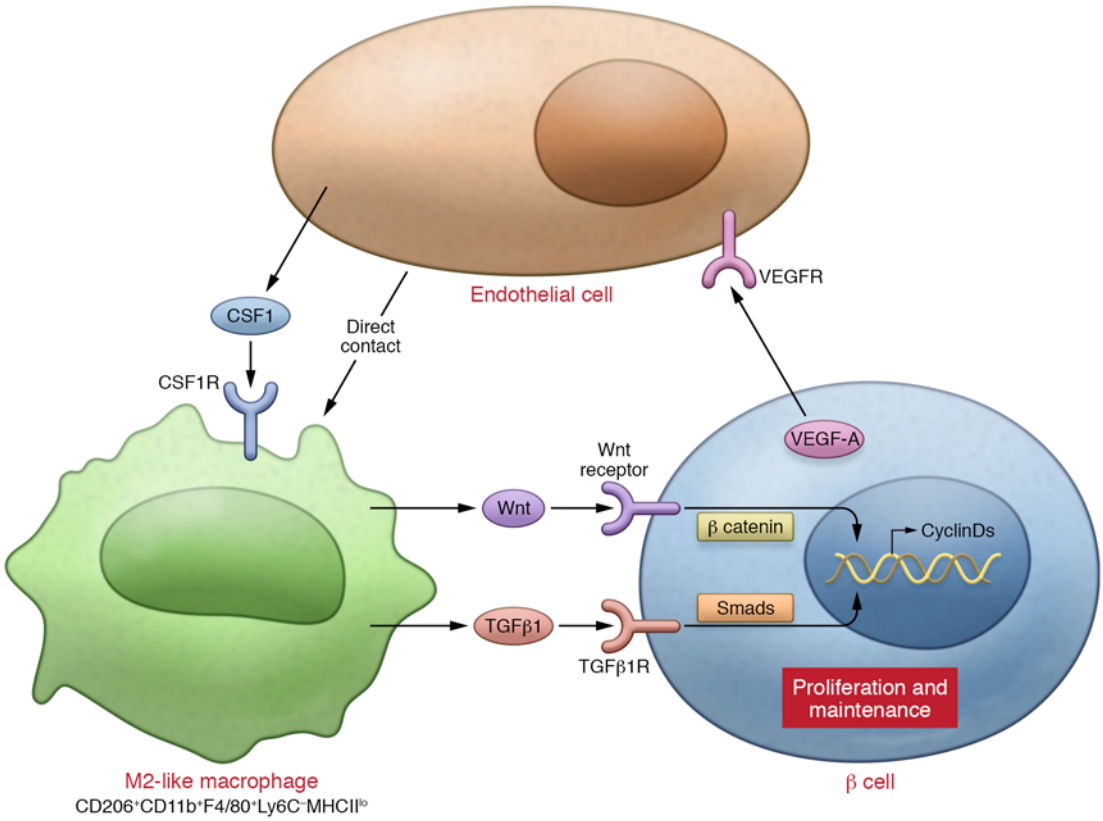

Figure 2. M2-like polarization of islet macrophages plays key roles in the proliferation and physiologic maintenance of β cells. Multiple cytokines (e.g., TGF-β1, VEGFA, and CSF1) secreted from multiple cell types within islets (e.g., macrophages, β cells, and endothelial cells) form networks that contribute to a microenvironment that promotes M2-like polarization of islet macrophages. CSF1 signaling is a common mediator that maintains and promotes M2-like activation of both islet-resident and recruited macrophages. M2-like macrophages play an indispensable role in the establishment of the microenvironment necessary for β cell health. CSF1R, CSF1 receptor.

inflammatory response in the islets, and also demonstrate a role for the autocrine or paracrine effects of IL-1β. In vivo, short-term intravenous ethyl-palmitate infusion induced *Ccl2*, *Cxcl1*, *Il1b*, and *Tnf* expression and reduced insulin and *Pdx1* expression within islets, mimicking the observations from *db/db* and KKAy mice (21) and individuals with T2D (46). FCM analysis of islets from these mice revealed that ethyl-palmitate infusion recruited $CD11b^+$ $Ly\text{-}6C^+$ classically activated M1-like monocytes/macrophages to the islets. $Tlr4^{-/-}$ and $Myd88^{-/-}$ mice were completely protected from both M1-like macrophage recruitment and β cell dysfunction. Bone marrow transplantation studies revealed that TLR4 in islet cells, but not in bone marrow cells, was required for M1-like macrophage recruitment. The short-term nature of the ethyl-palmitate experiment supports the causative contribution of M1-like macrophage-induced islet inflammation to palmitate-induced β cell dysfunction without the confounding effect of insulin sensitization (21).

These reports demonstrate that TLR-mediated sensing of palmitate in β cells is responsible for the initial chemokine secretion that induces macrophage recruitment. Thereafter, communication via proinflammatory cytokines and chemokines between M1-like macrophages and β cells form a vicious cycle that amplifies islet inflammation (Figure 1). These findings are consistent with the previous finding that antagonizing IL-1 signaling inhibits both macrophage recruitment and β cell dysfunction in GK rats (24).

Islet inflammation induced by IAPP in T2D. Amyloid deposits are commonly observed within islets from T2D patients (68). Islet amyloid deposits are composed of IAPP (69, 70), which is produced in β cells and secreted in a monomeric form along with insulin (71). In pathologic conditions, secreted hIAPP is converted to a β-sheet structure and oligomerized (72, 73). The monomeric form of IAPP does not itself affect β cell function, and only the aggregated form — particularly with non-fibrillar hIAPP oligomers, less with mature amyloid fibrils — exhibits toxicity toward β cell function in vitro (74, 75). Given this fact, it is noteworthy that the oligomerization of IAPP differs among species. For example, human and feline IAPP can be oligomerized but rat and mouse IAPP cannot (74, 76). Thus hIAPP transgenic (hIAPP-Tg) mice (77) exhibit islet amyloid deposition, while transgenic mice expressing rat IAPP (rIAPP-Tg) do not (78). Further analysis of hIAPP-Tg mice revealed that dietary fat promotes islet amyloid formation and that one year on a HFD caused β cell loss and impaired insulin secretion (79). Islets in hIAPP-Tg mice exhibited larger F4/80-positive areas and greater expression of chemokines (*Ccl2* and *Cxcl1*) and macrophage markers (*Emr1* and *Itgax*) than non-transgenic mice after one year on either a low-fat diet or HFD (80). Additionally, immunostaining studies revealed the presence of IAPP within the lysosomes of islet macrophages in hIAPP-Tg mice but not rIAPP-Tg mice, suggesting that macro-

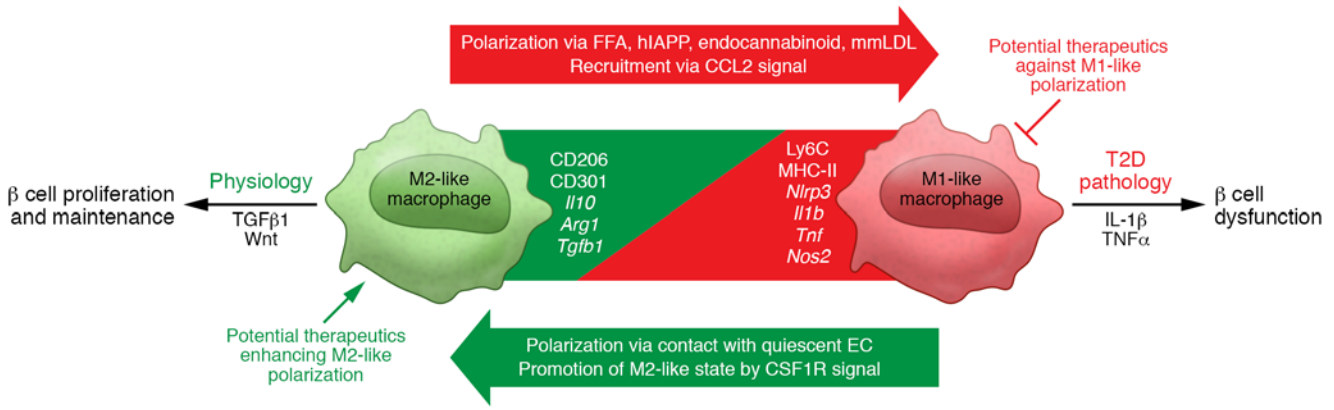

Figure 3. Overview of islet macrophage biology in the context of normal physiology and T2D pathology. M1-like macrophages are represented by surface expression of Ly6C and MHC-II and gene expression of *Nlrp3*, *Il1b*, *Tnf*, and *Nos2*. M1-like macrophages are induced by such factors as FFAs, hIAPPs, and endocannabinoids, and at least some M1-like macrophages are recruited through CCL2 signaling. M2-like macrophages are represented by surface expression of CD206 and CD301 and gene expression of *Il10*, *Arg1*, and *Tgfb1*. At least some M2-like macrophages self-renew or are recruited through CSF1 signaling. In the sterile islet inflammation observed in T2D, islet macrophages' polarization is seamlessly regulated as a continuum, rather than as distinct bimodal M1 and M2 polarization. Studies of the regulation of islet macrophage polarization, characterizations of subsets of heterogeneous islet macrophages, and analyses of the mechanisms by which these polarized macrophages exert their physiologic and pathologic effects on islet biology have the potential for translation to T2D therapeutics.

phages are involved in the toxic effects of islet amyloid deposition in T2D (78). Finally, in a human study using immunohistochemical analysis of islets, there were three-fold more $CD68^+$ cells per unit area in islets from diabetic patients with islet amyloid deposition than in islets from non-diabetic patients or diabetic patients without amyloid deposition (14).

Masters and colleagues have presented a clear perspective on how hIAPP activates macrophages and induces islet inflammation (20). This group focused on the finding that inflammasome activation is the critical event in the initiation of islet inflammation by hIAPP, as hIAPP induced IL-1β but had no effect on IL-6 or TNF secretion from mmLDL-primed dendritic cells in vitro. The significance of the inflammasome/IL-1 pathway to hIAPP-induced islet inflammation in vitro was further demonstrated by the finding that TNF secretion from macrophages and chemokine secretion from isolated islets induced by long-term stimulation with hIAPP was strongly suppressed by IL-Ra (81, 82). In vivo, IL-1Ra treatment improved glucose tolerance in recipients of hIAPP-Tg islets and conferred nearly complete protection from macrophage recruitment to the transplanted hIAPP-Tg islets (81). IL-1Ra also improved islet function without affecting insulin sensitivity in hIAPP-Tg KKAy mice (83).

In accordance with the earlier electron microscopic observation of amyloid within macrophage lysosomes (84), the involvement of phagolysosomes in hIAPP-mediated inflammasome activation was demonstrated in an experiment in which administration of the phagocytosis inhibitors cytochalasin D and bafilomycin A blocked inflammasome activation in macrophages in vitro (20). These mechanisms are consistent with the mechanisms of inflammasome activation in other amyloid-induced pathologies including Alzheimer's disease (85, 86). It was also shown that fibril aggregates accumulate within macrophage lysosomes, further supporting the involvement of hIAPP phagocytosis by macrophages (87). Immunostaining of islets from hIAPP-Tg mice fed a HFD for one year revealed that IL-1β colocalized with amyloid deposits and with macrophages, but not with β cells. These studies show that macrophages are the source of IL-1β within islets both in vivo and in vitro (ref. 20 and Figure 1).

Several studies have sought to determine the identity of the priming factors that induce production of pro–IL-1β and the inflammasome component NLRP3 (88), which leads to inflammasome activation within T2D islets. Among several factors reported to stimulate TLR4 in metabolic syndrome (58, 89), Masters and colleagues confirmed that mmLDL cholesterol (90) can prime macrophages via TLR4 for inflammasome activation by hIAPP (20). More recent reports indicate that soluble hIAPP species produced during early hIAPP aggregation are themselves able to activate the TLR2/MyD88 pathway to prime macrophages (82, 91), and palmitate priming of inflammasomes has also been reported (92). Additionally, an independent group confirmed the presence of inflammasome markers (*Nlrp3*, *Pycard*, and *Casp1*) and inflammatory cytokines (*Il1b*, *Tnf*, and *Il6*) within islets of hIAPP-Tg mice fed a HFD (80).

Depletion of islet macrophages from hIAPP-Tg mice fed a HFD improved systemic glucose tolerance and GSIS of isolated islets, and almost completely blocked islet inflammation, despite greater deposition of amyloid within islets, demonstrating the causal role of islet macrophages in hIAPP-induced islet inflammation and β cell dysfunction in vivo (23). hIAPP-induced IL-1β secretion and NLRP3/caspase-1 induction within isolated islets was completely abolished by clodronate liposome–mediated macrophage depletion (23), which demonstrates the contribution made by resident macrophages to inflammasome activation and IL-1β secretion induced by hIAPP in vitro. In this experiment, in contrast to complete inhibition of *Il1b* expression, macrophage depletion led to an 80% reduction in hIAPP-induced *Ccl2* expression, suggesting that nonphagocytic cells (likely β cells) contribute to islet chemokine production (81). Although there was no obvious infiltration of islets by different macrophages subsets, FCM analysis showed that macrophages within islets from hIAPP-Tg

mice fed a HFD express more CD11b, CD11c, and Ly-6C as well as more *Il1b* mRNA and less *Il10* mRNA than macrophages within islets from wild-type mice fed a HFD. From these findings it was speculated that hIAPP aggregation may affect the differentiation state of monocytes entering islets and that hIAPP polarizes resident macrophages toward a proinflammatory M1-like phenotype (ref. 23 and Figure 1).

Islet inflammation induced by endocannabinoid activation of inflammasomes in islet macrophages. Cannabinoid receptors are expressed in both nervous systems and the peripheral tissues. The most potent endocannabinoids (i.e., endogenous agonist for the cannabinoid receptors) are the lipid ligands anandamide and 2-arachidonoylglycerol. Endocannabinoids play multiple roles, including the regulation of appetite and mood in the nervous systems and the regulation of energy homeostasis in peripheral tissues (93, 94). The cannabinoid 1 receptor (CB1R) antagonist ibipinabant reportedly attenuates β cell loss in diabetic Zucker diabetic fatty (ZDF) rats (95). Investigation into this observation revealed a new mechanism for β cell loss involving CB1R signaling in islet macrophages. Jourdan and colleagues first confirmed that treating ZDF rats with the non–brain-penetrant CB1R inverse agonist JD5037 (96) preserved pancreatic β cell function and protected against β cell loss (22). Immunohistochemical analysis of the isolated islets revealed that JD5037 reduced macrophage infiltration into islets. mRNA analysis demonstrated that JD5037 reversed the M1-like polarity shift of islet macrophages, characterized by decreased expression of *Tnf*, *Nos2*, *Nlrp3*, and *Cnr1* and increased expression of *Tgfb1*, *Il10*, and *Arg1* within ZDF islets. Moreover, CB1R and NLRP3 were expressed in $CD68^+$ macrophages but not in insulin-producing cells. Macrophage depletion using clodronate liposomes demonstrated that macrophages are responsible for the increases in *Cnr1*, *Nlrp3*, *Txnip*, and *Tnf* expression and for the reduced insulin secretion capacity in ZDF rats. Additionally, the effect of macrophage CB1R on islet inflammasome activation and diabetes development was confirmed through macrophage-selective knockdown of *Cnr1* in ZDF rats. Finally, macrophage production of anandamide was stimulated by high glucose and palmitate in vitro. With these experiments, the authors demonstrated that in ZDF rats, autocrine activation of CB1R on macrophages by anandamide causes inflammasome activation and β cell dysfunction and apoptosis (Figure 1).

Beneficial and physiologic functions of islet macrophages

The studies summarized above suggest there is therapeutic potential in targeting cytokines from islet macrophages as well as the mechanisms responsible for M1-like polarization of islet macrophages. However, the fact that lean *Il1b*-deficient mice exhibit glucose intolerance with reduced islet insulin and *Pdx1* transcription (97) clearly indicates that these cytokines and the cells that secrete them also exert beneficial effects. Several analyses on the beneficial effects of low-concentration IL-1β on β cells have been performed, including investigation into the mechanisms involving the Fas-FLIP pathway (97) and insulin granule docking to the plasma membrane (98). Further studies of the physiologic and beneficial functions of islet macrophages and cytokines will enhance the safety and efficacy of therapeutics that target M1-like polarization and cytokine secretion. Beyond that, these studies would aid in the development of new therapeutic strategies aimed at enhancing the beneficial aspects of macrophage functions.

It has long been known that macrophages are present within healthy islets (99) and are important for the expansion of β cell mass (39, 40, 100). These studies demonstrated the indispensable role of CSF1/CSF1R signaling from duct epithelial cells for islet-resident macrophage development and the essential contribution made by islet-resident macrophages to efficient β cell proliferation during embryonic development and adulthood. Therefore, characterization of islet-resident macrophages has translational possibilities. Recent studies have shown that there are at least two distinct lineages of macrophages, and that lineage may affect their functional potential. The first macrophage lineage, which is derived from the yolk sac (YS), is established before the appearance of hematopoietic stem cells (HSCs) and persists as a population of $F4/80^{bright}$ macrophages that self-renew throughout adulthood in many tissues (101, 102). In some tissues, like intestine, YS-derived macrophages are replaced by HSC-derived cells; consequently, adult mice lacking CCR2, which is required for macrophage trafficking from the bone marrow, have markedly reduced tissue macrophages (103). The second macrophage lineage, which is derived from HSCs, gives rise to circulating monocytes that differentiate into $CD11b^{hi}F4/80^{lo}$ macrophages, which are dependent on the transcription factor Myb. Analysis of mouse pancreas showed that pancreatic $F4/80^{bright}$ macrophages found in proximity to β cells at E16.5 are Myb independent, indicating that islet macrophages originate from the YS (101, 104, 105). Recent studies also showed that islet macrophages are absent in *Csf1* mutant *op/op* mice in adulthood, are self-maintained independently from bone marrow–derived circulating monocytes, have a low proliferation rate (53), and are not affected by *Ccl2* and *Ccr2* deficiency (21), characteristics that are consistent with a YS origin. In contrast, lineage-tracing studies have shown that islet macrophages exhibit high positivity for HSC progeny markers (53). Therefore, the origin of islet resident macrophages remains to be confirmed by further analyses.

Recent studies have indicated that macrophages also play an important role in the maintenance of β cell mass and proliferation of β cells in response to increased workload or damage to islets. In a pancreatitis model it was shown that CSF1R signaling in M2-like macrophages is important for β cell proliferation and islet angiogenesis (41). In a study analyzing the streptozotocin model of diabetes, stromal cell–derived factor 1 recruited M2-like macrophages via CXCR4, after which these macrophages activated Wnt signaling in β cells, making M2-like macrophages indispensable for β cell replication (44). In the partial pancreatic ductal ligation (PDL) model, a well-established model of damage-induced islet proliferation, clodronate liposome–mediated macrophage ablation revealed the indispensability of $F4/80^+$ macrophages recruited to islets for β cell proliferation (42). The majority of the recruited macrophages were $CD206^+F4/80^+$ macrophages that exhibited increased *Arg1* expression and decreased *Nos2* expression, which is indicative of the alternatively activated M2-like phenotype. These macrophages contributed to the microenvironment necessary for β cell proliferation by releasing TGF-β1 (106), which induced SMAD7 in β cells. SMAD7 promotes replication of pre-existing β cells by increasing cyclin D1 and cyclin D2 and by inducing nuclear exclusion of p27 (ref. 42 and Figure 2).

Recently, studies of VEGF in β cells clarified the mechanisms underlying M2-like macrophage activation and the requirement for M2-like macrophages in β cell proliferation in mice. It was previously shown that *Vegfa* deficiency in β cells causes impaired islet vascularization and impaired insulin secretion (107), while *Vegfa* overexpression in β cells results in islet hypervascularization, inflammation, and β cell dysfunction (108). In a transient β cell–specific *Vegfa*-overexpressing mouse model, which demonstrates intra-islet endothelial cell proliferation accompanied by macrophage recruitment and decreased β cell proliferation and mass, withdrawal of the *Vegfa* overexpression stimulated β cell proliferation. Inhibiting macrophage recruitment through partial bone marrow ablation revealed the importance of M2-like $CD45^+CD11^+Gr1^-$ macrophage recruitment to islets for β cell proliferation (109). Interestingly, it was reported that endothelial cells could provide a niche for M2-like macrophage polarization through their direct contact with macrophages and also by providing paracrine factors, including CSF1 (110). It therefore seems that quiescent endothelial cells provide the local environment that enables M2-like activation of macrophages, and the environment established by these endothelial cells and M2-like macrophages promotes β cell proliferation (Figure 2).

To further analyze the origin and mechanisms of the M2-like macrophage activation required for β cell proliferation, Van Gassen and colleagues utilized adoptive transfer of $GFP^+CD11b^+Ly6C^{hi}$ monocytes in a PDL model (111). This study demonstrated that circulating $CD11b^+Ly6C^{hi}$ monocytes are recruited to the pancreas, where they differentiate from $CD11b^+Ly6C^{hi}MHCII^{hi}$ cells into $CD11b^+Ly6C^{lo}MHCII^{lo}$ M2-like or tissue-resident macrophage-like cells. When *Ccr2*$^{-/-}$ mice were used, though the CCL2/CCR2-mediated Ly-$6C^{hi}$ monocyte recruitment was blocked, there was compensatory proliferation of MHC-II^{lo} tissue-resident macrophages and PDL did not significantly alter β cell proliferation (42). Because CSF1R signaling reportedly mediates local macrophage replication and promotes differentiation of MHC-II^{hi} into M2-like MHC-II^{lo} macrophages in general settings (112), the effect of CSF1R neutralization was analyzed in the PDL model. CSF1R neutralization not only inhibited tissue-resident macrophage proliferation but also reduced differentiation of recruited MHC-II^{hi} macrophages into MHC-II^{lo} macrophages (ref. 111 and Figure 2).

These reports demonstrate that both macrophages recruited from bone marrow and islet-resident macrophages exhibit a capacity to support β cell proliferation through acquisition of an M2-like macrophage phenotype supported by CSF1R signaling. Furthermore, these studies suggest that multiple cytokines, including TGF-β1, VEGFA, and CSF1, secreted from multiple cell types, including macrophages, β cells, and quiescent endothelial cells, contribute to establishing a microenvironment that maintains the macrophage M2-like phenotype and β cell proliferation (Figure 2).

Conclusions

In addition to the contribution of inflammation to insulin resistance, the existence of islet inflammation and its causative involvement in β cell dysfunction in T2D is now well appreciated. Islet cells, including β cells, play an important role in the initiation of islet inflammation, as they have the ability to sense stimuli and secrete chemokines as well as hIAPP to activate macrophages. Most studies indicate that macrophages are the main source of proinflammatory cytokines within islets. Among these proinflammatory cytokines, IL-1β secreted from M1-like macrophages plays a crucial role in the initiation and amplification of islet inflammation. In comparison, M2-like macrophages are indispensible for both islet development and β cell proliferation in adults. Sterile factors stimulating macrophage activation, the origin of the macrophages, and the local milieu established by multiple cytokines from several cell types within islets are all key determinants of macrophage polarization and function in both normal physiology and T2D pathology.

The new understanding of β cell failure in T2D gained from studies of islet inflammation provides clues to translational possibilities. For example, as inflammasome/IL-1β signaling is the most common and impactful pathway activated in islets of multiple T2D models, strategies targeting IL-1 signaling have produced encouraging results in clinical studies (27, 28, 54, 55). In addition, because the correct dosage of IL-1β–neutralizing antibody is critical for recovery of β cell function in humans, a greater understanding of the macrophages' role in islet physiology should help to optimize IL-1–targeted strategies. Given that inflammatory pathologic changes have been observed in only a portion of T2D patients (12–14, 18), the identification of biomarkers that correlate with islet inflammation will help to enhance the effectiveness of therapeutics that target islet inflammation by allowing for appropriate patient selection. Also, in order to avoid systemically suppressing the function of M1-like macrophages, targeting the mechanisms of macrophage M1-like activation that are unique to islets could potentially lead to safe and effective strategies for suppressing islet inflammation (Figure 3).

It would also be desirable to support processes related to the normal physiology and beneficial aspects of islet macrophage behavior, such as their facilitation of β cell proliferation. The key components necessary to establish a physiological islet microenvironment remain unclear. The fact that a microenvironment supporting β cell proliferation is established through complex communication among several cell types via multiple cytokines makes development of translational approaches difficult. However, CSF1 signaling appears to be a core component, as its importance has been demonstrated in multiple settings, including development, a pancreatitis model, and a PDL model. Furthermore, targeting β cell proliferation may be effective in both T1D and T2D settings. Therefore, future studies of the beneficial aspects of islet macrophage biology could be highly productive (Figure 3).

An improved understanding of islet macrophage biology should enable development of strategies for blocking pathologic T2D islet inflammation to ameliorate β cell dysfunction while promoting physiologic immune cell function to enhance β cell proliferation, which may have broad translational implications (Figure 3).

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