Pancreatic islet inflammation: an emerging role for chemokines

J Jason Collier^{1,2}, Tim E Sparer³, Michael D Karlstad² and Susan J Burke⁴

¹Laboratory of Islet Biology and Inflammation, Pennington Biomedical Research Center, Baton Rouge, Louisiana, USA

²Department of Surgery, Graduate School of Medicine, University of Tennessee Health Science Center, Knoxville, Tennessee, USA

³Department of Microbiology, University of Tennessee, Knoxville, Tennessee, USA

⁴Laboratory of Immunogenetics, Pennington Biomedical Research Center, Baton Rouge, Louisiana, USA

Correspondence
should be addressed
to J J Collier

Email
Jason.collier@pbrcc.edu

Abstract

Both type 1 and type 2 diabetes exhibit features of inflammation associated with alterations in pancreatic islet function and mass. These immunological disruptions, if unresolved, contribute to the overall pathogenesis of disease onset. This review presents the emerging role of pancreatic islet chemokine production as a critical factor regulating immune cell entry into pancreatic tissue as well as an important facilitator of changes in tissue resident leukocyte activity. Signaling through two specific chemokine receptors (i.e., CXCR2 and CXCR3) is presented to illustrate key points regarding ligand-mediated regulation of innate and adaptive immune cell responses. The prospective roles of chemokine ligands and their corresponding chemokine receptors to influence the onset and progression of autoimmune- and obesity-associated forms of diabetes are discussed.

Key Words

- ▶ cytokine
- ▶ chemokine
- ▶ diabetes
- ▶ islet
- ▶ inflammation

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Introduction

Development of diabetes mellitus is currently classified based on the route by which hyperglycemia develops. Presently, type 1 diabetes (T1D) is described as an autoimmune disease that results when the function and/or mass of the insulin-producing β -cells is reduced to a degree that produces clinical symptoms (e.g., hyperglycemia, polyuria, etc.; see Atkinson *et al.* 2014). Type 2 diabetes (T2D) is described as a more slowly progressing disease characterized first by insulin resistance and glucose intolerance, two conditions that indicate 'pre-diabetes' (Johnson & Olefsky 2013, Cefalu *et al.* 2014, Cefalu 2016). The pre-diabetes stage progresses to overt clinical onset of diabetes when the function and mass of the islet β -cells decrease to the point where the ability to maintain glucose homeostasis is ultimately lost (Kahn 1998, Doria *et al.* 2008, Muoio & Newgard 2008). At present, the hallmark of each disease appears to be dysfunction of

the islet β -cell population, a reduction in total numbers of insulin-producing cells or both outcomes.

While the specific subclasses of diabetes are delineated by their individual etiological associations accompanying islet β -cell death and dysfunction, there are changes in the genetic programming of β -cells during progression to each form of diabetes that contribute to and influence disease progression (Cnop *et al.* 2014, Donath 2014, Lopes *et al.* 2014, Burke & Collier 2015, Sanchez *et al.* 2015). This transcriptional reprogramming is occasionally overlooked although it is an important contributor to disease progression and outcome. Precise signaling inputs control these genetic effects with the major alterations in gene transcription connected to the production of molecules that regulate cellular viability, influence immune cell recruitment, impact glucose-stimulated calcium dynamics and eventually reduce maximal glucose-mediated insulin

secretion. The end result is an overall diminution in either insulin-positive cell mass, circulating insulin levels or both. Despite distinct etiologies for T1D and T2D, understanding the pathophysiological mechanisms of each disease will likely benefit the development of therapies to treat both diseases. Thus, we begin by briefly outlining some of the noteworthy similarities between T1D and T2D to illustrate that significant parallels exist between these common endocrine diseases.

Parallels between T1D and T2D

Autoimmunity is a key driver of T1D (Castano & Eisenbarth 1990), while obesity is one of the most important risk factors for the development of T2D (Bray 1992). However, there are a number of commonalities between T1D and T2D that are worth considering. First, insulin resistance is a risk factor for both T1D (Fourlanos *et al.* 2004, Donga *et al.* 2015) and T2D (Kahn 1998, Samuel & Shulman 2016). Second, pro-inflammatory cytokines (e.g., IL-1 β , IFN- γ , TNF- α , etc.) contribute to the disease phenotype in both T1D and T2D (Dogan *et al.* 2006, Donath 2014, Burke *et al.* 2015b). Third, advanced glycation end products are present in both T1D (Coughlan *et al.* 2011, Forbes *et al.* 2011) and T2D (Nowotny *et al.* 2015). Fourth, insulin therapy is indicated for therapeutic intervention during specific stages of each disease (Atkinson *et al.* 2014, Home *et al.* 2014, Kreider & Lien 2015). Fifth, there are altered circulating levels of various chemokines in both T1D and T2D (Hanifi-Moghaddam *et al.* 2006, Shigihara *et al.* 2006, Takahashi *et al.* 2011, Sajadi *et al.* 2013, Corrado *et al.* 2014, Nunemaker *et al.* 2014, Burke & Collier 2015). Sixth, there are defects in insulin secretion and calcium usage in rodent and human islets associated with T1D and T2D (Boucher *et al.* 2004, Dula *et al.* 2010, Ramadan *et al.* 2011, Do *et al.* 2014, Burke *et al.* 2015b, Kenty & Melton 2015, Qureshi *et al.* 2015). Seventh, immune cells infiltrate the islets in both T1D and T2D (Gepts 1965, Ehses *et al.* 2007, Boni-Schnetzler *et al.* 2008, Richardson *et al.* 2009, Willcox *et al.* 2009, Burke *et al.* 2016). With this last point in mind, we note that excellent reviews exist discussing the immunology of diabetes (Castano & Eisenbarth 1990, Wallberg & Cooke 2013, Boldison & Wong 2016). Therefore, we will focus this review around pancreatic islet β -cell chemokine production with a discussion of two important chemokine receptor-signaling paradigms that fundamentally impact specific subsets of immune cells known to participate in the pathogenesis of T1D and T2D.

Chemokines: soluble secreted proteins that regulate immune cell movement and activity

Chemotactic cytokines (aka chemokines) are a family of small (8–10 kDa), secreted, signaling proteins that have biological impact through activation of their specific cell surface receptors. These receptor-mediated actions include the directed chemotaxis of a responsive target cell, changes in intracellular second messengers, and the ability to influence gene expression, protein localization, protein production and secretion of molecules relevant to immune cell function (Charo & Ransohoff 2006). Chemokines are currently classified by their structural characteristics and are divided into different families based on the spacing of their N-terminal cysteines (i.e., CC, CXC, CX₃C or C). Chemokines as a group are usually sub-classified into homeostatic or pro-inflammatory categories, with many present in both groups. These proteins direct leukocyte migration and influence immune cell activity. Their contribution to development, onset, maintenance and resolution of various disease processes constitute active research areas.

Chemokines are protein ligands for their cognate chemokine receptors, which are members of the much larger G protein coupled receptor (GPCR) superfamily. Upon ligand binding to the chemokine receptor, a conformational change leads to the exchange of GDP with GTP and the activation of the heterotrimeric G proteins (Pierce *et al.* 2002). These heterotrimeric G proteins consist of 21, 6 and 12 different G α , β and γ subunits, respectively, which contribute to the complexity of signaling outputs. The downstream effects of GPCR activation is dictated by signal strength and subsequent effects on the receptors: internalization (Marchese *et al.* 2008), intracellular location (Jiao *et al.* 2005), hetero/homo-dimerization (Trettel *et al.* 2003, Wilson *et al.* 2005, Martinez Munoz *et al.* 2009) and G protein subunit usage (Khan *et al.* 2013, Syrovatkin *et al.* 2016). In addition, the desensitization and inactivation of the intracellular signal also affects signaling outcomes. G protein receptor kinases (GRKs) and β -arrestins turn off the signaling of individual chemokine receptors.

Interestingly, β -arrestins can also induce their own downstream signaling outcomes (Luttrell & Lefkowitz 2002, Shenoy & Lefkowitz 2005, Defea 2008), with the levels of the desensitizing proteins and their isoforms affecting the overall signaling mechanisms. For example, CXCR1 and CXCR2 bind to GRK2 and GRK6, respectively, which produce differences in neutrophil

activation (Raghuwanshi *et al.* 2012). Discrete activation mechanisms allow for fine-tuning of the chemokine signaling pathways, an important observation bearing in mind there are ~50 associated chemokine ligands that impact ~20 different chemokine receptors. Most of the chemokine receptors display promiscuous interaction with multiple chemokines, which was initially thought as redundancy in the biological setting. However, in light of recent data on chemokine receptor activation linked to specific biological responses, a more complex picture has formed showing multiple different stimuli *at the same receptor* trigger differential downstream effects (Zweemer *et al.* 2014). In other words, one receptor can produce distinct outcomes depending on which ligand occupies the binding site. This updated paradigm is often referred to as biased signaling, biased agonism or functional selectivity (Wisler *et al.* 2014, Karin *et al.* 2016).

In the case of both T1D and T2D, we postulate that the most logical biochemical and immunological explanation for the initial and sustained entry of one or more immune cell populations into pancreatic tissue is directed chemotaxis by specific signaling molecules (e.g., chemokines). Because increased immune cell presence within pancreatic tissue occurs as part of the phenotype underlying T1D and T2D (Foulis & Stewart 1984, Foulis *et al.* 1991, Boni-Schnetzler *et al.* 2008, Donath *et al.* 2008, Hanafusa & Imagawa 2008), we and others have focused a significant portion of our research efforts into understanding the inflammation-associated processes related to islet chemokine production and secretion. Below, we provide a brief discussion of signaling through two representative chemokine receptors relevant to innate and adaptive immunity.

Chemokine ligand activation of chemokine receptors in innate and adaptive immunity

Pancreatic β -cells synthesize and secrete a variety of chemokines capable of recruiting leukocytes into pancreatic tissue (Sarkar *et al.* 2012). The expression of many of these chemokines is markedly enhanced by β -cell exposure to inflammatory signals (Sarkar *et al.* 2012, Cnop *et al.* 2014, Lopes *et al.* 2014, Burke & Collier 2015). Below, we focus on ligand-mediated signaling events associated with CXCR2 and CXCR3, which are two specific chemokine receptors reported to influence onset and progression of diabetes. These two receptors are also important because they exemplify enriched expression in neutrophils (CXCR2) and T-cells (CXCR3). Because β -cell exposure to inflammatory mediators

induces various chemokine ligands that bind to CXCR2 and CXCR3 (Burke *et al.* 2014, 2015a,b, 2016), we discuss what is known about the activation pathways of these two receptors and the downstream effector mechanisms that contribute to immune activation and subsequent β -cell destruction.

CXCR2

CXCR2 is highly expressed on neutrophils (Murphy & Tiffany 1991, Liu *et al.* 2010) and oligodendrocytes (Veenstra & Ransohoff 2012). It is expressed less consistently on T cells, basophils, mast cells and epithelial cells during wound healing. CXCR2 binds ELR-CXC chemokines (CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL7 and CXCL8) with high affinity. CXCR1 binds CXCL8 with high affinity, while CXCL6 and CXCL7 bind less well to CXCR1 than to CXCR2 (Waugh & Wilson 2008). In the case of CXCR2, this biased agonism (or functional selectivity) was initially thought of as redundancy for receptor activation. However, distinct and complex signaling crosstalk produces different phenotypic responses and is now thought to be important for physiological and pathophysiological outcomes (Zweemer *et al.* 2014).

For CXCR2 activation, specific chemokines bind and activate the receptor via the amino terminus and first extracellular loop. Site directed mutagenesis in these regions generates different activation and functional consequences (Katancik *et al.* 1997, Katancik *et al.* 2000). The receptor-associated signal is transduced to the second intracellular loop where the G proteins are bound to the aspartic acid, arginine and tyrosine (DRY) box and leads to the GDP to GTP exchange and G protein activation. After activation the carboxyl terminus is phosphorylated on the LLKIL motif, which leads to activation of the β -arrestins and internalization/desensitization (Raman *et al.* 2014). The receptor is then targeted to different endosomal compartments based on its phosphorylation state for degradation or recycling. These varied signals from CXCR2 stimulation initiate intracellular Ca^{++} flux, inhibition of apoptosis, migration, priming and adhesion depending on the downstream signaling pathway that is activated (Mocsai 2013). Neutrophil recruitment via CXCR2 is critical for the autoimmune responses targeting islet β -cells; inhibiting CXCR2 partially blocks this outcome (Citro *et al.* 2012, Diana & Lehuen 2014).

Moreover, neutrophils and CXCR2 signaling during diabetes development were shown in the NOD mouse model, which develops T1D spontaneously with many

features of the human disease. When CXCR2 was inhibited or neutrophils were depleted, disease progression was limited in these mice (Diana *et al.* 2013, Citro *et al.* 2015). Additional evidence showing that allosteric inhibitors of CXCR2/CXCR1 lead to prolonged survival of islet transplants in both mice and humans confirms the importance of neutrophil chemokine/chemokine receptor activation during disease onset (Citro *et al.* 2012). Using neutrophil elastase inhibitors, Talukdar *et al.* demonstrated that neutrophils also impact obesity-induced insulin resistance (Talukdar *et al.* 2012). Taken together, these data illustrate the promise of CXCR2- and/or neutrophil-based immunomodulatory strategies as possible treatments for pathological responses that contribute to the onset of T1D and T2D.

CXCR3

The interferon (IFN)-inducible, non-ELR CXC chemokine subgroup includes CXCL9 (Mig), CXCL10 (IP10) and CXCL11 (I-TAC), which are proteins that bind to CXCR3 (Groom & Luster 2011). This receptor is mainly found on NK cells and T cells and has a role in a variety of diseases, especially those with a Th component (Van Raemdonck *et al.* 2015). When CXCL10 is expressed in the islets of transgenic mice, an accelerated autoreactive immunological response was observed (Rhode *et al.* 2005). In addition, islet isografts were protected when CXCL10 expression was diminished (Bender *et al.* 2017). Serum CXCL10 levels are increased in both human subjects and in the NOD mouse model, linking elevated chemokine production with diabetes (Christen *et al.* 2003, Antonelli *et al.* 2014, Corrado *et al.* 2014). When CXCR3 is knocked out, there is a delay in the onset of virally induced diabetes (Frigerio *et al.* 2002) and in diabetes induced by multiple low doses of streptozotocin (Burke *et al.* 2016). By contrast, when CXCR3 null mice are crossed onto the NOD background, T1D development is accelerated (Yamada *et al.* 2012). What appear to be contrary findings could be due to genetic differences in the different mouse strains, the distinct models of T1D or functional selectivity of CXCR3 activation that is related to strain and/or the models of diabetes.

It is important to note that mice on a C57BL/6 background do not produce CXCL11 due to an insertion of 2 bases after nucleotide 39, which shifts the open reading frame of the CXCL11-encoding gene to a premature stop codon (Sierro *et al.* 2007). By contrast, the NOD mice retain the ability to produce CXCL11, which could explain differences in whole body phenotypes observed

between knockouts of CXCR3 when compared with C57BL/6 mice. In the viral model of T1D, knocking out ligands binding to CXCR3 showed redundancy instead of biased agonism, i.e., knocking out one of the CXCR3 ligands had no effect (Coppieters *et al.* 2013), leaving us to speculate how the *individual ligands* contribute to onset and progression of diabetes. Along these lines, the different chemokine ligands that bind CXCR3 show biased agonism during *in vitro* studies. Watts and coworkers used impedance measurements, BRET and CRE luciferase assays to show that CXCL9 induces β -arrestin binding and produces higher impedance measurements when compared with CXCL10 and CXCL11 (Watts *et al.* 2012). Conversely, Rajagopal and coworkers showed bias only for CXCR3 for internalization, with CXCL11 the more potent agonist when compared with CXCL9 and CXCL10 (Rajagopal 2013, Rajagopal *et al.* 2013). Although the signaling pathways activated after differential ligand binding have not been fully elucidated, there are some clues about CXCR3 signal transduction cascades that are informative. For example, the differential downstream signaling is due, at least in part, to differential G α subunit usage (Kouroumalis *et al.* 2005, Thompson *et al.* 2007). CXCL10 stimulation of CXCR3 also leads to activation of the ERK/Ras pathway and an increase in migration and signaling (Bonacchi *et al.* 2001).

Decoding the chemokine receptor signaling pathways could allow for drugs that have functional selectivity for some of the beneficial phenotypes while ideally eliminating the detrimental outcomes. One clue to the importance of specific pathways on diabetes development came from nucleotide-binding leucine-rich repeat and pyrin domain-containing protein 3 (NLRP3) knockout mice; NLRP3 functions as part of the innate immune system via pattern recognition responses (Martinon 2008). In NLRP3^{-/-} mice, expression of CXCR3, and the corresponding ligands, CXCL9 and CXCL10, were decreased (Hu *et al.* 2015). The expression of other chemokines and their receptors was also reduced. Importantly, diabetes took longer to develop in NLRP3^{-/-} mice, implying that these chemokines and associated receptors contribute to T1D development.

On a related note, the small molecule CXCR3 antagonist, SCH 546738, was shown to prevent autoimmune diseases (Jenh *et al.* 2012), while another CXCR3 inhibitor, NBI-74330, had distinct potencies for the different CXCR3 ligands (Heise *et al.* 2005). Whether this information regarding functional selectivity will be useful for the design of new therapeutics aimed at modulating human CXCR3 pathways during autoimmune diseases remains to be revealed. It will be of particular interest to

determine whether any of the existing molecules targeting chemokine receptors directly have therapeutic benefit in the different models of diabetes.

Islet-derived chemokines in T1D

Genes encoding chemokines are located within the known diabetes susceptibility locus *Idd4* in the NOD mouse, which is associated with T1D development (Grattan *et al.* 2002). While the initial trigger(s) prompting the onset of β -cell directed autoimmunity is unknown, enhanced production and release of chemokines from pancreatic islets coupled with the associated chemokine receptor activation on individual leukocytes is a very plausible component of the disease course. The following components are proposed to be contributors to T1D development: (1) secretion of cytokines and chemokines by resident immune cells (e.g., macrophages) in response to a specific pathogen or pathogenic signal (e.g., death of surrounding cells, viral infection, LPS, etc.); (2) the production of islet-derived chemokines increases in response to such signals, promoting the recruitment of additional immune cell populations (e.g., neutrophils, T-cells, etc.) into the pancreatic islets; (3) T-cell priming for one or more antigens (typically β -cell specific); (4) chemokine-mediated entry of primed lymphocytes into the pancreatic tissue; (5) continued production and release of cytokines (e.g., IL-1 β , IFN- γ , etc.) that regulate inflammatory responses, including immune cell activity, within pancreatic islets; and (6) sustained production of chemokines, including from pancreatic β -cells, creating a vicious feed forward cycle intended to either clear infection or promote tissue repair (physiological outcome) that transitions during chronic disease states (e.g., autoimmunity) to become pathophysiological. Portions of this model have been validated using transgenic mouse models where production of CCL2 or CXCL10 directly from β -cells stimulates immune cell entry into the pancreatic tissue (Grewal *et al.* 1997, Rhode *et al.* 2005, Martin *et al.* 2008b) and by reducing chemokine action via decoy receptor expression in NOD mice (Martin *et al.* 2008a).

T1D arises through multiple immune cell interactions, including macrophages, neutrophils and T-cells (Calderon *et al.* 2006, Diana *et al.* 2013). This means various innate and adaptive immune cells, and molecules secreted from such cells, all participate and contribute in some capacity to the disease pathology. Thus, signals contributing to immune cell recruitment, such as chemokines, are critical players in initiating, accelerating and/or maintaining disease trajectory. Consistent with this idea, IL-1 α and TNF- α are elevated in 'at risk' and new onset T1D subjects when compared with healthy controls (Rosa *et al.* 2008, Chatzigeorgiou *et al.* 2010, Zak *et al.* 2010). IL-1 α and TNF- α signal through the IL-1 receptor and TNF receptor, respectively, both of which activate the NF- κ B pathway. NF- κ B activation drives production of multiple chemokines in islet β -cells (Burke & Collier 2014, 2015) and reduces insulin secretion in rodent and human islets (Giannoukakis *et al.* 2000, Rehman *et al.* 2003, Rink *et al.* 2012, Burke *et al.* 2015b). Conversely, restricting NF- κ B activity in the multiple low dose streptozotocin (MLDS) model reduces chemokine production and protects against hyperglycemia (Eldor *et al.* 2006). In an alternative approach, blocking chemokine action using decoy receptors also revealed key roles for chemokine proteins in the pathogenesis of diabetes development (Martin *et al.* 2007, 2008a).

When examining rodent models of T1D (Tables 1 and 2), the production and release of CXCL9 and CXCL10 protein in islets from 4 and 10-week-old NOD mice correlated with the degree of insulinitis (Welzen-Coppens *et al.* 2013). Blocking CXCL10 activity with neutralizing antibodies prevented re-infiltration of pancreatic islets after a course of anti-CD3 therapy (Lasch *et al.* 2015). Because T-cells primed with autoantigens typically produce and secrete IFN- γ , targeted upregulation of CXCL10 by IFN- γ signaling in β -cells is likely contributing to T1D disease pathology (Burke *et al.* 2013a, 2016, Lundberg *et al.* 2016). The enhanced transcription of the CXCL10 gene is also consistent with heightened STAT1 activity in islet β -cells (Burke *et al.* 2013a, Lundberg *et al.* 2016). Moreover, pro-inflammatory cytokines (e.g., IL-1 β ,

Table 1 Islet chemokines in monogenic rodent models of obesity and diabetes.

Animal model (species)	Relevant human condition	Islet chemokine production	References
Akita (mouse)	ER stress/insulin insufficiency	No ^a	Burke SJ (unpublished observations) ^b
<i>db/db</i> (mouse)	Obesity/T2D	Yes	Eguchi <i>et al.</i> (2012), Burke <i>et al.</i> (2015a)
KKA ^y (mouse)	Obesity/T2D	Yes	Eguchi <i>et al.</i> (2012)
ZDF (rat)	Obesity/T2D	Yes	Jourdan <i>et al.</i> (2013)

^aMale mice at 8 weeks of age.

^bSJ Burke, MD Karlstad & JJ Collier, unpublished observations.

Table 2 Islet chemokines in polygenic rodent models of obesity and diabetes.

Animal model (species)	Relevant human condition	Islet chemokine production	References
BB (rat)	Autoimmunity/T1D	Yes	Kuttler <i>et al.</i> (2007)
GK (rat)	Insulin resistance/T2D	Yes	Ehses <i>et al.</i> (2009)
MLDS (mouse/rat)*	Insulin insufficiency/T1D	Yes	Martin <i>et al.</i> (2007, 2008a), Burke <i>et al.</i> (2016)
NOD (mouse)	Autoimmunity/T1D	Yes	Martin <i>et al.</i> (2008a), Welzen-Coppens <i>et al.</i> (2013), Burke <i>et al.</i> (2016)

*MLDS, multiple low doses of streptozotocin (STZ). STZ impacts multiple mouse and rat strains; thus, we have included it under the polygenic models.

IFN- γ , etc.) increase the expression of CXCL9 and CXCL11 in mouse, rat, and human β -cells using activated STAT1 as a key component of the signaling response (Burke *et al.* 2016). STAT1 is therefore a key driver of chemokine production (Burke *et al.* 2013a, 2016) and inducible nitric oxide synthase abundance and activity (Burke *et al.* 2013b, 2015b, Corbett *et al.* 1992, 1993, Heitmeier *et al.* 1997). Increased STAT1 expression is also strongly correlated with HLA class I (i.e. HLA-ABC) and HLA-F isoforms in insulin-containing islets (Richardson *et al.* 2016). Thus, NF- κ B and STAT1 likely cooperate to control inflammatory responses within pancreatic islets, which ultimately determine the autoimmune and auto-inflammatory outcomes (Fig. 1). The model shown in Fig. 1 offers an attractive conceptual framework from which to explain why individual immunomodulatory approaches may have underachieved from a therapeutic perspective.

One such example of a promising single immunomodulatory strategy that has thus far failed to produce remission, or prevent T1D, in mice is anti-IL-1 β neutralization (Gill *et al.* 2015). Although it was promising that human subjects with recent onset T1D receiving a targeted anti-IL-1 therapeutic (Anakinra) displayed reduced insulin requirements one and four months after diagnosis compared to controls (Sumpter *et al.* 2011), in a larger study, IL-1 neutralization strategies were not effective at meeting the clinical endpoints (Moran *et al.* 2013). It is worth noting, however, that one month after diagnosis the insulin-dose-adjusted A1c in patients given Anakinra was lower than controls (Sumpter *et al.* 2011).

Multiple explanations might exist to explain these initially disappointing findings. The first is that the neutralization or trap strategies do not completely block signaling through the IL-1 receptor, which has two known agonist ligands (i.e., IL-1 α and IL-1 β). The second explanation is that other signals, such as TNF- α or ligands that activate pattern recognition receptors (PRR), can still activate NF- κ B despite interventions that restrict signaling through the IL-1 receptor (Fig. 1). The third explanation is that signaling through specific receptors, such as IL-1R1, may have both physiological and pathological outcomes

and that receptor antagonism eliminates both positive and negative outcomes.

We note that NF- κ B activation by a variety of inflammatory pathways, in conjunction with elevated levels of STAT1 and/or enhanced STAT1 transcriptional responses, would likely support sustained production of soluble factors (e.g., chemokines) that influence immune cell recruitment. In addition, the powerful combination of NF- κ B and STAT1 activation regulates other key genes

Figure 1

Signals that activate NF- κ B and STAT1 enhance chemokine production within pancreatic islets that contribute to the inflammatory response influencing both insulin secretion and total β -cell mass. Many signals converge on the NF- κ B and STAT1 pathways to coordinately reprogram beta cells at a transcriptional level, leading to inflammation-based changes in insulin secretion, β -cell mass or both. PRRs can be either cell surface based, such as Toll-like receptor-2 and -4 or positioned intracellularly (e.g., Toll-like receptor-3, NOD1, NOD2, etc.). TNF- α and IL-1 signal through specific cell surface receptors (receptor not shown) and converge on the NF- κ B pathway. The interferon family of cytokines signals through cell surface receptors that activate JAK-STAT pathways. Activation of COX2 produces prostaglandins, which may influence leukocyte activity. Cytokine-mediated increases in iNOS elevate intracellular production of nitric oxide, which acts as a rheostat for insulin secretion. COX2, cyclooxygenase-2; IFNs, interferons alpha, beta and gamma; iNOS, inducible nitric oxide synthase; PRR, pattern recognition receptors.

relevant to islet β -cell function and dysfunction (Fig. 1). Consequently, in our view, combination therapies may end up providing the most benefit to prevention of autoimmune diseases, such as T1D, by restricting signaling of multiple pathways that collectively contribute to chemokine production, and other important mediators of inflammation, which ultimately regulate islet β -cell function and mass.

Islet-derived chemokines in T2D

Multiple models of rodent obesity and diabetes display elevations in islet chemokine production (Tables 1 and 2). In addition, staining of human pancreatic tissue revealed high levels of CXCL10 in islets from subjects with T2D (Schulthess *et al.* 2009) while RNA sequencing approaches showed the expression of multiple chemokines in human islets exposed to the fatty acid palmitate (Cnop *et al.* 2014). These data are consistent with enhanced leukocyte presence in human pancreatic tissue during obesity (Boni-Schnetzler *et al.* 2008, Donath *et al.* 2008). Schulthess and coworkers suggest that CXCL10 signals through TLR4 on β -cells as a mechanism for deleterious effects during obesity (Schulthess *et al.* 2009). While an intriguing possibility, these observations still await independent verification.

On the other hand, genetic deletion of the CX₃CR1 receptor reduces insulin secretion in response to the physiological signals glucose and GLP-1 (Lee *et al.* 2013). The chemokine CX₃CL1 (aka fractalkine), which signals through CX₃CR1, is decreased in islets during aging and obesity (Lee *et al.* 2013). It is therefore possible that particular chemokines promote β -cell health, either by recruiting specific immune cells, through direct effects on β -cells, or both. In addition to mechanisms that support leukocyte recruitment and production of pro-inflammatory cytokines, loss of these 'protective' or homeostatic chemokines might be a mechanism which also contributes to β -cell dysfunction during progression to T2D.

Obesity and islet inflammation

How does obesity trigger islet inflammation? Since no one mechanism has emerged to explain all observations, we put forth two conceptual possibilities. The first is that obesity negatively influences gut barrier function, allowing translocation of bacterial products initially into the mesenteric lymphatic system and then into

the systemic circulation (Fig. 2A). This elevation in lipopolysaccharide (LPS), or other molecules that signal through pattern recognition receptors, may contribute to the subclinical, low grade inflammation that drives insulin resistance and associated tissue dysfunction (Carneiro *et al.* 2008, Purohit *et al.* 2013, Cox *et al.* 2015). With this in mind, it is conceivable that islet resident macrophages become activated during obesity due to elevated levels of circulating LPS derived from increased intestinal permeability (i.e., 'leaky gut'). The presence of LPS increases macrophage cytokine production and also enhances macrophage sensitivity to IFN- γ (Held *et al.* 1999).

IFN- γ is a cytokine that activates macrophages and also promotes the differentiation of immature DCs into effector DCs that heavily influence Th1 responses (Boehm *et al.* 1997). These biological responses are intended to induce robust anti-bacterial activity, but may be dysregulated during obesity. Indeed, CXCL8, CXCL10 and IFN- γ increase in circulation with excess body weight (Strackowski *et al.* 2002, Sharabiani *et al.* 2011). Consequently, it is plausible that bacterial translocation from a leaky gut results in a low level of LPS that, in combination with elevations in other circulating factors linked with inflammation, promotes detrimental changes in pancreatic islets. Macrophage cytokine production is a key component that impacts pancreatic islet function and mass (Fig. 2A). While speculative, this mechanism fits with existing observations put forth in the literature (Balzan *et al.* 2007, Cani & Delzenne 2007, Cani *et al.* 2007).

The second conceptual possibility explaining how obesity leads to islet inflammation is lipid overload and associated metabolic trauma (Fig. 2B). In this model, lipids accumulate in lean tissues once storage in adipose tissue has been exceeded (Unger & Orci 2000, Unger 2003). Lean tissue responses to surplus fatty acids may vary. For example, fatty acids promote macrophage activation as well as chemokine production in pancreatic islets (Eguchi *et al.* 2012), potentially via cell surface (e.g., TLR4) or intracellular (e.g., NOD1, NOD2, etc.) pattern recognition receptor signaling (Fig. 1). Moreover, incomplete fatty acid oxidation products drive enhanced NF- κ B activity in macrophages, promoting pro-inflammatory actions (Rutkowski *et al.* 2014). If such incomplete fatty acid oxidation mechanisms also occur in β -cells, it could help to explain lipid-induced changes in β -cell insulin secretion as well as chemokine production that occur in obesity (Burke *et al.* 2015b, Eguchi *et al.* 2009,

Figure 2

Conceptual models for how obesity may influence islet inflammation and chemokine production. (A) 1. Increased intestinal permeability allows microbial products (e.g., LPS) to enter the circulation. 2. These molecules are 'sensed' by pattern recognition receptor enriched in immune cells, such as neutrophils (not shown) and macrophages (shown). An increase in macrophage activation leads to production and release of cytokines (e.g., IL-1 β). 3. Distinct pro-inflammatory cytokines (e.g., IL-1 β , IFN- γ , TNF- α , etc.) promote transcriptionally active NF- κ B and STAT1 proteins. 4. The NF- κ B and STAT1 pathways regulate chemokine production in beta-cells; release of β -cell derived chemokines influences innate and adaptive immune responses. (B) 1. When consumption of free fatty acids (FFAs) exceeds storage capacity in adipose tissue, these lipids begin to be stored in lean tissues (e.g. liver, muscle and pancreas). 2. FFAs activate cell surface receptors, altering intracellular signaling pathways. 3. Incomplete fatty acid oxidation may also activate signaling pathways, such as NF- κ B, that are linked with transcriptional changes. 4. Chemokine production is elevated in the obese, insulin resistant state, which would impact both tissue resident and infiltrating leukocytes.

2012). This is important because the increase in β -cell chemokine production by inflammatory signals coincides with diminutions in insulin secretion (Burke *et al.* 2015b).

Moreover, accumulation of excess lipid within β -cells impairs insulin secretion, at least in part, by interfering with metabolic coupling steps, such as pyruvate cycling (Boucher *et al.* 2004). Reducing the lipid burden or restoring pyruvate cycling improves islet β -cell secretory function (Shimabukuro *et al.* 1998, Boucher *et al.* 2004). Finally, intracellular lipid accumulation in the islet β -cell may also result in ER stress. Induction of mild ER stress induces β -cell proliferation (Sharma *et al.* 2015), while lipid signaling through pattern recognition receptors

likely reduces β -cell function and promotes production of molecules, such as chemokines and cytokines, that modulate immune cell recruitment and inflammation (Boni-Schnetzler *et al.* 2009).

Thus, multiple signaling mechanisms may exist to limit insulin secretion while augmenting chemokine production within pancreatic islets (and other tissues), driving increased immune cell infiltration and therefore influencing the status of pancreatic inflammation. Because increased numbers of immune cells have been observed within pancreatic islets from both rodents and humans during obesity validate these findings as conserved, important phenotypes (Ehse *et al.* 2007,

Boni-Schnetzler *et al.* 2008, Donath *et al.* 2008). However, it is important to note that any immune cell entry into the pancreatic tissue is often heterogeneous. In addition, changes in the tissue resident immune cell activation state (e.g., M2→M1), without enhancements in immune cell number, could also drive inflammation-mediated changes in tissue function (Burke & Collier 2014).

Consistent with changes in leukocyte number or activation state, increased presence of chemokine ligands has been documented in pancreatic islets of mice, rats and humans during obesity (Donath *et al.* 2008, Schulthess *et al.* 2009, Eguchi *et al.* 2012, Sajadi *et al.* 2013, Burke *et al.* 2015a). While there is no doubt that obesity is a major risk factor for the development of T2D, the molecular determinants explaining the increased risk are still being investigated. Our view is that pancreatic islet-derived chemokines play critical roles by regulating immune cell flux into the pancreatic islets, influencing the activation state of resident immune cells or both. These outcomes ultimately impact β -cell substrate metabolism linked with insulin secretion as well as influencing proliferation of insulin-producing cells.

Summary and conclusion

A role for elevated chemokine production within β -cells as a critical mechanistic determinant controlling islet inflammation during T1D and T2D development is emerging (Tables 1 and 2). Chemokines, and other proteins that regulate inflammation, can be induced by a variety of signals that activate either STAT1, NF- κ B or both signaling pathways (Fig. 1). The increased synthesis and secretion of discrete chemokines induce receptor-mediated signals that regulate immune cell entry into, and activity within, pancreatic tissue (Fig. 2). Importantly, chemokine production in pancreatic islets appears to be a common phenomenon between T1D and T2D. While rodent work has been incredibly informative towards understanding the genetics and phenotypes of the human disease, much remains to be uncovered. It is our hope that understanding the inflammatory signaling-induced upregulation of chemokine production and chemokine/chemokine receptor interactions will assist in the design of therapeutics relevant to improving β -cell mass and secretory function as a way to combat the growing incidence of T1D and T2D.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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