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Harnessing CXCL12 signaling to protect and preserve functional β-cell mass and for cell replacement in type 1 diabetes

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ABSTRACT

Type 1 diabetes (T1D) is a complex multifactorial disease characterized by autoimmune destruction of insulin-producing pancreatic β cells. Our understanding of the pathogenic mechanisms and natural history of T1D has evolved significantly over the past two decades; we can efficiently predict high-risk individuals, early diagnose the disease and stage progression. Fortuitously, novel *in vitro* differentiation protocols for generating functional β-like cells from human pluripotent stem cells have been developed. These advances provide a definitive roadmap to implement realistic preventive and β-cell replacement therapies in T1D. Immunoprotection and preservation of functional β-cell mass are a *sine qua non* for the success of these interventions. The chemokine, stromal cell-derived factor-1alpha, known as CXCL12-α, is an attractive therapeutic target molecule in this context. CXCL12-α signaling promotes β-cell development, survival and regeneration and can mediate local immunomodulation in the pancreatic islets. Interestingly, CXCL12-α is robustly expressed in maturing insulin-producing β cells and in adult β cells during periods of injury and regeneration. However, under normal physiological settings, CXCL12-α is repressed in terminally differentiated mature β cells and islets. Here, we provide a comprehensive overview of the role of CXCL12-α signaling in β-cell biology, physiology and immune regulation. We discuss CXCL12-α signaling mechanisms that could be harnessed to modulate β-cell autoimmunity, protect and preserve functional β-cell mass and for cell replacement therapy in T1D.

Contents

Abbreviations: DPP4, dipeptidyl peptidase 4; T1D, type 1 diabetes; INS, insulin; GAD65, glutamic acid decarboxylase 65-kDa isoform; IA2, islet antigen 2; ZnT8, zinc transporter 8; hESC, human embryonic stem cell; hiPSC, human induced pluripotent stem cell; GPCR, G protein-coupled receptor; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-activated kinase; PI3K, phosphoinositide 3-kinase; PLPβ, phospholipase β; JAK, janus kinase; STAT, signal transducer and activator of transcription; DAG, diacylglycerol; GRK, G protein-coupled receptor kinase; AKT, protein kinase B; LMO2, LIM domain only 2; PDX1, pancreatic and duodenal homeobox 1; NGN3, neurogenin 3; NOD, non-obese diabetic; DC, dendritic cell; MΦ, macrophage; MSC, mesenchymal stem/stromal cell; HSC, hematopoietic stem cell; HSPC, hematopoietic stem and progenitor cell; PGE2, prostaglandin E2; TSG-6, TNF-α-induced protein 6; IDO, indoleamine 2,3-dioxygenase; PD-L1, programmed death-ligand 1; GSIS, glucose-stimulated insulin secretion.

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1. Introduction

Type 1 diabetes (T1D) results from autoimmune destruction of insulin-producing β cells of the pancreatic islets of Langerhans (Katsarou et al., 2017). Without effective treatment, ensuing insulin deficiency leads to chronic hyperglycemia and associated metabolic and organ complications (Atkinson, Eisenbarth, & Michels, 2014). Clinical T1D diabetes typically eventuates loss of 70-90% of an individual's β cells (Eisenbarth, 1986; Johnson, 2016). With its incidence and prevalence on a global rise (Dabelea, 2009), T1D is one of the most common childhood autoimmune disorders (Eisenbarth, 1986; Johnson, 2016). Advanced treatment algorithms with newer and superior acting insulin analogs, insulin delivery systems and glucose monitoring technologies enable T1D patients to experience complication-free lives and improved life expectancies ("Introduction: Standards of Medical Care in Diabetes—2018" 2018; Sun et al., 2011). However, this is not curative, and daily monitoring with frequent insulin injections is cumbersome, causing discomfort. Thus, the economic and emotional burden of living with T1D is high (DiMeglio, Evans-Molina, & Oram, 2018). Even more importantly, it is virtually impossible to simulate the dynamic changes in insulin secretion from β cells in response to glucose fluctuations by exogenous insulin administration. Furthermore, insulin administration is problematic in a subset of patients who suffer hypoglycemia unawareness (Cryer, 1994). Ultimately, patients experience damage to fine nerve endings and blood vessels that result in organ complications such as nephropathy, neuropathy, retinopathy and cardiovascular complications (DiMeglio et al., 2018; Herold, Vignali, Cooke, & Bluestone, 2013).

The consensus is that an intricate interplay of genetic predisposition, intrinsic β-cell physiological mechanisms and environmental factors act in harmony to trigger loss of self-tolerance to β-cell antigens, resulting in selective autoimmune destruction of β cells (Atkinson et al., 2011; Bluestone, Herold, & Eisenbarth, 2010; Knip et al., 2005; Marre et al., 2016; Rewers & Ludvigsson, 2016). Thus, the pathophysiology of T1D is characterized by an inflammatory immune cell infiltration of the pancreatic islets of Langerhans. This immune infiltrate comprises predominantly $CD8^+$ T cells and to a relatively lesser extent, macrophages, $CD4^+$ T cells, dendritic cells (DCs) and B cells (Coppieters et al., 2012; Eisenbarth, 2010; Hanninen et al., 1992; Lernmark et al., 1995; Willcox, Richardson, Bone, Foulis, & Morgan, 2009). $CD4^+CD25^{+-}$ $FoxP3^+$ regulatory T cells (Tregs) and natural killer (NK) cells have been shown to be rare in the islet inflammatory lesion (Atkinson et al., 2014; Tang et al., 2008; Tang & Bluestone, 2008; Xufre et al., 2013). Tregs in T1D patients also appear to be functionally compromised (Brusko, Wasserfall, Clare-Salzler, Schatz, & Atkinson, 2005; Lindley et al., 2005). Autoantibodies with specificities against β-cell antigens including insulin (INS), glutamic acid decarboxylase 65-kDa isoform (GAD65), islet antigen 2 (IA2) and zinc transporter-8 (ZnT8) are commonly detected in the sera of patients and individuals at risk of developing T1D (Bingley et al., 2018; Ilonen et al., 2013; Krischer et al., 2015; Soeldner, Tuttleman, Srikanta, Ganda, & Eisenbarth, 1985; Tarn et al., 1988; Ziegler et al., 2013; Ziegler, Hummel, Schenker, & Bonifacio, 1999; Ziegler & Nepom, 2010). These autoantigens are associated with the secretory function of β cells, underscoring a contribution of the intrinsic unique physiology of the β-cell to disease pathogenesis (Atkinson et al., 2011; Kracht et al., 2017; Tersey et al., 2012). Diabetogenic $CD8^+$ T cells primarily mediate β-cell destruction via perforin/granzyme (Trivedi et al., 2016) and Fas/Fas ligand interactions (Pang et al., 2009; Roep, 2003). $CD4^+$ T cells promote β-cell destruction via secretion of cytotoxic pro-inflammatory cytokines including IFN-γ, IL-1β and TNF-α (Arif et al., 2004; Rabinovitch, 1998) and by activation of diabetogenic $CD8^+$ T cells, B cells and macrophages (Padgett et al., 2015; Thayer et al., 2011). Macrophages, B cells and dendritic cells (DCs) can promote β-cell destruction through antigen presentation to and activation of autoreactive T cells (Boldison & Wong, 2016; Martinez, Helming, & Gordon, 2009; Summers, Behme, Mahon, & Singh, 2003). Additionally, macrophages can produce pathogenic pro-inflammatory cytokines such as IL-1β and TNF-α (Arnush, Scarim, Heitmeier, Kelly, & Corbett, 1998; Calderon, Suri, & Unanue, 2006; Dahlen, Dawe, Ohlsson, & Hedlund, 1998). Apoptosis is considered the predominant pathway of β-cell death in T1D, although necrosis has also been implicated (Cnop et al., 2005; Eizirik & Darville, 2001; Eizirik & Mandrup-Poulsen, 2001; Wilcox, Rui, Hebrok, & Herold, 2016).

Logically, replacement or regeneration of functional β cells together with induction of immunological tolerance to or immunoprotection of β cells is a potential cure for T1D. Fortunately and fortuitously, our understanding of the pathogenic mechanisms and natural history of T1D (Atkinson et al., 2014; Atkinson, von Herrath, Powers, & Clare-Salzler, 2015; Bluestone, Herold, & Eisenbarth, 2010) and the developmental biology of the β-cell have advanced significantly over the past two decades (Melton, 2016; Pagliuca & Melton, 2013). We can efficiently predict individuals at high risk of developing T1D, early diagnose the disease and stage progression (Insel et al., 2015; Redondo, Oram, & Steck, 2017; Regnell & Lernmark, 2017; Sosenko et al., 2015; Ziegler & Nepom, 2010; Ziegler et al., 2013). Novel scalable *in vitro* differentiation protocols for producing glucose-responsive insulin-secreting β-like cells (so-called SC-β cells) from human embryonic and induced pluripotent stem cells (hESCs/hiPSCs) have been developed (Millman et al., 2016; Pagliuca et al., 2014; Rezania et al., 2014). These advances provide a definitive framework to implement practicable therapeutic interventions in T1D. We can design strategies aimed at: (1) preventing disease progression in at-risk individuals, (2) preserving and regenerating endogenous functional β-cell mass in newly diagnosed patients and (3) replacing functional β-cell mass in disease-established patients. Achieving immunological tolerance to/immunoprotection of endogenous or transplanted β cells while preserving or maintaining functional β-cell mass would be indispensable to the success of these interventions.

The chemokine, stromal cell-derived factor-1alpha (SDF-1α), known as CXCL12-α (referred to as CXCL12 henceforth), plays crucial roles in immune regulation and pancreatic β-cell biology that are relevant to the immunopathogenic and pathophysiologic mechanisms in T1D. CXCL12 is a major regulator of immune cell activation, trafficking, differentiation and function and can induce local immunomodulation in the pancreatic islet microenvironment. Furthermore, CXCL12 signaling promotes β-cell development, survival and regeneration. Accordingly, dysfunctional CXCL12 signaling and/or polymorphisms in the CXCL12 gene have been implicated in both murine and human autoimmune diabetes (Dubois-Laforgue et al., 2001; Ide et al., 2003; Leng, Nie, Zou, & Chen, 2008). Here, we provide an overview of the role of CXCL12 signaling in immune regulation and pancreatic β-cell biology. We discuss CXCL12 signaling in β-cell development, differentiation, survival, function and regeneration as well as immune regulation. We highlight signaling mechanisms of CXCL12 that could be harnessed to modulate β-cell autoimmunity, regeneration, to preserve functional mass and for cell replacement therapy in T1D.

2. CXCL12 signaling

CXCL12 is an 8-kDa homeostatic chemokine that is encoded by a single gene with six variant splice isoforms, including SDF-1α, SDF-1β, SDF-1γ, SDF-1δ, SDF-1ε, and SDF-1ϕ (Janowski, 2009; Yu et al., 2006). The predominant and best-studied isoform is CXCL12-α (Yu et al., 2006). CXCL12 refers to this isoform in this review. CXCL12 is highly conserved across species; between human and mouse, there is only one amino acid difference of a conserved valine for isoleucine at position 18 of its 68 amino acid residues (Shirozu et al., 1995). This high sequence homology enables reactivity of CXCL12 across different species (Nagasawa, Tachibana, & Kishimoto, 1998). The chemokine exerts its effects by binding to two distinct seven-membrane transpanning G protein-coupled receptors (GPCRs), namely CXCR4 and CXCR7 (Balabanian et al., 2005; Oberlin et al., 1996). CXCL12 and its receptors comprise the only chemokine signaling system in which loss of function

results in embryonic and/or perinatal lethality (Gerrits et al., 2008; Ma et al., 1998; Sierro et al., 2007; Zou, Kottmann, Kuroda, Taniuchi, & Littman, 1998). CXCL12 is the only chemokine ligand that binds CXCR4, and loss of function of CXC12 or CXCR4 phenocopy each other in embryonic development (Ma et al., 1998; Nagasawa et al., 1996; Zou et al., 1998).

CXCL12 induces several divergent intracellular signaling pathways that can culminate in many downstream effects including cell migration, survival and/or proliferation, increased intracellular calcium, gene transcription and cell differentiation (Ganju et al., 1998; Teicher & Fricker, 2010). Some of the signaling pathways mediated by CXCL12 via the CXCR4 and CXCR7 receptors are illustrated in Fig. 1. The chemokine can bind its receptors as a monomer or a dimer, which is influenced by its extracellular concentration (Drury et al., 2011). High concentrations favor CXCL12 dimer formation (Ray et al., 2012). Monomeric CXCL12 preferentially binds to CXCR4 (Drury et al., 2011; Ray et al., 2012). The chemokine has a higher binding affinity for CXCR7 compared with CXCR4 (Balabanian et al., 2005). *In vitro* studies indicate that monomeric CXCL12 signaling via CXCR4 induces calcium influx and chemotaxis, whereas dimeric CXCL12 can induce calcium influx but lacks chemotactic activity (Drury et al., 2011; Veldkamp et al., 2008; Veldkamp, Peterson, Pelzek, & Volkman, 2005). The CXCR4 receptor can signal as a monomer, homodimer (Percherancier et al., 2005; Toth, Ren, & Miller, 2004) or heterodimer with CXCR7 (Decaillot et al., 2011; Levoye, Balabanian, Baleux, Bachelerie, & Lagane, 2009). Activation of CXCR4 by CXCL12 can trigger classical heterotrimeric G protein (G_α, G_β and G_γ subunits) signaling as well as engagement of β-arrestin signaling (Quoyer et al., 2013). On the other hand, activation of CXCR7 or CXCR4-CXCR7 heterodimer preferentially elicits β-arrestin signaling independent of G protein (Decaillot et al., 2011; Rajagopal et al., 2010).

Activation of CXCR4 results in exchange of GDP for GTP in the G_α subunit, ($G_{\alpha i}$ specifically) resulting in dissociation of the trimer into an active GTP-bound $G_{\alpha i}$ and a $G_{\beta\gamma}$ dimer (Kehrl, 1998). Activated $G_{\alpha i}$ inhibits adenylyl cyclase activity, and triggers activation of mitogen-activated protein kinase/extracellular signal-regulated kinases (MAPK/ERK) and phosphoinositide-3 kinase (PI3K) signaling pathways, while dissociated $G_{\beta\gamma}$ dimer triggers activation of phospholipase C-β (PLCβ), leading to activation of phosphatidylinositol-4,5-bisphosphate (PIP2), diacylglycerol (DAG), inositol-1,4,5-triphosophate (IP3) and intracellular Ca^{2+} mobilization (Busillo & Benovic, 2007). CXCR4 can homodimerize upon CXCL12 binding and induce recruitment and activation Janus Kinase-Signal Transducer and Activator of Transcription (JAK/STAT) signaling pathway independent of G protein (Pfeiffer et al., 2009; Vila-Coro et al., 1999). Subsequent nuclear translocation of activated STAT proteins can induce several cellular changes such as gene expression (Vila-Coro et al., 1999). CXCR4 signal transduction culminates in cellular effects including cell migration, proliferation, survival, gene transcription, and differentiation (Ganju et al., 1998; Teicher & Fricker, 2010). Alternatively, activated CXCR4 can be phosphorylated by protein kinase C (PKC) and G protein-coupled receptor kinases (GRKs) (Busillo et al., 2010). The phosphorylated CXCR4 is rapidly internalized by β-arrestin for subsequent ubiquitylation and lysosomal degradation (Haribabu et al., 1997).

As aforementioned, activation of CXCR7 by CXCL12 does not trigger classical G protein-mediated signaling. Instead, it could lead to one of two downstream effects depending on the context. On one hand,

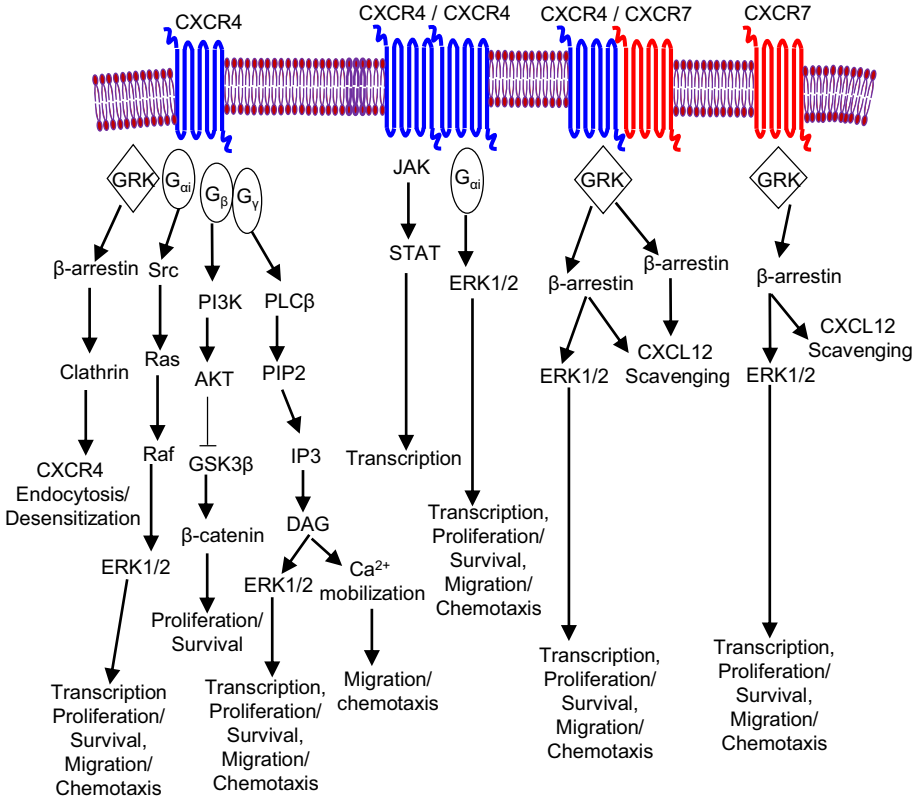

Fig. 1. CXCL12/CXCR4/CXCR7 signaling CXCL12 acts on CXCR4 and CXCR7, which are two distinct seven-membrane transpanning G-protein-coupled receptors. CXCR4 can transduce signaling as a monomer, homodimer or heterodimer with CXCR7. Binding of CXCL12 to CXCR4 can transduce classical G-protein-coupled signaling to activate ERK1/2, PI3K/AKT, PLCB/IP3 pathways, culminating in cell proliferation/survival and migration/chemotaxis or gene transcription. Activated homodimers of CXCR4 can transduce JAK/STAT signaling independent of G-protein and subsequent gene transcription as well as G-protein-coupled signaling and subsequent ERK1/2 pathways. Heterodimerization of CXCR4 with CXCR7 preferentially elicits β-arrestin-mediated signaling to activate ERK1/2 signaling or result in CXCL12 scavenging. CXCR7 transduces CXCL12-mediated signaling via β-arrestin independent of G-protein and activates ERK1/2 signaling or CXCL12 scavenging.

CXCL12 could be internalized and degraded, in which case CXCR7 serves as a decoy receptor or a sink for CXCL12 (Wang et al., 2012). On the other hand, activation of CXCR7 and/or CXCR4/CXCR7 heterodimer could lead to β-arrestin-mediated G protein-independent signaling, whereby activated CXCR7 or CXCR4/CXCR7 heterodimer induces recruitment of β-arrestin and subsequent activation of AKT and ERK1/2 independent of G proteins (Rajagopal et al., 2010; Torossian et al., 2014).

The plethora of possible signaling pathways mediated by CXCL12 imbues the chemokine with pleiotropic biological activities, depending on the cellular context as well as that of the tissue microenvironment.

3. Islet expression of CXCL12 and its cognate receptors

CXCL12 is expressed at a very low level, if at all, in terminally differentiated mature pancreatic islets (Pullen & Rutter, 2013), and at an even much lower level in all three major endocrine cell types including α, β and δ cells (Lemaire et al., 2017). CXCL12 is thus considered one of eleven so-called "β-cell or islet core disallowed or forbidden genes" that are ubiquitously expressed across tissues but selectively repressed in adult β cells and islets (Pullen et al., 2010; Pullen & Rutter, 2013; Rutter, Pullen, Hodson, & Martinez-Sanchez, 2015; Thorrez et al., 2011). Although repressed in adult β cells, CXCL12 is robustly expressed in insulin-producing developing and regenerating β cells (Kayali et al., 2003; Kayali et al., 2012; Liu, Stanojevic, Avadhani, Yano, & Habener, 2011; Yano, Liu, Donovan, Thomas, & Habener, 2007). In the fully developed and mature pancreas, expression of CXCL12 is restricted to the ductal epithelium and islet-surrounding vascular endothelial cells (Kayali et al., 2012; Yano et al., 2007).

CXCR4 is the most abundantly expressed chemokine receptor in the pancreatic islet (Amisten, Salehi, Rorsman, Jones, & Persaud, 2013), and predominantly co-expressed with insulin in β cells (Kayali et al., 2012; Yano et al., 2007). CXCR4 is expressed in α cells, albeit to a lesser extent, and only a minority of α cells co-express glucagon and CXCR4 (Yano et al., 2007). CXCR7 is expressed in human islets (Amisten et al., 2013), especially in β cells (Bergholdt et al., 2012). However, the CXCL12/CXCR7 axis is less studied compared with the CXCL12/CXCR4 axis in pancreatic islets.

4. CXCL12 signaling in β-cell development

The CXCL12/CXCR4 axis plays an integral role in the survival, proliferation, migration and differentiation of pancreatic progenitor cells into mature β cells (Katsumoto & Kume, 2011; Kayali et al., 2003; Kayali et al., 2012). Both CXCL12 and CXCR4 are simultaneously expressed throughout pancreatic endocrine cell differentiation and maturation (Kayali et al., 2012). CXCR4 is a marker for the definitive endoderm (DE) (D'Amour et al., 2005), from which the pancreas develops. *Cxcl12*, expressed in the DE, attracts *Cxcr4*-expresing angioblasts in the mesoderm. The recruited angioblasts produce LIM domain only 2 (*Lom2*), which induces pancreatic and duodenal homeobox 1 (*Pdx1*) expression in the endoderm to specify the pancreatic fate (Katsumoto & Kume, 2011, 2013). As pancreas development progresses, the specification of multipotent pancreatic progenitors into endocrine cell lineage is characterized by a transient expression of the transcription factor neurogenin 3 (NGN3) (Gradwohl, Dierich, LeMeur, & Guillemot, 2000; Gu, Dubauskaite, & Melton, 2002; Rukstalis & Habener, 2009). Then a complex interplay of unique transcription factors and extrinsic cues dictate differentiation of NGN3-positive endocrine progenitors into β cells or other endocrine cell fates (Oliver-Krasinski & Stoffers, 2008; Pan & Wright, 2011). The CXCL12/CXCR4 axis appears to be particularly important for β-cell differentiation and maturation beyond NGN3-expressing endocrine progenitors. For instance, ectopic overexpression of *Cxcl12* in chick embryogenesis caused excessive recruitment of angioblasts to the endoderm and generated higher *Pdx1*- and SRY box 9 (*Sox9*)-expressing pancreatic progenitors. This in turn, led to a larger pancreas with an expanded insulin-expressing area (Katsumoto & Kume, 2011). In contrast, inhibition of CXCR4 via AMD3100 treatment caused diminished *Pdx1*- and *Sox9*-positive pancreatic progenitors, a smaller pancreas and smaller insulin-expressing area (Katsumoto & Kume, 2011).

By using immunofluorescence staining, Kayali et al. demonstrated that CXCR4 expression is tracked through human fetal pancreas development, co-localizing predominantly with insulin-positive cells during later stages of endocrine cell development (Kayali et al., 2012). The authors demonstrated that pharmacological inhibition of CXCR4 using AMD3100 blocked differentiation and maturation of human fetal islet-like clusters into insulin-producing β cells *in vivo* (Kayali et al., 2012). They found that at the developmental stage when endocrine islet cells are formed, as marked by transient NGN3 expression, the NGN3-positive cells also express CXCR4. It is thought that approximately 80% of the NGN3-positive cells would differentiate into β cells (Gu, Brown, & Melton, 2003). It is therefore, conceivable that CXCL12 acts as a mitogen and/or a morphogen, recruiting differentiation factors for NGN3-expressing cells, promoting their survival, expansion and differentiation into mature β cells.

5. CXCL12 signaling in β-cell regeneration

There is limited capacity of adult pancreatic β cells to regenerate (Kopp, Grompe, & Sander, 2016; Rankin & Kushner, 2009; Tschen, Dhawan, Gurlo, & Bhushan, 2009; Zhou & Melton, 2018). The prevailing wisdom suggests three possible mechanisms of β-cell regeneration. These include replication of pre-existing β cells (Dor, Brown, Martinez, & Melton, 2004; Meier et al., 2006; Teta, Rankin, Long, Stein, & Kushner, 2007), β-cell regeneration from precursor cells (neogenesis) (Bonner-Weir et al., 2004; Li et al., 2010; Liu, Guz, Kedees, Winkler, & Teitelman, 2010; Xu et al., 2008) and trans-differentiation of non-β cells into β-cells (Chera et al., 2014; Chung, Hao, Piran, Keinan, & Levine, 2010; Thorel et al., 2010). Elegant genetic lineage tracing experiments in mice however, suggest that replication of pre-existing β cells in both homeostasis and during injury is the principal mechanism for replenishing or maintaining β-cell mass in adulthood (Dor et al., 2004). Studies on β-cell regeneration using rodent pancreas or β-cell-specific injury models have implicated a role for CXCL12 signaling in islet β-cell neogenesis and regeneration (Kayali et al., 2003; Liu et al., 2011). One of such models is the IFN-γ transgenic mouse, in which the insulin gene promoter drives IFN-γ expression. Locally produced IFN-γ causes lymphocyte infiltration and islet-cell destruction. This is accompanied by ductal hyperplasia and islet neogenesis (Gu & Sarvetnick, 1993, 1994; Sarvetnick, Liggitt, Pitts, Hansen, & Stewart, 1988). This model been suggested as an appropriate model for the study of the regeneration of functional β cells from ductal cells (Jones & Sarvetnick, 1997). However, it is important to note that the contribution of ductal cells as a source for β-cell regeneration remains an unresolved debate. A recent review that expatiates on these cells as sources for β-cell regeneration has been presented elsewhere (Aguayo-Mazzucato & Bonner-Weir, 2018). Kayali and co-workers demonstrated that in IFN-γ transgenic non-obese diabetic (NOD) mice undergoing islet neogenesis, expression of CXCL12 and CXCR4 is upregulated 3- and 4-fold, respectively (Kayali et al., 2003). They showed that CXCL12 and CXCR4 are both localized to primitive islet-cell clusters in the regenerating pancreas whereas the ducts express CXCR4 but not CXCL12. The ductal cells then migrate or bud off into the primitive islet-cell clusters on the CXCL12 gradient, and as they migrate or bud off, they undergo surface remodeling (Slack, 1995), changes in their adhesive properties (Cirulli et al., 2000) and terminal differentiation into β cells (Kayali et al., 2003). Consequently, monoclonal antibody inhibition of CXCR4 signaling significantly diminished PDX1-expressing ductal cells and the formation of new islets in this model (Kayali et al., 2003).

Another model for studying β-cell regeneration is β-cell ablation using β-cell-specific chemical toxins, such as streptozotocin (STZ) and

alloxan (Chera et al., 2014) or β-cell-specific genetic manipulation (Thorel et al., 2010). Such studies have demonstrated regeneration of new β cells from other endocrine cell types or proliferation of pre-existing β cells following near-complete ablation of β cells (Chen et al., 2009; Chera et al., 2014; Krishnamurthy et al., 2006; Thorel et al., 2010). The injured and regenerating β cells re-induce robust expression of CXCL12 (Liu et al., 2011), recapitulating their expression of the chemokine during development (Kayali et al., 2012). Injury to β cells by chemical toxins, metabolic stress and autoimmunity has been shown to cause α-cell expansion (Li, Karlsson, & Sandler, 2000; Nie et al., 2000; Thyssen, Arany, & Hill, 2006). Following injury to β cells, CXCL12 signaling is posited to promote α-cell hyperplasia and their subsequent trans-differentiation into β cells (Habener & Stanojevic, 2012; Stanojevic & Habener, 2015; Yano et al., 2007). An explicit model describing the mechanism of CXCL12 signaling-mediated α-cell-to-β-cell conversion has been presented elsewhere (Habener & Stanojevic, 2012; Liu et al., 2011).

6. CXCL12 signaling in β-cell survival and function

The PI3K/AKT and WNT/β-catenin signaling pathways are principal regulators of β-cell survival and proliferation (Fatrai et al., 2006; Georgia & Bhushan, 2004; Kushner et al., 2005; Rulifson et al., 2007). CXCL12 induces activation of both pathways in islets and β cells (Liu et al., 2011; Yano et al., 2007), and promotes survival and expansion of insulin-producing maturing β cells (Kayali et al., 2003). In terminally differentiated mature β cells, CXCL12 exerts a predominantly pro-survival and non-proliferative effect (Liu & Habener, 2009). Several studies have demonstrated both anti-apoptotic (Yano et al., 2007) and anti-necrotic (Grdovic et al., 2014) effects of CXCL12 on β cells. Yano and co-workers demonstrated that transgenic β-cell-specific overexpression of CXCL12 protects mice against STZ induction of β-cell apoptosis and hyperglycemia (Yano et al., 2007). The anti-apoptotic effect was associated with activation of AKT and increased induction of the anti-apoptotic protein BCL2 and inactivation of the pro-apoptotic protein BAD (Yano et al., 2007). In further studies, the authors demonstrated that through the activation of the PI3K/AKT pathway, CXCL12 enhances the WNT/β-catenin signaling pathway (Fig. 1), suppressing GSK3β to promote β-cell survival (Liu & Habener, 2009). They showed that CXCL12 prevents β-cell apoptosis from a variety of β-cell toxins including STZ, cytokines, and thapsigargin. It was recently demonstrated that a subpopulation of β cells that resists autoimmune destruction exists during T1D in NOD mice. This cell population was enriched for CXCL12, among other factors (Rui et al., 2017). Grdovic and co-workers showed that β-cell overexpression of CXCL12 considerably improves insulin expression and viability of isolated rat islets and Rin-5F β cells following exposure to hydrogen peroxide (Grdovic et al., 2014).

CXCL12 is a major substrate for degradation by dipeptidyl peptidase 4 (DPP4/CD26), which causes CXCL12 inactivation by removing the NH_2-terminal two residues of the chemokine (Christopherson, Hangoc, & Broxmeyer, 2002; Christopherson, Hangoc, Mantel, & Broxmeyer, 2004; Sadir, Imberty, Baleux, & Lortat-Jacob, 2004). Thus, CXCL12 is intrinsically unstable due to proteolytic degradation and has a half-life of less than 1 minute for truncation by DPP4 (Lambeir et al., 2001) and DPP4 inhibitor treatment increases the local levels of CXCL12 (Brenner et al., 2014; Theiss et al., 2013). Pancreatic islets highly express DPP4 (Bugliani et al., 2018) and this could inactivate the protective effects of CXCL12 in islets. Saxagliptin, a DDP4 inhibitor, has been reported to improve β-cell function in human clinical trials and to promote islet neogenesis in rodents (Henry et al., 2011; Takeda et al., 2012; Wu et al., 2015; Zhang, Wang, Huang, & Wang, 2011). Li et al. reported that saxagliptin treatment induced β-cell proliferation and improved β-cell function via suppression of DPP4-mediated inactivation of CXCL12 under high glucose conditions (Li et al., 2017). The authors demonstrated that this effect was independent of the DPP4-glucagon-like peptide 1 (GLP-1) axis, which is often considered to be the main pathway through which gliptins exert their insulinotropic effects (Holst & Deacon, 1998).

While the β-cell growth and pro-survival effects of CXCL12 are well established, the physiological relevance of its disallowance or forbiddance in terminally differentiated mature β cells and islets remains unclear. This is particularly important in the context of endocrine cell differentiation and hormone secretion. The continuous expression of CXCR4 in mature β cells and the pro-survival effects exerted by CXCL12 on β cells however, clearly indicate that CXCL12/CXCR4 signaling is active in mature β cells. Moreover, the co-expression of CXCR4 with insulin in mature β cells but not with glucagon in α cells suggests that CXCR4 signaling may be essential for insulin production but not glucagon. In support of this view, CXCL12 that is produced by β cells during injury has been shown to induce CXCR4-mediated production of prohormone convertase 1/3 (PC1/3, encoded by the PCSK1 gene) in α cells. PCSK1 in turn processes proglucagon to GLP-1 instead of glucagon in the α cells (Liu et al., 2011). We have recently shown that in human stem cell-derived β cells (SC-β cells) (Pagliuca et al., 2014), recombinant CXCL12 protein potentiates glucose-stimulated insulin secretion (GSIS). We observed that when SC-β cells are co-encapsulated with recombinant CXCL12 in alginate microcapsules and transplanted into diabetic mice, the mice achieve enhanced GSIS and accelerated reversal of hyperglycemia (Alagpulinsa et al., submitted for publication). Luther and co-workers reported that under normal physiological conditions, ectopic β-cell-specific overexpression of CXCL12 in pancreatic islets does not induce unwarranted leukocyte activation and infiltration in the islets (Luther et al., 2002). This suggests that the disallowance of CXCL12 in mature islets may not be a mechanism to prevent unnecessary leukocyte infiltration and/or lymphoid tissue formation in the islets. Furthermore, transgenic β-cell-specific overexpression of CXCL12 in mice did not cause metabolic dysfunction in adult mice (Yano et al., 2007). The plasma insulin or glucose levels and weight of the CXCL12-transgenic mice versus their littermate controls were indistinguishable (Yano et al., 2007). Taken together, it is most likely that the repression of CXCL12 expression upon β-cell terminal differentiation and maturation serves as a mechanism to regulate β-cell mass and function, and that its expression in mature β cells may not cause β-cell dedifferentiation or impaired insulin secretion. That said, the effect of β-cell-specific overexpression of CXCL12 on GSIS or glucose tolerance in isolated primary islets or mice in health has not been evaluated.

7. CXCL12 signaling in immune regulation and β-cell autoimmunity

7.1. CXCL12-mediated regulation of immune cell function and β-cell autoimmunity

CXCL12 plays a crucial role in modulating immune responses through the regulation of leukocyte activation, trafficking/migration, differentiation and function. Although generally perceived as a leukocyte chemoattractant in inflammation, CXCL12 appears to be involved primarily in immune surveillance under physiological conditions rather than inducing inflammation (Bleul, Fuhlbrigge, Casasnovas, Aiuti, & Springer, 1996). On the contrary, several studies indicate an anti-inflammatory, immunoregulatory and tissue repair function for CXCL12 during pathological inflammation and autoimmunity (Karin, 2010; Meiron, Zohar, Anunu, Wildbaum, & Karin, 2008; Sanchez-Martin, Sanchez-Mateos, & Cabanas, 2013). Polymorphisms in the CXCL12 gene, located near the T1D susceptibility locus IDDM10 as well as dysregulated CXCL12 signaling has been linked with T1D susceptibility in both humans and murine models of the disease (Dubois-Laforgue et al., 2001; Ide et al., 2003; Kawasaki et al., 2004). Differential expression and single nucleotide polymorphisms (SNPs) in the CXCR7 gene, one of the receptors for CXCL12, have also been associated with T1D (Bergholdt et al., 2012). We will provide an overview of the known immunoregulatory functions of CXCL12, which are summarized in Fig. 2.

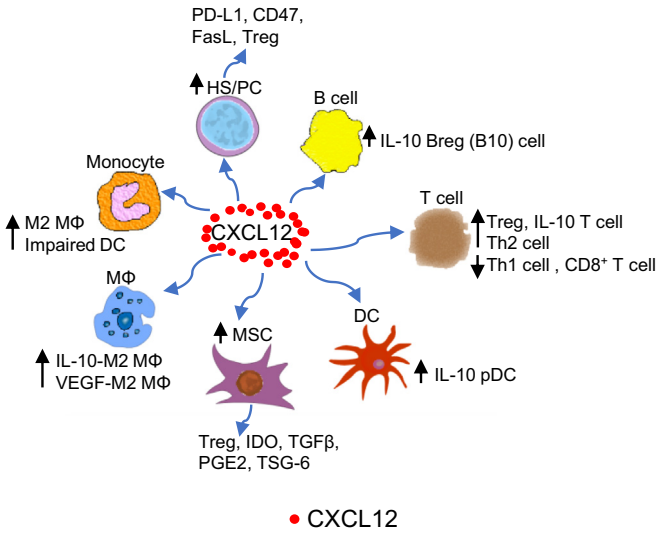

Fig. 2. Immune regulatory functions of CXCL12 This scheme shows the immunomodulatory effects of CXCL12 on different cell types. CXCL12 can recruit and retain Tregs, induce polarization of IL-10-producing T cells/Th2 cells, repel $CD8^+$ T cells and decrease Th1 cells/Th2 cells ratio. CXCL12 can also induce generation of plasmacytoid dendritic cells (pDCs) that produce IL-10. CXCL12 can induce differentiation and polarization of monocytes/macrophages toward anti-inflammatory M2 macrophages that produce IL-10 and/or VEGF and into DCs with impaired ability to stimulate antigen-specific T cell responses. Also, CXCL12 can recruit B cells and enhances their IL-10 production. CXCL12 also recruits and retains MSCs, which produce immunoregulatory molecules such as IDO, TGFβ, PGE2, and TSG-6 and while causing Treg expansion. Like its effect on MSCs, CXCL12 can recruit and retain HS/PCs, which express immunosuppressive molecules such as PD-L1, CD47 and FasL and cause Treg expansion.

We have shown that CXCL12 can recruit $CD4^+CD25^+$ $FoxP3^+$ regulatory T cells (Tregs) and repel effector T cells from a local anatomic site in a concentration-dependent manner (Poznansky et al., 2000; Vianello et al., 2006). In agreement with these findings, Glawe et al. reported that CXCL12 that is expressed in the islet endothelium repels autoreactive/diabetogenic T cells, preventing autoimmune diabetes (Glawe et al., 2013; Sharp et al., 2008), while Aboumrad et al. showed that T cells that are recruited by CXCL12 protect recipient mice from adoptive T-cell transfer of autoimmune diabetes (Aboumrad, Madec, & Thivolet, 2007). The pancreatic lymph nodes (PLNs) represent a major site for diabetogenic T-cell activation (Gagnerault, Luan, Lotton, & Lepault, 2002). Nti et al. observed that the PLNs of NOD mice exhibit decreased expression of CXCL12, accompanied by deficiency of Tregs in the PLNs (Nti et al., 2012). The authors demonstrated that restoration of normoglycemia in these mice via induction of allogeneic hematopoietic chimerism was characterized by increased CXCL12 expression in the PLNs and enhanced infiltration of Tregs (Nti et al., 2012). Leng et al. showed that the bone marrow of NOD mice has increased expression of CXCL12, resulting in polarized retention of Tregs in the bone marrow. They demonstrated that inhibition of CXCL12 singling the bone marrow via treatment with the CXCR4 antagonist, AMD3100, caused egress of the Tregs to the peripheries to prevent islet inflammation (Leng et al., 2008). In experimental autoimmune encephalitis (EAE), which is an animal model for multiple sclerosis or brain autoinflammation, Meiron et al. showed that CXCL12 restrains inflammation and autoimmunity by inducing local tissue polarization of T cells and macrophages into anti-inflammatory and immunosuppressive IL-10-producing regulatory cells (Karin, 2010; Meiron et al., 2008). Furthermore, CXCL12 has been shown to directly cause costimulation of IL-10 secretion in different human T cell types (Kremer, Kumar, & Hedin, 2007; Kumar et al., 2006). The chemokine can induce the generation IL-10-producing plasmacytoid dendritic cells (pDCs) (W. Zou et al., 2001) and DCs with impaired ability to induce antigen-specific T-cell responses (Sanchez-Martin et al., 2011). Depending on the cytokine milieu, macrophages can adapt a pro-inflammatory M1 macrophage phenotype or an alternative anti-inflammatory M2 macrophage phenotype (Davis et al., 2013). Multiple studies have demonstrated that local expression or delivery of CXCL12 protein induces differentiation and polarization of monocytes/macrophages toward the anti-inflammatory M2 macrophage phenotype (Brenner et al., 2015; Chatterjee et al., 2015; Sanchez-Martin et al., 2011). Thus, CXCL12 induces polarization of immunosuppressive macrophages that produce IL-10 and/or VEGF (Beider et al., 2014; Meiron et al., 2008; Sanchez-Martin et al., 2011). A subset of B cells known as regulatory cells, contribute to immune tolerance through STAT3-mediated production of IL-10 (known as B10 cells) (Lemoine, Morva, Youinou, & Jamin, 2009). Nishimura et al. demonstrated that local tissue delivery of CXCL12 induces recruitment of B cells that produce IL-10 (Nishimura et al., 2013). Furthermore, Shaim and co-workers showed that CXCL12 induces IL-10 production in chronic lymphocytic leukemia cells, which is a tumor of B cells, via CXCR4-STAT3 signaling (Shaim et al., 2017). Taken together, it is apparent that CXCL12 exerts anti-inflammatory and immunoregulatory effects during pathological inflammation and autoimmunity by modifying the differentiation and function of multiple immune cell types.

IL-10 and Tregs possess potent immunosuppressive, anti-inflammatory effects and impaired function of both has been implicated in T1D. CXCL12 thus represents a key mechanism for modulating T-cell expression of IL-10 and to induce islet-specific recruitment of Tregs in T1D. Although generally perceived as a chemoattractant for immune cells for inflammation, the prevailing research indicates an immunoregulatory and anti-inflammatory role for CXCL12 during pathological inflammation and autoimmunity. The functional outcome of CXCL12 signaling is clearly influenced by its local concentration and appears to be disease- and cell- or tissue-dependent.

7.2. CXCL12-mediated regulation of mesenchymal and hematopoietic stem cells

Mesenchymal stem/stromal cells (MSCs) and hematopoietic stem cells (HSCs) possess immunoregulatory properties and thus form an important component of the immune response (Abdi, Fiorina, Adra, Atkinson, & Sayegh, 2008; Fiorina, Jurewicz, et al., 2011). These cells produce several molecules that generally inhibit immune responses and induce expansion of Tregs (Fig. 2). HSCs have been shown to express immune inhibitory molecules such as PD-L1 (Ben Nasr et al., 2017; Fiorina, Voltarelli, and Zavazava, 2011; Zheng et al., 2011), CD47 (Chao, Weissman, & Majeti, 2012) and FasL (Akiyama et al., 2012; George et al., 1998). MSCs have also been shown to produce molecules including indoleamine 2,3-dioxygenase (IDO) (Le Blanc & Mougiakakos, 2012), TFGβ (Burr, Dazzi, & Garden, 2013; Murphy, Moncivais, & Caplan, 2013), prostaglandin E2 (PGE2) (Nemeth et al., 2009), anti-inflammatory factor TNF-α-induced protein 6 (TNAIP6 or TSG-6) (Lee et al., 2009), among other factors. The commonest MSCs and HSCs investigated for T1D treatment have been bone marrow-derived (Fiorina, Voltarelli, & Zavazava, 2011). Both MSCs and HSCs highly express CXCR4 (Son et al., 2006; Sugiyama, Kohara, Noda, & Nagasawa, 2006), which keeps them anchored in the bone marrow where CXCL12 is expressed at high levels (Sugiyama et al., 2006). This retention in the bone marrow appears to be aggravated in autoimmune diabetes where the expression of CXCL12 in the bone marrow is further enhanced (Leng et al., 2008). This results in polarized retention of MSCs, HSCs and Tregs in the bone marrow and promotes islet insulitis in NOD mice and in human patients (Ben Nasr et al., 2017; Ferraro et al., 2011; Leng et al., 2008). MSCs and HSCs can be recruited from local tissue or bone marrow to sites of injury/wound or inflammation including pancreatic islets (Sordi et al., 2010). The CXCR4-CXCL12 axis has

been harnessed to modulate the distribution and function of these cells to prevent or reduce β-cell autoimmunity (Abdi et al., 2008; Aguayo-Mazzucato & Bonner-Weir, 2010; Ben Nasr et al., 2017; Fiorina et al., 2009; Leng et al., 2008; Sordi et al., 2005). Ben Nasr et al. reported that hematopoietic stem and progenitor cells (HSPCs) of T1D patients express low levels of PD-L1 compared with healthy controls. The authors demonstrated that when HSPCs are engineered to express PD-L1, they home to the inflamed islet via a CXCL12 gradient to reverse autoimmune diabetes (Ben Nasr et al., 2017). Interestingly, Rui and co-workers identified a subpopulation of β cells that resist autoimmune attack in the NOD mouse. The authors showed that this population of β cells showed upregulation of immunomodulatory molecules including PD-L1, CXCL12 and Q1a (Rui et al., 2017).

8. Harnessing CXCL12 for β-cell replacement therapy

β-cell replacement by islet transplantation for the treatment of T1D has been demonstrated to restore physiologic glycemic control and insulin independence (Shapiro et al., 2000). However, the extreme scarcity of donor islets and the undesirable effects associated with chronic immunosuppression that is required to protect the replaced cells from immune rejection has severely limited this treatment (Foster et al., 2018; Markmann et al., 2016). The advent of *in vitro* differentiation protocols for generating glucose-responsive insulin-secreting β-like cells from human pluripotent stem cells (Millman et al., 2016; Pagliuca et al., 2014) could overcome these barriers. It may be argued that autologous β-cell products derived from iPSC would eliminate allogeneic immune rejection in the patient from whom the iPSC was derived. However, these cells would be vulnerable to autoimmune destruction in the context of T1D (Piemonti et al., 2013; Vendrame et al., 2010). Ultimately, strategies to induce immunotolerance or immunoprotection of replaced SC-β cells would be required for their successful clinical translation regardless of their source.

Bioengineering strategies to shield transplanted β cells from recipient immune attack is an extensively investigated area to avoid the need for systemic immunosuppression. SC-β cells particularly offer an advantage in this regard in that the iPSC from which they are derived could be engineered to make them immune-tolerant (Sneddon et al., 2018). CXCL12 could be manipulated in a dose- and tissue-dependent manner to induce the repulsion of effector T cells, a mechanism we termed “fugetaxis”, (Poznansky et al., 2000; Poznansky et al., 2002) and to concurrently recruit FoxP3$^+$ regulatory T cells (Vianello et al., 2006; Yan et al., 2011; Zou et al., 2004) at an anatomic site. These effects and other immunoregulatory mechanisms of CXCL12 (Karin, 2010) as described above could be harnessed to generate site-specific immunoisolation of transplanted islets from recipient immune destruction. In this respect, it is worth pointing out that tumor cells exploit high expression of CXCL12 to achieve effector T cell exclusion and to gain local immunological privilege (Feig et al., 2013; Joyce & Fearon, 2015). Indeed, we demonstrated that allogeneic islets that are engineered to express high levels of CXCL12 are immunoprotected and function long-term to induce prolonged glycemic correction in immunocompetent mice without systemic immunosuppression (Papeta et al., 2007). The high expression of CXCL12 caused scanty infiltration of cytotoxic T cells compared with control-treated alloislets (Papeta et al., 2007). We further demonstrated that simple coating of allogeneic islets with high concentration of CXCL12 (1 μg/ml) prior to transplantation results in prolonged islet survival and function. This approach exploits the fact that the native islet capsule contains fibronectin, to which CXCL12 can bind and be eluted dynamically to modulate T cell migration and immune responses (Pelletier et al., 2000). Transplanted allogeneic islets that were exposed to high concentrations of CXCL12 maintained normoglycemia in immunocompetent mice significantly longer compared to control PBS-treated islets. Explanted islets that were exposed to CXCL12 were characterized by infiltration of FoxP3$^+$ regulatory T cells but scanty effector CD3$^+$ T cells, whereas the reverse was true for control treatment. Fiorina et al. also demonstrated that antagonism of CXCR4 induces egress of host bone marrow HSCs to islet allografts transplanted under the kidney capsule to promote immunoprotection and prolonged survival of the islet allografts in immunocompetent C57BL/6 mice (Fiorina, Jurewicz, et al., 2011). The HSCs, which express CXCR4, migrated to the transplanted islets on a gradient of CXCL12 that is expressed in the islets. The mobilized HSCs expressed high levels of PD-L1, which was responsible for the immunoprotection of the allograft (Fiorina, Voltarelli, and Zavazava, 2011).

In addition to directly engineering islets to express immunomodulatory molecules for immunoprotection, encapsulation of islets in a semi-permeable membrane/capsule to prevent direct contact with the recipient’s immune system while allowing sufficient transport of oxygen, other nutrients and insulin transfer is an intensively investigated approach for decades. An encapsulation strategy that can promote survival, function and immune protection remains an aspiration. Islet encapsulation brings issues of inflammatory foreign body responses (FBRs), insufficient oxygen/nutrient access and altered insulin kinetics that cause early islet-graft failure (de Vos, Faas, Strand, & Calafiore, 2006; O'Sullivan, Vegas, Anderson, & Weir, 2011). Engineering encapsulation biomaterials to locally release immunomodulatory molecules that suppress local inflammation is thus being pursued to obviate host inflammatory responses to encapsulated cells (Desai & Shea, 2016). Macrophages have been identified as indispensable to the FBR to biomaterial implants; their activation at the site of the implant leads to a cascade of events including recruitment and differentiation of fibroblasts to myofibroblasts responsible for final fibrosis of the implant (Anderson, Rodriguez, & Chang, 2008; Doloff et al., 2017; Wynn & Ramalingam, 2012). The immunomodulatory capacities of CXCL12 to polarize macrophages toward M2 phenotype as well as T cells toward anti-inflammatory, IL-10-producing cells as (Desai & Shea, 2016) well its capacity to recruit regulatory cells to an anatomic site (Fig. 3) can potentially be exploited to dampen local inflammatory response to encapsulated islet implants. Accordingly, we have harnessed these functions of CXCL12 to achieve local immunoisolation and long-term function of alginate-microencapsulated allogeneic and xenogeneic islets in murine models to T1D without immunosuppression (Chen et al., 2015). We demonstrated that islet-containing microcapsules that incorporated CXCL12 were characterized by little infiltration of effector T cells, macrophages and fibroblasts but FoxP3$^+$ regulatory T cells. In contrast, effector T cells, macrophages and fibroblasts and shortened function of the encapsulated cells, marked control microcapsules without the incorporation of CXCL12 (Chen et al., 2015). We have recently extended this approach to achieve immunoisolation and long-term function of alginate-microencapsulated SC-β cells in immunocompetent C57BL/6 mice without systemic immunosuppression (Alagpulinsa et al., submitted for publication). We observed that incorporation of alginate with high concentration of CXCL12 (2 μg/ml) to encapsulate SC-β cells and transplantation into immunocompetent C57BL/6 diabetic mice caused little to no pericapsular fibrotic overgrowth, with glycemic correction >150 days. The recovered SC-β cells were viable and maintained their differentiation status. In contrast, when no CXCL12 or a low dose (0.2 μg/ml) of the chemokine was incorporated in alginate to encapsulate the cells, long-term glycemic control could not be achieved and the recovered microcapsules were characterized by dense fibrotic overgrowth without viable SC-β cells. The fibrotic overgrowth stained intensely for macrophages and myofibroblasts (CD68 and αSMA, respectively), which is reminiscent of the fibrotic FBR. Thevenot et al., also demonstrated that incorporation of CXCL12 into poly-lactic-co-glycolytic acid (PGLA) scaffolds led to a profound amelioration of the inflammatory response and prevented fibrosis of the implanted scaffolds (Thevenot et al., 2010). This effect was also characterized by sparse recruitment of macrophages as well as reduced production

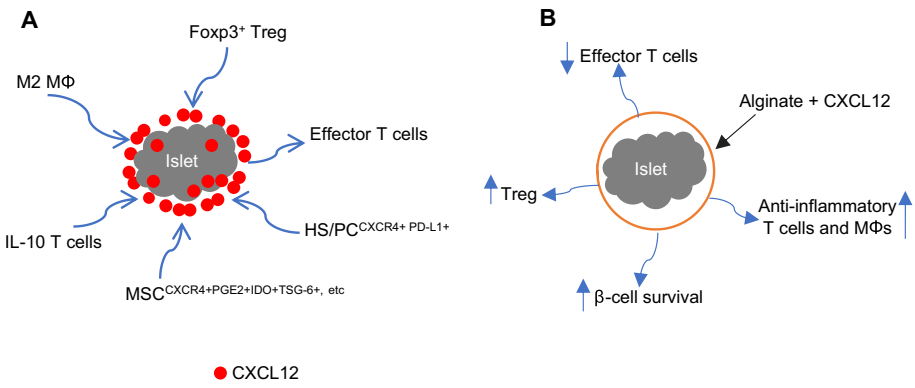

Fig. 3. Potential mechanisms of CXCL12-mediated immunoisolation and protection of unencapsulated (A) and alginate-encapsulated (B) pancreatic islets for transplantation. (A) Transgenic overexpression or coating of with CXCL12 can induce infiltration of Foxp3$^+$ Tregs, CXCR4-expressing MSCs and HS/PCs and switch macrophages and T cells in the local vicinity toward M2 phenotype and IL-10-producing cells while causing repulsion of effector T cells to create immunoisolation of the islet. (B) CXCL12 can be incorporated into alginate to encapsulate islets and CXCL12 can support β-cell survival, induce recruitment of Tregs and repel cytotoxic T cells while retaining and polarizing T cells and macrophages toward anti-inflammatory T cells and macrophages to minimize the inflammatory foreign body response to transplanted encapsulated cells.

of pro-inflammatory cytokines and chemokines at the site of the implant. These findings designate CXCL12 as an attractive molecule for immunomodulation of biomaterials for cell encapsulation to overcome the host inflammatory FBR.

9. Conclusions and perspectives

An overview of the roles of CXCL12 signaling in pancreatic β-cell biology and immune regulation in the context of T1D is presented in this review. CXCL12 signaling is an integral component of β-cell biology and physiology, and immune regulation, both in health and in disease. During embryonic development, the CXCL12/CXCR4 axis plays an important role in establishing the pancreatic fate as well as further potentiation of the differentiation of pancreatic progenitor cells into mature insulin-producing β cells. Importantly, CXCL12 activates the PI3K/AKT and the WNT/β-catenin pathways in β cells, two principal pathways that regulate β-cell mass. The chemokine exerts anti-apoptotic, pro-survival and regenerative effects on β cells. Interestingly, CXCL12 can orchestrate the induction of a local immune privileged site by skewing the function of diverse immune cell types toward anti-inflammatory and immune suppressive phenotypes and recruiting and retaining regulatory immune suppressive T cells as well as MSCs and HSCs. These effects can be harnessed to limit islet inflammation and prevent immune destruction of endogenous and transplanted β cells. Furthermore, these effects of the chemokine could be harnessed for immunomodulation of biomaterials such as alginate for islet encapsulation to prevent the inflammatory FBR in cell replacement therapy for T1D. CXCL12 signaling, especially via CXCR4, thus represents an attractive therapeutic target for modifying the disease course of T1D and for cell replacement therapy in T1D. These could be potentially achieved by using recombinant CXCL12, CXCL12 mimetics or agonists, CXCR4/CXCR7 agonists/antagonists as well as DPP4 inhibitors and with site-specific delivery technologies.

Despite its potential therapeutic benefits in the pancreatic islets, CXCL12 is considered a core-disallowed gene in terminally differentiated and mature β cells and islets. The physiological relevance of this forbiddance or disallowance requires investigation. Ectopic expression of CXCL12 in mature β cells has been shown to induce neither unwarranted leukocyte infiltration nor cause metabolic dysfunction in mice. Instead, this protects mice from diabetes. However, conditional and elective deletion or loss-of-function studies of the CXCL12/CXCR4/CXCR7 system are necessary to provide a definitive physiological function for CXCL12-dependent signaling in the pancreatic islet. The impact of CXCR4 and/or CXCR7 activation on mature endocrine cell differentiation and hormone secretion including insulin and glucagon need to be determined. Furthermore, the mechanisms and the tissue environmental factors through which CXCL12 differentially recruits and modify the polarization of immune cells need further investigation.

Conflict of interest statement

MCP is scientific founder of VicapSys, Inc. All other authors declare no conflict of interest.

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