CXCR3, CXCR5, CXCR6, and CXCR7 in Diabetes

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Abstract

Many studies have suggested that CXCR3, CXCR5, CXCR6 and CXCR7 chemokine receptors play a crtical role in the autoimmune process and in β-cell destruction in type 1 diabetes (T1D). In particular circulating CXCL10 level (the ligand of CXCR3) is high in T1D patients, and this suggests that CXCL10 may be a candidate for a predictive marker of T1D. Blocking the CXCL10/CXCR3 axis in newly onset of diabetes seems to be a possible approach for the therapy of T1D. Attempts have been done in modulating or blocking CXCR5, CXCR6 and CXCR7 chemokine receptors in experimental settings of T1D. Further studies are needed to investigate interactions between chemokines and cytokines in the pathogenesis and therapy of T1D.

Running Title: CXCR3, CXCR5, CXCR6, and CXCR7 in T1D.

Keywords: CXCL10, CXCR3, CXCR5, CXCR6, CXCR7, T1D.

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Introduction

Diabetes mellitus type 1 (also known as type 1 diabetes, or T1DM; or insulin-dependent diabetes or juvenile diabetes) is a form of diabetes mellitus that results from the autoimmune destruction of the insulin-producing β-cells in the pancreas. The subsequent lack of insulin leads to increased blood glucose. T1D is an immunologically mediated disease, in which a T helper (Th)1 immune response leading β-cells destruction has been shown [1], involving an expansion of autoreactive CD4+ Th cells and CD8+ T cells, autoantibody-producing B cells and activation of the innate immune system [2-4]. As the key immunological mechanisms of the pathogenesis of this disease are still unclear [5, 6], prevention and treatment of T1D are hampered.

Cytokines and chemokines, and their receptors, play an important role in the pathogenesis of T1D and are targets of new therapeutic approaches. Here, we review researches about (C-X-C motif) receptor (CXCR)3, CXCR5, CXCR6 and CXCR7 chemokine receptors in T1D.

CXCR3 and diabetes

CXCR3 and its chemokines

CXCR3 receptor is mostly expressed on activated Th1 cells. It is a seven-transmembrane G-protein coupled chemokine receptor, that is determinant in a variety of inflammatory and immunological disorders. (C-X-C motif) ligand (CXCL)10/interferon (IFN)-γ-induced protein 10 (IP-10), CXCL9/monokine induced by IFN-γ (MIG) and CXCL11/Interferon Inducible T cell α (ITAC) are its ligands. They are expressed by infiltrating leukocytes, endothelial cells, fibroblasts and by epithelial cells following stimulation by IFN-γ or Type I IFNs; their expression is synergistically enhanced by tumor necrosis factor (TNF)-α [7-11].

In transplant rejection, a critical role for CXCR3-dependent T cell recruitment is suggested by the strong upregulation of CXCR3 ligand expression and the prevalent expression of CXCR3 on infiltrating T cells during allograft rejection in human and animal models [12-14]. The potential importance of CXCR3-mediated leukocyte recruitment in the pathology of autoimmune disorders is indicated by the upregulation of CXCR3 ligands and the increased number of CXCR3+ lymphocytes evidenced in chronic inflammatory diseases, as rheumatoid arthritis (RA) [15-18], systemic lupus

erythematosus (SLE) (19), inflammatory arthritis (19), and multiple sclerosis [20, 21].

CXCL10/IP-10 is the main CXCR3 ligand and its secretion depends on IFN-γ, that is itself mediated by the interleukin (IL)-12 cytokine family [22]. Upon cytokines stimulation, CXCL10 is secreted by several cell types, including T lymphocytes, monocytes, fibroblasts, thyrocytes, preadipocytes, etc. Therefore, the presence of high levels of CXCL10 in peripheral liquids is a marker of host immune response, particularly Th1 orientated T cells.

Circulating levels of CXCL10 are increasing with age [23]. Furthermore, recent reports have shown that the serum and/or the tissue expressions of CXCL10 are increased in organ specific autoimmune diseases, such as Graves' disease or Graves' ophthalmopathy [24, 25], autoimmune thyroiditis [26, 27], or systemic rheumatological disorders like rheumatoid arthritis [28], systemic lupus erythematosus [29], systemic sclerosis [30, 31], psoriasis or psoriatic arthritis [32, 33], sarcoidosis [34, 35], HCV-related cryoglobulinemia [36, 37], other HCV immune mediated disorders [36-38] and also in liver and thyroid cancers [39, 40].

CXCR3 chemokines and diabetes

The evaluation of circulating levels of CXCL10 in T1D produced different results. Most of the studies observed high levels of serum CXCL10 in children and adults with T1D, expecially in newly diagnosed T1D [41-44]. Only two studies did not find a CXCL10 increase in T1D serum levels [45, 46].

The prototype Th1 chemokine (CXCL10) and the prototype Th2 chemokine (C-C motif) ligand 2 (CCL2)/monocyte chemotactic protein-1 (MCP-1) have been evaluated longitudinally in children with T1D at onset and follow-up [47]. Serum CXCL10 levels were significantly higher in T1D children than in relatives or control children, while CCL2 were similar in patients, relatives and control subjects. During the follow-up of T1D patients, CXCL10 was significantly reduced, while CCL2 did not change, with respect to baseline. This study first shows that in children with newly diagnosed T1D, raised serum CXCL10 and normal CCL2 concentrations indicate a predominant Th1-driven autoimmune process, which shifts toward Th2 immunity over the first 1-2 years from diagnosis [47]. Studies examining the sources of CXCL10 in T1D showed that it was produced by peripheral blood monocytes and leukocytes [48, 49].

CXCL10 is highly expressed in lymphocytes infiltrating the human islet; and β-cells, stimulated by cytokines (as IFN-γ and TNF-α), can modulate the autoimmune response releasing CXCL9, CXCL10 and CXCL11. These chemokines induce the migration of Th1 lymphocytes into the islet, that secrete more IFN-γ and TNF-α, stimulating a further chemokine production by the target cells, that perpetuates the autoimmune cascade. In agreement, CXCL10 has been identified as the prevalent chemokine expressed *in vivo* in the islet environment of prediabetic animals and T1D patients [50-52].
For the above mentioned reasons, the CXCL10/CXCR3 chemokine system plays a critical role in the autoimmune process and in β-cell destruction in T1D.

Effect of blocking CXCL10 in T1D

The effect of CXCL10 neutralization using a T1D model initiated by developmentally regulated presentation of β-cell antigens has been evaluated. The occurrence of diabetes was suppressed by CXCL10 neutralization, after the administration with cyclophosphamide in non-obese diabetic (NOD) mice, even if CXCL10 neutralization did not significantly inhibit insulitis and did not influence trafficking of effector T cells into the islets. As CXCL10 and CXCR3 were coexpressed on insulin-producing cells, CXCL10 was considered to affect mature and premature β-cells in an autocrine and/or paracrine manner. In fact, CXCL10 neutralization increased the proliferation of β-cells and β-cell mass, without inhibiting insulitis. For these reasons, it was suggested that CXCL10 neutralization could be considered a new therapeutic target for β-cell survival, during the early stage of T1D and also after islet transplantation [53].
Another paper evaluated the effect of CXCL10 neutralization in a "spontaneous diabetes" model of NOD mice, through CXCL10 DNA vaccination (pCAGGS-CXCL10), that induced the production of anti-CXCL10 antibodies (Ab) *in vivo* and suppressed the incidence of spontaneous diabetes, in young NOD mice, without inhibiting insulitis or altering the immunological response. pCAGGS-CXCL10 treatment increased the proliferation of pancreatic β-cells, causing an increase of β-cell mass in this spontaneous diabetes model as well. For these reasons, it has been suggested that CXCL10 neutralization could be useful for maintaining β-cell mass at any stage of autoimmune diabetes [54].

CXCR5 and diabetes

CXCR5, also known as Burkitt lymphoma receptor 1 (BLR1), belongs to the CXC chemokine receptor family, and it is a G protein-coupled seven transmembrane receptor for chemokine CXCL13, encoded by the CXCR5 gene [55].

The CXCR5 gene is specifically expressed in lymphatic tissues, such as follicles in lymph nodes as well as in spleen; it plays an essential role in B cell migration [56, 57]. CXCL13, also known as B lymphocyte chemoattractant (BLC), is selectively chemotactic for B cells belonging to both the B-1 and B-2 subsets. CXCL13 and its receptor CXCR5 control the organization of B cells within follicles of lymphoid tissues [58] and is expressed highly in liver, spleen, lymph nodes. In T lymphocytes, CXCL13 expression is thought to reflect a germinal center origin of the T cell, particularly a subset of T cells called Follicular B Helper T cells (or Tfh cells) [58].

Lymphocytes that invade nonlymphoid tissues often organize into follicle-like structures known as tertiary lymphoid organs (TLOs). These structures resemble those found in spleen or lymph nodes, but their function is unknown. TLOs are recognized in the NOD mouse model of T1D. In some cases, TLOs have been associated with the B lymphocyte chemoattractant, in fact CXCL13 is present in inflamed islets of NOD mice [59].

Ab blockade of this chemokine unraveled B lymphocyte organization in islet TLOs, without reducing their proportion in the islets. However, loss of B lymphocyte organization in islets did not provide disease protection against T1D [60]. Tfh cells exert an important role in autoimmune diseases; to investigate the role of Tfh cells in patients with T1D and the effect of anti-CD20 monoclonal antibody (rituximab) on Tfh cells in T1D patients, 54 patients with T1D and 37 healthy controls were enrolled, and 20 T1D were treated with rituximab. Increased frequencies of circulating Tfh cells together with enhanced expression of IL-21 were detected in T1D patients. After rituximab therapy, the frequencies of circulating Tfh cells and serum protein-tyrosine-phosphatase-2 autoantibody (IA2A) were decreased, together with the levels of IL-21, IL-6 and Bcl-6. Furthermore, β-cell function in 10 of 20 patients was improved. These data suggest that Tfh cells may participate in the T1D-related immune responses, and in the disease progression [61].

CXCR6 and diabetes

CXCL16 is a small CXC chemokine, composed of a CXC chemokine domain, a transmembrane domain and a cytoplasmic tail containing a potential tyrosine phosphorylation [62]. CXCL16 is

produced by dendritic cells found in the T cell zones of lymphoid organs, and by cells found in the spleen [62]. Cells that bind and migrate in response to CXCL16 include several subsets of T cells, and natural killer T cells [62]. CXCL16 interacts with the chemokine receptor CXCR6, also known as Bonzo [62, 63]. Expression of CXCL16 is induced by the inflammatory cytokines IFN-γ and TNF-α [63, 64].

Mesenchymal stem cells (BM-MSCs) are stromal cells with the ability to proliferate and differentiate into many tissues. A minority of BM-MSCs (2% to 25%) expressed a restricted set of chemokine receptors [CXCR4, chemokine (C-X3-C motif) receptor 1 (CX3CR1), CXCR6, (C-C motif) receptor (CCR) 1, CCR7] and, accordingly, showed appreciable chemotactic migration in response to the chemokines CXCL12, chemokine (C-X3-C motif) ligand 1 (CX3CL1), CXCL16, CCL3, and CCL19. A population of bona fide MSCs that also expressed CXCR4, CXCR6, CCR1, and CCR7 could be isolated from normal adult human pancreas [65]. Enteroviruses, particularly Coxsackie virus B4 (CVB4), are considered to be involved in the pathogenesis of T1D [66]. A study compared the characteristics of T cell immune response to CVB4 in children with T1D and healthy children with and without human leukocyte antigen (HLA) risk-associated haplotypes (HLA-DR3-DQ2 or HLA-DR4-DQ8) for T1D. Peripheral blood mononuclear cells (PBMCs) showed, in children with T1D, a decreased percentage of T cells expressed CCR2, CXCR6, IL-18R, and IL-12Rβ2-chain after *in vitro* stimulation with CVB4 in comparison with healthy children. Moreover, children with T1D had decreased IFN-γ secretion in CVB4-stimulated PBMCs, suggesting an impaired type 1 immune response against CVB4. This may lead to a delayed clearance of the virus and, at least partly, explain why children with T1D may be more prone to CVB4 infections and related complications, such as β-cell damage [67].

Since chemokines and chemokine receptors are key players in the migration of pathogenic T cells into the islets of NOD mice developing T1D, it was studied the expression of Th1- and Th2-associated chemokine receptors, and the two isoforms of CD45 leucocyte antigen on CD4(+) and CD8(+) lymphocytes from T1D and healthy children. It has been shown an increased expression of CCR7 and CD45RA and reduced CD45RO on CD8(+) cells among recent-onset T1D patients. The percentages of CD4(+) cells expressing CXCR3, CXCR6 and CCR5, and the secretion of CXCL10/IP-10, MCP-1, macrophage inflammatory protein (MIP)-1α and MIP-1β was lower among diabetics. The Authors suggest that low expression of Th1-associated receptors and secretion of chemokines, together with an

increased amount of CD8(+) cells expressing CD45RA and CCR7 in T1D patients therefore might represent suboptimal Th function in T1D, leading to impaired T cytotoxic responses [68].

A prior exposure to Complete Freund's Adjuvant (CFA) suppresses the severity of experimental autoimmune encephalomyelitis (EAE) and spontaneous diabetes in rodents. It has been shown that CFA adjuvant immunotherapy of EAE requires IFN-γ, which suppresses development of the Th17 response, and diverts autoreactive T cells away from the central nervous system toward immature myeloid cells expressing CXCL10 and CXCL16 in the lymph nodes, suggesting a similar mechanism in T1D [69].

CXCR7 and diabetes

CXCR7 belongs to G protein-coupled receptor family, and it is a chemokine receptor able to bind the chemokines CXCL12/stromal cell-derived factor-1 (SDF-1) and CXCL11 [70, 71].

Genome-wide association studies (GWAS) have heralded a new era in susceptibility locus discovery in complex diseases. For T1D, >40 susceptibility loci have been discovered. A study integrated T1D GWAS data with protein-protein interactions to construct biological networks of relevance for disease. A total of 17 networks were identified. To substantiate these networks, it was performed expressional profiling in human pancreatic islets exposed to proinflammatory cytokines. Three networks were significantly enriched for cytokine-regulated genes and, thus, likely to play an important role for T1D in pancreatic islets. Eight of the regulated genes (CD83, IFNGR1, IL-17RD, TRAF3IP2, IL-27RA, PLCG2, MYO1B, and CXCR7) in these networks also harbored single nucleotide polymorphisms nominally associated with T1D. Finally, the expression and cytokine regulation of these new candidate genes were confirmed in insulin-secreting INS-1 β-cells. These results provide novel insight to the mechanisms behind T1D pathogenesis and, thus, may provide the basis for the design of novel treatment strategies [72].

Conclusion

The above mentioned studies have suggested that CXCR3, CXCR5, CXCR6 and CXCR7 chemokine receptors play a crtical role in the autoimmune process and in β-cell destruction in TID. In particular serum CXCL10 is high in T1D patients, and this suggests that CXCL10 may be a candidate for a predictive marker of T1D. Blocking the CXCL10/CXCR3 axis in newly onset of diabetes seems to be a

possible approach for the therapy of T1D. Attempts have been done in modulating or blocking CXCR5, CXCR6 and CXCR7 chemokine receptors in experimental settings of T1D.

Further studies are needed to investigate interactions between chemokines and cytokines in the pathogenesis and therapy of T1D.

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P. Fallahi, A. Antonelli, S.M. Ferrari made substantial contribution in the design of the study, in the acquisition and interpretation of data, and wrote the paper. A. Corrado, A. Di Domenicantonio and G. Frenzilli made contribution in the acquisition of data and their analysis.

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