Review

Role of CXCL10 in cryoglobulinaemia

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ABSTRACT

Interferon (IFN)-γ-induced protein 10 (IP-10/CXCL10) and its receptor, C-X-C motif receptor 3, appear to contribute to the pathogenesis of hepatitis C virus (HCV)-related mixed cryoglobulinaemia (MC) (HCV+MC). The secretion of CXCL10 by cluster of differentiation (CD) CD4+, CD8+, and natural killer-T cells is dependent on IFN-γ, which is itself mediated by the interleukin-12 cytokine family. Under the influence of IFN-γ, CXCL10 is secreted by several cell types including lymphocytes, hepatocytes, endothelial cells, fibroblasts, etc. In tissues, recruited T helper 1 lymphocytes may be responsible for enhanced IFN-γ and tumour necrosis factor-α production, which in turn stimulates CXCL10 secretion from the cells, therefore creating an amplification feedback loop, and perpetuating the autoimmune process. High levels of CXCL10 in circulation have been found in HCV+MC, especially in patients with clinically active vasculitis. Furthermore, HCV+MC patients with autoimmune thyroiditis (AT) have higher levels than those without AT. Further studies are needed to investigate interactions between chemokines and cytokines in the pathogenesis, and to evaluate whether CXCL10 is a novel therapeutic target in HCV-related MC.

Introduction

Chemokines are small proteins which play a significant role in leukocyte trafficking (1) by producing chemotactic activity in cells expressing chemokine receptors. The chemokines are divided into two major (CX3C motif and CXC motif) and two minor (CC motif and C motif) subfamilies (2-4). In particular, the CXC subfamily only has one non-conserved aminoacid residue separating the N-terminal cysteines. Interferon (IFN)-γ-induced protein 10 (IP-10/CXCL10) is a member of the CXC subfamily. CXC chemokines bind to CXC chemokine receptors (CXCR). CXCL10 specifically activates CXCR3 which is a seven trans-membrane-spanning G protein-coupled receptor predominantly expressed on activated Th1 lymphocytes (5), Natural killer (NK) cells, macrophages, and B cells (6, 7). The IFN-γ-induced CXC chemokines [monokine induced by IFN-γ (Mig/CXCL9) and IFN-inducible T-cell chemoattractant (I-TAC/CXCL11)], also activate CXCR3. These CXC chemokines are preferentially expressed on Th1 lymphocytes (8-10), too. CXCL10 is highly expressed in a diverse range of human diseases. It has been shown to be involved in the pathological processes of three main human disorders, infectious diseases, inflammatory (11, 12) and autoimmune diseases (2), and cancer. Since CXCL10 plays a significant role in leukocyte homing to inflamed tissues, it exacerbates inflammation and causes significant tissue damage (2). CXCL10 is an ELR-negative CXC chemokine that attenuates angiogenesis and has anti-tumour actions (13, 14). However, an increased expression of CXCL10 and its corresponding receptor CXCR3 have also been associated with advanced human cancers, including malignant melanoma (15), ovarian carcinoma (16), B-cell lymphoma (17) and basal cell carcinoma (18), and thyroid cancer (19).

Under proinflammatory conditions CXCL10 is secreted from a variety of cells, such as activated neutrophils, monocytes, epithelial cells, endothelial cells, fibroblasts and keratinocytes in response to IFN-γ (18, 20). This crucial regulation of the IFN response, preferentially attracts activated Th1 lymphocytes to the area of inflammation and its expression is associated with Th1 immune responses (21-23).

Recent reports have shown that the serum and/or the tissue expressions of CXCL10 are increased in organ-specific autoimmune diseases, such as autoimmune thyroiditis (AT) (24, 25), Graves' disease (26), Graves' ophthalmopathy

(27, 28), type 1 diabetes (29-31), or systemic rheumatological disorders like rheumatoid arthritis, systemic lupus erythematosus, systemic sclerosis (32, 33), sarcoidosis (34, 35), psoriasis (36, 37), cryoglobulinaemia (38, 39). As the determination of high levels of CXCL10 in peripheral liquids is a marker of host immune response, especially Th1 orientated T-cells, here we review CXCL10 in hepatitis C virus (HCV) infection associated mixed cryoglobulinaemia (MC) (HCV+MC), to understand better the role of chemokines and cytokines and their interactions in the pathogenesis of this disease, and to evaluate whether CXCL10 could be considered a novel therapeutic target in HCV-associated mixed cryoglobulinaemia.

CXCL10 and hepatitis C virus infection associated mixed cryoglobulinaemia

MC is usually classified among systemic vasculitis, in the setting of small-vessel vasculitis (40-42) and it is classified in type 2 and type 3 according to the presence of polyclonal or oligo-monoclonal immunoglobulin M. Because expansion of rheumatoid factor-producing B-cells is the underlying disorder of MC, this condition is considered a 'benign' B-cell lymphoproliferative disease (43, 44). The mechanisms responsible for the lymphoproliferation surrounding MC remain unknown.

It is suggested that a key factor in the pathogenesis of HCV+MC is represented by the inhibition of the apoptosis of B-cells, leading to their progressive accumulation (45, 46).

Saadoun *et al*. (47) studied the local immune response in the liver, which is considered the principal site for immune reactions involved in MC pathogenesis. In that study, the cytokine profile of liver-infiltrating T lymphocytes from HCV patients with or without MC (of type 2) were compared. They showed that, although no differences were found in the proportion of cluster of differentiation (CD) $CD4^+$, $CD8^+$ liver T cells, the ability of freshly isolated liver T cells to produce type-1 cytokines in response to stimulation with phorbol myristate acetate and ionomycin for 6 hours was significantly higher in HCV+MC patients than in HCV-infected controls without MC.

This concept agrees with previous data obtained in peripheral blood mononuclear cells (48), ruling out the possibility of a discrepancy between the response of peripheral and liver T cells. Interestingly, in both studies [by Saadoun *et al*. (47) and Loffreda *et al*. (48)] a reduced expression of interleukin (IL)-10 (a strong inhibitor of IFN-production) is demonstrated regardless of the different sources. These observations suggest that the evolution of HCV infection toward MC is characterised by a strong Th1 response.

Several studies have shown an increased expression of IFN-γ (49) and IFN-γ inducible chemokines (50), in particular CXCL10, in hepatocytes and in lymphocytes of HCV-infected patients (51, 52), directly related with the degree of inflammation and with an increase of circulating levels of IFN-γ and CXCL10 (40, 53-56).

Furthermore, it has been shown that non-structural 5A and core proteins, alone or by the synergistic effect of cytokines, such as IFN- and tumour necrosis factor (TNF)-α, are able to upregulate CXCL10 and CXCL9 gene expression and secretion in cultured human hepatocyte-derived cells (57). This suggests that CXCL10 produced by HCV-infected hepatocytes could play a key role regulating T cell trafficking into a Th1-type inflammatory site as the liver tissue during chronic HCV infection, by recruiting Th1 lymphocytes, that secrete IFN-γ and TNF-α, that induce CXCL10 secretion by hepatocytes, thus perpetuating the immune cascade (58).

Furthermore, we have recently shown that circulating CXCL10 and CXCL11 are higher in patients with HCV+MC than in chronic HCV patients. Moreover, our studies demonstrate markedly high serum levels of CXCL10 and CXCL11 in patients with HCV+MC compared to healthy controls in particular in the presence of active vasculitis. A strong relationship between circulating IFN-γ and CXCL11 was shown, strongly supporting the role of a Th1 immune response in the pathogenesis of HCV+MC patients (59-64).

By comparison, the prototype Th2 chemokine (C-C motif) ligand (CCL)2 was not significantly different in patients with HCV+MC and active vasculitis than in MC patients and it suggests that the Th1 CXCL10 chemokine is specifically involved in the appearance of vasculitis in these patients (64).

On the whole, the above-mentioned data underline the importance of the activation of the Th1 immunity in the immunopathogenesis of HCV+MC, but suggest a complex dysfunction of the cytokine/chemokine network in these patients, involving also pro-inflammatory cytokines.

A high prevalence of papillary thyroid cancer (PTC) (65, 66) was first observed in 139 HCV-infected patients (2.2%), while no case was observed in 835 control subjects long-term residents of an iodine-deficient area (67), subsequently confirmed in other studies (68, 69). The prevalence of thyroid cancer was also investigated in a series of unselected 94 HCV+MC patients in comparison with a gender- and age-matched control group obtained from a sample of the general population (470 subjects). The prevalence of thyroid nodules was higher in control subjects than in MC patients (65.3% *vs*. 54.8%), even though not significantly. Two patients with PTC were found in the MC series, while no case was observed among controls (p=0.001, χ^2 p-value). In both MC patients with PTC lymphocytic infiltration was observed in the thyroid tissue (70).

Recently, other studies have confirmed an association between AT and thyroid cancer (71). Accordingly, features of AT were observed more frequently in HCV-infected patients than in controls suggesting that AT may be a predisposing condition for thyroid cancer (72). Moreover, in our HCV+MC patient series (70) both patients with PTC had lymphocytic infiltration of the thyroid (73).

The finding of an increased prevalence of thyroid cancer in HCV-infected patients is clinically relevant since about 15–30% of these patients may show an aggressive disease, for example with lung metastases, difficult to treat (59, 74-77).

Recently in PTC, rearrangements of the *RET* receptor (RET/PTC) and activat-

ing mutations in the *BRAF* or *RAS* oncogenes activate a common transcriptional programme in thyroid cells that includes upregulation of the CXCL10 chemokine, which in turn stimulates proliferation and invasion (78). More recently, we have shown that a more than ten times higher CXCL10 secretion has been induced by IFN-γ and TNF-α in PTCs with respect to normal thyroid follicular cells (19).
Furthermore, RET/PTC-induced gene expression in thyroid PCCL3 cells reveals early activation of genes involved in regulation of CXCL10 (79).

Conclusion

CXCL10 and its receptor, CXCR3, appear to contribute to the pathogenesis of HCV+MC. The secretion of CXCL10 by $CD4^+$, $CD8^+$ and NK-T cells is dependent on IFN-γ, which is itself mediated by the IL-12 cytokine family. Under the influence of IFN-γ, CXCL10 is secreted by several cell types including lymphocytes, hepatocytes, endothelial cells, fibroblasts, etc. In tissues, recruited Th1 lymphocytes may be responsible for enhanced IFN-γ and TNF-α production, which in turn stimulates CXCL10 secretion from the cells, therefore creating an amplification feedback loop, and perpetuating the autoimmune process. High level of CXCL10 in circulation have been found in HCV+MC, especially in patients with clinically active vasculitis. Furthermore, HCV+MC patients with AT have higher levels than those without AT. Further studies are needed to investigate interactions between chemokines and cytokines in the pathogenesis, and to evaluate whether CXCL10 is a novel therapeutic target in HCV+MC.

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