CXCL10 in psoriasis

Running Title: CXCL10 in PsO

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Abstract

Chemokine (C-X-C motif) ligand (CXCL)10 is involved in the pathogenesis of psoriasis. It has been demonstrated that chemokine (C-X-C motif) receptor (CXCR)3 and CXCL10 were detected in keratinocytes and the dermal infiltrate obtained from active psoriatic plaques and that successful treatment of active plaques decreased the expression of CXCL10. Elevated CXCL10 serum levels have been shown in patients with psoriasis, with a type 1 T helper cells immune predominance at the beginning of the disease, while a decline of this chemokine has been evidenced later, in long lasting psoriasis. Circulating CXCL10 is significantly higher in patients with psoriasis in the presence of autoimmune thyroiditis. It has been hypothesized that CXCL10 could be a good marker to monitor the activity or progression of psoriasis. Efforts have been made to modulate or inhibit the CXCR3/CXCL10 axis in psoriasis to modify the course of the disease.

Keywords: CXCL10, psoriasis, autoimmunity, CXCR3

1. Introduction

Psoriasis [also known as psoriasis vulgaris (PsO)] [1], affects 2–4% of the general population [2]; it is considered a chronic relapsing/remitting immune-mediated skin disease [1], and presents itchy red, scaly patches, papules, and plaques, with different severity, from minor localized patches to complete body coverage.

Five main types of PsO exist: plaque, guttate, inverse, pustular, and erythrodermic [3]. Plaques are commonly evident on the skin of elbows and knees, but also on scalp, palms of hands and soles of feet. Fingernails and toenails are commonly affected (psoriatic nail dystrophy) and can be present as an isolated sign. Psoriatic arthritis (PsA) is an inflammatory arthritis linked to PsO that affects up to 30% of the patients [1].

The causes of PsO are not completely known. PsO has been associated with an elevated risk of other immune-mediated disorders like Crohn's disease, ulcerative colitis and autoimmune thyroiditis [4]. It is a genetic disease, activated by environmental factors [1].

The epidermal layer of the skin typically grows rapidly in PsO [5]. The sequence of pathological events occurring in PsO determines an abnormal production and an overabundance of skin cells [6], that are replaced every 3–5 days in PsO instead of the common 28–30 days [7], probably owing to the premature maturation of keratinocytes, that is induced by an inflammatory cascade in the dermis [8]. These immune cells from dermis go to epidermis and release inflammatory molecules [tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, and IL-22] [9]. These released inflammatory signals seem to stimulate the proliferation of keratinocytes [9].

Markers of susceptibility for the development of PsO are considered gene mutations

of proteins that are associated with the capability of skin to serve as a barrier [10, 11]. In PsO, DNA is able to stimulate the receptors on some dendritic cells, to produce interferon (IFN)-α [12].

Dendritic cells connect adaptive and innate immune system, are highly present in psoriatic lesions [5], inducing T cells and type 1 helper T cells (Th)1 to proliferate. The number of dendritic cells can be reduced by targeted immunotherapy and psoralen and ultraviolet A (PUVA) therapy, inducing a Th2 cytokine pattern instead of a Th1/Th17 cytokine profile [9]. In PsO, T cells from dermis go to epidermis and release IL-17 and IFN-γ [13].

Until now, different treatments can help to control the symptoms [14, 15]; for example, a new generation of targeted immune therapies is still under investigation to advance treatment options for PsO, and are now needed for approval to targeted immunotherapies for PsO [16, 17].

In PsO, cytokines (as TNF-α, IL-1β, IL-6, and IL-22) [9] induce inflammatory signals and the secretion of chemokines [9].

Here, we review the role of the prototype Th1 chemokine chemokine (C-X-C motif) ligand (CXCL)10 in PsO.

2. REVIEW

2.1 CXCR3 chemokines

Chemokines are small molecules that form a subfamily of the cell signalling cytokines. They are divided into four groups (on the basis of the existing space between the first two cysteine residues in the N-terminal region). In chemokines of the C-X-C subgroup, the two N-terminal cysteines are separated by a single and variable amino acid; CXC chemokines are further sub-divided into two subgroups: 1)

ERL positive (ERL+): containing Glu-Leu-Arg sequence (ERL motif); 2) ERL negative (ERL-): without ERL motif [18, 19]. CXCL10/IFN-γ-induced protein 10 (IP-10), CXCL9/monokine induced by IFN-γ (MIG) and CXCL11/Interferon Inducible T cell α (ITAC) belong to this last subgroup. These chemokines act by binding to chemokine CXC receptor 3 (CXCR3) [19], a G protein coupled receptor with seven transmembrane domains, existing in two forms: a) CXCR3-A able to bind CXCL9, CXCL10 and CXCL11; b) CXCR3-B (a splice variant) also binds (C-X-C motif) ligand 4 (CXCL4) [20]. CXCR3 and its ligands (CXCL10 is the main) have an important role in the chemotaxis of inflammatory cells, are expressed on activated T lymphocytes, above all Th1, and natural killer (NK) cells [21], but also on surface of B lymphocytes (and other cells), and are important in the development of a Th1 orientated immune response.

Th1 activation induces IFN-γ and TNF-α production, which stimulates secretion of CXCL10 (encoded by CXCL10 gene) [22], by the lymphocytes themselves, but also by other cells, such as neutrophils, monocytes, endothelial cells, fibroblasts, thyrocytes, keratinocytes, etc; this process leads to an amplification feedback loop which initiates and perpetuates the immune cascade (**Figure 1**) [19, 23, 24]. For these reasons, high levels of CXCL10 in peripheral liquids are considered a marker of a Th1 orientated immune response.

In animal and human allograft rejection, CXCR3 ligand expression is upregulated and there is a prevalent expression of CXCR3 on infiltrating T cells; this let hypothesize the importance of CXCR3-dependent T cell recruitment in transplant rejection [25-27].

CXCL10 is an important chemoattractant for neutrophils, and prominent infiltration and microabscess formation by neutrophils is a distinctive hallmark feature of PsO.

Many studies have demonstrated a critical pathogenic role of neutrophils in PsO, overall in the initial phases, suggesting that blocking neutrophil function may have therapeutic benefit in this disease [28-30].

Macrophages are the source of another potential psoriasis trigger. Studies show folic acid receptors being over expressed in activated macrophages in PsO [31, 32].

However, folic acid's actions on inflammation are unusual. The endothelial-dependent response called flow-mediated vasodilation is principally regulated by release of nitric oxide (NO) from the endothelium. NO is synthesized from the amino acid L-arginine by endothelial nitric oxide synthase (eNOS). Five mg/day - but not 400 μg/day folic acid improves blood vessel cell function measured by flow-mediated vasodilation in coronary artery disease patients. The inactive monomer of eNOS results in a reduction of net NO synthesis and increases superoxide generation [33].

Dimeric eNOS is anti-inflammatory, and monomeric is pro-inflammatory, showing the dual nature of this mechanism. This suggests that high-dose folic acid can also have important anti-inflammatory potentials [34-36]. Etanercept-cleared psoriasis skin has 87% less inducible nitric oxide synthase (iNOS) from tissue using a reverse transcriptase PCR assay [37].

Attempts to treat PsO using this hypothesis to date have been mixed [38].

2.2 CXCL10 in autoimmunity

Stimulated by cytokines, various cell types (T lymphocytes, monocytes, fibroblasts, thyrocytes, preadipocytes, etc.) secrete CXCL10. Accordingly, a marker of host immune response, in particular Th1 orientated T-cells, is the presence of elevated levels of CXCL10 in peripheral liquids.

The increased IFN-γ and TNF-α production, related to the recruitment of Th1 lymphocytes, stimulates the CXCL10 secretion from the abovereported cells, creating an amplification feedback loop [19].

Serum CXCL10 levels increase with advancing age [39], and serum and/or tissue expression levels are augmented in organ specific autoimmune diseases, as type 1 diabetes [40-43], Graves' disease or Graves' ophthalmopathy [44-46], autoimmune thyroiditis [47, 48], or systemic rheumatological disorders, like rheumatoid arthritis [49], systemic lupus erythematosus [50], systemic sclerosis [51-53], PsO or PsA [4, 54, 55], sarcoidosis [56], HCV-related cryoglobulinemia [57-60], other HCV immune mediated disorders [61-65], and also in cancers [66-71].

2.3 CXCL10 in psoriasis

Inflammation and an elevated epidermal turnover are pathological characteristics of psoriatic plaques.

Pathological event in PsO begins with inflammatory cascade in dermis [72].

CXCL10 was evidenced in dermal infiltrate and keratinocytes obtained from active psoriatic plaques [73]; an effective therapy decreased its expression in plaques.

As it will be shown later fumaric acid esters and apremilast act in part reducing CXCL10.

Northern blot analysis confirmed these data with a CXCL10 cDNA probe. Activated T cells and HLA-DR keratinocytes have been previously revealed in active psoriatic plaques. As CXCL10 is detected in delayed cellular immune responses, the significance of ongoing cellular immune responses in the pathogenesis of PsO has been investigated [73].

Another study found a strongly elevated constitutive transcriptional activity of the CXCL10 gene in unstimulated keratinocytes, not stimulated by IFN-γ, TNF-α, or their combination [74].

Immunohistochemical techniques evidenced that the cellular infiltrate in acute psoriatic plaques is constituted by 5-8% CD3(-)CD56(+) NK cells, particularly localized in the mid and papillary dermis. NK lymphocyte migration towards CXCL10 is implicated in the pathogenesis of psoriasis [75].

IL-27, a member of IL-12 family, is a recently discovered cytokine, involved in the priming of Th1 cells. The pathophysiological significance of IL-27 has been reported and discussed in Th1/Th17-mediated inflammatory diseases in the paper by Shibata et al. [76]. Psoriatic patients had elevated serum IL-27 levels with respect to those of healthy controls, that significantly correlated with disease severity and with the levels of IFN-γ. These data suggest that the increased IL-27 levels may contribute to the enhanced Th1 activity in psoriatic patients. The infiltration of IL-27-secreting cells in the papillary dermis of skin lesions in PsO has been shown by immunohistochemical analysis, but not in skin lesions in the presence of atopic dermatitis (AD) or normal skin. These data indicate that IL-27 may specifically act in psoriatic lesions. IL-27 alone induced strongly the *in vitro* secretion of CXCL9, CXCL10, and CXCL11 in normal human keratinocytes, while it inhibited the TNF-α-induced secretion of IL-1α and CCL20. These chemokines selectively attract activated Th1 cells through CXCR3. The expression of these chemokines is upregulated in the keratinocytes of psoriatic skin lesions. These data lead to hypothesize that IL-27 might promote through CXCR3 chemokines (mainly CXCL10) the Th1-type inflammation in the absence of other inflammatory mediators in psoriatic patients and contribute to the onset of the disease [76].

Intra-epidermal cutaneous lymphocyte antigen (CLA)+ and integrin αEβ7+ T lymphocytes selectively expressed CXCR3, in PsO, suggesting an involvement of CXCR3 (and through it CXCL10) and CC chemokine receptor (CCR)4 in T lymphocyte trafficking to the psoriatic dermis and of CXCR3 in the following T cell homing to the overlying epidermis [77].

In PsO, keratinocytes have an intrinsically huge and different chemokine production profile, favoring the recruitment of distinct leukocyte subsets into the skin, and showed significantly more elevated levels of constitutive and induced IL-8 and a stronger induction of CXCL10, than in AD [78].

Immunohistochemical staining showed cutaneous CXCL10 was strongly expressed in lesional keratinocytes in PsO and focally in AD [79].

Keratinocytes functionally respond to IL-18 with the upregulation of major histocompatibility complex II and secretion of CXCL10, suggesting a key role of IL-18 in inflammatory skin diseases in the epidermis [80].

Prolactin and IL-18 enhance CXCL9, CXCL10, and CXCL11 in human keratinocytes, and promote type 1 T cell infiltration into psoriatic lesions through these chemokines [81, 82].

Circulating CCL2 and CXCL10 were tested in 68 PsA patients, and in gender- and age-matched (1:1) control subjects selected from the general population [53]. Circulating CXCL10 mean is significantly more elevated in PsA patients than in control subjects. Considering a high CXCL10 level as a value at least 2 SD above the mean value of the control group (>198 pg/ml), 49% of patients with PsA and 5% of controls showed high CXCL10. Considering CXCL10 circulating levels and disease duration, a significant inverse correlation was evidenced. Also mean circulating CCL2 levels were significantly higher in patients with PsA than in controls. In conclusion,

elevated CXCL10 and CCL2 levels are shown in PsA patients, with a Th1 immune predominance at the beginning of the disease, and a decline of CXCL10 in long lasting disease, with a significant increase of the CCL2/CXCL10 ratio. This suggests a switch from Th1 to Th2 immune response in long duration PsA [53].

However, discordant results have been recently reported [83].

In a previous paper [54] we evaluated circulating CXCL10 (α) and CCL2 (β) in a large series of patients with PsA, with/without autoimmune thyroid (AT) disorders, to connect the obtained levels to the clinical phenotype.

Considering an elevated CXCL10 level as a value of at least 2 SD above the mean value of the control group, 5% of control 1, 19% of control 2, 42% of PsA and 63% of PsA+AT, showed elevated levels of CXCL10. In conclusion, higher circulating CXCL10 and CCL2 have been demonstrated in PsA patients than in control subjects. Circulating CXCL10 in PsA is significantly higher in the presence of AT [54].

The involvement of IL-27 in the pathogenesis of PsO was investigated also in induced psoriasis-like inflammation on mouse back skin with topical application of imiquimod (IMQ), and subcutaneous injections of IL-27 or phosphate-buffered saline (PBS) [84]. Elevated levels of IL-27 mRNA and the presence of infiltration of IL-27-producing cells in the papillary dermis has been revealed in IMQ-treated skin. Injecting IL-27 in the IMQ-treated skin the disease worsened with respect to the injection of PBS. The mRNA levels of IFN-γ, CXCL9, CXCL10, CXCL11, and TNF-α increased with the injection of IL-27, but not those of IL-17F, IL-17A, IL-22, and CCL20. At last, IL-27 antagonism reduced the upregulation of IFN-γ, CXCL9, CXCL10, CXCL11, and TNF-α mRNA levels, and led the IMQ-treated skin to improve clinically and histologically. These data show IL-27 may act in a proinflammatory way, exacerbating the psoriasis-like skin inflammation IMQ-induced [84].

It was also demonstrated that a psoriasis-associated risk haplotype at the IL-12B locus causes an elevated expression of IL-12B by monocytes and correlated with increased circulating IL-12, IFN-γ and the IFN-γ-induced chemokine, CXCL10 [85].

2.4 CXCR3 and CXCL10 as targets of therapies in PsO

The PsO systemic treatment with fumaric acid esters has demonstrated its efficacy, even if their cellular and molecular mechanism of action is still unknown. The efficacy of dimethylfumarate on the release of CXCL1, CXCL8, CXCL9, CXCL10 and CXCL11 in human keratinocytes and peripheral blood mononuclear cells has been evaluated. It has been shown that dimethylfumarate dose-dependently inhibited CXCL1, CXCL8, CXCL9, CXCL10 and CXCL11 transcription. This could partly clarify the positive effects of the therapy with fumaric acid esters in PsO, as dimethylfumarate is able to inhibit the production of chemokines that may be critically involved in the development and perpetuation of psoriatic lesions [86].

In psoriatic skin, NO is highly produced by epidermal keratinocytes in response to IFN-γ and TNF-α [87]. A research investigated whether the NO donors, S-nitrosoglutathione (GS-NO) and (±)-(*E*)-methyl-2-((*E*)-hydroxyimino)-5-nitro-6-methoxy-3-hexenamide (NOR-1), could modulate the chemokine secretion by human keratinocytes activated with IFN-γ and TNF-α. GS-NO-treated psoriatic skin showed reduction of CXCL10, CCL2, and RANTES, but not IL-8 expression by keratinocytes. In keratinocytes, these data indicate that NO donors are able to negatively regulate the chemokine production [87].

The result of TNF/lymphotoxin blockade with etanercept (soluble TNFR) was investigated in 10 patients with PsO treated for 6 months [88], in particular the

histological response, inflammatory gene expression, and cellular infiltration in psoriasis plaques. IL-1 and IL-8, CXCL10, and CCL20 mRNA expression reduced rapidly and completely, that may be justified by a reduced infiltration of T cells, neutrophils, and dendritic cells. This causes an overturning of the epidermal hyperplasia and cutaneous inflammation typical of psoriatic plaques [88].

Apremilast is an orally administered phosphodiesterase-4 inhibitor, used in clinical studies of PsO. Apremilast inhibited *in vitro* the secretion of CXCL9, CXCL10, IFN-γ, TNF-α, and IL-2, IL-12 and IL-23 from human primary peripheral blood mononuclear cells, suggesting a new strategy for the treatment of PsO [89, 90].

Apremilast has been approved by both the United States FDA and European Medicines Agency for treatment of PsA [89, 91].

Recently, the two terpenes costunolide (CS) and dehydrocostuslactone (DCE), naturally present in many plants, have been demonstrated to be able to have anti-inflammatory effects on various human cell types. The role of CS and DCE in the regulation of proliferative and inflammatory responses to cytokines in human keratinocytes has been evaluated. CS and DCE reduced glutathione (GSH) intracellularly in human keratinocytes, and inhibited STAT3 and STAT1 phosphorylation and activation triggered by IL-22 or IFN-γ, respectively. As a consequence, CS and DCE reduced the IL-22- and IFN-γ-induced expression of inflammatory and regulatory genes in keratinocytes, including CCL2 and CXCL10. It was hypothesized that using CS and DCE in PsO therapy the pro-inflammatory actions of IFN-γ and IL-22 on keratinocytes may be reduced [92].

3. Conclusions

CXCL10 is involved in the pathogenesis of PsO. It was shown that CXCR3 and CXCL10 were evidenced in keratinocytes and the dermal infiltrate from active psoriatic plaques; an effective therapy of active plaques reduced CXCL10 expression in plaques. In PsO, elevated serum levels of CXCL10 have been demonstrated, with a Th1 immune predominance at the beginning of the disease, and a decline of CXCL10 levels in long lasting PsO. Circulating CXCL10 (α chemokine) levels in patients with PsO are significantly higher in the presence of AT. It has been hypothesized that CXCL10 could be a good marker to monitor the activity or progression of PsO.

Since CXCL10 is an important marker of inflammation in PsO, it is used to evaluate genetic susceptibility [85], or the anti-inflammatory activity of new therapies [89, 93]. Furthermore, selective and potent CXCR3 or CXCL10 antagonists have been evaluated in autoimmune diseases, and it has been suggested a possible use also in PsO [16, 17].

Figure Legend

Figure 1. Activated Th1 lymphocytes produce IFN-γ and TNF-α, which stimulate secretion of CXCL10, by the lymphocytes themselves, but also by other cells (dermal fibroblasts, keratinocytes, endothelial cells, etc); this process leads to an amplification feedback loop which initiates and perpetuates the immune cascade.

Conflict of interests

The authors declare no conflict of interests.

Financial disclosure

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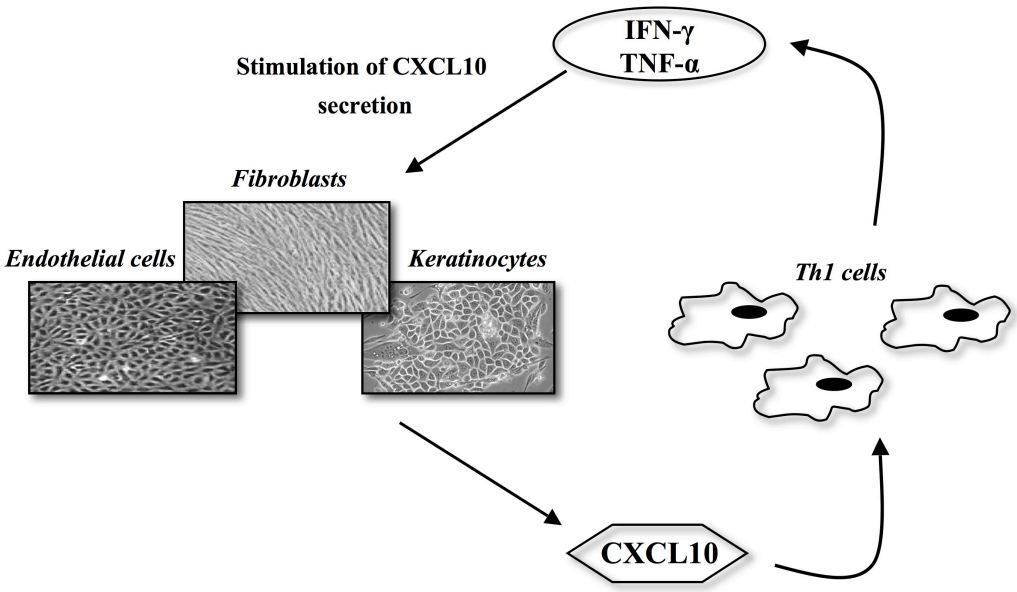
IFN-γ
TNF-α
Stimulation of CXCL10
secretion
Fibroblasts
Endothelial cells
Keratinocytes
Th1 cells
CXCL10

Figure 1

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