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CXCL13 as a new biomarker of systemic lupus erythematosus and lupus nephritis – from bench to bedside?

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Summary

Different studies over the last decade have linked the B cell-attracting chemokine CXC ligand 13 (CXCL13) to the autoimmune disease systemic lupus erythematosus (SLE). A pathogenetic role of this chemokine for disease manifestation in SLE was described initially in mouse models for SLE. Mechanisms of CXCL13 actions were also identified in SLE patients. Moreover, various clinical studies have identified CXCL13 serum levels as a useful biomarker in patients with SLE of different ethnicities for disease activity. In addition, CXCL13 seems to be a promising marker for the diagnosis of lupus nephritis, one of the most severe complications of SLE. However, its exact place within the mechanisms that lead to SLE remains to be defined. Further research is needed to resolve more details of the pathomechanism and the signalling pathway of CXCL13 in SLE. Blocking CXCL13 or the signal pathways of CXCL13 is seen as a promising therapeutic approach for SLE and will be addressed in the near future. This review summarizes all papers that linked CXCL13 to SLE and highlights its importance in the pathogenesis and diagnosis of SLE

Keywords: biomarker, CXCL13, lupus nephritis (LN), systemic lupus erythematosus (SLE)

Introduction

Systemic lupus erythematosus (SLE) is a complex autoimmune disease that is characterized by the production of pathogenic autoantibodies against nuclear structures. So far, more than 150 autoantibodies in SLE have been identified, including antibodies against double-stranded (ds) DNA, histones, nucleosomes and other chromatin components. One main pathogenic mechanism in SLE is the formation of autoantibodies and the deposition of antibody-containing immune complexes in blood vessels throughout the body (for a review see [1]). This is one explanation for the heterogeneous clinical manifestations of this disease and the multiple organ damage seen over time [2,3].

The aetiology of SLE is not resolved completely; however, genetic and environmental factors seem to influence the susceptibility of this disease. Indications for the importance of genetic background in SLE came from studies in families and research on identical twins; patients with SLE have relatives with autoimmune disease more frequently than others [4]. In terms of ethnicity, there is a significantly higher incidence of SLE in African Americans, Hispanics and Asian populations compared to the Caucasian population [5].

Genome studies revealed various SLE-associated loci and genes, including polymorphisms in *STK17A* gene [6] and variations in the regions *ITGAM*, *KIAA1542* and *PXK* [7]. However, SLE has a complex genetic and environmental background, and therefore none of these genes or environmental factors is likely to be entirely responsible for triggering the autoimmune responses.

Because women in child-bearing age are affected nine times more often than men, hormonal factors also seem to play an important role [8]. In accordance with this finding, application of oestrogens can lead to an exacerbation of SLE in humans and in murine models [9] [10]. Regarding environmental factors, it is known that ultraviolet light (UVB), various drugs, pollutants and vaccinations are triggers of SLE onset [11]. Furthermore, vitamin D deficiency and viruses such as Epstein–Barr virus (EBV), human herpes virus 8 (HHV 8), parvovirus B19 and human papilloma virus (HPV) are associated with SLE [12–14]. There is also growing evidence that cigarette smoking may induce a

short-term increased risk of SLE in genetically susceptible individuals [15]. It is likely that many more factors are involved in the pathogenesis of SLE and will be discovered in the near future.

Lupus nephritis (LN)

It is well known that various organs can be affected by SLE [16–18]; however, LN belongs to the most severe complication of SLE. Even though there has been a slight decrease in mortality, the treatment strategies of LN are still not satisfactory with respect to remission induction and unwanted toxic effects [19,20]. Autoantibodies against different intrarenal antigens, deposition of immune complexes, the formation of tertiary lymphoid tissue and a local antibody production are accepted pathogenic mechanisms in the development of LN [21]. These mechanisms can further lead to cell proliferation, production of extracellular matrices as well as secretion of chemokines and proinflammatory cytokines, leading to an infiltration of lymphocytes into the kidney [22]. The diagnosis of LN is made by kidney biopsy, which also allows an assignment to the five different classes of LN according to the 2003 International Society of Nephrology (ISN)/Renal Pathology Society (RPS) classification (for review see [23]). In the long term, up to 20% of patients with LN develop an end-stage renal disease (ESRD) that requires renal replacement therapies such as haemo- or peritoneal dialysis or kidney transplantation [24]. ESRD and dialysis are correlated with an increased morbidity and mortality and have a significant impact on the costs of the health system [25]. Immunosuppressive therapies as cyclophosphamide, glucocorticoids, azathioprine, mycophenolate mofetil (MMF) and biologicals have improved the disease outcome in the last decades. However, it remains difficult to achieve complete remission with these treatment strategies, and side effects of this medication are common [26,27]. As it is known that an early diagnosis and a contemporary treatment of the disease leads to an improved outcome of SLE patients, biomarkers that enable an early detection of SLE and LN in particular are of great clinical interest.

The chemokine CXCL13 and SLE

It is well known that different chemokines are involved in the pathogenesis of LN by orchestrating proinflammatory microenvironments, recruiting immune cell subsets into the kidney and by inducing local activation of immune effector cells [28]. Chemokines are *chemo*tactic cyto*kines* with a low molecular weight (8–10 kDa) that are, based on the pattern of their N-terminal cysteines, classified into four groups (CXC, CC, C and CX3C- chemokines). Signalling is induced by binding to their corresponding receptors on the target cells [29,30]. The primary functions of CXC chemokines are chemoattraction and activation of leucocytes in multiple immunological responses. In this review we highlight the role of the chemokine CXC ligand 13 protein (CXCL13) in SLE. A pathogenetic role of this chemokine for disease manifestation in SLE was described initially in a mouse model for SLE. Recently these findings were taken from bench to bedside and confirmed in different clinical studies on patients with SLE, and will be discussed later in this review.

The chemokine CXC ligand 13 protein (CXCL13), also known as B cell-attracting chemokine-1 (BAC-1) or B lymphocyte chemoattractant (BLC), is a CXC subtype member of the chemokine superfamily. The receptor of CXCL13 is CXCR5, which is normally expressed on mature B cells and follicular T helper cells (Tfh) [31]. It has been demonstrated that CXCL13 is sufficient to induce secondary lymphoid tissues in peripheral organs; supporting this finding, mice deficient in CXCL13 or its receptor, CXCR5, fail to form lymphoid follicles [32]. Of interest, $CXCR5^{-/-}$ and $CXCL13^{-/-}$ mice frequently lack inguinal, iliac and parathymic lymph nodes, whereas facial, superficial cervical and mesenteric lymph nodes are retained. Different studies suggest that CXCL13 is important in the context of autoimmunity, as B cell trafficking and the development of lymphatic organs, two features of SLE, are influenced by CXCL13.

CXCL13 and SLE: animal studies and *in-vitro* experiments

In 2001 Ishikawa *et al*. were the first to connect CXCL13 to SLE. They found, in New Zealand black (NZB) × New Zealand white (NZW) F_1 (BWF) mice, a mouse model that resembles SLE in humans, increased expression of CXCL13 in aged mice with LN compared to similarly aged control mice [33]. CXCL13-positive cells were detected within infiltrates in the kidneys with a reticular pattern of staining. These infiltrates in aged NZB/W mice are comparable to infiltrates in forms of SLE nephritis in patients. $CD11b^{+}CD11c^{+}$ dendritic cells were identified as a main source of CXCL13 expression. These dendritic cells were increased in the thymus and spleen of aged BWF1 mice and showed a chemotactic activity for B cells. This is of clinical relevance, as B cell infiltrates in kidneys correlate with a worse outcome [34].

In gene expression studies in NZB/W F_1 mice, different chemo- and cytokines were up-regulated during the course of disease. This indicates that cyto- and chemokines are involved in the development of LN. Of interest, CXCL13 was one of only two chemokines (of 61 tested inflammatory chemo- and cytokines) up-regulated at the mRNA-level in this experimental model for SLE before glomerular or interstitial infiltration was evident, but early immune complex deposition occurred [35]. These findings support the hypothesis that there is an ordered progression of inflammatory invasion in LN and that CXCL13 is a distinctive

early event in the development of LN. However, besides B cell attraction, little is known about the pathomechanistic effect of CXCL13 in LN. Recently, it was demonstrated in *in-vitro* experiments that incubation of human podocytes, which are epithelial cells of the kidney filtration barrier, with CXCL13 induces receptor stimulation of CXCR5 on podocytes and activates intracellular signalling pathways. The podocyte activation resulted in secretion of proinflammatory cytokines and chemokines into the culture supernatant. This cytokine/chemokine cocktail was sufficient to induce a neutrophil respiratory burst in isolated human granulocytes, indicating that CXCL13 may have local proinflammatory effects [36]. Moreth and co-workers discovered a mechanism by which CXCL13 is induced. They found that the proteoglycan biglycan can trigger CXCL13 expression via Toll-like receptors 2/4 in macrophages and dendritic cells in two mouse models of SLE, in Murphy Roths large (MRL)/lpr and NZB/NZW F_1 mice. Of interest, biglycan levels were markedly elevated in the plasma and kidneys in a mouse model for SLE. Plasma and renal levels of CXCL13 were increased by overexpression of soluble biglycan in these mice. As expected, CXCL13 led to an accumulation of B cells in the kidney enhancing albuminuria and organ damage. Consequently, biglycan-deficient mice had a significant decline in circulating and renal CXCL13 levels and a reduced number of B cells in the kidney. The overall outcome of biglycan-deficient mice, compared to control mice, was improved, exhibiting lower levels of autoantibodies and less renal damage [37].

CXCL13 and SLE: from bench to bedside

It is well accepted that intrarenal B cells are of significance in renal diseases. Shen *et al.* found, in a study that included 150 patients with LN, that intrarenal B cells were more likely to be associated with higher activity and chronicity indices compared to biopsies without B cell infiltrates [34]. Steinmetz and co-workers characterized intrarenal lymphoid clusters of 32 patients with LN and found different levels of B cell organization, ranging from scattered B cell infiltrates to lymph follicle-like structures with separate T and B cell zones and a central follicular dendritic cell network. Of interest, in regions of B cell infiltration a higher expression level of CXCL13 was found. Most B cells in this region expressed the corresponding receptor CXCR5, also supporting the hypothesis that CXCL13 induces B cell infiltration into the kidneys via its receptor CXCR5 [38].

In different clinical studies, CXCL13 serum levels were assessed in patients with SLE by enzyme-linked immunosorbent assay (ELISA) methodology. In one study on 91 Caucasian patients, CXCL13 serum levels were correlated with disease activity using the SLE Disease Activity Index (SLEDAI) and to lupus nephritis. It was found that serum CXCL13 levels correlated well with SLEDAI and median CXCL13 concentrations were higher in patients with renal involvement [39]. In a further study on 35 Asian patients with SLE, plasma concentrations of CXCL13 were assessed. This study correlated CXCL13 plasma levels positively and significantly with SLEDAI score. However, a correlation with LN was not investigated [40]. The most comprehensive study to date was performed by Lee *et al.* [41]. They measured CXCL13 concentrations in the serum of 425 Asian patients with SLE with and without LN. CXCL13 levels were again higher in patients with SLE compared to controls. In line with the results of the study on Caucasian patients, SLE patients with LN had significantly higher levels of serum CXCL13 than others. Moreover, when tissue expression of CXCL13 and its receptor CXCR5 in the kidneys were analysed, CXCL13 and its corresponding receptor were expressed highly in the renal cortex of patients with LN compared to healthy controls.

The above-mentioned studies included adult patients. Only one study in paediatric SLE patients has been published so far. The study by Ezzat *et al.* [42] included 40 patients with SLE ranging in age from 8–16 years (mean age 10·5 ± 2·04 years). In accordance with the studies on adult patients, the concentrations of circulating CXCL13 correlated well with SLEDAI and median CXCL13 concentrations were higher in patients with renal involvement compared with children and teenagers without renal involvement. The function of CXCL 13 and its cross-talk with kidney cells is summarized in Fig. 1.

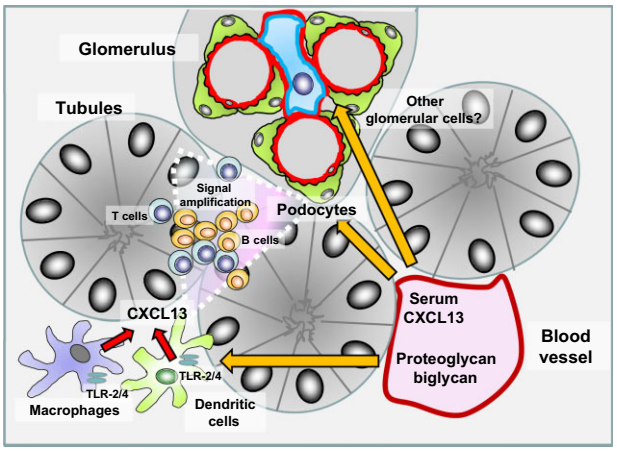

Fig. 1. Schematic overview of the functions of CXCL13 and its cross-talk with resident kidney cells. Circulating proteoglycans and biglycans induce dendritic cell and macrophage activation via Toll-like receptors (TLR) 2/4 and trigger CXCL13 production. CXCL13 levels increase in the serum and are measurable. CXCL13 recruits B cells to the site of production, e.g. in the kidney. Podocytes, which are epithelial cells in the glomerula of the kidney, respond to CXCL13 stimulation with a proinflammatory response that could amplify the signal and lead to further recruitment of inflammatory cells. A direct effect of CXCL13 on other resident kidney cells such as mesangial cells, endothelial cells and tubular cells is likely, but has not been investigated.

For clinical use, it is of great importance to know whether CXCL13 remains stable in blood samples at room temperature, at least from the time a blood sample is taken until its analysis. Therefore, we tested the stability of CXCL13 serum levels stored at room temperature and found that CXCL13 serum levels are stable at room temperature for a minimum of 24 h and are resistant to at least four freeze–thaw cycles. Hence, CXCL13 is a stable marker, and can be analysed in clinical serum samples even under suboptimal storage conditions [42]. There are, however, important limitations of CXCL13 for clinical use: CXCL13 serum levels can also increase in patients with severe active infections alone. In this context, serum samples of septic patients were analysed. It was found that serum CXCL13 levels in septic patients were comparably high, as in patients with active SLE [39]. Therefore, CXCL13 serum levels cannot be used to distinguish between an active autoimmune response and severe infectious diseases. It has to be considered that CXCL13 serum levels are also elevated significantly in other diseases, such as rheumatoid arthritis, Lyme borreliosis, Sjögrens' syndrome and idiopathic pulmonary fibrosis [43–46]. Therefore comorbidities should be ruled out for a source of CXCL13 production.

Conclusion and outlook

Taken together, data from *in-vitro* and *in-vivo* research suggest that CXCL13 is involved in the pathogenesis of SLE. Furthermore, CXCL13 serum levels correlated significantly with SLE disease activity in different studies in mice and on more than 500 patients (children, teenagers and adults of different ethnicities). Moreover, a positive correlation to LN was investigated and observed in most performed studies. Thus, CXCL13 levels can be seen as a new biomarker for SLE and LN in particular.

However, more studies are needed to resolve the pathomechanistic effects in more detail, as CXCL13 may also be an interesting target in the treatment of SLE. As well as CXCL13, other promising biomarkers for LN have been described recently. The urinary levels of tumour necrosis factor-like weak inducer of apoptosis (TWEAK) and monocytic chemoattractant protein-1 (UMCP-1), for example, seem to be promising biomarkers for LN [47,48]. Studies that evaluate a beneficial effect of a combination of these biomarkers are of interest, and are so far lacking.

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Disclosure

The authors declare no conflicts of interest.

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