

CXC LIGAND 13 IN RHEUMATOID ARTHRITIS AND ITS RELATION IN PATIENTS WITH SECONDARY SJOGREN'S SYNDROME

Thesis

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INTRODUCTION

Rheumatoid arthritis (RA) manifests as chronic inflammation affecting small and medium-sized joints in a symmetric fashion. It is one of the most common systemic autoimmune diseases that affects all ethnic groups throughout the world (*Wolfe, 1996*). The disease is characterized by acute painful inflammatory episodes, destructive changes of the joints that result in deformity and progressive functional impairment of joints (*Goronzy and Weyand, 2001*).

The pathogenesis of RA is characterized by an increased presence of monocytes, macrophages, and lymphocytes in the synovial fluid and tissue, leading to the release of cytokines and chemokines. These pro-inflammatory mediators subsequently activate different proteases (*Wolfe, 1996*), leading to the formation of inflammatory “pannus”. This hyperplastic invasive tissue culminating in the destruction of bone and cartilage leads to increased disability in RA patients (*Van der Heijde, 1995*). The severity of the disease is linked to existing joint damage as well as to the extent of ongoing inflammation; therefore, an effective assessment of

disease activity is important in the clinical management of the disease (*Wolfe, 1997*).

Although RA is properly considered a disease of the joints, abnormal immune responses can cause a variety of extra-articular manifestations. In some cases, production of rheumatoid factor (RF) with the formation of immune complexes that fix complement contributes to extra-articular findings (*Firestein, 2001*).

The most common extra-articular manifestation of RA is secondary Sjogren's syndrome (sSS) which occurring in approximately 35% of patients (*Manthorpe et al., 1981*), where involvement of exocrine lacrimal and salivary glands occurs. The decrease of salivary and lacrimal functions in RA is assumed to be related to the lymphocytic infiltrate present in the affected glands. sSS is manifested by dry eyes (keratoconjunctivitis sicca) and dry mouth (xerostomia) (*Andonopoulos et al., 1987*), the diagnosis of Sjogren's syndrome (SS) facing some difficulties, it was found that the average time between occurrence of their first symptoms and diagnosis of SS was 7.1 years (*Segal et al., 2009*).

Biochemical markers of joint tissue metabolism and disease activity in patients with RA would be useful for monitoring the varying disease course (*Gordon and Hastings, 1994*). Measurements of the acute phase reactants such as C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) have been the most widely used serological parameters for long term monitoring of disease activity (*Emery and Luqmani 1993*). However, differences exist between clinical inflammation and the level of ESR and serum CRP as these parameters could be normal in patients with apparent inflammation (*Van Leeuwen et al., 1994*).

Therefore, there are markers reflecting other aspect of disease activity in RA which were of clinical importance to identify patients at risk of more aggressive disease and progressive joint destruction early in the course of the disease in order to treat these patients more intensively with disease modifying anti-rheumatic drugs (DMARDs) (*Van Leeuwen et al., 1994*).

The chemokine CXC ligand 13 protein (CXCL13), also known as B-cell-attracting chemokine-1 or B-lymphocyte chemoattractant (BLC), is a CXC subtype member of the chemokine superfamily. Chemokines

have been shown to orchestrate migration and preferential sequestration of B and T cells in inflammatory lesions (*Kulkarni and Anders, 2008*).

BLC (CXCL13), a CXC chemokine, is the only chemokine which is known to specifically chemoattract B cells, belonging to both B-1 and B-2 subsets through the interaction with its receptor CXCR5. The gene for CXCL13 is located on human chromosome 4 in a cluster of other CXC chemokines (Gunn et al., 1998). CXCL13 and CXCR5 together control the organization of B cells within follicles of lymphoid tissues (*Ansel et al., 2000*) and is expressed highly in the liver, spleen, lymph nodes, and gut of humans.

Plasma levels of CXCL13 provide an accurate test for defining the disease activity in RA patients (*Rioja et al., 2008*).

Moreover, it was found that B cells have been implicated in the pathogenesis of SS and CXCL13 is a homeostatic chemokine that regulates B cell movement (*Rotondi et al., 2007*). Therefore CXCL13 may be pathogenetically involved in the progression and severity of sSS, thus measurement of CXCL13 in plasma of patients with sSS will indicate that the

patient has active auto-immune disease, but will not be specific for SS.

A prior study had been practiced on mice which had approved the specific role of salivary CXCL13 in diagnosis of sSS (*Kramer et al., 2011*). The current study may be the first one done on human using measurement of CXCL13 levels in the saliva to determine its role in patients with sSS, as saliva can be obtained easily, providing a non-invasive, rapid and economical method.

AIM OF THE WORK

The aim of this work is to measure the level of CXCL13 in the plasma and unstimulated saliva of RA patients in order to find out its role in the disease activity of rheumatoid arthritis patients and its relations to secondary Sjogren's syndrome.

RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease characterized by an inflammatory erosive synovitis. This chronic inflammatory condition induces changes in the cellular composition and in the gene expression profile of the synovial membrane, resulting in intimal lining fibroblast like synoviocytes hyperplasia and sublining infiltration with mononuclear cells, especially CD4+ T cells, macrophages, and B cells (*Wolfe, 1996*). With the thickening and proliferation of synovium, the joint is filled by the thickened and abnormally proliferated tissue called pannus, which spreads across the articular cartilage and subsequently causes erosions, and structural damage of the cartilage, bones and ligaments leading to disability and substantial loss of mobility due to pain and joint destruction (*Goronzy and Weyand, 2001*).

Although RA is known as a disease of the joints, it is important to recognize that it is a systemic disease often affecting extra - articular tissues throughout the body including the skin, blood vessels, heart, lungs and muscles (*Firestein, 2001*).

RA affects all ethnic groups throughout the world. Females are 3 times more likely to be affected than males (*Wolfe et al., 1968*). The reasons for this over representation of women are not clear, but genetic (X-linked) factors and hormonal aspects are likely to be involved (*Oslen and Kovacs, 2002*). The onset of disease can occur at any age but peak incidence occurs within the fourth and fifth decades of life (*Drosos, 2004*). Genetic factors contribute 50% to 60% of the risk of developing RA. The gene most strongly associated with RA is the human leukocyte antigen (HLA)-DRB1 gene in the major histocompatibility complex, where specific alleles within the DRB1*04 and *01 clusters encode the “shared-epitope” sequences within the expressed DRB1 molecule. HLA-DRB1 may contribute up to one-third of the genetic susceptibility to RA (*Begovich et al., 2004*).

Pathophysiology:

In a normal joint, synovia consists of synovial membrane (1 or 2 cell layers) and a lower layer of loose connective tissue. The cells lining the synovia are known as “A synoviocytes” (macrophage-like synoviocytes) and “B synoviocytes” (fibroblast-like synoviocytes). In the early stage of RA, hyperplasia of

the synovial membrane (a layer of 10 or more cells in thickness) occurs (**Arend, 2001**), RA synovial fibroblasts (RASFs) together with synovial macrophages, are the two leading cell types in the terminal layer of the hyperplastic synovial tissue that invades and degrades adjacent cartilage and bone. In this destructive process, RASFs actively drive inflammation and degradation of the joint by producing inflammatory cytokines and matrix-degrading molecules, matrix metalloproteinases (MMPs), include collagenases, stromelysin, gelatinases, and membrane-type (MT). Of these MMPs, collagenase-1 (MMP-1) cleaves collagens I, II, VII and X (**Ulf Müller-Ladner et al., 2007**), thus participating in joint destruction in concert with activated chondrocytes and osteoclasts. Villous projections protrude into the joint cavity, invading the underlying cartilage and bone where the proliferating tissue is called pannus.

The synovial hyperplasia, which consists mainly of an increase in cell numbers, especially in the synovial lining layer. To facilitate this growth, angiogenesis (blood vessel proliferation) is mandatory not only for synovial activation but also for subsequent joint destruction (**Distler et al., 2004**). One of the triggering factors appears to be involved is the

articular hypoxia, which stimulates both synthesis of proangiogenic factors but also the expression of chemotactic factors, MMPs such as MMP-1, MMP-3 and osteoclastogenic factors (*Kurowska et al., 2004*) In the synovial sublining region, edema, angiogenesis, and increased cellularity lead to a marked increase in tissue volume (*figure 1*). T and B lymphocytes, plasma cells, interdigitating dendritic cells (IDC) and follicular dendritic cells (FDC), and natural killer cells (NK cells) accumulate in rheumatoid synovium and can be distributed diffusely throughout the sublining or organized into lymphoid aggregates. The dominant cells, CD4⁺ T cells, are mostly of the memory CD45RO and display the chemokine receptors CXCR3 and CCR5 characteristic of Th1 cells. CD4⁺ T cells are especially enriched in aggregates, whereas CD8⁺ T cells are present in the periphery of the aggregates or scattered throughout the sublining (*Jean-Marc, 2008*).

Also B lymphocytes play several critical roles in the pathogenesis of rheumatoid arthritis. They are the source of the rheumatoid factors and anticitrullinated protein antibodies, which contribute to immune complex formation and complement activation in the joints. B cells are also very efficient antigen-presenting cells, and can contribute to T cell activation through

expression of costimulatory molecules. B cells both respond to and produce the chemokines and cytokines that promote leukocyte infiltration into the joints, formation of ectopic lymphoid structures, angiogenesis, and synovial hyper-plasia (*Gregg and Dennis 2003*).

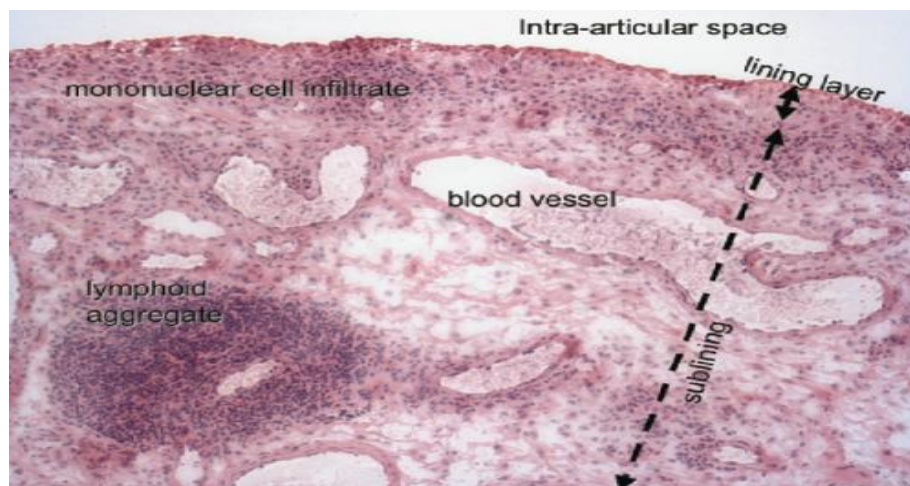


Fig. (1): Synovium in rheumatoid arthritis. Only modest synovial lining hyperplasia is present in this example, although sublining mononuclear cell infiltration, lymphoid aggregates, and vascular proliferation are prominent (*Jean-Marc 2008*).

Immunopathogenesis of Rheumatoid arthritis **(figure 3):**

The roles of small molecule mediators of inflammation (e.g., arachidonic acid metabolites), autoantibodies, cytokines, growth factors, chemokines,

adhesion molecules, and matrix metalloproteinases (MMPs) have been identified in concern to the pathogenesis of RA. Induction phase of RA is initiated by the innate immunity, as it can prepare the joint for subsequent recruitment of inflammatory and immune cells (**Firestein and Zvaifler, 2002**). Cigarette smoke, bacterial products, viral components, and other environmental stimuli can contribute to these responses. In some individuals, a predetermined propensity for immune hyperreactivity or autoreactivity might lead to a different outcome. The genome of these individuals encodes a variety of genes implicated in RA, including class II major histocompatibility complex (MHC) genes, protein tyrosine phosphatase-22 (PTPN22), cytokine promoter polymorphisms. Abnormal T cell selection also could contribute by allowing autoreactive T cells to escape deletion (**Firestein, 2008**).

T cells often constitute 30% to 50% of cells in RA synovia, and mostly CD4 +. About 5% of cells are B lymphocytes or plasma cells, although in some tissues the percentage can be considerably higher than CD4 + cells.

Chemokines play a key role in the organization of tissues into lymphoid structures such as aggregates and germinal centers. B cell attracting chemokine

(CXCL13) and chemokine ligand 21 (CCL21) seem to be especially important, and their expression in rheumatoid synovium correlates with the presence of this microarchitecture (*Manzo et al., 2005*). CXCL13, in particular, is produced by synovial follicular DCs (*Manzo et al., 2008*).

Aggressiveness of RASFs:

RASFs are potent effector cells in RA, generating enzymes that degrade cartilage and bone, and serving as a primary source of inflammatory cytokines in the synovium. These invading cells express VCAM-1, which potentially could facilitate adhesion to cartilage or chondrocytes, and proteases that digest the cartilage matrix. It was found that excessive production of interleukin 1 (IL-1) and underexpression of IL-10 contribute to the invasive properties of RA synoviocytes (*Pap et al., 2004*).

RASFs develop a unique aggressive phenotype that increases invasiveness into the extracellular matrix and further exacerbates joint damage. Recent advances in understanding the biology of RASFs, including their regulation of innate immune responses and activation of intracellular signaling mechanisms that control their behavior, provide novel insights into disease mechanisms (*Bartok and Firestein, 2010*).

B-cell regulation, autoimmunity and autoantibodies:

A significant percentage of RA synovial tissues have discrete lymphoid follicles populated by B cells in the sublining region. B cells are present in the aggregates and express the maturation marker CD20 and proliferation antigens such as Ki67. The formation of these structures depends on several soluble and membrane-bound cytokines, including lymphotoxin. B cells accumulate in lymphoid aggregates in RA synovium under the influence of a variety of chemotactic factors, including CCL21 and B cell – attracting chemokine-1 (CXCL13) (*Rioja et al., 2008*).

Antibodies directed against joint-specific and systemic auto-antigens are commonly detected in the blood of RA patients. Autoantibodies are also found in immune complex deposits in rheumatoid joints and probably contribute to the local inflammation by activating complement (*Edwards and Cambridge, 2006*).

Rheumatoid factors (RF):

Rheumatoid factors (RF) are autoantibodies directed against the Fc portion of IgG (*Dorner et al., 2004*). IgG and IgM RF are found in up to 90% of RA patients. Testing for IgM RF is about 70% sensitive and 80% specific for RA. However, these autoantibodies can