Serum BLC/CXCL13 Concentrations in Children with Systemic Lupus Erythematosus

Thesis

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Essam Shehata Mohamed Shokr

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LIST OF ABBREVIATIONS

ACR:	**American College of Rheumatology**
act:	**Activated T cell**
actTH2:	**Activated TH2 cell**
ANAs:	**Antinuclear antibodies**
Anti-dsDNA:	**Anti-double stranded-DNA**
APS:	**Antiphospholipid syndrome**
B:	**B-cells**
BAS:	**Basophil**
b-FGF:	**Basic fibroblast growth factor**
BLC:	**B-lymphocyte chemoattractant**
BMMC:	**Bone marrow-derived mast cells**
C:	**Cysteine**
CINC:	**Cytokine-induced neutrophil chemoattractant**
CLA	**Cutaneous lymphocyte-associated antigen**
CNS:	**Central nervous system**
cSLE:	**Childhood-onset SLE**
CVD:	**Cardiovascular disease**
CYC:	**Cyclophosphamide**
DC:	**Dendritic cells**
EC:	**Endothelial cells**
EDTA:	**Ethylene-diamine-tetra-acetate**
ELISA:	**Enzyme linked immunosorbant assay**
ELR:	**Glutamate / Leucine / Arginine**
ENA78:	**Epithelial derived neutrophil attractant**
Eos:	**Eosinophils**
EP:	**Epithelial cells**
ESR:	**Erythrocyte sedimentation rate**
ESRD:	**End-stage renal disease**
F:	**Fibroblast**
GBM:	**Glomerular basement membrane**
GCP:	**Granulocyte chemotactic protein**
GM-CSF:	**Granulocyte-Macrophages-colony stimulating factor**
GN:	**Glomerulonephritis**
GRO:	**Growth-related oncogene**
ICAM:	**Intercellular adhesion molecule**
ICs:	**Immune complexes**
IDECs:	**Inflammatory dendritic epidermal cells**
IFN-γ:	**Interferon-γ**

Igs:	**Immunoglobulins**
IL:	**Interleukin**
ILD:	**Interstitial lung disease**
immDC:	**Immature dendritic cell**
IP-10:	**Interferon-gamma-inducible protein-10**
IQR:	**Interquartile range**
I-TAC:	**Interferon-inducible T-cell alpha chemoattractant**
K:	**Keratinocytes**
KD:	**kilo Dalton**
L:	**Lymphocytes**
LARC:	**Liver and activation-regulated cytokine**
M	**Monocytes/Macrophages**
MAb:	**Monoclonal antibodies**
matDC	**Mature dendritic cell**
MCP:	**Monocyte chemoattractant protein**
M-CSF:	**Macrophage colony-stimulating factor**
MDC:	**Macrophage-derived chemokine**
MIP:	**Macrophage inflammatory protein**
MMF:	**Mycophenolate mofetil**
MMPs:	**Matrix metalloproteinases**
N:	**Neutrophils**
NAP:	**Neutrophil activating peptide**
NP:	**Neuropsychiatric**
PF4:	**Platelet factor-4**
PLT:	**Platelets**
PMN:	**Polymorphonuclear leukocytes**
rstT:	**Resting T cell**
RTX:	**Rituximab**
SDF-1:	**Stromal-derived factor**
SLE:	**Systemic lupus erythematosus**
SLEDAI:	**Systemic lupus erythematosus disease activity index**
T:	**T-cells**
TARC:	**Thymus and activation-regulated cytokine**
TECK:	**Thymus-expressed chemokine**
TGF:	**Transforming growth factor**
TIMP:	**Tissue inhibitors of matrix metalloproteinases**
TNF:	**Tumor necrosis factor**

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INTRODUCTION

Systemic lupus erythematosus (SLE) is an organ non-specific autoimmune disorder, with multiple immunopathogenic mechanisms being implicated in its development. The most conspicuous feature of the disease is an exaggerated synthesis of various types of autoantibodies, followed by the formation of immune complexes that deposit in tissues and elicit an inflammatory response. Apart from antibodies, dendritic cells, T cells and cytokines are substantially involved in the pathogenesis of SLE and class I interferons seem to play a crucial role. SLE is a genetically determined disease. HLA system and complement system genes, apoptosis regulating genes and IgG Fc-gamma receptor genes are among the multiple genes implicated in SLE. The role of hormones, both estrogen and progesterone and androgens, in SLE activity has been reported ***(Buc and Rovensky, 2009).***

Cytokines, chemokines, and growth factors are over expressed by renal parenchymal cells and by infiltrating mononuclear cells in human and experimental lupus nephritis. The importance of cytokines in the pathogenesis of lupus nephritis has been established using spontaneous mouse models of SLE. The actions of these cytokines are complex. There is a growing appreciation that the cytokine level and stage of kidney disease determines whether cytokine protects or promotes further tissue injury ***(Rovin, 2008).***

B-Lymphocyte Chemoattractant (BLC), also known as B Cell-Attracting chemokine 1 (BCA-1), or CXCL13, is a member of the CXC subtype of the chemokine superfamily. BLC is critical for secondary lymphoid tissue development and navigation of lymphocytes within the microcompartments of these tissues. The gene encodes a putative protein of 109 amino acids (aa), including a 21 aa leader peptide. At the aa level, human BLC exhibits 64% similarity to its mouse counterpart, and the gene has been mapped to chromosome segment 4q21 ***(Picchio et al., 2008).***

Within the human BLC protein sequence, an arginine residue separates the first two of four conserved cysteine residues that are characteristic of CXC chemokines. BLC is a pertussis toxin-sensitive chemoattractant for B cells in vitro. It is constitutively expressed in the B cell follicles of secondary lymphoid organs, and expression of BLC in these structures is dependent upon the activity of lymphotoxin α/β. ***(Ishikawa et al., 2002).***

BLC is also expressed in the pleural and peritoneal cavities, and in ectopic lymphoid follicles found within the synovium of patients with rheumatoid arthritis. The primary BLC receptor is the 7-transmembrane G-protein coupled receptor, CXCR5, also known as Burkitt's lymphoma receptor 1 (BLR-1). Cells that express CXCR5 and respond to BLC include B cells, follicular B helper T (TFH) cells, osteoblasts, podocytes and a subset of skin-derived dendritic cells. In CXCR5-transfected HEK

cells, BLC can stimulate elevations of Ca2+ and pertussis toxin-sensitive activation of MAP kinase signaling cascades. CXCR3, a known receptor for IFN-γ-inducible protein (IP-10), monokine induced by IFN-γ (MIG), and interferon-inducible T cell alpha chemoattractant (I-TAC), is also activated in vitro by BLC ***(Picchio et al., 2008).***

CXCL13-/- and CXCR5-/- knockout mice exhibit similar abnormalities including deficiencies in the development of most peripheral lymphoid organs. Both knockouts have decreased numbers of peripheral lymph nodes and Peyer's patches, and a disruption of polarized B and T cell microcompartments in spleen and Peyer's patches. Furthermore, CXCR5-/-B cell entry into Peyer's patches (homing) is impaired. BLC likely plays a complex role in antigen-induced movement of B cells within secondary lymphoid tissues. After antigen binding, B cells move from the follicle to the boundary of the T cell zone where they interact with helper T cells. Movement of B cells to the boundary of B and T cell zones is dependent upon the activity of CCR7, a receptor for the T cell zone chemokines MIP-3_/CCL19 and 6Ckine/CCL21. B cell translocation to the B/T cell boundary is inhibited by over-expression of CXCR5. B1 cells, in contrast to conventional circulating B cells (B2 cells), home to the peritoneal and pleural cavities. This activity is inhibited in CXCL13-/- mice and is accompanied by a marked reduction in antibody responses to peritoneal antigens. TFH cells, a CD4+ peripheral blood and tonsillar memory T cell subset, also express CXCR5

and migrate in response to BLC. Tonsillar TFH cells cultured with tonsillar B cells stimulates IgG and IgA antibody production. In vivo, BLC co-localizes with TFH cells in B cell zones of secondary lymphoid tissues suggesting a potential role in the regulation of humoral immunity ***(Ishikawa and Matsushima, 2007).***

A total of 15–20% of cases of SLE are diagnosed in children younger than 16 years (childhood-onset lupus). Although there have been few studies directly comparing childhood- to adult-onset lupus, there is substantial evidence to suggest that pediatric lupus patients display some differences in their disease profile compared with adult-onset populations. A higher prevalence of nephritis and a higher prevalence of progression to end-stage renal disease are distinguishing features of childhood-onset lupus ***(Tucker et al., 2008).***

Aim Of The Work

The aim of this study was to determine the expression of B-Lymphocyte Chemoattractant (BLC), also known as B Cell-Attracting chemokine 1 (BCA-1), or CXCL13 in the serum of SLE children. The results were compared with those obtained from a group of healthy age-and sex-matched children serving as controls, to clarify the clinical usefulness of such biomarker and its relevance to clinical and laboratory variables in terms of SLE disease severity, activity and response to therapy. This chemokine could hopefully be a reasonable target for therapeutic potentials of SLE.

SYSTEMIC LUPUS ERYTHEMATOSUS

Systemic lupus erythematosus (SLE) is an autoimmune rheumatic disease that results from the interaction of multiple environmental, immunological and genetic factors, causing inflammation and eventually damage in a wide range of organs and systems. Its prevalence ranges from approximately 40 cases per 100,000 individuals in Caucasians to more than 200 cases per 100,000 individuals among black people ***(Johnson et al., 2005)***. In common with other autoimmune diseases, it is more prevalent among women, by a factor of 9 ***(Abu-Shakra et al., 1995).***

Although mainly a disease of women of childbearing age, its prevalence is not confined within this population. A total of 15-20% of cases present in children less than 16 years of age. Although there have been limited studies directly comparing adult- and childhood-onset SLE (cSLE), it has been suggested that pediatric lupus patients have a more aggressive disease course and an increased rate of more unusual initial clinical presentations compared with their adult counterparts ***(Tucker et al., 2008).***

A total of 67 childhood-onset and 131 adult-onset SLE patients were followed-up prospectively for 9 years for disease activity and damage. Children with childhood-onset SLE had more active disease at presentation and over time, especially active renal disease, culminating in overall greater damage and a higher need for steroids and immunosuppressive drugs ***(Brunner et al., 2008).***

Differences in the serological and autoantibody profiles of children and adults with SLE have also been described. Lupus may be life-threatening when major organs are affected; however, in the majority of cases, it results in chronic debilitating ill health. Thus, the impact of the disease on growth and development, and its effects on the psychosocial adjustment of children, are important issues that need to be addressed by healthcare providers ***(Hersh et al., 2009)***.

Epidemiological Features of SLE

One of the main difficulties in comparing data from published studies on cSLE is the lack of agreement about the definition of a 'child', with the cut off for inclusion as a 'childhood-onset case' varying between 14 and 20 years of age. As disease expression in SLE is influenced by environmental factors and differs between racial and ethnic groups, when attempting comparisons, it is also important to use cohorts of adults and children with SLE from similar backgrounds. Furthermore, juvenile SLE patients are invariably referred to adult clinics and thus are treated by different physicians when their age exceeds a certain limit, which might render their long-term enrollment to studies problematic or alternatively confound the studies they participate in. Despite these limitations, some conclusions regarding epidemiological differences between adult- and childhood-onset disease can safely be drawn ***(Carreno et al., 1999).***

As already mentioned, in around 15% of individuals with SLE, the disease begins prior to 16 years of age. The diagnosis of lupus is uncommon before 10 years of age ***(Jimenez et al., 2003).*** The median age of diagnosis of SLE in children is 12.1 years, with the female to male ratio ranging from 2.3:1 to 9:1 ***(Benseler and Silverman, 2005).*** In

another series, the female to male ratio in children presenting with lupus before the age of 12 is 3-5:1, whereas this ratio for lupus presenting in the peri- or postpubertal age is approximately 5-7:1, approximately the same ratio as is seen in adults ***(Miettunen et al., 2004).***

The incidence and severity of childhood-onset SLE varies among different ethnic groups. In Caucasians, the incidence of lupus onset prior to 19 years of age is between 6 and 18.9 cases per 100,000 individuals, whereas in pediatric populations of African-American ancestry, it reaches 30 cases per 100,000 individuals, emphasizing the striking impact of race on the incidence of lupus (up to a threefold increase in the prevalence of disease is observed in non-Caucasians), comparable to adult-onset disease ***(Jimenez et al., 2003).***

Younger age, male sex, non-Caucasian race, low socioeconomic status, nephritis and CNS disease are considered to be risk factors for severe lupus; however, their association with a poorer prognosis in childhood- or adult-onset disease remains controversial ***(Bogdanovitc et al., 2004 and Mok, 2005).***

Clinical Manifestations

- **General Features:**

Comparing the clinical features of childhood- and adult-onset disease reveals similarities as well as important differences. In general, children with lupus tend to have more severe and more aggressive disease than adult SLE patients, and childhood-onset SLE often presents with major organ system involvement, including renal and neuropsychiatric (NP) disease ***(Klein-Gitelman et al., 2002).***