ROLE OF B-LYMPHOCYTE STIMULATOR (BLyS) IN THE PATHOGENESIS OF SYSTEMIC LUPUS ERYTHEMATOSUS.

M.D. THESIS

Submitted for partial fulfillment of M.D.degree in Clinical Pathology.

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LIST OF ABBREVIATIONS

Abbreviation	The full term
A	Adenine
Ab	Antibody
ACR	American College of Rheumatology
ANA	Anti-nuclear antibodies
Ag	Antigen
APS	Antigen presenting cells
APLS	Antiphospholipid syndrome
APRIL	A proliferation-inducing ligand
BCMA	B-cell maturation antigen
BCR	B cell receptor
BLyS	B lymphocyte stimulator
BM	Bone marrow
B-ME	B-mercaptoethanol
BR3	BAFF receptor 3
BtK	Bruton's tyrosine Kinase
C	Cytosine
C1q	Complement 1 q
C3	Complement factor 3
C4	Complement factor 4
CBC	Complete blood count
CD	Cluster of differentiation
c-DNA	Complementary DNA
CNS	Central nervous system
CREP	Cot producing cyclic adenosine monophosphate response element binding protein.
CRP	C-reactive protein
CSF	Cerebrospinal fluid
CSR	Class switch DNA recombination
CTLs	Cytotoxic T lymphocytes
DC	Dendritic cells
DLBCL	Diffuse large B-cell lymphoma
DNA	Deoxy-ribonucleic acid
ds-DNA	Double stranded DNA
EDTA	Ethylenediamine tetra- acetic acid
ELISA	Enzyme-linked immunosorbent assay
ESR	Erythrocyte sedimentation rate

FcγR	Receptor for IgG Fc fragment
Fol B	Follicular B lymphocytes
G	Guanine
g	Gram
GC	Germinal center
GSF	Granulocyte colony stimulating factor
Hb	Hemoglobin
HBSS	Hank's balanced salt solution
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HPF	High power field
IFIG	Interferon-inducible genes
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
KD	Kilo Dalton
LDH	Lactate dehydrogenase
LPS	Lipopolysaccharide
MCP-1	Monocytes chemoattractant protein-1
MHC	Major histocompatibility complex
mg	Milligram
MIF	Macrophage migratory inhibitor factor
min	Minute
ml	Milliliter
MM	Multiple myeloma
MQ	Macrophage
m-RNA	Messenger RNA
MZ	Marginal zone
n	Number
NF-kB	Nuclear factor kappa B
NIK	Nuclear factor Kappa B inducing kinase
PBMCs	Peripheral blood mononuclear cells
PCR	Polymerase chain reaction
PKCδ	Protein kinase Cδ
r	Correlation matrix and coefficient of correlation
RA	Rheumatoid arthritis
RBCs	Red blood cells
RF	Rheumatoid factor
RNA	Ribonucleic acid
RNP	Ribonucleoprotein

RT-PCR	Reverse transcription-PCR
SD	Standard deviation
sec	Second
SLE	Systemic lupus erythematosus
SLEDAI	Systemic lupus erythematosus disease activity index
Sm	Smith
SS	Sjogren's syndrome
T	Thymidine
T1	Transitional type 1 B cells
TACI	Transmembrane activator-1 and calcium modulator and cyclophilin ligand-interactor
TD	T cell dependent
Th	T helper
TG	Transgenic
TI	T cell independent
TLR	Toll-like receptor
Tm	Melting temperature
TNF	Tumor necrosis factor
TRAF	TNF receptor-associated factor
WBCs	White blood cells

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Abstract

Systemic lupus erythematosus (SLE) is a prototype of human systemic autoimmune diseases. The pathogenesis behind the disease remains unclear. It is a chronic disease characterized by multisystem organ affection; with recurrent remissions and exacerbations. Therefore, detecting changes in disease activity is of great importance.

B lymphocyte stimulator (BLyS) is a noval member of the tumor necrosis factor (TNF) ligand superfamily. It is a potent B cell survival factor, inducing B cell proliferation, differentiation, and immune modulation. Now, there are growing evidences that BLyS contributes to the pathogenesis of SLE.

Aim of the work:

The aim of this work is to study the expression of the BLyS mRNA using reverse transcription- polymerase chain reaction (RT-PCR) to examine the hypothesis that enhanced expression of BLyS gene is associated with increased disease activity.

Results:

Overexpression of BLyS gene was significantly correlated with the disease activity.

Keywords:

SLE – BLyS – Reverse – Transcription PCR.

Introduction
&
AIM OF THE WORK

INTRODUCTION

Systemic lupus erythematosus (SLE) is a prototypic systemic autoimmune disease characterized by multiple organ damage, high titers of autoantibodies, and various clinical manifestations often demonestrating a waxing- and-waning course. Womens, especially in their 20s and 30s, are affected more frequently than men (***Smith, and Gordon; 2010***).

The etiology of SLE remains unknown and is clearly multifactorial. Many observations suggest a role for genetic, hormonal, immunologic, and environmental factor (***Kaufman et al., 2006***).

The immunologic events triggering the onset of clinical manifestations have not yet been fully defined, but mounting evidence has implied that B cells are key players in the pathogenesis. B cell defects that have been defined include abnormal expression or function of key signaling molecules, dysregulation of cytokines with key B cell effects, and perturbations in B cell developmental stages. Many of these defects may contribute to or be reflective of abnormalities in B cell tolerance (***Anolik; 2007***).

One of the most striking cytokine abnormalities in SLE is the presence of high levels of B-lymphocyte stimulator (BLyS) in serum. BLyS belongs to the tumor necrosis factor (TNF) superfamily and is a prominant factor in B cell differentiation, homeostasis, and selection. It is specifically expressed on cells of myeloid lineage. High levels of BLyS may relax B cell selection and contribute to autoantibody production, exacerbating the SLE disease state (***Thien et al., 2004***).

A clear relationship between BLyS overproduction and SLE has been described through several previous studies.

Because of the heterogeneity of SLE patients and the significant challenges to clinicians and investigators who aim to test promising new therapeutic agents, ***Collins and his colleagues, (2006)*** examined the hypothesis that increased disease severity and activity characterize SLE patients with overproduction of BLyS, and defined a subgroup of SLE patients who will be the appropriate candidates for BLyS antagonist therapy (***Collins et al., 2006***).

AIM OF THE WORK

The aim of this work is to study the role of BLyS in SLE pathogenesis by detecting its mRNA expression in peripheral blood mononuclear cells (PBMCs) from SLE patients using conventional reverse-transcription polymerase chain reaction (RT-PCR) and correlation BLyS mRNA expression with different laboratory and clinical features of SLE.

Chapter 1

B Lymphocyte Stimulator

In the july 9, 1999, Human Genome Sciences announces the discovery and characterization of a noval immune stimulant, called B Lymphocytes Stimulator (BLyS).

Definition:

BLyS/BAFF is a noval member of the tumor necrosis factor family (TNF) of ligands that is involved in B cell survival and maturation. It has become a target for therapeutic trials in autoimmune diseases such as systemic lupus erythematosus (SLE) and rheumatoid arthritis ***(Mackay and Schneider, 2009)***

Synonyms:

1- BLyS (B lymphocyte stimulator)
2- BAFF (B lymphocyte activating factor belonging to the TNF family).
3- TALL-1 (TNF- and ApoL-related leukocyte-expressed ligand1).
4- $_{z}$TNF4.
5- THANK (TNF homologue that activates apoptosis, NF- $_{k}$B, and c-Jun NH2- terminal kinase).
6- DTL (Dendritic cell derived TNF – like molecule).
7- CD257.
8- TNFSF-13b (Tumor necrosis factor ligand superfamily, member 13b) ***(Mackay et al., 2002)***