

Abstract

The Association of Single Nucleotide Polymorphism of Interleukin-21 Gene and Serum Interleukin-21 Levels with Systemic Lupus Erythematosus

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Abstract: Background: Systemic lupus erythematosus (SLE) is a common autoimmune disorder which commonly results from the combined effects of a large number of genes. Variations in the DNA sequence in the Interleukin-21 (IL-21) gene may lead to altered IL-21 production and/or activity which can affect an individual's susceptibility to SLE. IL-21 is a novel class I cytokine produced by activated CD4⁺ T cells, natural killer T cells and T helper (Th) cells. There is increasing evidence that IL-21 contributes to the pathogenesis of SLE due to its biological activity.

Aim of the study: To investigate the association between single nucleotide polymorphism (SNP) of IL-21 rs2221903 gene and serum IL-21 levels with SLE and to detect the possible association between IL-21 serum levels and the pathogenesis of the disease.

Subjects and methods: This study was conducted on 30 SLE patients and 20 age and sex matched healthy controls. Serum IL-21 levels were measured using enzyme-linked immunosorbent assay (ELISA) technique and SNP of IL-21 rs2221903 gene was detected by genotyping assay, using real time polymerase chain reaction (RT-PCR). Results: Serum IL-21 levels were significantly higher in patients compared with controls ($p < 0.001$). Patients with high activity index of SLE had significantly higher levels of serum IL-21 (p value < 0.001). A statistically significant association was found between the T allele of SNP rs2221903 and SLE, but; no association between SNP of IL-21 rs2221903 genotypes and SLE or serum IL-21 levels could be detected.

Conclusion: IL-21 plays an important role in the immune-pathogenesis of SLE and could be used as a possible target for novel immunotherapy. The T allele of SNP rs2221903 suggests that the IL-21 gene may contribute to an inherited predisposition to SLE.

Key words: SLE, IL-21, single nucleotide polymorphism, T allele, Genotypes.

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List of Abbreviations

Abbreviation	
ADCC	antibody-dependent cell-mediated cytotoxicity
anti-ds DNA	anti-double-stranded DNA
anti-Sm	anti-Smith
AP-1	activated protein 1
APCs	antigen presenting cells
BAFF	B-cell activating factor
Bcl-6	B-cell lymphoma 6
BCR	B cell receptor
BILAG	British Isles Lupus Assessment Group
Blimp-1	B-lymphocyte induced maturation protein 1
CD 40L	CD40 ligand
CMV	cytomegalovirus
CR1	complement receptor 1
CD	Crohn's disease
CTL	Cytotoxic T lymphocyte
CTLA-4	Cytotoxic T Cell Antigen 4
CXCR3	CXC-chemokine receptor-3
CXCR5	CXC-chemokine receptor 5
UC	Ulcerative colitis
DCs	dendritic cells
DZ	dark zones
FDCs	Follicular dendritic cells
EAE	experimental autoimmune encephalomyelitis
ECLAM	European community Lupus Activity Measure
GCs	Germinal Centers
GM-CSF	granulocyte macrophage colony-stimulating factor
HLA	Human Leukocytic Ag
ICOS	inducible T cell co-stimulator
IFN γ	Interferon- gamma
IL	interleukin
IL-21	Interleukin-21

IL-21R	IL-21 specific receptor
IBD	Inflammatory bowel disease
JAKs	Janus kinase
KIRs	killer Ig-like receptors
Lef1	lymphoid enhancer binding factor 1
LZ	Light zone
lncRNAs	Long noncoding RNAs
lincRNAs	long intergenic ncRNAs
MAPK	mitogen-activated protein kinase
miRNAs	micro-RNAs
MS	Multiple sclerosis
ncRNA	Noncoding RNAs
NFAT	Nuclear factor of activated T cells
NK	Natural Killer cells
NKG2D	natural killer group 2, member D
NKT	Natural killer T
nt	nucleotides
NuHi	nucleohistone
PCs	plasma cells
PD1	death protein 1
PI3K	phosphoinositide 3-kinase
RA	Rheumatoid arthritis
ROR γ	receptor-related orphan receptory
SELENA	Safety of Estrogen in lupus Erythematosus National Assessment
SHM	somatic hyper mutations
SLAM	Systemic Lupus Activity Measure
SLE	Systemic lupus erythematosus
SLEDAI	Systemic Lupus Erythematosus Disease Activity Index
SNPs	single nucleotide polymorphisms
SOCS1	suppressor of cytokine signaling 1
STAT	signal transducer activator of transcription
T1D	Type I diabetes
Tcf7	transcription factor 7

TCR	T-cell receptor
TD	T-dependent antigens
Th1	T helper-1
Th2	T helper-2
TFH	T follicular helper
TGF- β	Transforming growth factor- β
TH17	T helper-17
Treg	regulatory T
UV	ultraviolet
α -GalCer	α -Galactosylceramide
γ C	common γ chain

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Introduction

Systemic Lupus Erythematosus (SLE) is one of the most relevant world-wide autoimmune disorders. The formation of autoantibodies and the deposition of antibody-containing immune complexes in blood vessels throughout the body is the main pathogenic mechanism of SLE leading to heterogeneous clinical manifestations and target tissue damage. (*J et al., 2014*).

SLE is characterised by the presence of an autoreactive CD4⁺ T cell subset and increased antibody production. CD4⁺ T cells that are made autoreactive in vitro, for example by inhibiting DNA methylation, are capable of stimulating autologous B cells, resulting in increased antibody production. This suggests an important role for T-cell dependent B-cell activation (*Sawalha et al., 2008*).

Many factors have been proposed in the pathogenesis of SLE, such as genetic factors, environmental factors, hormonal action, viruses and dysregulation of cytokine production. The etiology and pathogenetic mechanisms of SLE have not been clearly elucidated. Genetic factors seem to play a significant role in the susceptibility to SLE. However, familial aggregation and a higher rate of concordance for SLE in monozygotic than in dizygotic twins provide strong support for the role of genetic

factors in the pathogenesis of this disorder (**Costa-Reis and Sullivan, 2013 and Shai et al., 1999**). Subjects who have a first-degree relative with SLE are over six times more likely to develop the disease than those without such relatives (**Bengtsson et al., 2002**). Association studies have shown a genetic association with cytokine network (**Asano et al., 2013**). The unbalanced cytokine regulation which contributed to the pathogenesis of SLE development has been investigated. A number of cytokines which were associated with the pathogenesis of SLE including interleukin-12 (IL-12), IL-17, IL-27, IL-10 and tumor necrosis factor- α (TNF- α) have been reported (**Duarte et al., 2013**).

SLE patients have higher frequencies of lymph nodal T helper 17 (Th17) cells well as elevated levels of its cytokines including IL-23, IL-17, IL-21 and IL-6 (**Chen et al., 2012**).

Interleukin (IL)-21 is recognized as a member of the type I cytokine family, mainly produced by a range of differentiated CD4⁺ T cell subsets, such as Th17 and natural killer (NK) T cells (**Leonard et al., 2008**) and Th1 cells (**Zhu et al., 2010**). IL-21 specific receptor (IL-21R) is expressed on CD4⁺, CD8⁺ T cells, B cells, NK cells, dendritic cells, macrophages, and keratinocytes (**Spolski et al., 2008**). IL-21 performs diversely as follows: it promotes CD8⁺ T cell expansion, induces differentiation of naive T cells into Th17

cells, negatively regulates induced-regulatory T cells, plays a role in follicular T helper cell development and is necessary for germinal center formation. Furthermore, IL-21 plays a part in B cell activation and differentiation of plasma cells that produce IgG and is also involved in the maturation, activation, and survival of NK cells (**Ding et al., 2012**).

Recent genome-wide association (GWA) studies have shown that single nucleotide polymorphisms (SNPs) in the chromosome 4q27 region containing IL-2 and IL-21 are associated with chronic inflammatory disorders, including inflammatory bowel disease, celiac disease, psoriasis, diabetes, rheumatoid arthritis, and SLE, suggesting a common genetic background for these diseases (**Sarra and Monteleone, 2010**).

The involvement of IL-21 in the pathogenesis of SLE remains to be demonstrated. However, the ability of this cytokine to regulate in vitro and in vivo immunological pathways that are relevant in SLE and the evidence obtained in murine models of SLE suggest that IL-21 could play an important role in the production of pathogenic autoantibodies and end-organ damage in this disease. So, it is tempting to speculate that IL-21 inhibitors could be useful to control lupus-related clinical manifestations (**Sarra and Monteleone, 2010**).

Aim of the Work

The aim of this study is to find if there was an association between SNP of IL-21 rs2221903 gene and SLE, and whether it can serve as a novel genetic marker of susceptibility to the disease, and to measure IL-21 serum levels in SLE patients and its possible association with the pathogenesis of the disease in the Egyptian population.