T Helper 17 Cells In Systemic Lupus Erythematosus Patients By Real Time Polymerase Chain Reaction

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Abstract

The novel T helper 17 subset have been associated with several autoimmune disorders in humans. They secrete a distinctive set of immunoregulatory cytokines, including IL-17A, IL-17F, IL-22, and IL-21. T helper cells have been extensively studied in SLE. Th 17 cells were assessed in SLE by assessing the expression of the lineage specific transcription factor, retinoic acid related orphan receptor gamma t (RORγt) determined by Real Time PCR. Our results have shown a significant reduction of RORγt in SLE patients compared to control subjects.

Keywords

SLE – T helper 17 – RORγt - Real time PCR

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DEDICATION

I dediacte this work to my recently departed uncle. May you rest in peace.

Also, I would like to dedicate this work to my husband for his loving support.

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List of Abbreviations

Abbreviation	
ACL	Anticardiolipin Antibody
ACR	American College of Rheumatologists
AF2	Activation Function 2
ANA	Anti-nuclear Antibodies
Anti dsDNA	Anti double stranded DNA Antibodies
AntiSm Ab	Anti Smith Antibodies
APCs	Antigen Presenting Cells
BAFF	B Cell Activating Factor
BCR	B Cell Receptor
β-ME	β-Mercapto Ethanol
C_1q	Subcomponent of Complement 1
CC	CC Chemokine
CCL	CC Chemokine Ligand
CCR6	CC Chemokine Receptor 6
CD	Cluster of Differentiation
cDNA	Complementary Deoxyribonucleic acid
CIA	Collagen Induced Arthritis
CNS	Central Nervous System
con A	Concanavalin A
CpG	"—C—phosphate—G—" region of DNA
CR	Complement Receptor
CTLA-4	Cytotoxic T Lymphocyte Antigen 4
CXC	CXC Chemokine
CXCL	CXC Chemokine Ligand
DBD	DNA Binding Domain
DCs	Dendritic Cells
DLE	Discoid Lupus erythematosus
DN	Double Negative
DNA	Deoxyribonucleic acid
DVT	Deep Venous Thrombosis
EAE	Experimental Autoimmune Encephalomyelitis

Abbreviation	
EBV	Epstein Barr Virus
ECG	Electro-Cardiogram
EDTA	Ethylene-Diamine-Tetraacetic Acid
ELISPOT	Enzyme-Linked immunosorbent spot
ESR	Erythrocyte Sedimentation Rate
FAS	Apoptosis Stimulating Fragment
FcγRIIA	Fragment Crystallizable Gamma Receptor Class IIA
FcγRIIIA	Fragment Crystallizable Gamma Receptor Class IIIA
FOXP3	Forkhead Box Protein 3
G-CSF	Granulocyte-Colony Stimulating Factor
HLA	Human Leukocyte Antigen
ICOS	Inducible Co stimulator
ICOS-L	Inducible Co stimulator Ligand
IDO	Indolamine 2,3- dioxygenase
Ids	Idiotypes
IFNα	Interferon α
IFNγ	Interferon γ
Ig	Immunoglobulin
IL	Interleukin
IL-17R	Interleukin 17 Receptor
IL-23R	Interleukin 23 Receptor
ILT-3	Immunoglobulin Like Transcript-3
ILT-4	Immunoglobulin Like Transcript-4
iNKT	invariant Natural Killer T cells
IPEX	Immune dysregulation, Polyendocrinopathy, Enteropathy, X-linked syndrome
IRF5	Interferon Regulatory Factor 5
iTreg	Induced T regulatory cells
JAKs	Janus Kinases
LBD	Ligand Binding Domain
MBL	Mannose Binbing Lectin
MCP-1	Monocyte Chemotactic Protein 1

Abbreviation	
MHC	Major Histocompatibility Complex
MMP	Matrix Metalloproteinase
mRNA	Messenger Ribonucleic acid
NOS	Nitric Oxide Synthase
NPSLE	Neuro-psychiatric Systemic Lupus Erythematosus
NR2a, NR2b	N-methyl D-aspartate Receptor Subunits
NSAID	Non-steroidal Anti Inflammatory Drugs
nTreg	Natural T regulatory cells
PAMPs	Pathogen Related Molecular Patterns
PBMCs	Peripheral Blood Mononuclear Cells
PCR	Polymerase Chain Reaction
PD-L1	Programmed Death-Ligand 1
PHA	Polyhydroxyalkanoates
PRR	Pattern Recognition Receptors
PTPN22	Protein Tyrosine Phosphatase, non-receptor type 22
PWM	Pokeweed Miotgen
RA	Rheumatoid Arthritis
RNA	Ribonucleic acid
RNP	Ribo-nucleo Protein
ROREs	Retinoic acid related Orphan Receptor Elements
RORα	Retinoic Acid Related Orphan Receptor α
RORγt	Retinoic Acid Related Orphan Receptor gamma t
RQ	Relative Quantitation
SCLE	Subacute Cutaneous Lupus Erythematosus
SD	Standard Deviation
shRNA	Short Hairpin Ribonucleic Acid
SLE	Systemic Lupus Erythematosus
SLEDAI	Systemic Lupus Erythematosus Disease Activity Index
SmD1	Sm protein D1
STAT	Signal Transducers and Activators of Transcription
T-bet	T box transcription factor
TCR	T Cell Receptor

Abbreviation	
TGFβ	Transforming Growth Factor β
Th1	T Helper 1 cells
Th17	T Helper 17 cells
Th2	T Helper 2 cells
TLR	Toll Like Receptor
TNF	Tumour Necrosis Factor
Treg	T regulatory cells

INTRODUCTION AND AIM OF WORK

Introduction

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease, characterized by the production of multiple autoantibodies, complement activation and immune-complex deposition, causing tissue and organ damage. The disease occurs nine times more often in women than in men, especially between the ages of 15 and 50, and is more common in those of non-European descent **(Zhao *et al.*, 2010)**.

SLE is a multisystem disorder affecting virtually all systems. Joints, skin and blood are affected in 80 -100 % of cases. Kidneys, central nervous system and cardio-vascular system are affected in over 50% of cases, while thrombosis associated with presence of anti- cardiolipin antibody is present in 10 % of cases. The course of the disease is unpredictable, with periods of exacerbations (called *flares*) alternating with remissions **(Hahn and Tsao, 2009)**.

The T Helper subsets, characterized by different profiles of cytokine production, have been identified in both mice and humans. Th1 cells produce IFN-γ and are mainly devoted to protection against intracellular microbes, whereas Th2 cells produce IL-4, -5, -9, and -13 and are involved in the protection against gastrointestinal nematodes, but are also responsible for allergic disorders (**Romagnani, 1997**). Th0 (type 0) is a third type of T Helper that is able to produce both the cytokines of Th1 and Th2 **(Mosmann and Sad, 1996)**.

Th17 cells were identified as an independent lineage of CD4 T cells that secrete a distinctive set of immunoregulatory cytokines, including IL-17A, IL-17F, IL-22, and IL-21**(Unutmaz, 2009; Jetten, 2009)**. These cytokines collectively play roles in inflammation and autoimmunity and in response to extracellular pathogens. The expression of the lineage-specific transcription factor retinoic acid receptor-related orphan receptor RORγt leads to Th17 lineage commitment **(Manel et al., 2008)**.

Th 17 cells have also been incriminated in two murine autoimmune models, namely, experimental autoimmune encephalomyelitis (EAE) and collagen-induced arthritis (CIA) **(Harrington *et al.*, 2006)**.

IL-17 is a pleiotropic proinflammatory cytokine that enhances T cell priming and stimulates epithelial, endothelial and fibroblastic cells to produce multiple proinflammatory mediators, including IL-1, IL-6, TNF- and chemokines **(Kolls and Linden, 2004)**.

In humans, IL-17 and CD4+ Th17 cells have been implicated in multiple sclerosis **(Tzartos *et al.*, 2008)**, rheumatoid arthritis **(Chabaud *et al.*, 1999)**, psoriasis **(Wilson *et al.*, 2007)**, Crohn's disease and ulcerative colitis **(Homey *et al.*, 2000; Annunziato *et al.*, 2007)**.

The objective of our work is to study the role of Th17 cells in SLE patients by assessing the expression of the lineage specific transcription factor, RORγt determined by Real Time PCR, in the hope that we can better diagnose and treat SLE in the future.