

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

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Presented By

**Marianne Nabil Youssef Abadir**

**M.B., B.Ch.**

Supervised By

**Prof. Dr. Safaa Mostafa El Karaksy**

Professor of Clinical and Chemical Pathology

Faculty of Medicine

Cairo University

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**Dr. Mariam Onsy Farag Hanna**

Ass. Prof. of Clinical and Chemical Pathology

Faculty of Medicine

Cairo University

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**Dr. Hala Ahmed Raafat Youssef**

Ass. Prof. of Rheumatology and Rehabilitation

Faculty of Medicine

Cairo University

**FACULTY OF MEDICINE  
CAIRO UNIVERSITY**

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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 (ROR $\gamma$ t) determined by Real Time PCR. Our results have shown a significant reduction of ROR $\gamma$ t in SLE patients compared to control subjects.

### **Keywords**

SLE – T helper 17 – ROR $\gamma$ t - Real time PCR

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

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## List of Abbreviations

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

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

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

<b>Abbreviation</b>	
<b>TGF<math>\beta</math></b>	Transforming Growth Factor $\beta$
<b>Th1</b>	T Helper 1 cells
<b>Th17</b>	T Helper 17 cells
<b>Th2</b>	T Helper 2 cells
<b>TLR</b>	Toll Like Receptor
<b>TNF</b>	Tumour Necrosis Factor
<b>Treg</b>	T regulatory cells

# INTRODUCTION AND AIM OF WORK

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## 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- $\gamma$  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 ROR $\gamma$ t 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, ROR $\gamma$ t determined by Real Time PCR, in the hope that we can better diagnose and treat SLE in the future.