FLOW CYTOMETRIC T REGULATORY CELLS IN SYSTEMIC LUPUS ERYTHEMATOSUS EGYPTIAN PATIENTS

Thesis

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Background: T regulatory cells ($CD4^+$ $CD25^+$ T cells) play an important role in the immune system. Defects in their number or function have been reported in autoimmune diseases as systemic lupus erythematosus (SLE).

Materials and Methods: The study included thirty patients already diagnosed as SLE patients and ten normal individuals sex and age matched as a control group. Both groups were all subjected to determination of $CD4^+$ $CD25^+$ T cells frequency in their peripheral blood by flowcytometry.

Results: There was a significant statistical decrease in the frequency of$CD4^+$ $CD25^+$ T cells in the patient group. Also there was a significant negative correlation with ESR. No correlation was found with the other laboratory investigations.

Discussion: Decrease in Tregs($CD4^+$ $CD25^+$ T cells) frequency can be detected in active SLE patients. There a negative correlation between frequency of $CD4^+$ $CD25^+$ T cells and disease activity assessed by ESR, yet no statistical correlation was found with age, C3 levels, CRP nor corticosteroid dose therapy.

Conclusion: Tregs play an important role in the pathogenesis and the activity of SLE. Assessment of Tregs can be used in assessment of disease activity.

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LIST OF CONTENTS

Title	Page No.

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The ability to distinguish self from non-self is the hallmark of the immune system, thereby inhibiting autoimmunity but allowing effective responses against microbial antigens. The state of unresponsiveness known as immunologic self-tolerance is maintained through many mechanisms. Besides elimination of potentially self-reactive T cells in the thymus (central tolerance), there are mechanisms that exist to control T cells that have escaped thymic selection and entered the periphery (peripheral tolerance); these include anergy, clonal deletion, and ignorance. Additionally, a more active aspect of immunologic tolerance is mediated through naturally occurring $CD4^{+}CD25^{+}$ regulatory T cells (T reg) which play a critical role in suppressing the response of the immune system to self antigens**.**

Systemic lupus erythematosus (SLE) is a heterogeneous disease with a broad clinical spectrum ranging from primarily cutaneous lesions to severe systemic organ manifestations. It is associated with abnormal immune response including production of autoantibodies and immune complexes**.**

$CD4^{+}CD25^{+}$ T cells show decreased activity in SLE, which may be due to CD95L-mediated apoptosis, a process called activation-induced cell death, which may explain the loss of T reg in patients with active SLE.

AIM OF THE WORK

The aim of this study is to investigate for abnormalities of $CD4^+CD25^+$ T reg cells among Egyptian SLE patients, and to relate these abnormalities, if present, to clinical situation of the patients, treatment status and disease outcome.

SYSTEMIC LUPUS ERYTHEMATOSUS

Introduction:

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease that can be fatal, although with recent medical advances, fatalities are becoming increasingly rare. As occurs in other autoimmune diseases, the immune system attacks the body's cells and tissues, resulting in inflammation and tissue damage. SLE can affect any part of the body, but most often harms the heart, joints, skin, lungs, blood vessels, liver, kidneys, and nervous system. SLE is a chronic inflammatory disease believed to be a type III hypersensitivity response with potential type II involvement ***(James et al., 2005).***

The course of the disease is unpredictable, with periods of illness alternating with remissions. Lupus can occur at any age, and is most common in women, particularly of non-European descent; 90% of people diagnosed with SLE are women. Lupus is treatable through addressing its symptoms, mainly with corticosteroids and immunosuppressants; there is currently no cure. Survival in patients with SLE in the United States, Canada, and Europe is approximately 95% at five years, 90% at 10 years, and 78% at 20 years ***(Hahn, 2008)***. Even so, a patient in whom lupus is diagnosed at 20 years of age still has a 1 in 6 chance of dying by 35 years of age, most often from lupus or infection. Later, myocardial infarction and stroke have become important causes of death ***(Gladman and Urowitz, 2007).***

Epidemiology:

The prevalence of lupus ranges from approximately 40 cases per 100,000 persons among Northern Europeans to more than 200 per 100,000 persons among blacks ***(Johnson et al., 1995).*** Asian, Hispanic and Black populations have higher relative risk of SLE compared with white populations. Onset of SLE is rare before 5 years of age. The female to male ratio increases with advance in age from 4.5:1 in prepubertal to 4.79:1 in postpubertal children and 8:1 in adults ***(Supavekin et al., 2005).***

Classification of Lupus:

There are several types of lupus which include:

Drug-induced Lupus Erythematosus:

Drug-induced form of SLE occurs equally in either sex. Many drugs cause a variant of lupus called drug-induced lupus. The best known of these drugs are procainamide, hydralazine, and quinidine. Patients with drug-induced lupus usually present with skin and joint manifestations, besides renal manifestations while neurologic features are very rare ***(Rubin, 2002).***

Several major criteria have been used in the diagnosis of drug-induced SLE. These criteria include the development of SLE like symptoms during drug therapy, the rapid resolution of the clinical and serological features of SLE within weeks after discontinuation of drug therapy, the lack of disease recurrence

over the next 10 months and no clinical and laboratory evidence of SLE prior to the beginning of the drug administration ***(Verma et al., 2000)***. Furthermore, no preceding infection or other SLE-inducing events had to be shown, and the presence of anti-histone antibodies is a strong evidence that the symptoms were due to drug-induced SLE. In this connection, anti-histone antibodies are present in only 50% of patients with idiopathic SLE, but in 95% of patients with drug-induced SLE; while antidouble stranded DNA (anti-ds DNA) antibodies are usually absent in drug-induced SLE and present in 85% of patients with idiopathic SLE. Antinuclear antibodies (ANA) are not helpful in making this distinction, since they are present in more than 85% of patients affected with either idiopathic or drug-induced SLE ***(Pelizza et al., 2006).***

Lupus Nephritis:

Lupus nephritis is an inflammation of the kidneys caused by SLE. Apart from the kidneys, there is also damage to the skin, joints, nervous system and virtually any organ or system in the body. Sufferers of lupus nephritis may or may not have symptoms of kidney disease, but it can manifest itself through weight gain, high blood pressure, darker foamy urine or swelling around the eyes, legs, ankles or fingers. Furthermore, patients may suffer from other symptoms of lupus unrelated to kidney function. Such symptoms can include arthritis, fever, gastro-intestinal disturbances, headaches, fatigue, and fluid in the joints. The diagnosis of lupus nephritis depends on blood tests, urine

analysis, X-rays, ultrasound scans of the kidneys, and kidney biopsy ***(Weening et al., 2004)***.

Discoid Lupus Erythematosus:

Within the spectrum of diseases included in SLE, at one end is a disease confined mainly to the skin and referred to as discoid lupus erythematosus (DLE) and at the other end is a systemic disease with involvement of heart, lungs, brain, kidneys and other organs called systemic lupus erythematosus ***(Fitzpatrick et al., 2001)***. DLE is a chronic dermatological disease that can lead to scarring, hair loss, and hyperpigmentation changes in skin if it is not treated early and promptly. It has a prolonged course and can have a considerable effect on quality of life. Early recognition and treatment improves the prognosis. Because lesions are induced or exacerbated by ultraviolet exposure, photoprotective measures are important ***(Panjwani, 2009).***

Subacute Cutaneous Lupus Erythematosus:

Subacute cutaneous lupus erythematosus (SCLE) is a distinct form of lupus erythematosus that is characterized by non scarring, non–atrophy-producing skin lesions. It is associated with anti-Ro/SS-A antibodies. Most patients with SCLE are believed to have a chronic or relapsing but benign process, with few of the serious manifestations of SLE, such as central nervous system or renal disease. However, reports of severe systemic disease occurring in patients with SCLE

suggest that the full spectrum of LE associated disease is possible ***(Black et al., 2002).***

📝 *Neonatal Lupus:*

Neonatal lupus erythematosus (NLE) is an uncommon disease. It occurs in 1 in 20,000 live births. NLE has been reported more in females. With the heart disease, female to male ratio is 2:1 and with skin disease ratio is 3:1. The age of onset is from birth to 6 months of age ***(Liu et al., 2001)***. The mother produces IgG auto-antibodies against Ro (SSA), La (SSB) and / or U 1-ribonucleoprotein (RNP), and they are passively transported across the placenta. The presence of maternal anti-SSA/Ro and SSB/La antibodies increases the risk of bearing infants with NLE. These autoantibodies can be found alone or in combination. However anti Ro antibody is present in almost 95% of patients. Mothers of patients with NLE may have SLE, Sjogren Syndrome, Undifferentiated autoimmune syndrome or Rheumatoid arthritis ***(Kim et al., 2001).*** Mothers with primary Sjogren syndrome or undifferentiated autoimmune syndrome have a greater risk of delivering an infant with congenital heart block than those with SLE. NLE have 15-30% incidence of congenital complete heart block developing between 18th and 20th week of gestational age ***(Liu et al., 2001)***.

❖ Pathogenesis:

The pathogenesis of SLE is complex. There is an interaction between different factors including environmental, genetic, hormonal and immunological factors.

1- Environmental factors:

The disease trigger may be due to environmental factors. These factors may not only exacerbate existing lupus conditions but also trigger the initial onset. They include certain medications (such as some antidepressants and antibiotics), extreme stress, exposure to sunlight (UVrays), hormones, and infections. These stimuli cause the destruction of cells and expose their DNA, histones, and other proteins, particularly parts of the cell nucleus ***(Wang et al., 2008).***

2- Genetic factors:

Research indicates that SLE may have a genetic link. Lupus does run in families, but no single "lupus gene" has yet been identified. Instead, multiple genes appear to influence a person's chance of developing lupus when triggered by environmental factors. The most important genes are located in the HLA region; especially Class II (DR, DQ, DP) and class III (C2, C4) on chromosome 6, where mutations may occur randomly (*de novo*) or may be inherited ***(Forabosco et al., 2006)***. Other genes which contain risk variants for SLE include two regions on chromosomes 2, 4 and 16 ***(Hahn and Tasao, 2009).***

The concordance rate for lupus is 25% among monozygotic twins and approximately 2% among dizygotic twins ***(Sullivan, 2000).*** These rates indicate that a genetic contribution is important, but it is not sufficient to cause the disease.