STUDY OF INTERLEUKIN-18 (IL-18) AND INTERLEUKIN-10 (IL-10) PRODUCTION IN CORRELATION WITH DISEASE ACTIVITY IN SYSTEMIC LUPUS ERYTHEMATOSUS

A Thesis

Submitted to the Medical Research Institute
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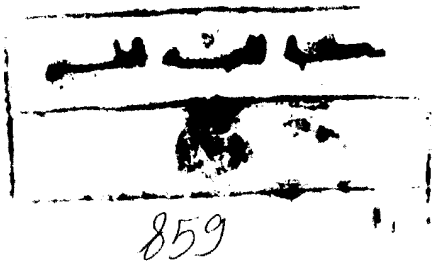

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LIST OF ABBREVIATIONS

ACP	:	Approximate cumulative prevalence
AICD	:	Activation induced cell death
Anti-nRNP	:	Anti-nuclear ribonuclear protein
Anti- Sm AB	:	Anti-Smith antibody
APC	:	Antigen-presenting cell
BCR	:	B cell receptor
CSIF	:	Cytokine synthesis inhibitory factor
DTH	:	Delayed type hypersensitivity
FCS	:	Fetal calf serum
GM-CSF	:	Granulocyt-macrophage colony stimulating factor
HLA	:	Human leukocyte antigen
IFN	:	Interferon
IL-18 BP	:	Interleukin-18 binding protein
IL-18R	:	Interleukin-18 receptor
Mo AB	:	Monoclonal anibody
MHC	:	Major histocomptability complex
NF-AT	:	Nuclear factor of activated T cell
NK	:	Natural killer cell
NO	:	Nitric oxide
PLCγ	:	Phospholipase Cγ isozyme
PTKs	:	Protein tyrosine kinase
SLAM	:	Systemic lupus activity measure criteria
SLEDAI	:	SLE disease activity index
TCR	:	T cell receptor
TGF	:	Transforming growth factor
TNF	:	Tumor necrosis factor


Introduction

INTRODUCTION

Systemic lupus erythematosus

Systemic lupus erythematosus (SLE) is a chronic, potentially debilitating or fatal autoimmune disease in which the immune system attacks the body cells and tissue, resulting in inflammation and tissue damage. SLE can affect any part of the body, but often harms the heart, joints (rheumatological), skin, lungs, blood vessels and brain/nervous system. Lupus is treatable, mainly with immunosuppression, though there is currently no cure for it. [1]

SLE reflects a general defect in immune regulation that results in hyperactive T cells and B cells. Tissue damage is widespread, from both cell-mediated immune responses and from direct cellular damage caused by auto-antibodies or by accumulation of immune complexes.[2]

It is also, a disease of unknown cause that may produce variable combinations of fever, rash, hair loss, arthritis, pleuritis, pericarditis, nephritis, anemia, leukopenia, thrombocytopenia, and central nervous system disease. The clinical course of SLE is characterized by periods of remissions and acute or chronic relapses. [3]

Epidemiology

Systemic lupus erythematosus can occur at any age but has its onset primarily between the ages of 16 and 55 years. [4]

SLE is up to 10 times more common in women than men, and typically has a predilection for women in their child-bearing years. [5]

Johnson *et al*, (1995) reported that the overall prevalence is estimated to be about 1 per 1000. A study from Birmingham, UK, found the prevalence to be 27.7/100.000 in the general population, but nearly 9 times higher in Afro-Caribbean females. [6] Data from a national health survey in the USA found the self-reported prevalence of SLE to be 241/100.000.[7] Recognizing that this may well be an over-estimate, combining self-reporting with evidence of a current prescription for anti-malarials, corticosteroids, or other immunosuppressive medications reduced this figure to 53.6/100.000.[7]

The prevalence of SLE is about the same world wide; the disease appears to be common in China, Southeast Asia, and among blacks in the Caribbean but is seen infrequently in blacks in Africa.[4]

Diagnostic criteria

The diagnosis of SLE is facilitated by determining whether the patient has 4 of the 11 clinical and/or laboratory criteria developed for the classification of SLE (Table1). [4]

Table (1): Criteria for classification of systemic lupus erythematosus.[4]

Criterion	Definition/examples
1- Malar rash	Fixed erythema over the malar eminences, tending to spare the nasolabial folds
2- Discoid rash	Erythematosus raised patches, may scare
3- Photosensitivity	Skin rash as a result of unusual reaction to sunlight
4- Oral ulcers	Usually painless
5- Arthritis	Non-erosive: Jaccoud's arthropathy
6- Serositis	a) Pleuritis-pleuritic pain, pleural rub, pleural effusion b) Pericarditis-ECG changes, rub, pericardial effusion
7- Renal disorder	a) proteinuria (> 3+ or 0.5 g/day) b) Cellular casts in urine
8- Neurological disorder	a) Seizures b) Psychosis
9- Haematological disorder	a) Haemolytic anemia b) Leukopenia c) Lymphopenia d) thrombocytopenia
10- Immunological	a) Anti-DNA antibodies b) Anti-Sm antibodies c) Anti-phospholipid antibodies
11- Anti-nuclear antibody	Exclude drug causes

Clinical manifestations

Systemic lupus erythematosus is highly variable in onset as well as in course. The initial symptoms may be nonspecific and include myalgia, nausea, vomiting, headaches, depression, easy bruising, more specific symptoms or any combination thereof. These symptoms may be mild or severe, fleeting or persistent. [4]

Table (2) shows the clinical features of SLE and their approximate cumulative prevalence (ACP). [4]

Table (2): Clinical features in systemic lupus erythematosus and their approximate cumulative prevalence (ACP). [4]

Manifestation	Approximate cumulative prevalence	
	At Onset	At Any Time
• *Nonspecific*		
Fatigue	-	90
Fever	36	80
Weight loss	-	60
Arthralgia/myalgia	69	95
• *Specific*		
1- Arthritis	-	90
2- Skin		
Butterfly rash	40	50
Discoid LE cells	6	20
Photosensitivity	29	58
Mucous ulcers	11	30
Alopecia	-	71
Raynaud's phenomenon	18	30
Purpura	-	15
Urticaria	-	9
3- Renal	16	50
Nephrosis	-	18
4- Gastrointestinal	-	38
5- Pulmonary	3	50
Pleurisy	-	45
Effusions	-	24
Pneumonia	-	29
6- Cardiac	-	46
Pericarditis	-	48
Murmurs	-	23
Electrocardiographic changes	-	34
7- Lymphadenopathy	7	50
8- Splenomegaly	-	20
9- Hepatomegaly	-	25
10- Central nervous system	12	75
Functional	-	Most
Psychosis	-	20
Seizures	-	20
11- Hematologic	-	90

Aetiology and pathogenesis

The cause of SLE remains unknown, although many observations suggest a role for genetic, hormonal, immunologic, and environmental factors (Fig.1). [4]

I- Genetic factors

The higher rate of the disease has been seen in monozygotic twins (25%) compared with dizygotic twin (3%). The increased frequency of lupus and other immunological disorders in relatives of lupus patients compared with healthy controls, and the higher prevalence of SLE in certain ethnic groups, leads to the suggestion that genetic factors play a role in the pathogenesis of SLE.[8]

It has been reported that in identical twins, if one of them has systemic lupus erythematosus, 50% to 60% of the other will also have the disease. [9] Also, in the first degree relatives of patients with SLE, the incidence of the disease is more than 200 times greater than in the general population. Some evidences suggest that asymptomatic first degree relatives with SLE have impaired suppressor T-cell function. [9]

The concordance of SLE in identical twins, the increase in frequency of SLE among first degree relatives, and the increased risk of developing the disease in siblings of SLE patients reflects a polygenic inheritance of the disease. [9]

The genes of the major histocompatibility complex (MHC) have been most extensively studied for their contribution to human SLE. Population studies reveal that the susceptibility to SLE involves human leukocyte antigen (HLA) class II gene polymorphisms. [10] An association of HLADR2 and DR3 with SLE is a common finding in patients. The HLA class II genes have also been associated with the presence of certain autoantibodies such as anti-Sm (small nuclear ribonuclear protein), anti-nRNP(nuclear ribonuclear protein), and anti-DNA antibodies. [11]

Among other MHC gene system, inherited complement deficiencies also influence disease susceptibility. The HLA class III genes, particularly those encoding complement components C2 and C4, confer risk for SLE in certain ethnic groups. Patients with homozygous C4a null alleles are at high risk of developing SLE. Moreover, SLE is associated with inherited deficiencies of C1q, C1r/s, and C4. A decrease in complement activity could promote disease susceptibility by impairing the neutralization and clearance of self and foreign antigens. When the antigen burden overwhelms the clearance capacity of the immune system, autoimmunity may emerge. [11]

II- Hormonal factors

The increased frequency of SLE in woman of childbearing age suggests that hormones influence the disease. Also, the production of DNA antibodies appears to be enhanced by estrogen. It has been reported that the female hormones promote B cell hyperactivity, whereas androgens may have the opposite effect. [8]

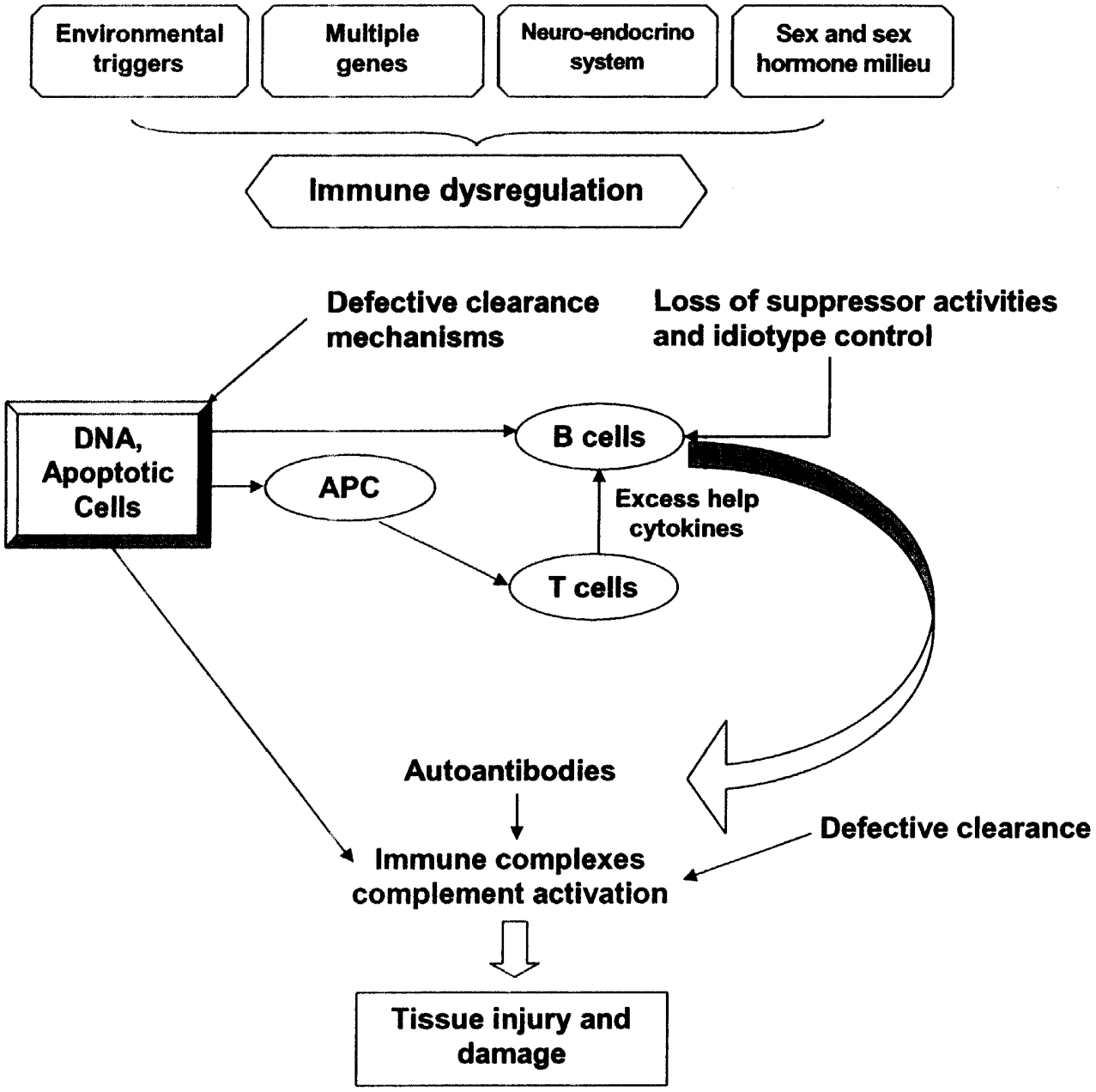

Figure (1): The pathogenesis of systemic lupus erythematosus. [4]
APC: Antigen -presenting cells.

Both physiological and supraphysiological concentrations of estrogens facilitate humoral responses, leading to increased B cell proliferation and antibody production. On the contrary, high doses of estrogens inhibit T cell responses, such as proliferation and IL-2 production. Estrogen also enhances the cell surface expression of CD40 ligand (CD40L) in cultured T cells from patients with SLE. These effects appear to be unique to patients with SLE, indicating that lupus T cells are more sensitive to estrogens.[12] Taken together, estrogens may aggravate SLE by prolonging the survival of autoimmune cells, increasing T helper type 2(Th2) cytokine production, and stimulating B cells to produce autoantibodies. The inhibition of the Th1 response and the enhancement of CD40L expression on lupus T cells may indirectly promote the Th2 response and lead to further B cell hyperactivity.[12]

III- Environmental factors

Environmental factors such as microorganisms (e.g. viruses) may stimulate specific cells in the immune system. Retroviruses have been suggested as one etiologic agent in systemic lupus erythematosus. Also, it has been reported that there is an association of SLE with Epstein-Barr virus infection. Furthermore, the anti-HCV antibodies were detected in the urine samples of SLE patients, but not in the sera of these patients.[13]

On the other hand, the ultraviolet (UV) light is known to exacerbate lupus skin lesions. The UV light may stimulate keratinocytes to secrete more IL-1, which in turn stimulate B cells to make more antibodies. Exposure of DNA to UV light increase thymine dimmer, which renders the DNA more immunogenic. The exposure of keratinocytes to UV light induces apoptosis. Also, the cell damage induced by UV light increases release of heat shock protein, which participates in activation of autoreactive T cells. Not all antibodies cause disease. In fact, all normal individuals make autoantibodies but in low levels.[14]

IV- Immunologic factors

Numerous immune system abnormalities occur in patients with SLE, the etiology of which remains unclear. Some of these immune defects are episodic, and some correlate with disease activity. SLE is primarily a disease with abnormalities of immune system regulation. These abnormalities are thought to be secondary to a loss of "self" tolerance; that is, SLE patients (either before or during disease evolution) are no longer totally tolerant of all their "self" antigens, and consequently an immune response develops to these antigens.[4]

The number of suppressor T cells decreases; these would normally be down-regulating (maintaining homeostasis) immune responses. Furthermore, mice with lupus and possibly humans with SLE have a (genetic) defect in apoptosis that result in abnormal programmed cell death. Cells break down abnormally, and certain (especially nuclear) antigens are processed by antigen-presenting cells (i.e. macrophages, B lymphocytes, dendritic cells) into peptides.[4]

The peptide-major histocompatibility complex stimulates the expansion of helper (i.e. CD4) autoreactive T cells that, through release of cytokines (i.e. interleukin-6, interleukin-4 and interleukin-10), cause autoreactive B cells to become activated, proliferate, and differentiate into antibody-producing cells and make an excess of antibodies to many nuclear antigens.[4]

Alternatively, T cells respond abnormally to nuclear material over expressed on apoptotic blebs of cells; or microorganisms may be broken down within antigen-presenting cells into" mimicry peptides' that have similar antigenic structures to self peptides. Thus, a characteristic immune profile develops in patients with SLE: the development of elevated levels of antinuclear antibodies (ANAs) especially to DNA, Sm, RNP, Ro, La, and other. [4]

Immunodeficiency in SLE

1- Innate (non-specific) immune system in SLE

♦ **Neutrophils**

Neutrophil problems can be divided into neutropenia, and neutrophil function problems. Neutropenia can occur as an autoimmune neutropenia related to SLE, or as a result of treatment for example with immunosuppressive drugs such as cyclophosphamide. Recurrent abscesses can also occur in diabetes mellitus, and nasal carriers of *Staphylococcus aureus*. Genetic defects of neutrophil function, such as chronic granulomatous disease are very rare. [15]

♦ **Complement deficiency**

The complement system plays an important part in defense against pyogenic organisms, especially gram-negative bacteria. In addition to playing an important role in host defense against infection, the complement system is a mediator in both the pathogenesis and prevention of immune complex diseases, such as systemic lupus erythematosus. [15, 16]

Complement deficiency is frequently seen in lupus but this is usually due to complement consumption as a result of classical complement pathway activation by immune complexes. Patients with SLE may have anti C1q antibodies, which have been shown to be related to lupus nephritis. They are strongly associated with severe consumptive hypocomplementaemia.[17] With respect to genetic deficiency, C4 null alleles are quite common and are associated with lupus. Homozygous genetic complement component deficiency is much rarer of course and those predisposing to SLE include C1q, C2, C4, i.e., the early parts of the classical pathway. The figure for C1q, C1s and C1r (often go together), and C4 association with susceptibility to SLE are 90, 67, and 80%, respectively.[17]

C3 deficiency is more typically characterized by recurrent pyogenic infections, membranoproliferative glomerulonephritis and rashes.[17] Furthermore, it was observed that in patients with SLE, complement consumption, with falling serum concentrations, often mirrors disease activity. [18]

It is possible that complement has both inflammatory and anti-inflammatory functions, the latter reflected by its role in clearing immune complexes from the circulation and removing them from tissues.[19] Complement also binds to cells that have undergone apoptosis[20] and helps to eliminate these cells from tissue.[21] If the complement system fails at this point, such waste material (consisting of partially degraded components of the cytoplasm and nucleus) could accumulate and evoke an autoimmune response.[22] There are three hypothesized steps to the development of systemic lupus erythematosus.[22]

Figure (2) illustrates The "Waste-Disposal" Hypothesis. The first step is the failure to clear autoantigens (i.e., defective waste disposal). This is the stage at which complement deficiency may have a pathogenic role. [22]

In Panel A, a macrophage is shown engulfing an apoptotic cell. There are a variety of ligands on apoptotic cells and receptors on macrophages that make this process extremely efficient. The binding of C1q, C-reactive protein, and IgM to apoptotic cells may promote the activation of complement, leading to the clearance of apoptotic cells by ligation of complement receptors. The binding of serum myeloid P component masks autoantigen on the surface of apoptotic cells and promotes their safe disposal. Once the macrophage has engulfed the apoptotic cell, it secretes the antiinflammatory cytokine transforming growth factor β (TGF-β). [22]

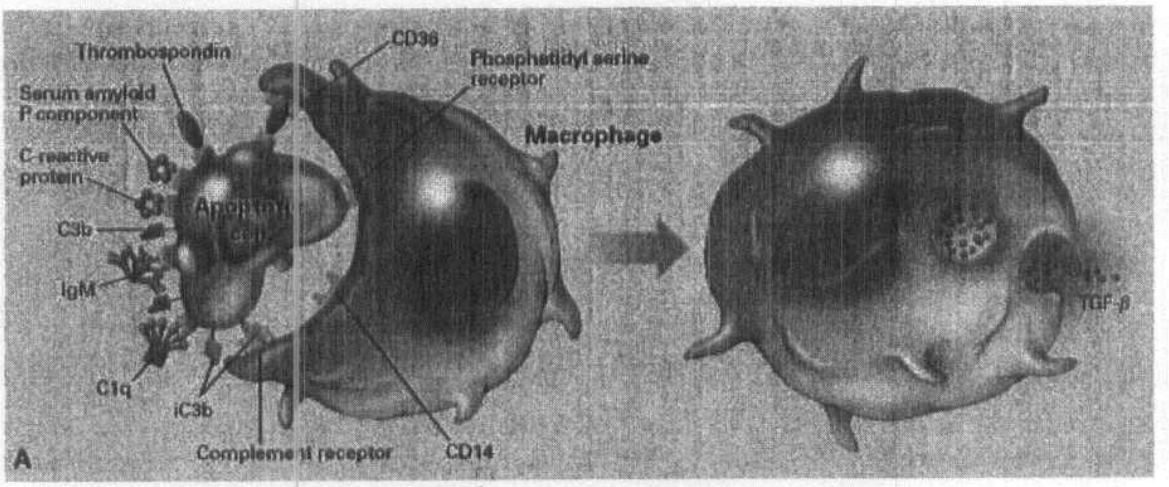

Figure (2): The "Waste-Disposal" Hypothesis. Panel A.

The second step is the uptake of autoantigen by immature dendritic cells in the presence of inflammatory cytokines, which causes these cells to mature into antigen-presenting cells, allowing the presentation of autoantigens to T cells. [22]

As shown in Panel B, when there is an excess of apoptotic cells and the failure of one or more of the normal systems of receptor–ligand recognition for the uptake of apoptotic cells, immature dendritic cells may take up apoptotic cells. If this occurs in the presence of inflammatory cytokines such as granulocyte–macrophage colony-stimulating factor (GM-CSF), tumor necrosis factor α (TNF- α), and interleukin-1, the dendritic cell may mature into an autoantigen-presenting cell. The dendritic cell is shown presenting autoantigens to a T cell in the presence of costimulatory molecules and cytokines. [22]

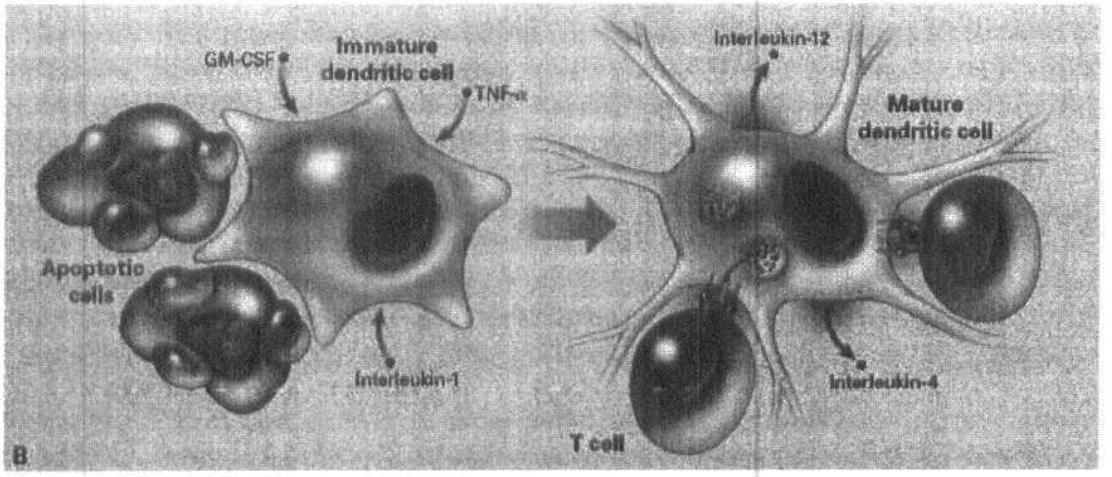

Figure (2): The "Waste-Disposal" Hypothesis. Panel B.

The third step is the provision by T cells of help to autoreactive B cells, which have taken up autoantigen by means of their immunoglobulin receptors. Such B cells mature into plasma cells that secrete autoantibodies. However, it is intriguing that in more than 90 percent of patients with a C1q deficiency, this defect alone appears to be sufficient to cause the expression of systemic lupus erythematosus. (22)

Panel C shows an autoreactive B cell that has taken up autoantigens from an apoptotic cell through its antibody receptors. The B cell is receiving help from an activated T cell, which is expressing costimulatory molecules and cytokines involved in the maturation of B cells, including an important member of the tumor necrosis family, B lymphocyte stimulator (BLyS), also referred to as TNF-4. The autoreactive B cell divides and matures into a plasma cell that secretes autoantibodies. It is likely that in the majority of patients, systemic lupus erythematosus develops only in the presence of abnormalities in more than one of these steps. (22)

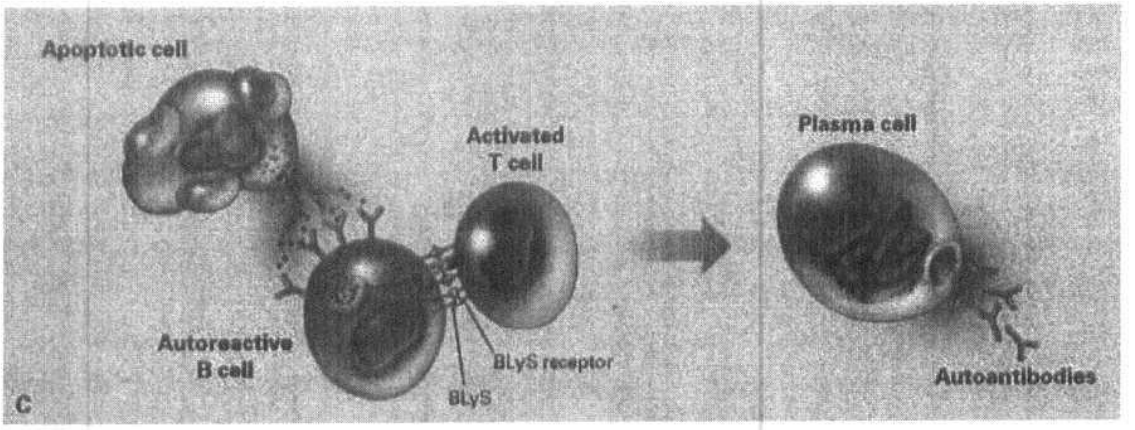

Figure (2): The "Waste-Disposal" Hypothesis. Panel C.

2-Adaptive (specific) immune system in SLE

A- Cellular elements of the adaptive immune system and T cell abnormalities

In SLE several functional defects have been recognized among the cells of the immune system, including T and B lymphocytes, natural killer (NK) cells and accessory cells (known as antigen presenting cells or APC_S). The consequence of this is the breakdown of immunological tolerance through the reversal of clonal anergy, activation of self-reactive autoaggressive T cells and defective T cell suppression. [23, 24]

In human SLE, the total number of T cells is usually reduced, probably owing to the effects of anti-lymphocyte antibodies.[25] Proliferative response of peripheral blood T cells to signals such as mitogens, specific antigens, and autologous cells are usually lower than those in healthy individuals.[26] However, the ability of T cells to help antibody production, and to suppress it, is probably the T cell function most pertinent to clinical SLE.[27] Because many of the pathogenic autoantibodies in patients with SLE are IgG, T cell help is necessary for their production and maintenance.[27]

The numerous functional abnormalities of human lupus T cells are heterogeneous, frequently conflicting and occasionally related to one another. [28]

Lupus T cells (T helper cells (CD_4^+) and T suppresser cells ($CD8^+$)) and T-cell lines exhibited significantly increased Ca^{2+} responses, when compared with the responses of T cells from normal subjects or patients with systemic autoimmune disease other than lupus.[29]

The Ca^{2+} pathway, critically determines the nuclear factor of activated T cells mediated transcription of genes such as those encoding the CD_{40} ligand (CD_{40} L)[30] and Fas ligand.[31] Therefore, it is not surprising, that lupus lymphocytes express increased CD_{40} L and Fas L on their surface as a result of their increased Ca^{2+} response.[32] Desia-Mehta et al,(1996)[33] found that hyperexpression of CD40L occurs not only in the T cells but also in the B cells of lupus patients.[33] Because both T and B cells also express CD_{40}, it is possible that increased CD_{40}-CD_{40}L interaction takes place, leading to increased stimulation of B cells and production of autoantibodies.[32]

The most important cellular elements of the adaptive immunity are the T helper (Th) lymphocytes. These cells are classified into different types based on their cytokine profile. Type 1($Th1_)$ cells produce interferon-gamma (IFN-γ), interleukin-2 (IL-2) and tumor necrosis factor-β (TNF-β) and promote the production of opsonizing and complement-fixing antibodies, macrophage activation, antibody-dependent cell cytotoxicity, and delayed type hypersensitivity. [34]

On the other side, type 2 (Th2) cells produce IL-4, IL-5, IL-6, IL-9, IL-10 and IL-13 and provide optimal help for humoral immune responses, including IgE and IgG_1 isotype switching and mucosal immunity, through induction of mast cells and eosinophil growth and differentiation. [35]

The balance between Th_1 and Th_2 cells is associated with various immune responses in infectious diseases or immunological disorders. [36] The establishment of the Th1/Th2 balance is determined early during immune responses and depends on many factors including antigen structure, the functional status of antigen-presenting cells , the strength of T cell activation, the presence of cytokines such as IL-12[37] and IL-4, co-stimulatory signals such as CD_{80} or CD_{86}, and the microenvironment.[36]

In patients with SLE, the elevated levels of Th_2 cytokines such as IL-4 and IL-10 have been expected to facilitate antibody production by B cells and play an important role in the development of autoantibody-mediated SLE disease. [38]

It has been reported that there was a positive correlation between the proportion of the Th_1-and Th_2 type cytokine production and the disease activity of SLE. [39] There was also a tendency of decreased number of the cells which produce the Th_1-type cytokines and of increased number of the cells which produce the Th_2-type cytokines. This might be associated with the pathogenesis of SLE and have an important role in the induction of the polyclonal B cell activation. [40]

B- Humoral (Antibody) deficiencies and B cell abnormalities

The most marked defect in SLE is the increase in number of activated B-lymphocytes, which contributes to the hypergamma- globulinaemia associated with reactivity to self-antigens. There is also an increase in interleukin-2 (IL-2) receptor levels on circulating B cells. [41]

Grimaldi et al, (2005) [42] found that the increased number of B cells is accompanied by T lymphocytopenia especially of cells bearing the $CD_4^+/CD_{45}R^+$ phenotype. This population of cells "helps" to induce suppression by providing a signal to the CD_8^+ (suppressor) cells and the reduction in this subset may contribute to the failure of the T cells to suppress the hyperactive B cells. [42]

B cell abnormalities can precede the development of SLE. Activated lupus B cells have higher intracytoplasmic calcium responses than controls.[43, 44]

There is also evidence that B cells in patients with SLE are more sensitive to the stimulatory effects of cytokines such as IL-6 than non-SLE B cells. [45]

Disease flares are often accompanied by high IL-10 and low IFN-γ production by peripheral blood cells, [46] suggesting that the deviation of cytokines toward these that favor antibody production and suppress direct T cell-mediated responses (such as regulation of the hyperactive B cells) is an important abnormality in SLE. [27]

The central immunological disturbance in patients with SLE is autoantibody production (Autoantibodies are antibodies that are directed against one's self).[47]These antibodies are directed at several self molecules found in the nucleus, cytoplasm, and cell surface, in addition to soluble molecule such as IgG and coagulation factors. [48]

Antinuclear antibodies are most characteristic and present in more than 95% of patients. Anti-double stranded DNA (ds-DNA) and anti-smith (anti-Sm) antibodies are unique to patients with SLE. In fact, their presence is included in the classification criteria of SLE. [49]

The sm antigen is designated as a small nuclear ribonucleoprotein (sn RNP) and is composed of a unique set of Uridine rich RNA molecules bound to a common group of core proteins and other proteins associated with the RNA molecules. Anti-sm antibodies react with sn RNP core proteins, whereas anti-DNA antibodies bind to a conserved nucleic acid widely present on DNA. [50] Anti-DNA antibody titres frequently vary over time and disease activity but anti-sm antibody titres are usually constant. [51]

Anti-DNA antibodies differ in their properties, including isotype, ability to fix complement, and capacity to bind to the glomeruli causing pathogenicity. [52] Only certain types of anti-DNA antibodies are pathogenic. [53] The involvement of anti-DNA antibodies in lupus nephritis is suggested by several pieces of circumstantial evidences. First, clinical observation in most patients indicate that active nephritis is associated with raised anti-DNA titres and reduced total hemolytic complement values.[54] Second, anti-DNA antibodies show preferential deposition in the kidneys, suggesting that DNA-anti-DNA antibody immune complexes are the main mediators of inflammation.[55] Although renal injury may result from immune complexes containing anti-DNA antibodies, circulating immune complexes have been difficult to characterize[55] because of their low concentration in serum. The formation of immune complexes in situ, instead of within the circulation, may be another possibility. [56] Anti-DNA antibodies may bind to pieces of DNA adherent to the glomerular basement antigens, such as C_1q, nucleosomes, heparan sulfate and laminin. [56] The binding of anti-DNA antibodies to these antigens may initiate local inflammation and complement activation, and may also anchor immune complexes to the kidney sites, whether or not they are formed in the circulation or in situ. [48]

In addition to anti-DNA and anti-sm antibodies, a variety of other autoantibodies are often detected (Table 3). [8]

Table (3): Systemic lupus erythematosus antibodies

Antibody	% of patients
ANA	94
Anti-Sm	9
Anti-RNP	21
Anti-Ro	32
Anti-La	12
Anti-dsDNA	60
Decreased C3	40
Rheumatoid factor (RF)	20
Anti-cardiolipin (IgG)	25
Anti-cardiolipin (IgM)	13
Lupus anticoagulant	14
Coombs\	18
Anti-thyroglobulin	10
Anti-thyroid microsomes	13

ANA (antinuclear antibody) was considered positive if present to a titre of ≤ 1:40.

* These data are based on 300 patients attending the University College/Middlesex hospital lupus clinic (1978 to 2000). [8]

Immune cell signaling defects in lupus

The aberrant T cell receptor (TCR) and B cell receptor (BCR) signaling cascade of lupus lymphocytes:

In normal lymphocytes, ligation of the antigen-receptor (Ag-receptor) complex by either Ags or anti-receptor antibodies causes the phosphorylation of variant polypeptides of the Ag-receptor complex at tyrosine residues. These become the anchoring sites for members of the Src or Syk family of protein tyrosine kinase (PTKs), leading to their activation and hence to amplification of the production of phosphotyrosines. One such PTK target protein is phospholipase cγ isozyme 1 or 2 (PLCγ) in T and B cells, respectively. Tyrosine-phosphorylated PLCγ translocates to the cell membrane where it cleaves membrane phospholipids and generates the second messengers diacylgycerol and 1, 4, 5-inositol trisphosphate receptor (Insp3). Binding of InsP3 to its receptor, located in the endoplasmic reticulum, causes the release of stored Ca^{2+}. In turn, Ca^{2+} activates the serine phosphatase calcineurin, which dephosphorylates the cytoplasmic (phosphorylated) from the transcription factor of activated T cells (NF-AT). Dephosphorylated NF-AT rapidly translocates to the cell nucleus and with the help of other factors initiates the transcription of early immune response genes (Figure 3). [32, 57]

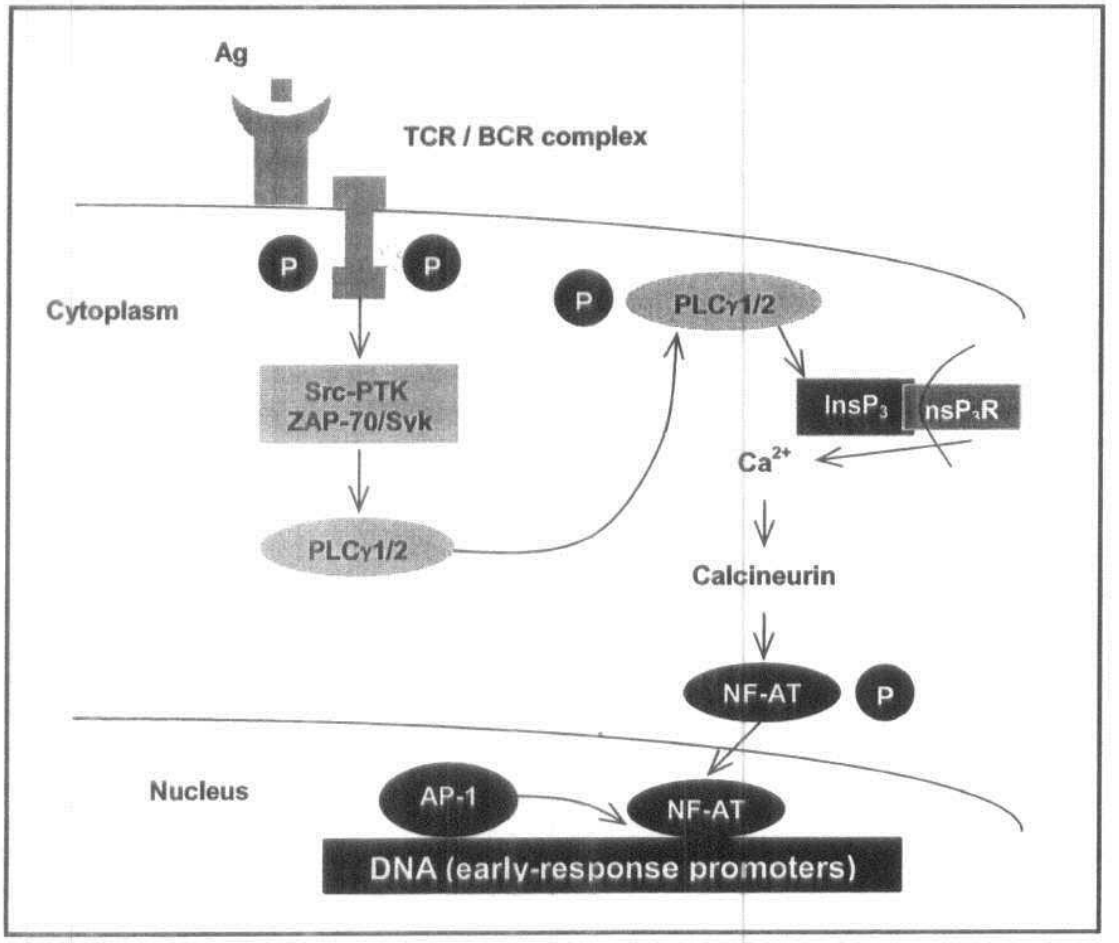

Figure (3): T cell receptor- or B cell receptor- mediated signaling events in normal lymphocytes. [32]

Figure (4) illustrates the quantitative differences of the TCR- or BCR-mediated signaling cascade between normal and lupus lymphocytes. Anti-receptor antibody-mediated signaling in lupus lymphocytes results in enhanced production of cytosolic tyrosine-phosphorylated proteins, followed by the release of increased amounts of InsP3 and by abnormally high fluxes of Ca^{2+}. Free Ca^{2+} is a second messenger that influences the transcription of Ca^{2+} calcineurin-NF-AT pathway-responsive genes. In lupus T cells particularly, TCR/CD3-mediated signaling is conducted in the absence of TCRζ chain.[32, 58]

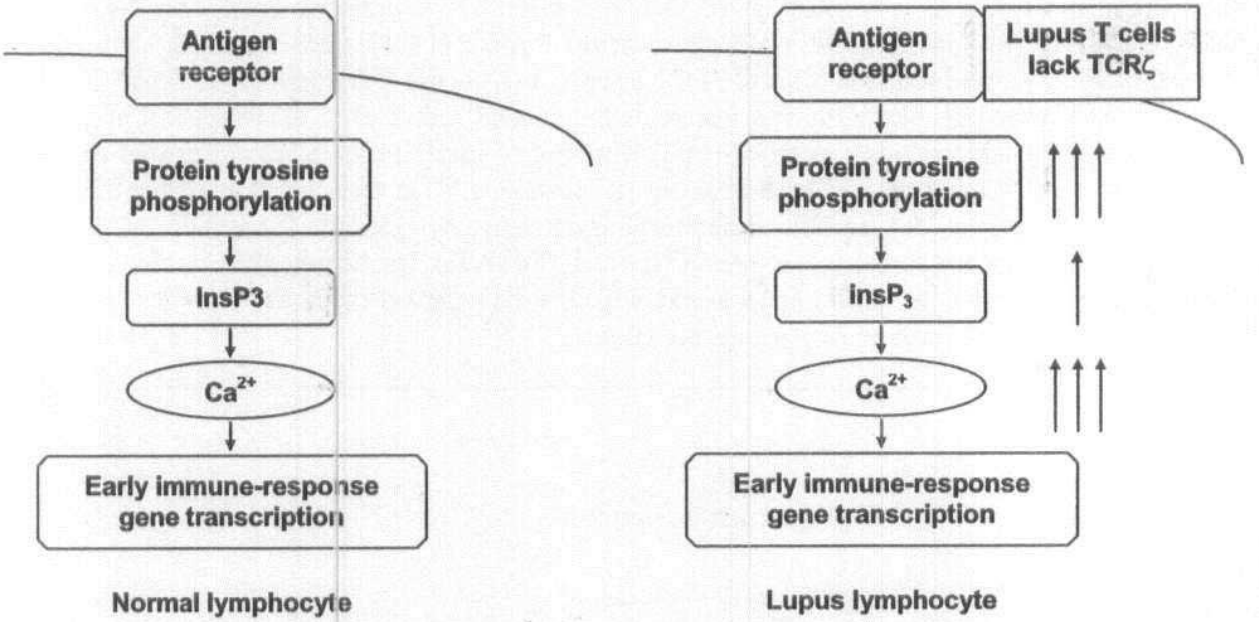

Figure (4): Quantitative differences of the TCR-or BCR-mediated signaling cascade between normal and lupus lymphocytes. [32]

Apoptosis in systemic lupus erythematosus

Apoptosis (programmed cell death) is a genetically controlled process that removes unwanted or damaged cells. [59] It is considered as a physiological mechanism for deleting cells from the body without inducing inflammation and subsequent damage to contiguous cells (Fig. 5). [60]

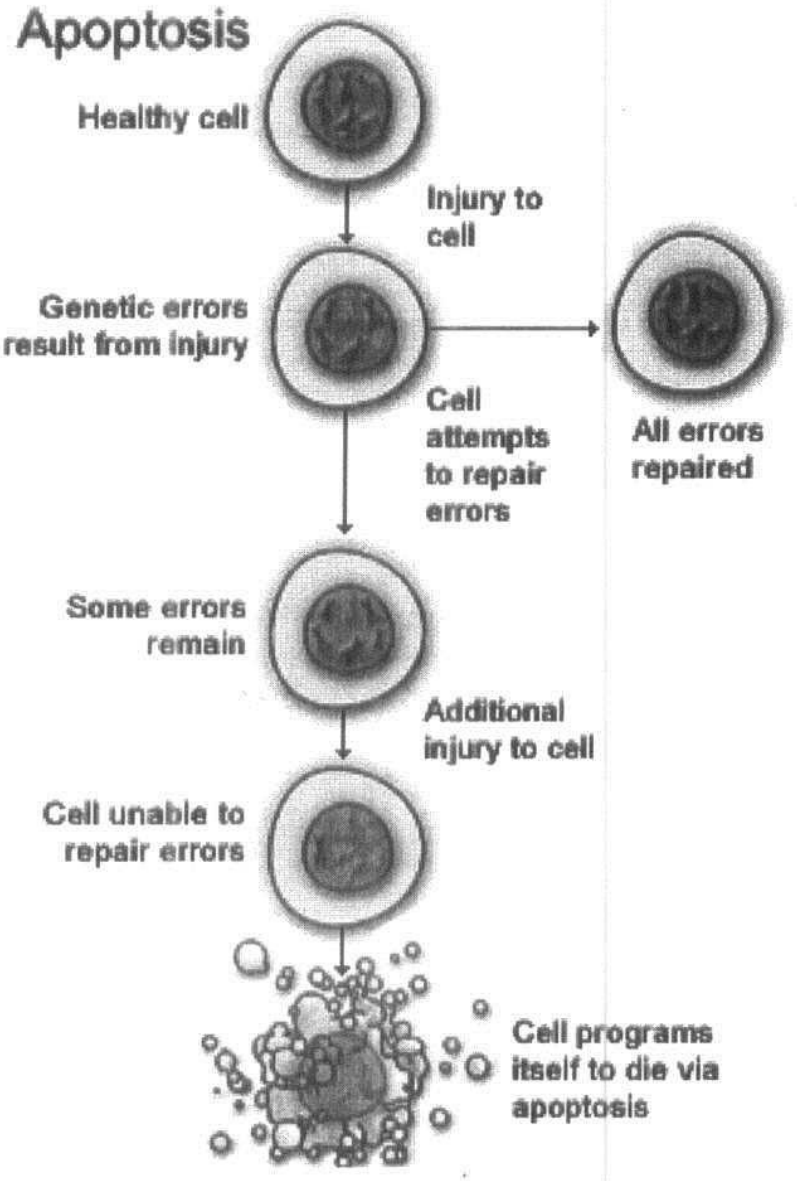

Figure (5): The normal process of apoptosis. [60]

The increased rate of apoptosis which was detected by many workers in SLE patients[61-62] would theoretically increase the chance of leakage of intracellular antigens that may either trigger an autoimmune response or participate in the formation of immune complexes.[61-62]

The production of autoantibodies, such as anti-chromatin autoantibodies, which through a complex formation with chromatin become deposited in basement membranes, especially in the skin and the kidney, where they may cause glomerulonephritis.(Figure 6).[63-67]

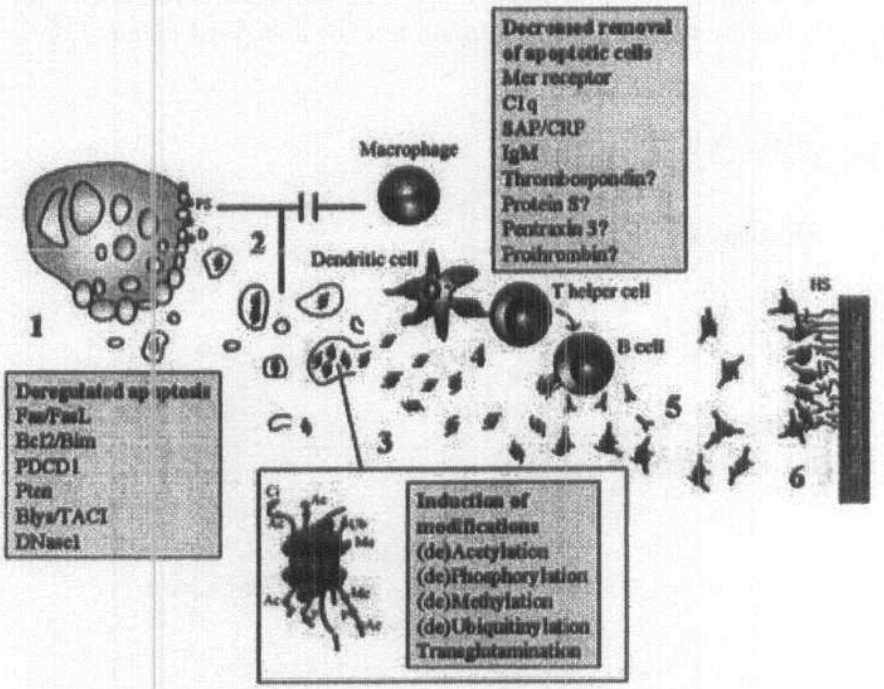

Figure (6): Hypothesis for the development of anti-chromatin responses in SLE. (1) Disturbed apoptosis either at the wrong time-point and/or at the wrong microenvironment. (2) Defective removal of apoptotic cells leading to the release of apoptotic blebs containing chromatin. (3) Apoptosis induced chromatin modifications. (4) Stimulation of the immune response by chromatin. (5) Formation of anti-chromatin/chromatin complexes. (6) Binding of the complexes to heparan sulphate in basement membranes due to the charge interaction between cationic N-termini of the core histones and the anionic charged heparan sulphate. [67]

Apoptotic defects in SLE involving at least two genes, Bcl-2 and Fas, have been extensively investigated in the pathogenesis of SLE. [68]

Bcl-2 is a proto-oncogene located on the inner mitochondrial membrane. Bcl-2 is a unique oncogene that it promotes lymphoid cell survival by interfering with apoptosis rather than by inducing cell proliferation. There is now a considerable evidence that Bcl-2 expression is enhanced in a proportion of peripheral T cells, but not in B cells of SLE patients, and that Bcl-2 levels correlate with overall disease activity. [69]

Fas (Apo-1/CD_{95}) is the cell surface protein responsible for induction of apoptosis in lymphocytes through induction of several signaling pathways. [70] The defective clearance of apoptotic cells in SLE could be the result of quantitative or qualitative defects of the early complement proteins, such as C2, C4 or C1q. [71]

Cytokine network in systemic lupus erythematosus

Cytokines are low-molecular-weight proteins which act as chemical modulators of the immune system, and as mediators of cell-cell interactions, with a wide heterogeneity of pro- and anti-inflammatory effects. [72]

Abnormal cytokine production has been implicated to be of pathogenic importance in systemic lupus erythematosus. [73] Cytokine production is not only changed in patients with SLE when compared with healthy controls but also changes with different disease phenotypes. [74]

The pattern of cytokine production in different disease states is therefore often described as a Th1, Th2, Th3 and Th0 response based on the cytokines produced. In SLE, it seems that the balance between proinflammatory and anti-inflammatory cytokines determine the degree and extent of inflammation and thus can lead to major clinical effects. Every proinflammatory cytokine is counteracted by either anti-inflammatory cytokines or cytokine antagonists and therefore it is the relative concentration of a cytokine to its inhibitor or antagonist that will determine its action. [74, 75]

Among the pro-inflammatory cytokines, TNF-α which can be induced by immune complexes and antibodies to double-stranded DNA (ds-DNA), has been found to be overexpressed in SLE, including lupus nephritis.[76] TNF-α can further induce the expression of other pro-inflammatory cytokines including interleukin-18 (IL-18), which participates in production of IFN-γ (Fig. 7).[77]

In study of Santiago-Raber et al,(2003)[78] IFN-γ was over expressed in mononuclear cells of lupus kidneys and might correlate with disease activity.[78] Funauchi et al,(1991)[79] showed an increase in numbers of low density (activated) B cells and a decrease in high density B cell numbers in SLE as compared with controls.[79] They showed that high density B cells from normal controls but not from SLE were inhibited in their proliferative response to IL-4 by IFN-γ while the response of total B cells to phytoheamagglutinin (PHA) or IL-2 induced T cell factors was increased by IFN-γ in both normal controls and in patients with SLE. They suggested that IFN-γ may be of the factors that promotes polyclonal B cell activation in SLE. [79]

High IL-2 levels were detected in the sera of 50% of patients with active SLE disease. [80] Impaired IL-2 release was demonstrated in mitogen stimulated(peripheral blood mononuclear cells)PBMCs of lupus. [81] This reduced production of IL-2 correlated with an increased spontaneous IgG production from lupus PBMCs. [74]

SLE rash is often triggered by ultraviolet light. Exposure to UV light can induce cells from the monocyte/macrophage fraction of PBMCs taken from patients with SLE to produce IL-6 suggesting that cytokine release may play a part in the exacerbation of SLE caused by photosensitivity. [82]

Pathogenic autoantibodies in lupus mice generally belong to the IgG_2 and IgG_3 subclasses, [83] which are predominantly dependent on type 1 cytokine, IFN-γ, and are suppressed by the type 2 cytokine, IL-4. [84]

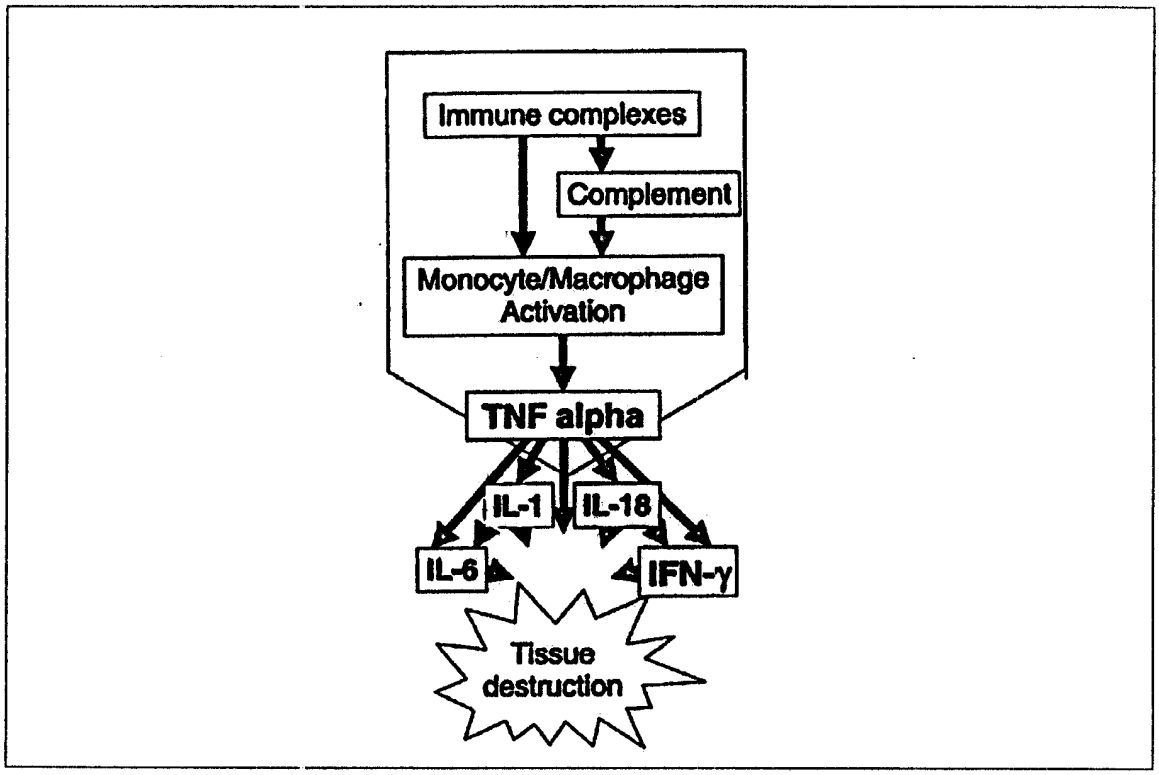

Figure (7): Proinflammatory cytokines in SLE inflammatory organ disease.[77]

IL-4, however, also promotes autoimmunity by inhibiting apoptosis of autoreactive B cells, [85] and inhibits autoimmunity by inducing the production of the regulatory cytokine, transforming growth factor beta (TGF-β). [86]

Type 1 and 2 cytokines can also directly participate in end organ damage. [87] For example, the type 1 cytokine IFN-□ can exacerbate organ inflammation, whereas type 2 cytokines can exacerbate tissue fibrosis.[88] It remains largely unresolved, however, which cytokines play the most vital roles in regulation of autoantibody production and in end organ damage during the development and progression of SLE.[89]

Th3 cells are regulatory cells that can act to induce immune tolerance and characteristically produce TGFβ, IL-4 and IL-10. [90]

Transforming growth factor-β (TGF-β) is a family of proteins (TGF-β1,2 and 3), involved in the generation of CD8+T suppressor cells, NK cells and regulatory T(Th3) cells for down-regulatory effects on antibody production. [91] It has been established that peripheral lymphocytes produce levels of active TGF-β that enable CD8+ cell to develop inhibitory activity[92], and that lymphocyte production in both the total and biologically active form of TGF-β is reduced in subjects with SLE.[93] Furthermore, decreased production of total TGF-β1 inversely correlates with disease activity, although that of active TGFβ1 dose not have such a correlation.[94] It has been shown that the lack of IL-2[95] or TNF-α, [96] increased amounts of IL-10,[92] and decreased protease activity in subjects with SLE[97] contribute to decreased active TGF-β production that blocks the generation of regulatory T cells.[91]

INTERLEUKIN-10

IL-10 (18 kd polypeptide) was originally described as a "cytokine synthesis inhibitory factor" (CSIF) produced by Th_2 clones.[98] IL-10 is a pleiotropic cytokine that can produced by various types of cells, including Th0, Th1, Th2, $CD8^+$ T lymphocytes, B lymphocytes, monocytes and keratinocytes.[99]

It has been well documented that IL-10 mediates its action on a wide variety of cell types, and has both stimulatory and inhibitory effects on the immune responses.[100] For instance, IL-10 is a potent suppressor of macrophages, Th1 cells and NK cells.[101] Furthermore, IL-10 has been demonstrated to play an important role in controlling autoimmune disease.[102] In contrast, IL-10 has been shown to stimulate the growth of mast cells, B and Th2 lymphocytes and promote adaptive immunity.[103] Moreover, many studies indicate that alterations in IL-10 regulation may result in accelerated T-cell apoptosis and aberrant T-cell-dependent B-cell function.[104,105]

Interleukin-10 receptor

IL-10 activity is mediated by its specific cell surface receptor complex, which is expressed on activated cells, in particular immune cells.[106]

The human IL-10 receptor (IL-10R) is a heterotetramer composed of two of each of the receptor chains ($IL\text{-}10R_1$ and $IL\text{-}10\ R_2$), which belong to the IFN-R-like or class II cytokine receptor.[107]

The $IL\text{-}10R_1$ chain plays a dominant role in mediating high affinity ligand binding and signal transduction, whereas the $IL\text{-}10R_2$ subunit is thought to be required for signaling only.[108] IL-10/IL-10R interaction in immune cells results in transcriptional activation of several hundred genes, some of them are more than 50-fold up regulated.[109]

The IL-10/IL-10R interaction activates the tyrosine kinase Jak_1 and Tyk_2, which are associated with the $IL\text{-}10R_1$ and IL-10R2, respectively. The receptor engagement and tyrosine phosphorylation activates the cytoplasmically localized inactive transcription factors STAT1, 3 and 5, resulting in translocation and gene activation.[110]

IL-10 signaling result in the inhibition of inflammatory processes by suppressing the expression of proinflammatory cytokines, chemokines, adhesion molecules, as well as antigen presenting and co-stimulatory molecules in monocytes/macrophages, neutrophiles, and T cells.[111]

As all of these inflammatory proteins are transcriptionally controlled by NF-kB, it was suggested that IL-10 may exert a significant part of its anti-inflamnatory properties by inhibiting this transcription factor (Fig.8).[112]

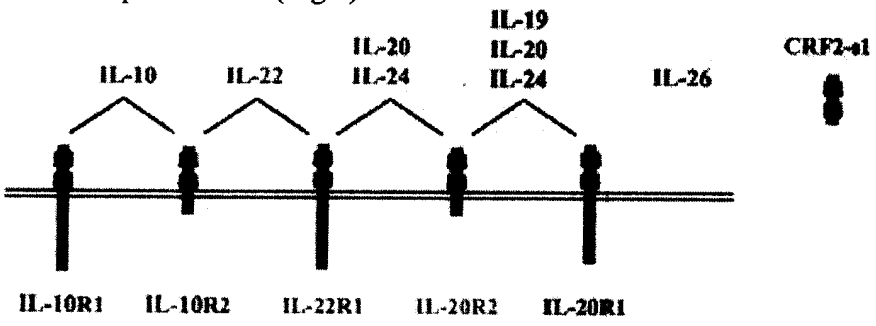

Figure (8): Ligand/receptor binding of the IL-10 family molecules.[112]

Effect of interleukin-10 on immune cells

A comprehensive summary of the effects of IL-10 on the various components of the immune system is shown in table (4). [113-122]

Table (4): General effects of IL-10 on various components of the immune system.

Cell Line Affected by IL-10	General Effect of IL-10
T-lymphocytes	Specifically inhibits Th1 cell cytokine synthesis in the mouse (especially when macrophages acted as the antigen-presenting cells). This function is antagonized by IL-4.
	Inhibitory effects on proliferation, survival, and cytokine production of human T-cells. For example, direct interaction of IL-10 with the IL-10 receptor on T cells acts to suppress transcription of the gene for IL-2, which inhibits T cell proliferation.
	Indirect inhibition of T cells. This inhibition is caused by the alteration of the antigen- presenting function of monocytes. IL-10 downregulates the expression of MHC class II antigen on monocyte, thereby decreasing the peptide/MHC complexes available for interaction and proper stimulation of T cells.
	Plays a role in causing T cells to undergo anergy.
	Inhibits IFN-γ synthesis by $CD8^+$ T cells without affecting the cytotoxic function of these $CD8^+$ T cells.
	Human IL-10 demonstrates the ability to stimulate chemotaxis, proliferation, differenciation and cytolytic activity of human $CD8^+$ T cells.
	Inhibits apoptotic death of IL-2 dependent T cells by prolonging cell survival. This prolongation of survival may occur through upregulation of Bcl-2 by IL-10.
Macrophages/Monocytes	Effects on morphology, phenotype and cytokine production.
	Causes deadherence and rounding up of monocytes.
	Inhibits the constitutive and inducible expression of MHC class II on monocytes.
	Able to block B7 and ICAM-1 expression in monocytes in response to IFN-γ.This act disrupts monocyte-T cell interaction.
	Prohibits human monocytes (at mRNA level) from producing IL-1α, IL-1 β, IL-6, IL-8, TNF-α, granulocyte macrophage (GM-CSF), and granulocyte colony-stimulating factor (G-CSF) following activation.
	Inhibits T cell production by preventing IL-12 expression from monocytes.

Table (4): Continue

Cell Line Affected by IL-10	General Effect of IL-10
	Inhibits its own production (IL-10) by monocytes, thus demonstrating the workings of a self-regulatory negative feedback loop.
	Inhibits macrophage activation by IFN-δ.
Natural Killer Cells (NK)	Inhibits monocyte-induced production of IFN-δ by NK cells. Indirectly causes this inhibition by suppressing monocyte production of IL-12.
	Enhances production of IFN-δ, TNF-α, and GM-CSF by IL-2-activated NK cells.
	Induces NK cytotoxic activity against NK-resistant tumor cell targets.
B-lymphocytes	Induces the expression of MHC class II antigen on resting B cells.
	Leads to enhanced viability of B cells *in vitro*.
	Induces apoptosis in germinal center B cells. Mechanism unknown.
	Stimulates differentiation of B cells into antibody secreting cells (especially after activation by CD40 antibody). Differentiation occurs when IL-10 acts with IL-2 and effectively increases the affinity of the B cells' IL-2 receptor.

Interleukin-10 in SLE

IL-10 has been presumed to be an important modulator of disease activity in human SLE. Patients with lupus produce large amounts of IL-10 [123], with enhanced gene expression in PBMCs [124], and its serum level correlates with disease activity. [125] While the precise role of IL-10 in the pathogenesis of lupus remains uncertain, studies have suggested that this cytokine is pathogenic both in humans and in mice. For example, continuous administration of anti-IL-10 delays onset of autoimmunity in (NZW x NZB) F1 mice. [126] Liorente et al, (2000) [127] reported that anti-IL-10 administration to six human lupus patients with active disease, and dependent upon steroids for treatment, led to a reduction in disease activity. [127]

Sung et al, (2006) [128] found that, in multicase SLE families, healthy individuals also exhibit high IL-10 levels and, as in patients, the cellular sources are monocytes and subpopulation of B lymphocytes. Some studies related the elevated levels of IL-10 to a genetic origin, while others pointed at environmental trigger. [128]Taken together, these findings suggested two possible causes for lupus IL-10 overproduction. The first would be due to an inherited defect of IL-10 synthesis by some immune cells, while the second cause would result from high IL-10 output by tissues damaged by the inflammatory process.[129]IL-10 may play a central role in the pathogenesis of SLE particular by inducing autoantibody production. Autocrine synthesis of IL-10 by B cells, and paracrine release by macrophages, has also been demonstrated. [129] Furthermore, IL-10 producing cells that do not belong to the immune system may underlie the deleterious effects of exposure to sunlight and pregnancy in the course of SLE. [129]

Enghard et al, (2006) [130] studies in NZB/WFI mouse model with clinical features of murine lupus suggested that the association between the frequencies of T cells expressing IFN-γ and IL-10 and clinical findings is a key role for these cells in the pathogenesis of lupus. [130]

A direct evidence of the role of IL-10 in human lupus clinical manifestation has been reported in a study performed in steroid dependent SLE patients who were treated with a murine anti-IL-10 monoclonal antibody (MO AB). This treatment brought about a rapid amelioration of clinical status in particular the cutaneous and articular symptoms, which was maintained for next 6 months of follow up. Besides the clinical improvement, a decrease in the biological markers of immune system and a partial restoration of T lymphocyte function were achieved. These results were striking for the sustained control of disease activity allowed significant decreasing of the corticosteroid dosage. [131]

INTERLEUKIN-18

Interleukin-18 is a pleiotropic cytokine initially discovered as IFN-γ inducing factor. IL-18 is a member of the IL-1 cytokine family that mediates important activities during both acquired and innate immune responses. [132] It is produced by activated macrophages and plays a role in the development of immunity against intracellular pathogens.[133] IL-18 is also produced by Kupffer cells, Keratinocytes, intestinal epithelial cells, osteoblasts and adrenal cortex. [134]

IL-18 is a Th_1 cytokine which initially characterized by its capacity to promote Th1 responses in synergy with IL-12. [135] IL-18 has been shown to drive either Th1 or Th2 responses, dependent on the cytokine microenvironment, suggesting a broader role in functional T cell differentiation than that originally recognized.[136] IL-18 enhances NK cell cytotoxicity and directly induces IFN-γ production by NK Cells.[137]

Interleukin-18 receptor

IL-18 exerts its effects via the IL-18 R(Fig.9), a heterodimeric complex consisting of a ligand-binding α-chain (IL-18 Ra/IL-1R-related protein) and an associating β-chain (IL-18 Rβ/accessory protein-like).[138] Both chains of the IL-18 R belong to the IL-1R family and consist of three Ig- like domains in the intracellular region.[139] IL-18 binding protein

(IL-18 Bp) is not a part of the IL-18 signaling complex, but, rather, antagonizes IL-18 activity. [140] It is a unique, secreted receptor-like molecule and consists of a single Ig-like domain. [141] Human IL-18 Bp is secreted constitutively in healthy subjects, with circulating concentrations of 2-5 ng/ml.[142] IFN-γ increases gene expression for IL-18 Bp in renal mesangial cells, which functions as a feedback loop and reduces IL-18 induced IFN-γ.[143]

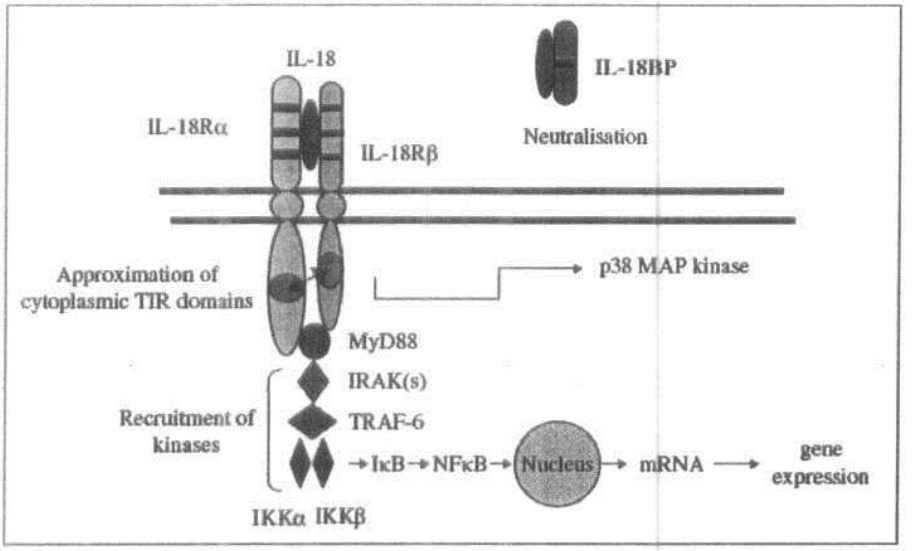

Figure (9): The structure of IL-18 receptor.

Effect of IL-18 on immune cells

In addition to the major role of IL-18 as a strong induction of IFN-γ production by Th_1 and NK cells in concert with IL-12, [136] it also induces GM-CSF, IL-4, IL-6, IL-13, and histamine, augments NK activity, and stimulates Fas ligand expression. [144] IL-18 also activates T cells to proliferate and synthesize IL-2, TNF-α, IL-2 Rα and suppresses the production of IL-10. [134]

Naïve T cells modestly but clearly express IL-18R, but they fail to produce a significant amount of IFN-γ in response to stimulation with Ag and/or IL-18. However, stimulation with IL-12 and IL-18 in presence and absence of Ag equally induces them to produce IFN-γ in a synergistic manner. Although, the level of IL-4 production by T cells stimulated with IL-12 and IL-18 is modest, additional stimulation with Ag markedly increases their IL-4 production. Moreover, naïve T cells can develop into Th2 cells and produce both IL-4 and IL-13 in response to stimulation with TCR engagement after being stimulated with IL-12, IL-18 and Ag (Fig.10). [134,145]

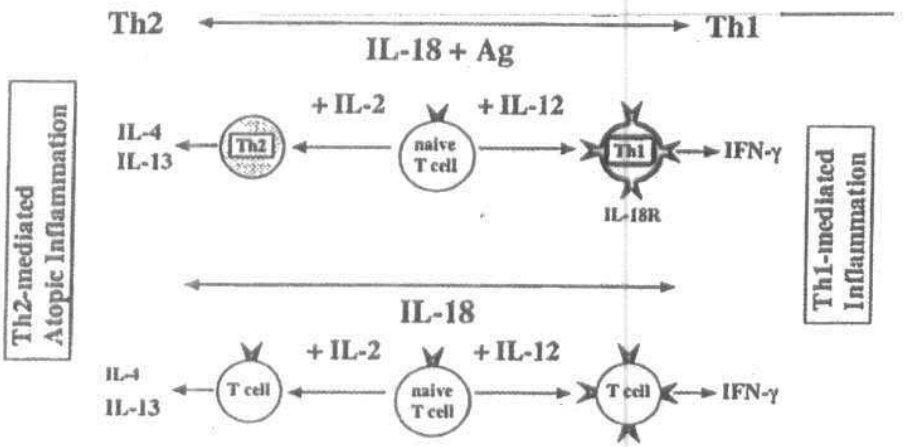

Figure (10): IL-18 stimulates Th_1 or Th_2 response depending on its cytokine milieu. [134]

Roles for IL-18 in various pathological conditions

Figure (11) summarizes several of IL-18's emerging functions in promoting or suppressing disease. In infectious diseases, IL-18 plays a crucial part in promoting the inflammatory response during bacterial sepsis, a response that can lead to hepatic injury and multiple organ failure. [146] On the other hand, like many proinflammatory cytokines; IL-18 appears to act in host defense. For example, in one report, pretreatment with IL-18 prevented or minimized the severity of experimentally induced bacterial infection. [147] IL-18 is also instrumental in the suppression of allergy, because it inhibits IgE production. [148] With regard to type 1 diabetes, IL-18 favors the development of insulitis in nonobese diabetic mice. [149] However, in other studies, IL-18 was shown to decrease insulitis. [150]

IL-18 may play an additional pathogenic role in rheumatoid arthritis, a disease that is driven by Th1 responses. IL-18 appears to synergize with IL-12 and IL-15 to activate production of IFN-γ and of IL-18 itself by synovial tissue. [134]

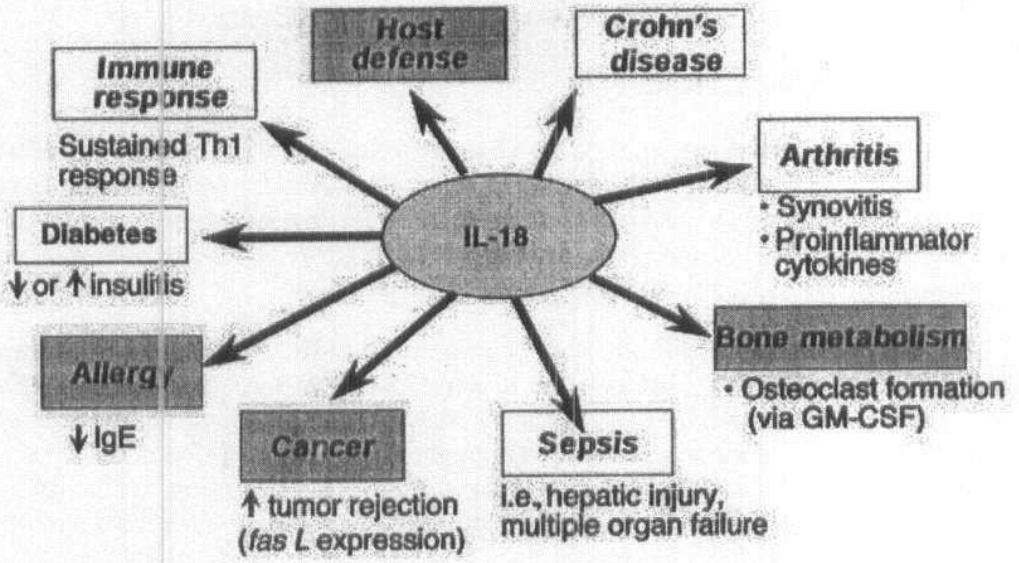

Figure (11): Potential roles for IL-18 in various pathological conditions. Yellow highlighting indicates a potentially beneficial effect of IL-18. [134]

IL-18 in SLE

IL-18 levels have been found to be elevated in serum of lupus patients and have correlated with disease activity. It has been postulated that IL-18 may act together with TNF-α and IL-1 to mediate the inflammatory mechanism in SLE patients. [151]

IL-12 has a synergistic effect with IL-18, and both cytokines are inducers of IFN-γ. Studies on animal models of SLE have strengthened the hypothesis that IFN-γ may play a central role in SLE. The presence of renal damage in SLE patients correlates with high levels of IFN-γ that is induced by IL-18 and IL-12. [152] In the absence of IL-12, however, IL-18 may also mediate the induction of type2 cytokine production. [137]

Shibatomi et al, (2001) [152] confirmed the importance of IL-18 in lupus disease activity but did not show any correlation between serum levels of IL-18 and IL-1 or TNF-α. This study suggested that IL-18 may not be important in SLE patients as a proinflammatory cytokine but it could play an important role in lupus pathogenesis through its action on NK cells. [152] NK cells may participate in immune regulation by controlling autoreactive T and B cells. It seems that the high levels of IL-18 found in autoimmune diseases could induce a particular apoptotic process called "activation induced cell death" of NK cells leading finally to a loss of control of autoreactive cells and development of autoimmune diseases.[153]

IL-18 accelerates spontaneous autoimmune lupus disease with characteristic glomerulonephritis and vasculitis. This is further exacerbated by the synergistic action of IL-12 and IL-18, a combination known to promote Th1 cell development include reciprocal enhancement of their receptor expression. It is also important to note that IL-18 and IL-12 plus IL-18 treatments led to a profound suppression of the production of IL-10 which is closely associated with the Th2 response and anti-inflammation in numerous systems. [154]

Aim of the Work

AIM OF THE WORK

The aim of the present study was designed to reveal the relationship of IL-10 and IL-18 production in patients with systemic lupus erythematosus in relation to disease activity.

Subjects and Methods

SUBJECTS AND METHODS

This study was conducted on two groups of subjects:

1. Thirty female patients suffering from systemic lupus erythematosus (SLE), diagnosed according to the Systemic Lupus Activity Measure (SLAM) criteria.[155] Those patients were selected from department of Internal Medicine, Medical Research Institute Hospital, Alexandria University.
2. Fifteen age and sex matched normal healthy individuals as a control group.

The exclusive criteria:

1. Patients were chosen free from any other chronic disease.
2. Patients were not treated by azathioprine, cyclosporine A, cyclophosphamide or cortisone therapy for at least 6-months before blood sampling.

All patients under study were subjected to:

- Full history taking including age, duration and activity of lupus disease.
- Clinical examination including SLE Disease Activity Index (SLEDAI).
- Laboratory investigations including complete urine analysis and tests for anti-dsDNA IgG antibodies (anti-DNA) [156], Anti-nuclear antibodies titre (ANA) [157], C-reactive protein (CRP) [158] and erythrocyte sedimentation rate (ESR). [159]
- Immunological investigations.

Immunological Investigations

Separation of Peripheral Blood Mononuclear Cells

Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation over histopaque 1077[160].The procedure was performed at room temperature under aseptic conditions.

Reagents

- Histopaque 1077 (Sigma, USA).
- RPMI-1640 tissue culture medium supplemented with L-glutamine (Sigma, USA).
- Fetal calf serum (FCS) (GIBCO, UK) inactivated by heating at 56°c for 30 minutes.
- Penicillin(100 IU/ml) and streptomycin(100µg/ml) (Sermoid,Germany).
- Phosphate buffered saline (PBS) PH 7.2-7.4.
- Sterile ammonium chloride solution (0.87%).
- Heparin-free preservative (Sermoid,Germany).

Procedures

1- Four ml blood were drawn by vein-puncture from each individual under study .All samples were collected on heparin-free preservative as an anticoagulant and mixed thoroughly.
2- Each sample was diluted with an equal volume of saline and well mixed in a sterile tube with a sterile Pasteur pipette.

3- The diluted blood was over layered very carefully by side wall of the tube on half volume of Histopaque1077- density gradient solution in a sterile centrifuge tube.
4- The tube was centrifuged at 1800 r.p.m for 30 minutes.
5- After centrifugation, the interface layer containing the mononuclear cells (the ring containing the mononuclear cells) was carefully aspirated by sterile Pasteur pipette and transferred to a clean sterile centrifuge tube.
6- If the sample was contaminated with red blood cells,10 ml of sterile ammonium chloride (0.87%)was added to the cell suspension and left for 5 minutes at room temperature to get rid of the red blood cells and then washed 3 times with RPMI-1640 culture medium.
7- The cells were washed in 10 ml normal saline and centrifuged at 1000 rpm for 10 minutes. The supernatant was then discarded.
8- The cells were resuspended in 2 ml RPMI culture medium (RPMI-1640 supplemented with 10% heat inactivated fetal calf serum,100 IU/ml penicillin and 100 µg/ml streptomycin).
9- The cells were counted by hemocytometer and adjusted to 2×10^6 cells/ml.

Viability

Principle

The viability of the separated cells was tested by dye exclusion technique, which is based on the impermeability of the viable cells to Trypan blue dye [161] .The dye intake by dead cells through ruptured cell membrane gives a blue color while viable cells keep their color as their cell membranes are intact.

Reagents

Trypan blue dye 0.2% (0.2 gm was dissolved in 100 ml distilled water and stored at room temperature).

Procedures

1- One drop of the cell suspension was mixed with one drop of 0.2% Trypan blue solution, left for 2 minutes and then examined microscopically. Non viable cells stain blue.
2- At least 200 cells were counted and the percentage of viable cells was calculated according to the following equation:

$$\% \text{ of viable cells} = 100 - \frac{\text{Number of dead cells}}{\text{Total cell count}} \times 100$$

Stimulation and culture of mononuclear cells

Principle

When mononuclear cells are cultured *in vitro* with certain mitogens (e.g. phytohemagglutinin (PHA)), the cells are stimulated and release more cytokines .

Procedures

All the steps of this technique were done under complete sterile condition. Only cells with a viability exceeding 95% were used for tissue culture.

1. One ml of the previously prepared cell suspension in supplemented RPMI medium(RPMI-1640 medium supplemented with L-glutamine, 10% fetal calf serum, penicillin(100 IU/ml) and streptomycin(100µg/ml)) was placed in a sterile tube and then freshly prepared Phytohemagglutinin (PHA) (10 µg/ml) (Biochrom AG, Berlin) was added and well mixed using a sterile Pasteur pipette. The suspension was placed in the culture plate (200µl/well).
2. Similarly, the cell suspensions without mitogen (PHA) were placed in the culture plate.
3. The culture plate was covered with its lid and incubated in CO_2 incubator at 37°C in an atmosphere of 5% CO_2 humidified air and left for 24 hours.
4. At the end of the incubation period, the cell culture supernatant was aspirated. Each culture supernatant sample with PHA and without PHA was harvested in an epindorf tube. The epindorfs were labeled and stored at -70°C until the time of cytokines determination.

Determination of cytokines:

I. Determination of interleukin-10

IL-10 concentrations (pg/ml) were determined in all samples using a quantitative sandwich-type enzyme immunoassay technique "IL-10 ELISA kit" (Immunotech, France) according to the manufacturers instructions.

Principle

The immunoenzymatic assay of IL-10 is a sandwich type assay with two immunological steps. In the first step the IL-10 is captured by a monoclonal antibody bound to the wells of a microtiter plate. In the second step a biotinylated monoclonal antibody is added together with streptavidin-peroxidase conjugate. The biotinylated antibody binds to the solid phase antibody-antigen complex and, in turn, binds the conjugate. After incubation, the wells are washed and the antigen complex bound to the well detected by addition of a chromogenic substrate. The intensity of the coloration is proportional to the IL-10 concentration in the sample or standard.

Reagents

- Microwell plate coated with monoclonal antibody (murine) to human IL10.
- IL-10 standard lyophilized (20 ng/ml).
- Biotinylated monoclonal antibody (murine).
- Diluent.
- Streptavidin-HRP conjugates.
- Wash solution (phosphate buffer solution).
- Substrate solution (TMB/H2O2).
- Stop solution (0.5 mol/L sulfuric acid).

Procedures

1. A dilution series of IL-10 standard were prepared using standard diluent: 2000, 400, 80, 16 and zero pg/ml, then 50 µl of each concentration was added to each of the standard wells.
2. 50 µl of the samples were added to each of the sample wells.
3. The micro-titer plate was then covered with adhesive cover foil and incubated for 2 hours at room temperature with gently shaking.
4. After incubation, the microwell plate was washed 3 times with wash buffer solution.
5. 50 µl of Biotinylated antibody and 100 µl of Streptavidin-HRP conjugate were added to each of the standard as well as the sample wells. Then the plate was covered with adhesive cover foil and incubated for 30 minutes at room temperature with gently shaking.
6. Each well was washed 3 times with wash buffer solution.
7. 100 µl of substrate solution was added into each well. Then the plate was covered with adhesive cover foil and incubated for 15 minutes in the dark at 18-25°c.
8. 50 µl of stop solution was added to each well. Then the optical density was measured at 450 nm using an ELISA reader.
9. The known standard concentrations of IL-10 (pg/ml) were plotted onto the X-axis of log-linear paper and the corresponding absorbancies on the Y-axis.
10. A standard curve was created by drawing the best fitting curve through the points. The concentration of IL-10 in the unknown samples was determined from the standard curve (Fig12).

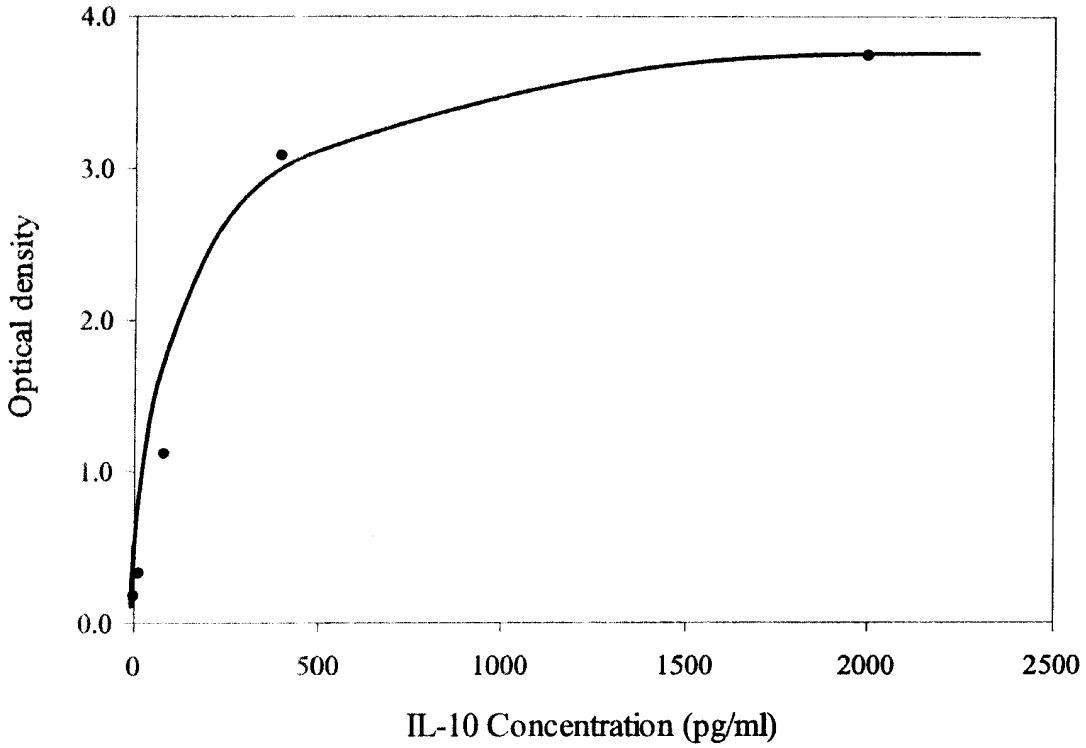

Figure (12): Standard curve of interleukin-10 (IL-10).

II.Determination of interleukin-18

IL-18 concentrations (pg/ml) were determined in all samples using a quantitative sandwich-type enzyme immunoassay technique "IL-18 ELISA kit" (MBL, Japan) according to the manufacturers' instructions.

Principle

The assay is based on a quantitative sandwich-enzyme-immunoassay principle using two monoclonal antibodies against two different epitopes of human IL-18. In the wells coated with anti-human IL-18 monoclonal antibody, sample to be measured or the standards are incubated. After washing, a peroxidase conjugated anti- human IL-18 monoclonal antibody is added into the microwell and incubated. After another washing, the peroxidase substrate is mixed with the chromogen and allowed to incubate for an addition period of time. An acid solution is then added to each well to terminate the enzyme reaction and to stabilize the developed color. The optical density of each well is then measured at 450 nm using a microplate reader. The concentration of human-IL-18 is calibrated from a dose response curve based on reference standards.

Reagents

- Microwell plate coated with anti-Human IL-18 antibody.
- Human IL-18 calibrator (lyophilized 20 ng/ml).
- Conjugate reagent (Peroxidase conjugate anti-Human IL-18 monoclonal antibody).
- Conjugate diluent.
- Wash solution (phosphate buffer solution).
- Substrate solution (TMB/H2O2).
- Stop solution (0.5 mol/L sulfuric acid).

Procedures

1- A dilution series of IL-18 standard were prepared using standard diluent: 1000, 400, 160, 64, 25.6 and zero pg/ml, then 100 µl of each concentration were added to each of the standard wells.
2- 100 µl of the samples were added to each of the sample wells.
3- The micro-titer plate was then covered with adhesive cover foil and incubated for 30 minutes at room temperature (20-25°c) on a shaker under gently shaking.
4- After incubation, the microwell plate was washed 4 times with wash buffer solution and completely aspirated.
5- 100 µl of conjugate solution was added to each well. Then the plate was covered with adhesive cover foil and incubated for 60 minutes at room temperature (20-25°C) on a shaker under gently shaking.
6- Each well was rinsed and washed 3 times with wash buffer solution.
7- 100 µl of substrate solution was added into each well. Then the plate was covered with adhesive cover foil and incubated for 30 minutes in the dark at room temperature (20-25°C).
8- 100 µl of stop solution was added to each well. Then the optical density was measured at 450 nm using an ELISA reader.
9- The known standard concentrations of IL-18 (pg/ml) were plotted onto the X-axis of a log-linear paper and the corresponding absorbancies on the Y-axis.
10- A standard curve was created by drawing the best fitting curve through the points. The concentration of IL-18 in the unknown samples was determined from the standard curve (Fig13).

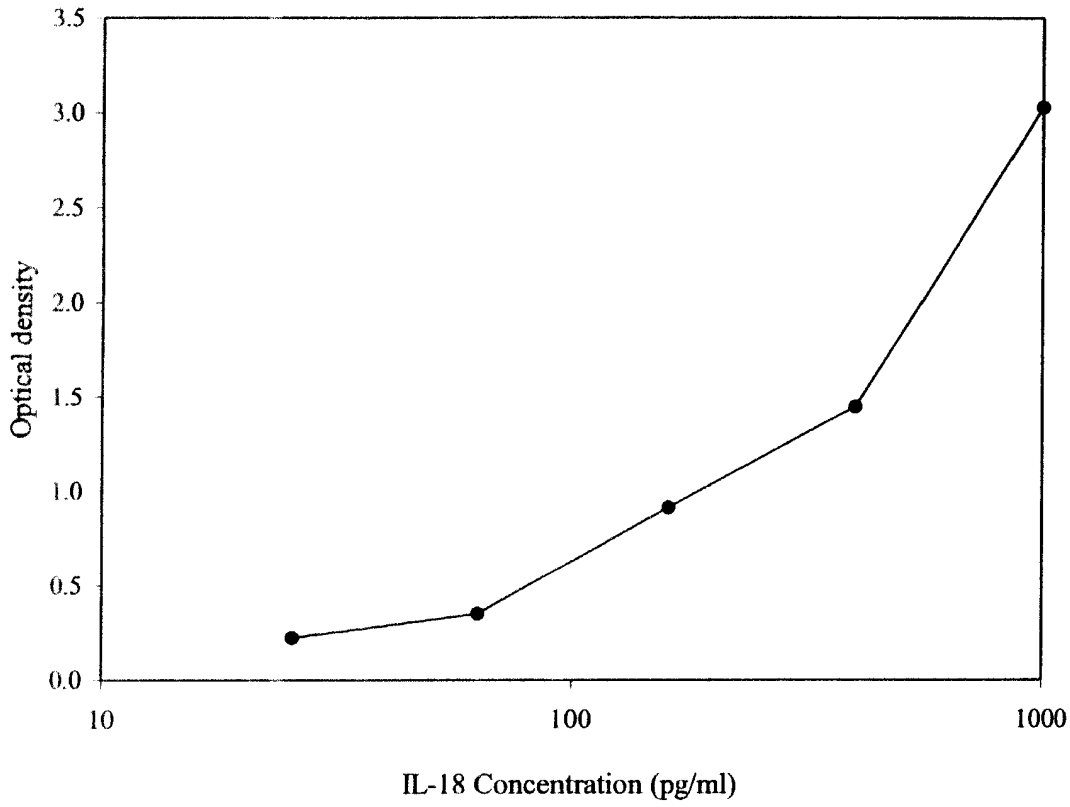

Figure (13): Standard curve of interleukin-18 (IL-18).

III. Determination of serum C3 and C4 levels

The serum levels of C3 and C4 (g/l) were quantitatively measured in all samples by means of immunoturbidimetry with the TurbidTimeSystem according to the manufacturers' instructions (Dade Behring, USA).

Principle and the explanation

The complement system is an integral part of the antigen-nonspecific immune defense. It can be activated via two reaction pathways, the classical pathway which is triggered primarily by cell-bound immune complex, and the alternative pathway which is activated primarily by foreign bodies such as micro-organisms. The complement component C3 is a key protein in both reaction pathways, whereas C4 belongs to the classical pathway of complement activation. Complement activation is associated with consumption of components C3 and C4 so that a reduction in their concentrations can allow diagnostic conclusions to be reached. In the SLE, the serum concentration of the C3 and C4 reflect the activity of the disease [162].

The proteins contained in human bodily fluids form immune complexes in an immunochemical reaction with specific antibodies. The increasing light absorption of the reaction mixture is then tracked photometrically. The maximum reaction velocity and the time required to reach maximum velocity is dependent on the concentration of the analyze in the sample. The evaluation is performed by comparing the measured reaction parameters with the values obtained for reference preparation. The reference curve is dependent on the lot (in the form of a barcode).

Materials

- Turbitime system (Behring).
- Turbiquant reagent for C3c (Behring).
- Turbiquant reagent for C4 (Behring).

Procedures

1- Two ml of blood was drawn by vein-puncture from each individual under study.
2- Each sample was placed on clean tube and let to coagulate for one hour.
3- The tube was centrifuged at 1000 rpm for 30 minutes.
4- After centrifugation, the serum layer was aspirated by Pasteur pipette and transferred to a clean epindorf. The epindorfs were labeled and stored at -8°c until the time of assay.
5- The serum samples were diluted 1:21 with isotonic saline.
6- The vial of reagent was placed in the reagent receptacle of the TurbiTimeSystem, and then 50µl of the diluted sample (for C3c) or 200 µl (for C4) was placed in a cuvette with a stirrer. The pipette tip should be near to the bottom of the cuvette when pipetting the sample.
7- The cuvette was inserted into the cuvette holder and then 500 µl of reagent (Turbiquant C3c or Turbiquant C4) was added. When pipetting the reagent, the pipette tip should be inserted approximately 1 cm into the cuvette.
8- After 30 seconds the results were given printed. The results were expressed as g/l.

IV. Determination of SLE disease activity index

The SLE Disease Activity Index is scored on 24 items. The six neurologic elements each scoring 8 points, include seizure, psychosis, organic brain syndrome, visual changes with retinal involvement, cranial nerve involvement and new cerebrovascular accident. Vasculitis also scores 8 points. Renal involvement with new-onset proteinuria, casts, hematuria, and pyuria would accumulate 16 points of activity, allowing 4 points for each item. Arthritis and myositis score 4 points each. Pleurisy, pericarditis, and mucosal membrane ulcerations each receive 2 points, as do the onset of a new rash and the occurrence of alopecia. Low complement level and abnormal ds-DNA add 2 points each; fever, thrombocytopenia, and leucopenia add 1 point each. [163]

Statistical analysis

The results were analyzed using SPSS version 10. All data are given as mean ± SE and compared by Student's t-test for values of IL-10, IL-18 in cell culture supernatant with and without PHA and C3, C4 between the two groups of study (normal control and systemic lupus patients). Paired t-test was used for the previous values in each group.

Differences between values were considered to be significant when P was less than 0.05 and highly significant at P less than 0.001.

Pearson's Correlation Coefficient gave linear correlation between variables within each group and the significant correlation was represented by Linear Regression.

Results

RESULTS

1- CLINICAL DATA

A- Age distribution

Age distribution study was carried out on 30 systemic lupus erythematosus (SLE) female patients, their age ranged from 15 to 55 years with a mean age of 32.25 ± 1.85 years (Table 5).

Table (5): Age distribution of patients with systemic lupus erythematosus.

Age (years)	Patient frequency	
	Number	%
15 -	6	20
25 -	12	40
35 -	8	26.7
45 – 55	4	13.3
Total	30	100%
Rang	15-55	
Mean	32.25	
SE	1.85	

B- Distribution of period of systemic lupus erythematosus disease

All of patients were on SLE for a period more than three months, ranged from 1 to 8 years with a mean of 3.8 ± 0.42 years (Table 6).

Table (6): Distribution of duration of systemic lupus erythematosus disease.

SLE (years)	Frequency	
	Number	%
1-	10	33.3
3-	9	30
5-	6	20
7-	5	16.7
Total	30	%100
Range	1-8	
Mean	3.8	
SE	0.42	

C- Systemic Lupus Eryrthematosus Disease Activity Index Score (SLE DAI)

SLEDAI ranged from 14-45 with a mean value of 26.5 ±1.6 (Table7).

Table (7): Systemic Lupus Erythematosus Disease Activity Index score (SLEDAI).

Case No.	SLEDAI
1	25
2	31
3	35
4	30
5	22
6	36
7	30
8	16
9	29
10	42
11	19
12	22
13	14
14	18
15	38
16	17
17	18
18	40
19	45
20	32
21	18
22	22
23	20
24	22
25	18
26	20
27	35
28	20
29	18
30	43
Range	14-45
Mean	26.5
SE	1.6

2- LABORATORY INVESTIGATIONS

In SLE patients, levels of anti-double strand DNA antibodies, anti-nuclear antibodies, C-reactive protein and erythrocyte sedimentation rate (ESR) are shown in (Table 8).

Table (8): Levels of Anti-double strand DNA antibodies, Anti–nuclear antibodies, C-reactive protein and ESR in SLE patients.

Case No.	Anti-dsDNA antibodies	Anti-nuclear antibodies	C-reactive protein mg/dl	ESR mm/h	
1	>1000	+4 (153.9)	14.9	31–66	
2	15.4 U/ml	7.1 U/ml	20.1	115–122	
3	+	+	12	112–124	
4	+	Strong +	16.3	74–101	
5	400 >75	38.37 >10	15	57–92	
6	+	+	13	70–103	
7	+	+	16.3	121–130	
8	+	+	17.8	90–110	
9	+	+	15.36	124–141	
10	+	+	12.1	130–162	
11	+	Strong positive	12.6	55–105	
12	+	+	11.75	23–40	
13	+	+	14.3	74–121	
14	+	+	13.5	70–100	
15	61.29	+ 1/460	14.4	110–125	
16	+	+	11.69	23–51	
17	+	+ 1/460	17.25	54–92	
18	+	+	15.2	114–122	
19	480 IU/ml	+ 44	14.5	70–115	
20	41.2	166	12.2	112–143	
21	>200	640	12.75	20–42	
22	+	+	13.45	62–104	
23	+	+	16.12	65–81	
24	+	+	12.69	15–32	
25	+	+	11.89	16–32	
26	+	+	13.58	15–31	
27	+	+	14.68	150–166	
28	+	+	13.22	32–56	
29	+	+	15.08	55–90	
30	+	+	15.08	168-115	
Range			11.69-20.10	15-168	31-166
Mean			14.30	74.2	97.1
SE			0.37	7.8	7

3- IMMUNOLOGICAL INVESTIGATIONS

1- Interleukin-10 level in peripheral blood mononuclear cells (PBMCs) culture supernatant of systemic lupus erythematosus (SLE) and normal control groups before and after stimulation with PHA.

In SLE patients, IL-10 level in PBMCs culture supernatant before stimulation with PHA ranged from 14.19 to 272.05 pg/ml with a mean value of 99.67 ± 13.4 pg/ml. It significantly increased (P=0.000) after stimulation with PHA to range from 38.40 to 501.43 pg/ml with a mean value of 203.09 ± 24.6 pg/ml.

In the control group, IL-10 level in PBMCs culture supernatant before stimulation with PHA ranged from 5.69 to 12.50 pg/ml with a mean value of 8.97± 0.6 pg/ml. It significantly increased (P=0.000) after stimulation with PHA to range from 21.03 to 43.69 pg/ml with a mean value of 32.44 ± 1.9 pg/ml.

A significant increase was observed in the mean value of IL-10 in SLE patient group compared to the normal control group before (p=0.000) and after (p=0.001) stimulation with PHA (Table 9, Fig. 14).

Table (9): The mean value of IL-10 level (pg/ml) in PBMCs culture supernatant of SLE patient and normal control groups before and after stimulation with PHA.

		Control group n =15	Patient group n = 30	Test of significant
Before stimulation	Range	5.69-12.50	14.19-272.05	t = 4.541* p = 0.000
	Mean	8.97	99.67	
	SE	0.6	13.4	
After stimulation	Range	21.03-43.69	38.40-501.43	t = 4.647 p = 0.001
	Mean	32.44	203.09	
	SE	1.9	24.6	
Paired t test		17.14*	7.67*	
P		(0.000)	(0.000)	

*: P is significant at the level < 0.05

t: Comparison between the mean value of IL-10 in PBMCs of SLE patient & normal control groups before and after stimulation with PHA.

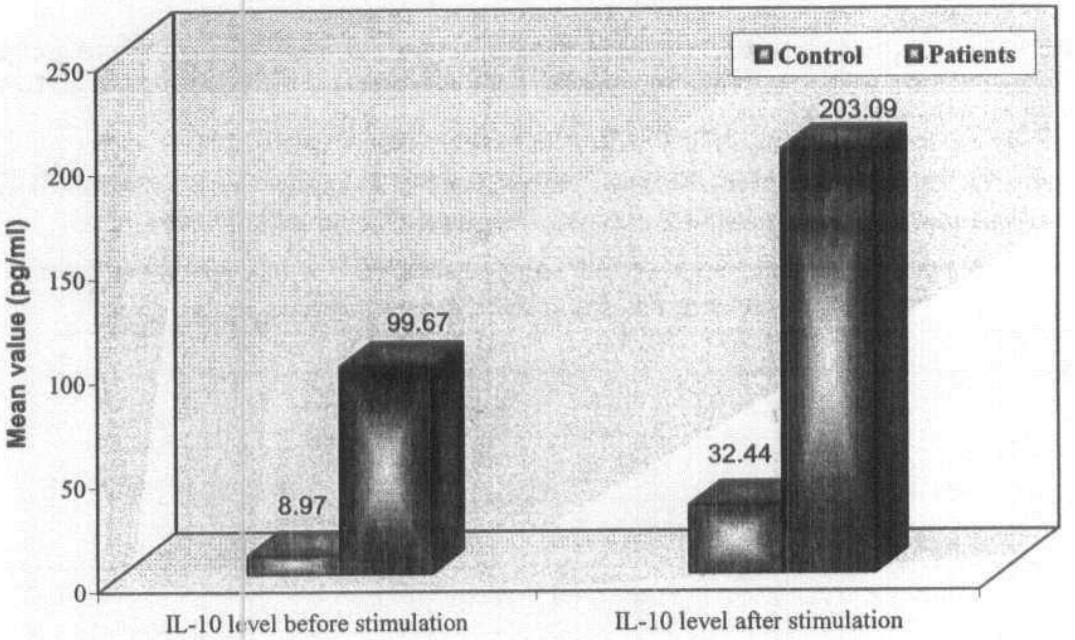

Figure (14): The mean values of IL-10 level in PBMCs of SLE and normal control groups before and after stiumlation with PHA.

2- Interleukin 18 level in peripheral blood mononuclear cells (PBMCs) culture supernatant of systemic lupus erythematosus (SLE) and normal control groups before and after stimulation with PHA.

In SLE patients, IL-18 level in PBMCs culture supernatant before stimulation with PHA ranged form 10.31 to 40.18 pg/ml with a mean value of 18.03±1.3 pg/ml. It significantly increased (P=0.000) after stimulation with PHA to range from 13.08 to 73.29 pg/ml with a mean value of 39.50 ± 3.7 pg/ml.

In the control group, IL-18 level in PBMCs culture supernatant before stimulation with PHA ranged form 2.48 to 10.66 pg/ml with a mean value of 7.11± 0.72 pg/ml. It significantly increased (P=0.000) after stimulation with PHA to range from 7.98 to 16.8 pg/ml with a mean value of 12.57 ± 0.74 pg/ml.

A significant increase was observed in the mean value of IL-18 in SLE patients compared to the normal control group before (p=0.000) stimulation and after (P=0.001) stimulation with PHA (Table 10, Fig. 15).

Table (10): The mean value of IL-18 level (pg/ml) in PBMCs culture supernatant of SLE patient and normal control groups before and after stimulation with PHA.

		Control group n =15	Patient group n = 30	Test of significant
Before stimulation	Range	2.48-10.66	10.31-40.18	t = 5.496* p = 0.000
	Mean	7.11	18.03	
	SE	0.72	1.3	
After stimulation	Range	7.98-16.8	13.08-73.29	
	Mean	12.57	39.50	t = 4.106* p = 0.001
	SE	0.74	3.7	
Paired t test		24.09*	7.479*	
P		(0.000)	(0.000)	

*: p is significant at the level < 0.05

t: Comparison between the mean value of IL-18 in PBMCs of SLE patient & normal control groups before and after stimulation with PHA.

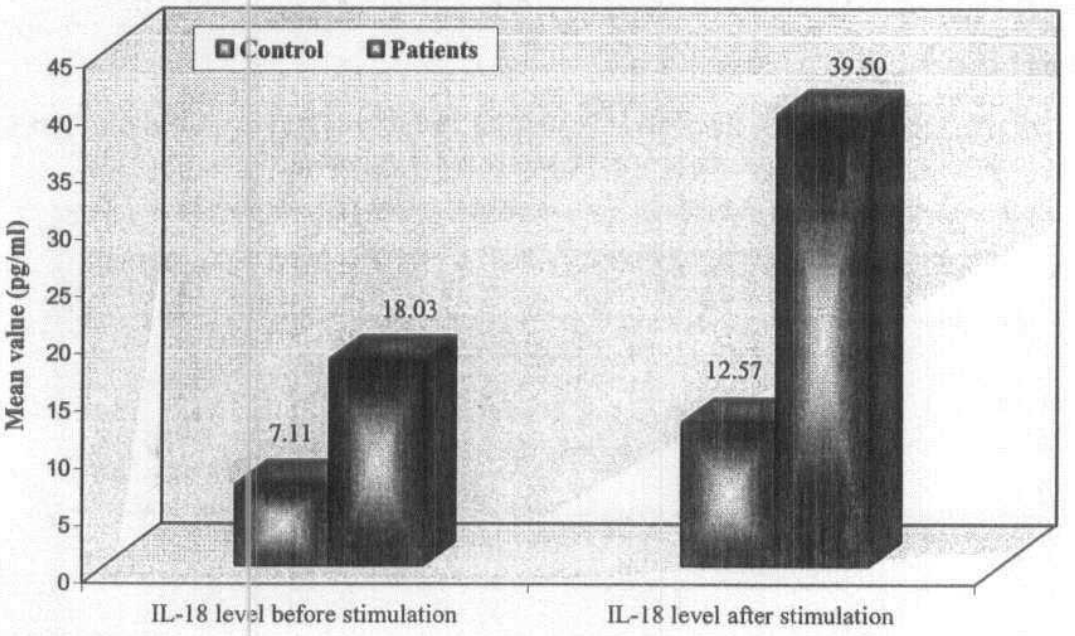

Figure (15): The mean values of IL-18 level in PBMCs of SLE and normal control groups before and after stiumlation with PHA.

3- Serum C_3 level

Serum C_3 level of SLE patient group varied from 22.40 to 147g/l with a mean value of 72.55±6.29 g/l, whereas serum C_3 level of normal control group varied from 92.40 to 174 g/l with a mean value of 126.96±6.02 g/l. A significant increase was observed in the mean value of serum C_3 level in normal control group than that of SLE patient (p=0.000) (Table 11, Fig. 16).

Table (11):Serum C3 level in SLE patient and normal control group.

		Control group n =15	Patient group n = 30	Test of significant
C3 (g/l)	Range	92.40-174	22.40-147	t = 6.925* p = 0.000
	Mean	126.96	72.55	
	SE	6.02	6.29	

*: p is significant at the level < 0.05
t: Comparison between the mean values of serum C3 level in SLE and normal control groups.

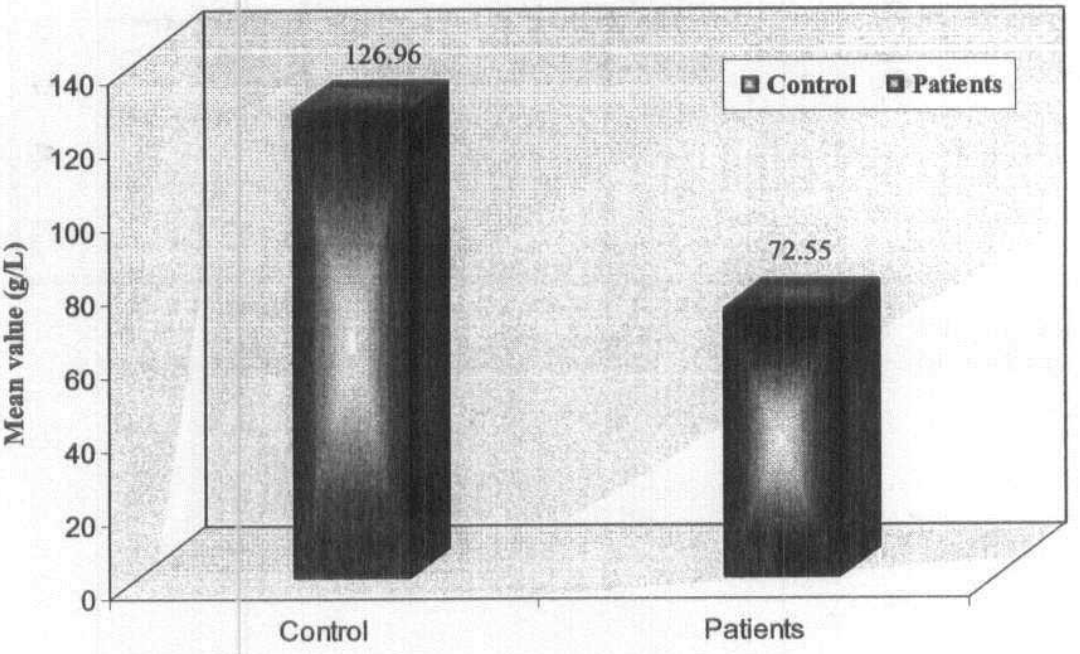

Figure (16): The mean values of serum C_3 level in SLE patients and normal control group

4- Serum C_4 level

Serum C_4 level of SLE patient group varied from 4.50 to 39 g/l with a mean value of 15.54 ± 1.7 g/l, whereas serum C_4 level of normal control group varied from 17.60 to 41.60 g/l with a mean value of 31.06 ± 2.23 g/l. A significant increase was observed in the mean value of serum C_4 level in normal control group than that of SLE patient group (p=0.000) (Table 12, Fig. 17).

Table (12): Serum C4 level in SLE patient and normal control groups.

		Control group n =15	Patients group n = 30	Test of significant
C4g/l	Range	17.60-41.60	4.50-39	t = 6.925* p = 0.000
	Mean	31.06	15.54	
	SE	2.23	1.7	

*: p is significant at the level < 0.05
t : Comparison between the mean values of serum C4 level in SLE and normal control groups.

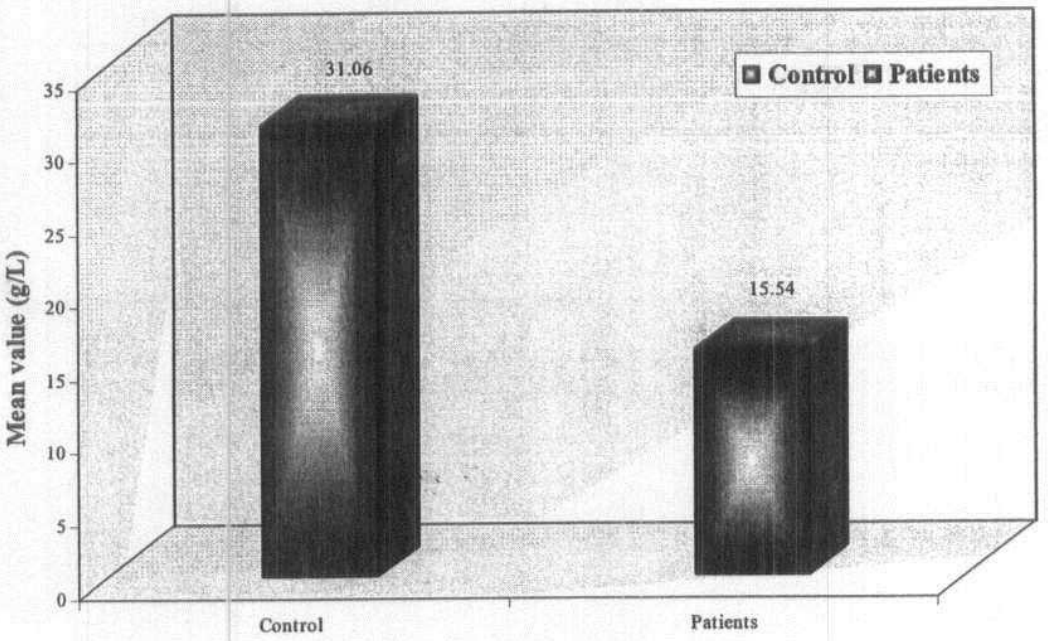

Figure (17): The mean values of serum C_4 level in SLE patients and normal control group

5- Comparison between serum C_3 and C_4 levels in SLE patient and normal control groups

The comparison between the mean value of serum C_3 and C4 levels is shown in table (13). There is a highly significant increase in the mean value of serum C_3 more than C4 level in SLE patient and normal control groups (p=0.000 for control and patient groups) (Table 13, Fig. 18).

Table (13):The mean value of serum C3 and C4 levels (g/l) in SLE patient and normal control groups.

		Control group n =15	Patients group n = 30	Test of significant
C3	Range	92.40-174	22.40-147	t =6.925* p =0.000
	Mean	126.96	72.55	
	SE	6.02	6.29	
C4	Range	17.60-41.60	4.50-39	t =5.081* p =0.000
	Mean	31.06	15.54	
	SE	2.23	1.7	
t test		14.4*	11.16*	
P		0.000	0.000	

*: p is significant at the level < 0.05

t : Comparison between the mean values of serum C3 and C4 levels in SLE patient and normal control groups.

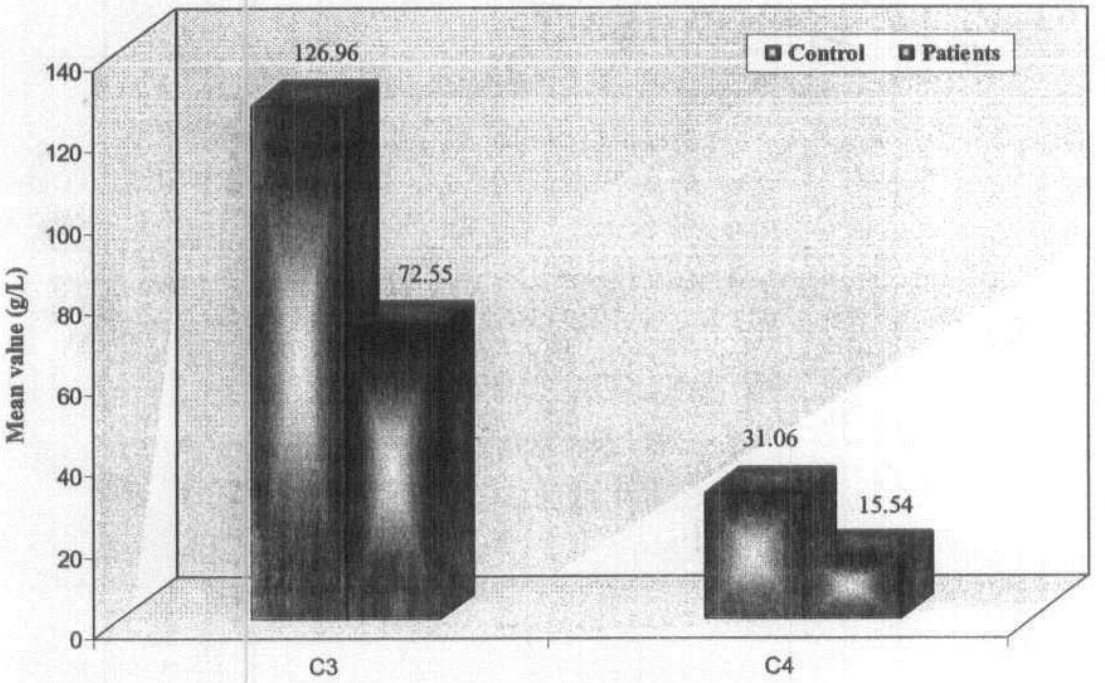

Figure (18): The mean values of serum C_3 and C_4 levels in SLE patient and normal control groups

4- CORRELATION ANALYSIS

The correlation studies were carried out between serum C3, C4 levels and IL-10, IL-18 levels in PBMCs before and after stimulation with PHA in SLE and normal control groups.

A-Correlation studies in SLE group

Table (14) shows the correlation between serum C3, C4 level and IL-10, IL-18 levels in PBMCs before and after stimulation with PHA in SLE group. It was found that there was a significant positive correlation between serum C3 and C4 levels (r=0.767, p=0.000) (Fig. 19). On the other hand, there was an insignificant negative correlation between C3 level and IL-10, IL-18 before and after stimulation with PHA. Serum C4 level also showed insignificant negative correlation with IL-18 before and after stimulation, while serum C4 level and IL-10 give insignificant positive correlation.

There was a significant positive correlation between SLEDAI and IL-10 level in PBMCs before(r=0.120,p=0.000) and after stimulation (r=0.182,p=0.000) with PHA (Fig. 20,21).

There was a significant positive correlation between SLEDAI and IL-18 level in PBMCs before(r=0.449, p=0.000) and after stimulation (r=0.0.553, p=0.000) with PHA (Fig. 22, 23).

Significant negative correlations were observed between serum C3, C4 levels and SLEDAI(r=-0.713, p=0.000 for C3& r=-0.670, p=0.000 for C4) (Fig. 24, 25).

B- Correlation studies in normal control group

There is an intermediate indirect correlation between all parameters that is statistically insignificant in normal control group (Table 15).

Table (14): Correlation analysis between serum C3, C4 levels, SLE disease activity index and IL-10, IL-18 levels in PBMCs before and after stimulation with PHA in SLE group.

		Activity index	C_3	C_4	IL-10 before	IL-10 After
C_3	r	-0.713				
	p	0.000*				
C_4	r	-0.670	0.767			
	p	0.000*	0.000*			
IL-10 Before	r	0.891	-0.056	0.050		
	p	0.000*	0.770	0.795		
IL-10 After	r	0.987	-0.208	0.170		
	p	0.000*	0.278	0.379		
IL-18 Before	r	0.729	-0.253	-0.248	0.125	0.146
	p	0.000*	0.268	0.278	0.511	0.441
IL-18 After	r	0.984	-0.345	-0.241	-0.140	-0.105
	p	0.000*	0.125	0.292	0.460	0.580

*: p is significant at the level < 0.05

Table (15): Correlation analysis between serum C3, C4 levels, SLE disease activity index and IL-10, IL-18 levels in PBMCs before and after stimulation with PHA in control group.

		C_3	C_4	IL-10 before	IL-10 After
C_3	r				
	p				
C_4	r	-0.118			
	p	0.677			
IL-10	r	-0.270	0.027		
Before	p	0.334	0.924		
IL-10	r	-0.188	-0.106	0.911**	
After	p	0.502	0.706	0.000	
IL-18	r	0.228	-0.077	-0.164	-0.164
Before	p	0.430	0.786	0.560	0.560
IL-18	r	0.263	-0.013	-0.275	-0.258
After	p	0.363	0.966	0.321	0.352

*: p is significant at the level < 0.05

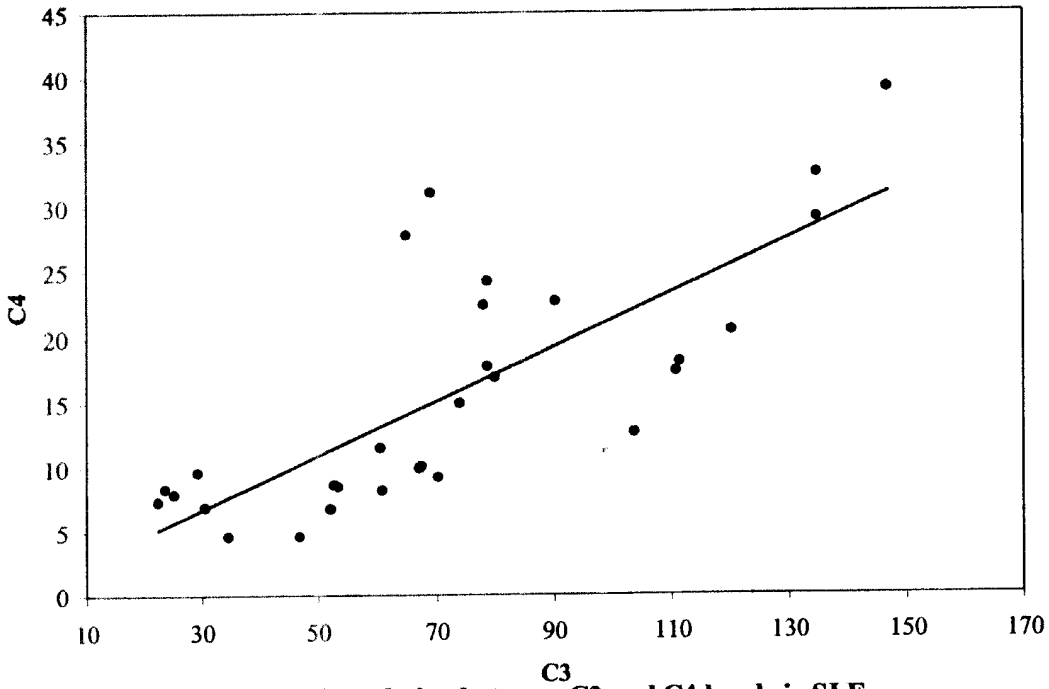

Figure (19) : Correlation between C3 and C4 levels in SLE patient group

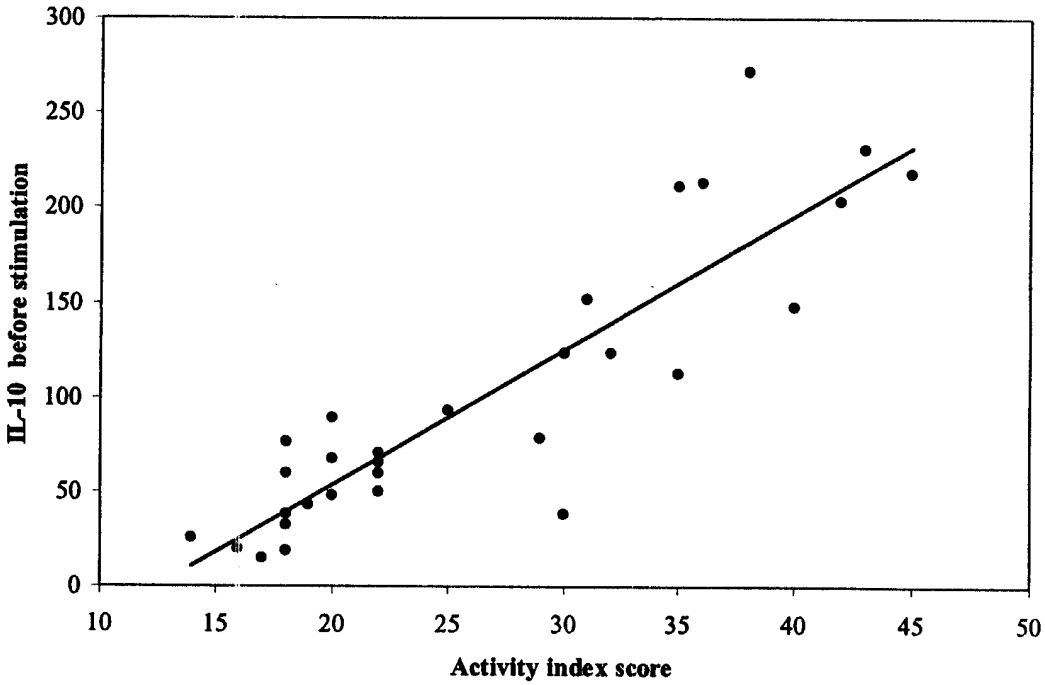

Figure (20) : Correlation between activity index score and IL10 level in PBMCs culture supernatant before stimulation in SLE patient group

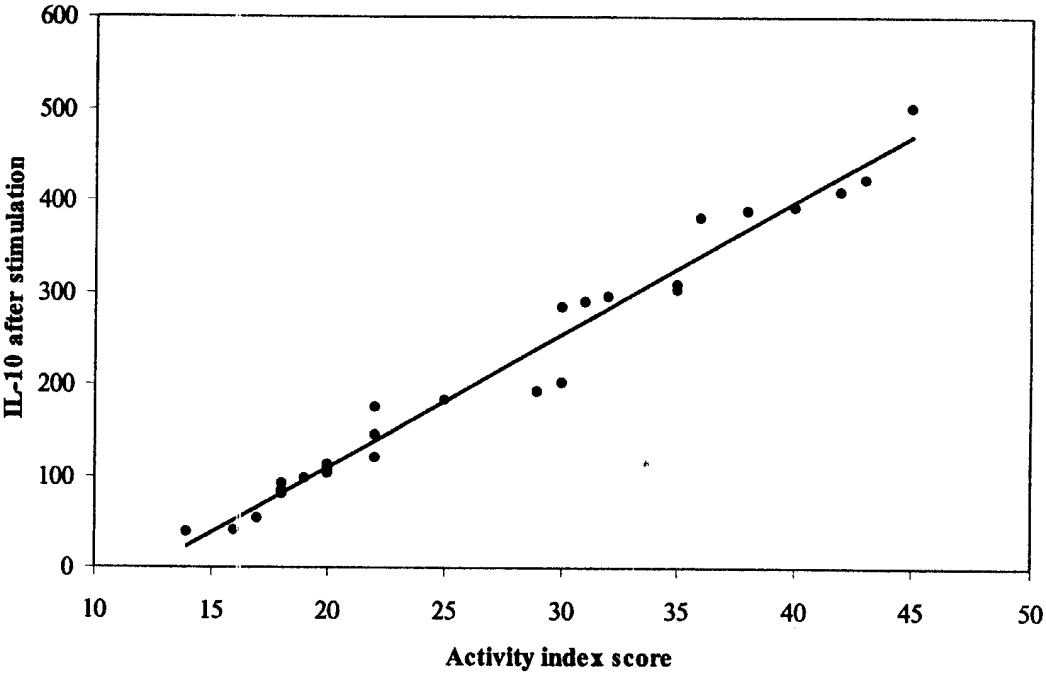

Figure (21) : Correlation between activity index score and IL-10 level in PBMCs culture supernatant after stimulation in SLE patient group

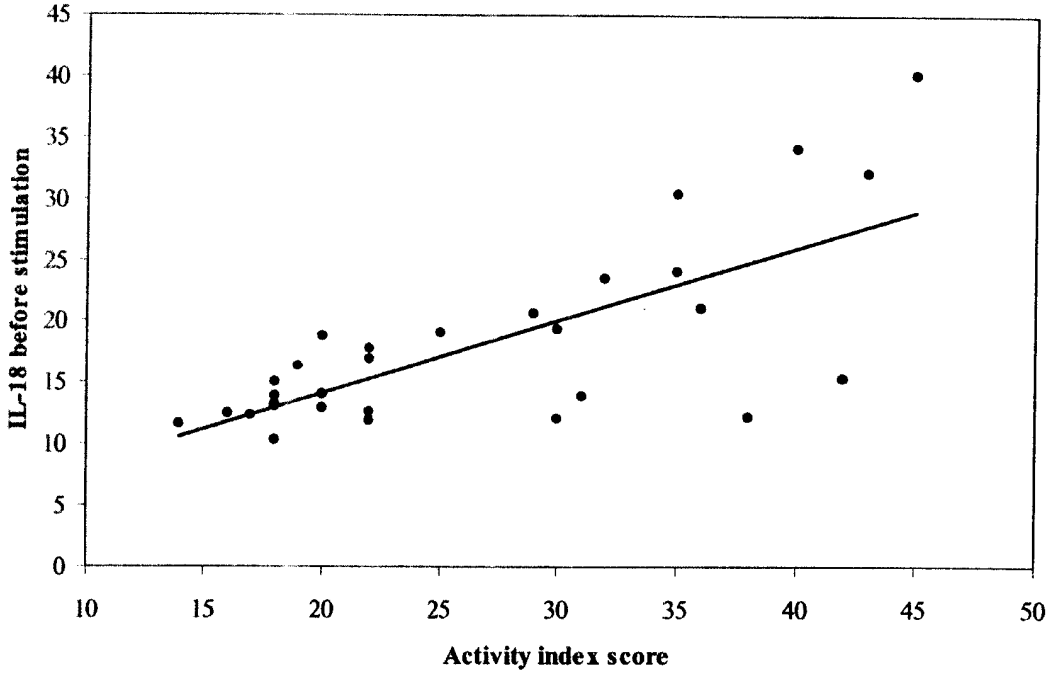

Figure (22) : Correlation between activity index score and IL-18 level in PBMCs culture supernatant before stimulation in SLE patient group

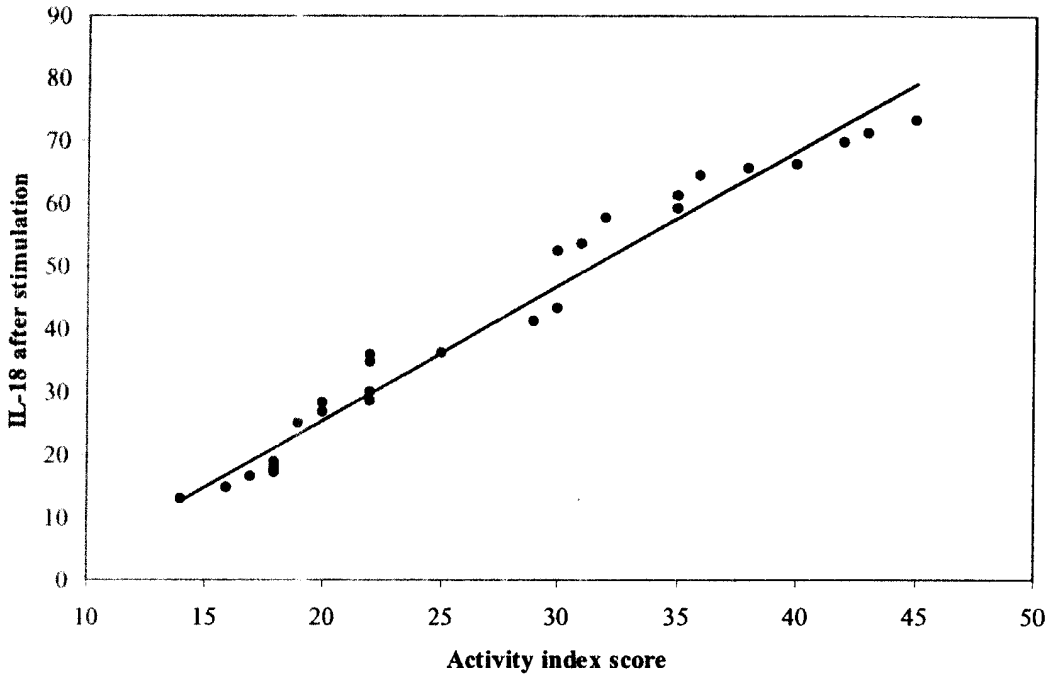

Figure (23) : Correlation between activity index score and IL-18 level in PBMCs culture supernatant after stimulation in SLE patient

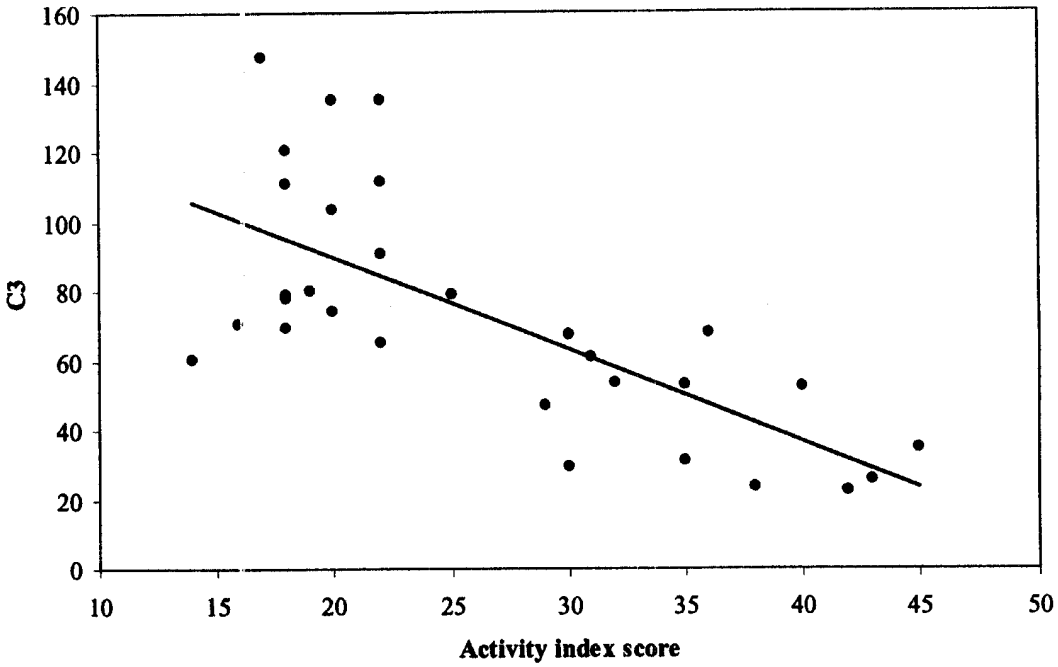

Figure (24) : Correlation between activity index score and serum level of C3 in SLE patient group

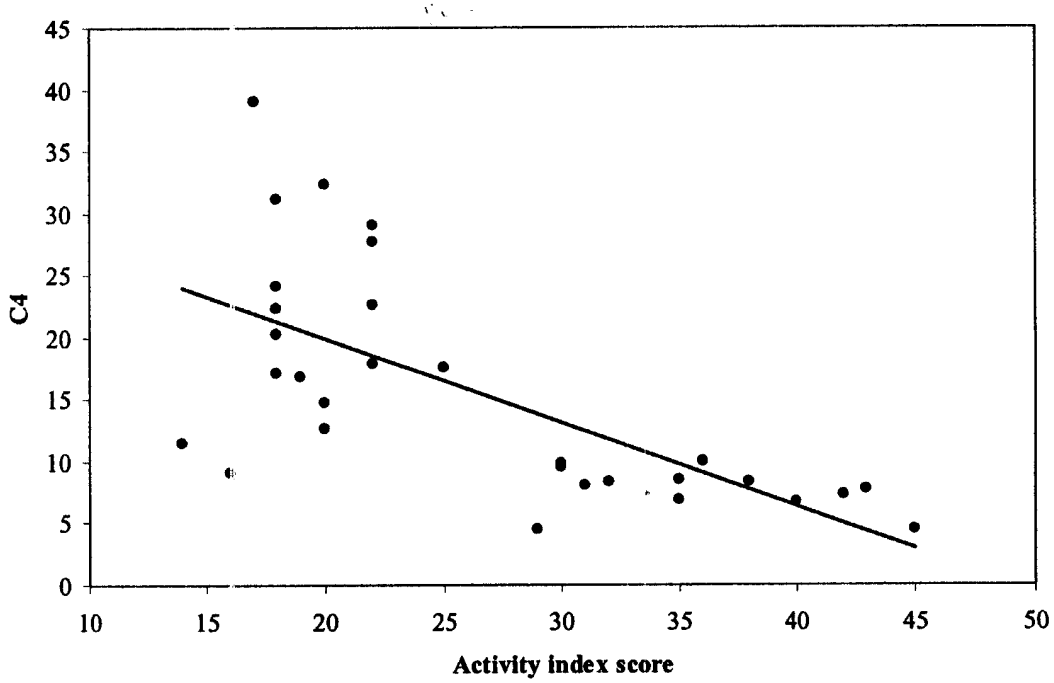

Figure (25) : Correlation between activity index score and serum level of C4 in SLE patient group


Discussion

DISCUSSION

Systemic lupus erythematosus is a disorder of immune regulation manifested by polyclonal B cell activation with autoantibodies and immune complexes that produce tissue damage. The pathogenesis of SLE is a multi-factorial with multiple susceptibility genes and environmental factors involved in its initiation. [1,163]

The complex cytokine network regulates T and B cell activation, differentiation and effector functions. These cytokines can be grouped into two broad categories, the Th1-type cytokines including IL-12, IL-2, INF-γ, IL-15 and IL-18. Th1 cytokines preferentially induce cell-mediated immunity, whereas the Th2-type cytokines including IL-4, IL-5, IL-6, and IL-10 primarily support antibody production or humoral immunity. [164,165] Imbalance between Th1 and Th2 cytokine production play a key role in the induction and development of several autoimmune diseases such as SLE.[164,166]

Serum levels of Th2 cytokines such as IL-4, IL-6 and IL-10 were found to be elevated with a decrease in the production of Th1 cytokines in many cases of SLE. Some reports showed an increase in the level of Th1 cytokines first followed later by the induction of Th2-type cytokines. [164,166] Such imbalance of Th cytokines may account for the polyclonal B-cell activation observed in SLE. [151]

Therefore, the aim of the present study was to investigate the relationship between IL-10 and IL-18 (as an example of Th2 and Th1 cytokines respectively) in SLE patients by measuring the levels of those cytokines in PBMCs culture supernatant of thirty SLE patients and fifteen age and sex matched healthy individuals. Correlation between these parameters and disease activity (SLE disease activity index) has been carried out.

Laboratory investigations including ESR, C- reactive protein, ANA and anti-ds-DNA antibodies (table 8) revealed that ESR and C-reactive protein elevated in SLE patient group. These data, reflecting a state of inflammation may be due to the immune complex deposition or the defective clearance of apoptotic cells by macrophages. Vibeke (2007)[167] found that the increased ESR, C-reactive protein, ANA and anti-ds- DNA antibodies detected in SLE patient group may be due to several mechanisms such as an excessive and uncontrolled differentiation and activation of autoantibodies-forming cells by T-cell help, defective CD8+ suppressive T cells and NK cells, elevated plasma level of cytokines such as IL-6 and IL-10, and decreased level of TNF-α and IL-1 which in turn impaired the removal of circulating autoantigens. [167]

Serum levels of C3 and C4 were measured in current study as indication of SLE disease activity. Serum levels of C3 and C4 were significantly increased in normal control group than that of SLE patient group ($p=0.000$) and a significant negative correlation between serum levels of C3 and C4 and disease activity index score ($p=0.000$ $r=-0.713$ for C3 and $p=0.000$ $r=-0.670$ for C4). Complement has a big part to play in the autoimmune processes in lupus. In SLE continued complement activity due to immune complexes causes damage via the membrane attack complex, promotion of phagocyte adhesion and activation (releasing an array of inflammatory mediators and cytokines) and promotes blood clot formation. [168] Deficiencies in the earlier parts of the cascade are associated with a reduced ability to remove the immune complexes generated in lupus, and so are often seen in lupus nephritis and vasculitis. [169] Low concentrations of complement components due to increased catabolism are found in a majority of patients with active and severe SLE.[170]

Sturfelt et al, (2005) showed that the decrease in C3 and C4 concentrations may be due to hypercatabolism which may result from deficiency of the alternative pathway regulatory proteins such as factor H and factor I. These regulatory proteins are responsible for downregulation of C3-convertase. [169] Also, the decreased C3 concentration may be due to reduced levels of complement receptor 1(CR1) or due to production of autoantibodies directed against C3 and C3 convertase. [169,171]

Interleukin -10 was first described as cytokine synthesis inhibitory factor (CSIF), an activity produced by mouse Th2 cells that inhibited activation and cytokine production by Th1 cell. [172] The ability of IL-10 to inhibit cytokine and nitric oxide production by both T cells and NK cells and expression of class II MHC [173] were found to be indirect via inhibition of accessory cell (macrophage/monocyte) function. [174]

Inflammatory bowel disease and other exaggerated inflammatory responses exhibited by IL-10 deficiency might indicate that a critical *in vivo* function of IL-10 is to limit inflammatory responses. [175]

In the current study, levels of IL-10 in PBMCs culture supernatant in SLE patients were significantly higher than those of controls both before and after stimulation with PHA ($p = 0.000$, $p = 0.000$) which is in consistent with many previous reports. [176-190]

Gröndal et al, (1999) detected an increased number of IL-10 producing cells in SLE patients and their family members in Icelandic population. [99]

Vilard et al, (1999) [75] reported that SLE is a disease characterized by an abnormally high production of IL-10. SLE patients exhibited an increased spontaneous synthesis of IL-10 by PBMCs compared with healthy controls. They demonstrated that the high IL-10 secretion by monocytes and lymphocytes was responsible for the heightened immunoglobulin production. In agreement with these observations, IL-10 was also found to be enhanced in patients with rheumatoid arthritis or Sjögren's syndrome; two disorders characterized by prominent B lymphocyte hyperactivity which results in increased production of immunoglobulins and the synthesis of autoantibodies. [75]

Several reports have suggested that IL-10 plays a role in the pathogenesis of SLE. Fiorentino et al, (1991) [121] firstly described IL-10 as a cytokine able to alter the balance of murine Th1/Th2 cell activity in favor of the Th2 type response. [121] IL-10 also appears to encourage lupus autoimmunity through its potent stimulation of B cell proliferation and differentiation. [103]

In animal models, immune complexes can stimulate the production of IL-10, thus shifting the immune response from a type 1 to type 2 patterns. [176] The possibility therefore exists that SLE immune complexes obtained *in vivo* also might exhibit type 2 cytokine inducing effect by stimulating macrophage production of IL-10 and IL-6. These cytokines might then further stimulate antibody production and subsequent immune complex formation. [176]

Also *in vitro* studies have suggested that hypergammaglobulinaemia in SLE is IL-10 dependent. [177] IL-10 enhances expression of MHC class II antigens and survival of resting mouse B cells [178] that may be an important mechanism for sustaining the production of pathogenic autoantibodies in SLE. [27]

Interestingly, Wu et al, (2002) [179] reported that synergistic effect may exist between IL-10 and bcl-2 genotypes in determining susceptibility to SLE. [179] Also IL-10 can function as a growth factor for γδ-TCR phenotype cells [180] , which give help for autoantibody production. [181]

Rönnelid et al, (2003) [182] suggested that the IL-10 effect is at least partially mediated through FCγRΠ, an immunocomplex binding receptor that has been implicated in SLE pathogenesis. So, B cell activation, antibody production, immunocomplex stimulated monocytes and type 2 cytokines create a vicious cycle that may help to maintain B cell hyperactivity in SLE. [182,191]

The most important critical aspect of the properties of IL-10 that would induce lupus autoimmunity is its ability to diminish macrophage activation and antigen presentation thereby directly and indirectly inhibiting T cell function. [183]

Filaci et al, (2001) [184] demonstrated that the functional activity of CD8+T suppressor cell is impaired in SLE patients with active disease. This alteration could be related to the abnormal cytokine secretion pattern of these cells. IL-10 inhibits generation and maturation of highly efficient APC that secretes IL-12, which induces differentiation of naive T cells to Th1 cells, responsible for production of CD8+T suppressor cells. [185] Also, high levels of IL-10 in patients with active SLE suppress TGF-β production by NK cells [94], which suppress B lymphocyte secretion of IgG via costimulation of CD8+ cells with IL-2. [94]

SLE patients have been reported to have both increased apoptosis and reduced clearance of apoptotic cell material. [186] Georgescu et al, (1997)[187] suggested that the increased apoptosis of SLE lymphocyte *in vitro*, is in part, due to effect of IL-10 and is mediated by FasL. [187] On the other hand IL-10 has been reported to protect B cells from apoptosis via stimulation of increased expression of the anti-apoptotic bcl-2. [171,188]

IL-10 also inhibits monocyte production of IL-12, a cytokine that has been reported to protect T cell from activation induced cell death (AICD).[189] Further support for the precise role of IL-10 in the pathogenesis of lupus comes from the finding that, continuous administration of anti-IL-10 delays onset of autoimmunity in NZW×NZB mice, an effect perhaps mediated by up regulation of TNF-α production.[190] Moreover, it has been reported that anti-IL-10 administration to six human lupus patients with active disease, and dependent upon steroids for treatment, led to a reduction in disease activity. [110]

Our result also revealed that, there was a significant positive correlation between IL-10 level in PBMCs culture supernatant of SLE patients and disease activity as shown in disease activity index. This result is in agreement with that of Lacki et al, (1997) [192] who reported that levels of IL-10 in SLE patients are significantly higher and there is a correlation of IL-10 levels with the clinical disease activity. [192]

Hagiwara et al, (1996) [39] suggested that disease severity is correlated with increased IL-10 secreting cells in peripheral blood of SLE patients. [39]

An *in vitro* study of Hagiwara et al, (1996) [39] has also indicated that the production ratios of IL-10/IL-2 and IL-10/IFN-γ in stimulated blood mononuclear cells were positively correlated with SLE disease activity. [39]

The disease severity in lupus patients was reported to correlate with an elevated ratio of cells secreting IL-10: IFN-γ and the number of cells secreting IFN-γ diminished with IL-10 administration in vivo. [177] These data showed that the expression of these cytokines might be coordinately regulated. As IL-10 is involved in the regulation of cytokine network, IL10 could not fully affect humoral immune activity in SLE. [177]

Tyrell et al (2001) [193] suggested that the effect of IL-10 on antibody production from peripheral blood mononuclear cells depends on the disease activity at the time of sampling. [193] There is also evidence that serum levels of particular cytokines may be associated with increased susceptibility to particular complications of SLE. [39, 99] IL-18 is a member of the IL-1 cytokine family that proved to play an important role in a number of autoimmune disease including (Rheumatoid Arthritis) RA, SLE and multiple sclerosis. [194] IL-18 is an important regulatory of the innate and acquired immune responses. It induces proliferation, cytotoxicity and cytokine production by Th1 and NK cells primarily in synergy with IL-12. [144]

In the current study, the mean values of IL-18 level in PBMCs culture supernatant before and after stimulation with PHA were significantly increased in SLE patients than those of normal control group (P=0.000, P=0.000).

This finding is in agreement with Esfandiari (2001) [195] who reported that SLE patients have higher levels of IL-18 than normal controls which is related to proinflammatory properties of IL-18. [195,196]

A proven role of IL-18 in autoimmune diseases was shown by the increased expression and potential proinflammatory activities of IL-18 in synovial tissues of inflammatory arthritis. [197] It induces the production of other proinflammatory cytokines, especially TNF-α in synergy with IL-15 and IL-12 amongst other cytokines. It may also amplify TNF-α release by enhancing cell-cell interactions between synovial T cells and macrophages. [194]

Leung, (2000) [198] explained that IL-18 is identified as a critical regulatory factor in the evaluation of Th1 immune responses because its role is to induce and promote IFN-γ from Th1 cell. [198] IL-18 also induces the expression of the Th2 cytokines IL-5and IL-13 but not IL-10. [197]

Dinarello (2000) [199] reported that in combination with other proinflammatory cytokines (like IL-12, IL-1 and TNF-α) IL-18 must be an important cytokine for initiating and progressing the catabolic response and fever in SLE. [199]

Dean et al, (2000)[74] reported in their review of cytokine patterns in SLE that lupus renal disease is associated with cytokine imbalance with an increase in proinflammatory cytokines especially IL-6 and IFN-γ. They also found a decrease in anti-inflammatory cytokines such as TGF-β, with an associated increase in TNF-α, IL-1Ra, IL-4 and IL-10. Accordingly, elevation of the proinflammatory IFN-γ inducing IL-18 is expected in lupus nephritis patients. [74]

Joosten et al, (2003) [200] found that IL-18 plays a role in the induction and perpetuation of chronic inflammatory synovitis.[200] Furthermore, Tomita et al, (2001)[201] found that overexpression of proinflammatory cytokines has been associated with neurodegeneration in lupus, and that proinflammatory cytokines are potential pathogenic factor in cerebellar disturbances in central nervous system. [201]

The current study found that, there was a significant positive correlation between IL-18 level in PBMCs culture supernatant and SLE disease activity as shown in the Disease Activity Index.

Our findings are in accordance with those obtained by Park et al, (2004) [202] who compared the changes of the IL-18 level and those of parameters reflecting the disease activity between the active stage and the stable stage of the disease. They found that the changes in IL-18 level correlated significantly with the changes of SLE disease activity.[202]

Wong et al, (2000) [151] showed that the circulating IL-18 concentration is significantly elevated in SLE patients and correlates with SLE DAI score. This may be related to the initiation of the inflammation, fever and organ damage that is commonly observed in SLE patients. [151] This finding was explained by the fact that IL-18 can enhance the expression of Fas ligand in NK cells and cytotoxic T lymphocyte (CTL) causing Fas-mediated apoptosis in epithelial cells and enhancing active tissue damage. [151]

Sun et al, (2000)[203] reported that, Anti-ds DNA is known to possess a dual effect on normal human mononuclear cells, first to enhance the release of proinflammatory cytokines to augment the inflammatory reactions, second to polarize the immune reaction towards Th2 pathway. This unique effect of Anti-ds DNA may play a role in the pathogenesis of SLE by augmenting the inflammatory response and autoantibody production commonly found in active SLE. [203]

Esfandiari et al, (2001) [195] found that IL-18 can accelerate spontaneous autoimmune lupus disease in animal models. The co-administration of IL-12 and IL-18 was associated with more severe systemic pathology characterized by glomerulonephritis and vasculitis, whereas administration of IL-18 alone was associated with the development of cutaneous lesions of SLE especially malar rash. [195]

Results reported in the current study as well as in the previous matching studies highlight the importance of IL-18 in the initiation of inflammation and organ damage observed in SLE patients. IL-18 might be a potential pathogenic cytokine in SLE that correlates with disease activity in lupus patients.

Getahun et al, (2006) [204] explained that anti-IL-18 antibody treatment in EAN C57BL/6 mice may not neutralize all IL-18 effects, i.e. it does not deplete IL-18 producing cells, but may rather exert immunomodulatory or even immunostimulatory effects. [204]

Our study revealed that in normal control group as well as SLE patient group, both IL-10 and IL-18 levels in PBMCs culture supernatant before stimulation with PHA are significantly lower than that after stimulation ($p=0.000$) meaning that lymphocytes were still respond to effect of PHA which acts on DNA synthesis to stimulate proliferation of cells that leads to release of more cytokines.

Our results also indicated that, there was a significant increase in the mean value of IL-10 more than IL-18 before and after stimulation with PHA ($P=0.000$).

Although early reports described defective Th1 and excessive Th2 responses in lupus, previous data suggested that both Th1 and Th2 cytokines can be elevated in lupus patients, this indicating that SLE is a complex disease driven by activation of different lymphokine systems at different time points, possibly explaining the heterogeneity of clinical manifestations. [58]

Zediak et al, (2003) [205] reported that, although IL-10 plays a role in inhibition of proinflammatory cytokines, it failed to inhibit production of IL-18 .One possible explanation is that during an immune response, IL-10 and IL-18 are cytokines that need to be coexpressed. This explanation is supported by previous studies reported that IL-10 and IL-18 synergize to enhance NK cell proliferation, cytotoxicity and INF-γ production. [205]

Also, IL-10 may synergize with IL-18 to potentiate Th1 responses a lower intensity than induced by IL-12 plus IL-18, in order to dampen the inflammatory response without abolishing it. [58]These discrepancies imply that Th cytokine response in SLE is very complex and requires further investigations. [119]

The use of cytokine antagonists like IL-18Bp which is a soluble inhibitory receptor for IL-18 or the use of other immunoregulatory cytokines represents a promising therapy for SLE and other diseases in which IL-18, IFN-γ and other proinflammatory cytokines represent potential pathogenic mediators. [72]

Summary

SUMMARY

Systemic lupus erythematosus is an autoimmune disease with variable clinical features in which immune dysregulation such as defective T cell functions and over autoantibodies production is a key feature in the pathogenesis.

The aim of the present work was to reveal the relationship of IL-10 and IL-18 production in SLE patients in relation to disease activity.

The study was conducted on 30 SLE patients collected from department of Medical Research Institute Alexandria University and 15 age and sex matched healthy individuals as a group of control.

The levels of IL-10 and IL-18 in PBMCs culture supernatant samples were detected with and without stimulation with PHA using ELISA technique.Correlations between these parameter and disease activity (SLE Disease Activity Index) were also investigated.

Our results revealed a significant negative correlation between serum levels of both C3&C4 and disease activity index score ($p=0.000$ $r=-0.713$ for C3 and $p=0.000$ $r=-0.670$ for C4). The decrease in C3 and C4 concentrations seemed to be due to hypercatabolism, which may result from deficiency of the alternative pathway regulatory proteins such as factor H and factor I.

The result of the present study also revealed that the levels of both IL-10 and IL-18 in the PBMCs culture supernatant were significantly increased in SLE patient group than normal controls before and after stimulation with PHA ($P=0.000$, $P=0.000$ before stimulation, $p=0.001$, $P=0.000$ after stimulation for IL-10 and IL-18 respectively). Our results also indicated that, there was a significant increase in the mean value of IL-10 more than IL-18 before and after stimulation with PHA ($P=0.000$). The disturbance of IL-10 and IL-18 production may be responsible for the increased apoptosis of lymphocytes and reduced clearance of apoptotic cell materials in SLE patients.

There were significant positive correlations between each of IL-10 levels and IL-18 levels in the culture supernatant before and after stimulation with PHA and disease activity index score ($p=0.000$ $r=0.891$, $p=0.000$ $r=0.729$ before stimulation, $p=0.000$ $r=0.987$, $p=0.000$ $r=0.984$ after stimulation for IL-10 and IL-18 respectively).

These findings clearly indicated the role of IL-10 in the pathogenesis of SLE. This role could be through alteration of Th1/Th2 cell activities in favor of the Th2 type response and stimulation of B cell activation and antibody formation. In addition, IL-10 would induce lupus autoimmunity via its ability to diminish macrophage activation and antigen-presentation, thereby directly and indirectly inhibiting T cell function.

Regarding IL-18, our data indicate that the role of IL-18 in the pathogenesis of SLE could be through promotion of Th1 cell development and activation which augment type 1 cytokine production. The elevated circulating IL-18 concentration may be related to the initiation of the inflammation, fever and organ damage commonly observed in SLE patients.

The use of cytokine antagonist like IL-18bp or the use of other immunoregulatory cytokines may represent a promising therapy for SLE.

Conclusion and Recommendations

CONCLUSION AND RECOMMENDATIONS

- Identification of the causes of uncontrolled IL-18 production and activity in autoimmunity would allow for novel therapeutic targets to effectively block autoimmune activation and inhibit concomitant tissue damage in autoimmune diseases including SLE.
- IL-10 plays an important role in down-modulating lupus through inhibition of inflammatory cytokine production that may have significant implications for the pathogenesis and treatment of human SLE.
- IL-10 and IL-18 play a critical role in both cellular and humoral immunity by participating in keeping the balance between Th1 and Th2 activities and between inflammatory and anti-inflammatory responses.
- Further studies on larger population are recommended for more understanding of the precise mechanism through which IL-10 and IL-18 are involved in the pathogenesis of SLE disease that could result in new therapeutic approaches for preventing and/or reversing the immune alteration in SLE patients.

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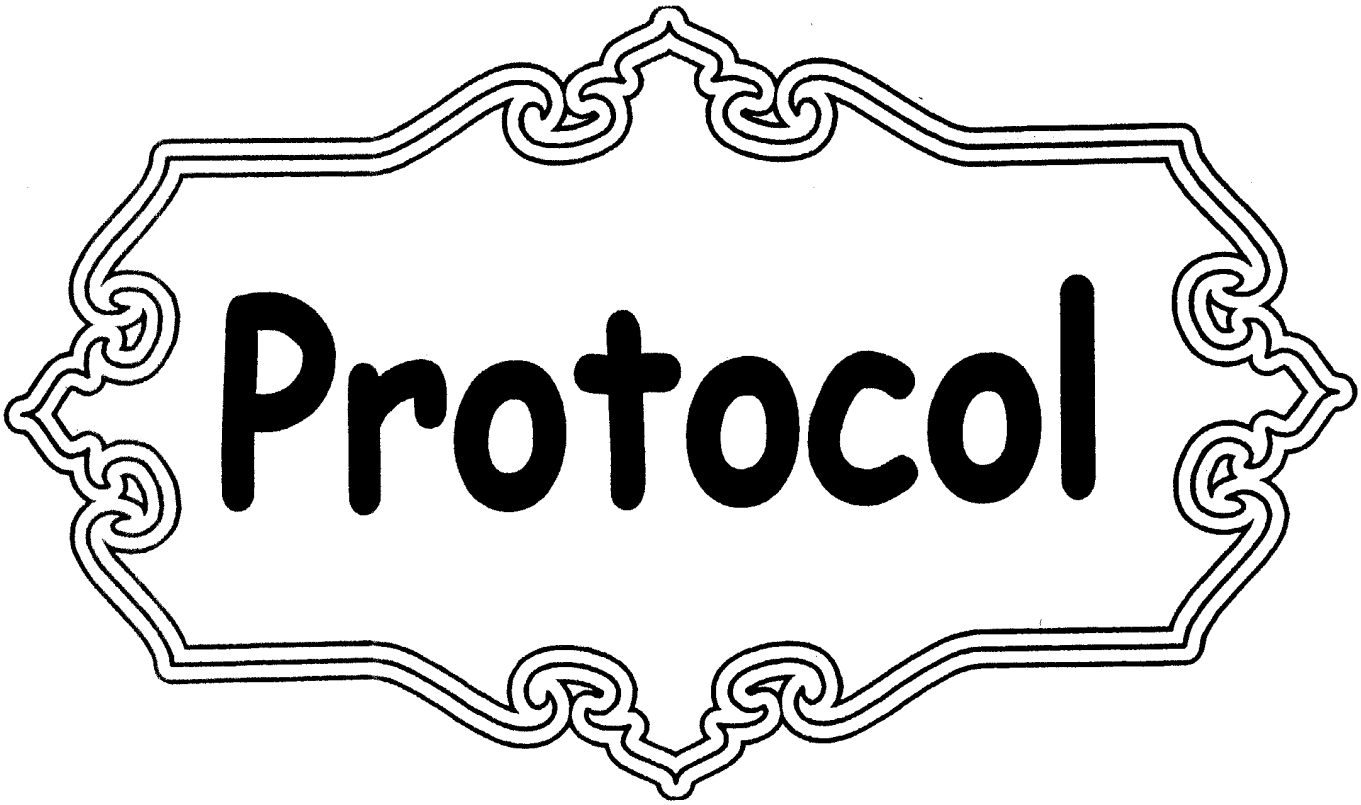
Protocol

بسم الله الرحمن الرحيم

STUDY OF INTERLEUKIN-18 (IL-18) AND INTERLEUKIN-10 (IL-10) PRODUCTION IN CORRELATION WITH DISEASE ACTIVITY IN SYSTEMIC LUPUS ERYTHEMATOSUS

دراسة إنتاج الإنترليوكين-١٨ والإنترليوكين-١٠ فى حالات الذئبة الحمراء وعلاقتهما بنشاط المرض

Protocol of a Thesis Submitted to
Medical Research Institute
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Master Degree

In

Immunology

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2003

خطة بحث مقدمة إلى
معهد البحوث الطبية
جامعة الإسكندرية
إيفاءً جزئيا للحصول على

درجـــة الماجستير

فـــى

المنـــاعـــة

من

منى محمد عبد العظيم صالح
بكالوريوس علوم
قسم بيولوجى
جامعـــة طنطا ــ ١٩٩٨

قسم المنـــاعـــة
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IL-18 that was originally termed IFN-γ inducing factor has been characterized as a multifunctional inflammatory cytokine. The role of IL-18 have been described in both physiological and pathological processes. As an immuno-potentiating factor, it has the capacity to induce IFN-γ production by T-Cells and natural killer cells.[9-12,13]

Interleukin-10 (IL-10) is a Th2 cytokine that is up-regulated in patients with SLE. IL-10 is produced by various cell types including B-lymphocytes and monocytes.[14] IL-10 is a potent stimulator of B-lymphocytes, promoting B-lymphocyte activation, proliferation, differentiation and autoantibody production.[15-17]

IL-10 has been recognized as an inhibitory cytokine, suppressing the production of pro-inflammatory cytokines as well as inhibiting cell-mediated immunity by antigen-presenting cells and T lymphocytes.[15-17]

In experimental mice with SLE, two stages of T-cell activation and cytokine secretion have been noticed; expression of Th1-derived cytokine first, followed by induction of Th2-derived cytokine.[10-12] Th1-derived cytokines are found to be regulated mainly by IL-18,[4] while Th2-derived cytokines are regulated by IL-10.[18] Therefore, it is possible that IL-18 could be responsible for an abnormality of Th1-derived cytokines in SLE patients.

AIM OF THE WORK

The aim of the present study is designed to reveal the relationship of IL-10 and IL-18 production in patients with systemic lupus erythematosus in relation to disease activity.

MATERIAL AND METHODS

- Thirty patients suffering from systemic lupus erythematosus, diagnosed according to the Systemic Lupus Activity Measure (SLAM) criteria,[19] will be conducted in the study as well as fifteen age and sex matched normal healthy individuals as a group of control.
- Patients will be recruited from the Department of Internal Medicine, Medical Research Institute Alexandria university.

The exclusion criteria include:

1- Patients will be chosen free from any other chronic disease.

2- Patients will not be treated by azathioprine, cyclosporin A, cyclophosphamide or cortisone therapy for at least 6- months before blood sampling.

- All patients will be submitted to:
  1- History taking including age, durations and activity of lupus disease.
  2- Clinical examination with stress on systemic manifestation of chest, heart, kidneys, skin and central nervous system involvement.
  3- Urine analysis and kidney function test to diagnose lupus nephritis.

Immunological study

(1) Immunological laboratory investigations:

1- Test for anti-DNA IgG antibodies,[20] Anti-nuclear antibodies (ANA)[21], C-reactive protein (CRP)[22], erythrocyte sedimentation rate (ESR)[23] and anti-platelet.[24]

2- Determination of serum C_3 and C_4 by turbidimetry to diagnose lupus activity.[25]

(2) Measurment of IL-18 and IL-10 levels:

- Mononuclear cells will be isolated from peripheral blood samples taken from both patients and normal controi group.[26]
- The separated mononuclear cells will be cultured for 24 hours.
- The mononuclear cell culture supernatant will be stored at-70°C.
- IL-10 and IL-18 levels will be measured in the cell culture supernatant samples by Enzyme-linked Immunosorbent Assay (ELISA) technique.[26, 27]

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Arabic Summary

الملخص العربى

الملخص العربى

يعتبر مرض الذئبه الحمراء أحد أمراض المناعة الذاتية حيث يرجع الكثير من الأعراض المرضية الاكلينيكية إلى إضطراب وظائف الجهاز المناعى ومن أهمها إختلال وظائف الخلايا الليمفاوية (ت) والزيادة المطردة فى إنتاج الأجسام المضادة الذاتية.

ونظرا لأهميه دور الوسيط المناعى (السيتوكين) إنترليوكين-١٨ المنشط للخلايا (ت) المساعدة وكذلك الإنترليوكين-١٠ المثبط لنشاط هذة الخلايا. فقد كان هدف هذا البحث هو دراسة إنتاج هذين العاملين فى مرض الذئبة الحمراء و إيجاد العلاقة بينهما ودرجة نشاط المرض.

وقد إشتملت الدراسة على ثلاثين مريضة بالذئبة الحمراء من المترددات على عيادة الأمراض الباطنة بمعهد البحوث الطبية جامعة الأسكندرية إلى جانب خمسة عشر متطوعة من الأصحاء المتماثلات فى السن كمجموعة مقارنة وقد خضعت مجموعتى المريضات و المتطوعات للفحص الإكلينيكى و الإختبارات المعملية الخاصه بالمرض.

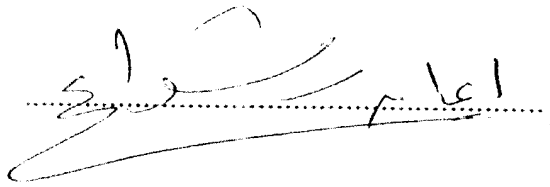
وقد تم جمع عينات الدم الطرفى لكل الأفراد تحت الدراسة و فصل الخلايا الاحاديه النواة و إستزراعها فى وجود العامل المحفز (الفيتوهيماجلوتينين) وبدونه لمدة ٢٤ ساعة. ثم قياس مستوى كلا من الإنترليوكين -١٠ و الإنترليوكين -١٨ فى مستخلص مزرعة هذة الخلايا.

وقد أسفرت النتائج عن زيادة ذات دلالة إحصائيه فى إنتاج كل من الإنترليوكين -١٠و الإنترليوكين -١٨ فى مجموعة المريضات عنها فى مجموعة المقارنة. كما أن إضافة الفيتوهيماجلوتينين لمزرعة خلايا الدم احاديه النواة ادت الى زيادة إحصائية فى إنتاج الإنترليوكين -١٠ و الإنترليوكين -١٨فى جميع العينات المستخدمة فى الدراسة من المجموعتين.

وقد أوضحت الدراسه أيضا أن هناك علاقة موجبة ذات دلالة إحصائية بين مستوى كل من الإنترليوكين -١٠ و الإنترليوكين -١٨ و معدل نشاط المرض.

وقد تبين من هذة الدراسة أهمية الدور الذى يقوم به كل من الإنترليوكين -١٠ و الإنترليوكين -١٨ فى إحداث التوازن بين نشاط الخلايا الليمفاوية المساعدة(ت) النوع-١ والنوع-٢ المنتجين لهما. وكذلك دورهما فى تنظيم التفاعلات الإلتهابية. وعلى هذا فإن دراسة العلاقات المركبة بين السيتوكينات بعضها و بعض على نطاق أوسع بغرض معرفة كيفية إعادة التوازن بين نشاط هذة الخلايا فى مرض الذئبة الحمراء تظل هى الهدف الرئيسى للحد من التفاعلات الإلتهابية فى هذا المرض. كما أن محاولة إستخدام أحد الوسيطين المناعيين تحت الدراسة أو كليهما فى منظومة علاج قد تكون ذات قيمة فى الحد من مضاعفات المرض والسيطرة عليه.

لجنة الإشراف



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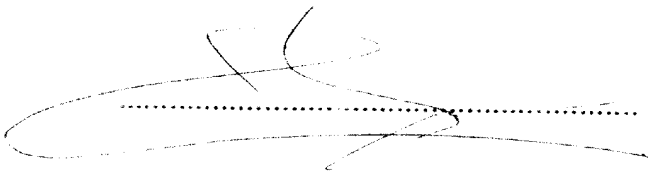

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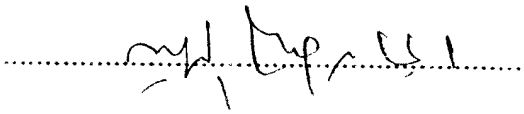

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دراسة إنتاج الإنترليوكين-١٨ والإنترليوكين-١٠ فى حالات الذنبة الحمراء وعلاقتهما بنشاط المرض

مقدمه من

منى محمد عبد العظيم صالح

بكالوريوس علوم

قسم بيولوجى (١٩٩٨)

للحصول على درجه

الماجستير

فى

المنـاعـة

لجنة المناقشة والحكم على الرسالة

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دراسة إنتاج الإنترليوكين-١٨ والإنترليوكين-١٠ فى حالات الذئبة الحمراء وعلاقتهما بنشاط المرض

رساله علميه

مقدمة إلى معهد البحوث الطبية-جامعة الإسكندرية

استيفاء للدراسات المقررة للحصول على درجه

الماجستير

فى

المناعة

مقدمه من

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٢٠٠٧