SERUM Th1 AND Th2 CYTOKINES IN SYSTEMIC LUPUS ERYTHEMATOSUS PATIENTS: IT'S RELATIONSHIP WITH DISEASE ACTIVITY AND ORGAN INVOLVEMENT

By

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DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF INTERNAL MEDICINE

UNIVERSITI SAINS MALAYSIA
MAY 2006

DEDICATION

I would like to dedicate my dissertation to my wife, Farhat Keenoo, whose support and encouragement throughout the process of writing this dissertation is invaluable.

I would also like to dedicate this dissertation to my parents for their love, inspiration, support and care.

ACKNOWLEDGEMENT

I would like to take this opportunity to express my gratitude to my supervisor, Prof. Madya (Dr) Kamaliah Mohd Daud for her supervision and guidance throughout the writing of this dissertation. I would also like to thank Dr Sukeri Mohamed for his help during the sample collection process in Hospital Kota Bharu.

In addition to that, I would like to express my thanks to Sheila, the research officer and Puan Malisa, the Immunology laboratory technician, for their help in sample collection and cytokine investigations.

This dissertation cannot be completed without the help given by medical clinic and medical ward staff at both Hospital Universiti Sains Malaysia and Hospital Kota Bharu.

I would also like to thank Dr Assoc. Prof. Syed Hatim Noor, Dr Tan Wim and Dr Sohaila for their guidance in statistical analysis of my research data and to their Department for the knowledge I have gained in biostatistics.

I would like to thank the Universiti Sains Malaysia for the knowledge and experience I gained throughout the Master program in Internal Medicine.

Finally, I would like to thank all my teachers for their knowledge, guidance and support.

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LIST OF ABBREVIATIONS

Ag	Antigen
ANA	Anti-nuclear antibody
APC	Antigen presenting factor
aPL	Antiphospholipid syndrome
ARA	American Rheumatism Association
AZA	Azathioprine
CD4	Cluster of differentiation 4
CPM	Cyclophosphamide
dsDNA	Double stranded deoxynucleic antibody
ESRD	End stage renal disease
EM	Electron microscopy
EIA	Enzyme immunoassay
ELISA	Enzyme linked immunoabsorbent assay
ESR	Erythrocyte sedimentation rate
GMB	Glomerular basement membrane
GFR	Glomerular filtration rate
HLA	Human leukocyte antigen
IF	Immunofluorescence
Ig	Immunoglobulin
IFN-γ	Interferon gamma
IL-2	Interleukin 2
IL-6	Interleukin-6
IL-13	Interleukin-13
Kg	Kilogram
LM	Light microscopy
LN	Lupus Nephritis
MHC	Major histocompatibility complex
mls	Mililitres
MMF	Mycophenolate mofetil
NIH	National institute of health
PBL	Peripheral B lymphocyte
PBMC	Peripheral blood mononuclear cell
PCNA	proliferating cell nuclear antigen
pg/ml	Picogram per mililitres
Rnp	Ribonucleoprotein
SLE	System lupus erythematosus
Sm	Smith
Th	T helper
TNF	Tumour necrosis factor
TGFβ	Transforming growth factor β
UV	Ultraviolet
UFEME	Urine for full examination and full examination
WT	Weigh
WHO	World health organization

ABSTRACT

Systemic lupus erythematosus (SLE) is a complex autoimmune disease that may result from defective functions of the immunoregulatory T cell circuits. Cytokines play an essential role in molding the quality of an immune response to foreign or self-antigens. With the growing literature regarding cytokine production in autoimmune diseases, an important role for an active Th1 response, characterized by production of IFN-γ, IL-2, IL-12 and TNF-α has become evident. On the contrary, there have also been repeated observations of high levels of type 2 cytokines (IL-4, IL-5, IL-10, IL-6 and IL-13) particularly in the systemic autoimmune diseases.

Objectives: To determine the circulating serum levels of two Th1 (IL-2, IFN-γ) and two Th2 (IL-6, IL-13) cytokines in patients with systemic lupus erythematosus (SLE), to compare serum level of IL-2, IFN- γ, IL-6 and IL-13 with the disease activity in SLE patients and to assess the relationship between serum level of cytokines (IL-2, IL-6, IL-13 and IFN-γ) and different organ involvement in SLE patients.

Methodology: We included 90 SLE patients and 30 healthy controls in this comparative cross sectional study carried out in Hospital Universiti Sains Malaysia (USM) and General Hospital Kota Bharu from October 2003 until September 2005 by selecting SLE patients in medical wards and outpatient clinics. Serum levels of cytokines were measured by ELISA (IL-2, interferon (IFN) γ, IL-6 and IL-13) as well as anti-dsDNA, ANA, C3 and C4 complement levels were determined. Disease activity was recorded according to the Systemic Lupus Erythematosus Disease Activity Index

(SLEDAI) and classified as high activity (SLEDAI > 8) or low activity (SLEDAI ≤ 8). Different organ affected at any time during the course of the disease was recorded.

Results: The mean age of the patients was 31.0±10.8 years. Out of 90 SLE patients, 81 were females (90%) and 9 males (10%). Majority was Malay (92.2%) and the rest was Chinese (7.8%). 52 patients (57.8%) were inactive SLE (SLEDAI score 0-8) and 38 patients (42.2%) active SLE (SLEDAI score >8). Serum levels of cytokines in SLE patients were significantly higher than in healthy control with the exception of IL-2 (IFN-γ $p<0.001$, IL-6 $p<0.001$, IL-13 $p=0.002$, IL-2 $p= 0.639$). There were also significant differences between active and inactive SLE patients with the exception of IL-2 (IL-6 $p <0.001$, IL-13 $p = 0.009$, IFN-γ $p <0.001$and IL-2 $p =0.087$). There was a positive correlation between Th1 (IFN- γ) and Th2 (IL-6 and IL-13) cytokines with the disease activity ((IFN-γ $p=0.002$, IL-6 $p<0.001$, IL-13 $p=0.006$, IL-2 $p= 0.151$). There was significant correlation between serum level of IL-13 and musculoskeletal involvement ($p =0.016$) and also IL-6 with haematological involvement ($p =0.003$).

Conclusion: The serum levels of Th2 (IL-6 and IL-13) and Th1 (IFN- γ) cytokines were significantly elevated and correlated with disease activity in SLE patients. Serum cytokine level could provide useful information about disease activity in SLE patients.

ABSTRAK

Pengenalan:

SLE adalah satu penyakit autoimun kompleks disebabkan oleh gangguan fungsi sel-sel T-sitokin yang memainkan peranan penting dalam membentuk kualiti balasan imun terhadap antigen sendiri atau asing. Terdapat banyak kajian mengenai peranan sitokin dalam penyakit autoimun terutamanya balasan aktif Th1 yang melibatkan sitokin IFN-γ, IL-2, IL-12 dan IFN-α. Walaubagaimanapun terdapat perhatian berlainan bahawa sitokin jenis kedua (IL-4, IL-5, IL-10, IL-6 and IL-13) juga memainkan peranan dalam penyakit autoimun ini.

Objektif:

Untuk menentukan paras serum 2 Th1 sitokin (IL-2, IFN-γ) dan 2 Th2 (IL-6, IL-13) sitokin dalam penyakit SLE, serta membanding paras serum sitokin IL-2, IFN-γ, IL-6 dan IL-13 dengan aktiviti penyakit SLE dan akhirnya untuk menentukan hubungan antara paras serum sitokin (IL-2, IL-6, IL-13 dan IFN-γ) dengan organ-organ yang terlibat dengan penyakit SLE.

Metodologi:

Kami mengenalpasti 90 pesakit SLE dan 30 individu sihat sebagai control. Kajian rentas dilaksanakan di Hospital Universiti Sains Malaysia (HUSM) dan Hospital Kota Bharu (HKB) daripada Okt 2003 sehingga September 2005 dimana pesakit dipilih melalui wad-wad perubatan dan klinik pesakit luar. Paras sitokin di ukur menggunakan teknik ELISA (IL-2, interferon IFN-γ , IL-6 dan IL-13) dan juga paras anti-dsDNA, ANA, komplemen

C3,C4 turut diukur. Aktiviti penyakit direkodkan berdasarkan Indeks Penyakit SLE (SLEDAI: SLE Disease Activity Index) dan diklasifikasikan sebagai akitiviti tinggi (SLEDAI > 8) atau aktiviti rendah (SLEDAI≤8). Pelbagai penglibatan organ semasa penyakit tersebut dihidapi turut direkodkan.

Keputusan:

Purata umur pesakit adalah 31.0±10.8 tahun. Daripada sembilan puluh pesakit SLE, lapan puluh satu adalah wanita (90%) dan sembilan pesakit adalah lelaki (10%). Majoriti adalah Melayu (92.2%) dan yang lain adalah Cina (7.8%). 52 pesakit (57.8%) adalah pesakit SLE inaktif (SLEDAI skor 0-8) dan 38 pesakit (42.2%) adalah pesakit SLE yang aktif (SLEDAI > 8). Paras serum sitokin IFN- γ, IL-6 dan IL-13 adalah lebih tinggi dikalangan pesakit SLE berbanding dengan individu yang sihat kecuali IL-2 (.IFN-γ $p<0.001$, IL-6 $p<0.001$, IL-13 $p=0.002$, IL-2 $p= 0.639$). Terdapat perbezaan signifikan antara pesakit SLE aktif dan bukan aktif kecuali IL-2 (IL-6 $p <0.001$, IL-13 $p = 0.009$, IFN-γ $p <0.001$and IL-2 $p =0.087$) Selain daripada itu, terdapat hubungan antara sitokin Th1, (IFN-γ) dan Th2 (IL-6, IL-13) dengan aktiviti penyakit (IFN-γ $p=0.002$, IL-6 $p<0.001$, IL-13 $p=0.006$, IL-2 $p= 0.151$). Akhirnya, terdapat hubungan signifikan antara paras serum IL-13 dengan penglibatan 'muskuloskeletal'($p =0.016$) dan juga IL-6 dengan system'hematologi'($p =0.003$).

Kesimpulan:

Paras serum sitokin Th2 (IL-6 dan IL-13) dan Th1 (IFN-γ) didapati bertambah dengan signifikan serta mempunyai hubungan dengan aktiviti penyakit dikalayan pesakit SLE.

Paras serum sitokin boleh menbekalkan maklumat bermanfaat mengenai aktiviti penyakit SLE.

CHAPTER ONE

Introduction

1.0 Systemic lupus erythematosus

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease characterized clinically by protean manifestations, most commonly including arthralgia, arthritis, rash, alopecia, oral ulcers, serositis, leukopenia, central nervous system and renal involvement (Lahita, 2004). It is an important prototype of systemic autoimmunity and it causes significant disability and premature death in those patients who suffer from it (Lahita, 2004).

1.1 Epidemiology

Primarily, SLE is a disease of young women of childbearing age between 15 to 40 years old and female to male ratio of 9: 1 during this period. Uramoto and colleagues in Minnesota, USA, reported that the incidence of SLE is more than tripled over the past four decades. (Uramoto, 1999). In Malaysia, it is estimated that more than 10,000 people have been diagnosed with SLE over the past 30 years, with the prevalence of 43 per 100 000 population (Wang et al, 1997). However, this number may be only the tip of the iceberg. The Malaysian SLE Association believes that there are many more SLE sufferers in Malaysia who have not been diagnosed (upusmalaysia.org, 2005). However, the onset of disease can range from infancy to old age. SLE affects approximately 1 in 2000 individuals in the general population, although the prevalence varies with race, ethnicity and

socioeconomic status (Ward, 1995). Estimation of the exact incidence of SLE in United State varies; it is reported that it was 124 cases per 100 000 (Hochberg, 1997). But some reports indicate that because of improved diagnostic measures, the incidence of SLE is increasing. It has been found that mean age at the onset of symptoms was 29 years and at the fulfillment of 4 ARA criteria for the diagnosis of SLE. Systemic lupus erythematosus (SLE) is the most diverse of the autoimmune diseases because it may affect any organ of the body and display a broad spectrum of clinical and immunological manifestations (Cervera, 2005). For example, in a study covering a span of greater than 40 years found that the pooled incidence of SLE is more than tripled from 1.51/100,000 during 1950 to 1979 to 5.56 /100,000 during 1980 to 1992 (Uramoto et al, 1999). A recent review of 19 studies published from 1995 to 2000, has been reported an even higher incidence rate 7.3/100 000 (Ruiz-Irastorza, 2000). The incidence of SLE is about 3 times higher in African American women than in white women (Fessel, 1988), while in the West, it has been shown that Blacks are more prone to get SLE compared to Caucasians (Fessel, 1988). In the United States morbidity and mortality appear to be greater among Hispanic than among Whites (Bonguel, 2002).

SLE is also common among Asian and Chinese and an increased prevalence of SLE has been noted in people of oriental race (Frank, 1980). The ethnic group at the greatest risk is African Caribbean Blacks (Molokhia, 2001) but in another study (Hopkinson, 1994) the highest rate is shown in African Caribbean (207/100,000), followed by Asian (48.8/100,000) and Whites (20.3/100,000).The mean time between the first manifestation and the final diagnosis of SLE was 2 years (Cervera, 2005). In the Euro- Lupus cohort, 90

patients (9%) developed the disease after the age of 50 and (9%) patients with SLE were men.

1.2 Aetiology

The exact patho-aetiology of systemic lupus erythematosus (SLE) remains elusive. An extremely complicated and multifactorial interaction among various genetic and environmental factors are probably involved (Mok, 2003). Systemic lupus erythematosus is a complex disorder that occurs as a consequence of a number of independent processes and factors. Environmental factors, such as viruses, exposure to chemicals, sunlight trigger inflammatory or immune activity. Multiple genes contribute to disease susceptibility (Mok, 2003). The concordance of the disease in identical twins is approximately 25–50% and that in dizygotic twins is around 5% (Pisetsky, 1997). The immune activation may begin as an appropriate response to an unwanted "invader". But, because of a combination of genetic factors, an individual with lupus develops an ongoing immune response that does not shut itself off appropriately. Genetic predisposition, sex hormones and environmental factors may play an important role in the pathogenesis of SLE (Belmont, 2000). The genetic susceptibility can be explained as follows: 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. Many different genes contribute to disease susceptibility. In a small proportion of patients (< 5%), a single gene may be responsible. For instance, patients with homozygous deficiencies of the early components of complement are at risk of developing SLE or a lupus-like disease (Walport, 1998). For most of the remaining patients, multiple genes are

required. Population studies reveal that the susceptibility to SLE involves human leucocyte antigen (HLA) class II gene polymorphisms. An association of HLA DR2 and DR3 with SLE is a common finding in patients of different ethnicities, with a relative risk for the development of disease of approximately two to five (Pisetsky, 1997). The HLA class II genes have also been associated with the presence of certain auto-antibodies such as anti-Sm (small nuclear ribonuclear protein), anti-Ro, anti-La, anti-nRNP (nuclear ribonuclear protein) and anti DNA antibodies(Schur, 1995). SLE is associated with inherited deficiencies of C1q, C1r/s and C2 (Atkinson, 1986). A decrease in complement activity could promote disease susceptibility by impairing the neutralisation and clearance of self and foreign antigens. When the antigen burden overwhelms the clearance capacity of the immune system, autoimmunity may ensure (Lau, 2003).

1.2.1 Sex hormone

Sex hormones play a part in the pathogenesis of SLE (Manolios and Schrieber, 1997). SLE is predominantly a female disease (Cervera, 1993). First onset of SLE before puberty and after menopause is uncommon (Formiga, 1999). The female predilection becomes less pronounced outside the reproductive age range. In addition, patients with Klinefelter's syndrome, characterised by hypergonadotrophic hypogonadism, are prone to the development of SLE (French, 1983). These observations suggest a role for endogenous sex hormones in disease predisposition. Abnormal oestrogen metabolism has been demonstrated in patients with SLE of both sexes, with an increase in 16α hydroxylation of oestrone, resulting in significantly raised 16α hydroxyestrone concentrations (Lahita RG, 1979). The 16α metabolites are more potent and feminising oestrogens. Women with SLE also have low plasma androgens, including testosterone, dihydrotestosterone,

dehydroepiandrosterone (DHEA), and dehydroepiandrosterone sulfate (Jungers, 1982). The concentrations of androgens correlate inversely with disease activity (Lahita, 1987). Low concentrations of plasma testosterone and raised luteinising hormone (LH) values (Lahita, 1987) have been found in some men with SLE. Some studies have found that exogenous exposure to estrogen, either through oral contraceptive or oestrogen replacement therapy may increase the incidence of SLE (Sanchez-Guerrero et. al, 1997). Taken together, oestrogens may aggravate SLE by prolonging the survival of auto-immune cells, increasing T helper type 2 (Th2) cytokine productions 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 (Mok, 2003).The effects of oestrogen is shown in table 1.1 and the role of hormones in SLE is summarized in table 1.2.

Table 1.1 Effects of oestrogen on immune function

Cell type	Effect	Dose
B cells	↑ B cell diffentiation and in vitro Ig production including anti-dsDNA (patients with SLE and healthy subjects)	**Physiological**
	↓ In vitro apoptosis of PBMCs and ↓TNF-α production (patients with SLE, not in healthy subjects)	**Physiological**
T cells	↓ Proliferative response to nitrogen and antigens	**High**
	↓ IL-2R expression and IL-2 production in activated peripheral blood T cells (healthy subjects)	**High**
	↑ Calcineurin mRNA values in cultured T cells (patients with SLE, not in healthy control or patients with other rheumatic diseases)	**Dose dependent**
	↑ CD40L Expression of peripheral blood T cell (patients with SLE, not in healthy controls)	**Physiological**
Monocytes	↑ IL-10 production (patients with SLE and healthy subjects)	**Physiological**
	↑ cNOS release	**Physiological**
Others	Adhessive molecule expression in endothelial cells	**High**

CD40L, CD40 ligand. cNOS, cytoplasmic nitric oxide synthase. dsDNA, double stranded DNA. Ig, immunoglobulin; IL, interleukin. IL-2R, interleukin 2 receptor. PMBC, peripheral blood mononuclear cells. SLE, system lupus erythematogus. TNF-α, tumour necrosis factor α

Adapted from a journal by Mok CC, Lau SC 2003. Pathogenesis of systemic lupus erythematosus, Clinical pathology 2003:56, 3.

Table 1.2 Role of hormones in human SLE

Role of hormones in human SLE
Susceptibility to SLE development • Low endogenous oestrogen concentrations are protective • Low androgen values in men increase risk • Use of exogenous oestrogen increases risk in women **Hormonal profile and HPA axis in patients with SLE** • Increase in metabolism of oestrogen to more potent metabolites (both sexes) • Low androgen values (both sexes). Androgen values correlate inversely with disease activity in women • Hyperprolactinaemia occurs in a subset of patients (both sexes). Prolactin concentrations correlate with disease activity in some studies. Bromocriptine is beneficial in mild SLE • Preliminary evidence for a defective HPA axis in untreated female patients with SLE **Hormones, SLE activity and prognosis** • Disease activity tends to reduce after menopause • Flares of SLE may occur during periods of rapid hormonal changes • Cyclical fluctuation of disease activity in women during the menstrual cycle • Patients with postmenopausal onset SLE have lower disease activity and better prognosis
HPA, hypothalamus – pituitary – adrenal; SLE, systemic lupus erythematosus.

Adapted from a journal by Mok CC, Lau SC 2003. Pathogenesis of systemic lupus erythematosus, Clinical pathology 2003:56,3.

1.2.2 Environmental factors

Possible environmental factors including ultra-violet radiation, drugs (e.g., procainamide, hydralazin and isonaizide), infectious agent and severe emotional/physical stress are implicated in triggering SLE activation. Several viral infections have been suspected to play at least a provoking role (Osmola, 2004). These factors can lead to flare up of lupus (Shapiro, 2004; Manolios, 1997).

Diagrammatic representation of different factors involved in Systemic lupus erythematosis pathogenesis is shown in figure 1.1:

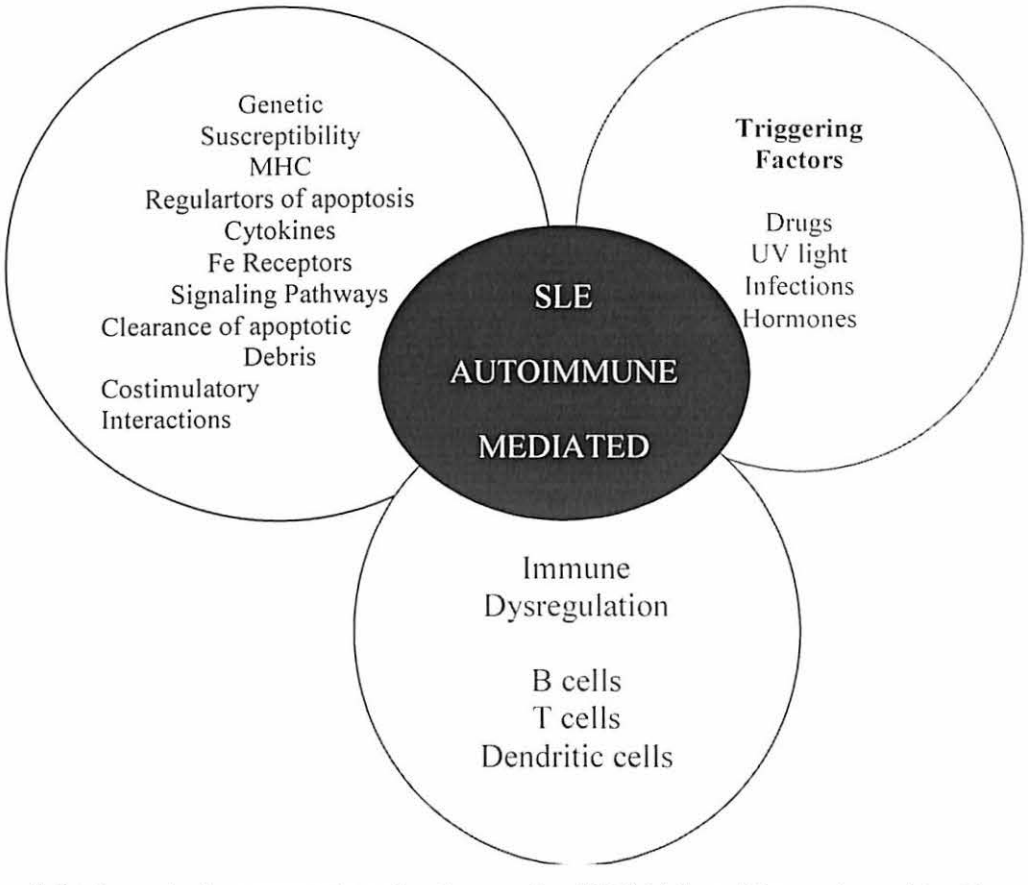


Figure 1.1 Theoretical representation of pathogenesis of SLE (Adapted from a journal by Giovanni F, Elena P, Betty D, Pathogenesis of SLE: implications for rational therapy, Disease Mechanism, Autoimmunity and inflammatory disease, Drug Discovery Today , Vol, 1, No. 3, 2004)

1.3 Immunopathogenesis

SLE is characterized by alterations in many different parts of the immune system. The basic pathological features of SLE are inflammation and blood vessel abnormalities, which include band or occlusive vasculopathy, vasculitis and immune complex deposition (Mok, 2003). Patients may share some central characteristics; a lack of tolerance and the production of pathogenic autoantibodies with immune complex formation.

1.3.1. Disturbances of the immune response

To better understand the pathogenesis of SLE, the contributions of various cell types have been examined. A model of possible T cell–antigen-presenting cell interactions is shown in figure 1.2.

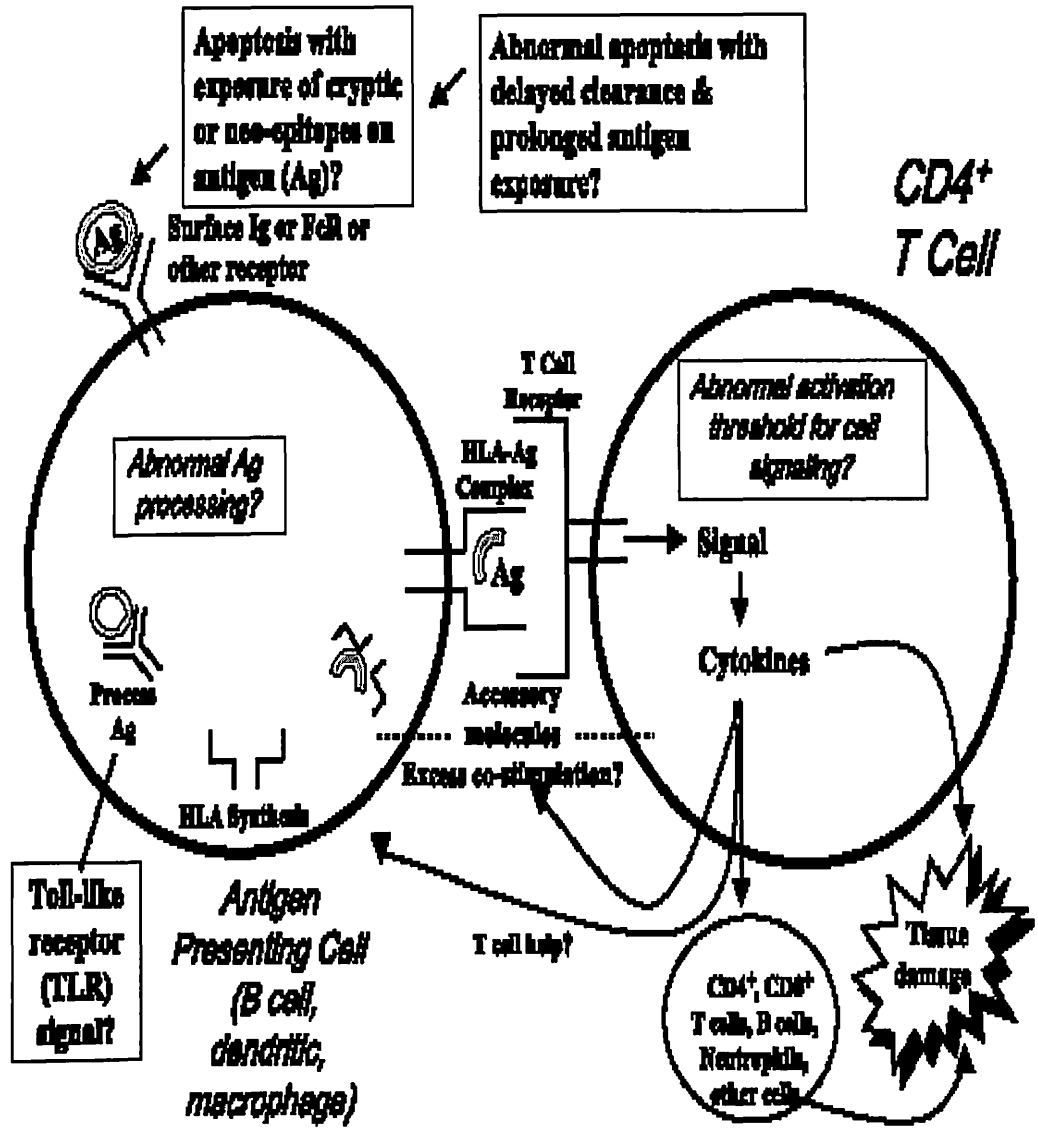


Figure 1.2. Model of possible T cell–antigen presenting cell interactions (Adapted from a journal by Robert W. Hoffman. T cells in the pathogenesis of systemic lupus erythematosus Clinical Immunology 2004; 113: 4-13

Figure 1.2 illustrates hypothetical points of the T cell-antigen presenting cell interaction which could lead to loss of immunological tolerance in SLE. It appears that there may be multiple immunological abnormalities that can lead to breaking of immunological self-tolerance and several of these may be simultaneously operative. In this model, self-antigen (Ag) is taken up by an antigen-presenting cell, processed and presented to T cells. T cell receptor signaling events lead to T cell activation and the production of soluble factors, such as cytokines, that may in turn provide help to autoantibody-producing B cells, assist in the recruitment of other cells to sites of inflammation or in some instances directly mediate tissue damage. Structural modification of antigen, abnormalities of antigen processing excess co-stimulation via accessory molecules, adjuvant-like signals through Toll-like receptors or abnormal activation threshold of T cell receptor could each contribute to breaking of immunological tolerance and be important in the pathogenesis of SLE.

Potential abnormal signaling points or pathways that could lead to loss of T cell tolerance in SLE are indicated. There is evidence for contributions by B cells, dendritic cells, nonlymphoid cells at sites of tissue injury and T cells to the development of SLE (Horowitz, 1997)

1.3.2 Immunotolerance

Current dogma suggests that triggering factors in a susceptible host result in the loss of self-tolerance and the development of autoreactivity. A fundamental process in autoimmune disease is the breakdown in immunological tolerance either central or peripheral Central tolerance involves thymic deletion of self reactive cells and upregulation of T-cells with low affinity of self-MHC. The peripheral T-cell tolerance includes anergy (loss of co-stimulatory signals) of reactive T-cells, ignorance of antigen by the immune system and suppression of autoreactive T-cells. Failure to become tolerant to self antigens, cross-reactivity and molecular mimicry, development of anti-idiopathic antibodies that cross-react with self antigens and polyclonal stimulation of naturally occurring autoantibody producing cells may underlie the initiation and maintenance of autoantibodies secretion (Manolios and Schrieber, 1997).

1.3.3 Apoptosis

Apoptosis is viewed as programmed cell death or cellular suicide. The main characteristic of apoptotic cells is that they, in contrast to necrotic cells, maintain their membrane integrity. The release of intracellular components is thereby prevented. Apoptotic cells are usually cleared by macrophages via a noninflammatory pathway (Voll, 1997). Apoptotic

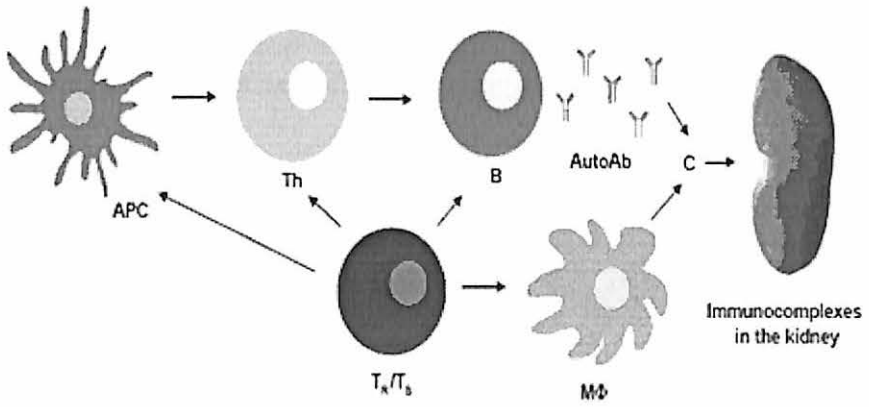
and necrotic cells are strong candidates as sources of autoantigens that drive the autoantibody response in autoimmune diseases. The fast and efficient uptake of dying cells is of main importance to prevent contact of the immune system with intracellular autoantigens (Rovere, 2000). Defects in the clearance of dying cells may contribute to the etiopathogenesis of systemic lupus erythematosus (SLE). Increased apoptosis or impaired clearance of apoptotic cell material has been implicated in the pathogenesis of human SLE. Impaired clearance functions for dying cells may explain accumulation of apoptotic cells and subsequently of secondary necrotic cells in various tissues of SLE patients. During cell death by necrosis or apoptosis, autoantigens are cleaved or otherwise modified. These modifications may render cryptic epitopes immune dominant. Dendritic cells may then acquire modified autoantigens, like apoptotic nuclei and chromatin and consequently start an immune reaction. Normally, apoptotic cells are swiftly removed by phagocytosis due to surface changes induced by the apoptotic process (Savill, 2002). This prevents the release of intracellular constituents, including nucleosomes, which are uniquely formed during apoptosis through cleavage of chromatin by nucleases. However, antibodies against nucleosomes are a hallmark of systemic lupus erythematosus (SLE) (Dieker, 2002). Therefore, disturbances in either apoptosis or the phagocytosis of apoptotic cells have been proposed to play a role in the development of autoimmunity, especially in SLE

1.3.4. Cellular defect

SLE is characterised by a myriad of immune system aberrations that involve B cells, T cells and cells of the monocytic lineage. This results in polyclonal B cell activation, increased numbers of antibody producing cells, hypergammaglobulinaemia, autoantibody production

and immune complex formation. It appears that excessive and uncontrolled T cells help in the differentiation and activation of autoantibody forming B cells is probably a final common pathway (Mok, 2003). The activation of B and T cells requires stimulation by specific antigens. Irritating chemicals such as pristine, bacterial DNA and cell wall phospholipids and viral antigens can induce anti-DNA antibodies in mice (Hahn, 1995). Moreover, self antigens, such as DNA–protein and RNA–protein complexes may induce autoantibody production (Klinman, 1991). Environmental antigens and self antigens are taken up by professional antigen presenting cells (APCs) or bind to induced antibodies on the surface of B cells. Both professional APCs and B cells process the antigens into peptides and present them to T cells through their surface HLA molecules. The activated T cells in turn stimulate the B cells to produce pathogenic autoantibodies. In addition to contact stimulation, the interaction of B and T cells is facilitated by several cytokines, such as IL-10. The number of B cells at all stages of activation is increased in the peripheral blood of patients with active SLE (Klinman, 1991). These B-cell abnormalities can precede the development of SLE. Activated lupus B-cells have higher intracytoplasmic calcium responses than controls (Liossis, 1996). There is also evidence that B cells in patients with SLE are more sensitive to stimulatory effects of cytokines such as IL-6 than non-SLE B cells (Linker-Israeli, 1991). Moreover, the phenomenon of epitope spreading has been demonstrated in both human and murine SLE (Monneaux, 2002). Thus, it appears that B-cells in patients with SLE are more prone to polyclonal activation by antigens, cytokines and other stimuli. Abnormalities in T cell function are also evident in patients with SLE. The total number of peripheral blood T cells is usually reduced, probably because of the effects of antilymphocyte antibodies (Bakke, 1983). There is an increase of T cell function towards B cell activation, leading to enhanced antibody production (Linker-Israeli, 1990).

Figure 1.3 summarises the different pathways in the control of autoimmune reactivity in SLE.



The control of autoimmune reactivity in SLE mediated by $CD4^+$ $CD25^+$ T_R and $CD8^+$ T_S cells T_R and T_S may control several events leading to production of the autoantibodies that fix the complement and cause tissue damage. APC, antigen presenting cell; Th, $CD4^+$ T helper cell; B, B cell; T_R, $CD4^+$ $CD25^+$ regulatory T cell; TS, $CD8^+$ suppressor T cell; Autoantibodies; Mφ, macrophage, C, complement.

Figure 1.3 A schematic model of control of autoimmune reactivity in SLE (Adapted from a journal by Antonio La Cava, Celia J. Fang, Ram. P. Singh, Fanny Ebling, Bevra H. Hahn, Manipulation of immune regulation in systemic lupus erythematosus. Autoimmunity Reviews, 2005; 4: 515– 519.

1.3.5. Role of antibodies

The presence of pathogenic auto-antibodies is a hallmark of SLE and reinforces the concept that B-cells have a key role in this disease. Auto-antibodies can be seen in healthy individuals, although these natural auto-antibodies are usually of a low affinity IgM isotype. They do not undergo maturation and do not cause autoimmune diseases or tissue damage (Tsokos, 2002). The auto-antibodies production in SLE is thought to be of pathogenic relevance, mainly by the formation of immune complexes, with deposition in target tissues such as glomeruli, heart, skin and vessels. The deposited immune complexes

then participate in inflammatory processes involving complement activation, eventually causing tissue damage. B cell are important in SLE as they produce antibodies against nuclear and cell surface antigens. B cell hyperreactivity is present in SLE and this results in the production of a variety of auto-antibodies, including those against nuclear antigens that contain chromatin (DNA histone) and uridylate-rich (U) small nuclear ribonucleoproteins (U-RNP or U-snRNP that are ribonucleoproteins contained within the spliceosome complex) (Maddison, 1977). Auto-antibodies against chromatin and ribonucleoprotein are serological hallmarks of SLE and their presence is used to help define the syndrome (Tan, 1982). While virtually 100% of SLE patients will have antibodies against a nuclear antigen (Holyst, 1998; Greidinger, 2003), only 30–50% of patients will have antibodies against one of the two nuclear antigens that are considered pathognomonic of SLE, double-stranded DNA (dsDNA) and the Sm antigen of the U-small nuclear ribonucleoprotein complex (Greidinger, 2003; Holyst, 1998). A higher percentage of SLE patients will have antibodies against single-stranded DNA and histones, although the presence of these antibodies is not specific for SLE. Anti-nuclear antibodies can be detected months to years preceding the development of SLE. However, the development of some auto-antibodies (such as those to ribonucleoprotein, Sm and dsDNA) is temporally closely linked to disease onset, suggesting that they play a role in the pathogenesis (Arbuckle, 2003).

1.3.6. Cytokines in SLE

Cytokine production in patients with SLE differs from both healthy controls and patients with other diseases such as rheumatoid arthritis (RA) (Gillian, 2000). Cytokines have been functionally divided into 2 subgroups: Th1, mainly interleukin IL-2, IL-12, interferon

(IFN)γ and tumor necrosis factor (TNF) α and β, which mainly activate the cellular machinery of the immune system; and Th2 (IL-4, IL-5, IL-6, IL-10, and IL-13) cytokines, which activate the humoral machinery (Funauchi, 1998; Mosmann, 1989). In patients with SLE, B-cell hyperactivity has been associated with a high production of Th2 cytokines. However, the participation of Th1 cytokines has been equally demonstrated (Theofilopoulos, 2001). Both Th1 and Th2 cytokines can participate in promoting or inhibiting auto-immune diseases; thus, a clear-cut distinction between Th1 and Th2 patterns is not without complexity (Amerio, 2002). It is important to note that cytokine production is not only changed in patients with SLE when compared with healthy controls but also changes with different disease phenotypes. For example, interleukin 6 (IL-6) seems to be increased in the cerebrospinal fluid (CSF) of patients with central nervous system (CNS) involvement in SLE but not in patients with SLE who lack neurological symptoms (Jara LJ, 1998). It may be that as in other inflammatory diseases, the balance of cytokines is more important in determining disease phenotype or severity rather than in determining disease susceptibility. The production of IFN-γ by peripheral blood mononuclear cells (PBMC) from patients with SLE is significantly correlated with global disease activity score, the Systemic Lupus Activity Measure (SLAM) (Spronk, 1993). It has been shown that there is a correlation between the subclass of antinuclear antibodies and the severity of SLE. All auto-antibodies found in subacute cutaneous lupus were of the IgG1 subclass while IgG2 and IgG3 auto-antibodies were also found in patients with systemic disease. IFN-γ is able to induce class switching to production of IgG2 and IgG3. IL-6 is necessary for bone marrow targeted plasma cell (CD38+, CD19+) survival and subsequent maturation.(Kawano, 1995) It has been suggested that IL-6 is involved in the autocrine

route that maintains B cell hyperactivity (Kawano, 1995). High levels of IL-6 have been shown to be implicated in the development of cardiopulmonary disease, which can take the form of pericarditis, valvular abnormalities (25%), pleural effusions, lupus pneumonitis, pulmonary hypertension and interstitial pneumonitis. Nine patients with lupus nephritis were shown to have increased plasma concentrations of IL-6 and sIL-6R as compared with five normal controls (Horii, 1993). IL-6 is detectable in the urine of patients with lupus nephritis and may constitute a useful diagnostic marker (Horii, 1993)

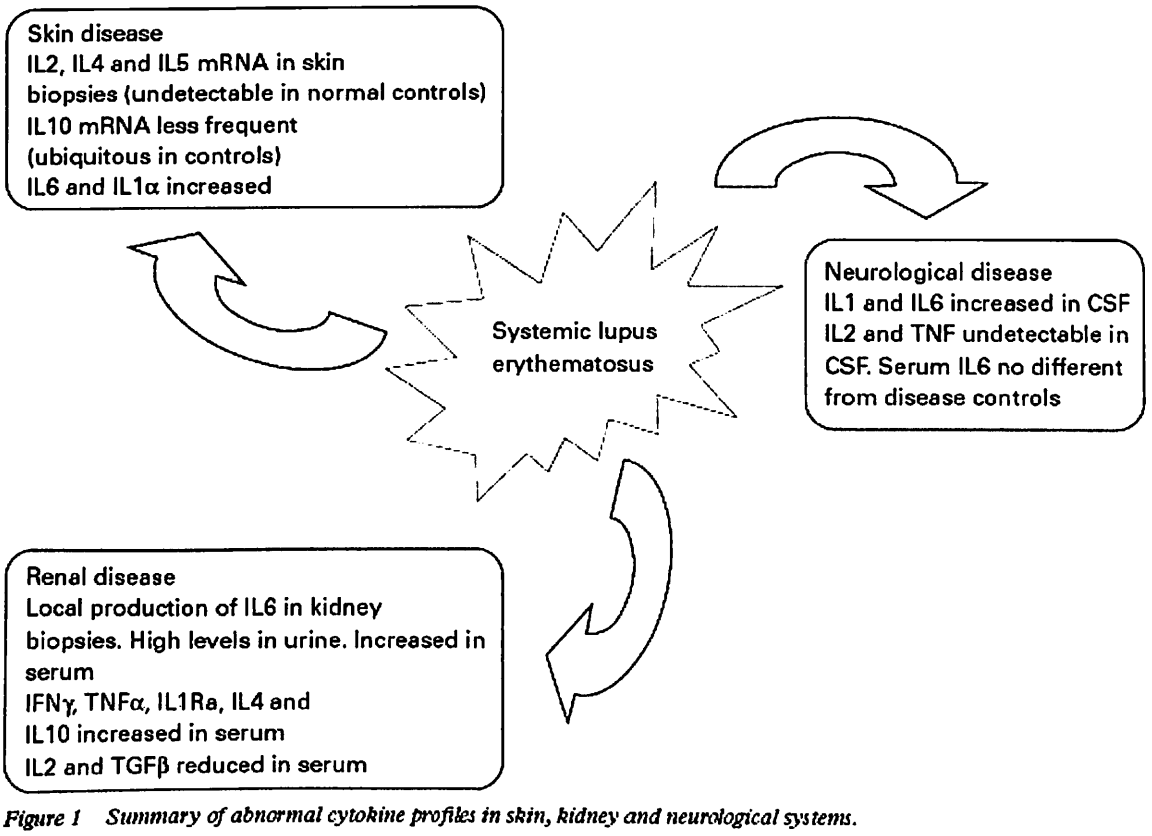


Figure 1 Summary of abnormal cytokine profiles in skin, kidney and neurological systems.

Figure 1.4 A summary of cytokines profile in skin, kidney and neurological system

(Adapted from a journal by Gillian S, Dean J. Esther, David A, Isenberg. Cytokines and systemic lupus erythematosus. *Ann Rheum Dis,* 2000; 59:243–251, 2000)

Derangement of T cell function has been widely demonstrated in SLE with abnormal cytokine profiles correlated to loss of immune tolerance, increased antigenic load and defective B cell suppression. Although early reports described defective Th1 and excessive Th2 responses in lupus, recent data suggest that both Th1 and Th2 cytokines can be elevated in lupus patients thus indicating that SLE is a complex disease driven by the activation of different lymphokine systems at different time-points, possibly explaining the heterogeneity of clinical manifestations (Amerio, 2002).

1.3.6.1 IL-2

IL-2 is a cytokine secreted primarily by Th1 that has been activated by stimulation with certain mitogen or by interaction of the T cell receptor complex with antigen/MHC complexes on the surfaces of antigen presenting cells. It can act as an autocrine factor by driving the expansion of the antigen specific cells, or as a paracrine factor by influencing the activity of other cells, either within or outside the immune system.

1.3.6.2 IFN-γ

IFN-γ is the principle Th1 effector cytokine within 2 main functions. It activates macrophages and enhances their microbiocidal activity. Apart from that, IFN-γ also stimulates the production of IgG antibodies which bind to high-affinity Fc receptors and complement proteins, therefore being the principle antibodies involved in the opsonisation and phagocytosis of particulate microbes. It also promotes the differentiation of CD8 T lymphocytes into active cytotoxic cells (Abbas et al. 1996)

1.3.6.3 IL-6

IL-6 is a cytokine produced by a variety of cells including Th2 during infection, trauma and immunological challenge. It promotes inflammatory events through the expansion and activation of T-cells, diffentiation of B-cells and the induction of acute-phase reactants by hepatocytes. On the other hand, it also has a protective role during disease process and counteracts the manifestation of certain inflammatory responses such as septic shock (Jones et al, 2001)

1.3.6.4 IL-13

IL-13 is a cytokine produced by Th2 and has an important anti-inflammatory role. Its inhibitory effect can lead to a decrease production of IL-1, IL-6, IL-8 and TNF (Chen et al 2001). Apart from that, it also affects B lymphocytes by increasing their proliferation and the expression of the CD23 surface antigen (Minty et al, 1993)

1.3.7 Complement system

Measurement of C3 and C4 levels may support the diagnosis of SLE. The presence of anti-dsDNA antibodies and hypocomplementaemia strongly suggests the diagnosis of SLE and identifies the patient at increased risk for glomerulonephritis. The complement system plays a dualistic role in the pathogenesis of SLE: on the one hand deficient function of complement may play a very important role in the deficient clearance of apoptotic cells and on the other 'normal' or excessive function of complement plays a major role in the pathogenesis of inflammation leading to tissue damage and to the symptoms in SLE patients.

Congenital deficiencies of C2, C4 and especially C1q have a very strong association with SLE-like disease. On the other hand, C3 deficiency has a much milder association with SLE. Yet C3 is the first common point in all 3 pathways of complement activation. Recently it has been shown that C1q receptors on the surface of macrophages are an extremely important mechanism in clearing apoptotic cells (Korb and Ahearn, 1997). So it could well be that SLE and SLE-like disease is mediated through an influence of C1q on macrophages resulting in an inefficient clearance of apoptotic cells. However, in patients with C1q deficiency the manifestations of SLE tend to be restricted to the skin and kidneys and anti-dsDNA antibodies tend to be not particularly high. So the primary event causing a defective clearance of apoptotic cells in most SLE patients is not yet clear. Autoantibodies against C1q may aggravate the defective clearance of apoptotic cells but they are unlikely to be the primary event in SLE. Also massive consumption of complement factors of the classical pathway may aggravate the deficiency of clearance of apoptotic cells. The second role complement plays in SLE patients is through its reaction with immune complexes. Immune complexes stimulate complement activation which in turn leads to inflammation.

1.4 Diagnostic criteria

The diagnosis of systemic lupus erythematosus is based on both clinical and laboratory criteria. The criteria set by the American College of Rheumatology (ACR) are most widely used (Hochberg, 1997). A person is suspected to have SLE if she/he fulfills 4 out of the 11 criteria as shown in table 1.3

Table 1.3: Revised criteria of the American College of Rheumatology for the classification of systemic lupus erythematosus	
1. Malar rash	Fixed erythema, flat or raised, over the malar eminences tending to spare the nasolabial folds.
2. Discoid rash	Erythematous raised patches with adherent keratotic scaling and follicular plugging; atrophic scarring can occur in older lesions
3. Photosensitivity	Skin rash as a result of unusual reaction to sunlight, by patient history or physician observation.
4. Oral ulcers	Oral or nasopharyngeal ulceration, usually painless, observed by a physician.
5. Arthritis	Non-erosive arthritis involving two or more peripheral joints, characterized by tenderness, swelling, or effusion.
6. Serositis	Pleuritis: convincing history of pleuritic pain or rub heard by a physician or evidence of pleural effusion or Pericarditis: documented by ECG or rub or evidence of pericardial effusion.
7. Renal disorder	Persistent proteinuria greater than 0.5 g/day or greater than 3+ if quantification not performed or cellular casts: can be red cell, haemoglobin, granular, tubular or mixed.
8. Neurological disorder	Seizures: in the absence of offending drugs or known metabolic derangements, e.g.uraemia, ketoacidosis or electrolyte imbalance or Psychosis: in the absence of offending drugs or known metabolic derangements, e.g. uraemia, ketoacidosis or electrolyte imbalance
9. Haematological disorder	Haemolytic anaemia: with reticulocytosis or Leucopenia: less than 4000/mm3 or Lymphopenia: less than 1500/mm3 or Thrombocytopenia: less than 1500/mm.
10. Immunological disorder	Anti-DNA: antibody to native DNA in abnormal titre or Anti-Sm: presence of antibody to Sm nuclear antigen or (c) Positive finding of antiphospholipid antibodies based on: (i) an abnormal serum level of IgG or IgM anticardiolipin antibodies; (ii) a positive test for lupus anticoagulant using a standard method; or (iii) a false-positive test for at least 6 months and confirmed by Treponema pallidium immobilisation or fluorescent treponami antibody absorption test
11. Positive ANA	An abnormal titre of antinuclear antibody by immunofluorescence or an equivalent assay at any point in time in the absence of drugs

1.5 Clinical signs and symptoms

1.5.1. Constitutional

The constitutional signs and symptoms of SLE often include fever, malaise, weight loss, symmetrical arthralgia, myalgia and headache (Greenberg, 1999). Most serious features of SLE are persistent fever and weight loss.

1.5.2. Musculoskeletal

Over 90 percent of SLE patients have polyarthralgias or polyarthritis because of the disease and it is the most common reason that SLE patients seek medical attention. Small joints of the hand and wrist are usually affected therefore soft tissue and tendon thickening causes swelling of these joints but the effusion is small unlike rheumatoid arthritis. SLE arthropathy is usually not erosive or destructive to bone. (Hay, 1995).

1.5.3. Mucocutaneous

More than 90 percent of patients with SLE eventually have cutaneous manifestations of the disease, including malar rash, discoid rash and alopecia. Approximately two-thirds of SLE patients have photosensitivity, defined as a skin rash due to an unusual reaction to sunlight (Provost, 1994). SLE can be acute, subacute or a chronic disease.

Some acute manifestations include bullous lesions and generalized erythema, which may or may not be photosensitive in nature (Sontheimer and Gillian, 1992). The most characteristic clinical feature of subacute cutaneous lesion is superficial, nonindurated and non-scarring photosensitivity inducing skin rash.

In chronic cutaneous SLE, patients may have a discoid rash with scarring. Oral, nasal and other mucus membrane lacerations may occur. Raynaud's phenomena occur in 10- 45 % of SLE patients and is the result of vasospasm and muscular damage, especially apparent with the classical white, blue and red color changes in the fingers Alopecia is observed in up to 45 % of SLE patients at some time in the disease or it can occur with some therapies for SLE. Hair loss may be diffuse or patchy associated with discoid lesions.

1.5.4. Serositis

Inflammatory serositis of the pleura, peritoneum and pericardium occur in 25 % of SLE patients. The patients can develop large pleural effusions, pericardial effusion or ascites. These effusions are typically inflammatory and exudative.

1.5.5. Haematological

Anaemia, leucopenia and thrombocytopenia are frequent manifestations of SLE and these patients often have normocytic normochromic anemia. Coomb's test is frequently positive and circulating anti-erythropoietin antibodies are reported as a possible mechanism of anemia in them (Tzioufas et. al, 1997).

Leucopenia with white blood cells (WBC's) count less than $4000/mm^3$ or lymphopenia with lymphocytes count less than $1500/mm^3$ on two or more occasions are part of the diagnostic criteria of SLE. However, other causes of decreased WBC's counts such as malignancy, infection and drug-induced leukopenia should be excluded before attributing the low WBC's count to SLE.

Thrombocytopenia with a platelets count less than 100,000/mm^3 in the absence of other causes is found in up to 25 % of SLE patients. However thrombocytopenia is often a marker of severe disease with poor prognosis (Reveille et. al, 1990).

1.5.6. Renal

The kidney is the most commonly involved organ in patients with SLE and is a major cause of mortality and morbidity. Lupus nephritis (LN) represents a major clinical manifestation and is present in 15% of patients at the time of diagnosis and in approximately 40% during the course of the disease (Cervera, 1993). Several studies show evidence of nephritis in approximately 50-70 % of renal biopsies from SLE patients (Golbus and McCune, 1994). The clinical manifestations of lupus nephritis that can be seen in SLE patients are asymptomatic haematuria and/or proteinuria, nephritic syndrome or chronic renal failure (Kong, 1996). The renal involvement is due to deposition of immune complexes containing anti-dsDNA antibodies in the kidney (Belmont, 2000). Renal biopsy is the gold standard for diagnosis and follow-up of LN. However, serial renal biopsies are not feasible due to their invasive nature and potential risks. Urinalysis, measurement of 24-h proteinuria and creatinine clearance only identifies lupus patients with overt kidney damage but these measurements are inadequate to classify the severity of nephritis and fail to detect early 'silent' disease (Calvani, 2005).

Cytokines normally produced within the renal parenchyma are overexpressed in LN by both resident and infiltrating cells (Neilson, 1997). Studies by Masutani et al (2001) and Mitsuteru et al (1999) both found predominance of Th1 cytokines over Th2 in the peripheral blood of patients with Class IV nephritis and SLE patients with proteinuria