Lecture No. 2

Systemic lupus erythematosus

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease characterized by the production of autoantibodies and a diversity of clinical manifestations. It most commonly presents in women during their child-bearing years, in which the immune system targets intracellular particles that contain both nucleic acids and nucleic acid binding proteins.

Etiology and Pathogenesis

Although the etiology of SLE is unknown, multiple factors are associated with the development of SLE, including genetic (HLA- DR2/DR3), racial, hormonal, immune abnormalities and environmental factors (ultraviolet light, viral infection involving molecular mimicry between organism and self for example anti-Sm autoantibody react with p24 gag protein of retroviruses and that anti- Ro recognizes a nucleocapsid protein on vesicular stomatitis virus). One proposed mechanism for the development of autoantibodies involves a defect in apoptosis or clearance of apoptotic cells, leading to a disturbance in immune tolerance. The redistribution of cellular antigens during apoptosis leads to a display of cytoplasmic and nuclear antigens on the cell surface, enhancing immune reactivity to antigens, which are normally protected intracellularly. Activation of antigen-presenting cells by IFN-α might promote presentation of autoantigens to self-reactive T cells. Immune complexes form in the microvasculature, leading to complement activation and inflammation. Antibody–antigen complexes deposit on the basement membranes of skin and kidneys. In active SLE, this process has been confirmed based on the presence of complexes of nuclear antigens such as DNA, immunoglobulins, and complement proteins at these sites.

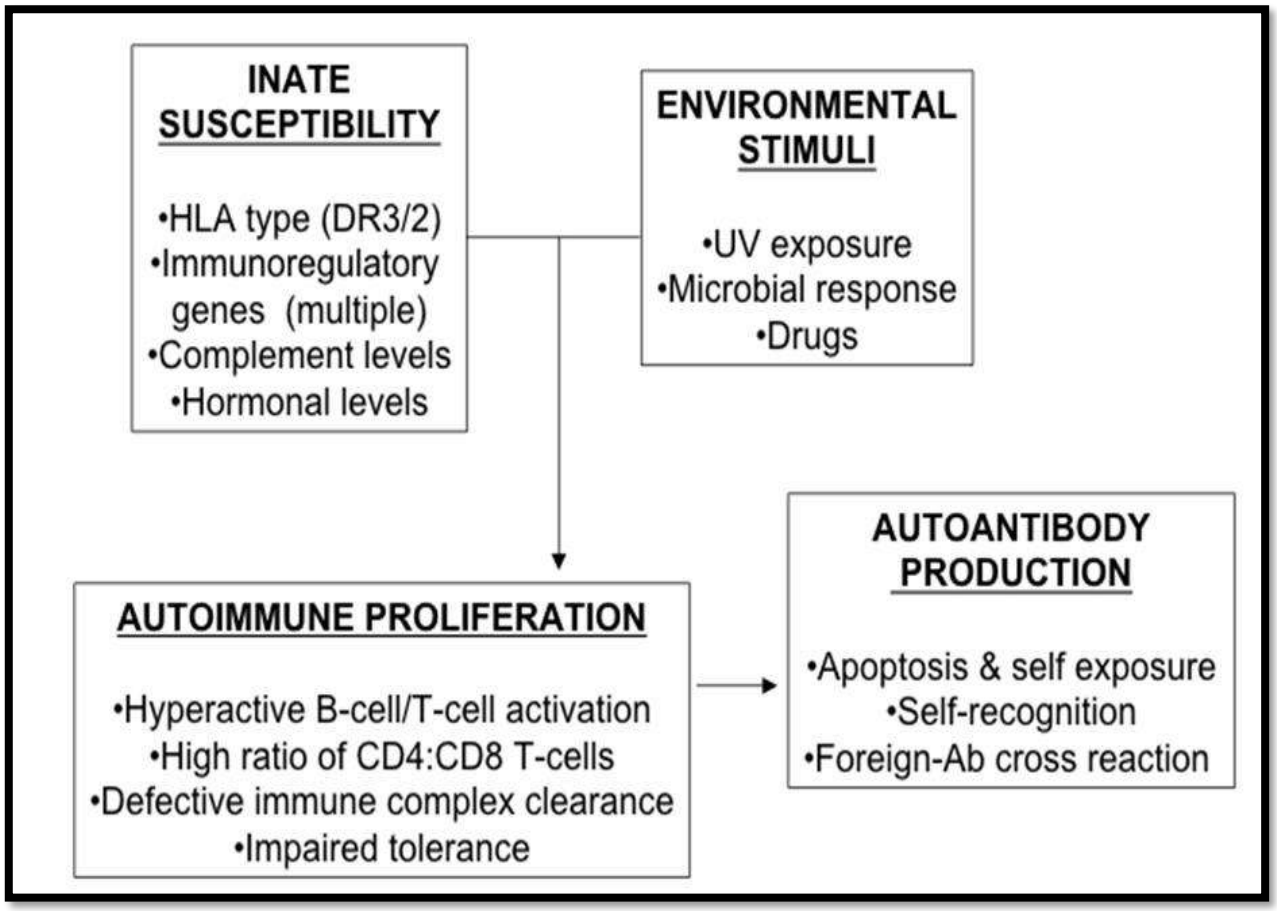
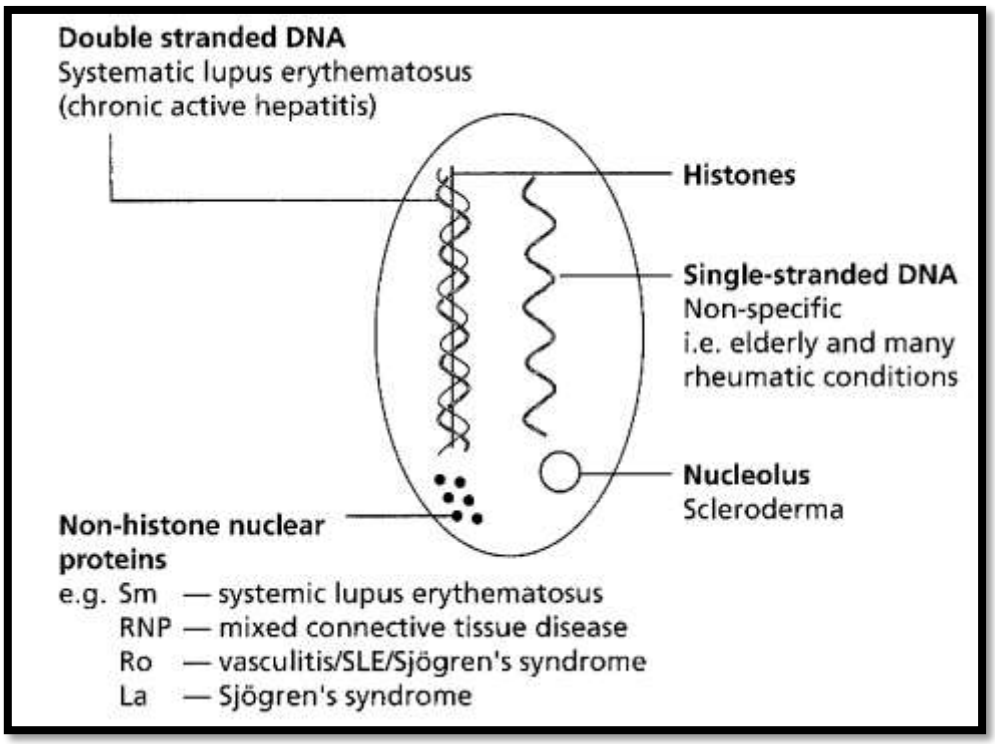


Figure: Etiology of SLE.



Nuclear antigens

Clinical Features

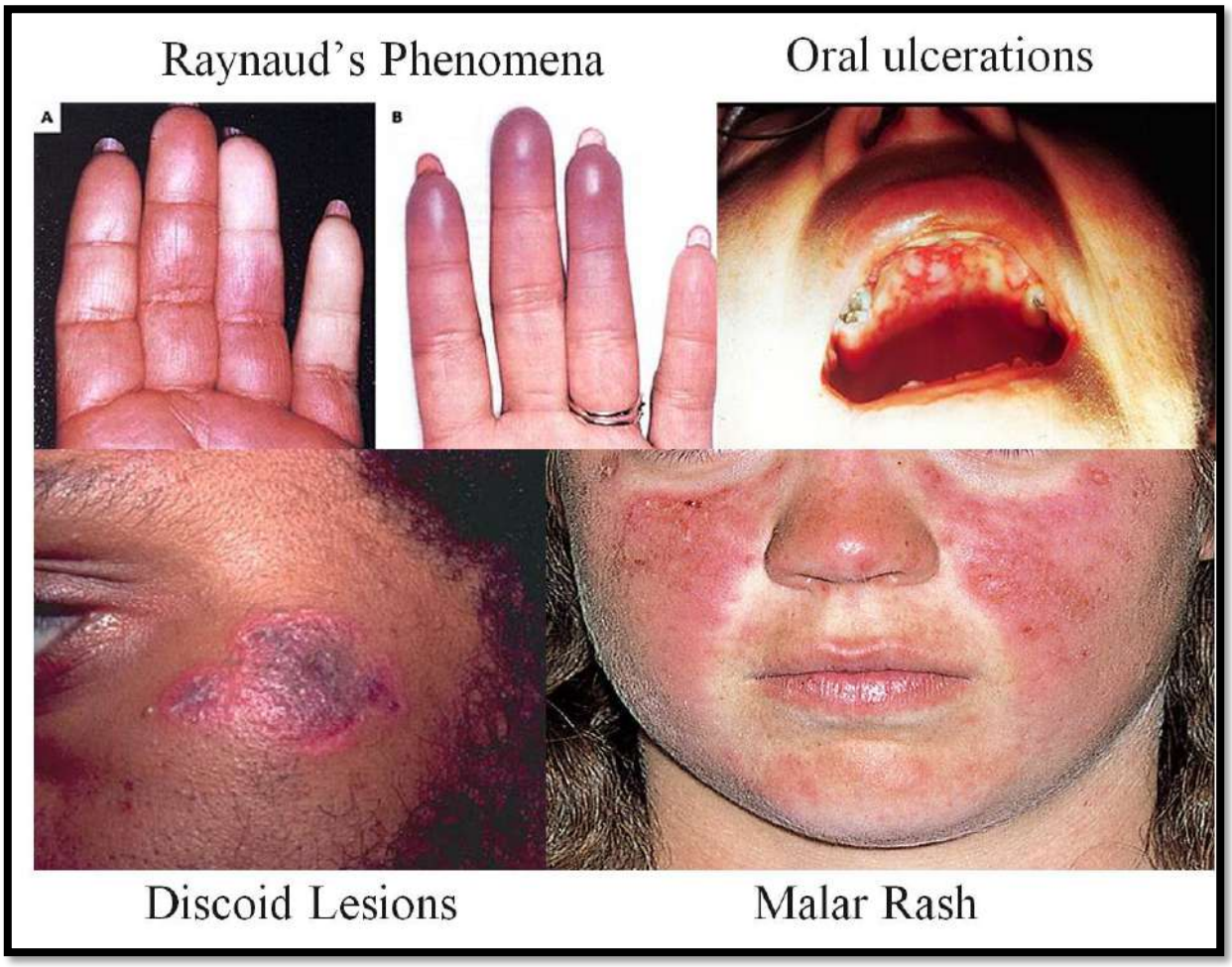
Systemic lupus erythematosus is a multisystem disease and can affect virtually all organs and system; whilst some manifestations are common, others are rare. Therefore, joint, skin and blood are affected in 80-100% of patients, kidneys, CNS and cardiopulmonary system in over 50%; while thrombosis, a typical lupus manifestation associated with possession of the anticardiolipin antibody, is present in 10% of patients. Systemic manifestations including fatigue, malaise, fever, anorexia, nausea and weight loss, are present in the great majority of patients.

The symptoms difference according to the infected organ and including arthritis, arthralgia, malar rash, an erythematous rash over the nasal bridge, photosensitivity, discoid lesions, headache, migraine, nephrotic syndrome, pleuritis and pericarditis.

Table 10.12 American College of Rheumatology criteria for diagnosis of systemic lupus erythematosus (SLE).*

Malar rash
Discoid rash
Photosensitivity
Oral ulcers
Non-erosive arthritis
Serositis (pleuritis/pericarditis)
Renal disease (persistent proteinuria/urinary casts)
Neurological disorder (seizures/psychosis)
Haemolytic anaemia/leucopenia/lymphopenia/thrombocytopenia
Antinuclear antibody
Antibodies to dsDNA/antibodies to extractable nuclear antigens/ antiphospholipid antibodies

* To establish a diagnosis of SLE a patient must have four or more of these criteria.



General laboratory findings

The most frequent laboratory alteration that is identified is normocromic normocytic anemia of chronic disorders. Occasinally a Coombs-positive haemolytic anemia is observed. Leukopenia (probably autoantibody mediated), especially lymphopenia, and thrombocytopenia are frequent. The erythrocyte sedimentation rate is typically elevated, while C-reactive protein tends to be normal. Urinalysis can show haematuria, proteinuria and renal casts in the presence of glomerulonephritis.

Immunological laboratory findings

All patients in whom SLE is suspected should be tested for antinuclear antibodies, including those to dsDNA and extractable nuclear antigens (ENA), and for antiphospholipid antibodies, as well as having their serum level of IgG and complement components, C3 and C4 measured, Antihistone antibodies are also present in patients with drug-induced SLE, most frequently associated with hydralazine and procainamide therapies.

The sero-immunological hallmark of SLE is **antinuclear antibodies (ANA)**, in the absence of ANA, the diagnosis of SLE is put into question, even though some 5% of patients may have an ANA-negative serology.ANA is currently detected by an indirect immunofluorescence technique, where diluted patients serum is applied to frozen tissue, especially liver, of rodent origin and cell lines of human origin, such as the HEp2 cell line derived from a laryngeal tumor, in which nuclei are prominent, are used as substrate to detect ANA. If the patient is ANA positive, the autoantibody will bind to nuclei. To reveal this binding, a second antibody tagged with fluorescent label is add. This second antibody will bind and ANA will then be seen by placing the preparation under a fluorescence microscope. Four patterns of fluorescence can be seen indicating different types of antinuclear antibodies.

Table. Immunofluorescence Patterns of Antinuclear Antibodies

Pattern	Antigen	Disease association(s)
Peripheral	Double-stranded DNA	SLE
Homogeneous	DNA-histone complexes	SLE and other connective tissue diseases
Speckled	Non-DNA nuclear antigens	
	Sm	SLE
	ribonucleoprotein	Mixed connective tissue disease, SLE, scleroderma, etc.
	SS-A, SS-B	Sjögren's disease
Nucleolar	Nucleolus-specific RNA	Scleroderma

ANA is a very sensitive test for SLE, being present in virtually all patients and frequently at high titers; its disease specificity is relatively low since it is frequently found in other rheumatic diseases, as well as in autoimmune liver disease, during viral infections and, occasionally, at low titers, in normal subjects.

DNA antibodies are the most important in SLE. They can react with **single-stranded DNA (ssDNA) or with double-stranded DNA (dsDNA)**.Although anti-ssDNA may be found in many diseases besides SLE, anti-dsDNA autoantibodies are found almost exclusively in

SLE (70%of the patients). While the disease specificity of dsDNA autoantibodies is high, that of ANA is low.

Anti-dsDNA autoantibodies are usually detected by very analytically sensitive technique, such as radioimmunoassay (RIA) or enzyme linked immunosorbent assay (ELISA).they can also be detected by immunofluorescence staining of an organelle called a kinotoplast in the flagellate *Crithdia luciliae,*which contains dsDNA.

In a patient with lupus nephritis, a kidney biopsy is frequently obtained for diagnostic reasons the glomeruli of such bioptic renal material contain antigen-antibody complexes. By applying a fluorescent antibody directed against human antibody (similar to that used in the second step of ANA detection) to frozen section of the kidney biopsy. This one step technique is known as direct immunofluorescence.

Extractable nuclear antigens (ENA) include **Sm** (Smith), **RNP** (ribonucleoprotein), **Ro** (Robert) also called SS-A (Sjogrens syndrome antigen A) and anti- **La** (Lane) or SS-B (Sjogrens syndrome antigen B).

Anti-ENA antibodies are include anti-Sm found almost exclusively in SLE, and anti-RNP more typically associated with mixed connective tissue disease than with SLE, anti- Ro and anti-La are found in Sjogrens syndrome. Other Anti-ENA antibodies are **anti-Jo-1**, anti **Scl-70** and **anticentromere**, which are associated mainly with polmyositis, systemic sclerosis and CREST syndrome respectively. Anti-ENA antibodies are normally detected by immunodiffusion or ELISA technique.

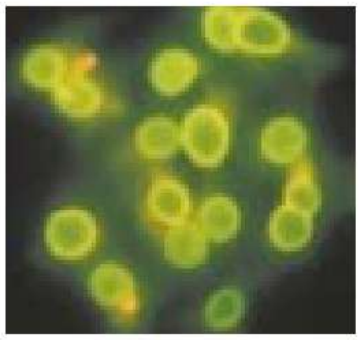
The lupus anticoagulant causes a prolonged clotting time in vitro but thrombosis in vivo. It is often found in associated with other autoantibodies to phospholipids, such as anticardiolipin antibodies and false positive tests for syphilis.

Assessment of the complement profile is of importance in management. Serial determinations of CH_{50}, a functional assay measuring complement hemolytic activity, and of the individual factors C3 and C4, inform on how much immune complexes are consuming complement.

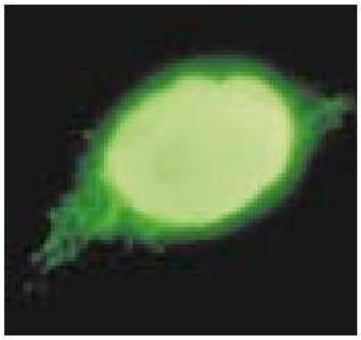
Table 10.13 Laboratory findings in untreated systemic lupus erythematosus (SLE)*.

Immunological test	%	Haematological	%	Others	%
dsDNA binding	70–85	Raised ESR	60	C-reactive protein–normal unless infection present	
Antinuclear bodies (high titre; IgG class)	95	Leucopenia	45	Proteinuria	30
Raised serum IgG level	65	Direct Coombs' test positive	40		
Low serum complement C3/C4 levels	60	Lupus anticoagulant	10–20		
Platelet antibodies	60				
Cryoglobulinaemia	60				
Antibodies to ENA:					
Sm	30				
RNP	35				
Ro	30				
La	15				
Antibodies to phospholipids	30–40				
Rheumatoid factor (low titre)	30				
Skin biopsy IgG, C3 and C4 deposits in normal skin	75				

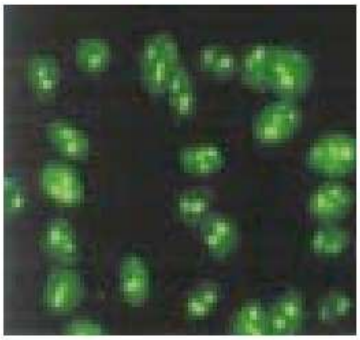
* Figures show percentage of patients with positive tests.
ESR, erythrocyte sedimentation rate; ENA, extractable nuclear antigens; RNP, ribonucleoprotein.



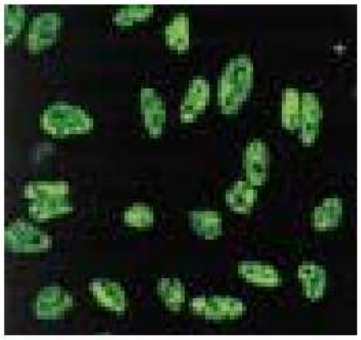
1. Rim pattern (150x magnif; anti-DNA)



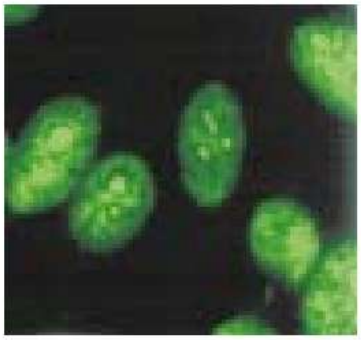
2. Homogenous pattern (435x enlarged; anti-DNA)



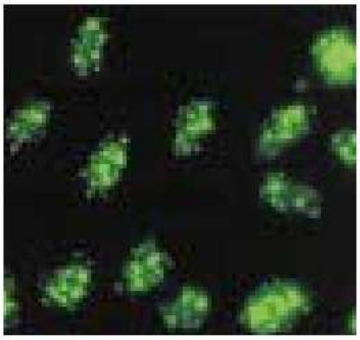
3. Nucleolar pattern (e.g. fibrillarin)



4. Coarse-speckled pattern (U1RNP/Sm)



5. Fine-speckled pattern (Ro/La)



6. Anti-centromere antibody pattern