

Interleukin 16 in Systemic Lupus Erythematosus and Lupus Nephritis

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Abstract

Background Systemic lupus erythematosus is a complex autoimmune disease with a wide spectrum of clinical manifestations, from mild to life-threatening conditions. Interleukin 16 is an immunomodulatory cytokine with reported roles in several autoimmune conditions and was identified as the most significantly enriched cytokine in both lupus erythematosus skin lesions and lupus nephritis urine proteomes.

Objective To review the biological characteristics of interleukin 16 and its reported roles in systemic lupus erythematosus, lupus nephritis, and other immune-mediated diseases, with emphasis on disease activity, renal histopathology, treatment response, and its potential utility as a urinary biomarker.

Conclusion Recent urinary proteomic studies have revealed a consistent elevation in interleukin 16 in the urine of patients with active lupus nephritis. An early decline in urinary interleukin 16 correlated with treatment response, and urinary interleukin 16 can distinguish between proliferative and pure membranous lupus nephritis. Its abundance also correlated with renal biopsy analyses, supporting its utility as a biomarker for monitoring intrarenal immune activity.

Keywords: *Systemic lupus erythematosus, Lupus nephritis, Interleukin 16, Disease activity*

Introduction

SLE is a complex autoimmune disease with a wide spectrum of clinical manifestations, from mild to life-threatening conditions. This disease leads to the inflammation of one or multiple organs including the skin, kidneys, joints and nervous system, contributing to the heterogeneity of clinical presentations. The reported incidence and prevalence of SLE vary widely across different countries and are strongly influenced by ethnicity, as well as environmental and genetic factors [1].

SLE pathogenesis is probably the result of complex interactions between ethnic, genetic, epigenetic, hormonal and environmental factors, which trigger an immunological disorder. However, only a small number of studies have examined whether environmental exposure interacts with genetic factors, despite the fact that a variety of exogenous triggers, including physical and chemical factors, have been proposed to influence the risk of SLE and lupus nephritis. [2].

Interleukin (IL) 16 is an immunomodulatory cytokine with reported roles in several autoimmune conditions[3].

IL-16 was also identified as the most significantly enriched cytokine in both LE skin lesion and LN urine proteomes [4].

Epidemiological Context

Epidemiological studies of SLE have been extensively conducted across the globe, revealing how various factors—including genetic predispositions, environmental influences, classification criteria, and the precision of

laboratory techniques employed for disease screening—can significantly impact the incidence and prevalence of SLE in different geographical regions [5].

Worldwide prevalence ranges from 15.87 to 108.92 per 100,000 people and incidence from 1.5 to 11.0 per 100,000 person-years, depending on geographic region and genetic background [5].

Epidemiological estimates of SLE vary substantially worldwide due to the variability in study design, environmental exposures, location and characteristics of the surveyed population, including race, sex and age [1].

People of African ethnicity had the highest incidence and prevalence of SLE, whereas those with Caucasian had the lowest incidence and prevalence [6].

However, notable differences exist between male and female SLE patients. Males tend to present with more severe forms of the disease and are generally diagnosed at an older age, often up to six years later than females [7].

Differences in SLE across sexes are influenced by genetic, hormonal, and immune response variations. The function of estrogen and its receptors in the pathogenesis is substantial, since they enhance immunological responses that worsen the disease, whereas androgens tend to improve it [8].

Pathogenesis of Systemic Lupus Erythematosus

The onset of SLE is linked to the collapse of immunological tolerance coupled with the interplay of genetic factors that predispose to SLE and a range of environmental triggers. This complex interaction culminates in the generation of autoantibodies with pathogenic potential. These lupus-associated autoantibodies collaborate with both innate and adaptive immune responses, promoting the accumulation of immune complexes across diverse tissues and organs. Such deposition initiates episodes of inflammation, both acute and chronic, ultimately inflicting damage upon the affected organs [9].

SLE exemplifies the interplay of genetic, epigenetic, environmental, and hormonal factors driving abnormalities in innate and adaptive immunity [10].

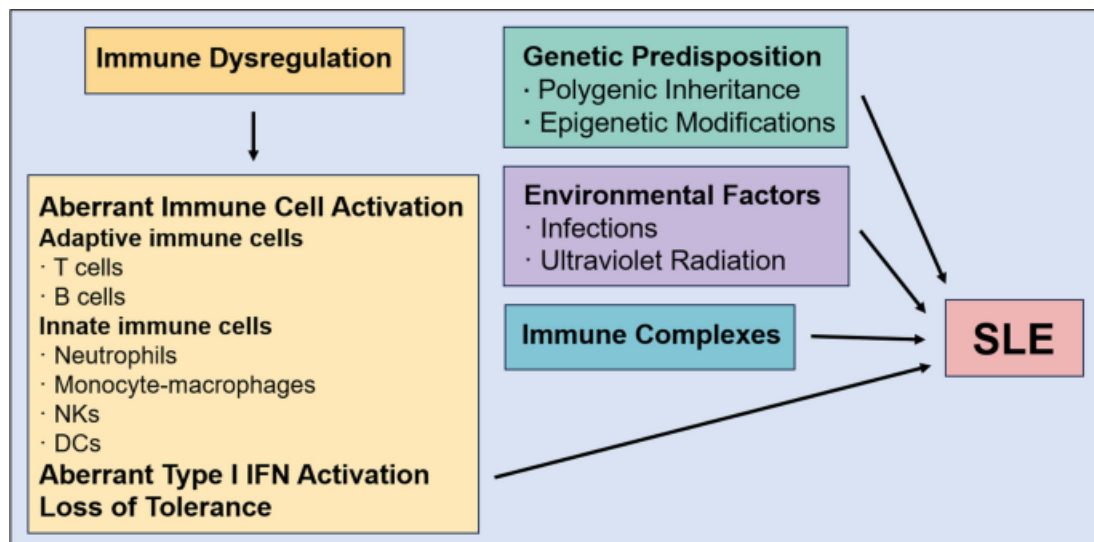


Figure 1 Pathogenesis of systemic lupus erythematosus

Source [9]

Genetic and Environmental Factors

Over 100 susceptibility loci in the human genome are linked to SLE and LN. Genetic variants are involved in loss of tolerance against nuclear autoantigens, abnormal lymphocyte and complement function and kidney damage. Genetic variants contribute to the racial disparities and clinical heterogeneity of SLE and LN [11].

SLE exhibits a strong familial accumulation with a much higher frequency among first-degree relatives of patients. SLE develops concordantly in approximately 25–50% of monozygotic twins and 5% of dizygotic twins.

In spite of the influence of heredity, most cases appear sporadically. Genetic and environmental factors are involved in the immune system dysregulation that can trigger the development of SLE [12].

Differences in disease presentation and progression between adult- (a SLE) and juvenile-onset (j)SLE patients may be caused by variable composition of inherited and acquired factors. Notably, compared to a SLE, an increased prevalence of common and rare genetic variants has been reported in jSLE, and increased genetic burden may result in early disease expression and more severe phenotypes [13].

The association among HLA, ethnicities and SLE is also evident. For example, HLA-DRB1*0301 was commonly found in Caucasians, while DRB1*1503 and DRB1*08 alleles were often discovered in African Americans and Hispanics. Interestingly, the presence of these alleles in certain ethnicities was linked to distinct manifestations of SLE in those populations [14].

In addition, HLA-DR2, HLA-DR3, several non-classical HLA genes, MHC class III genes and single-nucleotide polymorphism (SNP) MHC have also been reported to be associated with SLE [15].

It is currently known that different environmental factors could trigger SLE onset and flares in genetically susceptible individuals [16].

Exposure to environmental factors, such as ultraviolet B (UV-B) radiation, silica, cigarette smoke, oral contraceptives, infections and toxins, can impair immune tolerance in individuals with genetic susceptibility and cause aberrant autoimmunity activation [17].

Exposure to UV-B radiation induces the formation of reactive oxygen species and increases the expression of pro-inflammatory cytokines, such as IFN- α , IL-1, IL-6 and tumor necrosis factor (TNF)- α . In addition, UV-B also increases intercellular adhesion molecules (e. g. , ICAM-1 and LAF-1), IL-8 ligand secretion and chemokines (e. g. , chemokine (C-C motif) ligand [CCL]-5, CCL20, and CCL22), which recruits immune cells to areas of inflammation [17].

Exposure to UV-B radiation also causes activation of T lymphocyte cells that lasts longer, which is caused by upregulation of type I IFN and repression of Treg cells due to exposure to UV-B light [18].

II. Viral infections:

Several viruses have been reported to play a role in the pathogenesis of SLE including Epstein-Barr virus (EBV), cytomegalovirus (CMV), parvovirus B19, human immunodeficiency virus-1 (HIV-1) and hepatitis B virus vaccine [17].

One of the viruses known to influence the risk of SLE is EBV. In EBV infection, deficiencies in the complement pathway, components of the IFN pathway, and MHC-specific alleles may be involved. In addition, a process of molecular mimicry occurs in EBV infection. Antibodies to the EBV nuclear antigen (EBNA-1) can cross-react with SLE-associated autoantigens. In susceptible individuals, the immune response to EBNA-1 leads to the formation of cross-reactive antibodies, followed by epitope dissemination, which eventually develops into SLE [17].

Considering the sex and age predisposition of SLE, female sex hormones are undeniably involved in the pathogenesis of the disease [19].

Studies conducted on SLE-prone mice using gonadectomy/hormone deprivation and hormone supplementation have consistently confirmed this association, revealing that estrogen exacerbates the disease, while its removal ameliorates the disease in female subjects [20].

Sex hormones are involved in the development and function of innate and adaptive immune responses, and dysregulation of these mechanisms contributes to autoimmune abnormalities [21].

Estrogens are generally considered to have immune-stimulatory effects. Besides being present in reproductive tissues, estrogen receptors (ERs, ER α and ER β) are also widely expressed in most cells of the immune system, exerting influence on both innate and adaptive immune responses [22].

Pregnancy-associated changes in progesterone signaling has been considered important for innate immune surveillance and tolerogenic response, rendering progesterone a protective factor of SLE. Specifically, progesterone reduces the secretion of proinflammatory cytokines such as $\text{TNF}\alpha$, $\text{IL1}\beta$ and IL12 , leading to attenuated activities of primary players involved in both the innate and adaptive immune responses such as macrophages, DCs, CD4^+ and CD8^+ T cells [23].

Immune Dysregulation

The onset of SLE is linked to the collapse of immunological tolerance coupled with the interplay of genetic factors that predispose to SLE and a range of environmental triggers. This complex interaction culminates in the generation of autoantibodies with pathogenic potential. These lupus-associated autoantibodies collaborate with both innate and adaptive immune responses, promoting the accumulation of immune complexes across diverse tissues and organs. Such deposition initiates episodes of inflammation, both acute and chronic, ultimately inflicting damage upon the affected organs [9].

SLE exemplifies the interplay of genetic, epigenetic, environmental, and hormonal factors driving abnormalities in innate and adaptive immunity [10].

Immunological interplay driving SLE pathogenesis. In SLE, the pathogenesis of lupus nephritis involves an intricate interplay among B cells, T cells, DCs, and other immune components. Key aspects include: (1) Excessive production of autoantibodies by aberrant B cells leads to the formation of immune complexes, which deposit in the kidneys, activating the complement system and inciting inflammatory responses. (2) Autoreactive B cells present self-antigens to T cells, perpetuating immune dysregulation and fostering the release of pro-inflammatory mediators. (3) Dendritic cells exhibit aberrant recognition and presentation of self-antigens, thereby initiating and propagating autoimmune responses against renal tissue. (4) B cell differentiation into autoantibody-secreting plasma cells is dysregulated, resulting in the sustained production of nephritogenic autoantibodies. Elucidating these pathogenic mechanisms is pivotal for the development of novel therapeutic strategies aimed at halting or reversing the progression of lupus nephritis

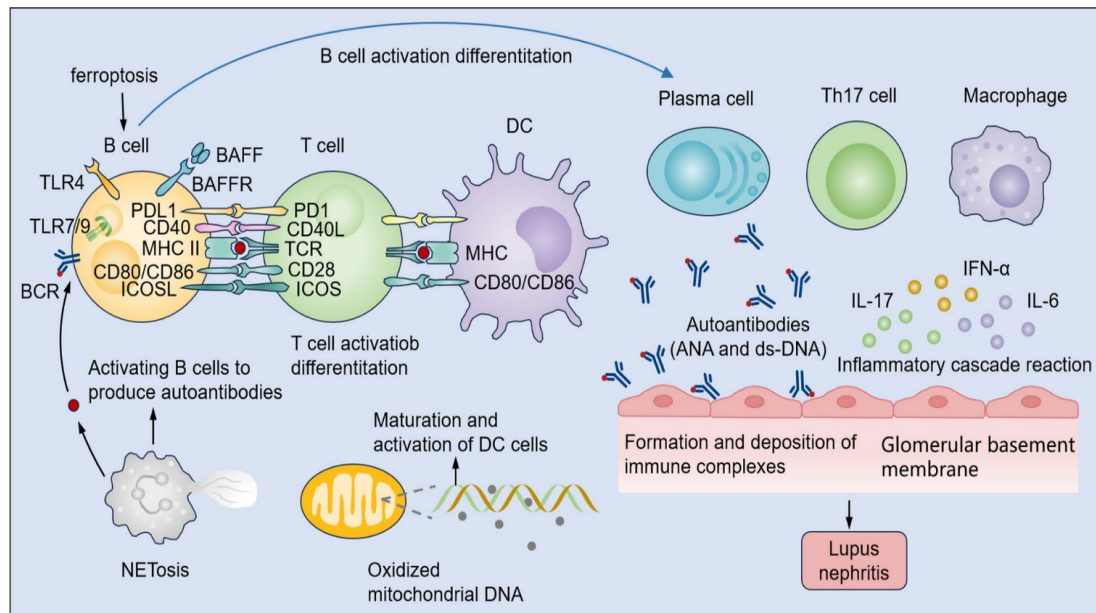


Figure 2 Immunological interplay driving systemic lupus erythematosus pathogenesis [9]

Innate Immune Activation

Macrophages are a heterogeneous population of immune cells, especially in such autoimmune diseases as SLE, under the influence of multiple factors. Thus, macrophages may exhibit different metabolic network characteristics in response to the effects of different autoantigens, profoundly affecting macrophage function [24].

Macrophages are members of the innate immune system that are crucial for inflammatory responses and host defense. They are mainly responsible for recognizing, phagocytosing, and degrading invading pathogens and cellular fragments and then presenting the processed antigenic components to other immune cells to drive the immune response [25].

Cross-linking of IgG immune complexes to the macrophage surface Fc γ receptor leads to increased cellular glycolysis and deepened tissue inflammation, suggesting glycolysis inhibition as a promising strategy to attenuate IgG-associated renal macrophage activation, pro-inflammatory cytokine secretion, and renal inflammation [24].

Macrophages, as pivotal players in the innate immune system, possess surface Toll-like receptors (TLRs) that, upon recognizing self-nucleic acids and other autoantigens, possess the potential to exacerbate autoimmune responses [26] [26].

Macrophages also play a pivotal role in activating and regulating the complement system, thereby significantly influencing the progression of LN [27] [27].

Macrophages possess the capability to modulate the functionality of neutrophils, encompassing the generation of NETs (neutrophil extracellular traps) and the regulation of LDGs (leukocyte-derived granules), subsequently influencing the inflammatory cascade within lymph nodes [28] [28].

Furthermore, during the early stage of inflammation, macrophages also amplify the inflammatory response by releasing a series of cytokines and chemokines that facilitate the recruitment of their effector cells. In addition to promoting inflammation, macrophages play important roles in tissue repair and fibrosis [29].

Neutrophils, one of the main constituents of innate immune system, are amongst the first responders to infectious agents and damaged tissues. They neutralize the invading pathogens by various mechanisms such as phagocytosis, degranulation, reactive oxygen species (ROS) production and (NET) formation [30].

Neutrophils also have contributory role in the pathogenesis and progression of autoimmune diseases by infiltrating sites of tissue inflammation. Respiratory burst and ROS production by neutrophils are considered risk factors for SLE and other autoimmune diseases. Another common contributing factor to SLE pathogenesis is NET formation which contains autoantigens recognized by adaptive immune system [31].

NETs are extracellular decondensed chromatin coated with different proteins such as histones, NE, MPO, high mobility group protein B1, proteinase 3 and LL-37 [32].

Alongside neutralizing invading pathogens, NETs have the capability to damage surrounding tissues because of their proteolytic enzymes or histone toxicity [33].

NET formation, termed “NETosis,” involves the release of chromatin from the neutrophil nucleus into the extracellular space [34].

During NET formation, nuclear and granule components which include DNA, RNA and granular proteins, such as myeloperoxidase, are expelled extracellularly into net-like structures that entrap and kill pathogens effectively [31].

In SLE, NETs are produced independently of pathogens and their clearance is impaired [35].

Components in serum from SLE patients, such as autoantibodies, are associated with increased NET formation [36].

NET formation leads to increased autoantigen exposure, interferon production, autoreactive B cell activation, autoantibody production and tissue damage [37].

Interferons (IFNs) are cytokines with pleiotropic roles in immune regulation that can be categorized into type I, II, and III based on sequence homology [38].

The IFN-I family consists of the IFN- α , IFN- β , IFN- δ , IFN- ϵ , IFN- κ , and IFN- ω subgroups, and 13 subtypes of IFN- α have been identified. Through binding to IFN- α receptor 1 (IFNAR1) and 2 (IFNAR2), which are universally expressed in all nucleated cells, IFN-I mediates its effects by stimulating Janus kinase (JAK)/signal

transducer and activator of transcription proteins (STAT) family proteins, whose activation triggers autoimmunity in SLE [39].

In patients with SLE, aberrant activation of IFN-I signaling, such as increased expression of STAT1, constitutive phosphorylation of JAK1 and STAT2, and an exaggerated response to IFN- β stimulation in immune cells, can be readily observed [40].

Because the generation of various autoantibodies, such as anti-double stranded DNA antibodies (anti-dsDNA Abs), is a critical process in the immunopathogenesis of SLE, IFN-I and IFN-I signaling facilitate the differentiation of extrafollicular B cells into short-lived antibody-forming cells [41].

IFN-I can promote the maturation of myeloid DCs, which promote the proliferation and differentiation of autoreactive T and B lymphocytes as well as the production of various cytokines, such as interleukin (IL)-6, IL-15, B lymphocyte stimulators, chemokines, and autoantibodies, and the formation of immune complexes that further trigger and exacerbate IFN-I autoimmunity through autocrine effects [42].

IFN-I may also affect chromatin remodeling and result in increased global epigenetic modifications, such as DNA hypomethylation and histone acetylation [43].

Although the extrarenal source of IFN-I is critical in the immunopathogenesis of lupus nephritis (30), importantly, local resident cells, such as mesangial cells, podocytes, tubular epithelial cells and endothelial cells, rather than infiltrating immune cells and hematologic cells, are the major sources of IFN- α in the kidney and are responsible for IFN- α -mediated renal damage in SLE patients [44].

Adaptive Immunity and Autoantibodies

Adaptive immune cells, including T lymphocytes, B lymphocytes, and plasma cells, play a central role in the disease's development. T cells may suffer a loss of self-tolerance, resulting in the stimulation of B cells by autoreactive cells. This activation leads to an overproduction of autoantibodies by B cells, which then form immune complexes. These complexes accumulate in tissues, initiating an inflammatory response. Innate immune cells, such as neutrophils, monocyte-macrophages, natural killer cells and DCs, also contribute significantly to SLE pathogenesis [45].

T follicular helper (TFH) and Th cells are an important pathogenic subset of CD4⁺ T cells in SLE. An increased TFH/Treg ratio can be observed in the peripheral blood of patients with active LN, especially in classes III and IV, and immunohistochemistry has revealed TFH1 cell infiltration in kidneys with LN [46].

There are two types of T cells, i. e. , $\alpha\beta$ ⁺ and $\gamma\delta$ ⁺ T cells, and $\alpha\beta$ ⁺ T cells can be further classified into CD4⁺ and CD8⁺ T cells. While CD8⁺ T cells take on the cell-killing activity [47].

CD4⁺ T cells are T helper (Th) cells capable of promoting CD8⁺ T cell development and B cell differentiation as well as antibody synthesis [48].

CD4⁺ T cells can be roughly subdivided into Th1, Th2, Th17 and regulatory T (Treg) cells, based on their distinct cytokine profilings. Furthermore, T-helper 17 (Th17) cells play a role in the pathogenesis of SLE, acting as the main producers of IL-17, a highly inflammatory cytokine. This cytokine promotes the recruitment of neutrophils, triggers the activation of the innate immune system, and enhances the functionality of B lymphocytes [49].

Regulatory T cells (Tregs) play a vital role in preserving peripheral tolerance towards self-antigens. In SLE, there is evidence of both quantitative and qualitative disparities in Tregs, yet research has yielded inconsistent findings, leaving their precise role in SLE yet to be fully defined. Tregs, owing to their suppressive effect on effector T lymphocytes, could be instrumental in foundational cellular therapies for SLE [50].

T-follicular helper (Tfh) cells, found in both germinal centers and extrafollicular areas, are associated with the expansion of autoreactive B-cell clones. This association has been observed in both mouse models and in individuals with SLE [51].

These cells have been observed to congregate with B cells in renal tissues, mirroring their aggregation in germinal centers during lupus nephritis [52].

CD8 + T cells contribute to the immune-mediated pathology observed in SLE. CD8 T lymphocytes in the peripheral blood of SLE patients exhibit functional deficits, such as an impaired ability to lyse target cells and a reduction in the synthesis of granzymes and perforins [53].

B cells contribute to the pathogenesis of SLE through their response to antigens and autoantibody production. The pathways implicated in the aberrant activation of B cells include the toll-like receptor (TLR) pathway, stimulation via beta cell-activating factor (BAAF), and B-cell receptor (BCR) mediated activation. The stimulation of B cells through the TLR pathway promotes loss of tolerance [54].

Transitional B cells are susceptible to TLR9 stimulation and produce autoreactive marginal zone B cells, as seen in SLE patients. An impairment of B cell tolerance can also occur through B cell stimulation from cytokines, particularly BAFF. SLE patients with high levels of BAFF exhibit significantly higher levels of antidsDNA, anti-histone, and anticardiolipin antibodies [55].

Furthermore, BCR is a crucial regulator of negative and positive selection, and continuous BCR sensing is necessary for B cell survival in healthy individuals. SLE patients with polymorphisms of the c-*Src* tyrosine kinase (*Csk*) gene exhibit a higher level of BCR-mediated activation of B cells and a higher concentration of serum IgM levels [56].

SLE is characterized as an autoimmune disease showing multiple organ involvement and complex clinical manifestations. The disease onset of SLE is related to heredity, infection, and ultraviolet radiation, etc. Numerous studies reported that B cells in SLE patients are aberrantly activated in response to self-antigens, producing a large number of auto-reactive antibodies (autoantibodies), potentially triggering an inflammatory cascade response and is closely related to the occurrence of SLE [57].

A hallmark of SLE is the presence of a broad spectrum of autoantibodies directed against nuclear, cytoplasmic, and cell surface antigens [58]

The role of autoantibodies in the pathogenesis of the disease has traditionally focused on deposition of immune complexes in the skin, the renal glomeruli and other tissues, along with focusing on direct targeting of antibodies to antigens deposited in situ [59].

The detection of specific autoantibodies is a prerequisite for the diagnosis of SLE, reflected in the inclusion of immunologic criteria in the 2019 EULAR/ACR classification criteria. Beyond their diagnostic value, autoantibodies also provide prognostic insights, helping stratify patients based on risk of organ involvement, disease activity, and long-term outcomes [60].

The pathophysiology of autoantibody production includes defects in apoptotic clearance, dysregulated type I interferon (IFN) signaling, B cell tolerance breakdown, and abnormal T cell help . Additional contributors include neutrophil extracellular trap (NET) formation, complement pathway dysfunction, genetic susceptibility, and epigenetic modifications [55].

Clinical Heterogeneity and Renal Involvement

SLE exhibits a broad spectrum of presentations ranging from mild symptoms to severe, life-threatening conditions. Adults diagnosed before 50 years of age usually present with cutaneous symptoms (malar rash) and renal abnormalities (lupus nephritis), displayed higher 10-year survival, and reported using more immunosuppressive therapy than patients getting diagnosed after 50 [61].

Preclinical lupus (PL) is a phase in developing SLE when the patient is at higher risk of developing SLE but is found asymptomatic on presentation. However, autoantibodies are mostly detectable in these patients' serum [62].

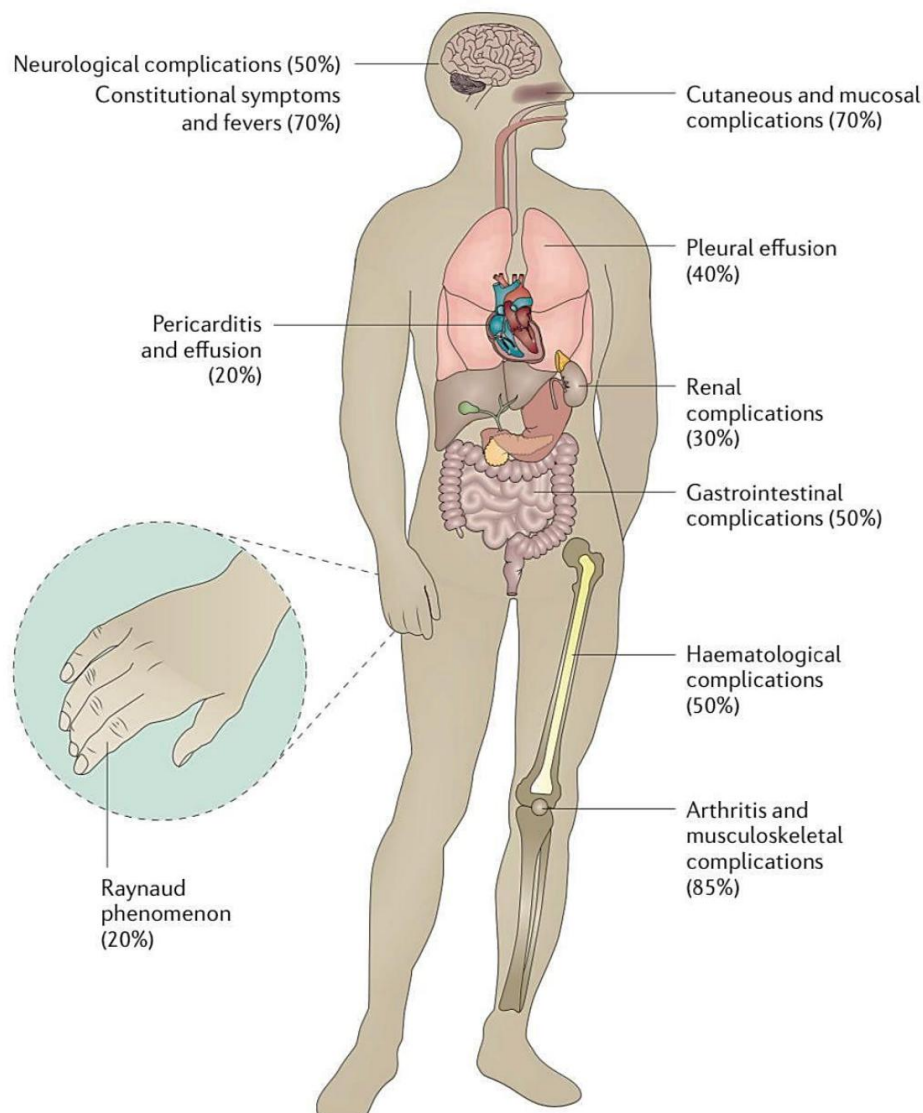


Figure 3 Clinical heterogeneity of systemic lupus erythematosus [59]

Lupus Nephritis

Lupus nephritis is the most common and severe complication of SLE. Approximately 40% to 60% of patients with SLE have lupus nephritis, and most cases occur within 5 years of SLE diagnosis. Despite significant advances in the understanding and treatment of lupus nephritis in recent years, treatment outcomes for lupus nephritis remain unsatisfactory and lupus nephritis remains a major cause of morbidity and mortality in patients with SLE [63].

If not recognized and treated urgently, LN significantly increases the risk of disease burden and irreversible kidney damage, leading to end-stage kidney disease (ESKD) [64].

To underline this, the recently updated EULAR recommendations for SLE highlight the need for 'regular screening for organ involvement (especially nephritis)' within one of the overarching principles for the management of the disease [65].

At the same time, the increasing availability of new serum and urinary biomarkers is changing the role of renal biopsy as a diagnostic and prognostic tool [66].

Risk factors for progressive kidney disease are not fully recognized but include neuropsychiatric lupus, pediatric onset, male sex, race other than Caucasian, poor socioeconomic status, hypertension, impaired renal

function at the time of renal biopsy, anemia, presence of anti-dsDNA antibodies, persistent hypocomplementemia, frequent relapses or incomplete remission [64].

The manifestation of lupus nephritis (LN) varies from subclinical laboratory abnormalities to overt nephritis, nephrotic syndrome, and rapidly progressive renal failure [67].

LN is characterized by inflammation of the kidney arising from complex interactions between the innate and adaptive immune responses and the kidney parenchyma [68].

The pathogenesis involves several genetic variants, including those that hinder the efficient elimination of dying cells, resulting in the persistence of nuclear antigens in the extracellular space. The released autoantigens act as triggers for innate and adaptive immune responses, while other genetic variants influence the magnitude of these immune reactions and the proper function of immune tolerance checkpoints, ultimately promoting the expansion of autoreactive T and B lymphocytes [69].

Consequently, circulating antinuclear antibodies, along with other autoAbs, lead to the formation of ICs that propagate systemic inflammation, leading to organ damage. The deposition of ICs in the renal microvasculature triggers inflammation and organ injury, accounting for the extensive morbidity and mortality associated with the disease [59].

Histologically, there are six distinct classes of nephropathy classified by the International Society of Nephrology/Renal Pathology Society (ISN/RPS) that represent different manifestations and severities of renal involvement in SLE [69]. The six histological classes are defined by specific microscopic lesions and distribution of immune complexes as shown in Table 1.

Table 1. International Society of Nephrology/Renal Pathology Society (ISN/RPS) histologic classification of lupus nephritis. [92].

The ISN/RPS Classification of Lupus Nephritis		
Class I	Minimal mesangial LN	Mesangial immune complex deposits with no mesangial hypercellularity
Class II	Mesangial proliferative LN	≥4 nuclei fully surrounded by matrix in mesangial areas away from the hilar region
Class III	Focal LN	<50% of glomeruli with endocapillary hypercellularity, fibrinoid necrosis, crescents, and/or segmental or global scars*
Class IV	Diffuse LN	≥50% of glomeruli with endocapillary hypercellularity, fibrinoid necrosis, crescents, and/or segmental or global scars*
Class V	Membranous LN	Subepithelial and/or intramembranous immune deposits, with or without mesangial deposits
Class VI	Advanced sclerosing LN	≥90% globally sclerosed glomeruli without residual activity

Classification Disease Activity and Damage

The Systemic Lupus International Collaborating Clinics (SLICC) classification criteria was introduced in 2012[7]. To be classified as SLE, the patient must meet at least 4 of 17 criteria, including at least 1 clinical and 1

immunological criterion or documented lupus nephritis (LN) with antinuclear antibodies (ANA) and/or anti-dsDNA. SLICC criteria is reported to have partially succeeded in improved performance over the ACR criteria, in that it had increased sensitivity (92–99% vs 77–91%) at the expense of reduced specificity (74–88% vs 91–96%) [70].

A novel SLE classification criteria jointly supported by European League Against Rheumatism (EULAR) and ACR was introduced in 2019 as shown in Table 2, and is based on two concepts, namely the presence of ANA as an entry criterion coupled with a scoring system based on variably weighed clinical and laboratory features. Performance characteristics in adult onset SLE found a sensitivity of EULAR/ACR to be similar to the SLICC criteria (98% vs 95%) [60].

Table 2. The 2019 EULAR/ACR classification criteria for systemic lupus erythematosus. [60].

<u><i>Clinical domains and criteria</i></u>	<u><i>Weight</i></u>
Constitutional Fever	2
Hematologic Leukopenia	3
Thrombocytopenia	4
Autoimmune hemolysis	4
Neuropsychiatric Delirium	2
Psychosis	3
Seizure	5
Mucocutaneous Non scarring alopecia	2
Oral ulcers	2
Subacute cutaneous OR discoid lupus	4
Acute cutaneous lupus	6
Serosal Pleural or pericardial effusion	5
Acute pericarditis	6
Musculoskeletal Joint involvement	6
Renal Proteinuria >0.5g/24h	4
Renal biopsy Class II or V lupus nephritis	8
Renal biopsy Class III or IV lupus nephritis	10
<u><i>Immunology domains and criteria</i></u>	
Antiphospholipid antibodies Anti-cardiolipin antibodies OR Anti-β2GP1 antibodies OR Lupus anticoagulant	2
Complement proteins Low C3 OR low C4	3
Low C3 and low C4	4
SLE-specific antibodies Anti-dsDNA antibody* OR Anti-Smith antibody	6

For the determination of disease activity in SLE, various scoring systems have been developed the Systemic Lupus Activity Measure (SLAM), Systemic Lupus Erythematosus Disease Activity Index (SLEDAI), Lupus Activity Index (LAI), British Isles Lupus Assessment Group index (BILAG), and the European Consensus Lupus Activity Measure (ECLAM) that mainly include clinical findings and laboratory parameters[71].

SLEDAI-2K is based on the presence of 24 descriptors in nine organ systems over the preceding 30 days. Descriptors of SLEDAI-2K are documented as present or absent. Each of the descriptors has a weighted score and the total score is the sum of all 24 descriptor scores (Table 3) [72].

Table 3. Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K). [72].

SLEDAI-2K (30 DAYS) DATA COLLECTION SHEET			
Study No.: _____		Patient Name: _____	
		Visit Date: _____ d _____ m _____ yr	
(Enter weight in SLEDAI-2K Score column if descriptor is present at the time of the visit or in the preceding 30 days)			
Weight	SCORE	Descriptor	Definition
8	☐	Seizure	Recent onset, exclude metabolic, infections, or drug causes.
8	☐	Psychosis	Altered ability to function in normal activity due to severe disturbance in the perception of reality. Include hallucinations, incoherence, marked loose associations, impoverished thought content, marked illogical thinking, bizarre, disorganized, or catatonic behavior. Exclude uremia and drug causes.
8	☐	Organic brain syndrome	Altered mental function with impaired orientation, memory, or other intellectual function, with rapid onset and fluctuating clinical features, inability to sustain attention to environment, plus at least 2 of the following: perceptual disturbance, incoherent speech, insomnia or daytime drowsiness, or increased or decreased psychomotor activity. Exclude metabolic, infectious, or drug causes.
8	☐	Visual disturbance	Retinal changes of SLE. Include cytoid bodies, retinal hemorrhages, serous exudate or hemorrhages in the choroid, or optic neuritis. Exclude hypertension, infection, or drug causes.
8	☐	Cranial nerve disorder	New onset of sensory or motor neuropathy involving cranial nerves.
8	☐	Lupus headache	Severe, persistent headache; may be migrainous, but must be nonresponsive to narcotic analgesia.
8	☐	CVA	New onset of cerebrovascular accident(s). Exclude arteriosclerosis.
8	☐	Vasculitis	Ulceration, gangrene, tender finger nodules, periungual infarction, splinter hemorrhages, or biopsy or angiogram proof of vasculitis.
4	☐	Arthritis	≥2 joints with pain and signs of inflammation (i.e., tenderness, swelling, or effusion).
4	☐	Myositis	Proximal muscle aching/weakness, associated with elevated creatine phosphokinase/aldolase or electromyogram changes or a biopsy showing myositis.
4	☐	Urinary casts	Heme-granular or red blood cell casts.
4	☐	Hematuria	>5 red blood cells/high power field. Exclude stone, infection, or other cause.
4	☐	Proteinuria	>0.5 gram/24 hours.
4	☐	Pyuria	>5 white blood cells/high power field. Exclude infection.
2	☐	Rash	Inflammatory type rash.
2	☐	Alopecia	Abnormal, patchy or diffuse loss of hair.
2	☐	Mucosal ulcers	Oral or nasal ulcerations.
2	☐	Pleurisy	Pleuritic chest pain with pleural rub or effusion or pleural thickening.
2	☐	Pericarditis	Pericardial pain with at least 1 of the following: rub, effusion, or electrocardiogram or echocardiogram confirmation.
2	☐	Low complement	Decrease in CH50, C3, or C4 below the lower limit of normal for testing laboratory.
2	☐	Increased DNA binding	Increased DNA binding by Farr assay above normal range for testing laboratory.
1	☐	Fever	>38° C. Exclude infectious cause.
1	☐	Thrombocytopenia	<100,000 platelets/ $\times 10^9/L$, exclude drug causes.
1	☐	Leukopenia	<3000 white blood cells/ $\times 10^9/L$, exclude drug causes.

The damage should be assessed annually using the SLICC/ACR Damage Index for SLE (Systemic Lupus International Collaborating Clinics/American College of Rheumatology) (Table 4) [73].

Table 4. SLICC/ACR Damage Index for systemic lupus erythematosus.. [73].

Item	Score	Item	Score
Ocular (either eye by clinical assessment)		Peripheral vascular	
Any cataract ever	0, 1	Claudication for 6 months	0, 1
Retinal change or optic atrophy	0, 1	Minor tissue loss (pulp space)	0, 1
Neuropsychiatric		Significant tissue loss ever (eg, loss of digit or limb) (score 2 if >1 site)	0, 1, 2
Cognitive impairment (eg, memory deficit, difficulty with calculation, poor concentration, difficulty in spoken or written language, impaired performance level) or major psychosis	0, 1	Venous thrombosis with swelling, ulceration or venous stasis	0, 1
Seizures requiring therapy for 6 months	0, 1	Gastrointestinal	
Cerebrovascular accident ever (score 2 if >1)	0, 1, 2	Infarction or resection of bowel below duodenum, spleen, liver or gallbladder ever, for any cause (score 2 if >1 site)	0, 1, 2
Cranial or peripheral neuropathy (excluding optic)	0, 1	Mesenteric insufficiency	0, 1
Transverse myelitis	0, 1	Chronic peritonitis	0, 1
Renal		Stricture or upper gastrointestinal tract surgery ever	0, 1
Estimated or measured glomerular filtration rate <50%	0, 1	Chronic pancreatitis	0, 1
Proteinuria >3.5 g/24 h	0, 1	Musculoskeletal	
or end-stage renal disease (regardless of dialysis or transplantation)	or 3	Muscle atrophy or weakness	0, 1
Pulmonary		Deforming or erosive arthritis (including reversible deformities, excluding avascular necrosis)	0, 1
Pulmonary hypertension (right ventricular prominence, or loud P2)	0, 1	Osteoporosis with fracture or vertebral collapse (excluding avascular necrosis)	0, 1
Pulmonary fibrosis (physical and radiographical)	0, 1	Avascular necrosis (score 2 if >1)	0, 1, 2
Shrinking lung (radiograph)	0, 1	Osteomyelitis	0, 1
Pleural fibrosis (radiograph)	0, 1	Tendon rupture	0, 1
Pulmonary infarction (radiograph)	0, 1	Skin	
Cardiovascular		Scarring chronic alopecia	0, 1
Angina or coronary artery bypass	0, 1	Extensive scarring of panniculum other than scalp and pulp space	0, 1
Myocardial infarction ever (score 2 if >1)	0, 1, 2	Skin ulceration (excluding thrombosis for >6 months)	0, 1
Cardiomyopathy (ventricular dysfunction)	0, 1	Premature gonadal failure	0, 1
Valvular disease (diastolic murmur, or systolic murmur >3/6)	0, 1	Diabetes (regardless of treatment)	0, 1
Pericarditis for 6 months or pericardiectomy	0, 1	Malignancy (exclude dysplasia) (score 2 if >1 site)	0, 1

Table 4. SLICC/ACR Damage Index for systemic lupus erythematosus.

Source: Gladman et al. [73].

Biology of Interleukin 16

IL-16 is a cytoplasmic protein cleaved by caspase-3 on various triggers. After cleavage, a mature C-terminal IL-16 is secreted and involved in chemoattraction, migration and activation of cluster of differentiation 4 (CD4) T cells and may also mediate effects by binding to C-C chemokine receptor type 5 (CCR5) and CD9 [74].

The N-terminal pro-IL-16 translocates to the nucleus and may impact cell cycle [75].

Physiologically IL-16 is expressed by cells of lymphoid organs: bone marrow, spleen, lymph nodes, thymus, tonsils and appendix, while tissues of the lower gastrointestinal tract express IL-16, but at a lower level. The majority of other tissues express mRNA for IL-16, and presumably can translate and secrete IL-16 under certain conditions. Among other tissue cells IL-16 has been found in cutaneous, cardiac, renal fibroblasts and bronchial epithelial cells [76]. The majority of the immune cell types can express IL-16 protein. The CD4⁺ and CD8⁺ positive T cells are the best studied expressors of IL-16, but also neutrophils, B-cells, eosinophils, dendritic cells, mast cells, macrophages and monocytes, natural killer cells all are found to express IL-16 [77].

Several receptors for IL-16 have been identified: CD4, CD9 and CCR5. CD4 is regarded as the major receptor as it is expressed by a range of hematopoietic and immune cells, as well on neuronal cells. The main ligand of IL-16 is D4 domain of CD4 [78].

Human T cells are the most studied source and target of IL-16. T cell activation by antigens, mitogens or vasoactive amines (like histamine or serotonin) may result in activation of casp-3 and cleavage of mature IL-16, which can be secreted. This process was described in both CD4⁺ and CD8⁺ T cells. While casp-3 is constantly active in CD8⁺ cells, continuously produced and cleaved IL-16 is stored in secretory vesicles, activation of casp-3 in CD4⁺ T cells must be induced by a stimuli, and thereafter IL-16 can be cleaved [74].

IL-16 has been implicated in multiple human conditions including inflammatory, autoimmune diseases, infections and cancers. Importantly, a recent major screening study for novel drug target evaluation identified IL-16 as a novel protein with causal evidence in multiple human diseases, a protein that has not been targeted so far [79].

Interleukin 16 in Other Immune Mediated Diseases

Rheumatoid Arthritis

High levels of IL-16 are found in the synovial fluid of RA joints and the lining layer of RA synovia [80].

It is considered that IL-16 is produced by fibroblasts and attracts CD4⁺ cells to the synovial infiltrates [81].

In vitro, dexamethasone treatment blocked RA patient derived immunoglobulins (IgG) induced T cell migration and IL-16 expression [82].

Psoriasis and Systemic Sclerosis

In psoriasis, a common immune-mediated skin disease, serum levels of IL-16 correlate with disease severity measured by psoriasis area and severity index (PASI). Cutaneous inflammatory infiltrates have abundant IL-16 carrying and producing cells, the majority of which express CD4 [83].

SSc (also known as scleroderma), a rheumatic disease manifesting as vasculopathy and fibrosis of connective tissue. The serum IL-16 levels in SSc patients are elevated. In the diffuse form, the amount of skin infiltrating IL-16 positive cells were higher than in those with the limited SSc form or controls, indicating that high IL-16 associate with a more severe SSc [84].

Vasculitis

Antineutrophil cytoplasmic antibody associated (ANCA) vasculitis, a group of rare, but life threatening, systemic diseases affecting small vessels, including those of vital organs, has been associated with high levels of circulating IL-16; in addition, circulating IL-16 levels correlated with disease damage [3].

In Takayasu vasculitis, inflammation affects the walls of large vessels and IL-16 expression was observed both in the circulation and intramural inflammatory infiltrates, and IL-16 levels correlated with amounts of CD4⁺ cells infiltrating large vessel walls, which induce arterial lesions [85].

Inflammatory Bowel Disease

Increased levels of IL-16 are observed in colonic mucosal biopsies in patients with Crohn disease and ulcerative colitis. Treatment with steroids efficiently reduced IL-16 expression in the gut mucosa to the levels as low as in controls [86].

Anti-IL-16 mAb treatment significantly reduced signs of disease in an animal colitis model (trinitrobenzenesulfonic acid (TNBS) -induced), such as weight loss, mucosal ulceration, myeloperoxidase activity ($P < 0.001$), as well as reduced mucosal levels of IL-1 β and tumor necrosis factor- α (TNF- α) [87].

Multiple Sclerosis

MS, a chronic inflammatory demyelinating and degenerative disease of the CNS, also appears to be affected by IL-16. In the brain lesions of relapsing and remitting (RRMS) lesions CD4+, CD8+, CD83+, DCs and B cells have been observed to express IL-16, as well as casp-3 [75].

Circulating levels of IL-16 correlate to markers of axonal damage (such as medium and heavy chains of neurofilaments) and cells carrying IL-16 are found in proximity to MS lesions [88].

While levels of serum IL-16 drop after successful treatment with IFN β -1a and was suggested as a marker of clinical response [89].

Anti-IL-16 Ab injection had therapeutic value in a mouse model of MS (EAE) [90]

Interleukin 16 in Systemic Lupus Erythematosus and Lupus Nephritis

IL-16, a potent T-cell chemoattractant, significantly correlates with renal activity and the NIH indices—histopathology-based scoring systems designed to quantify the severity and progression of LN [4].

Recent urinary proteomic studies have revealed a consistent elevation in IL-16 in the urine of patients with active LN. Moreover, an early decline in urinary IL-16 at 3 months correlated with treatment response to immunosuppression—a response determined based on UPCR, serum creatinine, and the dose of prednisone—outperforming traditional measures like UPCR [91].

In addition to its predictive value for treatment response, IL-16 can distinguish between proliferative LN and pure membranous LN with an AUC of 0.89. Additionally, urinary IL-16 abundance correlated with single-cell RNA sequencing analyses of renal biopsies, indicating that IL-16 is produced by infiltrating immune cells in LN kidneys, supporting its utility as a biomarker for monitoring intrarenal immune activity [4].

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