

INTRODUCTION

Systemic Lupus Erythematosus

Systemic lupus erythematosus (SLE) is one of the systemic autoimmune diseases characterized by the presence of autoantibodies, formation of immune complexes (ICs) and immune deregulation. It has chronic, relapsing and inflammatory features and often associated with febrile attacks. It mainly involves skin, joints, kidneys and serosal membranes leading to multi-organ damage. ⁽¹⁾

Incidence, Prevalence and Risk Factors:

The reported prevalence of SLE in the population is 20 to 150 cases per 100,000. In women, prevalence rates vary from 164 (white) to 406 (African American) per 100,000. Estimated incidence rates are 1 to 25 per 100,000 in North America, South America, Europe and Asia. The incidence was nearly tripled in the past 40 years of the 20th century and it is more common in urban than rural areas due to improved detection of the disease. It is also more common in Asians, immigrant Africans (Afro-Americans, Afro-Caribbeans, and Hispanic Americans) compared with Americans of European decent in the United States (US). In comparison, SLE occurs infrequently in Blacks in Africa. In New Zealand, the prevalence and mortality rates of SLE are higher in Polynesians than in Caucasians. ⁽²⁾

The disease is more common in women of childbearing age. This has been in part attributed to estrogen effect. In children, the female-to-male ratio is 3:1 but in adults, especially women of child-bearing age, the ratio ranges from 7:1 to 15:1 and in older individuals, especially post-menopausal women, the ratio is approximately 8:1. Also, women with early menarche or treated with estrogen-containing medications have a significantly increased risk for SLE. Most patients with SLE have disease onset between the ages of 16 and 55. Median ages at diagnosis are higher for white nations than black ones and for males than females. ^(3, 4)

Although the clinical status is poorer in those with less education, lower socioeconomic status, blacks and Mexican Hispanics in US have a poorer renal prognosis than Caucasians independent of socioeconomic status. This may be attributed to the lower response to cyclophosphamide (CYC) than whites. Moreover, blacks are more prone to have anti-Smith antibody (anti-Sm Ab), anti-ribonucleoprotein antibody (anti-RNP Ab), discoid skin lesions, proteinuria, psychosis and serositis. ^(4, 5)

Men with lupus tend to have higher frequencies of renal disease, skin manifestations, cytopenias, serositis, neurologic involvement, thrombosis, cardiovascular disease, hypertension, and vasculitis than women. In contrast, Raynaud's phenomenon, photosensitivity, and mucosal ulceration are less frequent manifestations in men than women. Most studies suggest that men have a higher one-year mortality rate. ⁽⁶⁾ Lupus seems to be milder in elderly patients but with greater prevalence of sicca symptoms, serositis, pulmonary involvement and musculoskeletal manifestations, also with greater prevalence of rheumatoid factor. ⁽⁷⁾

Pathogenesis:

The exact etiology of SLE remains obscure although it is clear that many factors are interacting as genetic, epigenetic, environmental, hormonal, and immunoregulatory factors.⁽⁸⁾

1- Genetics:

Genome-wide association studies (GWAS) have identified 30 to 40 gene loci with polymorphism that predispose to SLE.⁽⁹⁾ The most risky genetic polymorphisms include those encoding complement factors and the presence of a mutated three prime repair exonuclease 1 (TREX1) gene that degrades deoxyribonucleic acid (DNA) because each one can occur alone as an individual gene polymorphism and can achieve enough genetic susceptibility to disease development.⁽¹⁰⁾ Deficiency of complement (C) encoding genes causes deficiency of complement components such as C1q that is required to clear apoptotic cells or deficiency of C4A and C4B that have been linked to decreased elimination of self-reactive B cells.^(11, 12)

A combination of susceptibility genes or presence of susceptibility genes plus absence of protective genes are usually required to allow disease development. Each allele contributes only minimally and the cumulative effect of several genes is necessary to substantially increase the risk of SLE. Genes on different chromosomes are associated with development of the different clinical subsets such as nephritis (2q34), hemolytic anemia (11q14), discoid lupus and thrombocytopenia (11p13), vitiligo (17p12), or to the increased risk for end stage renal disease (ESRD).⁽¹³⁾

Most single-nucleotide polymorphisms (SNPs) associated with SLE fall within major histocompatibility complex (MHC) Locus. They include HLA-DR2 and HLA-DR3 and within HLA-DRB1 loci, HLA-DRB1*0301 and HLA-DRB1*1501 predispose to SLE whereas HLA-DRB1*1401 reduces the risk.⁽¹⁴⁾ Other associations of SNPs that predispose to SLE include TNIP1, PRDM1, JAZF1, UHRF1BP1 and interleukin-10 (IL-10). Other protective genes includes that encoding for toll-like receptor (TLR) 5, its absence can predispose to SLE.^(15, 16)

Moreover, certain SNPs linked to SLE have been identified for genes whose products may contribute to abnormal T-cell function in SLE as CD3- ζ and PP2Ac.⁽¹⁷⁾ In addition, genetic polymorphisms associated with interferon alpha (IFN- α) pathways that causes increased expression of IFN α -induced genes or increased sensitivity to IFN- α have been also linked to SLE.^(18, 19) Although these findings are promising, the loci identified so far can account for only about 15% of the heritability of SLE. In addition, an altered copy number of certain genes such as C4, fragment crystallizable gamma receptor type IIIB (Fc γ RIIIB) and TLR7, has been linked to disease expression.^(20, 21)

2- Epigenetics:

In addition, epigenetic modifications are important in pathogenesis of SLE as they represent the link between genetic and environmental risk factors. These include hypomethylation of DNA and post-translational modifications of histones (acetylation and methylation). Hydralazine and procainamide inhibit DNA methylation and can induce manifestations of lupus in healthy persons. Furthermore, the regulatory regions of some genes involved in the pathogenesis of SLE disease (ITGAL, CD40LG, CD70, and PPP2CA) have been reported to be hypomethylated. Recruitment of histone deacetylase 1 to the IL-2 promoter suppresses its expression. Trichostatin A, an

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inhibitor of histone deacetylase, normalizes the function of T cells from patients with SLE, and treatment of lupus-prone mice results in disease improvement. ^(22,23)

3- Environmental:

The environmental triggers of SLE include ultraviolet rays (UV), demethylating drugs, certain occupations, habits and pathogens. ^(24, 25) Infections include viral especially Epstein-Barr virus (EBV) which resides in B cells and stimulate IFN- α production and bacterial infections which act via bacterial unmethylated cytosine-phosphate guanosine (CpG) motifs. Patients with SLE also have a faster seroconversion to EBV, a higher viral load and a higher titer of antibodies to EBV. In addition, the molecular similarity between EBV nuclear antigen 1 and the common lupus autoantigen as Sjögren's syndrome-A antigen (Ro/SSA), and the inability of CD8+ T cells to control EBV-infected B cells that are present in SLE patients suggest that viruses may contribute to the expression of lupus. ^(26, 27) UV light may stimulate keratinocytes to express more small nuclear ribonucleoproteins (snRNPs) on their cell surfaces and to secrete more cytokines thereby stimulating B cells to make more antibodies. UV light decreases T cell DNA methylation, which may lead to overexpression of lymphocyte function-associated antigen-1 (LFA-1) converting them to autoreactive T cells resulting in autoantibody formation. ⁽²⁸⁾ In addition, many drugs have been reported to induce lupus like-disease and its associated autoantibody production. ⁽²⁹⁾

4- Hormones:

The basic role of hormones on the incidence and severity of SLE depends on their effects on immune responsiveness. The X chromosome may contribute independently from hormones because in castrated female and male mice that have been genetically manipulated to express XX, XO (female), XY, or XXY (male) combinations, the presence of two X chromosomes increases the severity of SLE. Also, among the genes known to contribute to the pathogenesis of SLE is CD40 which is located on chromosome X. ⁽³⁰⁾

The most important implicated hormones are estradiol and dehydroepiandrosterone (DHEA). Estrogen stimulates CD8+, CD4+ T cells, B cells, macrophages and the release of certain cytokines (eg, IL-1), stimulates the expression of both human leucocyte antigen (HLA) and endothelial cell adhesion molecules (ECAM) with the increases adhesion of peripheral mononuclear cells to endothelium. In addition estradiol reduces apoptosis in self-reactive B cells, thus promoting selective maturation of autoreactive B cells with high affinity for anti-DNA. Consequently, women are predisposed to make autoantibodies that eventually lead to clinically apparent SLE. However, the levels of estradiol and progesterone are lower during the second and third trimesters in patients with SLE than in healthy pregnant women, pregnancy may aggravate SLE. Also, a link between pregnancy outcome and the status of the disease at conception has been noted. ⁽³¹⁾ In comparison, androgens tend to share an immunosuppressive role which is evidenced by the low levels of serum DHEA in nearly all patients with SLE and treatment with DHEA has shown some clinical benefit. ⁽³²⁾ Although it is clear that hormones affect the autoimmune development, the use of oral contraceptives does not increase lupus flares in patients with stable disease. Pregnancy in patients with SLE presents a clinical challenge that requires the involvement of relevant specialists. ⁽³¹⁾

5- Immunoregulatory Factors:

The SLE is mainly a disease with abnormalities in the immune system. Both innate and adaptive immune responses are responsible for the continuous production of autoantibodies in patients having SLE. ⁽³³⁾ Loss of self tolerance, formation of auto antibodies and ICs against self-antigens are the main stay in this disease. Phagocytosis and clearing of ICs within the apoptotic cells are defective in patients with SLE allowing persistence of antigens and ICs leading to persistent activation of B cells and plasma cells making more autoantibodies. ^(34, 35)

Abnormal T cell function can be related to many factors, one of them is the production of IL-17. Normally, IL-17 is produced mainly by the activated T cells in response to certain bacteria and fungi. However, a high percentage of CD4+ T cells and the increased number of blood CD3+CD4-CD8-T cells in patients with SLE produce IL-17, and these cell types home to the kidney in patients with lupus nephritis (LN). ⁽³⁶⁻³⁸⁾ In addition, the expression of the adhesion molecule CD44 is abnormally increased in T cells from patients with SLE and can migrate at increased rates into inflamed organs. The expression of CD44 variant 3 and CD44 variant 6 is increased in T cells from patients with SLE, and these cells infiltrate the kidneys in such patients. ⁽⁹⁾

B cells are central to the expression of the disease as they process and present antigens and auto antigens to T cells in addition to their production of auto antibodies. Furthermore, in active SLE, a marked disease activity-dependent reduction in the number of native B cells is observed, and the number of plasma cells is increased in the peripheral blood. Compromise of tolerance checkpoints, along with other factors, may lead to increased production of auto antibodies. Also, the number of DNA binding B cells is increased in antigen-exposed and antigen-unexposed B cells and correlates with disease activity. ⁽³⁹⁾ Furthermore, the increased signaling of B-cell receptors in SLE may be facilitated by the limited suppression mediated by Fc γ IIB. ⁽⁴⁰⁾ This can be attributed to the presence of germ-line variants of sialic acid acetyltransferase that are of reduced activity, thus amplifying the signaling of B lymphocyte antigen receptors and the variant produces negative selection of auto reactive lymphocytes. ⁽⁴¹⁾

The activation of innate immunity occurs mainly via interactions between TLR7 and ribonucleic acid (RNA) and via TLR9 and DNA leading to activation of dendritic cells and release of type I and type III IFNs and tumour necrosis factor alpha (TNF- α). ⁽⁴²⁾ Stimulation of TLR7 or TLR9 reduces the immunosuppressive activity of glucocorticoids, suggesting that nucleic acid containing ICs that induce TLR signaling may limit effectiveness of glucocorticoids and may account for the high doses required for therapy. ⁽⁴³⁾ Correspondingly, blocking stimulation of TLR7 or 9 may be useful therapeutically and restore sensitivity to glucocorticoids such as antimalarial drugs which are used to treat some manifestations of SLE (e.g., hydroxychloroquine). They act via blocking TLR7 and 9 signaling that are involved in the IFN- α response. ⁽⁴⁴⁾ However, the activation of adaptive immune response occurs via T cell and B cell activation through interaction with self-antigens or antigens released by the apoptotic cells. T cells release IFN- γ , IL-6, IL-10, IL-17, while natural killer (NK) cells and T cells fail to release adequate quantities of tumour growth factor beta (TGF- β). These cytokine patterns favor continued autoantibody formation. ^(45, 46) Increased B lymphocyte activating factor (BAFF) expression may promote autoimmunity. Stimulation by BAFF is mainly important for the survival of T-dependent B cells. Increased BAFF, also termed B lymphocyte stimulating factor (BLyS) production, is promoted by increased TLR activation and increased type I and II IFNs. ⁽⁴⁷⁾

Diagnosis of Systemic Lupus Erythematosus:

SLE is characterized by having a wide spectrum of clinical manifestations at presentation including general symptoms, mucocutaneous, haematological, renal, neurological, gastrointestinal, pulmonary and cardiac manifestations. It runs an unpredictable course with a characteristic remission and relapse.⁽⁴⁸⁾ Diagnosis of SLE is based mainly on clinical practice. The American College of Rheumatology (ACR) classification of SLE is based on 11 criteria and the presence of any 4 or more of the 11 criteria, serially or simultaneously or during any interval of observation, is diagnostic of SLE.⁽⁴⁹⁾ (Table I)

Revision of the originally proposed classification criteria of ACR has taken place to overcome many evident pitfalls. The current Systemic Lupus International Collaborating Clinics (SLICC) classification criteria have been validated in comparison to the previously revised non validated ACR criteria. However the SLICC classification criteria retain specificity, they perform better than the revised ACR criteria in terms of sensitivity but not specificity. The validated SLICC classification criteria are more consistent with SLE pathogenesis and are simple to apply. Criteria are cumulative and need not to be present concurrently.^(49, 50) The SLICC is based on four of the clinical and immunological criteria including at least one clinical criterion and one immunologic criterion; or the patient has biopsy-proven nephritis compatible with SLE and with antinuclear antibody (ANA) or anti- double stranded deoxy nucleic acid (anti-dsDNA) antibodies.⁽⁵⁰⁾ (Table II)

Assessment of disease activity:

Assessment of disease activity in lupus patients is of extreme importance to tailor treatment. Individual patient evaluation should include a specific and separate estimation of disease activity, cumulative organ damage, patient-related quality of life (QoL), and drug toxicities. The included parameters are clinical, serological and sometimes radiological items. Assessment of each parameter differs from one score to another based on the detailed description of each parameter and the given priority according to disease manifestations. Some scores describe each parameter in a simple clinical definition, others define each parameter in a more detailed and precise description while graded activity of each of the indicated items is determined by others.⁽⁵¹⁾

General manifestations as fever, fatigue and weight loss are variably included. Neuropsychiatric manifestations as seizures and psychosis are indicated. Musculoskeletal manifestations as those of arthritis and myositis, mucocutaneous manifestations as mucosal ulcers, photosensitivity and discoid lupus were also checked. Renal manifestations as pyuria, proteinuria, haematuria, urinary casts or even a histological evidence of active lupus nephritis is variably assessed by the different scores. Vasculitis that includes major or even minor cutaneous or visceral affection is included in others. Cardiovascular and respiratory manifestations as those of pleurisy, pericarditis, myocarditis and arrhythmias, are also differently assessed by the different scores.⁽⁵¹⁾

The serological parameters include haematologic and immune systems. Haematologic parameters as anaemia, leukopenia and thrombocytopenia are included. Immune parameters as hypocomplementaemia and positive anti-ds DNA are included by some study scores. Radiological parameters are those used to prove evidences of manifestations like chest x-ray and electrocardiographic (ECG) changes were also

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included. The need for hospitalization and the need for immunosuppressive drug modification were considered in some indices. Improvement in a disease activity score represents a clinical benefit to the patient and should not be associated with worsening in any disease manifestation.⁽⁵¹⁾

Many indices have been developed to objectively measure lupus disease activity and several of these have been validated. The most widely used indices are the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI), the British Isles Lupus Assessment Group (BILAG) index, the European Consensus Lupus Activity Measurement (ECLAM), the Systemic Lupus Activity Measure (SLAM), the SLE activity Index Score (SIS) and the Lupus Activity Index (LAI). Most of these give a global score, which does not distinguish multiple mild manifestations from those with one or two severe features. Global scores do not tell whether the disease is improving, stable or worsening and does not identify new organ involvement. However, the BILAG index which is a more comprehensive transitional index gives graded categorical scores for each system. Nevertheless, all these indices are sensitive to changes in disease activity, reliable and correlate well with each other. All these indices have been validated and have excellent reliability, validity and responsiveness to change.⁽⁵²⁻⁵⁹⁾

Table I: The American College of Rheumatology (ACR) criteria for classification of systemic lupus erythematosus: ⁽⁴⁹⁾

Criterion	Definition
Malar rash	Fixed erythema, flat or raised, over the malar eminences, tending to spare the nasolabial folds.
Discoid rash	Erythematous raised patches with adherent keratotic scaling and follicular plugging; atrophic scarring may occur in older lesions.
Photosensitivity	Skin rash as a result of unusual reaction to sunlight, by patient history or physician observation.
Oral ulcers	Oral or nasopharyngeal ulceration, usually painless, observed by a physician.
Arthritis	Non-erosive arthritis involving 2 or more peripheral joints, characterized by tenderness, swelling, or effusion.
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.
Renal disorder	Persistent proteinuria > 0.5 gm per day or > than 3+ if quantitation is not performed. OR Cellular casts: may be red cell, hemoglobin, granular, tubular, or mixed.
Neurologic disorder	Seizures: in the absence of offending drugs or known metabolic derangements (e.g., uraemia, acidosis, or electrolyte imbalance). OR Psychosis: in the absence of offending drugs or known metabolic derangements (e.g., uraemia, acidosis, or electrolyte imbalance).
Hematologic disorder	Hemolytic anemia with reticulocytosis, OR Leucopenia: less than 4,000/mm ³ total on 2 or more occasions, OR Lymphopenia: less than 1,500/mm ³ on 2 or more occasions, OR Thrombocytopenia: less than 100,000/mm ³ in the absence of offending drugs.
Immunologic disorder	Positive LE cell preparation, OR Anti-DNA: antibody to native DNA in abnormal titer, OR anti-Smith antibody: (Anti-Sm Ab) presence of antibody to Smith nuclear antigen, OR False positive serologic test for syphilis known to be positive for at least 6 months and confirmed by Treponema pallidum immobilization or fluorescent treponemal antibody absorption test.
Antinuclear antibody (ANA)	An abnormal titer of antinuclear antibody by immunofluorescence or an equivalent assay at any point in time and in the absence of drugs known to be associated with "drug-induced lupus" syndrome.

Table II: The Systemic Lupus International Collaborating Clinics (SLICC) classification criteria of systemic lupus erythematosus: ⁽⁵⁰⁾

Clinical criteria	Immunological criteria
<ol style="list-style-type: none"> 1. Acute cutaneous lupus: <ul style="list-style-type: none"> • lupus malar rash (do not count if malar discoid); • bullous lupus; • toxic epidermal necrolysis variant of SLE; • maculopapular lupus rash; • photosensitive lupus rash; OR subacute cutaneous lupus 2. Chronic cutaneous lupus: <ul style="list-style-type: none"> • classical discoid rash; • localized (above the neck); • generalized (above and below the neck); • hypertrophic (verrucous) lupus; • lupus panniculitis (profundus); • mucosal lupus; • lupus erythematosus tumidus; • chillblains lupus; • discoid lupus/lichen planus overlap 3. Oral ulcers: Palate, buccal, tongue or nasal ulcers (in the absence of other causes) 4. Nonscarring alopecia (in the absence of other causes) 5. Synovitis: Involving two or more joints, OR characterized by swelling or effusion tenderness and at least 30 minutes of morning stiffness 6. Serositis: (in the absence of other causes) <ul style="list-style-type: none"> • Typical pleurisy for more than 1 day or pleural effusions or pleural rub; • Typical pericardial pain for more than 1 day or pericardial effusion or pericardial rub or pericarditis by electrocardiography 7. Renal: Urine protein/creatinine (or 24-hour urine protein) representing 500 mg of protein/24 hour or red blood cell casts 8. Neurologic: (in absence of other known causes) <ul style="list-style-type: none"> • Seizures • psychosis • mononeuritis multiplex • myelitis • peripheral or cranial neuropathy • acute confusional state 9. Hemolytic anemia 10. Leukopenia < 4,000/mm³, OR lymphopenia < 1,000/mm³ (at least once and in absence of other known causes) 11. Thrombocytopenia: < 100,000/mm³ (at least once and in absence of other known causes) 	<ol style="list-style-type: none"> 1. ANA: above laboratory reference range 2. Anti-dsDNA: above laboratory reference range, or >2-fold the reference range if tested by enzyme-linked immunosorbent assay (ELISA) 3. Anti-Sm: presence of antibody to Sm nuclear antigen 4. Antiphospholipid antibody: <ul style="list-style-type: none"> • Positive test result for lupus anticoagulant • False-positive test result for rapid plasma reagin • Medium- or high-titer anticardiolipin antibody level (IgA, IgG, or IgM) • Positive test result for anti-β₂-glycoprotein I (IgA, IgG, or IgM) 5. Low complement: Low C3, low C4, low CH50 6. Direct Coombs test: (In the absence of hemolytic anemia)

Lupus Nephritis

Kidneys are one of the most commonly affected organs by SLE. It was found that approximately 35% of adults with SLE have clinical evidence of nephritis at the time of diagnosis with an estimated total of 50–60% developing nephritis during the first 10 years of disease. The prevalence of nephritis is significantly higher in African Americans and Hispanics than in whites, and is higher in men than in women. Renal damage is more likely to develop in nonwhite groups. Overall survival in patients with SLE is approximately 95% at 5 years after diagnosis and 92% at 10 years after diagnosis. The presence of LN significantly reduces survival to approximately 88% at 10 years, with even lower survival in African Americans.^(6, 60)

The LN can be defined as clinical and laboratory manifestations criteria as persistent proteinuria with urine protein/creatinine ratio of ≥ 0.5 and/or cellular casts including red blood cells (RBC's), hemoglobin, granular, tubular, or mixed). The ACR criteria has recommended that a spot urine protein/creatinine ratio of ≥ 0.5 can be substituted for the 24-hour protein measurement, and “active urinary sediment” (≥ 5 RBC's/high-power field (hpf), ≥ 5 white blood cells (WBC's)/hpf in the absence of infection, or cellular casts limited to RBC or WBC casts) can be substituted for cellular casts. Renal biopsy in patients with LN with the presence demonstrating IC-mediated glomerulonephritis (GN) is compatible with LN.^(61, 62)

Pathogenic Mechanisms of Lupus Nephritis:

There is heterogeneity of the pathogenic mechanisms that are implicated in LN. This includes; deposition of autoantibodies and renal ICs, complement activation, activation of Fc γ receptors and TLRs, several immune cell stimulation, release of cytokines and chemokines, activation of transcription factors and lastly release of reactive intermediates. All these factors ultimately result in either renal regeneration or fibrosis.⁽⁶³⁾

The IC deposition is the main pathologic feature of tissue injury in SLE. They are formed of antinuclear antibodies to self nuclear materials that are present in blood and tissues in excess amounts beyond the capacity of the clearing complement cascade leading to a lack of tolerance that regulates autoreactive lymphocytes.⁽⁶⁴⁾ There are three postulated mechanisms for formation of glomerular ICs. One of them is the deposition of preformed circulating ICs, the second one which is called the “planted antigen” theory, is the cross-reactivity between autoantibodies and glomerular antigens such as laminin, annexin II or heparin and the third one is autoantibody binding to antigens trapped in the glomerular basement membrane (GBM).^(65, 66) Circulating DNA and nucleosomes can deposit in the GBM based on charge/charge interactions and serve as antigen for autoantibodies. In addition, they can bind to glomerular nucleosomes that have been formed from necrotic intrinsic glomerular cells.⁽⁶⁷⁾ Following formation of these ICs, there is down regulation of DNAase I in the kidney with further accumulation of nucleosomal material in the glomerulus.⁽⁶⁸⁾ ICs with a specific predilection to kidney tissues and contribute to development of LN are those containing cationic anti-DNA antibodies, antinucleosomes antibodies, anti-C1q antibodies and anti-chromatin antibodies.⁽⁶⁹⁾

In kidney, ICs deposit in the subendothelial space and mesangium before deposition in the GBM, subepithelial space, underneath proximal tubular epithelial cells

(PTECs) and interstitium.⁽⁶⁹⁾ Deposition of cationic immune deposits in the mesangial or subendothelial compartments can initiate the recruitment of inflammatory cells and the activation of resident mesangial and endothelial cells. While the GBM acts as a barrier for leukocyte infiltration and immune deposition in the subepithelial area, it is associated with podocyte injury and proteinuria.⁽⁷⁰⁾ These complexes can lead to further activation of immune pathways by co-stimulation of Fc γ receptors and TLRs and also by activation of complement cascade.⁽⁶⁷⁾

The C has a dual role in lupus. Although activation of the classical complement pathway seems to be protective, alternative complement pathway activation is considered a key component of complement-mediated damage in LN.⁽⁷¹⁾ Deficiency of early complement pathway components as C1, C2 and C4 carries a high risk for lupus due to the accumulated ICs and apoptotic bodies, while activation of the alternative complement pathway triggers an amplification loop that accelerates cleavage of C3 to C3b. C3b covalently binds to cellular surfaces, with release of anaphylatoxins C3a and C5a with and formation of membrane attack complex.⁽⁶³⁾ In addition, blockade of the alternative complement pathway either genetically or pharmacologically results in significantly decreased severity of renal disease in murine lupus models.⁽⁷²⁾ Also, elimination of the natural inhibitor of the alternative pathway, Factor H, leads to acceleration of lupus-like renal disease.⁽⁷³⁾ Furthermore, complement may cause tubular epithelial damage in lupus patients. During proteiuria, C3 is activated via the acidic urinary PH and urea with formation of membrane attack complexes on the epithelial side of tubular cells resulting in damage to the tubular epithelium.⁽⁶³⁾

The IC deposition in the kidney causes infiltration of inflammatory cells that interact with renal resident cells promoting tissue injury. Local cytokine, chemokine and adhesion molecule production leads to more infiltration of inflammatory cells with further inflammation, tissue injury and fibrosis. The presence of autoantigenes in apoptotic blebs activate both phagocytic cells such as dendritic cells and also autoreactive B cells leading to production of IFN- α and autoantibodies. In addition, defective clearance by macrophages secondary to polymorphisms in either the Fc receptor genes or the inactivated complement component 3 receptor 3 subunit (C3bi receptor) genes, integrin alpha M (ITGAM) results in accumulation of ICs with subsequent deposition in tissues. Peripheral blood T cells from patients with SLE express adhesion molecules such as CD44⁺. CD44⁺ T cells associated with phosphorylated ezrin, radixin, and moesin (pERM) signalling partner (CD44⁺pERM⁺ cells) are specific to kidney tissues. Many of these cells are CD3⁺CD4⁺CD8⁻ and secrete IL-17 leading to recruitment of polymorphnuclear cells and development of LN.^(1, 63)

Resident renal cells participate in disease expression. Mesangial cells, interstitial cells and podocytes acquire antigen presenting and secretory properties when exposed to IFN- γ as they secrete proinflammatory cytokines. In addition, mesangial cells from lupus-prone mice secrete alpha actinin (α -actinin) antibodies enforcing the inflammatory response. kallikrein seems to ameliorate murine and human LN, kallikrein gene polymorphisms and promoter gene SNPs are associated with development of nephritis in patients with SLE. Also, the vascular events that characterize SLE can be attributed to development of accelerated atherosclerosis. This results from the associated hypertension and metabolic syndrome, presence of antibodies to lipoproteins and oxidized lipoproteins. Endothelial cells get injured by the ICs and inflammatory molecules. This can lead to expression of adhesion molecules to attract lymphocytes

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and monocytes that adhere and infiltrate the subendothelial space or become detached to freely circulate in the blood.⁽¹⁾

The released immunogenic nucleic acids during apoptosis alter B cell tolerance through hypomethylation, oxidation, and high content of CpG. The immunogenicity of self-DNA is minimized by CpG suppression, CpG methylation, and inhibitory motifs that act together with the inaccessibility to TLRs. This immunogenicity is enhanced through many factors such as UV light, mitochondrial hyperpolarization and adenosine tri-phosphate (ATP) depletion. The DNA in SLE serum is in a hypomethylated state either due to a mutated TREX1 gene or reduced methyltransferase activity that is either inherent or drug-induced such as procainamide and hydralazine that inhibit DNA methylation and can also induce a lupus-like syndrome. The RNA autoantigens present in SLE sera are rendered immunogenic by the high content of uridine (U) and guanosine (G). Also, the small nuclear RNA (snRNA) bound to snRNPs is rendered immunogenic because it is rich in U and G content and the presence of reactive oxygen species (ROS).⁽⁷⁴⁾

Defective apoptotic cell clearance generates ANAs. Binding of ANAs to their specific TLRs induces intracellular inflammatory signalling pathway with activation of numerous transcription factors leading to aggressive autoimmune response in tissues and release of intense inflammatory cytokines. The interaction between TLR and ligands directly activates DCs and B cells with expansion of effector T and B lymphocytes specific for these autoantigens with the impairment of T regulatory cell (Treg) generation, differentiation and function, which contributes to the breakdown of peripheral tolerance and development of autoimmunity in SLE.⁽⁷²⁾ (Figure 1)

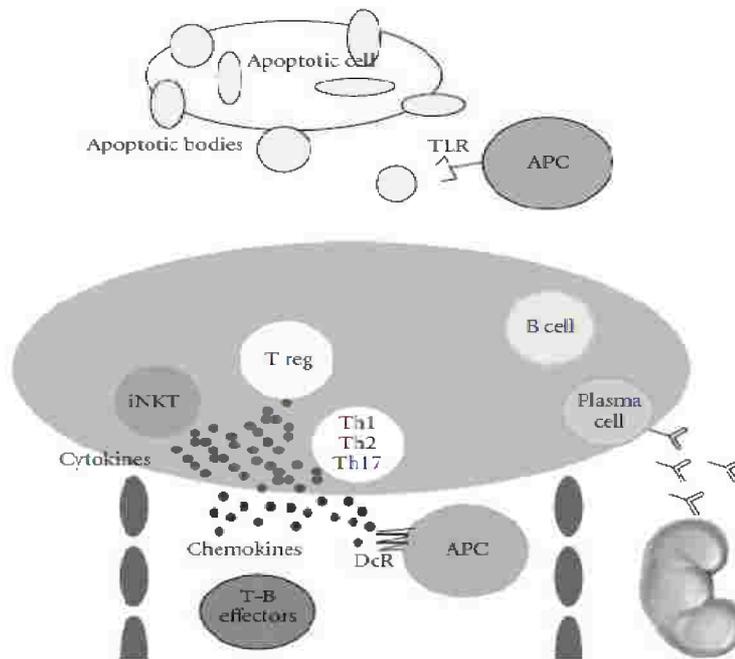


Figure 1: Mechanisms involved in the pathogenesis of systemic lupus erythematosus and lupus nephritis. iNKT: invariant natural killer T cell, T reg: regulatory T cell, APC: antigen presenting cell, Th: T helper cell.⁽⁷²⁾

Pathology of Lupus Nephritis:

Renal biopsy is now considered the gold standard in management of LN.⁽⁷⁵⁾ According to ACR guidelines renal biopsy should be considered in any patient with SLE who has clinical evidence of active nephritis especially during the first episode to evaluate disease for activity and chronicity, to detect tubular and vascular changes, to predict long-term outcomes and to guide treatment aiming to decrease the risk of progression to ESRD. In addition, it may identify additional or alternative causes of renal disease such as tubular necrosis related to medications, hypovolemia, or hypotension.⁽⁷⁶⁻⁷⁸⁾ A repeat renal biopsy should be considered in cases with persistent or worsening proteinuria, development of active sediments or increasing serum creatinine during treatment in patients with previous non-proliferative LN.⁽⁷⁹⁾

The LN can be classified by current International Society of Nephrology/Renal Pathology Society (ISN/ RPS) classification into six classes.⁽⁸⁰⁾ (Table III)

Class I Minimal mesangial Lupus Nephritis:

This class shows mesangial immune deposits by immunofluorescence microscopy (IF) but at the same time, they look normal by light microscopy (LM).⁽⁸⁰⁾

Class II Mesangial proliferative Lupus Nephritis:

This class shows pure mesangial hypercellularity of any degree or mesangial matrix expansion by LM, with mesangial immune deposits. There may be few isolated subepithelial or subendothelial deposits visible by IF or electron microscopy (EM), but not by LM.⁽⁸⁰⁾ (Figure 2 A)

Class III Focal Lupus Nephritis:

This class shows the proportion of glomeruli with active (A) and with sclerotic or chronic (C) lesions. It includes focal, active or inactive, segmental or global and endo- or extracapillary GN involving <50% of all glomeruli, typically with focal subendothelial immune deposits, with or without mesangial alterations. So it can be subdivided into the following:⁽⁸⁰⁾ (Figure 2 B)

III A : Active lesions: focal proliferative LN.

III A/C: Active and chronic lesions: focal proliferative and sclerosing LN.

III C : Chronic inactive lesions with glomerular scars: focal sclerosing LN.

Class IV Diffuse Lupus Nephritis:

This class shows the proportion of glomeruli with fibrinoid necrosis and/or cellular crescents. It includes diffuse, active or inactive, segmental or global and endo- or extracapillary GN involving $\geq 50\%$ of all glomeruli, typically with diffuse subendothelial immune deposits, with or without mesangial alterations. This class is divided into diffuse segmental (IV-S) LN when $\geq 50\%$ of the involved glomeruli have segmental lesions, and diffuse global (IV-G) LN when $\geq 50\%$ of the involved glomeruli have global lesions. Segmental is defined as a glomerular lesion that involves less than half of the glomerular tuft. This class includes cases with diffuse wire loop deposits but with little or no glomerular proliferation.⁽⁸⁰⁾ (Figure 2 C, D, E)

IV-S (A) : Active lesions of diffuse segmental proliferative LN.

IV-G (A) : Active lesions of diffuse global proliferative LN.

IV-S (A/C): Active and chronic lesions of diffuse segmental proliferative and sclerosing LN.

IV-G (A/C): Active and chronic lesions of diffuse global proliferative and sclerosing LN.

IV-S (C) : Chronic inactive lesions with scars of diffuse segmental sclerosing LN.

IV-G (C) : Chronic inactive lesions with scars of diffuse global sclerosing LN.

Class V Membranous Lupus Nephritis:

This class shows global or segmental subepithelial immune deposits or their morphologic sequelae by LM and by IF or EM, with or without mesangial alterations. Class V LN may occur in combination with class III or IV in which case both will be diagnosed; may show advanced sclerosis. ⁽⁸⁰⁾ (Figure 2 F, G)

Class VI Advanced Sclerotic Lupus Nephritis:

It shows $\geq 90\%$ of glomeruli globally sclerosed without residual activity. In all settings, tubular atrophy (mild, moderate or severe), interstitial inflammation and fibrosis, vascular lesions can be present. ⁽⁸⁰⁾ (Figure 2 H)

It has been described that SLE can be presented with lupus vascular changes as vasculitis, bland vasculopathy and haematological disorders, especially thrombotic thrombocytopenic purpura (TTP) and anti-phospholipid syndrome (APS). Haemolytic-uraemic syndrome (HUS) is less frequent in SLE. The patient presented with worsening of renal function with increase hematuria and loin pain and high mortality. Peripheral blood picture shows thrombotic microangiopathy with schistocytes. Renal biopsy showed vascular fibrinoid necrosis with narrowing of small arteries/arterioles. ^(81, 82) Samson et al ⁽⁸³⁾ reported this association during an episode of severe lupus nephritis in a young woman, who was successfully treated with steroids, cyclophosphamide and especially plasma exchange with plasma replacement.

Table III: International Society of Nephrology /Renal Pathology Society Classification of Lupus Nephritis 2003: ⁽⁸⁰⁾

Class I	Minimal mesangial LN.
Class II	Mesangial proliferative LN.
Class III	Focal LN (<50% of glomeruli) III (A): active lesions III (A/C): active and chronic lesions III (C): chronic lesions
Class IV	Diffuse LN (>50% glomeruli) Diffuse segmental (IV-S) or global (IV-G) LN IV (A): active lesions IV (A/C): active and chronic lesions IV (C): chronic lesions
Class V	Membranous LN.
Class VI	Advanced sclerosing LN ($\geq 90\%$ globally sclerosed glomeruli without residual activity)

Active and chronic lesions:(Table IV)

Active lesions:

The active lesions could be glomerular lesions, active vascular lesions. Active glomerular lesions include endocapillary hypercellularity with or without leukocyte infiltration and with substantial luminal reduction, karyorrhexis which is the presence of apoptotic pyknotic fragmented nuclei, fibrinoid necrosis which is a lesion characterized by fragmentation of nuclei or disruption of the GBM and often associated with the presence of fibrin-rich material, rupture of GBM as well as cellular or fibrocellular crescents. Active vascular lesions include hyaline thrombi that are intracapillary eosinophilic material of a homogeneous consistency which by immunofluorescence has been shown to consist of immune deposits. Also, necrotizing arteritis is one of the active vascular lesions that may be present alone or in combination. Furthermore, both tubular degeneration with necrosis and interstitial inflammation are active lesions that may be present separately or in combination. ^(80, 84, 85)

Chronic lesions:

The chronic glomerular lesions include glomerular sclerosis whether segmental or global, fibrous adhesions or fibrous crescents. In addition, vascular sclerosis, tubular atrophy, interstitial fibrosis may be present separately or in combination. ^(80, 84, 85)

Table IV: Active and Chronic Lesions In Lupus Nephritis: ⁽⁸⁰⁾

Active lesions	Chronic lesions
A. Glomerular: <ol style="list-style-type: none">1. Endocapillary hypercellularity with or without leukocyte infiltration and with substantial luminal reduction.2. Karyorrhexis.3. Fibrinoid necrosis.4. Rupture of GBM.5. Cellular or fibrocellular crescents.6. Large subendothelial deposits seen by LM (wire loops).7. Intraluminal immune aggregates (hyaline thrombi). B. Vascular: <ol style="list-style-type: none">1. Hyaline (IC) deposits.2. Necrotizing arteritis. C. Tubular degeneration and necrosis. D. Interstitial inflammation.	A. Glomerular: <ol style="list-style-type: none">1. Glomerular sclerosis (segmental, global).2. Fibrous adhesions.3. Fibrous crescents. B. Vascular sclerosis. C. Tubular atrophy. D. Interstitial fibrosis.

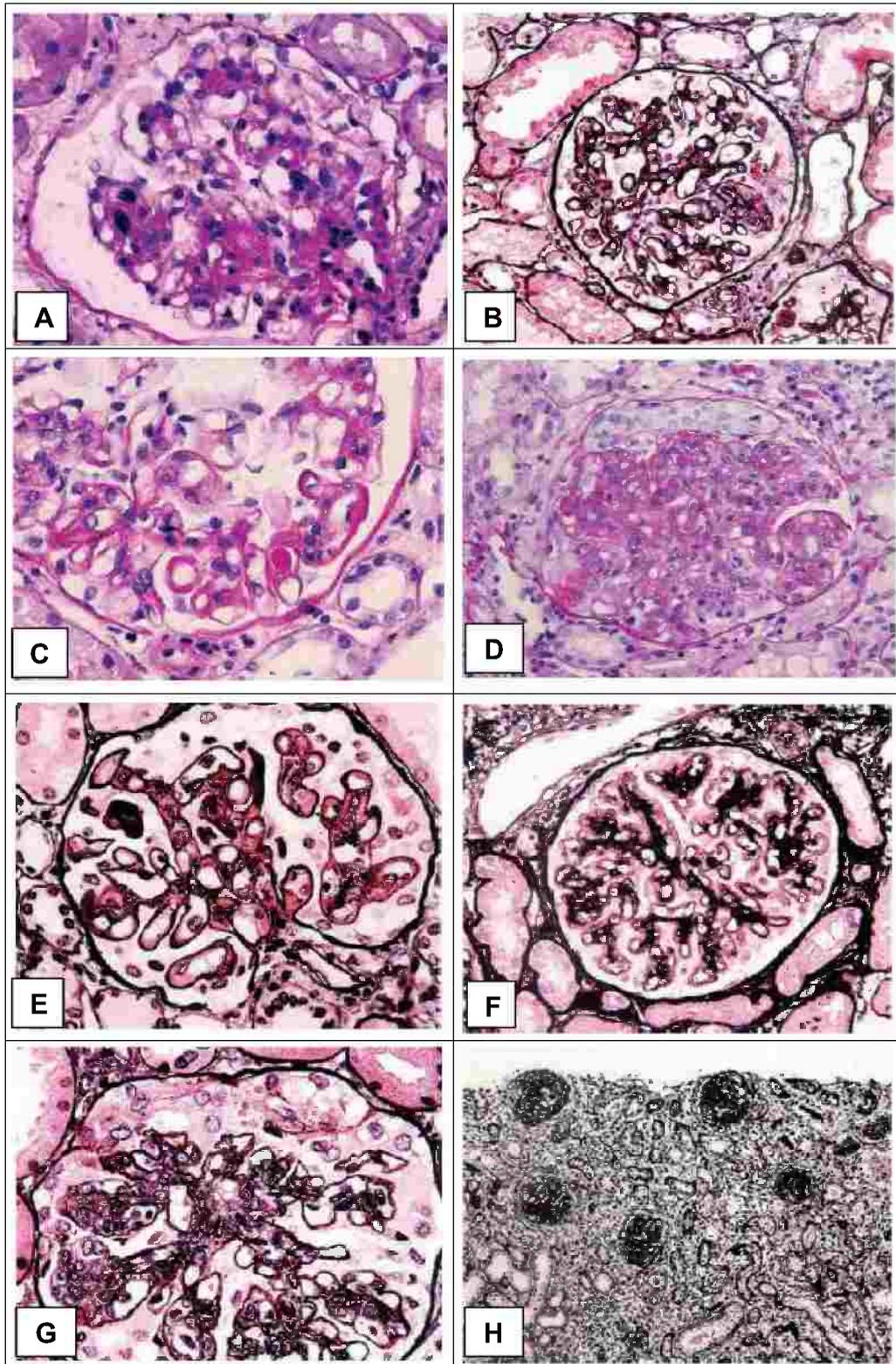


Figure 2: Histopathological light micrograph of lupus nephritis (LN). A-class II LN. {Periodic Acid Schiff (PAS)} B-class III (A) LN. (methenamine silver). C- class IV-S (A) LN. (PAS) D-class IV-G (A/C) LN. (PAS). E- class IV-G (A) LN. (methenamine silver). F- class V LN. (methenamine silver). G- class IV and V (A/C) LN. (methenamine silver). H- class VI LN. (methenamine silver) ⁽⁸⁰⁾

Management of Lupus Nephritis:

General Management Plan:

Treatment of LN should be based on the classification of type of LN by ISN/RPS criteria. Class I and class II generally do not require immunosuppressive treatment. In general, patients with class III and class IV require aggressive therapy with glucocorticoids and immunosuppressive agents. Class V when combined with class III or IV should be treated in the same manner as class III or IV while class V alone “pure membranous LN requires also aggressive therapy with glucocorticoids plus mainly MMF. On the other hand, class VI generally requires preparation for renal replacement therapy.⁽⁶²⁾ Both vasculitis and thrombotic microangiopathy are treated by plasma exchange in addition to glucocorticoids and immunosuppressive drugs.⁽⁸³⁾ Rituximab is reserved for those patients who are refractory to the standard agents.⁽⁸⁶⁾ Prevention of bland vasculopathy by early treatment of LN and regular follow up together with treatment of the anticipated hypertension are the main target.⁽⁸⁷⁾ All LN should be treated with a background of hydroxychloroquine unless there is a contraindication.⁽⁸⁸⁾ All LN patients with proteinuria ≥ 0.5 gm per 24 hours (or equivalent by protein/creatinine ratios on spot urine samples) should receive either angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARBs). Treatment with either ACE inhibitors or ARBs reduces proteinuria by approximately 30% and significantly delays doubling of serum creatinine and progression to ESRD in patients with non diabetic chronic renal disease (CKD). The use of combination ACE inhibitors/ARBs therapies is controversial. These classes of medications are preferred than calcium channel blockers and diuretics alone in preserving renal functions in CKD. In addition, careful control of hypertension with a target level of $\leq 130/80$ mm Hg.⁽⁸⁹⁻⁹¹⁾ Also, patients with low-density lipoprotein cholesterol ≥ 100 mg/dl should receive statins for the accelerated atherosclerosis related to the disease. In addition, women of child-bearing potential with active or prior LN receive counselling regarding pregnancy risks conferred by the disease and its treatments.^(62, 92, 93)

Induction Treatment of Class III and IV Lupus Nephritis:

Mycophenolate mofetil (MMF) (2–3 gm total daily oral dose) or intravenous CYC along with glucocorticoids should be used for induction of improvement in patients with ISN/RPS class III/IV LN. MMF and CYC are considered equivalent. Data show good results for induction therapy with MMF of 3 gm total dose daily for 6 months followed by maintenance with lower doses of MMF for 3 years.⁽⁹⁴⁾ However MMF has been similar in efficacy in all races studied to date, long-term studies with MMF are not as abundant as those with CYC. Asians compared to non-Asians might require lower doses of MMF for similar efficacy as regards response rates plus glucocorticoids. In addition, there is evidence that African Americans and Hispanics with LN respond less well to intravenous CYC than do patients of white or Asian races.^(95, 96) Thus, MMF and mycophenolic acid (MPA) may be an initial choice more likely to induce improvement in patients who are African American or Hispanic. The exact suggested dose of MMF varied based on the presence or absence of crescents in renal biopsy and the stability of serum creatinine in patients with proteinuria for whom a renal biopsy cannot be obtained. Some evidence suggests that MPA and enteric-coated mycophenolate sodium are less likely to cause nausea and diarrhea than MMF.^(97, 98) MMF and MPA are likely to be equivalent in inducing improvement of LN.^(98, 99)

There are 2 regimens of intravenous CYC; the first is the low-dose “Euro-Lupus” CYC (500 mg intravenously once every 2 weeks for a total of 6 doses) followed by maintenance therapy with daily oral azathioprine (AZA) or daily oral MMF and the second regimen is the high-dose CYC (500–1,000 mg/m² intravenously once a month for 6 doses) followed by maintenance treatment with MMF or AZA. However, previous studies showed that 30 months of high-dose intravenous CYC (the “National Institutes of Health” regimen) in which CYC was given monthly for 6 doses, then quarterly for an additional 2 years was more effective in preventing renal flare than the shorter 6-month regimen, the more current 3 to 6 month regimens followed by AZA or MMF maintenance are showing good long-term results at a stronger evidence. ^(94, 99)

Intravenous CYC at the low-dose “Euro-Lupus” dose is recommended for white patients with Western European or Southern European racial backgrounds; it showed no difference in efficacy with less serious infections. Ten years of follow up comparing low- and high- dose regimens showed similar rates of LN flares, ESRD, and doubling of the serum creatinine. ⁽¹⁰⁰⁾ Intravenous pulse glucocorticoids (500-1,000 mg methylprednisolone daily for 3 doses) in combination with immunosuppressive therapy is recommended followed by daily oral glucocorticoids (0.5-1 mg/kg/ day) followed by a taper to the minimal amount necessary to control disease. ⁽⁶²⁾

Almost all patients should be followed monthly for 6 months after initiation of induction treatment and before making major changes in treatment unless there is clear evidence of worsening at 3 months. MMF was preferable to CYC for patients who express a major concern with fertility preservation; since high-dose CYC can cause permanent infertility in both women and men and some women with LN treated with high-dose CYC developed sustained amenorrhea related to age. However 4.3 to 4.5% of patients had menopause during the CYC treatment. An emphasis had been made on being sure that a patient is not pregnant before prescribing MMF or MPA as the drug is teratogenic and the medications should be stopped for at least 6 weeks before pregnancy is attempted. ^(100, 101)

Induction Treatment of Crescentic Lupus Nephritis:

Either CYC or MMF should be used for induction of improvement in patients with class IV or IV/V plus cellular crescents along with intravenous pulses of high-dose glucocorticoid and initiation of oral glucocorticoids at the higher-range dosage, 1 mg/kg/day orally. The use of high-dose intravenous CYC for treatment of crescentic LN is favoured, although the presence of crescents indicates a poorer prognosis even with appropriate treatment. It was found that MMF (1 gm twice daily) is at least as effective as high doses of CYC in crescentic class IV LN. ^(102, 103)

Induction Treatment of Class V Lupus Nephritis:

Patients with pure class V LN and with nephrotic range proteinuria should be started on prednisone (0.5 mg/kg/day) plus MMF 2–3 gm total daily dose. In a retrospective analysis of patients with class V nephritis, MMF 2–3 gm total daily dose orally plus daily prednisone for 6 months resulted in improvement similar to that with intravenous CYC (0.5–1.0 mg/kg intravenous monthly for 6 months) plus prednisone, with 0–30% of patients having nephritic range proteinuria after 6 months. ^(104, 105)

There is evidence for the use of calcineurin inhibitors for their efficacy as an induction agent and in refractory disease. There is evidence to support the use of cyclosporine or tacrolimus in LN. In a prospective trial, tacrolimus was equivalent to

high-dose intravenous CYC in inducing complete and partial remissions of LN over a 6-month period.^(106, 107) In another 4-year-long prospective trial, cyclosporine was similar to AZA in preventing renal flares in patients receiving maintenance therapy.^(108, 109)

There is evidence in open-label trials that LN may respond to rituximab treatment. Prospective, randomized, placebo-controlled trials did not show a significant difference between rituximab and placebo.^(86, 110, 111) Recently, belimumab, a human IgG1 monoclonal antibody that binds soluble human BLyS and inhibits its biologic activity (BAFF inhibitor), has obtained the US Food and Drug Administration (FDA) approval in the treatment of seropositive active SLE patients in spite of prior therapies.⁽¹¹²⁻¹¹⁴⁾

Maintenance Therapy:

Either AZA or MMF should be used for maintenance therapy. Two recent prospective trials have been emerged.^(99, 115) It was found that MMF was statistically better than AZA in time to treatment failure and in each element of the composite score. Severe adverse events occurred in significantly more patients receiving AZA than receiving MMF.⁽¹¹⁵⁾ In the other study there were no statistically significant differences in any outcome measures.⁽⁹⁹⁾ In case of failure to respond after 6 months of treatment with glucocorticoids plus MMF or CYC, a switch of either the immunosuppressive agent to the other with these changes accompanied by intravenous pulses of glucocorticoids for 3 days is recommended.⁽⁶²⁾ In addition, rituximab can be used in a patient whose nephritis fails to improve or worsens after 6 months of one induction therapy or after the patient has failed both treatments.⁽¹¹¹⁾

Follow up monitoring:

Thorough and regular follow up monitoring of LN is recommended on a regular basis to allow early interventional modification of treatment according to the new situation in order to prevent or delay the progression to ESRD.⁽⁶²⁾

Toll-Like Receptors

The TLRs are found to be evolutionarily conserved from plants to mammals. *Toll* means “great” in English. In mid 1980’s, Anderson, et al⁽¹¹⁶⁾ coined it for a protein that played a critical role in the early embryonic development of *Drosophila*. Later it was found that this protein was also essential for the host innate immunity against fungal infection in adult flies.^(116, 117) In 1997, Medzhitov, et al⁽¹¹⁸⁾ were the first to report the cloning of a mammalian TLR homologue (identified as TLR4) of *Drosophila Toll* and termed it as “Toll-like receptor”.

The TLRs are one such signaling pathway that sense invading microbial pathogens and play crucial roles in the activation of innate and adaptive immunity. However, excessive TLR activation can disrupt immune homeostasis and may be responsible for the development of autoimmune and inflammatory diseases.⁽¹¹⁹⁾

Types of Toll Like-Receptors:

The TLRs, 13 of them have been discovered to date. Among them TLR1-9 are ubiquitously expressed in both human and mice. Human cells solely express TLR10, whereas mouse do not express TLR10 but do express TLR11, 12 and 13. Although various types of TLRs have common role in immunity, they basically differ in their ligand specificity, the usage of adaptor proteins, cell localization, and cellular responses.⁽¹²⁰⁾ (Figure 3)

Structures of Toll Like-Receptors:

All TLRs share similar domain architecture. They are transmembrane proteins, each one is composed of 3 domains; the first one is an extracellular leucine-rich repeat (LRR) which mediates the sensing of pathogens, the second one is an intracellular Toll/interleukin-1 receptor-like (TIR) domain which shows high similarities to the intracellular domain of the mammalian IL-1 receptor thus TLRs and IL-1 receptor trigger similar transduction cascades. It is required for downstream signal transduction. The third one is a transmembrane helix protein.^(121, 122) TLRs exist as homodimers or heterodimers with the capacity to engage their respective ligand.⁽¹²³⁾ Ligand binding induces a conformational change resulting in interaction or close juxtaposition of the two cytosolic TIR domains, thus providing an interface for an adapter protein with subsequent signal transduction leading to secretion of proinflammatory cytokines and type I IFNs through induction of immune and inflammatory genes.^(123, 124) These TLR responses are important in the functioning of both the innate and adaptive arms of immunity.⁽¹²⁵⁾ (Table IV, Figure 3)

Location and Function of Toll Like-Receptors:

The TLRs are located either on the cell membrane or on the endosome. On the cell membrane TLR1, 2, 4, 5, and 6 are located. Among them, TLR2 forms heterodimer with TLR6/TLR1 to recognize ligands such as peptidoglycan, lipoteichoic acid, lipoprotein from Gram-positive bacteria, lipoarabinomannan from mycobacterium, and zymosan from the yeast cell wall, diacylated mycoplasmal lipopeptide⁽¹²⁶⁾ and triacylated lipopeptides.⁽¹²⁷⁾ To enhance the ligand induced cellular signaling processes, TLR2 is found to interact with cluster of differentiation 14 (CD14).⁽¹²⁸⁾

TLR4, the first identified TLR mediates immune responses to Gram-negative bacteria (e.g. *Escherichia coli*)⁽¹²⁹⁾ by binding with the bacterial lipopolysaccharide (LPS) and its toxic moiety, lipid A.⁽¹³⁰⁾ In this scenario, TLR4 alone cannot confer response to LPS, hence it requires functionally interacting partners, such as myeloid differentiation protein-2 or lymphocyte antigen 96 (MD-2),⁽¹²⁶⁾ and CD14 for the activation of nuclear factor kappa-light-chain enhancer of activated B cells (NF- κ B) to produce proinflammatory cytokines.⁽¹³¹⁾ Though bacterial outer wall components like peptidoglycan and LPS are recognized by TLR1/2, 6 and 4, the recognition of bacterial flagellin is mediated by TLR5 from both Gram-positive and Gram-negative bacteria. The cellular responses to flagellin are same as peptidoglycans and LPS. TLR5 uses myeloid differentiation primary response 88 (MyD88) as adaptors to produce proinflammatory cytokines such as TNF- α . TLR5 is the only TLR which binds with flagellin.^(132, 133) (Table IV, Figure 3)

Some of the TLRs are intracellular, i.e., they are located in endosomes. Such nucleic acid sensing TLRs include TLR3, 7, 8, and 9, that are primarily involved in the recognition of nucleic acids (Table IV, Figure 3). Double-stranded RNA (dsRNA) a replication intermediate of most viruses, and polyinosinic-polycytidylic acid [poly (I: C)], a synthetic analog of dsRNA are known ligands for TLR3.^(134, 135) In addition, these TLRs can recognize some single-stranded RNA (ssRNA) containing viruses as west Nile virus, respiratory syncytial virus and encephalomyocarditis virus. Also it can sense dsDNA containing viruses as herpes simplex virus and mouse cytomegalovirus.⁽¹³⁶⁾ TLR9 recognizes bacterial and viral DNA and synthetic oligodeoxynucleotides (ODN) containing CpG motifs.⁽¹³⁷⁾ These are sequences of bases containing unmethylated cytosine (CpG DNA) and they are considered active and immunogenic when these unmethylated CpG DNA bases are surrounded by specific sequences.⁽¹³⁸⁾ These ligands trigger MyD88-independent pathway using TIR receptor domain-containing adaptor molecule-1 (TICAM1); also termed TRIF adaptor protein (TIR domain-containing adaptor inducing interferon- β), thereby leading to the production of IFN- β , which plays a potent role in the maturation of dendritic cells (DCs). Also, it can moderately activate B cells.^(136, 139)

TLR7 expressed in plasmacytoid DCs (pDCs) binds with small synthetic molecules such as loxoribine and imidazoquinoline compound R848.⁽¹⁴⁰⁾ However, ssRNA from vesicular stomatitis virus and influenza viruses are the natural ligands for TLR7.⁽¹⁴¹⁻¹⁴³⁾ Unlike TLR3; TLR7 uses MyD88 as adaptor protein to induce the production of IFN- α , TNF- α , and IL-12. In addition, guanosine and uridine rich ssRNA from human immunodeficiency virus type 1 (HIV)-1 are found to be identified by both TLR7 and 8.⁽¹⁴⁰⁾ However, exposure to free DNA in the cell can lead to the activation of specific receptors that lead to autoimmune and inflammatory diseases. Such DNA sensing receptors include TLR9 and retinoic acid inducible gene (RIG)-I-like receptors (RLRs). Unmethylated CpG-ODN and single stranded CpG ODN are known to bind with TLR9.⁽¹⁴⁴⁾ TLR9 in line with TLR7 and 8 also recruits MyD88 as the signaling protein for NF- κ B activation.^(137, 145)

Several TLRs have been reported in B cells such as TLR 2, 7, 9.⁽¹⁴⁶⁾ Endothelial cells express TLR7 for primary human dermal endothelial cells (HDMEC) and TLR8 for immortalized skin-derived endothelial cells (HMEC-1) under resting conditions but can express all 10 TLRs in proinflammatory conditions.⁽¹⁴⁷⁾ B cells, pDCs, monocytes, and macrophage lineage express TLR9 mRNA or protein.^(145, 148, 149) Both types DCs, the pDCs and myeloid dendritic cells (mDCs) express TLR1/2/6, and their activation

leads to DC maturation and secretion of various cytokines such as IL-6, IL-8, IL-10, IL-12 and TNF- α .^(149, 150)

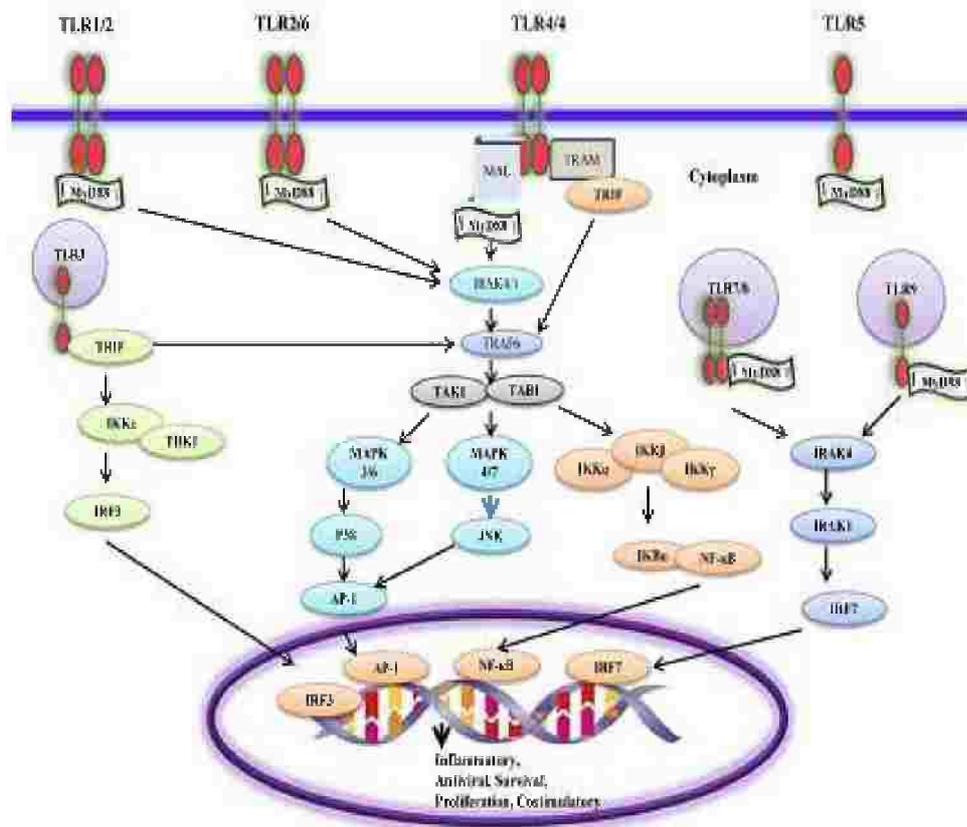


Figure 3: Differential use of adaptors by the different types of toll-like receptors (TLRs) and the transcription factors they activate.⁽¹⁵¹⁾

Role of Toll-Like Receptors in Innate adaptive and autoimmunity Diseases:

The innate immune system utilizes unique sets of molecules, collectively called pattern recognition receptors (PRRs) to recognize danger signals from pathogens and damaged cells. At least three distinct types of PRRs have been identified; TLRs that recognize nucleic acids on cell membranes or endolysosomal compartments, RLRs that recognize RNA or DNA in the cytoplasm and nucleotide binding and oligomerization domain, the nucleotide binding domain and leucine-rich repeat containing gene family (alternatively named NOD like receptors, NLRs) that monitor the cytosolic compartments closely interacting with TLR signalling pathways.^(152, 153)

The TLRs are found to recognize most gram-negative bacteria and certain gram positive bacteria and stimulate their own intrinsic signalling pathway and each one can induce specific biological responses such as dendritic cell maturation, cytokine production and development of adaptive immune response.^(154, 155) They are capable of binding to structures common to pathogens called pathogen-associated molecular patterns (PAMPs) and damage associated molecular patterns (DAMPs) which are expressed by most types of microbes such as bacteria, viruses, fungi and protozoa, also they can be aberrantly stimulated and contribute to the induction of infection and various autoimmune diseases, through binding directly to autoantigens or through binding to the activated complement components present in the ICs.⁽¹⁵⁵⁻¹⁵⁷⁾ (Table V)

Table V: Types of toll like receptors (TLRs), their locations, damage-associated molecular pattern molecules (DAMPs), pathogen-associated molecular pattern molecules (PAMPs), adaptors, secretory products, and the diseases in which they have been implicated: ⁽¹⁵¹⁾

TLRs	Immune cell expression	Ligands (DAMPs)	Ligands (PAMPs)	Signal adaptor	Major secretory products	Disease indications
TLR1 + TLR2 Cell surface	Monocytes, Macrophage, Dendritic cells, B cells	Heat Shock Proteins (HSP), High mobility group proteins (HMGB1), Proteoglycans, ECM	Triacylated lipoproteins, PAMP3, CSK4, Peptidoglycans, Lipopolysaccharide	TIRAP, MyD88, Mal	Inflammatory cytokines IL-6, TNF- α	cancer colon, Candidiasis
TLR2 + TLR6 Cell surface	Monocytes, Macrophages, Dendritic cells, B cells	Same listed above	Diacylated lipoproteins	TIRAP, MyD88, Mal	Inflammatory cytokines	Colon cancer, Gastric cancer, Hepatocellular carcinoma
TLR3 Endosome	B cell, T cell, Natural killer cell, Dendritic cell	mRNA and tRNA	dsRNA, Poly I:C, tRNA, siRNA	TRIF	Inflammatory cytokines, Interferon	Cancer: Breast, Colon, Melanoma, Hepatic, West Nile virus
TLR4 Cell Surface	Monocytes, Macrophage, Dendritic cells, B cells	HSP, High HMGB1, Proteoglycans phospholipids, b-defensin 2, Amyloid-b, Ox-LDL, ECM	Lipopolysaccharide, Viral envelop proteins	TRAM, TRIF, TIRAP, MyD88, Mal	Inflammatory cytokines, Interferon	Cancer: Breast, Colon, Gastric, Lung, Hepatic, Ovaries, Sepsis, autoimmune encephalomyelitis, Atherosclerosis, COPD, Asthma
TLR5 Cell surface	Monocytes, Macrophage, Dendritic cells	n.d.	Flagellin	MyD88	Inflammatory cytokines	Cancer: Gastric, Cervical, squamous cell
TLR7 Endosome	Monocytes, Macrophages, Dendritic cells, B cells	ssRNA	ssRNA, Imidazoquinolines (R848), Guanosine analogs (Loxoribine)	MyD88	Inflammatory cytokines, Interferon	Chronic lymphocytic leukemia
TLR8 Endosome	Monocytes, Dendritic cells, Mast cells	ssRNA	ssRNA, Imidazoquinolines (R848)	MyD88	Inflammatory Cytokines, Interferon	Systemic lupus erythematosus (SLE)
TLR9 Endosome	Monocytes, Dendritic cells, B and T cells	Chromatin IgG complex	CpG DNA, CpG ODNs	MyD88	Inflammatory Cytokines, Interferon	Cancer: Breast, Gastric, Hepatic, Cervical, Glioma, Malaria, Prostate, SLE

The host cells internalize both microbial and host-derived molecules by the endocytic pathway, while phagocytic cells internalize foreign particles by phagocytosis. Phagocytic uptake requires actin cytoskeletal reorganization and depends on guanosine triphosphatases (GTPases) so as to allow protrusions from membranes to wrap around the foreign and engulf it. Finally, phagosomes fuse with late endosomes (endolysosomes) for further processing. Nucleic acids are abundant in the host but usually the host-derived nucleic acids are not detected by endosomal TLRs as they are not delivered to the endosome except in autoimmune disease. Viral nucleic acids display features that allow discrimination from host nucleic acids including long dsRNA, 5-triphosphate RNA and unmethylated CpG DNA. However, physical separation i.e. compartment-based ligand recognition is the key of distinguishing self from foreign nucleic acids.⁽¹⁵⁸⁾

Internalization of nucleic acids to the endolysosomes stimulate endosomal TLRs which results in further translocation of TLR3, TLR7 and TLR9 from the endoplasmic reticulum where they have been synthesized to the endolysosomes. This translocation is regulated by three proteins; UNC93B1 (Unc-93 homolog B1) that is encoded by the UNC93B1 gene and is involved in innate and adaptive immune response. Deficiency of the encoded protein has been associated with herpes simplex encephalitis. Another two proteins, Gp96 (A heat shock protein 90 paralogue), it is present in the endoplasmic reticulum; it is one of the essential immune chaperones that regulate both innate and adaptive immunity. It plays a critical role in folding of proteins in the secretory pathway, and protein associated with toll-like receptor 4 PRAT4 [(PRAT4A and PRAT4B)], these proteins are two of the essential immune chaperones that regulate trafficking of TLRs. These events lead to more delivery of active TLRs to the endolysosomes where they are ready for ligand recognition.⁽¹⁵⁸⁾

The released IFNs produce powerful modulation of cell physiology including cell proliferation, survival, differentiation, protein translation and metabolism in addition to inhibition of nucleic acid replication. Therefore, their production is tightly regulated by tight regulation of gene expression through adequate control of transcription as well as post-transcriptional mRNA stability and translation. Furthermore, many elements of the regulatory pathway governing both the signalling pathway and IFN gene expression are themselves regulated by IFN providing an intricate network of overlapping feed-forward and feed-back regulatory loops. So, the homeostatic control of basal expression levels controlled by autocrine/paracrine cytokine signalling.⁽¹⁵⁹⁾

TLRs are activated by bacteria, virus and other pathogens. Sometimes the stimulation of one agent can inhibit other agent responses, causing the susceptibility to the main agent.⁽¹⁶⁰⁾ In a previous study, mice infected with virus showed increased susceptibility to bacterial infection and died because of the decreased production of a gene encoding p40 subunit of IL-12b upon activation of TLRs.⁽¹⁶⁰⁾ There is active crosstalk between G proteins and TLRs via secreted factors that can inhibit TLR signaling to escape host response.⁽¹⁶¹⁾ TLR4 signaling promotes an epithelial-mesenchymal transition in human hepatocellular carcinoma induced by LPS. TLR signaling contributes to germinal center antibody responses, and TLR4 has an essential role in early skin wound healing.⁽¹⁶²⁾

It is reasonable to assume that the association between infection and autoimmunity is often caused by TLR-mediated induction of proinflammatory cytokine and chemokine expression, and upregulation of co-stimulatory molecule expression by

antigen presenting cells (APCs). Experimental evidence continues to support a role for TLRs in the development of systemic autoimmune diseases like SLE, scleroderma, Sjögren's syndrome and multiple sclerosis and they show a characteristic set of ANAs and RNP-specific antibodies.⁽¹⁶³⁾

Several reports have shown the consequences of excessive activation of TLRs. Chronic exposure to TLR ligands further complicates the situation, leading to atherosclerosis⁽¹⁶⁴⁾ and tumor metastasis.⁽¹⁶⁵⁾ Hence, it is necessary to control the excess activation of TLRs. Naturally; cells possess regulatory checks in the form of negative regulatory proteins. One such regulatory loop is phosphoinositide 3-kinase (PI3K), a family of serine/threonine kinases, that phosphorylates phosphatidylinositol 4,5-bisphosphate (PIP2) and promotes cell survival, proliferation, and protein synthesis.⁽¹⁶⁶⁾

The TLR stimulation can lead to the pathogenic molecules that the targeted of these molecules either in a positive or negative way is required. TLRs are constantly monitored by physiological negative regulatory loops like membrane signaling molecule PIP2, B-Cell adaptor for PI3K (BCAP), Polo like kinases (PLKs), tumor necrosis factor alpha-induced protein 2 (TNFAIP2), TNFAIP3 and many others. Over activation of TLRs leads to various diseases such as sepsis and inflammatory bowel diseases. On the other hand, positive effects of TLRs are essential in bridging the connection of innate and adaptive arms of immune responses.⁽¹⁵¹⁾ (Figure 4)

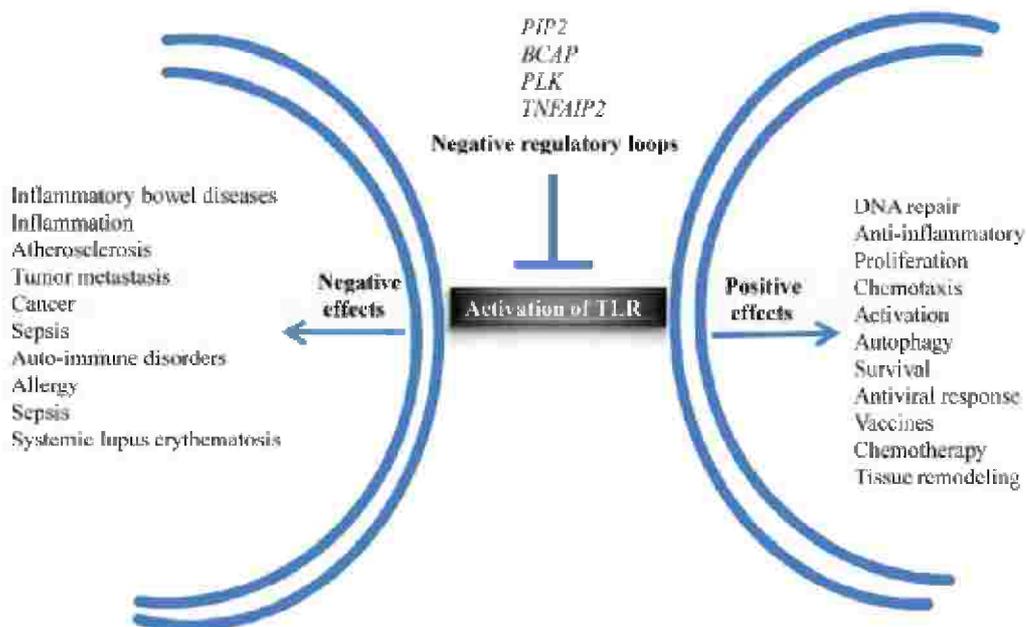


Figure 4: Roles of toll like receptors (TLRs) in inflammation and anti-inflammation. PIP2: Phosphatidylinositol 4,5-diphosphate, BCAP: B-Cell adaptor for Phosphoinositide 3 kinase (PI3K), PLK: Polo like kinases, TNFAIP2: tumor necrosis factor alpha-induced protein 3.⁽¹⁵¹⁾

Interferon lambda

The IFNs are considered a key sign of alarm in response to viral infection. They are powerful tools to directly and indirectly modulate the functions of the immune system.⁽¹⁶⁷⁾ They are classified within the class II cytokine family. The class II family of cytokines consists of three types of IFNs (types I, II and III) as well as the IL-10-related cytokines.⁽¹⁶⁸⁾ Thirteen different IFN- α subtype together with IFN- β , IFN- ω , IFN- κ and IFN- ϵ/τ constitute the type I IFN family.⁽¹⁶⁹⁾ While, type II IFN is IFN- γ and type III IFNs consist of IFN lambdas (IFN- λ 1, IFN- λ 2 and IFN- λ 3) which are also called IL-29, IL-28A and IL-28B, respectively. IFN- λ 3 shares considerable amino acid sequence homology with IFN- λ 2 (96%) but less with IFN- λ 1 (81%).⁽¹⁶⁸⁾

Type I IFN can be produced by all nucleated cells mainly pDCs and signals through IFN- α receptor (IFNAR). While type III IFNs are produced from a limited group of cells (epithelial cells) and signals through a different receptor IFN- λ receptor (IFNLR). They represent a newly identified group of the class II cytokine family. While they are functionally related to type I IFNs, they are structurally related to the IL-10 cytokine family. The lambda IFNs signal through a cytokine receptor complex which is unique for IL-28 and IL-29 though designated IL-28 receptor alpha (IL-28R α , IFN- λ R1 or IFNLR1), also it has a second short receptor chain; (IL-10R2) which is shared with receptors for IL-10 related cytokines such as IL-22 and IL-10.⁽¹⁶⁹⁾ (Figure 5)

Lambda Interferon receptor and intracellular signalling pathway:

Sensing self and foreign nucleic acids starts at PRRs and results in a downstream signalling cascade that leads to promoter stimulation of type I, II and type III IFNs.⁽¹⁵⁹⁾ The IFN- λ genes are clustered together on human chromosome 19 (19q13.13 region).⁽¹⁷⁰⁾ Induction of IFN- λ gene family shows Interferon regulatory factor (IRF) and NF κ B binding sites at their promoters. IFN- λ 1 was regulated similarly to IFN- β that is dependent on IRF3 and NF κ B while IFN- λ 2 and IFN- λ 3 regulation resembled IFN- α and is dependent on IRF7. However, they can be regulated differently than type I IFN genes at least in some cell types infected with viruses, and there is evidence that IRF and NF κ B proteins function independently on IFN- λ gene promoters rather than in concert.⁽¹⁵⁹⁾

Once IFN- λ binds to the IFN- λ R1 chain and the binary complex formed by the association of IFN- λ with the IFN- λ R1 chain, it causes a rapid conformational change that facilitates recruitment of the second receptor chain; IL-10R2 to the complex. Once assembly of the ternary complex is complete, the receptor-associated Janus tyrosine kinases; Janus kinase1 (Jak1) and non-receptor tyrosine-protein kinase 2 (Tyk2) mediate trans-phosphorylation of the receptor chains which results in the formation of phosphotyrosine-containing peptide motifs on the intracellular domain of the IFN- λ R1 chain that provide transient docking sites for latent preformed cytosolic signal transducers and activators of transcriptions (STAT) proteins including STAT1, STAT2 and in some cell types STAT3, STAT4, and STAT5 also. The activated STATs are tyrosine-phosphorylated and form homo- and heterodimers that recruit IRF9 to form a trimeric transcription factor complex known as IFN-stimulated gene factor 3 (ISGF3). This complex consists of 3 proteins; STAT1, STAT2, and IRF9. Once assembled, ISGF3 then translocates to the nucleus where it binds to IFN-stimulated response elements (ISRE) in the promoters of various IFN-stimulated genes (ISGs).⁽¹⁷⁰⁾

Introduction

Therefore, it is the proteins encoded by the ISGs that mediate the biological activities induced by IFNs as antiviral, immunostimulatory and antiproliferative effects and up-regulation of MHC class I antigen expression on many cell types. (Figure 5) ^(159, 171)

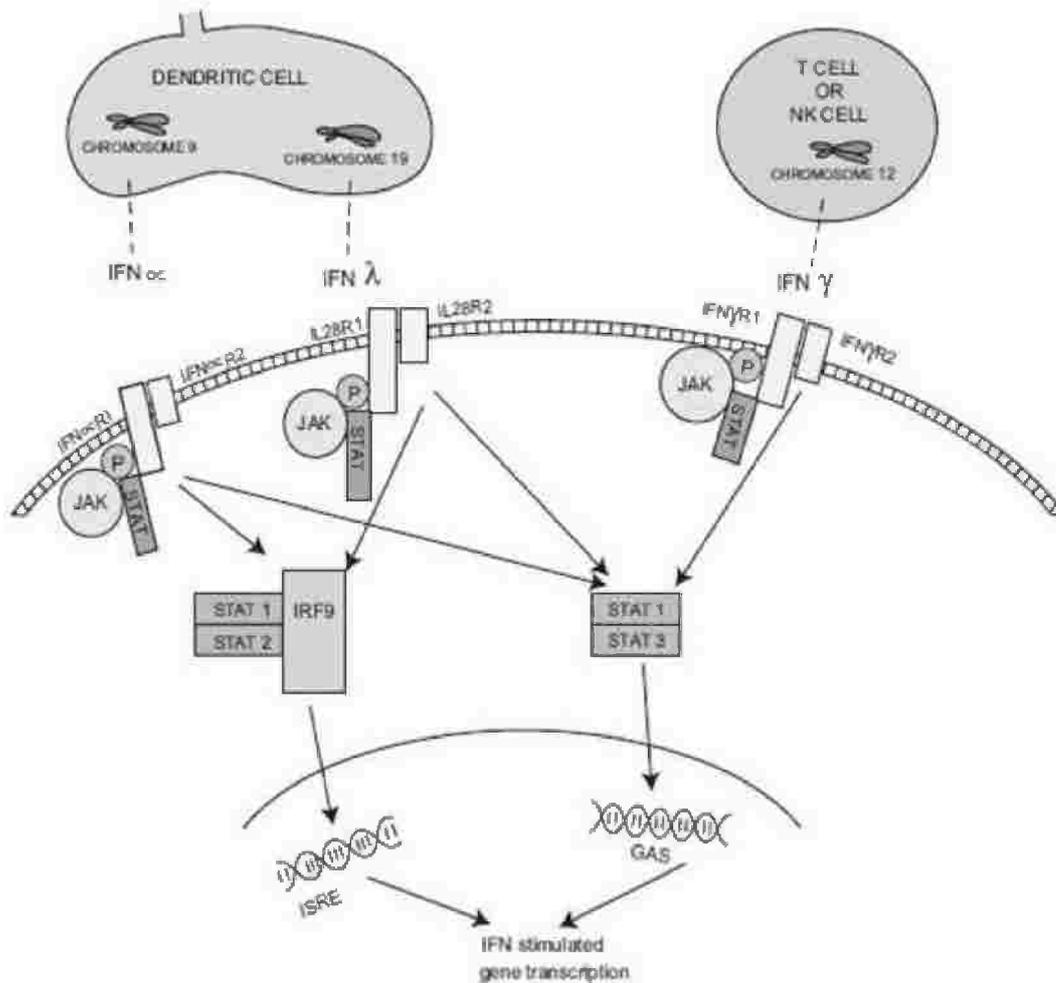


Figure 5: The signalling pathway of type III interferons (IFNs) compared with type II and type I IFNs. STAT: Signal transducers and activators of transcription, GAS: gamma-activated sequences, IRF9: interferon regulatory factor 9, ISREs: interferon stimulated regulatory elements. ⁽¹⁶⁸⁾

Biological role of IFN lambda:

As, normally, DCs have been found to be efficient stimulators of B and T lymphocytes as well as key controllers of immunity. However monocytes represent the circulating pool of macrophages and dendritic cell precursors, they behave like DCs in SLE. In addition, SLE blood is a dendritic cell-inducer. This is dependent on IFN- α and correlates with disease activity. Uncontrolled DC maturation could lead to activation and expansion of autoreactive T cells with loss of central tolerance. Furthermore, IFN- α stimulates human plasma cell differentiation with production of autoantibodies and activates CD8⁺ T cells to generate nucleosomes.⁽¹⁷²⁾

Although signaling events induced by lambda IFNs are similar to those of type I IFNs, lambda IFNs interact with a different, unique receptor complex that has a more restricted expression pattern compared with receptors for type I IFNs. For that reason, type III IFNs has higher tissue specificity than type I IFNs and they mediate many of the same biological activities since they activate the same intracellular signalling pathway.⁽¹⁷⁰⁾ The IFNLR, which is restricted to cells of epithelial origin as that of intestine, lung and vagina suggesting possible protection of these organs from viral infection by IFN- λ treatment. In addition, hepatocytes and some immune cells express IFNLR.⁽¹⁷³⁾ IFN- λ has been shown to inhibit hepatitis B virus (HBV) replication in a differentiated murine hepatocyte cell line. IFN- λ also inhibits replication of subgenomic and full-length hepatitis C virus (HCV) replicons in the human hepatoma cell line, Huh7.^(174, 175) Clinical trials of IFN- λ for HCV treatment were based on the hypothesis that limited IFN- λ receptor tissue distribution would decrease the viral load in infected hepatocytes^(175, 176) without the toxicity that accompanies IFN- α treatment. This expectation has so far been met,⁽¹⁷⁷⁾ lending support to the emerging paradigm that both IFN- α/β and IFN- λ contribute to host antiviral defense, with IFN- α/β being also a major regulator of immune cell function with both beneficial and detrimental sequelae. In addition, IFN- λ 1 and IFN- λ 2 were considered to inhibit HIV-1 infection in macrophages. Consistent with its antiviral activity, IFN- λ induces expression of several classical biomarkers of the antiviral response, including double-stranded RNA-activated protein kinase, 2',5'-oligoadenylate synthetase, and the Mx proteins.⁽¹⁷³⁾ Furthermore, the discovery of IFN- λ with antiviral properties but more restricted receptor expression might represent a suitable alternative for cancer therapy. In fact, lambda IFNs revealed potent antitumor activity in murine models of cancer and have been proposed as novel tools for cancer treatment.⁽¹⁶⁹⁾

IFN λ has been shown to decrease the production of Th2-type cytokines (IL-4, IL-5, IL-13, IL-14, IL-15), potentially favouring a Th1 immune pathway,^(178, 179) increase Treg and augment CD8⁺ T cell cytotoxicity and memory responses in macaques vaccinated with an HIV antigen.⁽¹⁸⁰⁾ However, others have shown that immune cell subsets (monocytes, NK, T cells) are unresponsive to IFN λ 1 and IFN λ 2 and postulated that this is due to the production of a soluble receptor produced by peripheral blood mononuclear cells.^(181, 182)

The ability of IFN- α to up-regulate induction of IFN- λ may be due to the ability IFN- α to up-regulate expression of the TLR and IRF7 genes.⁽¹⁸³⁻¹⁸⁵⁾

Recently, it was found that IFN- λ 1 has, besides its antiviral and antitumor activities, a relevant role in immunomodulatory responses. Specifically, it was noted that this cytokine regulates the development of Th1 and Th2 cells. This is shown by the reciprocal control of IL-4 and IFN- λ secretion; this is demonstrated by IL-4 stimulation

of monocytes to release high levels of IL-1 receptor antagonists that act directly on pDCs to augment their IFN- λ 1 production and function. Also, it was found that there is a markedly IFN- λ 1-dependent diminished IL-13 secretion in T cell cultures where IL-4 had been added. Therefore, IFN- λ 1 appears to be an inhibitor of human Th2 responses directed towards IL-13. Therefore, there is a mechanism in regulating IFN- λ 1 secretion and pDC function in which IFN- λ 1 emerges as a cytokine with an immunomodulatory role for Th2 generation. Furthermore, IFN- λ leads to generation of partially mature DCs with a tolerogenic phenotype; these DCs express high levels of MHC class I and MHC class II with low levels of costimulatory molecules and are able to migrate to lymph nodes upon injection into immunodeficient mice. In addition, they can induce mature DCs capable of inducing an IL-2-dependent proliferation of CD4+CD25+Foxp3+ Treg cell population.⁽¹⁶⁹⁾ In addition, IFN- λ 1 may participate in the pathogenesis of other autoimmune diseases such as SLE and might be related to the disease activity.⁽¹⁷¹⁾