Human Clinical Trials in Lupus Nephritis

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Summary: Improved patient survival after treatment of lupus nephritis with corticosteroids, immunosuppressants, and renal replacement therapy allows greater emphasis on long-term management issues. In particular, the recent focus has been on therapies to treat nephritis with fewer adverse effects compared with cyclophosphamide and immunosuppressive regimens. Issues complicating clinical trial design in lupus nephritis have severely limited comparisons across trials. These issues, including recognition and stratification of high-risk populations, comparable remission and response criteria, and appropriate use and interpretation of activity and damage indices have been the subject of much discussion and emerging consensus. Mycophenolate mofetil (MMF) has been used in the field of transplantation for more than 10 years. After initial anecdotal reports describing the benefits of MMF in the treatment of lupus nephritis, randomized controlled trials have established a role for MMF in the treatment of lupus nephritis. A host of newer agents including rituximab, abatacept, and monoclonal antibodies blocking costimulatory targets are in current clinical trials for lupus nephritis. As long-term outcomes in lupus nephritis improve, the toxicity of therapy and risk of relapse become increasingly important determinants of the choice of therapeutic agents. Semin Nephrol 27:115-127 © 2007 Elsevier Inc. All rights reserved.

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Systemic lupus erythematosus (SLE) is a multisystem autoimmune disorder characterized by the production of autoantibodies, a striking female predominance, and the frequent development of immune complex–mediated glomerulonephritis. The diagnosis of SLE is a clinical diagnosis based on combined clinical, pathologic, and laboratory findings enumerated in the criteria established by the American College of Rheumatology in 1982 (Table 1).[1] The 1987 modification recognized antiphospholipid antibodies in place of the LE cell prep criterion because most institutions no longer perform this test.[2] These criteria are useful in establishing a diagnosis of SLE, although the requirement that a patient show at least 4 of 11 signs or symptoms applies only to clinical research. In fact, many parameters in frequent clinical use, such as hypocomplementemia and renal biopsy results, are not included.[3] These criteria currently are undergoing reassessment by an international rheumatology group.

Renal disease caused by SLE significantly affects 25% to 40% of patients and is mediated largely by the renal deposition of immune complexes. The diagnosis of lupus nephritis (LN) usually is made after a renal biopsy in the presence of proteinuria and/or hematuria, positive serologies, and extrarenal manifestations of SLE. The presence of renal disease remains the most important predictor of morbidity and mortality in patients with SLE.[4,5] SLE affects predominantly young females of childbearing age with a peak incidence between ages 15 and 40. The incidence and prevalence of SLE and LN differ among different ethnic groups. African Americans have a 3-fold increased incidence of SLE, develop disease at younger ages, more frequently express anti-Smith and ribonuclear protein (RNP) antibodies, and have increased mortality when compared with Caucasians.[6,7]

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Table 1. The 1982 Revised Criteria for the Classification of SLE

Criteria	Definition
Malar rash	Fixed erythema, flat or raised, over the malar eminences, tending to skip over 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
Oral ulcers	Oral or nasopharyngeal ulceration, usually painless
Arthritis	Nonerosive arthritis involving 2 or more peripheral joints, characterized by tenderness, swelling, or effusion
Serositis	Pleuritis—convincing history of pleuritic pain, rubbing heard by a clinician, or evidence of pleural effusion Or pericarditis—documented by EKG or rub, or evidence of pericardial effusion
Renal disorder	Persistent proteinuria >.5 g/d or >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 (eg, uremia, ketoacidosis, or electrolyte imbalance) Or psychosis—in the absence of offending drugs or known metabolic derangements (eg, uremia, ketoacidosis, or electrolyte imbalance)
Hematologic disorder	Hemolytic anemia with reticulocytosis Or leukopenia—<4,000/mm^3 total on 2 or more occasions Or lymphopenia—<1,500/mm^3 on 2 or more occasions Or thrombocytopenia—<100,000/mm^3 in the absence of offending drugs
Immunologic disorder	Positive lupus erythematosus cell preparation Or anti-DNA—antibody to native DNA in abnormal titer Or anti-Sm—presence of antibody to Sm 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	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 SLE

Data from Tan et al.[1]

African Americans also develop nephritis earlier in their course of SLE. In an inception cohort of lupus patients in the southeastern Untied States, the difference in renal disease in African Americans versus Caucasians within a median of 13 months from diagnosis was significant (31% of African American patients versus 13% of Caucasian patients).[8] Hispanics also have greater frequency and severity of nephritis compared with Caucasians.[9] The proportion of patients receiving dialysis for end-stage renal disease for LN is increasing in the United States.[10,11]

Several demographic, serologic, and genetic risk factors are associated with an increased risk of developing kidney disease. Patients with LN are more likely than SLE patients without renal involvement to have a family history of SLE, anemia, high anti–double-stranded DNA (dsDNA) antibody titers, and hypocomplementemia.[12] Age at disease onset and sex also are important: patients with onset of SLE at younger than age 16 develop LN more frequently than adults (~85% versus 40%), as do males compared with females.[8] However, onset of SLE in older patients is not milder because race confounded initial reports that elderly patients were less likely to develop LN, and greater morbidity and

mortality is seen in this group.[13] The presence of anti-Sm autoantibodies has been associated with more severe expressions of SLE, including LN, although this was not seen in all patient cohorts.[14] The presence of anti-dsDNA and antihistone autoantibodies are associated with an increased risk of proliferative LN.

TYPES OF SLE NEPHRITIS

LN is an immune complex–mediated glomerulonephritis, varying in its expression from mild asymptomatic proteinuria to an overt nephrotic syndrome or acute nephritis associated with rapidly progressing azotemia. Most patients with SLE have deposition of immunoglobulin and complement, even in the absence of clinically significant renal dysfunction. Location, quantity, and host response to the immune reactants result in a spectrum of renal lesions categorized into different classes of LN. Despite these general correlations, there is substantial overlap in the clinical presentation of patients with the various histopathologic findings and it remains difficult to diagnose the type or severity of renal disease based on clinical grounds alone. For this reason, a renal biopsy is very useful—if not essential—in the management of patients with suspected LN. It provides an invaluable guide to therapy by clarifying the clinicopathologic syndrome, and assessing the relative degrees of active inflammation and chronic scarring. It also may identify unsuspected causes for an acute non–SLE-related worsening in renal function such as the development of a thrombotic microangiopathy or a drug-induced tubulointerstitial nephritis.

OLD AND NEW CLASSIFICATIONS OF LN

The classification of LN into discrete classes has been a critical step in facilitating communication between and among pathologists and clinicians, and to define homogenous groups of patients enrolled in clinical trials.

The initial classification of lupus nephritis was proposed in 1974 (by the World Health Organization [WHO]) and was modified in 1982 and 1995. An international panel of pathologists, rheumatologists, and nephrologists recently has proposed a new revision for the classification of LN (International Society of Nephrology/Renal Pathology Society classification of LN 2003). Overall the new proposal strongly resembles the 1974 classification, but differs notably in defining class III and IV lesions, requiring description of the activity and/or chronicity of these lesions and whether the glomeruli are involved globally or segmentally. These changes obviate the separate activity and chronicity indices. The definition of class V lesions is clarified. WHO class I, the mildest pathologic expression of LN, is associated with normal renal histology and is not included in the 2003 classification. Class II, characterized by immune complex deposition confined to the mesangium, with (class IIB) or without (class IIA) varying degrees of focal to diffuse mesangial hypercellularity, now are categorized as class I and II, respectively. Focal proliferative (class III) or diffuse proliferative (class IV) LN remain defined as less than 50% glomerular involvement vs 50% or more involvement, respectively, and descriptors of segmental versus global involvement of the glomeruli are required. Some investigators[15] report that these patterns represent distinct clinical subsets with differing outcomes, others[16] report that no differences are seen.

In the WHO classification, class V LN was categorized further as class Va when membranous changes are found exclusively, Vb when there is concurrent mesangial hypercellularity, Vc when there are focal endocapillary proliferative changes, and Vd in the presence of diffuse proliferative changes. Clinically, patients with class Vc and Vd nephritis follow a clinical course resembling that of focal or diffuse proliferative lupus glomerulonephritis (class III and IV), whereas patients with class Va and Vb have a predominantly nephrotic course similar to that of idiopathic membranous nephropathy. For these reasons, classes Vc and Vd have been abandoned by the 2003 International Society of Nephrology/Renal Pathology Society classification. Patients with features of membranous and proliferative lesions are reported as having class V in addition to class III or IV lesions. Because of the typically relapsing pattern of LN, the nephritis eventually results in extensive glomer-

ular sclerosis, adhesions, fibrous crescents, interstitial fibrosis, and arteriosclerosis (class VI) in both systems. Recent evaluation of the new classification compared favorably with the WHO criteria.[17]

Transformation between classes of LN increases the complexity of managing patients with LN. It is common for a class III lesion to progress to class IV LN. Both class III and IV lesions can transform into membranous (class V) LN, either spontaneously or with immunosuppressive therapy. It is less common, but possible, for membranous lesions to transform into more proliferative lesions. Repetitive clinical evaluations may not define these changes clearly, and repeated renal biopsies sometimes are needed. Another concern is the frequent relapses of LN shortly after discontinuation of immunosuppressive therapy despite clinical parameters suggesting remission. This raises the difficult issue of the role of repeat renal biopsy to define pathologic and clinical remission.

Some patients with SLE develop a thrombotic microangiopathy that may be associated with antiphospholipid antibodies or with an overlap syndrome with systemic sclerosis. Thrombotic thrombocytopenic purpura is increased in frequency in SLE, in up to 10% of patients with class IV LN.

Laboratory Findings

Antinuclear antibodies are more than 90% sensitive but only 70% specific for SLE because they also are found with other rheumatic diseases, infections, neoplasms, and among older people. Conversely, up to 10% of patients who meet the diagnostic criteria for lupus do not have a positive antinuclear antibody test result. Tests for antibodies to nuclear or cytosolic antigens other than DNA are more specific for SLE. For example, antibodies to the Sm antigen are very specific for lupus, but are found in only 20% to 30% of patients. The total hemolytic complement (CH-50), C4, and C3 typically are low during active disease. Because some patients with SLE have a genetic decrease in the synthesis of complement components (especially C4), a low complement concentration does not always indicate active disease. Longitudinally repeated measurements of these factors are more helpful in determining the relative state of disease activity of a patient.

Racial differences in lupus expression remain poorly understood. African Americans and Hispanics with LN are more likely to progress to end-stage kidney disease than Caucasians. Factors influencing renal outcomes may include socioeconomic and psychosocial variables. In the LUpus in MInorities: NAture versus Nurture (LUMINA) study, however, the worst outcome for African American patients with LN was independent of health care access, compliance with medications, and socioeconomic status.[18,19] Mortality rates from SLE have been relatively stable among Caucasians but have increased among African Americans since the 1970s.[6] Mortality rates in lupus patients on dialysis do not differ from the overall dialysis population, although lupus patients more typically are younger, female, and have lower incidence of diabetes.

Pathophysiologic Studies of Human LN

Many recent studies focus on genes that may induce or modify the progression of human lupus nephritis. For example, it has been reported that the angiotensin-converting enzyme insertion/deletion polymorphism is important in SLE.[20] However, in a meta-analysis of 13 prior studies consisting of 1,411 patients with lupus and 1,551 control patients, investigators found no association of the angiotensin-converting enzyme insertion/deletion polymorphism with SLE or LN in the total sample, or in any ethnic group. Trends for the deletion allele in lupus in Caucasian patients were not statistically significant.[21] Studies of polymorphisms in the interleukin (IL) genes in SLE, including *IL-12* and *IL-23* genes, have a pathophysiologic rationale, and these genes have been implicated in SLE. Yet a review study of a large group of 559 Spanish patients and 603 ethnically matched healthy controls showed that these polymorphisms did not play a relevant role in the susceptibility or severity of lupus.[22] A recent carefully controlled study reported that acetylation of polymorphisms was not an important risk factor in patients with lupus. A number of genes have been associated with susceptibility for autoimmune diseases in general, including

lupus, rheumatoid arthritis, and type I diabetes. A study of 16 different genes and the single-nucleotide polymorphism associated with type I diabetes susceptibility were examined in lupus patients. Although these genes were associated with pathways considered central to the development of type I diabetes, there were no positive findings in lupus. This study included 754 families with type I diabetes and a case-control collection of 1,500 to 4,400 cases. This type of large registry is required before any genes of interest can be considered important.[23]

Two recent positive genetic studies suggest that there are genes associated with autoimmunity in general rather than with a particular disease. The first of these in Nature Genetics examines a variant of the FcR receptor family associated with several autoimmune diseases including lupus.[24] A single-nucleotide polymorphism in the promoter region of this receptor resulted in autoimmune susceptibility by regulating FcR receptor-III expression on B cells. In a separate study, data are emerging from an analysis of families with multiple autoimmune diseases as part of a genetic consortium, Multiple Autoimmune Disease Genetics Consortium. This consortium includes families with lupus, type I diabetes, Hashimoto's thyroiditis, inflammatory bowel diseases, and 4 other autoimmune diseases. Notably, a single-nucleotide polymorphism in an intracellular tyrosine phosphate conferred risk for 4 separate autoimmune disease phenotypes including lupus, rheumatoid arthritis, type I diabetes, and Hashimoto's thyroiditis.[25]

Defects in the regulation of B and T cells in lupus have long been recognized. Investigation of B-cell tolerance in human lupus using tonsillar biopsy specimens[26] showed that autoreactive B cells in patients with lupus avoid normal allelic exclusion checkpoints, and that these B cells could participate in germinal cell reactions and expand with memory and plasma cell compartments. The regulation of T-cell function has been examined as well. It is well known that IL-2 production is abnormal in patients with lupus and IL-2 is important in the maintenance of T-cell tolerance. In these patients, the correction of a specific catalytic subunit of protein phosphatase IIa normalized IL-2 production and could prove to be a novel tool to correct T-cell IL-2 production in patients with lupus.[27] Another report suggested that a specific binding protein, or cyclic adenosine 5′-monophosphate (cAMP) response element modulator, binds to the IL-2 promoter and represses transcription of the IL-2 gene in human beings.[28] The T-cell Th1 or Th2 response was examined in 100 patients with lupus and 10 healthy controls to look for urinary excretion of transcription factor important in the Th1/Th2 balance in patients with lupus nephritis. By using urinary messenger RNA expression patterns, patients with active lupus appeared to have an expression pattern indicating a prominent Th1 type of T-lymphocyte activation.[29] These studies about B and T cells advance the understanding of the basic science of lupus and help to define future targeted therapies.

Many autoimmune diseases appear to have a seasonal variation. It is known, for example, that anti-neutrophil cytoplasmic antibodies (ANCA) vasculitis appears more frequently in late fall, winter, and early spring and is statistically less common in the summer months.[30] Similarly, there appears to be a clustering of thrombotic microangiopathy in the winter months. In an interesting biopsy study, patients with diffuse proliferative lupus glomerulonephritis had a higher incidence during the summer and fall than during the winter and spring. In contrast, there were a higher number of patients with membranous lupus during the winter and spring than in the summer. These studies suggest an infectious or viral pathogenic trigger causing this seasonal variation of lupus nephritis subtypes.[31]

Clinical Variants and Prognosis of Lupus and the Kidney

There have been a number of studies that examined the factors associated with poor outcomes in patients with LN. In the past, these studies have emphasized African American race, entry creatinine level, and pathologic indices. In a case-control study of 213 patients with LN, of whom 47% were Hispanic, 44% were African American, and 9% were Caucasian, one quarter of this population developed a

primary end point of death, end-stage kidney disease, or doubling of their serum creatinine level. Of these, 34% of African American, 20% of Hispanic, and 10% of Caucasian patients reached a primary composite end point. As anticipated, a proliferative glomerulonephritis with a higher activity index, higher baseline blood pressure, higher serum creatinine level, lower hematocrit, and a lower serum complement C3 were all independently important predictors. However, by multivariate analysis, only the chronicity index, the mean arterial blood pressure, and the baseline serum creatinine level proved to be associated independently with an adverse outcome.

Conversely, there also have been studies of patients with good long-term outcomes including a Canadian study that examined which patients with LN attain sustained remission. Sustained remission was defined stringently as normal renal function, urine protein excretion of less than .5 g/d, and inactive urine sediment without any significant immunosuppressive therapy for at least 3 years. A total of 35 patients were identified, 16 with a sustained remission of LN and 19 control patients with LN who did not have a sustained remission. Of the patients in remission at the final follow-up evaluation, creatinine clearance was significantly better than the controls, disease activity measured by activity score was lower, and cumulative damage indices did not increase after patients entered remission. As expected, in the control population these variables continued to increase. Patients who did well tended to be female, older, had higher nonrenal activity scores at the time of diagnosis, and did not require the use of azathioprine. In this population of Canadians, remission of LN occurred in many patients and was sustained without maintenance immunosuppressive therapy. In a similar kind of study by Moroni et al,[32] 32 patients with biopsy-proven LN who entered remission were followed-up for a median of 203 months. Fifteen patients never relapsed, whereas 17 developed lupus exacerbations within a median of 34 months after stopping therapy and required re-treatment. The only difference between the 2 groups was a longer median duration of therapy (57 months initially) versus 30 months in those patients who ended up having a relapse. The duration of remission was 24 months, or 12 months before stopping therapy. In this Italian series, some patients with lupus attained and maintained remission without any specific therapy for years, and even in those patients with new flares remission was attainable again. The longer the initial treatment and a remission before withdrawal of therapy seemed to decrease the risk of relapse. Whether these patients represent an anomaly or a typically Caucasian population from Halifax, Nova Scotia, or Milan, Italy, remains to be studied in a broader group of patients. It has long been noted that there are patients, typically Caucasian, who do very well with long-term follow-up evaluation.[33] In a study performed by Dooley et al[34] of Caucasian versus African American patients with diffuse proliferative glomerulonephritis treated with intravenous cyclophosphamide, Caucasian patients did well during the time of the study and continued to do much better despite being off all immunosuppressive therapy in some cases for years.

When patients with lupus (typically younger) progress to end-stage kidney disease, they are considered for kidney transplantation. Patients with LN as the cause of their kidney dysfunction have been considered at a higher risk for adverse outcomes relative to patients with nonlupus kidney failure. In a case-control study, 33 patients with lupus compared with 70 matched controls who underwent kidney transplant from 1982 to 2004 were examined. In these 2 populations, patient and graft survivals were very similar; however, the risk for thrombotic episodes was greater in patients with lupus and especially in those who were antiphospholipid antibody positive. These data strongly suggest that in patients with a kidney transplant, the presence of antiphospholipid antibody should suggest the consideration of anticoagulation therapy.[35]

Therapy Studies

Therapeutic decisions for individual patients with LN should be based on consideration of their clinical presentation, laboratory features, and histologic findings on biopsy. In general, patients with mesangial lupus nephritis (class

II) do not require immunosuppressive treatment beyond that required for their extrarenal manifestations of disease. In patients with advanced glomerulosclerosis, the risks of immunosuppression likely outweigh the potential benefits.

Therapy of Proliferative LN

Patients with very mild focal proliferative lupus nephritis (WHO class III lesions without crescents and karyorhexis, normal and stable glomerular filtration rate (GFR), modest proteinuria, and no demographic risk factors for poor outcome) may be followed-up closely without the immediate institution of aggressive immunosuppression. However, when there is necrosis, karyorhexis, or crescent formation in addition to the focal proliferative disease, the long-term outcome is similar to that of diffuse proliferative glomerulonephritis (class IV) and should be treated in the same fashion.

Patients with more severe focal proliferative or diffuse proliferative glomerulonephritis (WHO classes III and IV, respectively) are at high risk of progressive loss of renal function and warrant aggressive immunosuppressive therapy. In patients with proliferative lesions, the use of cytotoxic drugs (cyclophosphamide or azathioprine) in addition to corticosteroids has been shown to improve renal survival over treatment with corticosteroids alone.[36] A delay of therapy is associated with an increase in renal scarring that is poorly responsive to immunosuppressive therapy.[37]

There has been an explosion of therapeutic studies in LN with conventional and experimental approaches. A number of studies emphasize the importance of blood pressure control. A trial of 12 patients with LN who remained proteinuric despite glucocorticoids or immunosuppressive therapy were administered an angiotensin-receptor blocker for 6 months. As would be expected, proteinuria decreased significantly, serum albumin and cholesterol improved significantly, and systolic blood pressure decreased significantly.[38] Once immunomodulating therapy is stopped in some patients with lupus, angiotensin-converting enzyme inhibitors or angiotensin-receptor blockers reduce proteinuria and the rate of renal function deterioration in these patients. In a study from Hong Kong of Chinese patients, angiotensin-receptor blockade improved proteinuria, improved albuminuria, and reduced systolic blood pressure.[39]

Other studies have emphasized the toxicity of continuous cyclophosphamide therapy. The role of cumulative dose of cyclophosphamide versus route of administration (oral or intravenously) on toxicity was investigated. Patients with diffuse proliferative LN were treated with cyclophosphamide and prednisone. A total of 212 patients (89% female) who had lupus for an average of 37 months were randomized to receive daily oral cyclophosphamide versus intravenous bolus cyclophosphamide. At the last dose, almost 60% of patients had responded completely and 26% had responded partially. In a logistic regression analysis, the cumulative dose of cyclophosphamide and the total histologic chronicity score predicted a complete response. Seventy-three percent of patients ended up on maintenance immunosuppression, primarily azathioprine, for 3 years. During this time, 66 patients had a renal flare resulting in renal insufficiency, renal failure, or death. In general, renal survival rates were quite good with 88% of patients showing renal survival at 5 years, 83% at 10 years, and 71% at 15 years. As can be expected, ovarian toxicity was more common in the oral cyclophosphamide regimen, with increasing age and higher cumulative doses of cyclophosphamide. However, the cumulative dose rather than the route of cyclophosphamide administration determined ovarian toxicity. These results suggest that oral cyclophosphamide, in which the cumulative dose accrues most quickly, should be reserved for only high-risk patients who have failed other therapies.[40]

Several studies have examined the role of mycophenolate mofetil in the therapy of LN. The first randomized trial in a Chinese population of patients with LN compared oral cyclophosphamide with mycophenolate mofetil. At the end of 1 year, mycophenolate mofetil was at least as effective as the cyclophosphamide, with fewer side effects.[41,42] This study prompted a series of randomized trials examining the usefulness of mycophenolate mofetil therapy in response to con-

cerns that were raised about the short duration of the trials, the ethnically restricted study population, and the small numbers of patients included. These studies showed that mycophenolate mofetil and intravenous cyclophosphamide were equally efficacious.[43-45] A subsequent study from Hong Kong examined the long-term use of mycophenolate mofetil when compared with cyclophosphamide and azathioprine. In a randomized trial of patients with diffuse proliferative LN, the role of mycophenolate mofetil as a continuous induction maintenance regimen was examined in 33 patients, compared with 31 patients assigned randomly to cyclophosphamide and azathioprine treatment. Both arms received prednisolone. More than 90% of patients in both groups responded well with either complete or partial remission to their induction treatment. There was improvement in serology and proteinuria in both groups. Adverse events occurred in 6.3% of the mycophenolate mofetil group and 10% of the cyclophosphamide/azathioprine-treated group, who doubled their baseline creatinine level during the follow-up period. The odds ratio for relapse was similar in both treatment arms; that is, 11 patients in the mycophenolate mofetil group and 9 patients in the cyclophosphamide/azathioprine group relapsed. Mycophenolate mofetil was associated with fewer infections that required hospitalization and fewer infections overall, and this was an important finding of this study. Moreover, no patients in the mycophenolate mofetil group reached a composite end point of end-stage kidney disease, whereas 4 patients in the cyclophosphamide/azathioprine group reached end-stage kidney disease. Survival analysis was not significantly different, but these data again point to the fact that mycophenolate mofetil is an effective induction and maintenance treatment therapy, at least in Chinese patients with LN.[46] A similar randomized control study of pulse intravenous cyclophosphamide and mycophenolate mofetil in proliferative nephritis was conducted in Malaysia. This was a small study of only 44 patients from 8 centers who were treated either with cyclophosphamide or mycophenolate mofetil. Both therapies appeared to be effective as induction therapy for moderately severe proliferative LN, and the data revealed very few differences in the 2 groups of patients. In this study, however, the adverse side effects were similar.[47]

In the United States, Contreras et al[48] studied sequential therapy for induction using cyclophosphamide followed by remission maintenance with mycophenolate mofetil, or azathioprine, or intravenous cyclophosphamide. In this study, the mycophenolate mofetil–treated patients had the best long-term outcomes, with a reduced number of adverse events, especially when compared with the intravenous cyclophosphamide regimen. Although azathioprine and mycophenolate mofetil therapies were similar, mycophenolate mofetil was slightly better at long-term remission maintenance than azathioprine.

Another trial recently emerged from the United States sponsored by the Food and Drug Administration (FDA) that examined induction of remission in diffuse proliferative lupus nephritis with mycophenolate mofetil or intravenous cyclophosphamide.[49] This study involved a number of centers that represented the cross-section of patients with LN in a 24-week, randomized, open-label, noninferiority trial that compared oral mycophenolate mofetil with monthly intravenous cyclophosphamide. Mycophenolate mofetil was given at a starting dose of 1,000 mg/d and then increased to 3 times a day for a total of 3,000 mg/d. The primary end point was complete remission within 24 weeks, defined as the normalization of renal abnormalities. The secondary end point was partial remission within 24 weeks. A total of 140 patients were recruited and divided equally into the 2 treatment arms. Of these, 56 of 71 patients receiving mycophenolate mofetil and 42 of 69 patients receiving cyclophosphamide had satisfactory responses at 12 weeks, and 22% of the mycophenolate mofetil– and 5.8% of the cyclophosphamide-treated patients had complete remission. Partial remissions occurred in almost 30% of the mycophenolate mofetil– and 25% of the cyclophosphamide-treated patients. When the complete and partial remissions were considered together, the mycophenolate mofetil–treated patients appeared to have a superior response compared with the cyclophosphamide-treated patients. However, nearly half of the patients in both treatment arms did not

achieve either complete or partial response at 6 months. There were fewer adverse consequences with mycophenolate mofetil including a reduction in the number of infections and hospitalizations. Three of the cyclophosphamide-treated patients died, whereas none of the mycophenolate mofetil–treated patients died. As one would expect, there were more gastrointestinal side effects with this form of therapy, especially diarrhea.[49] This study, performed in a cross-sectional population in the United States, suggests that mycophenolate mofetil is useful for induction therapy.

Is mycophenolate mofetil effective in the treatment of membranous lupus nephropathy? In a retrospective study of 10 patients with membranous lupus who had been treated previously with a variety of agents, mycophenolate mofetil proved to be a useful agent. Urinary protein excretion and serum albumin level improved, although there was no change in the serum creatinine level.[44] Similarly, data extracted from the FDA mycophenolate mofetil/cyclophosphamide trial revealed similar results, with membranous nephropathy improving with mycophenolate mofetil therapy.[50] These studies, coupled with the obvious toxicity of cyclophosphamide, have availed the widespread use of mycophenolate mofetil as induction therapy for LN. Despite this, caution in adopting this new therapy remains important. These trials all have been of relatively short-term duration. The advantage of the cyclophosphamide-based National Institutes of Health treatment regimen is the advantage of decades of long-term follow-up evaluation. Cyclophosphamide treatment has allowed many patients to stop all immunomodulating therapy and to remain remission-free. It is not known whether patients given mycophenolate mofetil therapy will have to endure endless therapy. Anecdotal experience suggests that when tapering mycophenolate mofetil, patients with LN experience a flare. Many patients desire therapy with mycophenolate mofetil to preserve fertility. A female patient who is in complete remission and who wants to conceive a child will need to stop mycophenolate mofetil. What is the best approach to conversion from mycophenolate mofetil? Is azathioprine the correct drug? Should immunomodulating therapy be stopped altogether? Are there predictors that will help the clinician and patient understand the relative safety of mycophenolate mofetil tapering or cessation? Most importantly, what are the long-term consequences of mycophenolate mofetil–based therapy at 5, 10, or even 15 to 20 years? Yet another trial is underway involving centers across the globe that randomly induces a remission with 6 months of either cyclophosphamide or mycophenolate mofetil and then remission is maintained with either azathioprine or mycophenolate mofetil for a total of 24 additional months. This trial has recruited about half of the needed subjects.

In this era of treatment with mycophenolate mofetil or cyclophosphamide for glomerular disorders, it is interesting to examine what happens to patients who become critically ill and arrive in an intensive care unit. In a prospective study from Taiwan, 51 such patients with lupus were studied who had a mortality rate of 47%. The most common cause of admission was pneumonia with acute respiratory distress syndrome. Intracranial hemorrhage while in the intensive care unit, or gastrointestinal bleed, or concurrent septic shock was associated with a greater risk of dying. These results underscore the worry that our success in immunomodulating this disease may give rise to overimmunosuppression and death.[51]

In the past 18 months, a number of experimental agents have been tried in the treatment of lupus as well. Twelve different studies were reviewed wherein varying doses of rituximab and anti-CD20 antibody aimed at depleting B cells, or varying dosing intervals, were considered. Each of these trials used therapy in addition to rituximab, and there were vastly different measures of efficacy. The duration of follow-up evaluation ranged from 3 to 24 months, and a whole host of side effects was noted. What are the take-home messages from this review? First, it is noted from these and other anecdotal experiences that B-cell depletion occurs within 1 to 3 months of therapy and that this response coincides with a clinical response. B-cell depletion may last from 3 to 12 months, and the clinical benefits may last even longer. If B-cell depletion is not attained, clini-

cal efficacy will not occur. Moreover, serologic markers of dsDNA antibodies or C3 complement levels may not normalize, even in patients who clinically respond. There is no question from these anecdotal studies that a prospective randomized trial using rituximab is critical for understanding the use of this drug in LN.[52] In addition to rituximab, there are a number of studies underway of new agents that target immune cells, including the humanized anti-CD20 antibody, or epratuzumab (anti-CD22). A number of drugs block the costimulatory pathways between B and T cells by blocking the CD40 ligand pathway, B-lymphocyte stimulator (BLyS), or B-cell-activating factor (BAFF), or by blocking the B7 interaction with cytotoxic T lymphocyte-associated antigen 4-immunoglobulin (CTLA-4Ig).[53] An oral toleragen directed against anti-ds DNA antibodies remains controversial,[54] though these autoantibodies remain central pathogenic agents in lupus nephritis.[55] Blockade of the complement system with monoclonal antibodies directed against C5 and CDR1 also are under study.[56] None have been evaluated sufficiently to warrant their use outside of controlled clinical trials. Two of these agents, rituxamab and abatacept, recently were FDA approved for the treatment of rheumatoid arthritis and there are increasing anecdotal case reports and small series in LN, often of patients failing established therapy.

Laboratory Findings

For many years, the course of disease was followed-up serologically by serial determinations of titers of anti-dsDNA antibodies and complement levels. In a large study of 487 patients with lupus and a history of LN, anti-dsDNA antibodies were measured at baseline and then on a repetitive basis.[54] This study was performed as part of the LJP 394 tolerogen trial, including patients in the placebo versus the drug-treatment arm. In this study, dsDNA antibody titers correlated with the risk of renal flare. The incidence of renal flare was lower in patients who had sustained reductions in anti-dsDNA antibodies than in patients who had stable or increasing levels of these same antibodies. The data suggest that anti-dsDNA antibodies are a useful marker for following-up many patients with this disease. A problem remains: anti-dsDNA antibody assays are nonstandardized. There are at least 4 different routine assays for anti-dsDNA antibody detection, and depending on which one of these assays is used, the validity of anti-DNA antibody testing remains of concern. When a reference laboratory switches their anti-dsDNA assay, the results may greatly confound the interpretation of whether a patient is or is not at greater risk for relapse.[55] Other autoantibody testing, including anti-Smith and anti-RNP antibodies, are important in the diagnosis of disease. In fact, the anti-Smith antibody is highly specific for lupus and is almost diagnostic in the correct clinical setting.[56,57]

Several studies have examined the role of anti-C1q autoantibodies in LN, and the role of these antibodies in the pathogenesis of disease. Sixty-one patients with lupus, 40 who had biopsy-proven LN, were compared to controls including patients with other diseases associated with glomerulonephritis. Anti-C1q antibody titers correlated with disease activity and the flare of glomerulonephritis.[58] In a study of 151 patients with active lupus, there was a higher prevalence of anti-C1q antibodies than in those with no evidence of kidney disease.[59] Anti-C1q antibodies were absent in lupus patients without nephritis and lupus patients whose nephritis was quiescent. In 33 of 83 control patients with lupus but no history of kidney disease, 9 patients with anti-C1q eventually developed LN with a mean disease-free interval of only 9 months. So, in this study, the presence of anti-C1q antibodies in patients with lupus was associated with kidney disease and the development of disease involvement, suggesting that monitoring these antibodies may predict a lupus flare. C1q has a number of functions and plays a role in the clearance of immune complexes and apoptotic antibodies.[60,61] It is known that anti-C1q antibodies occur in SLE and a number of other autoimmune diseases.

Many attempts have been made in many kinds of glomerular diseases to use experimental microarray or proteomic methods as diagnostic or disease-modifying factors. These studies can be separated into those analyses that use

microarray data of RNA expression or proteomic data of either serum or urine. Urinary cellular messenger RNA was examined in lupus patients for a number of factors, including chemokine receptor CXRC3, interferon-producing protein 10, gamma transforming growth factor â, and vascular endothelial growth factor using quantitative real-time polymerase chain reaction analysis. A significant reduction in many of these factors was observed in patients who responded to therapy when compared with patients who were resistant to treatment. Measurement of these factors may predict disease severity. These were preliminary observations in only 26 patients, but it raises the possibility that this approach may be useful in monitoring patients.[62]

Proteomic studies also may reveal new possibilities. In particular, adiponectin, an adipocyte-derived cytokine with anti-inflammatory properties, was found in the urine and serum of patients with LN. A preliminary proteomic evaluation of urinary biomarkers in lupus had shown high levels of adiponectin. A detailed prospective examination of adiponectin in urine and plasma of patients with renal and nonrenal SLE was assayed prospectively looking for the relationship with lupus flares. Plasma adiponectin levels were higher in patients with lupus flares than in normal controls or patients who had non–renal-related lupus flares. This was true even after accounting for race and body mass index. Urine adiponectin levels increased more with renal flares, but not with nonrenal flares. Most importantly, urine adiponectin levels increased 2 months before overt kidney flare. The urine adiponectin level correlated with the plasma levels and the magnitude of proteinuria. In all urinary studies, the clearance of the protein must be adjusted for kidney function. In this case, urine adiponectin did not correlate with kidney function. In a further analysis, immunohistochemical identification of adiponectin was examined by kidney biopsy and was found on podocytes and tubules of lupus kidneys. The study suggests that urinary adiponectin may be a biomarker of lupus kidney flares.[63]

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