Management of Lupus Nephritis: An Update

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Systemic lupus erythematosus (SLE) is a multifaceted autoimmune disease characterized by the production of pathogenic immunoglobulins, such as antinuclear and antiphospholipid autoantibodies. The kidney is one of its major target organ, with up to 60% of adult SLE patients experiencing renal involvement, many with focal or diffuse proliferative glomerulonephritis, either as initial manifestation or during the waxing and waning course of the disease (1).

The aim of this concise review is to update the reader on the management of proliferative and membranous lupus nephritis (LN), on the basis of the results of pivotal controlled trials and of the hopes for more targeted immunointervention raised by recent advances in the understanding of the disease. Therefore, we start with a brief introductory glance on the mechanisms underlying LN.

LN: An Autoantigen-Driven Immune Response

There is overwhelming evidence that LN is caused by glomerular immune deposits, as indicated by the presence of immunoglobulins and complement breakdown products in virtually all kidney biopsy specimens obtained from patients with active LN. A major advance in lupus research has been the discovery that the disease is—at least in part—the result of an autoantigen-driven immune response (2).

Anti-dsDNA Antibody Response is Driven by Histone-Specific T-Helper Cells

On the basis of the classical view that anti-dsDNA antibodies are pathogenic in SLE, T-helper cells from lupus mice and patients have been cloned for their ability to induce the production of anti-dsDNA antibodies when cultured with autologous B cells. Sequencing of the T cell receptor β chain genes of these T-helper cell clones revealed a recurrent motif of anionic residues in the junctional region of the CDR3 loop, suggesting that the corresponding autoantigens were rich in cationic residues (3,4). It therefore was anticipated and proved

that the relevant antigens were peptides derived from the nucleosome, the DNA packaging unit, made of a 146-bp DNA loop wound around a cationic histone octamer core (5,6). This first set of experiments indicated that the so-called anti-dsDNA antibody B cell response in lupus was actually driven by histone-specific T-helper cells. As illustrated in Figure 1, right, the following picture can been sketched (7). B cells trap circulating DNA-binding proteins, such as nucleosomes, through their DNA-recognizing membrane-bound Ig. The complex is endocytosed and processed, and cationic peptides are presented in an MHC class II–restricted way to histone-specific T-helper cells. In the presence of appropriate co-stimulatory signals, such as CD40–CD40L (CD154) or CD28–B7.1/B7.2 (CD80/CD86), this cognate interaction results in B cell activation and proliferation, in particular through the production of cytokines.

Impaired Clearance of Apoptotic Bodies

The next step was to understand how nucleosomes are released and why they become immunogenic in SLE. Thus, it was demonstrated that blebs that appear on the surface of apoptotic cells, such as keratinocytes exposed to UV light, contained nucleosomes as well as other lupus target antigens, such as the ribonucleoproteins Ro and La, thereby suggesting that apoptotic waste could be a source of autoantigens (8). Most interesting, the clearance of apoptotic bodies by macrophages or other phagocytes is impaired in lupus patients (9), and apoptotic material was found to be tightly associated with dendritic cells in lupus lymph nodes (10). Taken together, these results suggest that autoantigens are processed by professional antigen-presenting cells and presented to autoantigen-restricted T-helper cells. Nucleosome-containing apoptotic material, rather than being engulfed and eliminated by macrophages without inducing immune and inflammatory responses, therefore could become immunogenic in SLE (Figure 1, left). Consistently, defective removal of apoptotic cells in C1q knockout mice (11) or serum amyloid P knockout mice (12) is associated with a lupus-like disorder. The reason that removal of apoptotic material is skewed toward dendritic cells in lupus patients is currently unknown. In this respect, the recent observation that IFN-α present in lupus sera favors the maturation of circulating monocytes in dendritic cells might be relevant (13).

Nucleosome/Antinucleosome Complexes

On the effector side, the role of nucleosome/antinucleosome complexes has been recently hypothesized (14). Thus, specific

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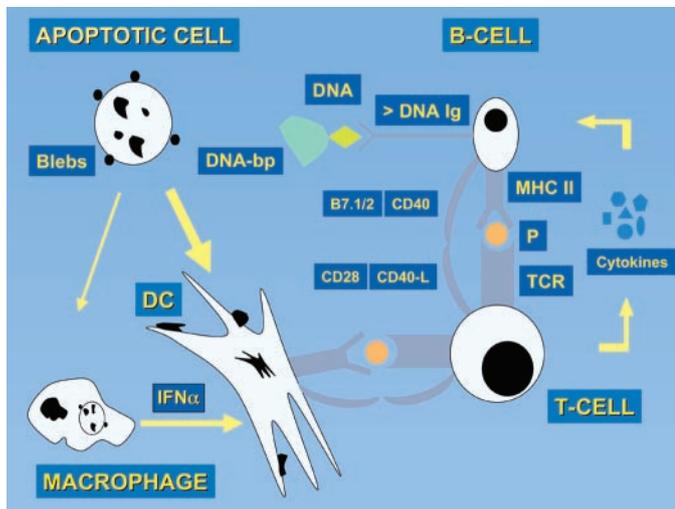
Figure 1. Autoantigen-driven immune response in systemic lupus erythematosus (SLE). B cells trap circulating DNA-binding proteins (DNA-bp), such as nucleosomes, through their membrane-bound anti-DNA antibody (> DNA Ig). The complex is endocytosed, processed, and presented, with MHC class II restriction (MHC II), to histone-specific T-helper cells. In the presence of optimal co-stimulation (through CD40-CD40L [CD154] and/or CD28-B7.1/B7.2 [CD80/CD86]), B cells become fully activated, in particular through the production of T cell–derived cytokines (right). Clearance of apoptotic bodies by phagocytes is impaired in SLE, and autoantigen-containing apoptotic material is processed by dendritic cells (DC) and presented to T-helper cells. IFN-α favors the maturation of circulating monocytes in dendritic cells (left). Adapted from Hermann *et al.* (7), with permission.

antinucleosomal antibodies (not reacting with dsDNA or with histones but with the complex of the latter and the former) are detected in the sera of LN patients, and their titers correlate with renal disease activity (15). Renal perfusion of nucleosome/antinucleosome complexes induces glomerular immune deposits and proteinuria in mice (16,17). Finally, nucleosomal antigens are detected in the glomerular basement membrane (GBM) of LN patients (18). Among other hypotheses, the cationic histone part of the nucleosome/antinucleosome complexes could bind to the negatively charged heparan sulfate molecule expressed on the GBM. Such nucleosome-mediated binding of antibodies to the GBM could then initiate glomerulonephritis, through complement activation but also through complement-independent mechanisms induced by Fc/Fc receptors interaction.

Beyond the Role of Antinuclear Antibodies

The pathogenesis of LN is probably much more complex than the above-mentioned mechanisms. Thus, Waters *et al.* (19), using a very elegant genetic approach, could demonstrate that breaking tolerance to dsDNA, nucleosome, and other nuclear antigens is not required for the development of nephritis in NZM2328 lupus-prone mice. In these animals, the *Cgnz1* locus on chromosome 1 is linked to nephritis, whereas the *Adnz1* locus on chromosome 4 is linked to antinuclear antibody

production. Congenic mice in which the genetic interval that contains *Adnz1* was replaced by that from C57L/J non–lupus-prone mice, still experienced nephritis, although their serum was negative for antinuclear, anti-dsDNA, and antinucleosome antibodies, thereby indicating that antinuclear antibody production and nephritis are under independent genetic control.

The genetic dissection of SLE will bring new insights into the pathophysiology of LN. Genome-wide screens performed in multiplex SLE families have already allowed the identification of multiple susceptibility loci and of candidate genes, such as PDCD-1 that encodes a protein that plays a role in lymphocyte activation and activation-induced cell death (20). In lupus-prone mice, congenic dissection is a powerful strategy to analyze the respective contribution of individual susceptibility loci to a polygenic trait. Congenic animals that bear a given susceptibility locus, such as *Sle1*, *Sle2*, *Sle3*, on a resistant background (B6) have been obtained. Most interesting, although each of the *Sle*-congenic strains displayed immune alterations, none developed fatal LN. Only multicongenic animals experienced full-blown disease (21,22). Fine mapping will further allow identification of disease-associated genes or pathways that could be specifically targeted.

Therapeutic Goals in LN: Still Unmet Expectations

Optimal management of LN remains a challenge because of the heterogeneity of the disease at presentation and its unpredictable course. Large multicenter studies are needed to gather enough patients to test new hypotheses, keeping in mind that very long-term follow-up (at least 5 yr) is required before conclusions on death and ESRD rates can be drawn. This prerequisite, brought to light by the pioneering studies conducted by the National Institutes of Health (NIH) group (23–25), is sometimes forgotten by investigators and/or their pharmaceutical industry partners, who look for prompt assessment of treatment efficacy.

Although there is no consensus on outcome definitions, such as remission and relapse of LN, most clinicians will agree on the following therapeutic goals for a patient with newly diagnosed lupus nephritis: (*1*) to achieve prompt renal remission, (*2*) to avoid renal flares, (*3*) to avoid chronic renal impairment, and (*4*) to fulfill these objectives with minimal toxicity. Although patient and renal survival rates have improved over the past decade (26), it should be stressed that current immunosuppressive regimens still achieve suboptimal results. First, the rate of renal remission after a first-line therapy is at best 81% in recent prospective studies (25,27–29). Second, renal relapses occur in one third of LN patients (30), mostly when patients are still immunosuppressed (31). Third, between 10 and 20% of LN patients experience ESRD 5 to 10 yr after disease onset, although these figures are lower (between 5 and 10%) in recent studies (28,29,32). Finally, treatment-related toxicity remains a major concern, such as metabolic and bone side effects of high-dose glucocorticoids (GC) (33–35), severe infections, or premature ovarian failure in women who receive high-dose cyclophosphamide (CYC) (36,37).

Numerous prognostic factors have been identified (38–40). Among others, nonwhite race (*e.g.*, black, Afro-Caribbean, Hispanic), poor socioeconomic status, uncontrolled hypertension, a high activity and chronicity index on kidney biopsy, renal impairment at baseline, poor initial response to therapy, and nephritic relapses have been associated with poor outcome. Lack of compliance to therapy, in particular to high-dose oral GC, is a trivial but underestimated (and mostly unconfessed!) cause of treatment failure. In a few cases, unrecognized association of proliferative LN with a thrombotic microangiopathy linked to the antiphospholipid clotting syndrome may further worsen the prognosis (41).

Taken together, LN still has a negative impact on lupus patients' survival as indicated by the long-term data collected between 1990 and 2000 by the investigators of the European Working Party on Systemic Lupus Erythematosus in a prospective series of 1000 European patients, whose overall survival rate at 10 yr was 88 and 94% for patients with and without renal involvement, respectively (42).

GC and Cytotoxic Drugs

Nonspecific immunosuppression by GC and cytotoxic drugs remains the gold standard treatment of LN, based on their well-known inhibitory effects on the immune system, their efficacy in murine models, and their wide availability at relatively low cost. Although caution should be applied in interpreting the data, three meta-analyses of controlled trials have shown the superiority of combined therapy with GC and cytotoxic drugs *versus* GC used alone. Thus, already in 1984, the analysis by Felson and Anderson, performed on eight trials including 263 LN patients (mainly enrolled at the Mayo Clinic and the NIH), indicated that patients who were given combined therapy with GC and cytotoxic drugs (azathioprine [AZA] and CYC considered together; oral CYC by that time) had less ESRD, fewer deaths from renal cause, and decrease in renal function compared with patients who were given GC alone (43). Bansal and Beto (44), in a pooled analysis of 19 clinical trials that included 440 LN patients, including most NIH studies with intravenous (IV) CYC, found that cytotoxic drugs used in conjunction with GC were statistically more effective than GC alone, with less ESRD (-13.2%) and total mortality (-12.9%), when AZA and CYC were considered together. Twenty-five studies (909 LN patients) were included in the last meta-analysis published by Flanc *et al.* (45), who found that patients who were given CYC combined with GC run a lower risk of doubling of serum creatinine. In conclusion, at the risk of expressing a tautology, the current state of knowledge indicates that patients with proliferative LN should be treated with GC and a cytotoxic drug, at least unless effective noncytotoxic therapy becomes available.

Induction versus Maintenance Therapy

An advance in the therapy of LN has been the understanding that cytotoxics should be used sequentially. After the oncologic experience, the concept is to induce remission of LN by a short course (a few months) of vigorous immunosuppression (*e.g.*, high-dose GC combined with fortnightly or monthly IV CYC) and to maintain remission by long-term administration (a few years) of either the same cytotoxic drug given less frequently (*e.g.*, quarterly IV CYC) or a potentially safer immunosuppressant (*e.g.*, AZA), with the hope to minimize toxicity without compromising efficacy. This fashionable concept, also recently applied in the field of primary systemic vasculitis (46), should not disguise (*1*) that there is no unanimously accepted definition of remission for LN (a reasonable consensus would be a 24-h proteinuria <0.5 g and <10 red blood cells/high-power field in the absence of renal impairment); (*2*) that the time frame to achieve remission and thereby the length of the induction phase is set somewhere between 3 and 12 mo, again without consensus; (*3*) that 20% of LN patients will never reach renal remission; (*4*) that the final outcome (*i.e.*, patient survival and prevention of chronic renal impairment with minimal toxicity and optimal quality of life) is the result of both remission and maintenance phases; and (*5*) that there is still considerable debate regarding which drug(s) should be used for induction and maintenance.

Cyclophosphamide

In the late 1970s and the early 1980s, daily oral CYC therapy combined with GC was considered the gold standard for LN (47,48). Later, given the toxicity of long-term continuous exposure to CYC (mainly for the bladder, the bone marrow, and the ovaries), oral CYC became supplanted by the intermittent pulse IV route, the more so as results from the NIH trials indicated that a high-dose long-term IV CYC regimen (0.75 to 1.0 g/m^2), prescribed monthly for 6 mo and then quarterly for up to 1 yr after remission, was superior to GC in the long run. This being said, several clinicians still consider that a short course of oral CYC could be prescribed in the acute phase, an option proposed in two recently published studies (27,49).

NIH Trials

In the first IV CYC NIH trial, Austin *et al.* (23) found that only patients who were given high-dose long-term IV CYC (and not those who were given oral CYC, AZA, or a combination of both) had a lower probability of ESRD compared with patients who were given oral GC alone. A subsequent analysis of the same trial revealed that all CYC-containing regimens (IV and oral) did better than GC alone in the long run (50). In a second trial, Boumpas *et al.* (24) showed that patients who had severe LN and were given a long-course (30 mo) IV CYC regimen but not those who were given a short course (6 mo) had a lower probability of doubling of serum creatinine (DSC) compared with patients who were given IV methylprednisolone (MP) pulses as immunosuppressive treatment. Not surprising, patients who were given a short-course IV CYC regimen (*i.e.*, not receiving any cytotoxic therapy after 6 mo) had a higher relapse rate than those who were given a long course. In the last NIH trial, referred to as the "Pulse Plus Study," combination therapy of IV MP and IV CYC was shown to achieve a higher rate of renal remission than IV MP alone (25). After a median follow-up of 11 yr, none of the 20

patients who received combination therapy experienced ESRD (32).

Shortcomings of the NIH Regimen

The shortcomings of the NIH regimen have already been underlined, including by the NIH investigators themselves: No effect on survival rates, no differences in outcome between IV CYC and other regimens including a cytotoxic drug (the significant differences were against GC), high rate of gonadal toxicity (ranging from 38 to 52% of women at risk) (36,37), increased risk of severe infections, significant percentage of treatment failures, and high rate of renal relapse despite incisive therapy (51). Two additional concerns should be stressed. The first is patients' preference. As suggested by a recent survey, patients are more and more reluctant to receive high-dose IV CYC. Female lupus patients were asked which treatment they would prefer if they experienced LN. Not surprising, 98% of them would choose AZA, instead of CYC, if AZA and CYC would confer an equal probability of renal survival, but making the assumption that CYC offers 100% renal survival at 5 yr—which is not true—still 31% of the patients would prefer AZA, given the pregnancy issue (52). A second concern deals with renal disease severity at diagnosis. Thus, in a series of 46 prospectively followed patients with biopsy-proven proliferative LN diagnosed in our academic hospital (mixed rheumatology and nephrology recruitment), only 18% experienced renal impairment at baseline, a striking difference with the series studied by Boumpas et al. (24) in which two thirds of the patients had abnormal renal function at presentation. These data suggest that renal disease might be less severe in European SLE patients, as a result of a different ethnic background and/or of early diagnosis and treatment of kidney involvement. Compared with tertiary centers such as the NIH, most European Clinics function as secondary referral centers, given their relatively easy (including geographic) accessibility. As a consequence, studies that test whether less incisive immunosuppressive regimens can be prescribed are justified. This was the major aim of the Euro-Lupus Nephritis Trial in which we tested whether low-dose IV CYC followed by AZA could achieve good clinical results, on the basis of the experience of the St. Thomas's Hospital group in London, United Kingdom. Over the past 15 yr, Hughes and D'Cruz (53,54) have indeed prescribed IV minipulses of CYC, at a fixed dose of 500 mg, weekly or fortnightly for a short induction period (a few months), followed by AZA as maintenance therapy. This regimen was used to treat LN but also other systemic rheumatic diseases (55), with encouraging results in open studies and minimal toxicity.

Euro-Lupus Regimen

In the Euro-Lupus Nephritis Trial, patients (84% white) with biopsy-proven proliferative LN were randomly assigned to a high-dose IV CYC regimen ($n = 46$; six monthly and two quarterly pulses with doses titrated according to the white blood cell count nadir) or a low-dose IV CYC regimen ($n = 44$; six fortnightly pulses at a fixed dose of 500 mg), each of which was followed by AZA. After a median follow-up of 41 mo, the rates of treatment failures did not differ between the groups. Severe infectious episodes were much less common, although the difference was not statistically significant (28). The outcome analysis was recently updated: After a median follow-up of 73 mo, there is still no significantly greater cumulative probability of ESRD or doubling of serum creatinine in patients who were given a low-dose IV CYC regimen, and control kidney biopsies performed in a small number of patients revealed a significant drop of the activity index and an absence of progression of the chronicity index in both groups. The follow-up of the Euro-Lupus Nephritis Trial provided an opportunity to identify prognostic factors that could predict long-term renal outcome in a large prospective cohort of patients. It is interesting that patients with renal impairment at 6 yr had a much less favorable initial response to immunosuppressive therapy at 3 and 6 mo, the most striking difference between patients with good (normal renal function) and poor (impaired renal function) long-term outcomes being their initial 24-h proteinuria reduction. The positive predictive value of a 75% drop in 24-h proteinuria at 6 mo for a good long-term renal outcome was 90%. These data suggest that long-term renal outcome can be predicted by early response to therapy (Houssiau et al., Arthritis Rheum, in press). Although some criticisms can be raised vis-à-vis this study (too small numbers of patients in each group to run a true equivalence trial, absence of standard NIH control arm), the data suggest that a short-course remission-inducing regimen of low-dose IV CYC followed by AZA—now referred to as the "Euro-Lupus regimen"—might achieve good long-term clinical results, even if AZA is probably not the optimal maintenance therapy given a renal relapse rate of 35% at 5 yr (28,31).

Mycophenolate Mofetil

Mycophenolate mofetil (MMF) is the prodrug of mycophenolic acid, an inhibitor of inosine monophosphate dehydrogenase. This enzyme controls the de novo synthesis of guanosine nucleotides, a pathway essential for DNA synthesis in lymphocytes (56). MMF displays other inhibitory properties on mesangial cell proliferation (57), expression of adhesion molecules on endothelial cells (58), and inducible nitric oxide synthase expression in the renal cortex (59). The first indication that MMF could be of interest in LN came from murine models of the disease. In NZB/W mice, MMF delayed deterioration of renal function and prolonged survival (60), and in MRL/lpr mice, the drug was found to reduce albuminuria and renal histologic changes (61). Several controlled trials therefore have been designed to test the potential of MMF in LN.

In the pioneering trial performed by Chan et al. (27), LN patients from the Queen Mary Hospital in Hong Kong were assigned, as induction therapy, either MMF ($n = 21$; 2 g/d for 6 mo and 1 g/d for 6 additional months) or oral CYC ($n = 21$; 2.5 mg/kg per d for 6 mo) followed by AZA (2.5 mg/kg per d for 6 mo). After 1 yr, all patients were maintained on low-dose AZA (1 to 1.5 mg/kg per d). Although no differences in early response could be observed between both groups (complete remission in 81% of patients who were assigned to MMF and in 76% of patients who were given CYC/AZA), further fol-

low-up indicated more early relapses in the patients who were given MMF as induction therapy (62), possibly related to a low MMF dosage and/or its early withdrawal.

MMF was also compared with IV CYC as induction therapy in two controlled trials, one performed in China, the other in the United States. Hu *et al.* (63) found that MMF ($n = 23$; 1 to 1.5 g/d for 3 to 6 mo; then, 0.5 to 1 g/d) was more effective in reducing proteinuria, hematuria, serum autoantibody titers, and glomerular immune deposits compared with IV CYC ($n = 23$; 0.75 to 1 g/m^2 monthly for 6 mo, then quarterly for 1 yr). Similar results were observed in a short-term American multicenter trial (64), in which MMF ($n = 71$; maximum tolerated dosage, up to 3 g/d) was compared with a standard NIH IV CYC regimen ($n = 69$; six monthly pulses) as induction therapy for severe LN. Complete remission at 6 mo, defined as a normal serum creatinine, proteinuria <0.5 g/d, and an inactive urinary sediment, was more frequently achieved in the MMF group (20%) than in the IV CYC (6%). Consistently, crossover to the other arm as a result of treatment toxicity or inefficacy occurred in 20% of IV CYC patients and in only 8% of MMF patients. As expected, severe pyogenic infections were more frequent in the IV CYC group compared with the MMF group (13 *versus* 6%).

MMF was also tested as maintenance therapy in the study performed in Miami by Contreras *et al.* (29), in which all patients (only 5% white) were given four to seven monthly IV CYC pulses before being assigned one of three different remission-maintaining regimens: Quarterly IV CYC pulses ($n = 20$), AZA ($n = 19$; 1 to 3 mg/kg per d), or MMF ($n = 20$; 0.5 to 3.0 g/d) for ~2 yr. Although the cumulative rate of renal survival did not differ statistically among the three groups, the most striking differences were (*1*) an increased mortality in patients who were given maintenance therapy with quarterly IV CYC pulses (*versus* those who were given AZA), (*2*) an increased drug-related morbidity in IV CYC patients (*versus* AZA and MMF patients), and (*3*) an increased relapse rate in IV CYC patients (*versus* MMF patients). No statistically significant differences were observed between AZA and MMF.

Taken together, although the MMF studies performed in LN can be criticized (small numbers of patients and/or short follow-up and/or peculiar ethnic background), the drug is clearly filling a slot either as remission-inducing or as remission-maintaining therapy, or possibly both, the more so as its toxicity profile is relatively safe in LN patients, the main side effects being gastrointestinal events such as diarrhea, nausea and vomiting, minor infectious episodes, and rare cases of leucopenia (27,29). How MMF compares with AZA for maintenance therapy is currently unknown. This critical issue (especially given the differential cost between the two therapies; MMF is 10 times more expensive than AZA) is currently addressed by MAINTAIN, an European-based trial comparing the two drugs as maintenance therapy after a 3-mo course of low-dose IV CYC.

Autologous Stem Cell Transplantation

On the basis of the observation that patients who had rheumatic diseases and underwent allogeneic bone marrow transplantation for a hematologic malignancy were sometimes cured from their autoimmune disease, investigators have treated patients with severe SLE by autologous stem cell transplantation (ASCT). The procedure consists of (*1*) harvesting the patient's CD34-positive hematopoietic stem cells (HSC), (*2*) inducing myeloablation by high-dose IV CYC combined with antithymocyte globulin or lymphoid irradiation, and (*3*) reconstituting the patient's hematopoietic system by infusion of autologous cryopreserved HSC. Groups from North America and Europe have now reported on the results of this procedure. Remission of disease activity, defined as a Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) <3 at 6 mo, was seen in 66% of the assessable patients who were registered in the European Blood and Marrow Transplant/European League Against Rheumatism database, 32% of whom relapsed to some degree (65). It is interesting that six of the 15 patients reported by Traynor *et al.* (66,67) experienced active LN before ASCT, and most of them markedly improved their 24-h proteinuria. Toxicity, however, remains a major concern as procedure-related mortality is as high as 12%, although better patient selection and further tuning of the conditioning regimen might improve these figures.

Immunoablative Doses of CYC

As an alternative to ASCT, Petri *et al.* (68) recently proposed using immunoablative doses of IV CYC without HSC rescue (50 mg/kg CYC for 4 consecutive days followed by 5 μg/kg granulocyte colony-stimulating factor until the neutrophil count is superior to 1×10^9/L for 2 consecutive days). This regimen is not myeloablative because HSC resist CYC as a result of expression of an enzyme, aldehyde dehydrogenase, that prevents the conversion of aldophosphamide into phosphoramide mustard, the active alkylating agent. Rescue by autologous HSC therefore is not needed. Nine of the 14 patients reported by Petri *et al.* experienced LN, and marked improvement of 24-h proteinuria was observed. Importantly, there were no deaths in this series of patients. A randomized trial to compare immunoablative doses of CYC with the NIH IV CYC regimen is ongoing.

Plasma Exchanges

On the basis of the pathophysiology of LN, extracorporeal removal of relevant autoantibodies seems a logical approach, either by unselective removal of plasma or by specific adsorption of immunoglobulins (or anti-dsDNA antibodies). In a controlled trial performed in patients with severe LN, Lewis *et al.* (69) evaluated the additional value of plasmapheresis over a standard regimen of GC and CYC. After a mean follow-up of >2 yr, no differences were observed regarding death, renal impairment, and proteinuria changes. On the basis of this study, plasma exchanges cannot be recommended in addition to immunosuppressive therapy, although plasmapheresis might be beneficial in some selected patients with life-threatening disease.

Toward More Targeted Immunointervention

On the basis of a better understanding of the pathophysiology of LN, several newer therapies, directed against B cells (rituximab [RTX], LJP 394), co-stimulatory signals (anti-CD40L [CD154] antibodies, CTLA4Ig), or cytokines, are currently being tested, aiming at more targeted approaches. Thus far, none has successfully reached the bedside on a large scale.

RTX

RTX is a chimeric mouse/human IgG1κ anti-CD20 B cell–depleting monoclonal antibody that is widely used in non-Hodgkin's lymphoma (70) and is currently being tested in several rheumatic and other diseases (71). RTX induces stringent and usually prolonged depletion of peripheral CD20-positive cells. Because plasma cells are CD20 negative, their number and function are not affected by RTX, thereby probably explaining why the antibody has little effect on serum total Ig titers. Allergic reactions are a potential side effect of RTX as a result of anti-mouse antibody responses. So far, only very small and uncontrolled studies have been performed in SLE patients, with, however, some interesting preliminary data. Thus, in an open study, the global activity score improved in six patients who were given two injections of 500 mg of RTX combined with two injections of 750 mg of CYC and with oral GC (72). In a phase I/II trial, 16 SLE patients were given RTX (one infusion of 100 mg/m^2 or one infusion of 375 mg/m^2 or four weekly infusions of 375 mg/m^2). B cell depletion was achieved in 10 patients, the intensity of which was found to correlate with serum RTX titers and, interestingly, with the high-affinity FcγRIIIa genotype (73). Clinical benefit was noted in good depleters only (71). Interesting results have been obtained in LN patients with refractory disease, with a parallel drop in serum anti-dsDNA antibody titers (D. Isenberg, personal communication). RTX therefore raises many hopes, and controlled trials are currently being designed, including in LN.

LJP 394

An ideal therapy would consist of selectively eliminating pathogenic autoantibody-producing B cells, sparing the non-autoimmune B cell compartment. This was the rationale for the development of LJP 394, an investigational immunomodulatory agent made of four dsDNA helices (20mer dC/dA oligonucleotides) attached to an inert scaffold composed of a triethyleneglycol core (74). By binding to anti–dsDNA-producing B cells, this so-called "B cell toleragen" is purported to induce their peripheral deletion, thereby reducing anti-dsDNA antibody production, with the hope to reduce the incidence of renal flares. Although the agent is well tolerated and indeed reduces serum anti-dsDNA antibody titers in SLE (75), its clinical efficacy is not proved. In a recently published trial, time to renal flare did not differ between LJP 394 and placebo, except when a subset analysis was performed on patients with high-affinity anti-dsDNA antibodies (76). A new trial therefore was designed to test efficacy in patients with high-affinity antibodies at baseline, but renal flares were not statistically less frequent in the LJP 394 group (12 *versus* 16% in the placebo arm) (77).

CD40-CD40L (CD154) Blockade

Two monoclonal antibodies directed against CD40L (CD154) have been developed, (BG9588 and IDEC-131) and tested in small preliminary clinical trials, with disappointing results in SLE, including LN (78–80). More important, severe thrombotic events have been observed, mainly with BG9588, thereby leading to an arrest of development.

CTLA4Ig

CTLA4Ig, now also referred to as abatacept, is a fusion protein between the external domain of human cytotoxic T lymphocyte–associated antigen 4 (CTLA4) and the constant region of the human IgG1 heavy-chain. CTLA4, expressed on activated T cells, is the high-avidity receptor for CD80 (B7.1) and CD86 (B7.2; expressed on antigen-presenting cells and B cells) and binds much more avidly to the latter molecules compared with CD28. By binding to CD80 and CD86, CTLA4Ig prevents engagement of CD28 on T cells and thereby appropriate co-stimulation. A complementary explanation for the inhibitory effects of CTLA4Ig on the immune system was recently provided by Grohmann *et al.* (81), who demonstrated that the compound stimulates dendritic cells (through B7.1/B7.2 expressed on their surface) to produce indoleamine 2,3-dioxygenase, an enzyme that breaks down tryptophane, thereby depriving T cells from this amino acid and compromising their function. Already 10 yr ago, a dramatic effect of a murine CTLA4Ig fusion protein was demonstrated on survival of NZB/W lupus mice, even when treatment was delayed until mice were severely nephritic, an experimental design obviously closer to the clinical setting (82). In humans, CTLA4Ig reduces the signs and the symptoms of rheumatoid arthritis in patients with active disease despite methotrexate therapy, and its toxicity profile looks safe (83). Needless to say, SLE is top on the list for the next clinical trials with abatacept.

Cytokine Blockade

Many cytokines, especially IL-10 (84–86), Blys (87,88), and IFN-α (89,90), play a role in the pathophysiology of SLE, probably not as initiating events but rather as mediators of inflammation and damage (91). On the basis of these observations, several trials are currently (or will be soon) designed to test whether blockade of the corresponding cytokines is beneficial, as suggested in a preliminary trial with an anti–IL-10 mAb (92).

Membranous LN

Pure membranous LN is categorized as class V LN according to the classification criteria recently proposed by the International Society of Nephrology (ISN)/Renal Pathology Society (RPS) (93) and may be associated with class III (focal) or IV (diffuse) LN. It can not be considered as a benign disease, given a substantial risk of ESRD and the morbidity associated

with hypercoagulability and hyperlipidemia as a result of prolonged nephrotic syndrome.

Immunosuppressive treatment of class V LN is poorly standardized as a result of the lack of published controlled trials, *e.g.*, comparing GC used alone or in combination with cytotoxic therapy. In this respect, a controlled trial is in the pipeline at the NIH comparing alternate-day GC prescribed alone or in combination with either IV CYC (given bimonthly) or cyclosporine A (CsA). The preliminary analysis at 1 yr favors combined therapy, especially with IV CYC (94). Other immunomodulatory agents have been tested in class V LN. Hu *et al.* (95) retrospectively studied the efficacy of CsA, in combination with GC, in a group of 24 class V LN patients: Complete and partial remissions were achieved by 52 and 43% of the patients, respectively. Moroni *et al.* (96), in a small retrospective analysis, reported that MP and chlorambucil (given alternated month for 6 mo) may induce a more stable remission of nephrotic syndrome and may better prevent renal impairment in comparison with GC alone. In a recent open-label trial, Mok *et al.* (97) treated 38 patients with GC and AZA. At 12 mo, 67 and 22% of the patients achieved complete and partial remission, respectively. After a mean follow-up of >7 yr, none had doubled their serum creatinine. Regarding MMF, it should be stressed that some patients with class V LN were included in the already quoted American induction trial indicating superiority of MMF over IV CYC (64). Moreover, MMF was shown to reduce 24-h proteinuria in an uncontrolled pilot study performed in class V LN patients (98). Finally, the results obtained by Remuzzi *et al.* (99,100) with RTX in idiopathic membranous nephropathy raise the possibility that anti-CD20 therapy might display positive effects in lupus membranous nephritis.

Nonimmunosuppressive Therapy of LN

The critical importance of optimal nonimmunosuppressive care to LN patients must be underlined. Although no specific data exist for LN, BP values should be maintained below 130/80 mmHg, as recommended in other chronic glomerular diseases. In patients with nephrotic-range proteinuria, proteinuria-sparing measures must be applied. Dyslipidemia should be incisively treated, the more so as premature atheroma and cardiovascular disease have become the greatest killer in SLE in the past decade (101–105). GC-induced osteoporosis, another concern in SLE patients (33,34), requires preventive treatment, such as the prescription of calcium salts and vitamin D3 supplements, indeed even antiresorptive therapy by bisphosphonates in selected cases.

Renal Replacement Therapy

In patients with severe and progressive renal impairment, it might be wiser to avoid additional immunosuppression to minimize drug-induced toxicity, the more so as renal replacement therapy is mostly well tolerated in SLE patients. It should be stressed, however, that morbidity is higher in those with the antiphospholipid syndrome, mainly as a result of thrombotic events, and that extrarenal lupus flares may sometimes require reintroduction of immunosuppressive therapy (106). Renal transplantation is the treatment of choice in lupus patients with

ESRD. It is as successful in LN patients as in the general population, according to the European Renal Association–European Renal Dialysis and Transplant Association Registry (107) and the U.S. Renal Data System (108), at least after adjustment for confounding factors such as black race. Recurrence of LN in a transplanted kidney is thought to be unusual (1 to 3%), but these reassuring figures have recently been challenged by a study performed in a series of 54 SLE patients who received a transplant, 30% of whom experienced a recurrence of LN, mostly of class II, however (109).

Conclusion

Since the review on LN by J.S. Cameron published in the *Journal of the American Society of Nephrology* in 1999 (1), several controlled trials that have opened exciting perspectives in LN. On the all, one might say that the *ratio* of our successes over our failures has improved, not only because new drugs, such as MMF, are available but also because we have learned to use the old ones more gently.

Patients who have SLE should be followed in dedicated clinics, and early kidney involvement should be detected by very regular assessments of proteinuria. Although debated, a baseline renal biopsy should be performed in patients with significant proteinuria to discriminate between different types of LN; to exclude other disease manifestations, such as a thrombotic microangiopathy; and to measure activity and chronicity indices. Patient education deserves a special comment: The critical importance of compliance to therapy and of obsessional follow-up must be stressed straightforwardly.

When faced with a patient with newly diagnosed class III or IV LN, a reasonable choice in 2004 is still to prescribe a 3- to 6-mo IV CYC course as initial therapy, in addition to GC. Although MMF is a new star twinkling in the sky, we still miss long-term follow-up data on patients who are given the drug as initial therapy. For maintenance, the choice is currently between AZA and MMF. A high-dose long-course quarterly IV CYC pulse regimen is probably not justified anymore as remission-maintaining therapy in most LN patients, mainly because of its gonadal toxicity, except when this concern is not applicable or when anticipated lack of compliance to daily oral immunosuppressive treatment should be prevented by the use of IV therapy. Once in remission, patients should be assessed for renal function, urinalysis, and 24-h proteinuria on a quarterly basis at least 5 yr after diagnosis, given the high recurrence rate of LN. For relapsing patients, a new induction course with IV CYC is probably justified, followed by maintenance therapy with another immunosuppressant than the one on which the patient failed. For refractory cases (and why not as a short-course induction therapy?), biologics raise great hopes, although further studies are obviously required. Treatment of membranous LN will remain debated until results of controlled trials become available. On the basis of the current data, it seems wise to treat these patients with a combination of GC and cytotoxics or CsA.

Intriguing is that some issues have been overlooked in clinical trials. Thus, little attention is drawn to the initial dose and tapering regimen of GC, a critical issue given their side

effects, sometimes improperly attributed to the cytotoxic drug. We eagerly need a consensus on outcome definitions, such as remission and relapse of LN. Although glomerular lesions logically influence our treatment strategy, we do not know how to handle interstitial and vascular renal disease. The influence of ethnicity, quoted from paper to paper to explain divergent results in different patient populations, has never been properly addressed. Finally, why would therapy not be tuned, on a patient per patient basis, according to response to therapy? Rather than apply a standardized regimen, a flexible approach could be adopted (110), such as prolonging the induction phase or early switching to another cytotoxic drug in case of insufficient response. By the time of the genetic revolution, it is hoped that gene expression studies, *e.g.*, by microarrays performed on circulating lymphocytes or indeed kidney biopsy specimens, will provide the clinicians with surrogate markers for long-term outcome, but this is probably still a dream.

Acknowledgments

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