

Rituximab in Idiopathic Membranous Nephropathy: A One-Year Prospective Study

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Abstract. Currently available monoclonal antibodies against the B cell surface antigen CD20 have been employed to explore whether specific inhibition of B cells may help improve the outcome of idiopathic membranous nephropathy (IMN) and may avoid the side effects of steroids and immunosuppressants. This prospective, observational study evaluated the 1-yr outcome of eight IMN patients with persistent (>6 mo) urinary protein excretion > 3.5 g/24 h given four weekly infusions of the anti-CD20 antibody rituximab (375 mg/m²). At 3 and 12 mo, proteinuria significantly decreased from mean (\pm SD) 8.6 \pm 4.2 to 4.3 \pm 3.3 (–51%, P < 0.005) and 3.0 \pm 2.5 (–66%, P < 0.005) g/24 h, albumin fractional clearance from 2.3 \pm 2.1 to 1.2 \pm 1.7 (–47%, P < 0.05) and 0.5 \pm 0.6 (–76%, P < 0.003), and serum albumin concentration increased from 2.7 \pm 0.5 to 3.1 \pm 0.3 (+21%, P < 0.05) and 3.5 \pm 0.4 (+41%, P

< 0.05) mg/dl. At 12 mo, proteinuria decreased to \leq 0.5 g/24 h or \leq 3.5 g/24 h in two and three patients, respectively. Proteinuria decreased in the remaining patients by 74%, 44%, and 41%, respectively. Body weight, diastolic BP, and serum cholesterol progressively decreased in parallel with an improvement of edema in all patients. Renal function stabilized (Δ 1/creatinine: +0.002 \pm 0.007). CD20 B lymphocytes fell below normal ranges up to study-end. No patient had major drug-related events or major changes in other laboratory parameters. Rituximab thus promotes sustained disease remission in patients otherwise predicted to progress to ESRD, and it is safe. The long-term risk/benefit profile of this novel, disease-specific approach seems much more favorable to that of commonly employed immunosuppressive drugs.

Idiopathic membranous nephropathy (IMN) is an immune-mediated disease of deposits of immunoglobulins G and complement components on the subepithelial layer of the glomerular capillary wall (1). Data from studies in animals suggest that the immune deposition resulting from B cell activation promotes injury to the glomerular filtering barrier and consequent proteinuria (2). Thus, agents that limit or prevent B cell production of nephritogenic immunoglobulins should block at an early step the sequence of pathogenic events and eventual progressive renal dysfunction in IMN. So far, however, therapeutic approaches to IMN relied on steroids and immunosuppressant drugs, which are not fully specific and carry the risk of severe toxic effects. This may explain why the outcome of IMN has not substantially improved over the past 30 yr and why up to 40% of patients still progress to end-stage renal failure despite treatment with glucocorticoids and alkylating agents. The long-term effectiveness of cyclosporine is also

questionable due to the high relapse rates and associated toxic effects of the drug (3,4).

Availability of monoclonal antibodies against the cell surface antigen CD20 of B cells allowed to explore whether more specific B cell inhibition (5) may help improving the outcome of IMN and avoiding the side effects of steroids and immunosuppressants. The rationale for using such an approach further relies on the evidence that the Th2 pathway for antibody response is activated and that the inhibition of B cells and of pathogenic antibodies is strictly associated with beneficial effects of immunosuppressive drugs in experimental membranous nephropathy (6,7). In eight patients with IMN and long-lasting persistent proteinuria, the intravenous infusion of the anti-CD20 monoclonal antibody rituximab achieved a 60% reduction in urinary proteins with notably modest side effects and no major adverse events, at least over 20 wk after treatment administration (8). We here report the long-term follow-up of these patients with the aim to assess the risk/benefit profile of specific B cell inhibition. We prospectively evaluated the time course of different efficacy/safety parameters, including proteinuria, serum creatinine concentration, and lymphocyte subpopulations, and we carefully recorded all major or minor adverse events occurring over 1 yr of follow-up. The results of this observational study formed the basis of the present report.

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Materials and Methods

Patients

Subjects with biopsy-proven IMN, creatinine clearance > 20 ml/min per 1.73 m² and persistent urinary protein excretion rate > 3.5 g/24 h for at least 6 mo, who were on full-dose ACE inhibitors (Ramipril, 5 to 10 mg/d), and who had no remissions from disease onset were eligible for study participation. The study protocol was approved by the Ethical Committee of the Clinical Research Center for Rare Diseases “Aldo & Cele Daccò”–Villa Camozzi of the Mario Negri Institute for Pharmacological Research. Patients gave written informed consent according to the declaration of Helsinki.

Treatment

Low sodium (< 2 , 3 g/d Nat) intake and a controlled protein (0.8 g/kg body weight) diet were recommended to all patients. All patients received a similar conservative treatment that included loop diuretics to control edema, full-dose ACE inhibitor therapy combined with beta-blockers and calcium channel blockers as deemed appropriate to control BP and proteinuria, and statins to control hypercholesterolemia. No change in diet and concomitant treatment was introduced throughout the whole study period. In addition to conservative therapy, patients received four weekly intravenous infusions of rituximab (375 mg/m²).

Follow-Up

In all patients, proteinuria and other clinical and laboratory parameters were evaluated at study entry and every month up to month 3 and at months 6, 9, and 12. Full blood cell count, lymphocyte subpopulations, including CD20 B cells, CD₃, CD₄, CD₈, NK cells, and CD₄/CD₈ ratios, and Ig (IgG, IgA, IgM) levels were also monitored at the same time points with the exception of month 9.

Outcome Variables and Statistical Analyses

Changes in proteinuria and in the reciprocal of serum creatinine concentration (1/cr) over time ($\Delta 1/\text{cr}$) were the primary efficacy variables of the study. Within-patient comparisons were performed by means of Repeated Measures ANOVA followed by paired *t* test. For subject no. 04, proteinuria was not available at months 6 and 9. For the statistical evaluation, these two missing values were replaced by the highest value of proteinuria found for that subject during follow-up. The multiple comparisons issue was addressed using Bonferroni adjustment. Linear time trend was analyzed by PROC GLM using POLYNOMIAL option for repeated measures analysis of the SAS Software (Release 8, SAS Institute Inc., Cary, NC). Orthogonal polynomials to each subject were fitted and then ANOVA on polynomial

coefficients was performed. Proteinuria and albumin fractional clearances were log-transformed before statistical analyses due to their skewed distribution. Data were expressed as mean \pm SD, unless differently stated. A *P* value of less than 0.05 was considered as statistically significant.

Results

Baseline Characteristics

Eight patients (three males) aged 52.8 ± 19.6 yr and with a known disease from 29.7 ± 13.5 mo entered the study. They had heavy proteinuria, hypoalbuminemia, mild to moderate renal insufficiency and hypercholesterolemia. Their BP was well controlled. Main baseline clinical and laboratory characteristics in individual patients and in the study group as a whole are given in Tables 1 and 2, respectively.

Outcome

As previously reported (8), proteinuria significantly decreased by month 1 after rituximab infusion. Then, proteinuria remained well below basal values throughout the whole study period (the reduction was statistically significant also after Bonferroni adjustment), the maximal reduction (66%) being achieved at study end (Table 2, Figure 1) (*P* < 0.05 for linear trend). Two females achieved full remission (proteinuria ≤ 0.5 g/24 h), and three females and one male partial remission (proteinuria ≤ 3.5 g/24 h or proteinuria reduction $> 50\%$ versus basal), respectively. The reduction in proteinuria was paralleled by a progressive and significant reduction in albumin fractional clearance (the reduction was statistically significant also after Bonferroni adjustment) and by a progressive and significant increase in serum albumin concentration that approximated normal ranges at study end (Table 3, Figure 2). At month 12, proteinuria and albumin fractional clearance decreased by 66% and 76%, respectively, and serum albumin increased by 41% versus basal. Total serum cholesterol also progressively decreased throughout the whole study period and the reduction versus basal values achieved the statistical significance at study end (Table 3). Proteinuria reduction was also paralleled by a progressive and significant decline in body weight that reflected an amelioration of edema in all patients. Systolic BP was stable as well, while diastolic BP tended to decline progressively and the reduction versus basal values was

Table 1. Main clinical and laboratory characteristics at study entry (month 0) of individual patients with idiopathic membranous nephropathy (IMN)

Parameter	Patient							
	1	2	3	4	5	6	7	8
Age (yr)	55	70	28	75	69	24	40	59
Gender (male/female)	F	F	F	M	F	F	M	M
Disease duration (mo)	14	35	46	24	24	13	33	49
Histology stage (1–4)	2	2	1	2	1	2	2	2
Urinary protein excretion (g/24 h)	16.0	7.0	4.8	5.6	5.7	9.1	14.1	6.7
Serum creatinine (mg/dl)	1.7	1.6	1.0	3.7	1.1	0.9	0.9	1.2

Table 2. Time course of urinary protein excretion rate (g/24 h) in individual patients with IMN from 6 mo before study entry (month -6) to study end (month 12)

Patient	Month								
	-6	0	1	2	3	6	9	12	
1	16.8	16.0	9.5	9.1	8.9	8.7	6.4	4.1	
2	7.2	7.0	2.9	2.6	3.1	3.3	4.4	2.6	
3	4.2	4.8	1.4	2.7	1.0	0.6	0.4	0.5	
4	5.6	5.6	3.5	4.8	5.6	5.6	5.6	3.2	
5	4.5	5.7	2.8	1.7	2.3	1.7	1.1	1.2	
6	8.5	9.1	3.2	5.9	1.5	1.4	0.7	0.4	
7	14.0	14.1	3.8	4.1	9.4	8.0	7.5	8.0	
8	6.5	6.7	3.0	2.0	3.1	3.0	2.8	4.0	

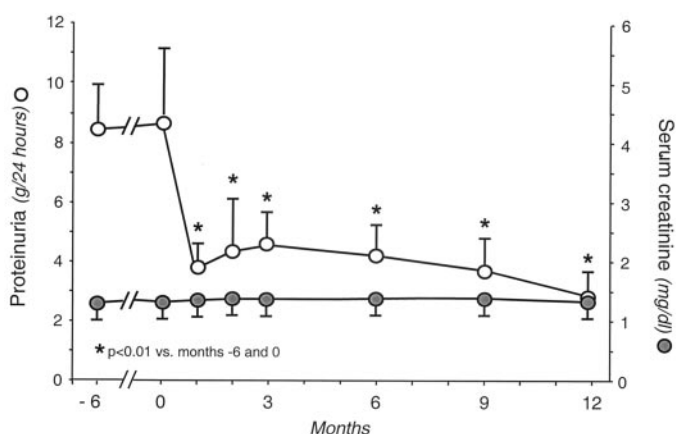


Figure 1. Time course of serum creatinine concentration and 24-h urinary protein excretion rate in eight patients with idiopathic membranous nephropathy (IMN) from 6 mo before rituximab administration to study end (month 12).

marginally significant at study end (Table 3). Renal function stabilized in all patients (Δ 1/creatinine: $+0.002 \pm 0.007$). Serum concentrations of IgG, IgA, and IgM did not change significantly throughout the whole study period (data not shown).

Lymphocyte Subpopulations

CD20 B lymphocytes decreased to undetectable numbers by month 1 after rituximab infusion and remained below normal ranges up to study end (Table 4). We recorded no significant changes in total white blood cell, platelet, and lymphocyte counts and in lymphocyte subpopulations including CD₃, CD₄, CD₈, NK cells, and CD₄/CD₈ ratios (Table 4).

Safety

Three events occurred in three patients during the first rituximab administration, none of which related to drug-induced immune-suppression. One of these events was reported as a possible episode of larynx spasm. Due to this concern, the patient was preventively treated with an intravenous pulse of methylprednisolone. This event, however, was characterized

only by a sensation of constriction to the neck and was not associated with any sign of larynx dysfunction such as dyspnea, tirage, or whizzings. An acute coronary syndrome was also unlikely because an ECG performed during the episode failed to show any sign of myocardial ischemia. An *a posteriori* reevaluation of the episode led to the conclusion that it most likely reflected just an anxiety reaction of the patient. This was consistent with the fact that the episode occurred during the first exposure to the experimental procedure (just after the patient had been informed that the study drug may occasionally cause breathing disturbances), almost instantaneously subsided during steroid infusion, and did not recur during the following three further exposures to the study drug.

Quite better characterized — and surely related to rituximab infusion — was an episode of skin rash that occurred in the second patient. This was accompanied by pruritus and promptly subsided with methylprednisolone infusion. This event, however, was classified as mild because it was not associated with any other sign of anaphylaxis such as hypotension or respiratory dysfunction. To limit the risk of recurrence, a pulse of methylprednisolone (125 mg, intravenously) was infused shortly before each further rituximab infusion.

Generalized chills that occurred in the third patient were not associated with any other adverse reaction and recovered within few minutes just by reducing the rate of rituximab infusion. They did not recur during the subsequent rituximab administrations.

Discussion

We found that patients with IMN, long-lasting nephrotic syndrome, and mild to moderate renal insufficiency had their urinary protein excretion significantly reduced by rituximab treatment during a 1-yr observation period as compared with pretreatment values. Concomitantly, renal function stabilized in all patients. Treatment was well tolerated, with no adverse event possibly related to persistent immunosuppression occurring throughout the whole study period.

Proteinuria reduction translated into increases in serum albumin concentration, amelioration of edema and hypercholesterolemia, and in the trend to a progressive decrease in diastolic BP throughout the whole study period. This outcome was

Table 3. Main clinical and laboratory parameters in eight patients with IMN from rituximab administration (month 0) to study end (month 12)

Parameter	Months												
	0	1	2	3	6	9	12						
Systolic BP (mmHg)	131 ± 11	125 ± 10	129 ± 11	129 ± 11	131 ± 9	131 ± 14	128 ± 4						
Diastolic BP (mmHg)	83 ± 7	78 ± 10	81 ± 6	80 ± 6	78 ± 9	78 ± 7	74 ± 11						
Body weight (kg)	78 ± 16	76 ± 15	75 ± 15 ^b	75 ± 15 ^b	74 ± 14 ^b	75 ± 15	75 ± 15						
Ser (mg/dl)	1.5 ± 0.9	1.5 ± 0.8	1.4 ± 0.7	1.4 ± 0.8	1.4 ± 0.8	1.3 ± 0.7	1.4 ± 0.7						
CrCl (ml/min per 1.73 m ²)	69 ± 31	68 ± 30	68 ± 30	71 ± 26	75 ± 32	76 ± 28	71 ± 26						
Urinary protein excretion (g/24 h)	8.6 ± 4.2	3.7 ± 2.4 ^a	4.1 ± 2.5 ^a	4.3 ± 3.3 ^a	4.1 ± 3.1 ^a	3.6 ± 2.7 ^a	3.0 ± 2.5 ^a						
Albumin fractional clearance (mg/dl)	2.3 ± 2.1	1.3 ± 1.4 ^a	1.3 ± 1.9 ^b	1.2 ± 1.7 ^b	1.0 ± 1.0 ^a	0.8 ± 0.7 ^a	0.5 ± 0.6 ^a						
Serum albumin (mg/dl)	2.7 ± 0.5	3.0 ± 0.4 ^a	3.1 ± 0.3 ^b	3.1 ± 0.3 ^b	3.3 ± 0.3 ^a	3.4 ± 0.3 ^b	3.5 ± 0.4 ^b						
Serum cholesterol (mg/dl)	236 ± 75	239 ± 82	221 ± 60	238 ± 48	210 ± 57	208 ± 59	184 ± 45 ^b						
Serum triglycerides (mg/dl)	186 ± 183	195 ± 121	204 ± 139	221 ± 138	206 ± 132	195 ± 121	165 ± 115						

^a P < 0.01 and ^b P ≤ 0.05 versus month 0.

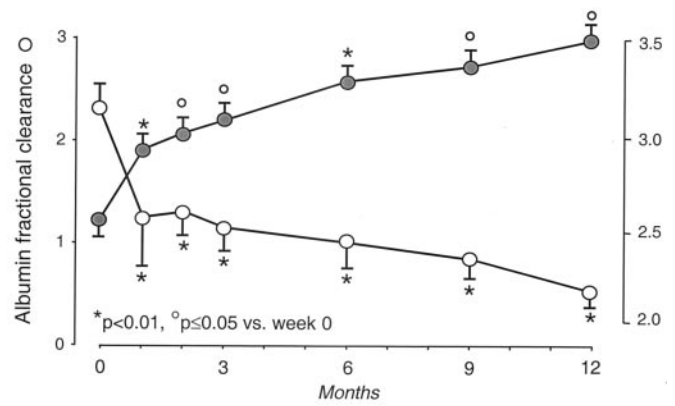


Figure 2. Time course of serum albumin concentration and albumin fractional clearance in eight patients with IMN from rituximab administration (month 0) to study end (month 12).

remarkably good as compared with the usual outcome of patients with persistent heavy proteinuria that almost invariably have a rapidly worsening renal function with relentless progression to ESRD (9). Of note, two and three patients achieved full (≤ 0.5 g/24 h) or partial (≤ 3.5 g/24 h) remission of proteinuria, respectively. In the remaining three patients, the antiproteinuric effect of rituximab was also consistent (-74% , -44% and -41% at 1 yr as compared with basal, respectively), despite residual proteinuria still in nephrotic ranges. Findings that both full responders, two of the three partial responders, and the one with proteinuria reduction $> 50\%$, but none of the two relatively poor responders (proteinuria reduction $< 50\%$) were female, suggests that the female gender may predispose to a better response to rituximab treatment. Given the heterogeneous course of the disease and the small number of patients considered in this study, the case for spontaneous remission rather than for therapeutic effect of the study drug cannot be formally ruled out, particularly in the two young female patients with full response. However, we found spontaneous remission very unlikely in either of the above cases and in the whole study group. First, proteinuria uniformly decreased in all patients given rituximab infusion and within a discrete period after treatment administration. Moreover, the magnitude of proteinuria reduction while on rituximab was relatively consistent, and patients had nephrotic-range proteinuria for at least 6 mo before study entry without previous history of spontaneous remissions. Finally, no other known confounder influenced the course of the disease, because no change in diet, BP, and lipid control or use of drugs that may directly affect proteinuria and disease progression, such as ACE inhibitors, angiotensin II receptor antagonists, and statins, were introduced throughout the whole study period. On the other hand, extensive evidence that a consistent reduction in urinary proteins almost invariably translates into improved long-term outcome of chronic nephropathies (10–15), can be taken to suggest that amelioration of proteinuria most likely accounted for the renoprotective effect of rituximab treatment in this specific clinical setting. Findings that CD20 depletion from the circulation precluded to progressive proteinuria reduc-

Table 4. Main hematology parameters in eight patients with IMN from rituximab administration (month 0) to study end (month 12)

Parameter	Months					
	0	1	2	3	6	12
White blood cell count ($10^3/\mu\text{l}$)	7.3 ± 3.1	7.8 ± 3.4	7.7 ± 3.8	8.2 ± 4.2	7.9 ± 3.5	8.3 ± 3.9
Lymphocytes (% of white blood cell count)	26 ± 10	28 ± 6	27 ± 7	30 ± 7	23 ± 8	23 ± 12
Lymphocyte subpopulations (% of lymphocytes) ^c						
CD 20 (8 to 16%)	9.3 ± 2.8	0.0 ± 0.0^a	0.4 ± 1.1^b	1.5 ± 2.1^b	3.6 ± 2.8^b	6.3 ± 3.8
CD3 (63 to 77%)	75 ± 4	83 ± 7	83 ± 6	84 ± 7	81 ± 5	77 ± 4
CD4 (37 to 51%)	54 ± 4	54 ± 12	55 ± 3	57 ± 8	55 ± 7	53 ± 5
CD8 (28 to 38%)	21 ± 4	26 ± 6	24 ± 4	26 ± 6	24 ± 4	24 ± 4
Natural Killers (10 to 15%)	12 ± 5	15 ± 8	17 ± 7	11 ± 8	15 ± 7	13 ± 5

^a $P < 0.01$ and ^b $P \leq 0.05$ versus month 0.

^c Normal ranges in brackets.

tion and persisted for the entire observation period can be taken to indicate that indeed the effect of rituximab of reducing urinary proteins and protecting against renal function deterioration is the consequence of its inhibitory actions on B cell differentiation and Ig secretion that were documented in several recent studies (5,16–21). It is tempting to speculate that *in vivo* rituximab ameliorated the clinical course of disease by inhibiting autoreactive B lymphocyte clones committed to form nephritogenic immunoglobulins. Of note, after the completion of the present study one of our patients had progressive recovery of circulating CD20 lymphocytes to normal ranges that was paralleled by progressively increasing proteinuria to pretreatment values over four more months of further follow-up. A longer follow-up is needed to assess whether the slow rise in CD20-positive lymphocytes found in some patients during the last few months of the study period may inevitably prelude to and play role in increases in proteinuria. We cannot exclude that rituximab may act by yet fully unknown mechanisms possibly independent of depletion of circulating CD20 positive cells. Regardless of the above, the reduction in circulating CD20 cells we observed up to 1 yr after rituximab infusion was overall remarkable in most patients, in agreement with evidence in healthy volunteers — and in patients with idiopathic thrombocytopenic purpura, hemolytic anemia, and arthritis — that B cells do not recover in the circulation for 9 to 12 mo (5,16–21).

Because of its human component, rituximab has a very low immunogenicity (21). Should recovery of CD20 cells occur, it would reflect long-term reversibility of the CD20-depleting effect rather than neutralization of the chimeric antibody and should allow successful retreatment. In fact, an additional course of rituximab was effective and well tolerated in patients with relapsing non-Hodgkin lymphoma, without substantially different side effects to those seen with the first course (22). This may well apply to membranous nephropathy. Only longer

follow-up of these patients will tell whether CD20 cell recovery may occur and be inevitably followed by relapse. Whether repeated courses of rituximab infusion and/or combining another immunosuppressive drug (6) may help achieve permanent disappearance/inactivation of the clone(s) of autoreactive cells is open to speculation.

In agreement with previous findings (8), rituximab treatment did not lower the serum levels of total IgG in patients with membranous nephropathy. Conceivably, this can be explained by kinetics of nephritogenic autoantibodies, which are cleared upon binding to antigens. Immune deposits indeed form rapidly in advance of proteinuria in the animal counterpart. Rapid waves of Ig deposition are thought to occur in patients with IMN (23). This should translate into relatively low plasma concentrations and short half-life of nephritogenic antibodies whose amount, however, is also minimal relative to total IgG. The role of a B cell–dependent pathway leading to formation of pathogenic antibodies and to full manifestation of human membranous nephropathy has been recently demonstrated in an elegant study of neutral endopeptidase (NEP) 1 antigen (24). The nature of pathogenic antigen and antibodies in IMN remain yet elusive, and studies at the moment can only be focused on potentially relevant Ig subclasses. In this respect, IgG4 were found to concentrate in the glomerular deposits (25,26), while serum levels were selectively low for IgG2 (27). Identifying the specific antibodies and documenting their disappearance from the circulation after effective B cell inhibition would greatly enhance our understanding of the role of B cell–related mechanisms in IMN. As to the lack of effect on total serum Ig, the same finding was reported in other clinical conditions, whereas rituximab treatment, either alone or in combination with immunosuppressive drugs, did suppress CD20 cells and/or disease-associated antibodies (28–30). Total Ig levels were also maintained in patients with hematologic malignancy, such as chronic lymphocytic leukemia, despite

low CD20 lymphocyte counts (31). Of note, Ig levels were preserved, again despite low B cells, in patients with membranous nephropathy and given fludarabine (32). We cannot exclude that rituximab may affect mechanisms of inhibition of B cell function other than antibody production alone. They include antigen-presenting properties and interaction with T lymphocyte subsets known to play pathogenic roles in experimental membranous nephropathy (6).

So far, no major drug-related side effects or major adverse events have been reported in patients given rituximab for immune-mediated diseases (16–21,33), and our group of patients is no exception. Indeed no opportunistic infection or other adverse event possibly related to persistent immunosuppression occurred throughout the whole study period. Of the three adverse events observed during the first rituximab administration, one — referred as suspected larynx spasm — most likely reflected an anxious reaction of the patient to the experimental procedure and did not recur during the subsequent treatment exposures. The second one was a well-characterized and clearly drug-related skin rash that however was mild in intensity and nonserious in nature, promptly responded to intravenous steroids, and was effectively prevented by prophylactic steroid administration in the occasion of the subsequent rituximab infusions. The third one was an episode of generalized chills that subsided just by reducing the rate of rituximab infusion and did not recur during the subsequent treatments.

In conclusion, rituximab infusion reduced proteinuria and prevented disease progression in IMN patients with long-lasting nephrotic syndrome and was well tolerated. This effect was most probably mediated by specific drug-related effects on autoreactive B cell clones. Thus, rituximab offers a new approach with a narrow, disease-specific mechanism for the treatment of IMN. This is in contrast to the current treatment options of nonspecific immunosuppression and to the conservative approach of ACE inhibition, BP control, and lipid control. In view of the major side effects (Cushing syndrome, leukopenia, cancer, renal toxicity, and opportunistic infections) associated with the use of steroids, alkylating agents, and cyclosporin, and lack of success with both the immunosuppression and conservative approaches, the risk/benefit profile of rituximab seems much more favorable. Further studies will clarify whether combination with other immunosuppressive drugs may serve to magnify the antiproteinuric effect of rituximab treatment. Large, randomized trials with longer follow-up are also needed to verify the long-term tolerability of rituximab therapy and its specific renoprotective potential *vis-à-vis* more conventional immunosuppressive regimens and, ideally, conservative treatment alone.

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