**Research letters**

# Rituximab for idiopathic membranous nephropathy

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**Treatments for idiopathic membranous nephropathy, a common cause of nephrotic syndrome, can be very toxic. In view of the pathogenic potential of B cells in this disease, we studied the effects of four weekly infusions of rituximab (375 mg/m$^2$)— the monoclonal antibody to B-cell antigen CD20—in eight patients who had idiopathic membranous nephropathy with persistent nephrotic syndrome. At weeks 4 and 20, urinary protein decreased from mean (SE) 8·6 g/24 h (1·4) to 3·8 (0·8) and 3·7 (0·9), respectively (p<0·0001). At week 20, albuminuria and albumin fractional clearance decreased by 70% and 65%, and serum albumin increased by 31%. CD20 B lymphocytes fell below normal ranges up to study end. The short-term risk-benefit profile of rituximab seems more favourable to that of any other immunosuppressive drug used to treat idiopathic membranous nephropathy.**

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Current therapeutic approaches to idiopathic membranous nephropathy (IMN), the most common cause of nephrotic syndrome in adults in many countries, still rely on steroids and immunosuppressant drugs, which are not fully specific and carry the risk of severe toxic effects. Over the past 30 years, the outcome of IMN has not substantially improved; up to 40% of patients can develop end-stage renal failure after treatment with glucocorticoids, alkylating agents, and other drugs. The long-term effectiveness of ciclosporin is also questionable due to the high relapse rates and associated toxic effects of the drug.[1,2]

Advances in the understanding of pathogenetic mechanisms of disease in IMN should help to find specific approaches that are more likely to be effective and safe in the long term. Proteinuria is a major independent indicator of disease progression. Data from studies in animals suggest that the typical subepithelial immune deposits in glomeruli are caused by B-cell-mediated reactions, which promote injury to the glomerular filtering barrier and result in proteinuria.[3] Thus, although IMN autoantigens remain elusive and the role of B cells has not been fully explained in man, agents that specifically interfere with B cells would ideally represent the first step toward selective therapy. The success of such treatments would also provide evidence for the role of a B-cell-related mechanism in IMN.[4]

| | Week | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | –24 | 0 | 1 | 2 | 3 | 4 | 8 | 12 | 16 | 20 |
| **Patient** | | | | | | | | | | |
| 1 | 16·8 | 16·0 | 13·6 | 8·4 | 7·6 | 9·5 | 9·1 | 8·9 | 8·7 | 5·8 |
| 2 | 7·2 | 7·0 | 4·5 | 3·3 | 3·9 | 2·9 | 2·6 | 3·1 | 2·7 | 3·3 |
| 3 | 4·2 | 4·8 | 4·8 | 1·8 | 2·5 | 1·4 | 2·7 | 1·0 | 0·6 | 0·4 |
| 4 | 5·6 | 5·6 | 4·4 | 3·1 | 3·5 | 3·5 | 4·8 | 5·6 | 5·7 | 5·6 |
| 5 | 4·5 | 5·7 | 2·9 | 2·8 | 2·7 | 2·8 | 1·7 | 2·3 | 1·9 | 1·7 |
| 6 | 8·5 | 9·1 | 5·8 | 4·7 | 2·3 | 3·2 | 5·9 | 1·5 | 1·4 | 1·0 |
| 7 | 14·0 | 14·1 | 11·0 | 8·2 | 8·3 | 3·8 | 4·1 | 9·4 | 7·8 | 8·0 |
| 8 | 6·5 | 6·7 | 3·0 | 2·5 | 2·9 | 3·0 | 2·0 | 3·1 | 3·5 | 3·5 |
| **Mean (SE)** | 8·4 (1·6) | 8·6 (1·5) | 6·3* ( 1·4) | 4·4* (0·9) | 4·2* (0·9) | 3·8* (0·9) | 4·1* (0·9) | 4·3* (1·1) | 4·0* (1·1) | 3·7* (0·9) |

*p<0·0001 versus week –24 and week 0.

Table 1: **Time course of 24 h urinary protein excretion rate (g/24 h) in individual patients from 24 weeks before study entry (week 0) up to study end (week 20)**

We investigated the efficacy and safety profile of rituximab (Roche SpA, Monza, Italy), a monoclonal antibody against the cell surface antigen CD20 of B cells,[5] in three men and four women with IMN. Patients' average age was 52 years (range 24–75). They had creatinine clearance greater than 20 mL/min/1·73 m$^2$, persistent urinary protein excretion rate greater than 3·5 g/24 h for at least 6 months, and were on full-dose angiotensin-converting-enzyme (ACE) inhibitors and without remission over 29·7 (13–49) months from renal biopsy. Patients gave written informed consent to study participation according to the declaration of Helsinki. Seven patients were on diuretics or statins (or both), two were on non-dihydropyridine calcium-channel blockers, three were on aspirin, and one was on oral anticoagulant therapy.

We gave the patients intravenous infusions of rituximab (375 mg/m$^2$) every 4 weeks. No change in diet or concomitant treatment was introduced during the 20 weeks of follow-up. Proteinuria (primary efficacy variable) and other clinical and laboratory variables were measured at baseline, weekly during the treatment period, and every 4 weeks from week 4 to week 20. Data with skewed distribution were log-transformed before analysis. Comparisons were by paired Student's *t* test and correlations by Spearman's rank.

During the treatment period, urinary proteins significantly and non-linearly decreased in all patients (tables 1 and 2). Two patients achieved full remission (proteinuria ⩽1 g/24 h) and three achieved part remission (⩽3·5 g/24 h). At study end, proteinuria had decreased by 62%, albuminuria by 70%, and albumin fractional clearance by 65%, whereas serum albumin had increased by 31% versus baseline, associated with a reduction in serum cholesterol (table 2). At each visit from week 8 to 20, serum albumin increase was associated with a decrease in albumin fractional clearance (Spearman's $r$=−0·73, p=0·04; −0·69, p=0·06; −0·76, p=0·03; −0·78, p=0·02, at weeks 8, 12, 16, and 20, respectively).

CD20 B lymphocytes decreased to undetectable numbers by fluorescence-assisted cell-sorter analysis after the first dose of rituximab, and remained well below normal ranges up to the study end (table 2). We recorded no significant changes in total white blood cell, platelet, and lymphocyte counts, or in serum concentrations of IgG, IgA, and IgM.

Side-effects (generalised chills that spontaneously subsided in one patient, and a skin rash and a larynx spasm that recovered within 20 min after 125 mg methylprednisolone intravenous injection in two cases) were transient and infusion-related, and they occurred only during the first rituximab administration.

The finding that the depletion of circulating CD20 cells was closely associated with the reduction of proteinuria and albuminuria after the first treatment suggests a cause-effect relation. This effect translated into increases in serum albumin and amelioration of oedema and hypercholesterolaemia.

| | Week | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 4 | 8 | 12 | 16 | 20 |
| Systolic blood pressure (mm Hg) | 131 (2) | 126 (4) | 129 (4) | 129 (4) | 128 (5) | 130 (5) |
| Diastolic blood pressure (mm Hg) | 83 (3) | 77 (4) | 81 (2) | 80 (2) | 76 (3) | 78 (3) |
| Bodyweight (kg) | 78 (6) | 76 (5) | 75 (5) | 75 (5) | 73 (5) | 73 (5) |
| Serum creatinine (μmol/L) | 124 (26) | 124 (26) | 124 (26) | 124 (26) | 124 (26) | 115 (26) |
| Creatinine clearance (mL/min/1·73 $m^2$) | 68·7 (10·1) | 65·6 (9·3) | 68·1 (10·6) | 70·7 (9·3) | 79·4 (10·8) | 69·5 (6·7) |
| Serum albumin (g/L) | 26 (2) | 29 (1) | 30 (1) | 31 (1) | 32 (1) | 34 (1) |
| Serum cholesterol (mmol/L) | 6·1 (0·7) | 6·3 (0·8) | 5·7 (0·5) | 6·2 (0·4) | 5·6 (0·6) | 5·2 (0·5) |
| Serum triglycerides (mmol/L) | 2·1 (0·4) | 2·2 (0·5) | 2·3 (0·5) | 2·5 (0·5) | 2·2 (0·6) | 2·2 (0·6) |
| Urinary protein excretion rate (g/24 h) | 8·6 (1·5) | 3·7 (1·0*) | 4·1 (1·0*) | 4·3 (1·0*) | 4·0 (1·0*) | 3·6 (1·0*) |
| Urinary albumin excretion rate (μg/min) | 3560 (706) | 2158 (639) | 2194 (511) | 2009 (535) | 1393 (534) | 1060 (349) |
| Albumin fractional clearance | 2·3 (0·7) | 1·2 (0·5) | 1·3 (0·3) | 1·1 (0·3) | 1·0 (0·3) | 0·8 (0·3) |
| White blood cell count ($10^3$/μL) | 7·3 (0·7) | 7·7 (1·2) | 7·7 (1·3) | 8·2 (1·5) | 7·1 (0·4) | 6·7 (0·6) |
| Lymphocytes (%) | 26·2 (3·7) | 27·6 (2·1) | 26·2 (2·3) | 24·6 (2·8) | 25·2 (1·6) | 28·1 (2·7) |
| CD20 (% of lymphocytes) | 9·3 (1·0) | 0·0 (0·0*) | 0·4 (0·4*) | 1·4 (0·7*) | 1·6 (0·5*) | 3·7 (0·2) |

Data are mean (SD). *p<0·0001 versus week 0.

Table 2: **Main clinical and laboratory variables at baseline (week 0), at the end of the treatment period (week 4), and every 4 weeks up to study end**

Spontaneous remissions were unlikely, since remissions occurred uniformly within a discrete period after rituximab infusion, the magnitude was relatively consistent, and patients had established nephrotic-range proteinuria for at least 6 months before study entry without signs of remission.

How could rituximab exert such a striking antiproteinuric effect? In Heymann's nephritis in animals, the experimental counterpart of IMN, antibodies against the podocyte-associated antigen megalin promote formation of subepithelial immune deposits and proteinuria. The effect of rituximab on CD20 lymphocytes is consistent with the prediction that a similar mechanism is directed against unknown or not yet fully characterised human autoantigens in IMN. In-vitro binding of CD20 to antibodies against CD20 inhibits B-cell differentiation and immunoglobulin secretion. Rituximab has been shown to block the humoral response against a single hapten in a primate model. In IMN, rapid improvement in proteinuria despite no great change in immunoglobulin concentrations might be attributable to selective inhibition of the autoreactive clones that produce nephritogenic immunoglobulin. This selective inhibition might arise during T-cell dependent activation and antibody production, requiring MMC class II and CD40 molecules, which are functionally coupled to CD20 and have pathogenic roles in experimental membranous nephropathy.

Persistent CD20 cell depletion and proteinuria reduction up to the study end at 16 weeks after the last treatment administration (at study week 20) are consistent with evidence in healthy volunteers—and in patients with idiopathic thrombocytopenic purpura, autoimmune haemolytic anaemia, and rheumatoid arthritis—that B cells do not recover in the circulation for 9–12 months. The consequent suppression of autoantibody production might underlie the long-term therapeutic effects of rituximab in these clinical conditions. Assuming a similar response in IMN, one can infer that rituximab should retain a substantial antiproteinuric effect for up to 9–12 months, resulting in substantial renoprotection.

The lack of major adverse events after rituximab treatment in IMN patients is consistent with data showing no increased incidence of serious or opportunistic infections in healthy volunteers and patients with cancer exposed to the same regimen. In adverse events, therefore, rituximab is superior to any immunosuppressive drugs so far used for IMN.

So far, clinically unimportant low-titre human antichimeric antibodies have been found in only one of 161 patients treated with rituximab for lymphomas, myelomas, Waldenstrom's macroglobulinaemia, hairy cell leukaemia, idiopathic thrombotic thrombocytopenic purpura, autoimmune haemolytic anaemia, rheumatoid arthritis, and cryoglobulinaemia. Thus, because of its human component, rituximab has a very low immunogenicity that would not preclude retreatment, if needed. Although we cannot exclude here that adverse events might occur after retreatment, no such event was reported in studies describing effects of rituximab in patients with immune mediated diseases.[4] Cost is another possible limit to be analysed with respect to less well tolerated treatments with major drug-related events.

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 ciclosporin, the risk-benefit profile of rituximab seems much more favourable than that of any other immunosuppressants in IMN.

*Contributors*

G Remuzzi had the idea, designed the study, and finalised the manuscript; P Ruggenenti contributed to the study design and wrote the first draft of the manuscript; M Abbate helped with the pathophysiology interpretation and contributed in writing; C Chiurchiu and V Brusegan managed the patients; and M Bontempelli studied the lymphocyte subpopulations.

*Conflict of interest statement*

None declared.

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