PAPER

Treatment of IgM antibody associated polyneuropathies using rituximab

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J Neurol Neurosurg Psychiatry 2003;**74**:485–489

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Received 6 August 2002
Accepted in revised form 23 November 2002

Objectives: Polyneuropathies with associated serum IgM antibodies are often difficult to treat. Rituximab is a monoclonal antibody directed against the B cell surface membrane marker CD20. Rituximab eliminates B cells from the circulation, and, over time, could reduce cells producing autoantibodies. This study tested the ability of rituximab to produce changes in serum antibody titres, and improvement in strength, in patients with neuromuscular disorders and IgM autoantibodies.
Methods: Over a period of two years, the authors evaluated changes in strength, measured by quantitative dynamometry, and concentrations of several types of serum antibodies in patients with polyneuropathies and serum IgM autoantibodies. Twenty one patients treated with rituximab were compared with 13 untreated controls.
Results: Treatment with rituximab was followed by improved strength (an increase of mean (SEM) 23% (2%)of normal levels of strength), a reduction in serum IgM autoantibodies (to 43% (4%) of initial values), and a reduction in total levels of IgM (to 55% (4%) of initial values). There was no change in levels of serum IgG antibodies. There were no major side effects, even though B cells were virtually eliminated from the circulation for periods up to two years.
Conclusions: In patients with IgM autoantibody associated peripheral neuropathies, rituximab treatment is followed by reduced serum concentrations of IgM, but not IgG, antibodies, and by improvement in strength. Additional studies, with placebo controls and blinded outcome measures, are warranted to further test the efficacy of rituximab treatment of IgM associated polyneuropathies.

Current treatments for IgM antibody related polyneuropathies are relatively few, and are very costly or toxic. Functional improvement in motor neuropathies associated with IgM binding to GM1 ganglioside may occur with IVIg therapy.[1–3] Benefit from single treatments occurs in 70% of patients with multifocal motor neuropathy, but usually lasts only weeks to months.[4 5] Long term intermittent IVIg treatment may produce continued improvement in strength, but can cost over $100 000 per year and may not eliminate the underlying pathogenic process.[6 7] Neuropathies with IgM binding to MAG or GM1 ganglioside may improve for several years after cyclophosphamide treatment.[8–11] Improvement is associated with reductions in levels of IgM autoantibodies. However, cyclophosphamide treatment may be associated with severe toxicity, including haemorrhagic cystitis and an increased long term risk of neoplasia.

In an effort to find a new treatment that improves function and reduces titres of IgM antibodies in these syndromes, but with fewer potential side effects than traditional chemotherapeutic agents, we have been studying the anti-B cell drug rituximab.[12] Rituximab is a chimeric mouse-human monoclonal antibody directed against the B cell surface membrane protein CD20.[13] Treatment with rituximab rapidly eliminates B cells from the circulation. Rituximab treatment is effective in the treatment of non-Hodgkin's B cell lymphoma,[14] and, possibly, in Waldenström's macroglobulinaemia.[15] Our initial data, in a series of five patients with IgM antibody associated neuropathies, showed that rituximab treatment was followed by an increase in strength over a six month period.[12] Based on this initial study, we treated 21 patients with IgM antibody associated neuropathies using rituximab. We followed up clinical and immunological outcome markers over a period of two years, and compared the results with 13 untreated controls.

METHODS

Treatment group

Twenty one rituximab treated patients followed up at the Washington University Neuromuscular Center were evaluated with history, physical examination, and laboratory testing. All patients had a slowly progressive course that evolved over two years or more. Fourteen patients had motor neuropathies characterised by asymmetric weakness (most prominent distally), wasting and hyporeflexia.[16] On electrodiagnostic testing 11 patients had motor conduction block with focal reduction in the area of compound motor action potentials of more than 50% in at least one nerve. All patients had evidence of motor axon loss with reduction in the size of compound motor action potentials in some areas of weakness. All 14 patients had increased serum titres of IgM anti-ganglioside antibodies. Serum IgM bound to GM1 ganglioside in nine patients, GalNAc-GD1a ganglioside[17] in eight patients, and both in three patients. Seven patients had a symmetric sensory motor, demyelinating neuropathy with small fibre and large fibre sensory loss, often with distal weakness predominantly in the legs, and high titres of serum IgM binding to myelin associated protein (MAG).[16 18] The major symptom requiring treatment in six of these patients was instability of gait. On electrodiagnostic studies all patients had symmetrically prolonged distal latencies and slowing of motor and sensory nerve conduction velocities. No patient had motor conduction block. All had evidence of distal loss of sensory and motor axons. No patient had been treated with immunomodulating drugs within two years of rituximab treatment. Twelve patients with motor neuropathies had previously been treated with intravenous immunoglobulin. In nine patients there was insufficient functional benefit to continue treatment. In three patients intravenous immunoglobulin treatments initially produced useful increases in strength for months to years, but then became less effective and were discontinued. Three

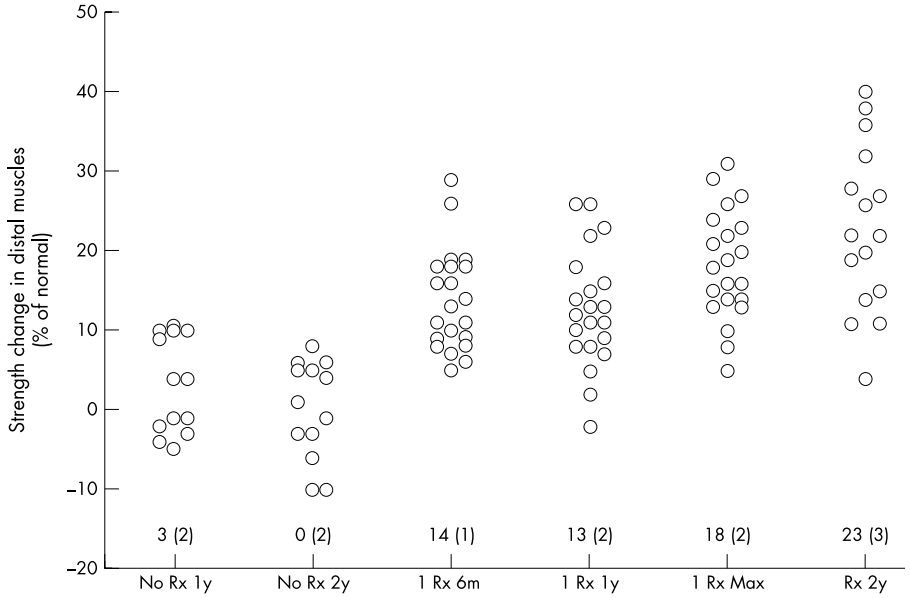

Figure 1 Strength in control and rituximab treated patients. No change in strength was found in untreated patients after one and two years of study. In rituximab treated patients, strength was improved at the six month time point, and at one and two years. The maximum improvement in strength during the first year averaged 18% (2%) of normal strength. At two years improvement averaged 23% (3%) of normal strength. No Rx 1y = strength change in untreated patients followed up for one year. No Rx 2y = strength change in untreated patients followed up for two years. 1 Rx 6m = strength change in 21 patients followed up for six months after the initial series of treatments. 1 Rx 1y = strength change in patients followed up for an average of one year (8 to 15 months) after the initial series of treatments with no additional treatments. 1 Rx Max = maximal strength change in patients followed up for an average of one year after the initial series of treatments. Rx 2y = strength change in 16 patients treated initially, and then again during the second year. Changes in strength are expressed as percentages of expected strength in a normal adult man or woman.

patients had been treated with intravenous cyclophosphamide during periods dating from 3 to 10 years before beginning this study.

Control group

Thirteen untreated patients followed up at the Washington University Neuromuscular Center were evaluated with standard clinical care that included history, physical examination, and laboratory testing, and followed up for two years. Eight patients had motor neuropathies (as above) with increased titres of serum IgM binding to GM1 or GalNAc-GD1a ganglioside. Five patients had a symmetric sensory motor, demyelinating neuropathy with high titres of serum IgM binding to MAG. No patient had been treated with immunomodulating drugs within two years of the period of evaluation. The absence of treatment was related to patient choice, lack of insurance coverage, or evaluation and follow up before rituximab became available. Six of the control patients were subsequently included in the rituximab treatment group.

Strength testing

Quantitative strength testing bilaterally in the same distal muscle groups (wrist extension, first dorsal interosseus, abductor policis brevis, grip, and foot and toe dorsiflexion), a total of 12 measurements, was performed by hand held dynamometry at least every six months.[9 19] Measurements were all made by the same examiner (AP), who was blinded to any results of prior examinations. Results were divided by the expected strength of an adult of the same sex, multiplied by 100 and averaged to derive a mean percentage of normal. Changes of strength of 12%, or more, of normal, in an individual, are significant ($p<0.05$).[9] At the onset of study periods, mean (SEM) distal strength, as a percentage of normal, was not different ($p=0.39$) comparing treated (57% (4%) of normal) and control, untreated groups (63% (6%) of normal).

Laboratory testing

We measured IgM binding to GM1 and GalNAc-GD1a gangliosides and MAG using our standard ELISA methods.[20–22] Quantitative immunoglobulins, serum M proteins (by immunofixation), and circulating B cells (CD-19$^+$) in the peripheral circulation (by flow cytometry) were also evaluated in all patients. Human anti-chimeric antibodies to rituximab and serum rituximab concentrations were measured (by Genentech[23]) in 10 of the earliest treated patients. For comparison of serum samples obtained over time from an individual patient, antibody titres in the different samples were measured in the same assay. Antibody titres were measured and calculated by individuals who were blinded to the patient's treatment status.

Rituximab treatment

Twenty one patients initially received a single course of four intravenous infusions of rituximab (375 mg/m^2) weekly for one month. Patients were pretreated with acetaminophen and diphenhydramine. We designed a protocol for a second year of treatment after several patients, who initially noted symptomatic benefit, complained of recurrent weakness beginning from three to nine months after the initial set of treatments. Eight to 15 months after the initial set of treatments, 16 of the 21 patients received a second set of treatments, initially two weekly intravenous infusions of rituximab (375 mg/m^2 each) and then a single infusion (375 mg/m^2) every 10 weeks, extending through the two year time point after the initial treatment. Four patients noted continued symptomatic benefit from the initial set of treatments and chose not to be retreated. One patient, after modest symptomatic benefit during the first six months after his initial rituximab treatments, experienced a precipitous decline in strength in some muscles over two months. He elected to be retreated using a combination of rituximab and cyclophosphamide (follow up data not shown).

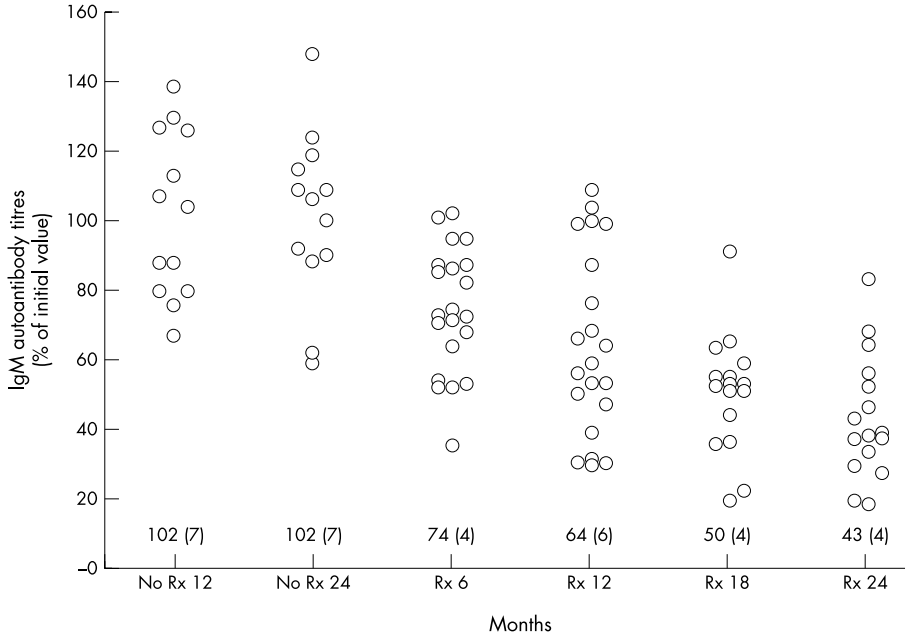

Figure 2 Autoantibody titres in control and rituximab treated patients. Change (percentage of initial value) of IgM autoantibody titres against GM1 or GalNAc-GD1a gangliosides, or MAG in control and rituximab treated patients. No change in titres was found in untreated patients after one and two years of study. In rituximab treated patients, IgM autoantibody titres were reduced at the 6 to 24 month time points. No Rx 12 = change in IgM autoantibody titres in untreated patients followed up for 12 months. No Rx 24 = change in IgM autoantibody titres in untreated patients followed up for 24 months. Rx 6 = change in IgM autoantibody titres in patients followed up for six months after the initial series of treatments. Rx 12 = change in IgM autoantibody titres in patients followed up for an average of 12 months after the initial series of treatments. Rx 18 = change in IgM autoantibody titres at 18 months in patients treated both initially, and then again during the second year. Rx 24 = change in IgM autoantibody titres at 24 months in patients treated both initially, and then again during the second year.

Statistical analyses

Results are expressed as mean (SEM). Data were analysed using Fisher's exact test and *t* test. Study protocols were approved by the Washington University institutional review board.

RESULTS

Strength testing (fig 1)

In the untreated group none of the 13 patients improved by 12% of normal strength at any time during the two years of follow up. The mean (SEM) change in strength was 3% (2%) at one year and 0% (2%) at two years. In the treated group, one year after the initial course of rituximab, strength improved by at least 12% of normal in 86% of patients (18 of 21; $p<0.001$ *v* untreated patients). The mean change in strength in treated patients at one year was an improvement of 13% (2%) ($p<0.001$ *v* untreated patients). In the subgroup of five patients who did not receive a second course of rituximab treatment, the mean change at one year was an improvement of 17% (6%). After two years of rituximab treatment, strength improved by at least 12% of normal in 81% of patients (13 of 16; $p<0.001$ *v* untreated patients). The mean change in strength in the treated group at two years was an improvement of 23% (2%) ($p<0.001$ *v* untreated patients). No differences in the degree of improvement in strength were found comparing treated patients in the motor (22% (3%) at two years) and MAG neuropathy (24% (6%) at two years) subgroups ($p=0.81$).

Antibody testing (figs 2 and 3)

In untreated patients after two years, there was no change in the levels of IgM or IgG directed against specific antigens (102% (7%) and 101% (9%) of initial values), or total IgM or IgG levels (105% (9%) and 96% (4%) of initial values). In treated patients only IgM antibodies showed changes in titres. Titres of serum IgM directed against specific antigens (GM1 or GalNAc-GD1a gangliosides, or MAG) fell to 64% (6%) of initial values at one year, and to 43% (4%) at two years. There were no significant differences between motor (45% (5%) at two years) and MAG neuropathy groups (43% (11%) at two years). Total IgM fell to 74% (3%) of initial values at one year, and to 55% (4%) at two years. Serum IgG titres against tetanus were 114% (9%) of initial values at one year and 100% (8%) at two years. Total IgG was 105% (4%) of initial values at one year and 102% (3%) at two years.

B cells

Before rituximab treatment, levels of circulating B cells averaged 178 (41) $\times 10^3/\mu l$ and 13%(2%) of total WBCs. One week after the initial treatment levels of circulating B cells averaged 1 (1) $\times 10^3/\mu l$ and 0.1% (0.05%) of total WBCs. Three months after the initial treatment there were no detectable circulating B cells in any of the 21 patients. One year after the initial treatment, and without any additional treatments, circulating B cells were found in 7 of the 10 patients in whom they were measured, and averaged 75 (24) $\times 10^3/\mu l$ and 5% (2%) of total WBCs. In three patients followed up without additional treatment for two years, levels of B cells had returned to their initial values.

Other studies

There were no major side effects during or after the rituximab infusions. Minor side effects included transient lowering of blood pressure, a truncal rash and chills in two patients each. These occurred only with the initial treatment. In the hypotensive patients, slowing the rituximab infusion rate was followed by normalisation of the blood pressure. One patient developed transiently increased weakness during the first week after the initial rituximab treatments. Strength then returned to pretreatment values over a period of two weeks. There were no infections of class II, or greater, severity. None of the 10 patients studied developed human anti-chimeric antibodies to rituximab over the two year study period. Rituximab levels averaged 42% (1%) after one week and 11%

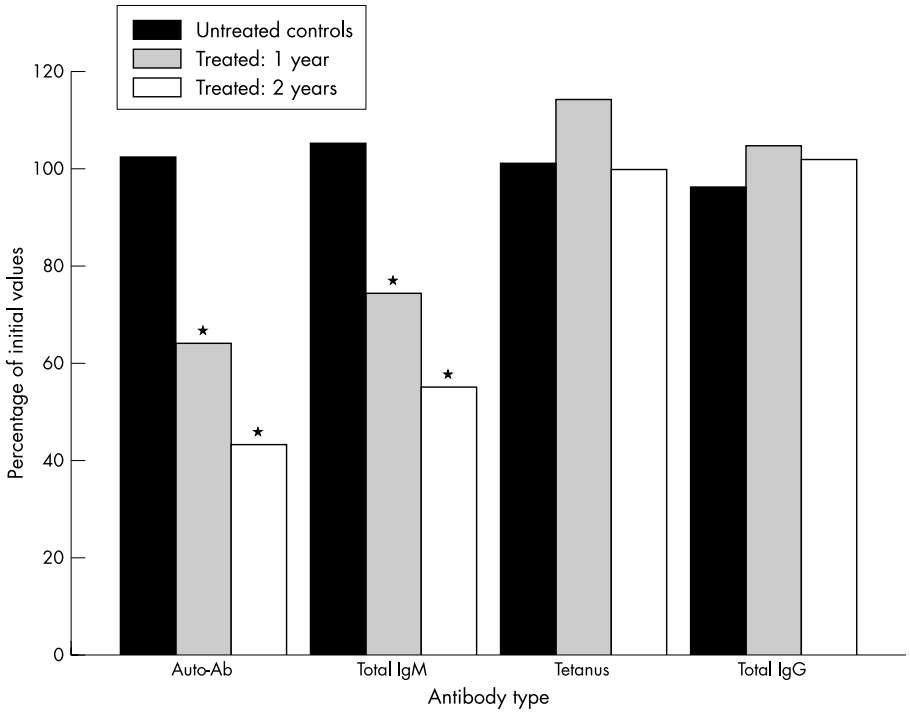

Figure 3 Antibody titres in rituximab treated and untreated patients. Changes (percentage of initial value) in different antibody titres in rituximab treated and control patients. Specific (against GM1 or GalNAc-GD1a gangliosides, or MAG) and total IgM antibodies were reduced in Rituximab treated patients compared with controls. Specific (against tetanus) and total IgG antibodies were unchanged in rituximab treated patients and not different from controls. * Different from untreated controls ($p<0.001$). Auto-Ab = titre of specific serum IgM antibodies (against GM1 or GalNAc-GD1a gangliosides, or MAG). Total IgM = level of total serum IgM. Tetanus = level of serum IgG antibodies against tetanus toxin. Total IgG = level of total serum IgG. Black bars = untreated patients with two year follow up. Grey bars = treated patients with one year follow up. White bars = treated patients with two year follow up.

(2%) after 10 weeks, compared with values taken 12 hours after infusion. In individual patients 10 weeks after infusions, mean rituximab levels ranged from 5% to 20% of initial values.

DISCUSSION

Our results suggest that rituximab is a well tolerated, comparatively safe, and probably efficacious treatment for patients with IgM antibody related polyneuropathies. Rituximab eliminated virtually all circulating B cells within one week of the initial treatment. After a single course of four treatments over one month, B cell levels remained undetectable in all patients at three months and remained below normal values for one year. The mechanisms maintaining the prolonged B cell suppression could be related to the long time normally needed for renewal of the peripheral B cell pool in adults, and to persistent rituximab in serum, with measurable levels persisting at least 10 weeks after an infusion, or tissues. The half life of exogenously administered IgG is generally thought to range from three to six weeks. The serum half life of rituximab in our treated patients was about one week immediately after the infusion, and five weeks thereafter. These data are in the general range of half life previously determined for rituximab.[23]

Treatment with rituximab was associated with very little morbidity. As previously reported,[13] there were occasional episodes of light headedness, chills, and hypotension associated with the initial rituximab treatment. This was treated by slowing the rate of infusion, and did not occur with any subsequent infusions. Despite elimination of most, or all, detectable circulating B cells for periods of more than a year, there were no prominent infections. This may be partially related to the lack of effect of rituximab on IgG antibodies and their associated protective humoral immunity. Continued monitoring and experience will be required to determine the side effects and safety considerations attendant to prolonged, continuous administration of rituximab and continued absence of circulating B cells.

After treatment with rituximab, patients developed increased strength and reduced titres of IgM antibodies. In the treatment group the mean improvement in strength after two years was 23% (2%) of normal levels, a degree of change significantly greater than occurred in untreated controls, who averaged a change of 0% (2%) of normal at two years. Most treated patients reported useful improvement in activities of daily living, such as hand strength or coordination, or gait steadiness. Our experience suggests that continued treatment with rituximab will often be necessary to optimise clinical improvement, at least for the first one to two years. During the year after the initial set of four treatments, over 50% of patients noted loss of, at least some of, their initial symptomatic benefit and requested retreatment with rituximab.

Rituximab treatment also showed a prominent effect on IgM antibody titres. Both specific serum IgM autoantibodies and total levels of circulating IgM were progressively reduced over the two year period of treatment, while levels of IgM antibodies in untreated patients were unchanged. There was no change in specific IgG antibodies to tetanus, or total levels of IgG in treated or untreated patients. The long half life of IgM antibodies and lack of change in IgG antibodies was surprising given the virtually complete elimination of B cells, which are the plasma cell precursors, from the circulation. One explanation for the slow reduction in antibody titres is that the antibody producing (plasma) cells, especially those synthesising IgG, could have a long life span. Alternatively, Rituximab might have failed to eradicate some non-circulating B cells in bone marrow, spleen, or lymph nodes. Residual B cells might provide a continued source for new plasma cells and synthesis of IgM and IgG.

Our results provide information regarding the possible spectrum of utility of rituximab in antibody associated disorders. The reduction in levels of IgM after rituximab treatment suggest that the drug may be effective in the treatment of disorders that are caused by a general increase in IgM levels or specific IgM antibodies. The improvement in measurements of strength in our patients, with IgM antibodies directed against a specific neural antigen, is consistent with this idea. Waldenström's macroglobulinaemia, with its general increase in serum IgM levels, may also improve after treatment with rituximab.[15] The lack of change in levels of IgG suggests that rituximab may be a less likely candidate for the treatment of IgG antibody mediated disorders. There is one report of clinical improvement and reduction in IgG anti-acetylcholine receptor antibody titres in myasthenia gravis after treatment with rituximab.[24] However, the syndrome was not a typical case of myasthenia gravis, as it was preceded by a bone marrow transplant. In addition, the results of that study are difficult to interpret as the patient was treated concurrently with corticosteroids and total serum IgG was not changed after the treatment. Rituximab has also been reported to be effective in treating idiopathic thrombocytopenic purpura with IgG platelet associated antibodies.[25] The mechanism by which rituximab might produce improvement in idiopathic thrombocytopenic purpura remains to be determined. Total levels of IgG were not changed, and levels of IgG platelet associated antibodies fell more rapidly than could be explained by elimination of B cells, and were not clearly associated with patient outcome.

We conclude that rituximab treatment is effective in reducing levels of IgM antibodies in patients with IgM associated peripheral neuropathies. Our results also suggest that treatment is followed by improvement in strength. A further study of the efficacy of intermittent treatment using rituximab in IgM associated polyneuropathies, with placebo controls and blinded outcome measurement, is warranted. Outcome measures should include changes in strength in distal musculature, scales of functional benefit, and levels of IgM antibodies over a period of one to two years. Long term risks and benefits, and the mechanisms by which rituximab selectively reduces IgM, but not IgG, antibodies also deserve further study.

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Competing interests: none declared.

Funding: this investigator initiated study was funded, in part, by a grant from Genentech. Partial support for this study was provided by the Washington University General Clinical Research Center (USPHS grant MO1 RR00036).

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