Rituximab for Refractory Wegener's Granulomatosis
Report of a Prospective, Open-Label Pilot Trial

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Rationale: **Standard therapy for Wegener's granulomatosis is fraught with substantial toxicity and not always effective. B lymphocytes have been implicated in the pathogenesis of Wegener's granulomatosis. Their depletion has been proposed as salvage therapy for refractory disease. Earlier encouraging reports are confounded by concomitant immunosuppressive medications and include only limited available biomarker data.**

Objectives: **To evaluate the efficacy and safety of rituximab for remission induction in refractory Wegener's granulomatosis.**

Methods: **A prospective open-label pilot trial was conducted with 10 patients monitored for 1 yr. Included were patients with active severe antineutrophil cytoplasmic antibody (ANCA)–associated vasculitis, ANCA positivity, and resistance to (or intolerance of) cyclophosphamide. The remission induction regimen consisted of oral prednisone (1 mg/kg/d) and four weekly infusions of rituximab (375 mg/m^2). Prednisone was tapered and discontinued over 5 mo. Failure to achieve remission, a clinical flare in the absence of B lymphocytes, and inability to complete the glucocorticoid taper were considered treatment failures.**

Main Results: **Three women and seven men (median age, 57 yr; range, 25–72 yr) were enrolled. All had ANCA reacting with proteinase-3. The median activity score at enrollment was 6 (range, 5–10). All patients tolerated rituximab well, achieved swift B-lymphocyte depletion and complete clinical remission (activity score, 0) by 3 mo, and were tapered off glucocorticoids by 6 mo. Five patients were retreated with rituximab alone for recurring/rising ANCA titers according to protocol. One patient experienced a clinical flare after B lymphocyte reconstitution.**

Conclusion: **In this cohort, rituximab was a well-tolerated and effective remission induction agent for severe refractory Wegener's granulomatosis.**

Wegener's granulomatosis (WG) and microscopic polyangiitis (MPA) are primary systemic small vessel vasculitides with predilection for the respiratory tract and kidneys (1). Most patients who suffer from generalized or severe disease activity have circulating antineutrophil cytoplasmic antibodies (ANCAs) reacting either with neutrophil proteinase-3 (PR3) or myeloperoxidase (MPO). To this day, the combination of glucocorticoids and cyclophosphamide remains the standard therapy for patients with severe WG or MPA (2, 3). Depending on the definition, remission can be induced in about 70 to 90% of patients with this regimen (2–5). Nevertheless, ANCA-associated vasculitis has a high relapse rate, and some patients do not respond satisfactorily to this treatment. The prolonged and repeated use of cyclophosphamide is associated with substantial toxicity, which ultimately limits or prohibits its use in some patients.

B lymphocytes are critical for the regulation of immune responses and production of antibodies. They function as antigen-presenting cells, express costimulatory molecules, produce cytokines, and regulate the differentiation and activation of T lymphocytes and dendritic cells. The role of B lymphocytes in the pathogenesis of autoimmune diseases is now well established (6, 7). Initial studies implicating B lymphocytes as active participants in the pathogenesis of WG provided the rationale for using cyclophosphamide to treat this disease (8–10). More recently, the number of activated peripheral blood B lymphocytes has been linked to disease activity and to the extent of organ involvement (11). B lymphocytes are also instrumental for the production of autoantibodies including ANCA, which in turn have multiple proinflammatory effects that can contribute to the development of tissue injury and vasculitis (12–15).

Rituximab is a chimeric monoclonal antibody directed against CD20, a cell surface antigen expressed almost exclusively on cells of B-lymphocyte lineage (16). Binding of the antibody to CD20 results in selective depletion of B lymphocytes by a variety of different mechanisms (17–19). This agent has become an important component of standard treatment regimens for non-Hodgkin's B-cell lymphoma (20). Because of the prominent role ascribed to B lymphocytes in autoimmune diseases (6, 7), rituximab is increasingly being investigated as a therapeutic agent for these nonmalignant indications (21, 22). Early reports of its successful use in autoantibody-mediated autoimmune diseases were followed by promising results achieved by B-lymphocyte depletion in multisystem autoimmune diseases, such as rheumatoid arthritis, which until recently were thought to be predominantly T-lymphocyte mediated (23–30).

Encouraged by early successes with the compassionate use of rituximab in patients with refractory Wegener's granulomatosis (31, 32), this prospective open-label pilot trial was performed to formally test the hypotheses that in patients with refractory ANCA-associated vasculitis selective B-cell depletion with anti-CD20 therapy (rituximab) will be effective for induction of remission, will allow tapering and discontinuation of glucocorticoids without relapse, and will result in disappearance of ANCAs. Some of the results have been previously reported as abstracts (33, 34).

METHODS

Trial Eligibility

This investigator-initiated trial was approved by the Institutional Review Board of the Mayo Clinic Rochester (Rochester, MN). Patients were eligible for participation if they fulfilled all of the following inclusion criteria: (*1*) a biopsy-proven diagnosis of WG or MPA and clinical features fulfilling the American College of Rheumatology criteria and Chapel Hill Consensus definitions (1, 35), (*2*) active severe (life- or organ-threatening) disease with a Birmingham Vasculitis Activity Score for WG (BVAS/WG) of 3 or more (36), (*3*) positive ANCA reacting with proteinase-3 (PR3-ANCA) or myeloperoxidase (MPO-ANCA) at

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the time of enrollment, and (*4*) refractory disease. Refractory disease was defined as meeting the first three criteria while receiving a maximally tolerated dose of cyclophosphamide or having contraindications for the repeated use of cyclophosphamide for treatment of the severe disease flare (history of documented cytopenias or hemorrhagic cystitis that prompted discontinuation of cyclophosphamide in the past, or malignancies other than nonmelanoma skin cancers within the last 5 yr). This definition of refractory disease is consistent with the definition used by the European Vasculitis Study Group and allows for comparability of trial results with those of other reports (37, 38). In addition, patients had to be 18 yr or older, able to provide informed consent, and have an absolute white blood count of 1,000/μl or greater, and premenopausal women had to use an acceptable method of birth control for the duration of the trial.

Patients with an active infection, history of HIV infection, known type I hypersensitivity or anaphylactic reactions to murine proteins, uncontrolled cardiac arrhythmias, or New York Heart Association classification III or IV heart disease were not eligible for participation in the trial. All immunosuppressive drugs were discontinued at the time of enrollment, but no washout time was required before initiation of the treatment protocol.

Disease Assessments and Definitions

Patients underwent complete physical examinations, chest roentgenograms, and laboratory testing at baseline, 1 wk after completion of the last study drug infusion, and at 3, 6, 9, and 12 mo. The Medical Outcomes Study 36-item Short-Form Health Survey (SF-36, version 2) was completed by the patients at each study visit to assess their quality of life (39).

Disease activity was measured at each study visit, using the BVAS/WG instrument (36). The BVAS/WG is a validated disease-specific activity index, which captures all possible organ manifestations of the disease (36). According to the BVAS/WG instrument, disease activity with the potential to cause irreversible organ damage or representing an immediate threat to the patient's life is considered severe disease. Features indicative of severe disease are designated as "major items" and include scleritis, alveolar hemorrhage, respiratory failure, active glomerulonephritis, central or peripheral nervous system involvement, or any disease item deemed severe enough to require treatment with cyclophosphamide. Each one of these items is assigned three points. All other disease items, such as nasal ulceration, or pulmonary nodules or cavities, are designated as "minor items" and assigned one point. In an effort to reduce redundancy of some of the original component items, and to optimize capture of clinical features unique to Wegener's granulomatosis, the BVAS/WG instrument was adapted from an existing disease activity score intended for use in all forms of systemic vasculitis (36, 40). On average, BVAS/WG scores are about half of those scored with the original instrument (41).

In accordance with the definitions used in the Wegener's Granulomatosis Etanercept Trial (3), complete remission was defined as having a BVAS/WG score of 0, and sustained remission as having a BVAS/WG score of 0 for at least 6 mo. Any rise in BVAS/WG score of one point or more was considered a relapse.

Treatment Protocol

The remission induction regimen consisted of the combination of glucocorticoids and rituximab. All patients received oral prednisone at a dose of 1 mg/kg/d, not to exceed 80 mg/d. The use of intravenous methylprednisolone, 1 g/d for 3 d, preceding the oral prednisone was allowed if deemed clinically indicated by the treating physician. No later than 4 wk after initiation of therapy, the prednisone dose was reduced to 40 mg/d. Subsequently, the daily prednisone dose was reduced to 30, 20, 15, 10, 7.5, 5, and 2.5 mg, and then to 0, every 2 wk. This standard tapering regimen resulted in complete discontinuation of prednisone over the course of 5 mo. In the absence of a relapse, patients were to remain off glucocorticoids for the remainder of the study.

The open-label rituximab regimen consisted of four weekly infusions, each at a dose of 375 mg/m^2 of body surface. Patients received 650 mg of acetaminophen and 50 mg of diphenhydramine orally before each infusion. In the event of rigors, chills, or a sensation of throat tightness during an infusion, the infusion was interrupted, 50 mg of intravenous meperidine was given, and infusion was resumed at a slower rate once symptoms had resolved. All infusion reactions were recorded as adverse events.

After successful depletion of B lymphocytes, remission induction, and glucocorticoid taper, patients were retreated with rituximab after return of B lymphocytes if they experienced a clinical relapse, or if they remained in clinical remission but became ANCA positive again or experienced a significant increase in ANCA level (fourfold titer rise by immunofluorescence or doubling of PR3-ANCA ELISA units compared with the ANCA nadir achieved after remission induction). In the event of a clinical relapse the entire remission induction regimen was repeated. Patients experiencing a rise in ANCA levels alone without clinical evidence of reactivation of the disease were retreated with four infusions of rituximab without glucocorticoids.

Concomitant Medications

The use of all other immunosuppressive agents or interventions, including plasma exchange, intravenous gamma globulin, methotrexate, azathioprine, mycophenolate mofetil, leflunomide, cyclosporine-A, anti–tumor necrosis factor agents, or others, was prohibited for the duration of the trial. All patients received *Pneumocystis jiroveci* pneumonia prophylaxis with trimethoprim-sulfamethoxazole (80 mg/400 mg daily) and osteoporosis prophylaxis.

Outcomes

Primary outcome measures were the clinical response, the ability to complete the prednisone taper without recurrence or worsening of disease activity, and ANCA levels. Treatment failures were defined as death resulting from persistent disease activity, inability to be weaned off glucocorticoids because of persistent or progressive disease activity, inability to complete the full course of rituximab therapy because of treatment-related adverse events, and disease relapse in the absence of detectable peripheral blood B lymphocytes. Secondary outcome measures included adverse events and quality of life. C-reactive protein and erythrocyte sedimentation rate, total lymphocyte and lymphocyte subset counts, and quantitative immunoglobulin levels were tertiary outcome measures.

Laboratory Measurements and Methods

Immunoglobulin G ANCAs were measured monthly throughout the study. Serum samples were processed by the Mayo Clinic Rochester Clinical Immunology Laboratory according to previously described routine procedures (42). Screening for PR3-ANCAs and MPO-ANCAs was performed by a direct ELISA method. Samples yielding positive results by ELISA were further tested by indirect immunofluorescence, using ethanol-fixed neutrophil cytospin preparations. Peripheral blood B lymphocytes were measured at each study visit (baseline and at 1, 3, 6, 9, and 12 mo) by fluorescence-activated cell sorting, using CD19 as marker. The lower detection limit was one B cell per microliter. Immunoglobulins A, M, and G, as well as immunoglobulin G subclasses, were also determined at baseline and at each subsequent study visit.

Statistical Analysis

Intention-to-treat analysis was used for all the major outcomes including all 10 subjects. Comparisons were performed with JMP version 5.0 (SAS, Cary, NC). A Student paired *t* test was used for comparisons of repeated measurements taken in the same patient at different time points. For variables that required nonparametric analyses (BVAS/WG, ANCAs), comparisons were made by Wilcoxon signed rank test. The eight scales of the SF-36 were scored with published software and analyzed as *z* scores calculated on the basis of norms from a U.S. general population sample (39). Data are presented as means ± SD or as medians with interquartile ranges (box plots). Statistical significance was taken at the $p < 0.05$ level.

RESULTS

Demographics and Disease Assessment at Baseline

Three women and seven men (median age, 57 yr; range, 25–72 yr) were enrolled between January 16, 2003, and November 17, 2003. Disease duration, treatment preceding enrollment, treatment

regimens they were failing, existing contraindications for cyclophosphamide use, organ involvement, and BVAS/WG scores at the time of enrollment are listed for each patient in Table 1. All patients met the definition of refractory disease. Three patients had persistent severe disease (at least one major BVAS/WG item indicating potentially life- or organ-threatening disease activity) with a BVAS/WG score of 5 or 6, while receiving a maximally tolerated cyclophosphamide dose. The other seven patients had severe disease relapses with BVAS/WG scores ranging from 5 to 10. Treatment of these severe relapses would require the use of cyclophosphamide as part of the remission induction regimen, which was precluded because of preceding cyclophosphamide treatment complications.

The median BVAS/WG score at enrollment was 6 (range, 5–10). Seven patients had active glomerulonephritis documented either by renal biopsy (n = 3) or by the presence of red blood cell casts in the urine (n = 4). Of the four patients with active lung disease, one had early signs of alveolar hemorrhage presenting as acute onset of hemoptysis and bilateral patchy alveolar infiltrates on computed tomography. The other three had typical bilateral cavitating and noncavitating lung nodules and mass lesions. The eye involvement of two patients consisted of optic neuritis in one patient who had previously lost vision in the contralateral eye due to the disease, and of orbital inflammatory pseudotumor and episcleritis in another. Central nervous system involvement consisted of meningeal involvement in one patient and pituitary involvement with panhypopituitarism in another. All patients had ANCAs reacting with proteinase-3 at the time of enrollment. Even though all the patients had severe disease according to the accepted definition (3), none of the trial participants required intensive care unit admission.

TABLE 1. DEMOGRAPHICS AND CLINICAL FEATURES OF TRIAL SUBJECTS

Patient No.	Contra-indications to CYC	Age (*yr*), Sex	Time since Diagnosis (*mo*)	No. Prior Relapses*	Immunosuppressants Previously Used†	Failing Therapy at Time of Rituximab	Active Organ Involvement at Start of Rituximab	Rituximab Dose in First Infusion Series (*mg*)	BVAS/WG (*Baseline/6 mo*)	Status as of 12/31/04
1	Failed CYC	58, M	6	0	P, CYC	CYC 150 mg qd; P 60 mg qd	Lung, kidney, joints	3,884	6/0	Stable remission at 19 mo. Preemptive retreatment at 9 mo
2	Failed CYC	56, M	17	1	P, CYC	CYC 2 g iv qm; P 20 mg qd	Lung, joints, skin, CNS (pituitary)	3,480	6/0	Stable remission at 17 mo. Preemptive retreatment at 9 mo. Prednisone 6 mg qd as hypopituitarism replacement therapy
3	Failed CYC	68, F	86	3	P, CYC, AZA, MTX	CYC 75 mg qd	ENT, lung, kidney, joint, PNS	2,535	5/0	Retreatment for flare at 9 mo. Stable remission 4 mo after retreatment
4	Cystitis	25, F	28	2	P, MTX, CYC	MMF 500 mg bid	ENT, eye, CNS (meningeal)	3,524	7/0	Stable remission at 23 mo
5	Cystitis	32, M	104	1	P, CYC, AZA	AZA 200 mg; P 20 mg bid	ENT, lung,‡ kidney, joints, skin	4,200	10/0	Stable remission at 20 mo. Preemptive retreatment at 9 mo
6	Cytopenia	60, M	18	1	P, CYC, etanercept	AZA 50 mg; Etanercept 25 mg bwk	ENT, kidney	3,540	6/0	Stable remission at 22 mo. Preemptive retreatment at 9 mo
7	Cytopenia	71, M	145	5	P, CYC, AZA, etanercept	Etanercept 25 mg bwk	ENT, kidney, joints	3,120	5/0	Stable remission at 16 mo
8	Cytopenia	51, F	40	5	P, CYC	MMF 1,000 mg qd; P 20 mg qd	Kidney, joints, skin	3,240	5/0	Stable remission at 18 mo. Preemptive retreatment at 9 mo
9	Cytopenia	72, M	164	3	P, CYC		ENT, kidney, joints	3,180	10/0	Stable remission at 18 mo. Preemptive retreatment at 16 mo, outside of trial
10	Cytopenia	56, M	78	2	P, CYC, AZA	AZA 100 mg qd	ENT, eye	2,924	5/0	Stable remission at 15 mo

Definition of abbreviations: AAV = antineutrophil cytoplasmic antibody–associated vasculitis; AZA = azathioprine; bid = twice daily; bwk = twice per week; BVAS/WG = Birmingham Vasculitis Activity Score/Wegener's granulomatosis; CYC = cyclophosphamide; ENT = ear, nose, and throat; F = female; iv = intravenous; M = male; MTX = methotrexate; MMF = mycophenolate mofetil; P = oral prednisone; PNS = peripheral nervous system; qd = daily; qm = monthly.

* Not including first bout of disease activity and not including the flare of disease leading to enrollment.

† Used to treat the disease before the failing regimen listed in the next column.

‡ Alveolar hemorrhage.

Clinical Efficacy

Peripheral blood B lymphocytes became undetectable within 1 wk after completion of the rituximab infusion series (Figure 1B). At the 6-mo follow-up visit, B lymphocytes were still undetectable (0% and fewer than 10/μl) in all patients. At 9 mo, B-lymphocyte counts had recovered (5% or more, or at least 10/μl) in seven patients. Among the remaining patients, B lymphocytes normalized by 12 mo (n = 1) and 15 mo (n = 2). All patients experienced prompt improvement of symptoms, and by 3 mo all had achieved complete remission (BVAS/WG = 0; Figure 1A). Renal function was preserved or improved in all seven patients who had active renal involvement at the time of enrollment (Figure 2A).

All patients completed the glucocorticoid dose taper without interruptions according to protocol and discontinued glucorticoids no later than 5.5 mo after enrollment. One patient, whose disease resulted in irreversible pituitary insufficiency, continued to take 6 mg of prednisone as part of continued hormonal replacement therapy (patient 2). This patient was also counted as having completed the glucocorticoid taper. Nine of the 10 patients remained in complete remission without glucocorticoid therapy for the duration of the trial. According to protocol, five patients received preemptive retreatment with rituximab alone for an increase in ANCA levels after return of B lymphocytes. The second rituximab infusion series was initiated in each of the patients at the 9-mo visit.

One patient (patient 3) suffered a severe disease relapse at 9 mo, when B cells had returned and ANCA levels had risen significantly. Remission was restored again in this patient with the same rituximab and oral prednisone remission induction regimen. At completion of the trial her BVAS/WG score had returned to 0, and the prednisone dose was down to 15 mg/d.

Laboratory Features

PR3-ANCA levels dropped in all patients and turned negative in six patients after remission induction therapy (Figure 1C). The patients who did not turn PR3-negative were those with the highest baseline values. However, these patients still experienced an average 9.6-fold decline in PR3-ANCA level. In patients with PR3-ANCA level increases after successful remission induction, these increases were associated with, or followed, reconstitution of B lymphocytes. Two patients remained PR3-ANCA negative for the remainder of the trial, despite return of B lymphocytes.

Erythrocyte sedimentation rate (Figure 2B) and C-reactive protein levels (Figure 2C) paralleled the inflammatory disease

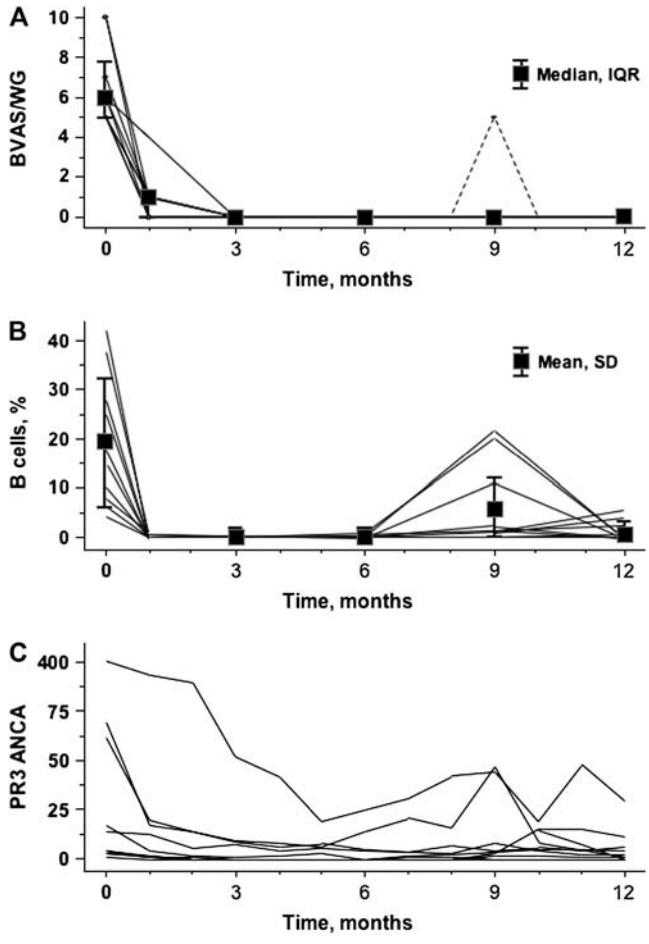

Figure 1. Response to treatment. (*A*) All 10 patients experienced a prompt decline in the Birmingham Vasculitis Activity Score for Wegener's granulomatosis (BVAS/WG). By 3 mo all patients had achieved complete remission with a BVAS/WG of 0. Only one patient experienced a clinical flare at 9 mo. This flare responded promptly on repeating the remission induction regimen. (*B*) Rituximab therapy resulted in peripheral blood B-lymphocyte depletion in all patients, and B-lymphocyte recovery occurred between 6 and 12 mo. (*C*) Monthly proteinase-3–antineutrophil cytoplasmic antibody (PR3-ANCA) levels measured in all patients. IQR = interquartile range.

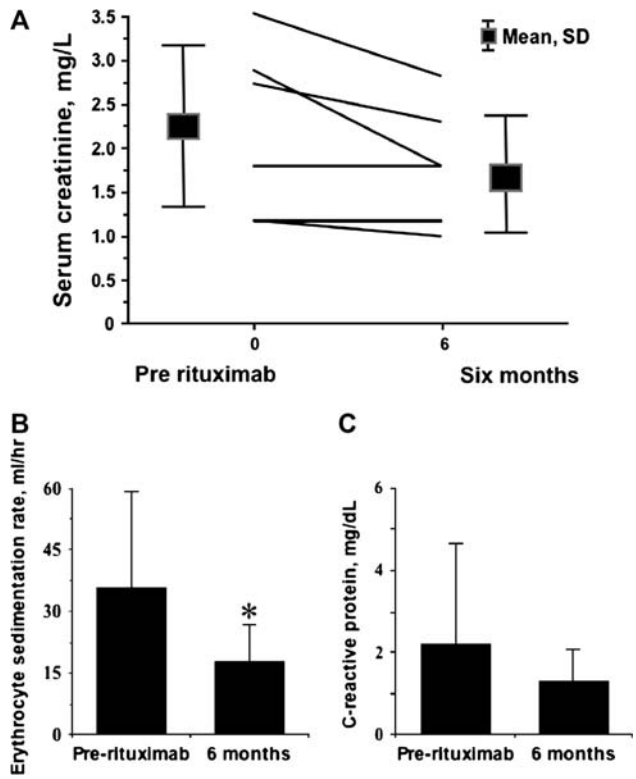

Figure 2. (*A*) Renal function remained stable or improved in all seven patients with active renal involvement at enrollment. Shown are mean (± SD) serum creatinine levels at enrollment and at 6 mo. Patients with high serum creatinine all had chronic renal insufficiency as a result of previous episodes of disease activity. (*B*) Reduction in sedimentation rate and (*C*) C-reactive protein levels occurred in all patients, but only the mean differences in erythrocyte sedimentation rate were statistically significant.

activity. Immunoglobulin M levels dropped in all patients after rituximab therapy and recovered with B-cell reconstitution (IgM nadir [mean ± SD], 22.1 ± 14.52 mg/dl; normal, 60–300 mg/dl). Immunoglobulin G levels and subclass levels showed only a minimal decline compared with baseline levels (IgG nadir [mean ± SD], 525.3 ± 170.73 mg/dl; normal, 600–1,500 mg/dl). Immunoglobulin A levels were not affected by therapy (IgA nadir, 122.1 ± 58.9 mg/dl; normal, 50–400 mg/dl). Human antichimeric antibodies were measured in all patients at baseline and at 3, 6, and 9 mo, and none were detected.

Adverse Events

All patients completed the first rituximab infusion series without any adverse event. One patient experienced rigors and chills with the first infusion of a subsequent retreatment series of infusions. Because the patient complained of dyspnea at the same time, he was observed in the hospital overnight even though no changes in hemodynamic parameters or oxygen saturation were observed during the episode. The remainder of the infusion dose was uneventfully given the next day, and the next three infusions were completed without adverse reactions.

Two patients (patients 2 and 6) developed herpes zoster eruptions during the first 3-wk rituximab infusion phase, followed by postherpetic neuropathy. One patient (patient 6) subsequently also developed influenza while B lymphocyte depleted. The patient was treated with oseltamivir, and the infection resolved over the next 10 d. Five patients experienced a total of 13 upper respiratory tract infections (rhinitis, sinusitis, cough with yellow and green nasal discharge, and phlegm production, with or without fevers). All episodes were treated with broad-spectrum antibiotics and resolved promptly within days.

Quality of Life

Quality of life, as assessed by the SF-36, improved significantly from baseline to 1 yr for the domains of Role-Physical ($p = 0.006$), Social Functioning ($p = 0.022$), and Vitality ($p = 0.019$). Although not statistically significant, increases from baseline to 1 yr were also observed for the remaining SF-36 domains (Table 2). For the domain of Physical Functioning, the change from baseline to 1 yr was found to be significantly correlated with the BVAS/WG score at enrollment (Spearman rank correlation, $r = +0.73$, $p = 0.026$).

TABLE 2. MEDICAL OUTCOMES STUDY 36-ITEM SHORT-FORM HEALTH SURVEY SCORES AT BASELINE AND AT 1-YR FOLLOW-UP*

	Score		
SF-36 Subscale	Baseline	1 yr	p Value†
Bodily pain	−1.0 ± 1.3	−0.2 ± 1.1	0.140
General health perceptions	−1.7 ± 0.6	−1.2 ± 1.0	0.198
Mental health	−0.5 ± 1.6	+0.1 ± 1.5	0.120
Physical functioning	−1.2 ± 1.3	−1.0 ± 1.4	0.572
Role—emotional	−0.7 ± 1.3	+0.3 ± 1.0	0.052
Role—physical	−1.7 ± 0.8	−0.6 ± 1.2	0.006
Social functioning	−1.1 ± 1.5	−0.2 ± 1.3	0.022
Vitality	−1.3 ± 1.1	−0.6 ± 1.1	0.019

Definition of abbreviation: SF-36 = Short-Form 36.

SF-36 subscale scores are analyzed as *z* scores calculated on the basis of norms from a U.S. general population sample (38). In all cases, data are presented as means ± SD.

* n = 10.

† Paired *t* test.

DISCUSSION

Clinical Efficacy

Symptoms improved within weeks after initiation of therapy in all patients. Undoubtedly, the immediate clinical response can be attributed largely to high-dose glucocorticoid therapy. However, in the absence of B lymphocytes, all patients completed the protocolized prednisone dose taper without signs of recurring disease activity, and all but one patient remained in stable clinical remission after completion of the successful glucocorticoid taper. For the majority of trial participants this was the first sustained glucocorticoid-free remission in years. All patients remained in remission for as long as peripheral blood B lymphocytes were undetectable. During the trial, five patients were retreated with rituximab alone for rising ANCA levels after reconstitution of their B lymphocytes. Taken together, these observations suggest that rituximab is an effective agent for both remission induction and remission maintenance.

B-Lymphocyte Depletion

In this trial, the clinical response was linked to effective B-lymphocyte depletion, which was achieved in all patients. It is also consistent with our previous experience in ethnically similar patients (32). In other disorders, including systemic lupus erythematosus, the degree of B-cell depletion after rituximab infusions has been less predictable. The B-lymphocyte depletion response of patients has been linked to the rituximab dose, ethnicity, FcγRIIIa receptor polymorphisms, and the development of human anti-chimeric antibodies (30, 43). All these factors may be interrelated. In the previously reported lupus cohort, black patients were reported to experience less complete B-cell depletion and a higher likelihood to develop human antichimeric antibodies than were white patients (30). Like most patients with WG, all participants in our trial were white, and none developed human antichimeric antibodies. Furthermore, the body surface area–based dosing regimen applied in our study differs from the dosing regimen used in reported rheumatoid arthritis and systemic lupus erythematosus trials (two fixed doses of 500 or 1,000 mg, 2 wk apart) (27, 29). Clear data linking weight-based dosing to efficacy of rituximab have not been reported. Yet, several of our patients would have received substantially lower cumulative doses using the fixed 1,000 mg twice-dose regimen (Table 1).

In rheumatoid arthritis and systemic lupus erythematosus, rituximab was used initially in conjunction with intravenous cyclophosphamide and glucocorticoids (26–28). This combination regimen was derived originally from earlier experience in lymphoma therapy, which indicated a higher efficacy of rituximab if given together with cyclophosphamide (44). A randomized trial in rheumatoid arthritis confirmed the efficacy of rituximab in this disorder, but did not clarify whether the addition of cyclophosphamide has a meaningful clinical benefit (29). Previous anecdotal experience and this trial indicate that B-lymphocyte depletion with rituximab in conjunction with a tapering glucocorticoid regimen may be sufficient to induce a sustained remission in WG (31, 32, 45). In addition, all reported clinical relapses, as well as the relapse observed in this trial, were preceded by reconstitution of B-lymphocyte counts (32, 45, 46). This suggests that B lymphocytes are indeed of paramount importance in the pathogenesis of WG.

ANCA and Immunoglobulin Levels

ANCA levels dropped in all our patients, whereas the total immunoglobulin G levels remained stable for the duration of the trial, even in patients who received two infusion series. This is consistent with previous observations in a variety of autoimmune

diseases, where autoantibody levels are suppressed but immunoglobulin G and protective antibody levels remain unaffected by rituximab therapy (31, 32, 47–49). In rheumatoid arthritis, the clinical response to rituximab therapy seemed to be contingent on the presence of rheumatoid factor, and it was associated with a decrease in autoantibodies (49, 50). In our study, patients who did not turn completely ANCA-negative were those with the highest PR3-ANCA levels at enrollment. Subsequent PR3-ANCA level increases coincided or followed B-lymphocyte reconstitution. These findings suggest that, like in rheumatoid arthritis, autoantibody production is dependent on B lymphocytes. This is in contrast to total immunoglobulin G and protective antibodies, which are produced by long-lived plasma cells and unaffected by rituximab therapy (49). We cannot entirely exclude the possibility that glucocorticoids contributed to the drop in PR3-ANCA levels, and the number of patients included in this study is too low to conclude whether PR3-ANCAs are produced by short-lived, antigen-specific, B-cell precursor-dependent plasma cells or by long-lived plasma cells. However, if the glucocorticoid effect were primarily responsible for the drop in antibodies, this effect would have been reflected in the immunoglobulin G levels, which were not affected by this therapeutic regimen (51). Furthermore, in rheumatoid arthritis, decreases in autoantibody levels have been reported in patients who did not receive high-dose glucocorticoids in conjunction with rituximab (50). Similarly, ANCA level decreases were observed in patients retreated preemptively with rituximab alone, both as part of this trial, and in patients treated on a compassionate use basis off-label (32).

The ANCA response in WG and autoantibody responses in rheumatoid arthritis seem to be different from the effect of rituximab on anti–double-stranded DNA antibody levels in systemic lupus erythematosus (30). In the latter dose escalation trial, varying doses of rituximab were given in conjunction with varying doses of prednisone (ranging from 0 to 40 mg/d), and no effect on anti–double-stranded DNA antibody levels was detected. It is possible that in different diseases, autoantibodies may be produced by different types of plasma cells. It is even possible that, depending on duration or clinical subsets of disease, the predominant cellular source of antibodies may be different between patients within the same disease group, or even change in the same patient over time. A variable effect of rituximab on autoantibody levels can hence be expected. These relationships deserve further study in large well-characterized patient cohorts.

Safety

Rituximab was well tolerated in this study cohort. Infusion reactions are the most common adverse effects reported with rituximab use (52). In this trial only one mild infusion reaction (rigors and chills) with the first infusion of a repeat series was encountered. None of the patients experienced infusion reactions with the first infusion. It is possible that the concomitant high-dose glucocorticoid therapy mitigates potential mild infusion reactions.

Two of the patients experienced herpes zoster eruptions. B-lymphocyte depletion has the potential to aggravate or reactivate latent viral infections (53). However, both of our patients had been treated with other immunosuppressive agents preceding enrollment into the trial, and herpes zoster eruptions are a recognized problem in patients with WG on treatment (54, 55).

The inability of patients to mount a humoral response to vaccines for the duration of B-lymphocyte depletion is of particular concern with respect to annual influenza vaccination (56–58). Trial subjects did not receive influenza vaccinations, but were carefully instructed to report immediately for testing in the event of symptoms arising during influenza season. One patient was infected with influenza, and the infection resolved promptly without complications within days under oseltamivir therapy. Because of concerns about bacterial infections potentially taking a more complicated course in the setting of B-lymphocyte depletion, the trial participants were treated empirically with broad-spectrum antibiotics (usually levofloxacin) if they reported symptoms of respiratory tract infections associated with the production of yellow or green nasal discharge or phlegm. No complicated bacterial infections were encountered in this cohort. Consequently, no obvious increased risk for complicated infections over other immunosuppressive regimens used for the treatment of WG became apparent. However, it must be acknowledged that the small sample size of this pilot study and the short observation period of 12 mo do not allow meaningful conclusions regarding the long-term safety of prolonged B-cell depletion in patients with WG.

Limitations

Although the results of this pilot trial are extremely encouraging, the study has limitations inherent to its design. First, the open-label design of this trial allows for the possibility that some of the more subjective components of the BVAS/WG disease activity scoring instrument are affected by bias on the part of both the assessing physicians and the trial subjects. Second, because this was a study in patients characterized as refractory, it is not appropriate to extrapolate the results to patients with newly diagnosed ANCA-associated vasculitis, who are candidates for standard therapy.

Third, although the presence of active glomerulonephritis is a bad prognostic indicator and by definition denotes severe WG (5), the absolute reduction in renal function observed in the trial participants was modest. Most likely this is because they were all well-established patients, who were monitored closely, and recurrent disease activity was consequently detected early. Hence, the efficacy of rituximab for renal activity of WG merits further study.

Finally, even though unlikely, it cannot be excluded with certainty that the immunosuppressive therapy received by some patients immediately preceding the trial contributed to the perceived beneficial effects of rituximab. However, in contrast to our previously reported cases (32), only one of the participants was given intravenous methylprednisolone (patient 5 for alveolar hemorrhage), and none were treated by plasma exchange. In contrast to cases reported by others, none of our patients received any other concomitant immunosuppressive drugs (46, 59–62). Thus, of all reports on the use of rituximab in ANCA-associated vasculitis, this cohort is the least confounded by other immunosuppressive therapies.

In conclusion, rituximab appears to be effective for the induction and maintenance of stable remission off glucorticoids in patients with WG, who failed to respond to cyclophosphamide or have contraindications for cyclophosphamide. Rituximab was well tolerated in these patients, and the short-term safety profile is encouraging. The efficacy and safety of rituximab in comparison with cyclophosphamide for remission induction in patients with newly diagnosed or acutely relapsing ANCA-associated vasculitis (WG or microscopic polyangiitis) are now being studied formally in an ongoing double-masked, double placebo–controlled multicenter trial (*see* www.clinicaltrials.gov).

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