

Safety and Efficacy of Rituximab in Patients with Rheumatoid Arthritis Refractory to Disease Modifying Antirheumatic Drugs and Anti-Tumor Necrosis Factor- α Treatment

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ABSTRACT. Objective. To determine the safety and efficacy of rituximab treatment in patients with active seropositive rheumatoid arthritis (RA) who had experienced an inadequate response to treatment with anti-tumor necrosis factor- α agents and/or traditional disease modifying antirheumatic drugs.

Methods. Rituximab was administered to 17 patients as weekly infusions for 4 consecutive weeks. Patients continued their baseline therapy and were followed for 28 weeks.

Results. All patients were evaluable for safety, and 13 for efficacy. Profound B cell depletion occurred by 12 weeks and was sustained at 24 weeks, whereas T cell, complement, and immunoglobulin levels remained within normal ranges. Rituximab was well tolerated, with no infusion related reactions and only mild/moderate adverse events. American College of Rheumatology 20% response (ACR20) was achieved in 55% of patients by Week 5, 75% by Week 8, 50% at Week 16, and 67% at Week 28. Corresponding ACR50 and ACR70 responses were achieved in 36% and 18%, 25% and 17%, 42% and 25%, and 33% and 17% of patients at Weeks 5, 8, 16, and 28, respectively. There were significant improvements over baseline in tender and swollen joint counts ($p < 0.0001$), physician's global assessment of disease activity ($p = 0.0001$), and patient assessed pain ($p = 0.0005$) and disability ($p = 0.0386$). Erythrocyte sedimentation rate ($p = 0.0361$) and rheumatoid factor titers ($p < 0.0001$) also decreased significantly.

Conclusion. These results support the hypothesis that B cells play an important role in RA pathophysiology, and suggest that rituximab is effective and well tolerated, with a rapid onset of clinical benefit, in patients with refractory, seropositive active RA. (J Rheumatol 2005;32:2109–15)

Key Indexing Terms:

RHEUMATOID ARTHRITIS
TREATMENT EFFICACY

ADVERSE EFFECTS

RITUXIMAB
B CELLS

Rheumatoid arthritis (RA) is a chronic, systemic, idiopathic autoimmune disease of unclear etiology. It is characterized by symmetrical synovitis; painful, swollen, and inflamed synovial joints; and the erosion of articular cartilage and bone with resulting progressive joint destruction, deformity, and consequential functional disability¹. Untreated, the longterm prognosis for many patients with RA is poor. Even with treatment, 80% of patients with RA experience some

level of disability throughout the course of their disease², as well as a reduced life expectancy³.

Historically, treatment regimens for RA have been based on the use of nonsteroidal antiinflammatory drugs (NSAID), corticosteroids, and disease modifying antirheumatic drugs (DMARD) such as hydroxychloroquine, sulfasalazine (SSZ) and methotrexate (MTX). Introduction of biologic agents, notably the anti-tumor necrosis factor- α (TNF- α) agents that target the T cell mediated production of TNF- α , has benefited many patients who have had an inadequate response to DMARD, especially when used in combination with MTX. Despite this improvement, a substantial proportion of patients fail to benefit adequately, are unable to maintain a response¹, or develop intolerable toxicity. There is a need to explore less toxic, more efficacious alternative treatment options for this disease.

The emerging recognition that B cells contribute significantly to the pathophysiology of some autoimmune diseases^{4,5}, and to RA in particular^{6,7}, has been fueled by encouraging clinical results from B cell depletion therapy with the chimeric anti-CD20 monoclonal antibody, ritux-

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imab⁸⁻¹². We describe evidence from a Phase I/II, 28 week open-label trial in which a cohort of patients with active, advanced RA despite treatment with anti-TNF- α agents and/or DMARD were treated with rituximab to assess safety of this treatment.

MATERIALS AND METHODS

Subjects eligible for enrollment were adult (≥ 18 yrs) patients who had active, seropositive RA with at least 2 swollen and tender joints and functional class I, II, or III, as defined by the American College of Rheumatology (ACR) revised criteria, despite at least 3 months' treatment with at least one DMARD; all gave signed, informed consent. This study was approved by the Institutional Review Boards of the VA Northern California Health Care System and the University of California, Davis.

Patients were excluded if they had a history of hypersensitivity to murine proteins or a history of significant renal, hepatic, cardiac, or psychiatric impairment. Active infection (including seropositive hepatitis C) and a malignancy (except adequately treated basal cell or squamous cell carcinoma of the skin or *in situ* carcinoma of the cervix) within the previous 5 years were also exclusions. Female patients who were pregnant or were planning conception during the subsequent 2 years were also excluded.

Treatment. Patients continued with their baseline DMARD and other concomitant medications at a stable dose throughout the study period. Rituximab was administered as an intravenous (IV) infusion weekly for 4 consecutive weeks according to a dose escalation schedule that was prospectively agreed to, prior to the availability of Phase IIa data¹², by US federal regulatory authorities, as a condition of approval of the investigational new drug (IND) application for this study.

Prior to each infusion, patients received acetaminophen 650 mg orally (po), diphenhydramine 50 mg po or IV, and dexamethasone 10 mg IV to attenuate possible infusion related symptoms. During Week 1, the dose of rituximab was 100 mg, which was infused at a rate of 50 mg/h with incremental increases of 50 mg/h after 30 min as tolerated. During the second week, 375 mg/m² of rituximab was infused at an initial rate of 100 mg/h with incremental increases of 100 mg/h every 30 min as tolerated, to a maximum infusion rate of 400 mg/h if the first dose was well tolerated. During Weeks 3 and 4, a dose of 500 mg/m² rituximab was infused at the same incremental rates as tolerated during Week 2.

During infusions, patients were closely observed for infusion related symptoms such as transient fever, rigors, hypotension, and dyspnea, in which event the infusion could be slowed or temporarily stopped and restarted when symptoms resolved. However, there were no such symptoms necessitating divergence from the above schedule.

No further rituximab infusions were administered during the subsequent followup period, although patients continued their baseline therapy according to clinical need.

Clinical assessments. At baseline, a thorough history and physical examination was conducted and recorded, together with baseline demographic data. At baseline and at Weeks 5, 8, 16, and 28, the numbers of tender joints [tender joint count (TJC)] and swollen joints (SJC) were recorded for each patient. At these timepoints the global assessment score of disease activity was recorded for each patient by the physician and by the patient using a 100 mm horizontal visual analog scale (VAS) score, where the left-hand extreme was labeled "no disease activity" and the right-hand extreme was labeled "maximum disease activity." Similarly, at each assessment timepoint patients recorded their level of pain on a 100 mm VAS where the left-hand extreme was labeled "no pain" and the right-hand extreme "pain as bad as it could be." In addition, patient assessed disability was evaluated using the Multidimensional Health Assessment Questionnaire¹³.

Blood samples were obtained at baseline and at followup visits for assessment of laboratory measures including erythrocyte sedimentation rate (ESR), rheumatoid factor (RF), peripheral B and T cell counts, complement C3 and C4 levels, and immunoglobulin isotype (IgA, IgG, and

IgM) and human anti-chimeric antibody (HACA) titers. Samples were processed and analyzed using standard techniques.

Statistical considerations. The primary outcome measures for this pilot study were safety and efficacy, as determined by ACR score, joint scores, and global assessment scores. The proportion of patients achieving ACR20, ACR50, and ACR70 were determined at each assessment timepoint. Differences in clinical scores were determined using the Freidman test for matched nonparametric data. Multiple pairwise comparisons of different timepoints were not performed due to the small sample size.

RESULTS

Seventeen patients received rituximab infusions and were evaluable for safety. Three patients were lost to followup before post-baseline assessments could be made: one patient withdrew consent, another patient relocated, and the third was unable to attend followup visits. There were no adverse events in these subjects. Of the 14 patients that completed treatment, 13 patients completed 28 weeks of followup after the final rituximab infusion and were evaluable for efficacy. Demographic and baseline characteristics of these patients are shown in Table 1. All patients had received at least one previous DMARD (range 1–7 drugs); all had received MTX, and 46%, 38%, and 38%, respectively, had received prior hydroxychloroquine, SSZ, and/or gold salts. In addition, 6 (46%) patients had received at least one prior anti-TNF- α agent. All patients exhibited active symptoms that required further intervention for their RA.

At baseline, 23% of patients received rituximab as their sole treatment for RA and 23% received rituximab in combination with a stable dose of corticosteroid and/or a NSAID. The remainder (54%) received rituximab in combination with at least one DMARD with or without steroids and/or NSAID. In total, 6 of 13 patients assessable for efficacy (46%) continued a low, but stable dose (average 8 mg/day, range 5–10 mg/day) of steroids for the duration of the study. Of those patients receiving concomitant DMARD, 46% received MTX and 31% received hydroxychloroquine. One patient received concomitant SSZ and one patient concomitant infliximab.

Safety outcomes. Rituximab was well tolerated by all patients. There were no adverse events during the infusions of rituximab. Post-infusion, one patient developed National Cancer Institute (NCI) common toxicity criteria grade 2

Table 1. Demographic and baseline characteristics of the research subjects.

Age, mean (range), yrs	66 \pm 8 (53–82)
Sex, male, %	77
Duration of RA, mean (range), yrs	17 \pm 9 (1–31)
Previous DMARD, mean (range), n	2.5 \pm 1.3 (1–7)
Swollen joints, mean (range), n	19 \pm 7 (6–26)
Tender joints, mean (range), n	19 \pm 7 (6–27)
Erythrocyte sedimentation rate (mean), mm/h	50 \pm 32
Patients on concomitant steroids, n (%)	6 (46)
Average daily steroid dose, mg (range)	8 (5–10)

Plus-minus values are means \pm SD.

transient diarrhea one week after the first rituximab infusion, which spontaneously resolved within one day, and one patient reported a NCI grade 1 dry mouth and headache 7 days after the fourth infusion of rituximab. One patient developed bronchitis and another patient developed flu-like symptoms, both NCI grade 1, at 2 and 7 days, respectively, after the fourth infusion of rituximab. All events resolved without sequelae.

Efficacy outcomes. Thirteen patients were evaluable for efficacy after 28 weeks' followup. All patients exhibited clinical improvement as measured by the ACR criteria. By Week 5 (that is, one week after the last of 4 weekly infusions of rituximab), 55% of patients had achieved an ACR20 level of response, 36% an ACR50, and 18% an ACR70 response. By Weeks 8, 16, and 28, 75%, 50%, and 67% of patients, respectively, had achieved an ACR20 response. ACR50 and ACR70 responses were also achieved in a substantial proportion of patients throughout the time course of the study (Figure 1).

The ACR scores reflect significant improvements in the mean number of tender and swollen joints between baseline and endpoint in response to therapy ($p < 0.0001$ for each indicator; Figure 2). These improvements were clinically relevant as measured by the physician's global assessment, which also demonstrated that highly significant improvements were maintained over the entire study period ($p = 0.0001$; Figure 3). Although the patients' global assessment scores, which started from a low baseline, did not show the same level of change as that of the physicians' global assessments, there were significant reductions from baseline in patient assessed pain as measured by the VAS score ($p = 0.0005$), and in disability as measured by the Health Assessment Questionnaire Disability Index ($p = 0.0386$)

from Week 5, which were also maintained below the baseline scores throughout the study period (Figures 4a and 4b).

Laboratory measures of disease activity were concordant with clinical improvement. One week after completion of treatment with rituximab, decreases over baseline were evident for the ESR and the RF titers, and by Week 28 the mean ESR had diminished by 40% ($p = 0.0361$ overall; Figure 5A) and the mean RF titer had fallen by 73% ($p < 0.0001$ overall; Figure 5B) of baseline values, respectively.

Circulating B cells were profoundly depleted by Week 12 and this depletion was sustained for the remainder of the study period (Figure 6A; Table 2). In contrast, T cell levels and complement C3 and C4 levels were not significantly affected (data not shown).

Although circulating B cells were essentially depleted, the mean immunoglobulin isotype titers remained within normal ranges (Figures 6B, 6C, 6D). The greatest proportional fall was seen with IgM (Figure 6C), which corresponded with the decrease in RF titers (Figure 5B). There were no serious infections reported during the course of the study.

Human anti-chimeric antibody concentrations were assessed in 13 patients at baseline and at least 2 additional timepoints over a 9 month period after completion of therapy using an assay technique described by Maloney, *et al*¹⁴ and confirmed by immunodepletion upon addition of rituximab to the assay. Of the 13 patients assessed, 3 (23%) were positive for HACA with a titer of ≥ 5 ng/ml (range 15–297 ng/ml). Of 3 patients that developed positive HACA titers, one had a minimal response and the other 2 had sustained responses meeting criteria for ACR20 and ACR70. There was no significant additional toxicity in the patients that became HACA-positive.

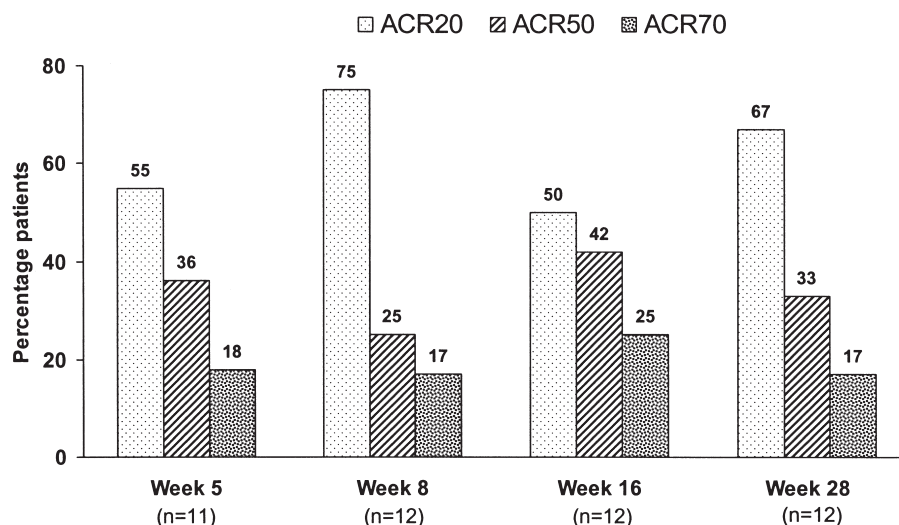


Figure 1. ACR responses in patients with RA treated with rituximab. Data for one patient at Weeks 5, 8, 16, and 28 and one patient at Week 5 were unavailable for calculation of ACR scores at these timepoints.

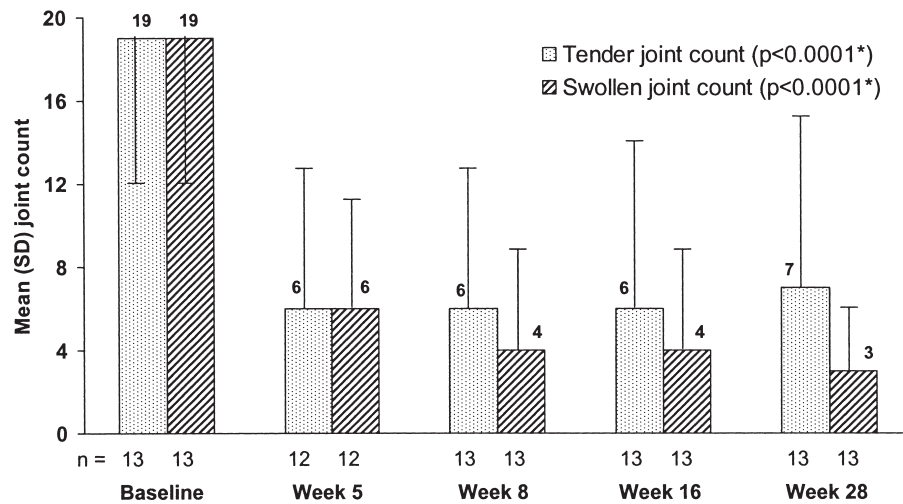


Figure 2. Improvements in tender and swollen joint count in patients with RA treated with rituximab. *Overall change over time (Friedman test, $n = 12$).

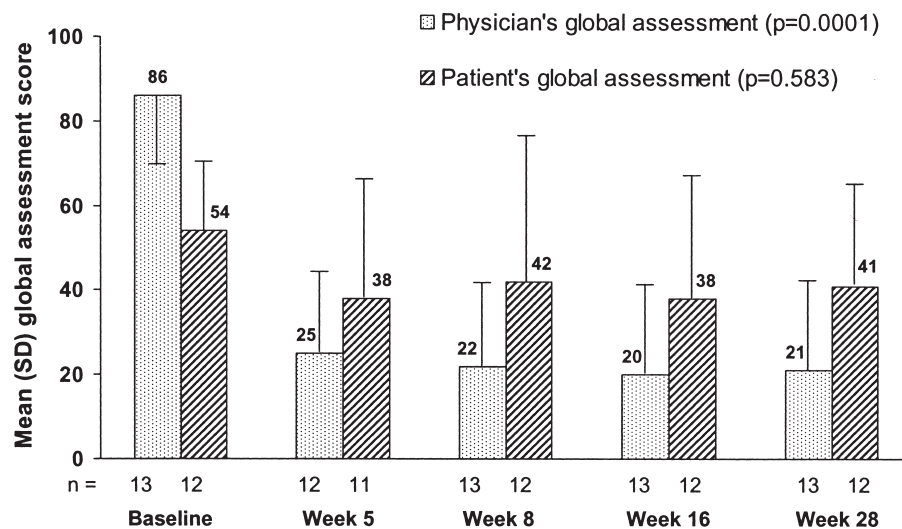


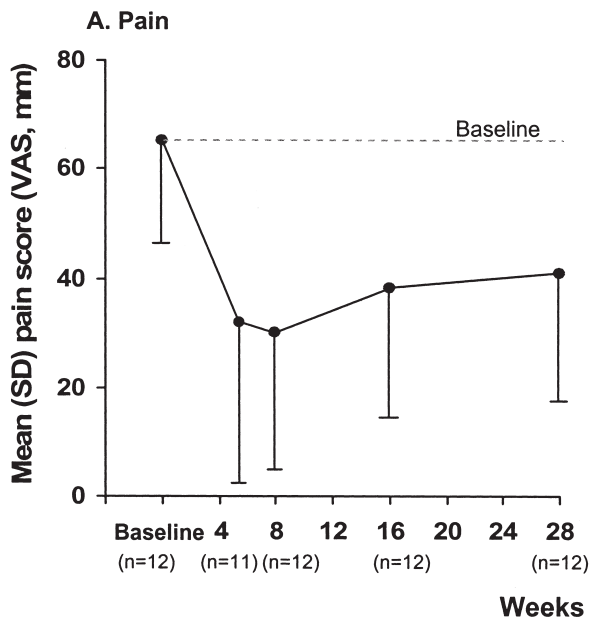
Figure 3. Changes in global assessment scores in patients with RA treated with rituximab. p: Overall change over time (Friedman test, $n = 12$ for physician global, $n = 11$ for patient global).

DISCUSSION

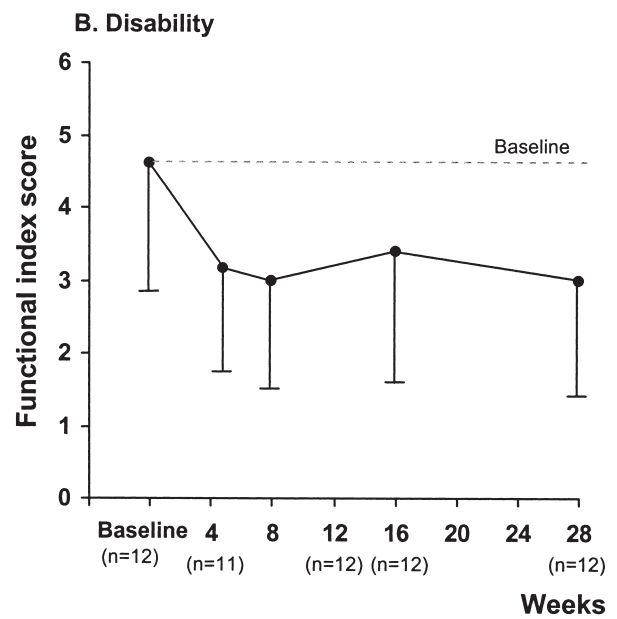
The results of this 28 week open-label study showed that rapid, substantial, and durable clinical benefit can be achieved with a short course of rituximab in patients with active RA who have had inadequate response to previous DMARD therapy. The clinical benefit of rituximab was expressed not only in terms of achievement of ACR20, ACR50, and ACR70 levels of response, but also in significant reductions from baseline in the number of tender and swollen joints, and improvements in the physicians' global assessments of disease activity and the patients' own assessments of pain and disability. These clinical improvements were concordant with improvements in laboratory indices of disease activity (ESR and RF), were apparent within 5 weeks of the start of treatment with rituximab, and were sustained at Week 28.

Based on the ability of rituximab to specifically deplete CD20-positive B cells, our results also provide further evidence that B cells play an important role in the pathophysiology of RA, which has been hypothesized to include the production of autoantibodies such as RF, antigen presentation, regulation of T cell activation, and production of proinflammatory cytokines⁴⁻⁷. The results confirm previous findings that B cell depletion correlates with improvement in clinical symptoms^{9,11,12}.

Rituximab is a chimeric murine/human monoclonal antibody that selectively binds CD20 on mature B cells. Rituximab has been used successfully in the treatment of non-Hodgkin's lymphoma (NHL)^{15,16} — from which an extensive safety database has accrued, with over 370,000 patient exposures since 1997¹⁷ — and in other autoimmune disorders such as systemic lupus erythematosus (SLE)^{18,19}.

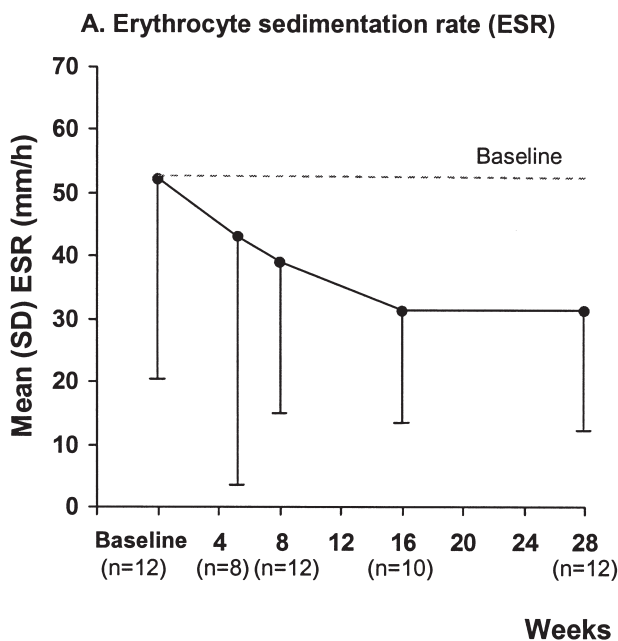


p=0.0005 overall change over time
(Friedman test, n=11)

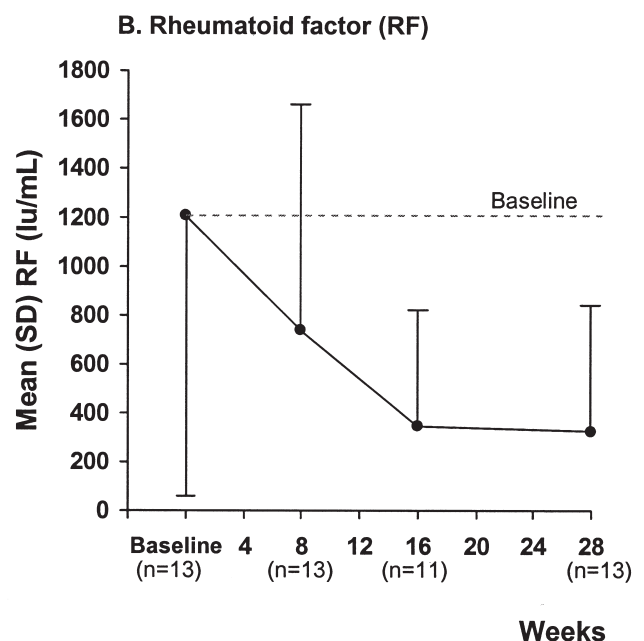


p=0.0386 overall change over time
(Friedman test, n=11)

Figure 4. Improvements in pain and disability scores in patients with RA treated with rituximab. A. Pain was self-assessed using VAS score. B. Disability was self-assessed using the Health Assessment Questionnaire disability index.



p=0.0361 overall change over time
(Friedman test, n=7)

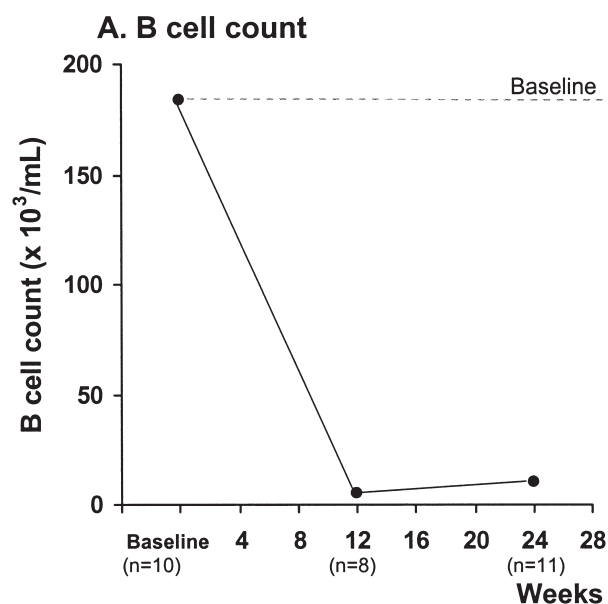


p<0.0001 overall change over time
(Friedman test, n=11)

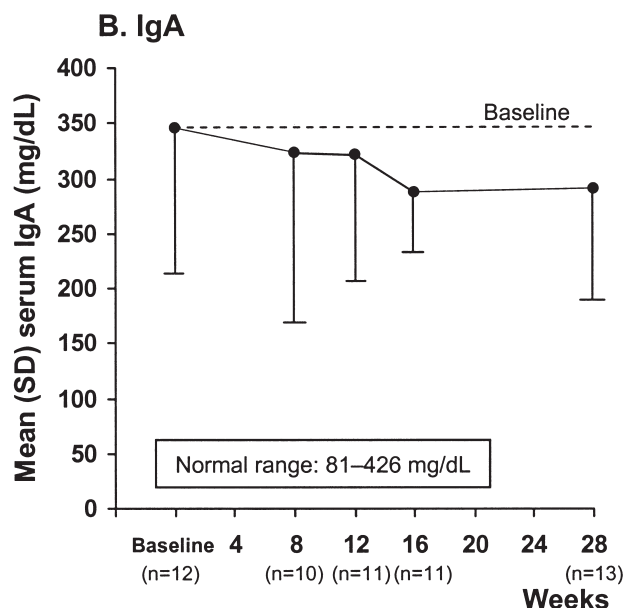
Figure 5. Effect of treatment with rituximab on erythrocyte sedimentation rate and rheumatoid factor titers.

Early encouraging results with rituximab in RA^{8,10} were based on a regimen derived from 3 components of the R-CHOP regimen (rituximab, cyclophosphamide, hydroxydoxorubicin, vincristine, and prednisolone), which has produced good results in NHL²⁰. Thus, these early pilot trials

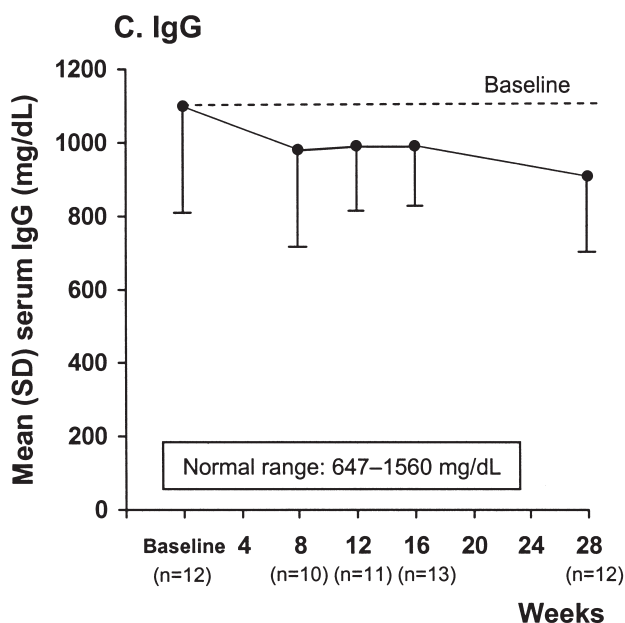
used a regimen of rituximab, cyclophosphamide, and a short course of high-dose steroids. The use of cyclophosphamide in the treatment regimen for RA has now been supplanted by MTX following the first randomized controlled trial, which showed that MTX was a more appropriate comedication for



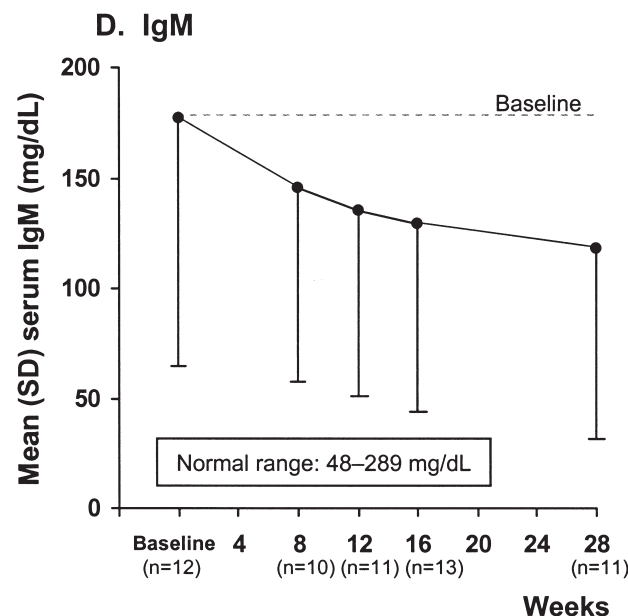
p=0.003 overall change over time
(Friedman test, n=7)



p=0.0439 overall change over time
(Friedman test, n=7)



p=0.0284 overall change over time
(Friedman test, n=9)



p=0.022 overall change over time
(Friedman test, n=7)

Figure 6. B cell depletion with minimal effect on immunoglobulin isotypes in patients with RA following treatment with rituximab.

RA in patients who had had an inadequate response to disease-modifying therapies¹². Both these regimens, however, used a high induction dose of corticosteroids, comprising a total dosage of 910 mg methylprednisolone administered both intravenously and orally over the first 14 days. The question remains about how much this high-dose induction regimen of corticosteroid is necessary to achieve the encouraging clinical profile of rituximab in RA described in the

randomized controlled trial. In addition, in that trial a transient but significant decrease in T cell levels was observed, potentially confounding the interpretation of the relationship between rituximab-mediated B cell reduction and clinical efficacy. There is little question that steroids in the doses given in the previous studies with rituximab are likely to contribute to early improvements in efficacy. Our open-label data and those of others^{9,11} suggest that early and sustained

Table 2. Lymphocyte counts over the 28-week treatment period in RA patients treated with rituximab.

	Baseline	Mean Levels, 10 ³ /ml			p*
		Week 8/12	Week 16	Week 24/28	
B cells	184	5	—	10	0.003
T cells	954	924	—	829	0.093

* Friedman test.

good clinical and laboratory responses and tolerability with rituximab are not contingent upon the use of high-dose steroids. In these cases, substantial clinical responses were noted with rituximab in RA patients with low-dose (≤ 10 mg/day) or no steroids. Further trials are warranted to explore the role of high-dose steroids in a rituximab-based regimen for RA.

While the development of HACA in this study was high (23%), and contrasts with that seen in larger studies in patients with RA (4.3%)¹² and NHL ($< 1\%$)²⁰, it may reflect the underlying nature of this disease or the difference in dose and schedule. No additional toxicity was observed in HACA-positive patients, and efficacy was consistent with the remainder of the study population; however, interpretation is difficult given the small sample size, and it will be assessed and verified in larger prospective randomized clinical trials.

All the patients in the present and previous studies treated with rituximab had had an inadequate or nondurable response to previous treatment with disease-modifying therapy and had the capacity to benefit from an alternative approach. All the trials, studies, and reports to date, including our series, on the use of rituximab in RA share several common features: that rituximab is well tolerated — in our study there were no serious infusion related reactions or later events — and that the clinical responses resulting from therapy are durable following profound and long-lasting depletion of B cells, without affecting circulating T cell levels. Further, B cell depletion with rituximab does not appear to increase the risk of opportunistic or latent infection in these patients. This observation may be due to the plasma cell-sparing effect of rituximab, such that immunoglobulin concentrations appear to be minimally affected, at least up to 6 months. No patient in our study has received further courses of rituximab, and the optimal dosing strategy with rituximab — upon the return of symptoms, at fixed intervals, or upon the return of B cells — requires further investigation.

The tolerability and safety of rituximab in our study is emphasized in that there is no indication of serious adverse events when rituximab was given as the sole disease-modifying drug (6 patients) or in combination with MTX, hydroxychloroquine, and/or SSZ (7 patients); one of our patients was also taking concomitant infliximab.

Our study supports the role for B cells in the pathogenesis of RA, and indicates that therapies that result in B cell depletion are associated with therapeutic efficacy.

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