B Cell Targeted Therapies in Autoimmune Diseases

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ABSTRACT. In addition to rheumatoid arthritis (RA), B cells are likely to play a significant role in the development of other autoimmune rheumatic diseases, such as systemic lupus erythematosus (SLE), myositis, and vasculitis. Small-vessel vasculitis subtypes may be immune complex-mediated (cryoglobulinemia) or antineutrophil cytoplasmic antibody (ANCA)-associated; ANCA may be involved in the pathogenesis of vasculitis. In SLE, both antibody-associated and antibody-independent processes are almost certainly involved. B cell activity and autoantibody production are increased, while patients often have reduced peripheral B cells and abnormal B cell profiles. B lymphocyte stimulator (BLyS) protein regulates B cell activation and differentiation. For these reasons, B cells and the molecules that activate them are potential therapeutic targets in these diseases. Recent clinical trial data from small studies of rituximab (RTX) in SLE suggest that treatment improved clinical variables and measures of disease activity in patients, including those with central nervous system SLE. With retreatment, patients whose B cells were successfully depleted continued to show improvement in clinical and laboratory variables. Preliminary data suggest that treatment with RTX may be effective in ANCA-associated vasculitis. In addition a recent study showed significant benefit with myositis. Although these studies contain small cohorts of patients, they demonstrate that B cell-modulating therapies show promise in treatment of a variety of autoimmune diseases. (J Rheumatol 2006;33 Suppl 77: 24-28)

Key Indexing Terms:
AUTOIMMUNE SYSTEMIC LUPUS ERYTHEMATOSUS
MYOSITIS
VASCULITIS
B LYMPHOCYTIC STIMULATOR

INTRODUCTION

The B cell appears to play an important role in the pathogenic process of several autoimmune diseases. In addition to antibody production, recent studies have revealed that B cells perform many diverse functions within the immune system that contribute to autoimmunity[1]. It is therefore appropriate to consider how B cell-mediated effects might be reduced or prevented by B cell-targeted therapies. Selective B cell depletion with anti-CD20 therapy is a promising, novel treatment option for patients with refractory autoimmune diseases. This article will describe the effects of B cell depletion, following treatment with monoclonal antibodies such as rituximab (RTX) in various autoimmune diseases.

RTX is a therapeutic monoclonal antibody against CD20, a surface antigen expressed by B cells. In 1997, RTX was first approved for treatment of patients with relapsed or refractory low-grade or follicular, CD20-positive, B cell non-Hodgkin's lymphoma. The role for RTX has since been extended to the treatment of more aggressive lymphomas when used as an adjuvant to more conventional chemotherapy. RTX has also been used as an adjuvant following bone marrow transplantation. Recent data suggest that RTX is effective in some difficult to treat diseases, including autoimmune hemolytic anemia, pure red cell aplasia, and cold agglutinin disease[2-4]. These are conditions traditionally refractory to conventional therapies. As a single agent, RTX produces response rates of 30% to 50% in patients with idiopathic thrombocytopenic purpura[5]. RTX is currently being evaluated in a variety of autoimmune diseases, most notably RA, but also SLE, dermatomyositis (DM), Wegener's granulomatosis (WG), and Sjögren's syndrome (SS). These studies will be reviewed in more detail below.

B cells may also be therapeutically targeted by other mechanisms. B lymphocytic stimulator (BLyS), also known as B cell activating factor, is essential for the development of B cells. Monoclonal antibodies against BLyS or soluble receptor immunoglobulin (Ig) antagonists are being explored for the treatment of autoimmune diseases including SLE.

B CELL TARGETED THERAPY FOR RHEUMATOID ARTHRITIS

RTX is a novel targeted therapy for the treatment of RA that appears to be highly effective, safe, and well tolerated. The first report of its intentional use in RA was in 5 patients[6]. It was then extended to open-label use in 22 patients[7]. These studies suggested that selective depletion of B cells using RTX could lead to sustained benefits for patients with active RA. Interestingly, along with a clinical benefit, there was a striking fall in IgM rheuma-

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This educational activity is sponsored by The University of Texas Southwestern Medical Center at Dallas, which is accredited by the Accreditation Council for Continuing Medical Education (ACCME) to provide continuing medical education for physicians. This continuing education activity is supported by an educational grant from Genentech, Inc. and Biogen Idec.

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toid factor (RF) and anti-cyclic citrullinated peptide (CCP) antibody levels even though total Ig and antibody responses to bacterial antigens were unchanged[8].

As described in detail in Dr. Cohen's article elsewhere in these proceedings[9], a Phase IIa 24-week, double-blind, randomized, controlled trial was carried out to confirm the initial observations of clinical benefit in RA[10]. In total, 161 patients with active RA were randomized to one of 4 treatment groups: RTX monotherapy; RTX plus methotrexate (RTX + MTX); RTX plus cyclophosphamide (RTX + CYC); or MTX alone. RTX was administered as two 1000-mg infusions on Days 1 and 15. All groups, including the control group, also received a 17-day course of treatment with corticosteroids. An analysis at 24 weeks showed that the proportion of patients achieving 20% response according to American College of Rheumatology criteria (ACR20) was significantly greater ($p \leq 0.025$ for all 3 comparisons) in all the RTX groups compared with the MTX control group (RTX alone, 65%; RTX + CYC, 76%; RTX + MTX, 73%; MTX alone, 38%). Both ACR50 (43% vs 13%; $p = 0.005$) and ACR70 responses were also significantly higher for the RTX + MTX group compared with the MTX group (23% vs 5%; $p = 0.048$). ACR50 response (24 weeks) was 33% with RTX alone, 43% with RTX + MTX, 41% with RTX and CYC. The RTX groups showed no significant safety differences compared with the MTX arm. The majority of adverse events were of mild to moderate intensity.

Levels of total serum Ig fell, but mean values remained within normal limits; IgG anti-tetanus and anti-pneumococcal capsular polysaccharide antibodies, measured by enzyme-linked immunosorbent assay, did not change significantly; however, IgA RF, IgG RF, and IgG anti-CCP decreased significantly more than the corresponding total serum Ig levels.

By combining 3 drugs that are capable of reducing B cells (steroids, CYC, and RTX or steroids, MTX, and RTX), maximal yet selective depletion of B cells could be achieved. These results suggest a benefit that is comparable to what can be achieved with anti-tumor necrosis factor therapy, but with the advantage of just 2 courses of treatment per year and lower cost.

SYSTEMIC LUPUS ERYTHEMATOSUS

Rituximab. The results of B cell depletion with RTX in patients with RA suggested to other investigators that similar autoimmune diseases such as SLE might also respond to this therapeutic approach. The first data showing that RTX was useful in patients with SLE were published in 2002[7,11]. In a Phase I/II trial of 12 patients (later expanded to 18), Anolik, *et al* treated patients with clinically active but non-organ-threatening SLE [Systemic Lupus Erythematosus Activity Measure (SLAM) score ≥ 6][11]. The doses of RTX were as follows: low dose = 1 infusion of 100 mg/m^2 (first 6 subjects), medium dose = 1 infusion of 375 mg/m^2 (next 6 subjects), and full dose = 4 weekly doses of 375 mg/m^2 (final 6 subjects). Significant B cell depletion in peripheral blood was observed, which correlated with clinical improvement. No changes in anti-dsDNA/C3 were claimed, although scrutiny of the data showed that levels of 4 of only 8 patients whose anti-dsDNA antibody levels were high did in fact fall. RTX was well tolerated. These investigators also evaluated the fate of discrete B cell subsets at baseline and during depletion with anti-CD20 monoclonal antibody therapy, as well as during the B cell recovery phase[12]. Compared with healthy controls, SLE patients (n = 22) displayed several abnormalities in peripheral B cell homeostasis at baseline, including naive lymphopenia; expansion of a CD27, IgD (double negative) population; and expansion of circulating plasmablasts. These abnormalities resolved after effective B cell depletion with RTX.

B cell depletion using RTX in combination with CYC and corticosteroids has also been reported to benefit patients with severe SLE[7]. In the initial report of an open-label study, each of 6 female patients with active SLE resistant to standard immunosuppressive therapy received two 500-mg infusions of RTX, and two 750-mg infusions of CYC, as well as high-dose oral corticosteroids during a 2-week period. One patient had not improved at 3 months and was then lost to followup. At 6 months, the 5 remaining patients showed clinical improvement. The urine protein creatinine ratios also fell, suggesting improvement in renal function. Therapy was well tolerated, with no significant adverse events reported.

In a followup, longterm, open-label study, 24 patients with SLE refractory to standard immunosuppressive therapy (mean of 3 agents) were started on B cell depletion therapy[13]. Patients were treated with RTX + CYC + oral steroids. Their mean age was 31 years (range 17-40), and they had a mean disease duration of 9 years (range 4-12). Disease activity in these patients was assessed every 1 to 2 months using the British Isles Lupus Assessment Group (BILAG) system and estimates of anti-dsDNA antibodies and serum C3 levels. The global BILAG score ($p < 0.00001$), serum C3 ($p < 0.0005$), and dsDNA binding ($p < 0.002$) all significantly improved from the time of B cell depletion to 6 months after treatment (Figure 1). Analysis of the regular BILAG assessments showed that improvements occurred in each of the 8 organ systems. In addition, the mean dose of steroids was reduced.

The results of a similar study focusing on the clinical outcome and safety profile of repeated B cell depletion in 7 patients with refractory SLE have recently been reported[14]. Retreatment with RTX (18 cycles in total, up to 3

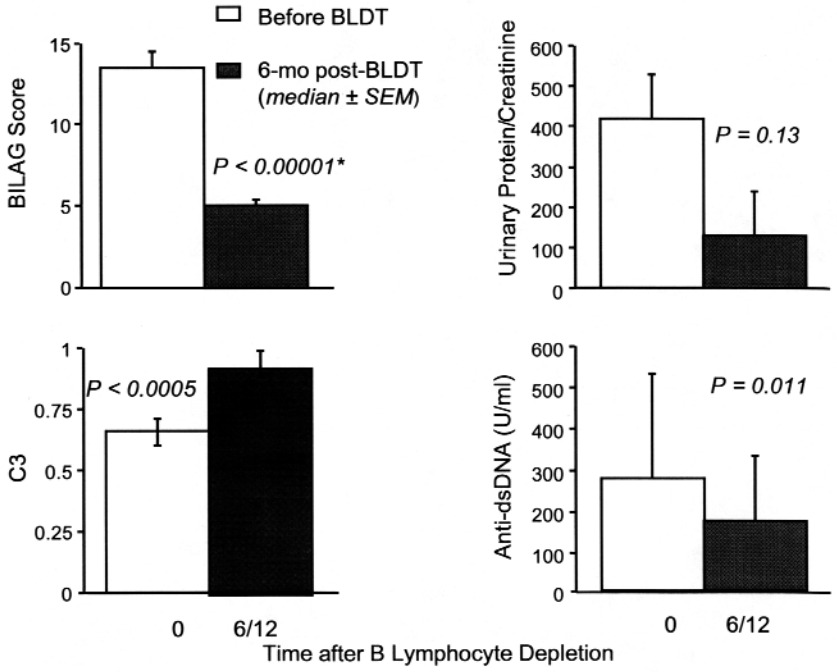

Figure 1. Clinical and laboratory variables in patients with refractory SLE treated with rituximab. BLDT: B-lymphocyte depletion therapy; BILAG: British Isles Lupus Assessment Group. *Paired t test. From Leandro, *et al. Rheumatology Oxford* 2005;44:1542-5, with permission.

cycles per patient) in patients with severe SLE was safe and appeared to be effective for 6 to 12 months on average[15] (Figure 2).

Belimumab. As discussed elsewhere in these proceedings, RTX is a method of B cell modulation that functions by depleting B cells. Another method that has been examined in clinical trials is the blocking of B cell activation. Belimumab is a human monoclonal antibody that selectively recognizes and inhibits the biological activity of BLyS, which is required for the maturation of B cells. B cell maturation antigen induces antigen presentation in B cells[16]. B cell activating factor has been shown to be elevated in patients with RA, SLE, and SS[17].

Belimumab is a potential treatment for SLE, RA, and other autoimmune diseases. A Phase I, randomized, placebo-controlled, double-blind study in patients with moderate-mild SLE (n = 70) who were followed for 84 to 105 days has been conducted[18]. The goals of the study were to evaluate safety, tolerability, immunogenicity, and pharmacology of 4 different doses of belimumab. Patients were randomized to 1, 4, 10, or 20 mg/kg (n = 57) versus placebo (n = 13). Single intravenous (IV) dose or 2 infusions 21 days apart were administered. There was one infusion reaction (high-dose) and one case of a patient who developed neutralizing antibodies. The reported half-life was 13 to 17 days. A 12% to 47% reduction in CD20+ cells in all treatment arms was observed. This was followed by one of the largest lupus studies conducted to date, a Phase II multicenter, double-blind, placebo-controlled, dose-ranging study in a large cohort of patients with SLE (n ~ 350). Unfortunately, the study did not meet its primary endpoint[19].

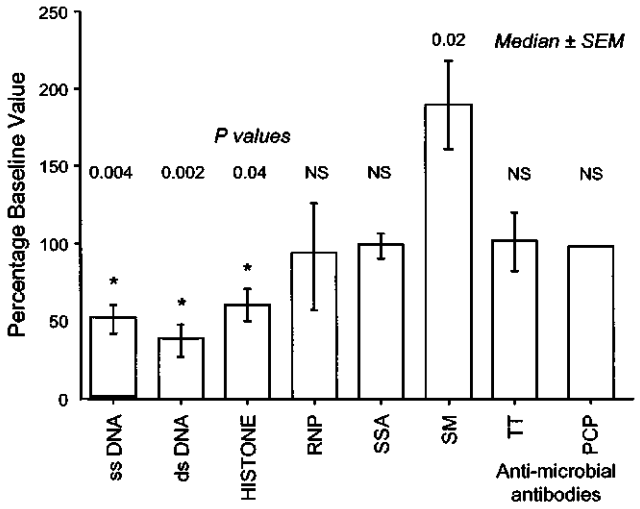

Figure 2. Antibody levels 6 months after B lymphocyte depletion (BLDP). RNP: ribonuclear protein; TT: tetanus toxoid; PCP: pneumococcal vaccine. *Significant.

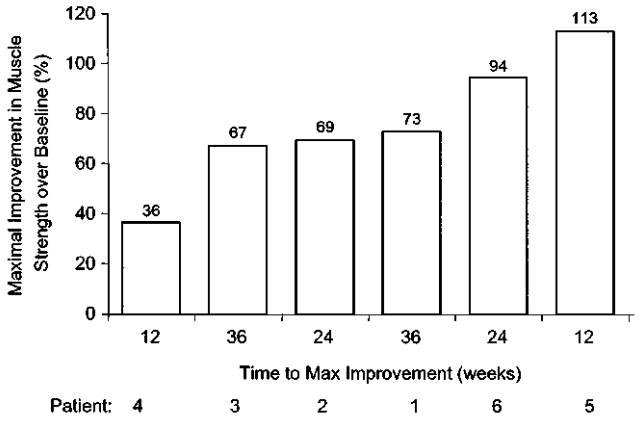

Figure 3. Open-label study of rituximab in patients with dermatomyositis. From Levine. *Arthritis Rheum* 2005;52:601-7, with permission.

DERMATOMYOSITIS

In addition to SLE, the use of B cell depletion as a form of treatment has now extended to other autoimmune and rheumatic diseases, one of which is DM, a chronic and debilitating inflammatory disorder of skin and muscle affecting both children and adults. The pathogenesis of the disease remains poorly understood, and treatment options are limited.

Levine and colleagues evaluated the safety and efficacy of RTX in an open-label, uncontrolled pilot trial of 7 adult patients with DM who had failed at least one standard therapy[20]. Patients received 4 infusions of IV RTX given weekly (the first 3 patients received 100 mg/m^2; the last 4 received 375 mg/m^2). Efficacy was assessed by quantitative muscle dynamometry, creatine phosphokinase (CPK), and lung function tests. Six patients were available for followup. All 6 evaluable patients achieved CD20+ B cell depletion. Improvements in muscle strength were observed in all evaluable patients (Figure 3). Other markers of disease activity in DM showed improvement, including a decrease in CPK (baseline range 128_5600 U/l to 57–1168 U/l) and an improvement in forced vital capacity (maximum improvement range 33%–44%). Other symptoms of DM, including rash and alopecia, improved in patients so affected. RTX was well tolerated, and no serious treatment-related adverse events were reported.

WEGENER'S GRANULOMATOSIS

Standard therapy for WG, which includes high-dose steroids, is not always effective and may be characterized by substantial toxicity. B lymphocytes have been implicated in the pathogenesis of WG, and B lymphocyte depletion has recently emerged as a promising approach to the treatment of WG[21]. In an open-label, uncontrolled trial of 11 patients with ANCA-associated vasculitis (10 with severe WG, one with microscopic polyangiitis), patients were treated with RTX 375 mg/m^2 weekly (x 4) + oral steroids ± IV steroids (3 patients with renal disease also had plasma exchange). Efficacy was assessed by Birmingham Vasculitis Activity Score, cytoplasmic-specific ANCA levels, and renal function testing. All 11 achieved CD20+ B cell depletion, and remission was achieved in all patients (Table 1). Improvement in renal function also was observed. RTX was well tolerated in this population.

Table 1. Open-label study of rituximab in patients with Wegener's granulomatosis. Values are mean ± SD unless otherwise indicated.

	Baseline	6 Months
BVAS, range	3 ± 11	0 in 10 patients
ESR, mm/h	49 ± 48	19 ± 22
cANCA, units	4.1 ± 3	0 in 8 patients
Creatinine clearance, ml/min	34 ± 25	46 ± 33

BVAS: Birmingham Vasculitis Activity Score; ESR: erythrocyte sedimentation rate; cANCA: cytoplasmic-specific antineutrophil cytoplasmic antibody.

SJÖGREN'S SYNDROME

SS is a difficult disease to treat. It is a chronic autoimmune rheumatic disorder of the exocrine glands with associated lymphocytic infiltrates of the affected glands, most notably the salivary and lacrimal glands. Studies of B cell activating factor indicate that diminished apoptosis and disturbed B cell maturation may be responsible for the occurrence of autoreactive B cells and B cell hyperreactivity. A Phase II open-label study of RTX treatment in patients with active primary SS of short duration (early primary SS) and patients with primary SS and mucosa-associated lymphoid tissue (MALT)-type lymphoma (MALT/primary SS) has recently been reported[22]. Patients (8 patients with primary SS, 7 with MALT/SS) were treated with 4 infusions of RTX (375 mg/m^2) given weekly after pretreatment with prednisone and antihistamine. Significant improvement in subjective symptoms and salivary gland function was noted; however, 4 of the 15 patients developed human anti-chimeric antibodies (HACA) that resulted in a serum sickness-like disease in 3. The development of HACA reported here is higher than has been described in other autoimmune disorders and requires further investigation of the possibility to overcome this with the use of steroids.

CONCLUSION

While these data must be interpreted with caution, they suggest that B cell depletion with RTX is a promising treatment approach for a range of autoimmune disorders. More randomized clinical trials are needed to confirm open-label data, but B cell depletion looks promis-

ing for RA, SLE, WG, and possibly myositis and SS. Current data obtained from studies of RTX-based therapy for autoimmune diseases show good tolerability and sustained improvement in disease symptoms. Future research is likely to be focused on the optimization of responses with RTX-based therapy. Early observations suggest that this approach is likely to yield significant clinical benefits in a wide range of organ-specific and systemic autoimmune diseases. In addition, studies with RTX have shown that B cell modulation is a valid target for autoimmune diseases. This opens the door to exploring other mechanisms of B cell modulation for therapy.

REFERENCES

1. Zouali M, Sarmay G. B lymphocyte signaling pathways in systemic autoimmunity: implications for pathogenesis and treatment. Arthritis Rheum 2004;50:2730-41.
2. Quartier P, Brethon B, Philippet P, Landman-Parker J, Le Deist F, Fischer A. Treatment of childhood autoimmune haemolytic anaemia with rituximab. Lancet 2001;358:1511-3.
3. Zecca M, De Stefano P, Nobili B, Locatelli F. Anti-CD20 monoclonal antibody for the treatment of severe, immune-mediated, pure red cell aplasia and hemolytic anemia. Blood 2001;97:3995-7.
4. Berentsen S, Tjonnfjord GE, Brudevold R, et al. Favourable response to therapy with the anti-CD20 monoclonal antibody rituximab in primary chronic cold agglutinin disease. Br J Haematol 2001;115:79-83.
5. Stasi R, Pagano A, Stipa E, Amadori S. Rituximab chimeric anti-CD20 monoclonal antibody treatment for adults with chronic idiopathic thrombocytopenic purpura. Blood 2001;98:952-7.
6. Edwards JC, Cambridge G. Sustained improvement in rheumatoid arthritis following a protocol designed to deplete B lymphocytes. Rheumatology Oxford 2001;40:205-11.
7. Leandro MJ, Edwards JC, Cambridge G, Ehrenstein MR, Isenberg DA. An open study of B lymphocyte depletion in systemic lupus erythematosus. Arthritis Rheum 2002;46:2673-77.
8. Cambridge G, Leandro MJ, Edwards JC, et al. Serologic changes following B lymphocyte depletion therapy for rheumatoid arthritis. Arthritis Rheum 2003;48:2146-54.
9. Cohen SB. Updates from B cell trials: efficacy. J Rheumatol 2006;33 Suppl 77:12-7.
10. Edwards JC, Szczepanski L, Szechinski J, et al. Efficacy of B-cell-targeted therapy with rituximab in patients with rheumatoid arthritis. N Engl J Med 2004;350:2572-81.
11. Anolik J, Campbell D, Felgar R, Rosenblatt J, Young F, Looney RJ. B lymphocyte depletion in the treatment of systemic lupus (SLE): phase I/II trial of rituximab (Rituxan®) in SLE. [abstract]. Arthritis Rheum 2002;46 Suppl:S46.
12. Anolik JH, Barnard J, Cappione A, et al. Rituximab improves peripheral B cell abnormalities in human systemic lupus erythematosus. Arthritis Rheum 2004;50:3580-90.
13. Leandro MJ, Cambridge G, Edwards JC, Ehrenstein MR, Isenberg DA. B-cell depletion in the treatment of patients with systemic lupus erythematosus: a longitudinal analysis of 24 patients. Rheumatology Oxford 2005;44:1542-5.
14. Ng KP, Leandro MJ, Edwards JC, Ehrenstein M, Cambridge G, Isenberg DA. Repeated B cell depletion in treatment of refractory systemic lupus erythematosus. Ann Rheum Dis 2005; Epub ahead of print.
15. Cambridge G, Leandro MJ, Isenberg DA, Teodorescu M, Ehrenstein MR, Edwards JCW. B cell depletion therapy in systemic lupus erythematosus: effect on autoantibody and anti-microbial antibody profiles [abstract]. Arthritis Rheum 2004;50 Suppl:S227.
16. Yang M, Hase H, Legarda-Addison D, Varughese L, Seed B, Ting AT. B cell maturation antigen, the receptor for a proliferation-inducing ligand and B cell-activating factor of the TNF family, induces antigen presentation in B cells. J Immunol 2005;175:2814-24.
17. Pers JO, Daridon C, Devauchelle V, et al. BAFF overexpression is associated with autoantibody production in autoimmune diseases. Ann NY Acad Sci 2005;1050:34-9.
18. Furie R, Stohl W, Ginzler E, et al. Safety, pharmacokinetic and pharmacodynamic results of a phase 1 single and double dose-escalation study of LymphoStat-B (human monoclonal antibody to BLyS) in SLE patients [abstract]. Arthritis Rheum 2003;48 Suppl:S377.
19. Human Genome Sciences. LymphoStat-B (human monoclonal antibody to B lymphocyte stimulator) [press release]. Rockville, MD: Human Genome Sciences; 2005. Available at: www.hgsi.com/products/LSB.html. Accessed February 14, 2006.
20. Levine TD. Rituximab in the treatment of dermatomyositis: an open-label pilot study. Arthritis Rheum 2005;52:601-7.
21. Keogh KA, Ytterberg SR, Fervenza FC, Carlson KA, Schroeder DR, Specks U. Rituximab for refractory Wegener's granulomatosis: Report of a prospective, open-label pilot trial. Am J Respir Crit Care Med 2006;173:180-7. Epub 2005 Oct 13.
22. Pijpe J, van Imhoff GW, Spijkervet FK, et al. Rituximab treatment in patients with primary Sjogren's syndrome: an open-label phase II study. Arthritis Rheum 2005;52:2740-50.