Review

B Cell-Targeted Therapy in Autoimmune Disease: Rationale, Mechanisms, and Clinical Application

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ABSTRACT. B cells play a critical role in the pathogenesis of rheumatoid arthritis (RA) and other autoimmune diseases. Recently, a number of biologic agents that target B cells have been tested as therapies for these conditions. These agents either deplete B cells, by targeting cell-surface antigens such as CD20, or block B cell function, for example by inhibiting the activity of B cell survival factors such as BLyS. Of this group of agents, the first in clinical use has been rituximab, a chimeric monoclonal antibody that depletes B cells by binding to the CD20 cell-surface antigen. Initially introduced as a treatment for non-Hodgkin’s lymphoma, rituximab is now approved for the treatment of RA. In this review we explore the rationale behind B cell-targeted therapy, highlight the results of clinical trials with rituximab in RA and other autoimmune diseases, and describe other emerging therapies directed at B cells. (J Rheumatol 2008;35:1245–55)

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Rheumatoid arthritis (RA) is a systemic autoimmune disease affecting about 1% of the US population1, and it is seen with similar frequency worldwide. Even though the etiology of RA is not fully understood, there is strong evidence for a significant role for B cells, as well as T cells, macrophages, dendritic cells, and numerous proinflammatory cytokines. We examined the B cell and its role in the pathogenesis of RA and other autoimmune conditions, with particular attention to the development of agents that modulate or ablate B cells, and their effectiveness and safety in treating these conditions.

Pathogenesis of RA

The pathogenesis of RA is highly complex and involves an ongoing interaction of numerous types of cells and cell mediators (Figure 1)2. It is believed that antigenic triggers, set off by genetic, environmental, and hormonal factors, start a self-perpetuating cascade of autoimmune inflammatory processes in the synovial and other compartments3-5. Cells invading the synovium organize themselves into sophisticated microstructures. In some patients, T and B cells form aggregates, which eventually develop into germinal centers6. Antigen-activated CD4+ T cells activate monocytes, macrophages, and synovial fibroblasts to secrete the proinflammatory cytokines interleukin 1 (IL-1), IL-6, and tumor necrosis factor-α (TNF-α)7. Effects of these cytokines include osteoclast activation, induction of cell-surface adhesion molecules, and mediation of matrix metalloproteinase (MMP) secretion from fibroblasts7. Activated T cells stimulate B cells to differentiate into plasma cells, which produce autoantibodies including rheumatoid factor (RF) and anti-cyclic citrullinated peptide antibodies (anti-CCP). A possible pathogenic role of RF involves the activation of complement through the formation of immune complexes2. Both RF and anti-CCP have been detected in patients some time before the onset of clinical RA, but anti-CCP has been detected earlier8,9. A study of serial measurements in blood donors found that the median time from the first immunoglobulin M (IgM)-RF or anti-CCP positivity to onset of RA symptoms was 2.0 years and 4.8 years, respectively9. Individuals who are homozygous for shared epitope susceptibility genes, particularly those who are also exposed to environmental risk factors, have a markedly and selectively increased risk of anti-CCP-positive RA10. Hence, it is likely that anti-CCP-positive RA and anti-CCP-negative RA are etiologically distinct disease entities10. A recent comprehensive genetic analysis of RA showed that a genetic variant at the TRAF1–C5 locus on chromosome 9 is associated with an increased risk of anti-CCP-positive RA11. Several studies have shown that the presence of anti-CCP is a predictive marker for erosive progression of RA12-14. Anti-CCP-positive patients develop significantly more severe radiologic damage than do anti-CCP-negative patients14. Anti-CCP may also have a role in the pathogenesis of RA: citrullination of intraarticular proteins in response to inflammation might be the initial event leading to autoantibody production in RA15.

Activated macrophages, B and T cells, and fibroblasts, together with their expressed cytokines, stimulate angiogenesis, explaining the increased synovial vascularity seen in patients with RA. In addition, synovialvascular endothelial
cells become activated and express adhesion molecules that promote the influx of inflammatory cells into the joint\(^2\). Overall, a large number of different cell types contribute to the formation of an inflamed and hyperplastic synovium, with consequent progressive joint damage.

The extensive involvement of B cells in the pathogenesis of RA makes them an attractive therapeutic target. In addition to being the source of RF and anti-CCP antibodies\(^1\), B cells are up to 1000 times more efficient in their role as antigen-presenting cells (APC) than other cells\(^1\). Murine experiments showed that B cells produced autoantibodies and that this activity was critical to the pathogenic process\(^2\). Specifically, B cell-activated T cells in isolation produced a mild form of arthritis; however, when this event was coupled with B cell autoantibody generation, severe arthritis developed. These results suggest the existence of a synergistic relationship between B and T cells that leads to progressive and destructive arthritis. The central role of B cells as autoantibody producers and efficient APC has been further emphasized in studies using another murine model (human RA synovium–SCID mouse chimeras); in these experiments, T cell activation in rheumatoid synovitis was shown to be dependent on B cells, while alternative APC such as dendritic cells or macrophages were not able to sustain T cell activation\(^1\).

Growing evidence suggests that activated B cells can also influence immune responses by producing cytokines\(^1\). For example, experiments have shown that cytokine-producing B cells can influence the initiation of immune responses at ectopic sites\(^2\), and regulate the T cell response generated in secondary lymphoid tissues\(^2\).

The multiple pathogenic roles of autoimmune B cells in RA are further strengthened by their ability to self-perpetuate\(^4\). Antibodies recognizing a foreign antigen should provide survival signals to the B cell, whereas antibodies recognizing self should provide death signals. Therefore, a B cell carrying a useless or dangerous antibody should normally die. However, in autoimmunity, the antibody — either as a B cell receptor (BCR) and/or in soluble form — interacts with antigen(s) in ways that subvert normal antibody-dependent survival signals. One example is IgG RF, which is able to self-associate to form complement-fixing multimeric immune complexes, which can provide RF-specific B cells with a positive survival signal\(^4\).

Targets in the B cell lineage: CD20

Improved understanding of the pathogenesis of autoimmune diseases has allowed identification of cellular and molecular markers that could be targeted in order to affect the activity of...
B cells. Hybridoma antibody technology has played a major role in developing such agents. Early studies identified CD20 as a specific marker for B cells. CD20 is a 297-amino acid, 33 to 35-kDa transmembrane phosphoprotein expressed on mature and precursor B cells. The function of CD20 is not well understood, partly because the natural ligand of CD20 is unknown and because CD20 knockout mice have no obvious B cell deficits. However, CD20 has been proposed to function as a store-operated calcium channel, which is activated by receptor-stimulated calcium depletion of intracellular stores. CD20 is expressed throughout the maturation process of B cells, but not on stem cells or fully mature plasma cells. Therefore, the protective immunologic memory derived from plasma cells should be preserved following depletion of CD20-positive B cells. Other advantages of targeting CD20 include the lack of CD20 internalization after antibody binding, the stable expression of CD20, and the fact that CD20 is rarely shed from the cell surface, thereby encouraging sustained binding to therapeutic agents.

B-cell-targeted therapies in RA: Clinical data
The majority of our information about the effects of B cell-targeted therapy in RA derives from trials with rituximab, which has been available for the treatment of lymphoma since 1997. Results of trials with other agents that target the same B cell surface marker, ofatumumab, ocrelizumab, and TRU-015 are now emerging, as are data from agents that target B cells via different mechanisms.

Rituximab. Rituximab, a genetically engineered chimeric monoclonal antibody, selectively targets B cells bearing the CD20 surface marker. This selective binding induces cell death, although the mechanisms involved are not fully understood. Experimental evidence supports the involvement of at least 3 mechanisms:

1. Complement-dependent cytotoxicity (CDC), which involves the complement system protein C1q, formation of circular pores in the cell membrane resulting in compromised membrane integrity, and ultimately cell lysis.
2. Antibody-dependent cellular cytotoxicity (ADCC), which leads to membrane damage and cell lysis via the recruitment of macrophages, natural killer cells, and cytotoxic T cells.
3. Apoptosis, induced directly through the binding of rituximab to CD20.

Data obtained using lymphoma cells suggest that the major mechanisms of rituximab-induced B cell death involve complement-dependent cytotoxicity and antibody-dependent cellular cytotoxicity.

Rituximab was originally approved in 1997 for the treatment of relapsed or refractory low-grade or follicular CD20+ B cell non-Hodgkin’s lymphoma. Approval for use in RA in the US followed in 2006 after demonstration of efficacy in clinical trials. Early case reports and studies in small numbers of patients were published between 1999 and 2002. An open-label study involving 5 patients with refractory RA treated with rituximab, cyclophosphamide, and prednisolone provided the first demonstration that rituximab was efficacious and well tolerated in RA. In a subsequent open-label study, also involving 5 patients with refractory RA, De Vita and coworkers reported that rituximab was effective in the absence of high-dose corticosteroids, thus providing strong evidence for a role of B cells in the etiology of RA.

Rituximab efficacy. The first randomized, double-blind, controlled, Phase II study of rituximab in RA enrolled 161 patients who had active RA despite treatment with methotrexate (MTX); these patients were thus not expected to respond to MTX monotherapy, which was one of the 4 treatment arms. The other 3 treatment arms were rituximab plus MTX, rituximab in combination with cyclophosphamide, and rituximab monotherapy. The rituximab dose in these 3 arms was 1000 mg on Days 1 and 15. All 4 treatment arms also received a course of corticosteroids. All rituximab treatment regimens achieved superior efficacy over MTX alone. At Week 24 the proportions of patients meeting the primary endpoint (American College of Rheumatology 50% response; ACR50) were 43% for rituximab-MTX, 41% for rituximab-cyclophosphamide, 33% for rituximab alone, and 13% with MTX alone. Similar trends were observed for ACR20 and ACR70, and significant responses were maintained through Week 48. Rituximab continued to have a beneficial effect for at least 2 years. Higher proportions of patients receiving rituximab plus MTX completed 2 years of followup without the need for further treatment (45%), compared to those receiving placebo plus MTX (15%), rituximab alone (10%), or rituximab plus cyclophosphamide (22%). Patients were eligible for repeat treatment if they had had ≥ 20% improvement in swollen joint count and tender joint count and had residual disease activity defined as a swollen joint count and a tender joint count ≥ 8; the need for repeat treatment was determined by the treating physician. The improvements in physical function [Health Assessment Questionnaire Disability Index (HAQ-DI)] reported in this study reflected the benefit of active therapy.

The randomized, double-blind, Phase Ib Dose-ranging Assessment iNternational Clinical Evaluation of Rituximab in RA (DANCER) study evaluated patients with moderate or severe RA despite ongoing MTX treatment who had previously had an inadequate response to at least one but not more than 5 disease modifying antirheumatic drugs (DMARD) (other than MTX) and/or biologic agents (infliximab, adalimumab, etanercept, or anakinra). In this study, both standard (1000 mg × 2) and reduced (500 mg × 2) doses of rituximab in combination with MTX yielded significant ACR20, ACR50, and European League Against Rheumatism (EULAR) “good/moderate” responses compared with placebo plus MTX (Figure 2). ACR70 and EULAR “good” responses were more frequent in patients receiving the standard dose (1000 mg × 2). Efficacy was not affected by the concomitant use of high-dose corticosteroids between infusions. Both doses of rituximab were reported to be well tolerated, with a

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low incidence of serious adverse events. Results from an open-label extension of this trial indicated that retreatment was effective following a full cycle of repeat rituximab therapy in previous responders who had relapsed during the followup period. Improvements in patient-reported outcomes, such as physical function, pain, and fatigue, have also been reported for the extension phase of the DANCER trial.

Randomized Evaluation of Long-term Efficacy of rituximab in RA (REFLEX) was a randomized, double-blind, Phase III study designed to determine the efficacy and safety of rituximab in patients with active RA who had an inadequate response to one or more anti-TNF agents (including etanercept, infliximab, or adalimumab). All patients also received folate, intravenous methylprednisolone, and oral prednisolone during the 2-week treatment period. In combination with MTX, rituximab therapy was shown to be superior to placebo plus MTX in terms of ACR (Figure 3) and EULAR scores. In subsequent analyses, all patient-reported outcomes such as fatigue, disability, and health-related quality of life showed significant and clinically relevant improvements in patients who had received rituximab.

Results of longterm followup of the original Phase II/III clinical trials have demonstrated that repeated treatment with rituximab leads to continued clinical improvement in patients with active RA. In a safety and efficacy analysis of the ongoing clinical program in which 1053 patients have been exposed to rituximab (total exposure to treatment of 2438 patient-years, with 120 patients having exposure exceeding 3 years), ACR responses were comparable or improved following Course 3 compared with Course 1 and Course 2 both in patients who had previously had an inadequate response to DMARD (DMARD-IR) and in patients who had previously had an inadequate response to TNF inhibitors (TNF-IR). The results also indicated that the most appropriate retreatment interval was 6–12 months, with median time periods between the treatment courses of 48.7 weeks (DMARD-IR) and 37.9 weeks (TNF-IR) for Course 1–Course 2, and 56.2 weeks (DMARD-IR) and 42.1 weeks (TNF-IR) for Course 2–Course 3. Overall tolerability reported in this study was very good, and there were no safety concerns beyond those seen in the original clinical trials. Analysis of physical function and quality of life data from this study also showed favorable longterm results for rituximab, with encouraging results recorded for both the HAQ-DI and the SF-36.

Rituximab safety. The results of clinical trials conducted to date have shown that rituximab is well tolerated in the majority of patients with RA. The overall safety profile for 161 patients in the 24-week double-blinded Phase II study was consistent with that reported previously for rituximab in patients with lymphoma. However, the incidence of infusion-related adverse events was reduced in RA compared with lymphoma (36% vs 78%, respectively). Most infusion reactions are mild to moderate and generally respond to stopping the infusion, treating the reaction, and proceeding at a slower rate of infusion. Examples of infusion reactions include hypotension, hypertension, fever and chills, rash, and throat discomfort. The overall incidence of infections was similar in both rituximab and control groups at Weeks 24 and 48. One rituximab-treated patient died of pneumonia. Followup safety data recorded over 2 years showed no differences in the occurrence of adverse events leading to withdrawal, serious adverse
events, or infections in the rituximab treatment groups compared to the placebo plus MTX group.

In the DANCER trial, adverse events were recorded in 81% and 85% of patients in the rituximab 500 mg × 2 (n = 124) and 1000 mg × 2 (n = 192) infusion groups, respectively, compared with 70% of patients in the placebo group (n = 149). The majority of adverse events (82%) were classified as mild to moderate. Severe adverse events (National Cancer Institute Common Toxicity Criteria grade 3) occurred in roughly 18% of patients in each study arm. The most frequently reported adverse events were associated with the first infusion. Acute infusion reactions were more commonly associated with rituximab therapy (32%–37%) than with placebo treatment (14%). Premedication using intravenous (IV) glucocorticoids (100 mg methylprednisolone) lowered both the incidence and the severity of these acute infusion reactions.

The REFLEX trial also reported encouraging safety results. The frequencies of adverse events were similar for both placebo (n = 209) and rituximab-treated (n = 311) patients (88% and 85%, respectively), and the majority of adverse events were classified as mild to moderate. Infusion-related adverse events were more common among rituximab patients (29%) than among placebo patients (23%). Acute infusion reactions during the first infusion were more common in the rituximab group than in the placebo group, and fewer reactions occurred during the second infusion (Figure 4). Although 2 rituximab patients (0.6%) in this trial experienced a serious adverse event during infusions, most infusion reactions were mild or moderate in severity.

Available longterm safety data for repeat treatment rituximab regimens corresponding to 2438 patient-years in patients with an inadequate response to one or more TNF inhibitors or DMARD show similar rates of infection and overall incidence of adverse events following additional rituximab courses as compared to the first course, other than declining infusion reaction rates. A recent open-label extension study of RA patients who had previously completed 16–24 weeks in a double-blind rituximab trial reported a decreasing incidence of infusion-associated adverse events during repeat courses of rituximab; the proportion of patients with adverse events within 24 hours of the first infusion decreased from 31% for Course 1 (n = 1039) to 14%–19% for Courses 2 to 4 (Course 2, n = 570; Course 3, n = 191; Course 4, n = 40). The repeat courses of rituximab were not associated with any additional safety events beyond those reported in the double-blind phase of the trials. Finally, a pooled analysis of safety data from this open-label extension trial (n = 1039) revealed no new safety indications beyond those originally reported in the original trials. In this pooled analysis, the rate of serious infections (5.03 events/100 patient-years; defined as a serious infectious adverse event or any infection requiring IV antibiotics) did not appear to increase with prolonged peripheral B cell depletion, and was consistent with that reported in the general RA patient population. However, a further analysis revealed that some patients developed at least one episode of lower immunoglobulin levels, and there is a trend toward higher rates of serious infection in this population, although the statistical analysis was unable to detect a difference. No cases of tuberculosis were reported, and there was no indication of an increased risk of malignancy with additional courses of treatment.

Application of rituximab therapy for RA in clinical practice. Rituximab, in combination with MTX, is indicated for adult patients with moderate to severe active RA who previously showed no response, lost response with time, or had adverse effects when using one or more TNF antagonist therapies. Although the 500-mg dose of rituximab was as efficacious as the 1000-mg dose in the Phase Ib DANCER trial, only the 1000-mg dose was evaluated in the Phase III REFLEX trial. Hence, the standard Food and Drug Administration-approved dose consists of two 1000-mg IV infusions separated by 2 weeks. In order to reduce the incidence of infusion reactions, glucocorticoids such as methylprednisolone (100 mg IV) may be administered 30 minutes before the start of the rituximab infusion. Additional premedication therapies that may be helpful include acetaminophen and diphenhydramine.

Administration of rituximab leads to almost complete depletion of peripheral B cells within 2 weeks of the first dose. (Because the presence of rituximab in the patient’s plasma can interfere with flow cytometric enumeration of CD20+ B cells, B cell depletion is measured from the count of CD19+ peripheral B cells, as CD19 and CD20 are coexpressed on B cells.) The majority of patients show peripheral B cell depletion for at least 6 months, with a small proportion (4%) of patients achieving prolonged peripheral B cell depletion lasting more than 3 years.

There are increasing data concerning the timing of retreatment courses using rituximab; experience to date suggests that...
the optimal (median) interval between courses is 6–12 months. A recent study investigated predictors of Disease Activity Score (DAS28) after a second course of rituximab. Two independent predictors of DAS28 at Week 12 after Course 2 were identified. By taking into account a patient’s DAS28 after Course 1, every point (1.0) deterioration in DAS28 before repeat treatment resulted in a 0.28 ± 0.09-point higher DAS28 after Course 2. Therefore, optimal timing for retreatment, in order to achieve the greatest response in the subsequent course, appears to be prior to worsening of DAS28 score/disease activity.

Evidence from patients with lymphoma and systemic lupus erythematosus (SLE) suggests that B cell depletion reduces the immune response to vaccination, therefore it is recommended that required vaccinations be administered prior to beginning treatment with rituximab.

Emerging anti-CD20 therapies in RA

Encouraging initial results have been reported from early clinical trials of other anti-CD20 agents.

In an ongoing double-blind, randomized, placebo-controlled Phase II dose-ranging trial of ofatumumab (HuMax-CD20; a fully human monoclonal IgG1 antibody) in patients with active RA, ACR20 responses were reached at Week 24 in 41%–50% of ofatumumab-treated patients compared with 9% in the placebo group. All ofatumumab dosage groups showed a rapid and sustained peripheral CD19+ B cell depletion.

In a recent combined Phase I/II study of 237 patients with moderate to severe RA, ocrelizumab, a human monoclonal antibody, was well tolerated and demonstrated clinical activity at all doses (10, 50, 200, 500, 1000 mg) at Week 24. The most frequent adverse events were infusion-associated Grade 1/2 headaches, nausea, chills, pyrexia, and dizziness. B cell depletion was observed with all doses, with earlier B cell repletion noted at lower doses.

TRU-015 is a CD20-directed SMIP™ drug candidate. Unlike the other anti-CD20 agents, TRU-015 is not a monoclonal antibody, but rather a single-chain polypeptide known as a small modular immunopharmaceutical. In a randomized, double-blind, placebo-controlled Phase II dose-ranging trial, the 800-mg and 1600-mg doses of TRU-015 achieved significant improvement in RA disease activity compared with placebo.

Other B cell-targeted therapies

A number of other B cell-specific agents are being developed that target other B cell surface markers (e.g., CD22) or receptors that modulate B cell function, such as BAFF (synonymous with BLyS) and APRIL (a proliferation-inducing ligand). Even though these agents are still in clinical trials, some have shown encouraging efficacy data in RA and other autoimmune conditions. Differences in response to these various agents are shedding further light on B cell pathophysiology in these conditions.

Belimumab (BmAb) is a fully human monoclonal antibody that inhibits the activity of BAFF. In a recent double-blind Phase II study, BmAb or placebo was intravenously administered to 283 subjects with active RA previously treated with DMARD and biologic therapies. BmAb was well tolerated, and ACR20 responses at 24 weeks showed modest effect, favoring BmAb over placebo (29% vs 16%). There were also significant reductions in B cell counts at Weeks 20–24 in all active treatment groups compared to placebo.

Atacicept (TACI-Ig; a soluble receptor fusion protein antagonist of the B cell maintenance and survival factors BLyS and APRIL) was shown to induce dose- and time-dependent decreases in mature B cells in peripheral blood and lymphoid tissues in preclinical studies. In a Phase Ib study in patients with active moderate to severe RA, atacicept was well tolerated and positive trends in ACR20 and DAS28 scores were observed. Predictable declines in immunoglobulin levels, related to targeting of plasma cells, as well as earlier lineage B cells, were noted. Larger Phase II trials are needed to provide an understanding of the efficacy of this new agent.

BR3-Fc, a recombinant human fusion protein that blocks BAFF, is being evaluated in clinical trials. The results from a safety and pharmacokinetic/pharmacodynamic study of BR3-Fc in RA was recently reported, in which BR3-Fc reduced B cell levels by a median of about 55%.

B cell-targeted therapies in other autoimmune diseases.

Systemic lupus erythematosus: Clinical data

Rituximab. Uncontrolled, open-label studies of 6–12 months’ duration involving small numbers of patients (n < 20) with SLE resistant to standard immunosuppressive therapy have generally shown rituximab to be efficacious and well tolerated. Significant clinical responses were documented using the British Isles Lupus Assessment Group (BILAG) clinical index and the SLE Disease Activity Index (SLEDAI). An
open-label, Phase I/II dose-escalation trial of rituximab added to ongoing therapy also reported good safety and efficacy\(^72\); following treatment with a single 100 mg/m\(^2\) infusion, or a single 375 mg/m\(^2\) infusion, or 4 weekly infusions of 375 mg/m\(^2\), 11/17 patients had profound B cell depletion; low doses were associated with nonresponse and the development of human antichimeric antibodies (HACA). Interestingly, lack of response was associated with low-affinity FC\(\gamma RIIa\) inheritance\(^72\). A recent open-label study introduced a different dosing regimen in 15 patients with active and refractory SLE\(^73\); rituximab was administered as 4 weekly infusions of 500 mg or 2 infusions of 1000 mg every other week. The treatment was well tolerated; B cells decreased rapidly by 14 days in all patients and remained depleted until 6 months post-treatment. Nine of the 13 patients who completed the study achieved a major or partial clinical response (BILAG score), while 3 patients developed HACA associated with the disappearance of serum rituximab. A longterm followup study of 38 SLE patients with B cell depletion following rituximab therapy showed that one-third of the patients remained well without requiring further standard immunosuppressive agents\(^74\). Most flares occurred 6–12 months post-treatment and the authors concluded that the autoantibody profile could be used to identify which patients would have a more sustained response. In another study, 16 female patients with severe refractory SLE were treated using a protocol consisting of 4 weekly infusions of rituximab 375 mg/m\(^2\) combined with 2 (0.5 mg/m\(^2\)) infusions of cyclophosphamide\(^75\); systemic followup was performed 1, 2, 4, and 6 months after treatment, and then every 2–3 months. The treatment was found to be very effective, with a complete response or remission occurring in nearly 50% of patients. Although the median time to achieve a SLEDAI-50 clinical response (a 50% improvement in the SLEDAI score) was 3 months, the median duration of B cell response was 7 months.

The results from the above studies have promoted an increased biologic understanding of the subtle differences that can be expected in the use of rituximab for the treatment of SLE compared to RA. For example, in the study by Leandro and coworkers\(^70\), patients initially responded well to rituximab, with consistently low B cell counts and a marked improvement in lupus activity. However, relapse was common, indicating the need for either maintenance immunosuppression or retreatment. The dose-escalation study performed by Looney and colleagues\(^72\) reported peripheral B cell depletion to < 5 cells/µl in 11/17 evaluable patients. General lupus activity symptoms in these patients improved significantly, and the beneficial effects persisted for more than 12 months. However, although the high-dose group showed a trend toward better B cell depletion, some patients in the lower-dose group also depleted well, while some in the higher-dose group did not. Therefore, factors other than dose seem to influence B cell depletion. Finally, in a study of B cell abnormalities in SLE, B cell depletion therapy with rituximab was shown to dramatically improve abnormalities in B cell homeostasis, including naïve lymphopenia, with a decreased proportion of autoreactive memory B cells after treatment\(^76\). These investigators suggested that, for maximal clinical efficacy and induction of longterm remissions with rituximab, full reestablishment of B cell tolerance with elimination of autoreactive memory and plasma cell populations will be necessary.

There is growing evidence to support the clinical efficacy of rituximab therapy in patients with lupus nephritis. In an open-label study, patients received 4 weekly infusions of rituximab 375 mg/m\(^2\) combined with oral prednisolone. Rituximab therapy was well tolerated and resulted in B cell depletion lasting 1–7 months\(^77\). Complete remission of nephritis was defined as normal serum creatinine and albumin levels, inactive urine sediment, and 24-hour urinary protein < 500 mg. Partial remission was defined as > 50% improvement in all renal parameters that were abnormal at baseline. Partial remission was observed in 8/10 patients (median 2 months); 5 of these patients subsequently achieved complete remission, which was sustained for 12 months in 4 patients. Interestingly, clinical remission was associated with a decrease in T-helper cell activation, supporting the idea that B cells have additional roles in promoting autoimmunity by directly influencing T cells. In another study, rituximab (4 × 375 mg/m\(^2\)) in combination with cyclophosphamide and high-dose corticosteroids was administered to 7 female patients with severe refractory lupus nephritis; histologic changes in renal biopsies taken before and after treatment were analyzed\(^78\). Nephritic signs and symptoms improved in all patients, with 3 attaining complete remission and 2 partial remission, while histologic grade and severity improved in most biopsy specimens.

B cell recovery in peripheral blood and lymphoid tissue was evaluated during a longterm followup of 15 patients who had previously been treated with rituximab in the Phase I/II dose-escalation study described above\(^72,79\). In the 3 patients who were in clinical remission at 5 years after treatment, total memory B cell levels (both switched memory and IgM memory) remained significantly lower than those in healthy controls (mean percentage of peripheral blood B cells 6.3% vs 30.5%), whereas among the remaining patients (short-term responders and nonresponders) total memory B cell levels were significantly higher (51.1%) and not significantly different from those in healthy controls. Delayed memory B cell recovery correlated with the degree of expansion of peripheral blood transitional B cells during B cell reconstitution post-treatment. However, tonsil biopsy tissues revealed active germinal center reactions despite low levels of peripheral blood memory B cells, suggesting that peripheral blood memory cell reconstitution lags behind a slow secondary lymphoid tissue recovery\(^79\).

Two patients with severe lupus receiving rituximab therapy off-label with fatal progressive multifocal leukoencephalopathy (PML) were recently described\(^80\). PML is a rare form of central nervous system viral disease caused by activation of a
latent virus present in up to 80% of healthy adults. PML has been observed in lupus patients not receiving rituximab and has not been reported in RA patients treated with rituximab. As of August 2007, there were 23 reported cases of PML in patients who were receiving rituximab for the treatment of hematologic malignancies. The majority of these patients were treated with rituximab in combination with multi-agent chemotherapy or as part of hematopoietic stem cell transplant.

Other B cell-targeted therapies. A trial in lupus with BmAb failed to meet primary efficacy endpoints in the intent-to-treat population. However, when the subset of patients with well-defined disease markers was analyzed, modest efficacy was shown. It is not clear to what extent B cell ablation will be necessary to achieve full efficacy. Patients from this trial were entered into a 1.5-year open-label followup. BmAb was found to be well tolerated in combination with SLE standard-of-care therapy during this period.

In a Phase Ib study in patients with SLE, atacicept was well tolerated, and considerable improvements in SLEDAI score were observed in some patients.

Sjögren’s syndrome
Rituximab. B cell depletion has also been noted after rituximab therapy in patients with Sjögren’s syndrome (SS). In an open-label trial, 15 patients diagnosed with primary SS were administered 4 weekly infusions of rituximab 375 mg/m². Treatment resulted in a rapid decrease in peripheral B cell levels, while levels of IgG remained stable. Four of the patients developed HACA. Serum-sickness-like symptoms were noted in a few patients. Patients showed a significant improvement in subjective symptoms and, where applicable, increases in residual salivary gland function. Parotid biopsies obtained before and after rituximab therapy in several patients provided the first histologic evidence for a significant reduction in lymphoid infiltration — with a decrease in B/T cell ratio and an increase in parenchymal function. In a followup study of this group, rituximab therapy was found to be effective for 6–9 months, with retreatment resulting in a similarly effective clinical response. A recent retrospective study involving 16 female patients with systemic complications of primary SS reported good efficacy and tolerance for rituximab; treatment was effective in 4/5 patients with lymphomas and in 9/11 patients with systemic disorders. However, only a minority of patients showed an improvement in symptoms of dryness. In addition, rituximab treatment allowed corticosteroid use to be reduced in 11 patients.

Other B cell-targeted therapies. Epratuzumab, an anti-CD-22 agent, has been evaluated in a small study of 15 patients with primary SS; efficacy results following IV epratuzumab 360 mg/m² biweekly for 4 doses were promising, with improvement of sicca symptoms and B cell levels showing a median decrease of 57%. Epratuzumab therapy appeared to be well tolerated in this trial.

Vasculitis
B cell therapy is becoming increasingly prominent in the treatment of vasculitis. Initial open-label studies using rituximab in hepatitis C virus (HCV)-related and non-HCV-related cryoglobulinemia demonstrated varying degrees of efficacy and safety. In a recent study, 8 patients with type II mixed cryoglobulinemia who had failed corticosteroid, immunosuppressant, or antiviral therapy were administered 4 weekly infusions of rituximab 375 mg/m², then a maintenance infusion every 3–4 months for 1 year. Although cryoglobulinemia persisted in all patients, skin ulcers were partially or completely cleared; peripheral neuropathy diminished in 2 patients and stabilized in 5; and arthralgias were attenuated in 5 patients. Of the 4 patients with glomerulonephritis, 3 demonstrated significant creatininemia and proteinuria reduction, and proteinuria stabilized in 1 patient.

Preliminary data suggest that rituximab is effective and well tolerated in the treatment of antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis. Although conventional therapy with cyclophosphamide and glucocorticoids can be effective, problems persist with toxicity and a high relapse rate. In a recent open-label study, 10 patients with refractory Wegener’s granulomatosis received daily oral prednisone 1 mg/kg and 4-weekly infusions of rituximab 375 mg/m². All patients tolerated rituximab well, and achieved rapid B cell depletion, with complete remission at 3 months. Proteinase-3-ANCA levels dropped in all patients and turned negative in 6 patients after remission induction therapy. The patients who did not turn proteinase-3-negative were those with the highest baseline values. Five patients who were retreated with rituximab monotherapy due to recurring/rising ANCA titers and B cell repletion were able to maintain remission.

Conclusion
The role of B cells in RA has been progressively elucidated in recent years. Therapies targeted against B cells are now delivering encouraging results in patients refractory to other treatments. Clinical experience with rituximab, the first approved B cell-targeted therapy, has shown that B cell depletion is an effective and generally well tolerated treatment option for RA. The combination of rituximab with MTX appears to be most effective. Rituximab is effective in patients who have failed to respond to anti-TNF-α agents, as well as in anti-TNF-γ-naïve patients. The original rituximab clinical trials are now in extension phases, and data obtained to date indicate continued efficacy in patients receiving subsequent courses of treatment over periods of more than 3 years. Safety data are similarly remaining stable over this period, even though an increasing number of patients may display a transient or sustained decrease of immunoglobulin levels. Patient-reported outcomes data are also encouraging, with significant improvement reported in quality of life and physical functioning. To date, most of the causally-associated adverse events reported...
with rituximab have been infusion-associated reactions, which decrease with subsequent infusions and courses. The rates of infections, including serious infections, are generally within the range expected for patients with RA, but this is an area where surveillance is clearly important as patient safety is tracked over continued courses of therapy. While hepatitis B reactivation has not been observed with rituximab in patients with autoimmune diseases to date, the oncology experience demands that patients be screened for hepatitis B infection before starting B cell depletion therapy. To date, there has not been an increase in malignancy beyond expected background rate. There have been no case reports of drug-induced reactivation has not been observed with rituximab in patients who have not adequately responded to traditional autoimmune disease. Antigenicity appears to be a minor issue. Preliminary data with other agents that target CD20 or modulate B cell activity are also showing efficacy. Preliminary data indicate that the targeting of B cells may also prove useful in other autoimmune diseases such as SLE, SS, and vasculitis. These results point to promising new therapeutic options for patients who have not adequately responded to traditional immunomodulatory therapies.

REFERENCES


