Review
B cell targeted therapies

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Abstract

Although the precise pathogenesis of rheumatoid arthritis (RA) remains unclear, many cell populations, including monocytes, macrophages, endothelial cells, fibroblasts and B cells, participate in the inflammatory process. Ongoing research continues to evaluate the critical roles played by B cells in sustaining the chronic inflammatory process of RA. These findings have contributed to the development of targeted therapies that deplete B cells, such as rituximab, as well as inhibitors of B lymphocyte stimulation, such as belimumab. In a phase I trial, belimumab treatment significantly reduced $CD20^+$ levels in patients with systemic lupus erythematosus. Phase I and phase II trials of rituximab found that rituximab plus methotrexate achieved significantly better American College of Rheumatology 50% responses for patients with RA than those patients receiving monotherapy with methotrexate. These clinical trial data present promising evidence for B cell targeted therapies as future therapeutic options for RA.

Introduction

Advances in our understanding of the pathogenesis of rheumatoid arthritis (RA) have allowed clinicians to target selectively the pathogenic elements of this disease. Until recently, treatment focused on targeting cytokines such as tumor necrosis factor-α and interleukin (IL)-1. However, it may be more appropriate to move treatment upstream from the cytokines to target the cellular processes that drive rheumatoid disease. Recent studies have shown that antibody-producing B cells not only generate antibodies but also present antigen to T cells, resulting in many of the cellular events and inflammatory processes of RA.

In an overview of studies of the roles played by B cells and B cell depletion in RA, Tsokos [1] suggested that B cells contribute to the expression of autoimmunity, and that antibodies that target B cells, such as anti-CD20, may substantially abrogate expression of disease. Tsokos suggested four possible mechanisms of action by which anti-CD20 could deplete B cells. First, after the antibody binds to the extracellular domain of the CD20 antigen, it may activate complement and lyse the targeted cell. Second, anti-CD20 antibody may permit antibody dependent, cell-mediated cytotoxicity, which occurs after the Fc portion of the antibody – the part of the antibody responsible for binding to cell receptors – is recognized by appropriate receptors on cytotoxic cells. Third, the antibody may alter the ability of B cells to respond to antigen or other stimuli. Finally, anti-CD20 antibody may initiate programmed cell death or apoptosis of B cells. All of these mechanisms of B cell depletion may be involved to variable degrees, depending on which B cell pool is affected. For example, Fc receptor mediated cytotoxicity is prominent in the destruction of B cells in peripheral blood, whereas complement activation is involved in the killing of B cells in lymphoid organs [1].

Thus, the new concept is not only to target cytokines but also to target the cellular elements, such as B cells, that cause or perpetuate RA. How, then, may B cells be targeted? There are several ways to target B cells. First, one may target cytokines that promote B cell function and survival, such as IL-6 and B lymphocyte stimulator (BLyS). This may be done in several ways. One can produce an antibody to BLyS or use a soluble receptor such as transmembrane activator and calcium-modulator and cyclophilin ligand interactor-immunoglobulin (TACI-Ig) to block positive signaling through BLyS receptors. Second, B cells may be depleted by monoclonal antibodies that inhibit CD19, CD20, CD21, or CD22 B cell surface antigen. Finally, the co-stimulatory molecule may be targeted, preventing B cells from contributing to the inflammatory process through processing autoantigen and presenting it to T cells [2], as well as by producing cytokines and autoantibody. Treatment with anti-CD20 antibody destroys mature B cells in central lymphoid organs, the synovium, and the peripheral blood.

ACR = American College of Rheumatology; BLyS = B lymphocyte stimulator; RA= rheumatoid arthritis; SLAM = Systemic Lupus Activity Measure; SLE = systemic lupus erythematosus; SLEDAI = Systemic Lupus Erythematosus Disease Activity Index; TACI-Ig = transmembrane activator and calcium-modulator and cyclophilin ligand interactor-immunoglobulin.

Silverman and Carson [2], in their analysis of B cells in RA, found that B cells are also very efficient antigen presenting cells, contributing to T cell activation through expression of co-stimulatory molecules. In addition to being the precursors of antibody-secreting plasma cells, the B cells play a critical role in RA in terms of the afferent arm of the immune response. Thus, B cells can act as highly efficient antigen presenting cells, which assist in the activation of autoreactive T cells.

B cells both respond to and produce the chemokines and cytokines that promote leucocyte infiltration into the joints, formation of ectopic lymphoid structures, angiogenesis, and synovial hyperplasia. The success of B cell depletion therapy in RA may depend on disruption of some or all of these diverse functions.

B lymphocyte stimulator inhibition: belimumab

Therapies that inhibit BLyS currently under development include soluble receptors such as TACI-Ig and anti-BLyS monoclonal antibodies. Furie and coworkers [3] conducted a phase I investigation of belimumab (LymphoStat-B, Human Genbome Sciences Inc., Rockville, MD, USA), a fully human monoclonal antibody that inhibits soluble BLyS. This randomized, double-blind study was designed to evaluate the safety, tolerability, immunogenicity and pharmacology of four different doses (1, 4, 10 and 20 mg/kg) of LymphoStat-B or placebo, administered as a single intravenous infusion or two infusions 21 days apart. Study participants had stable systemic lupus erythematosus (SLE) of mild-to-moderate disease activity and were receiving a stable standard-of-care SLE treatment regimen for 2 months before enrollment.

Patients were followed for 84–105 days to assess peripheral B cell concentrations, serologies, and disease activity (using the Safety of Estrogens in Lupus Erythematosus: National Assessment [SELENA] Systemic Lupus Erythematosus Disease Activity Index [SLEDAI]), and to monitor adverse events, pharmacokinetics, and safety. Data from placebo patients ($n = 13$) in single-blind or double-dose cohorts were pooled and compared with those receiving LymphoStat-B ($n = 57$) in each of the four single or double dose cohorts.

The pharmacokinetics of single doses were dose proportional. The lengthy half-life (13–17 days), slow clearance (4.00 ± 1.56 ml/day per kg) and are consistent with a fully human monoclonal antibody. All LymphoStat-B cohorts exhibited significant reductions in CD20$^+$ cells (12–47%) at one or more visits from days 42–105 compared with placebo.

Furie and coworkers found that LymphoStat-B was well tolerated at all doses, with no study withdrawals. The overall incidence of adverse events was similar between LymphoStat-B and placebo groups. No increased incidence of infections occurred in the treatment group, and none of the infections reported was attributed to the study agent.

Six patients experienced serious adverse events, with similar frequencies observed in the placebo and treatment groups. None were deemed to be related to the study agent. Severe (grade 3 and 4) laboratory abnormalities or adverse events occurred infrequently. One patient experienced an infusion reaction at the highest single dose, while another developed neutralizing antibodies to LymphoStat-B.

Reductions in anti-dsDNA or immunoglobulin levels compared with placebo were observed in some LymphoStat-B cohorts. No change in SLE disease activity was observed over this short exposure. The investigators concluded that there was a significant reduction in peripheral B cells with LymphoStat-B treatment, consistent with its ability to bind and inhibit the biological activity of BLyS.
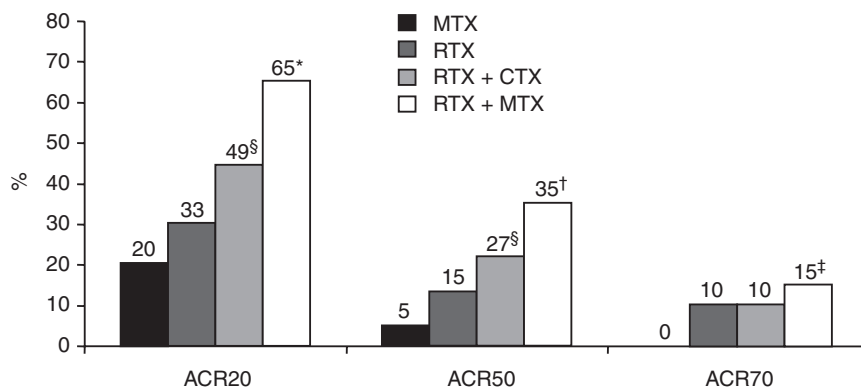
Rituximab
Primary analysis

Rituximab, a genetically engineered chimeric anti-CD20 monoclonal antibody, is currently approved for the treatment of relapsed or refractory, low grade, or follicular CD20$^+$ B cell non-Hodgkin's lymphoma. Rituximab selectively depletes B cells that bear the CD20 surface marker via multiple mechanisms that include antibody-dependent cellular cytotoxicity and complement-dependent cytotoxicity, and via the induction of apoptosis.

Edwards [4] hypothesized that depletion of B lymphocytes could represent a new treatment for RA. In order to assess better the efficacy of this B cell targeted therapy, a randomized, double-blind, controlled study was carried out to examine the effect of selective depletion of B cells with rituximab in patients with RA [5].

A total of 161 patients with active RA who had failed to respond to treatment with methotrexate (at least 10 mg/week for at least 16 weeks) were randomly assigned to one of four treatment regimens: oral methotrexate as a control arm; intravenous rituximab alone (1000 mg on days 1 and 15); intravenous rituximab plus cyclophosphamide (750 mg on days 3 and 17); or rituximab plus methotrexate. All patients received 100 mg methylprednisolone just before each treatment (or intravenous placebo), as well as prednisone 60 mg/day on day 2 and days 4–7, and 30 mg/day on days 8–14.

Clinical assessments were performed at baseline (day 1) and at weeks 12, 16, 20 and 24, using the American College of Rheumatology (ACR) core set of disease activity measures: swollen and tender joint counts (66 joints evaluated), patients' evaluation of pain based on a scale of 0 (no pain) to 100 (unbearable pain), patients' global assessment of disease activity on a scale from 0 (disease inactivity) to 100 (maximal disease activity), physicians' assessment of disease activity, assessment of physical function reported by patients utilizing a health assessment questionnaire, and laboratory evaluation of serum C-reactive protein levels and erythrocyte sedimentation rate.

Figure 1

Rituximab in rheumatoid arthritis. Phase IIa: American College of Rheumatology (ACR) responses at 48 weeks (NRI). *$P < 0.0001$; †$P = 0.003$; ‡$P = 0.03$; §$P = 0.01$; P values using Fisher's exact test, comparing MTX with each RTX group. CTX, cyclophosphamide; MTX, methotrexate; NRI, nonresponder imputation; RTX, rituximab. With permission from Edwards and coworkers [5]. Copyright © 2004 Massachusetts Medical Society. All rights reserved.

Patient responses were assessed at week 24 for the primary analyses and at week 48 for the exploratory analyses. The primary end-point was the proportion of patients with an ACR50 response at week 24. Secondary end-points were ACR20 (a 20% improvement according to ACR criteria) and ACR70 (a 70% improvement according to ACR criteria) responses, as well as a response based on the European League Against Rheumatism criteria.

At week 24, the proportion of patients with an ACR50 response was significantly greater for the group of patients taking the rituximab–methotrexate combination (43%; $P = 0.005$) and the rituximab–cyclophosphamide group (41%; $P = 0.005$) than for those receiving methotrexate alone (13%). Investigators also noted that the number of patients achieving an ACR50 response in the group receiving rituximab alone was greater than that in the control group, but this failed to reach statistical significance ($P = 0.059$).

Notably, the mean change from baseline in Disease Activity Score at week 24 reflected significant improvement over methotrexate alone in all rituximab groups ($P = 0.002$). The clinical parameters employed in the calculation of the Disease Activity Score include number of tender joints, number of swollen joints, erythrocyte sedimentation rate, and the patient's subjective assessment of disease activity.

Exploratory analysis: a 48 week study
An exploratory analysis of ACR responses at 48 weeks found that, of patients in the rituximab–methotrexate group, 35% ($P = 0.002$) and 15% ($P = 0.03$) had ACR50 and ACR70 responses, respectively, as compared with 5% and 0% in the methotrexate control group [5,6]. In addition, 27% of patients in the rituximab–cyclophosphamide arm achieved an ACR50 response ($P = 0.01$; Fig. 1).

In terms of pharmacodynamic outcomes, treatment with rituximab was associated with nearly complete depletion of peripheral blood B cells throughout the entire 24-week period. Although such a profound reduction in B cells may suggest greater susceptibility to infection, the overall incidence of infection was similar in the control and rituximab groups at 24 and 48 weeks. By week 24, one patient in the control group and four in the rituximab groups had suffered a serious infection. An additional two serious infections – including a fatal bronchopneumonia – were reported during the extended 48-week period in the rituximab group. There was no accumulation of any particular type of infection in rituximab treated groups.

Despite B cell depletion, immunoglobulin levels did not change substantially. Patients in the rituximab groups experienced substantial and rapid reductions in rheumatoid factor levels, whereas those in the methotrexate alone group experienced only a moderate reduction in rheumatoid factor levels [5] (Fig. 2).

Adverse events
Overall, 73–85% of patients in all treatment groups reported at least one adverse event, with hypertension, hypotension, nasopharyngitis, arthralgia, back pain, hyperglycaemia, cough, flushing and headache reported most often. Of patients in each group, 30–45% experienced events associated with initial infusion, although 85–95% of adverse events related to rituximab infusions were characterized as mild or moderate.

Infusion reactions occurred during the first infusion in approximately one-third of patients in the groups receiving rituximab and the placebo group (36% and 30%, respectively). The rate of first infusion reaction in patients with

Figure 2
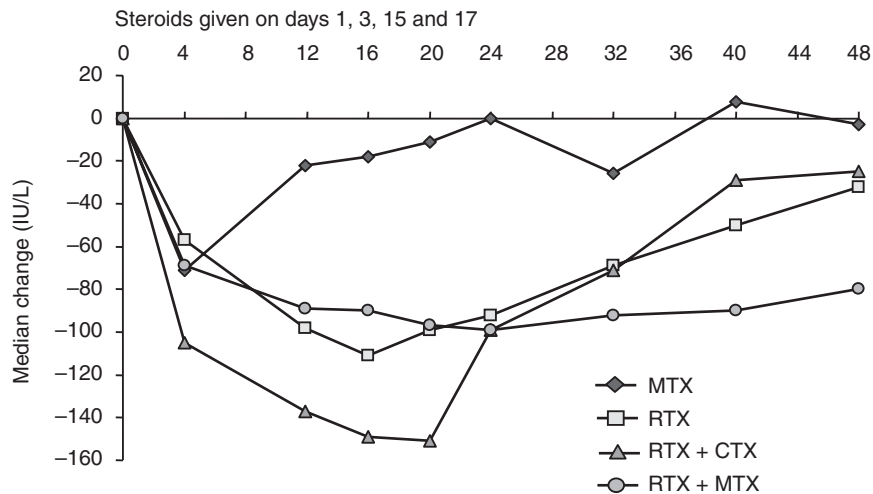
Rituximab in rheumatoid arthritis. Phase IIa: changes in total rheumatoid factor (median). CTX, cyclophosphamide; MTX, methotrexate; RTX, rituximab. Data from Edwards and coworkers [5] and from Emery and coworkers [6].

RA was considerably lower than rates seen in patients with non-Hodgkin's lymphoma (36% versus 70–80%). According to the 48-week data, the most common infusion related reactions reported by patients were hypotension (14% versus 10% in placebo group), hypertension (9% versus 5%), flushing (6%), pruritus (6%), and rash (6%). Six patients during the primary 24-week trial period and an additional three at 48 weeks withdrew from the trial because of adverse events, including exacerbation of ongoing RA, hypotension and bronchopneumonia, staphylococcal septicaemia, renal impairment and rash.

Rituximab in systemic lupus erythematosus
Albert and coworkers [7] conducted a phase I study to determine the safety and efficacy of B cell depletion with the anti-CD20 monoclonal antibody rituximab as treatment for SLE. Seven patients with active and persistent SLE who had failed at least one immunosuppressive agent enrolled in this pilot study. Each patient received 4-weekly infusions of rituximab at 375 mg/m^2.

Six out of seven patients exhibited a clinical response, defined as improvement in SLEDAI. All but one patient had greater than 99% B cell depletion lasting more than 3 months. The patient who exhibited only 95% B cell depletion had no clinical response. Interestingly, this patient also showed responses to immunizations, whereas two other patients with complete B cell depletion (and clinical response) failed to achieve adequate immune responses. Among clinical responders, 50% had a brief remission (6 weeks to 6 months), and the other 50% had a more prolonged remission (6–9 months).

Among longer term responders, the steroid dose was either stabilized or lowered; one patient had been in remission for 14 months without the need for steroid therapy. All brief responders returned to an immunosuppressive regimen. There was a trend toward a decrease in the percentage of B cells expressing CD86 among all patients. There was no distinctive pattern of other cell surface marker expression among responders and nonresponders. No consistent changes were found in serum chemistry, serum complement levels, autoantibody titres and immunoglobulin levels.

A study conducted by Anolik and coworkers [8] found that, compared with normal control individuals, lupus patients exhibited several abnormalities in peripheral B cell homeostasis at baseline, including naïve lymphopenia, and expansion of circulating plasmablasts. Remarkably, these abnormalities partially resolved after effective B cell depletion with rituximab and immune reconstitution [8].

The frequency of autoreactive VH4.34 memory B cells also decreased 1 year following treatment, despite the presence of low levels of residual memory B cells at the point of maximal B cell depletion and persistently elevated serum autoantibody titres in most patients. This study demonstrates that, in SLE patients, specific B cell depletion therapy with rituximab dramatically improves the abnormalities in B cell homeostasis that are characteristic of this disease.

Looney and coworkers [9], in a phase I/II study, examined the potential use of rituximab in B cell depletion for patients with SLE. In order to establish the safety and efficacy of rituximab in this patient population, those investigators performed a

dose escalation trial of rituximab added to ongoing therapy in SLE. They administered rituximab as a single infusion of 100 mg/m^2 (low dose), a single infusion of 375 mg/m^2 (intermediate dose), or as four infusions (1 week apart) of 375 mg/m^2 (high dose). CD19$^+$ lymphocytes were measured to determine the effectiveness of B cell depletion. The Systemic Lupus Activity Measure (SLAM) score was used as the primary outcome for clinical efficacy.

Looney and coworkers demonstrated that rituximab was well tolerated in this patient population, with most experiencing no significant adverse effects. Three patients had serious adverse events requiring hospitalization (one *Staphylococcus aureus* abscess of the thigh, one localized case of herpes zoster and a transient ischaemic attack), although none was deemed related to rituximab administration. The majority of patients (11 out of 17) had profound B cell depletion (CD19$^+$ B cell count <5/µl). In these patients the SLAM score was significantly improved at 2 and 3 months compared with baseline ($P = 0.0016$ and $P = 0.0022$, respectively). This improvement persisted for 12 months, despite the absence of a significant change in anti-dsDNA antibody and complement levels. Six patients developed human antichimeric antibodies at a level of 100 ng/ml or greater (range of peak levels 631–9930 ng/ml). These human antichimeric antibody titres were associated with African American ancestry, higher baseline SLAM scores, reduced B cell depletion and lower levels of rituximab at 2 months after initial infusion.

Conclusion

Recent reports underscored the critical role played by peripheral blood B cells in self-sustaining chronic inflammatory processes. A novel B cell targeted approach, via inhibition of BlyS, has shown early promise in the treatment of SLE. A recent phase I study of 70 patients found that treatment with the BlyS inhibitor belimumab reduced CD20$^+$ cells to between 12% and 47% compared with placebo [3]. Moreover, several clinical trials have provided a substantial body of evidence that the B cell depleting agent rituximab, alone or in combination with other disease-modifying anti-rheumatic drugs, produces profound and prolonged depletion of B cells and confers clinically meaningful benefits to patients with RA and SLE.

Edwards and colleagues [5] showed that, at 24 weeks, significantly more patients receiving rituximab plus methotrexate and rituximab plus cyclophosphamide had ACR50 responses than patients receiving methotrexate alone [5]. Moreover, in a phase I trial of rituximab conducted in seven patients with SLE [7], six (85%) showed an improvement in SLEDAI, as well as a 99% depletion of B cells for more than 3 months. Notably, of the clinical responders in long term remission (6–9 months), the steroid dose remained stable or was lowered. In fact, one patient was in remission for 14 months without the need for any steroid therapy.

Table 1

Unanswered questions about B cell therapies

What is the duration of clinical efficacy after a single course of rituximab? Belimumab?

Is there a need for concomitant corticosteroids with each course of corticosteroids?

What are the long-term implications of CD20$^+$ B cell depletion?

Is there a risk for increased occurrence of serious infections with B cell targeted therapies? Opportunistic infections?

What role might biologics play in combination with B cell targeted therapies?

Important questions remain regarding this emerging form of therapy (Table 1). Research suggests that there is restoration of B cells, usually 6–18 months after depletion therapy. Long-term rituximab treatment for RA is likely to involve maintenance doses, possibly in combination with immuno-suppressive or immunomodulatory agents (e.g. biologic response modifiers). Further studies are needed to address the need for a regimen that can be used for maintenance therapy. Therefore, with B cell targeted therapies, as with other biologics, close monitoring of immunocompetence and risks for serious adverse events and opportunistic infections is required in patients undergoing treatment for RA or other autoimmune disorders.

Taken together, the data presented suggest that B cell targeted therapies are likely to be promising additions to our therapeutic armamentarium, in the treatment both of RA and other autoimmune diseases.

Competing interests

References

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