

## Review

**B cell depletion in autoimmune disease**

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**Abstract**

The CD20 cell marker appears early in the process of B cell development. In this review we focus on the results of attempts to utilize B cell depletion based on the use of a chimeric monoclonal antibody (MAb) specific for human CD20, rituximab, for the treatment of patients with autoimmune diseases. In 1997, rituximab was approved for the treatment of low-grade B cell non-Hodgkin's lymphoma. Following these encouraging results, rituximab started to be used experimentally in other diseases presumed to be due to B cell pathology. The first autoimmune disease to be treated effectively was chronic idiopathic thrombocytopenia. More recent success has been demonstrated in patients with rheumatoid arthritis and systemic lupus erythematosus.

**Keywords:** autoimmune disease, B cell depletion, rituximab

**Introduction**

B lymphocytes arising from haematopoietic stem cells pass through a series of intermediate stages (pro-B, pre-B, immature B and mature B cells); this gives rise to plasma cells, which produce immunoglobulins. Although the majority of immunoglobulins are concerned with defence against the multitude of pathogens that surround and invade our bodies, some, by virtue of their recognition of self targets (autoantibodies), have the capacity to cause self harm or autoimmune disease. The issue of the relative contributions of T cells, B cells, cytokines and other elements within the immune system has been debated for decades, but the past 5 years have seen an upsurge of interest in the notion that B cells are an integral part of the problem in autoimmunity and that blocking them may be beneficial. The work of Mathis and colleagues [1] suggesting a role for B cells in the development of a form of experimental arthritis, and the studies conducted by Edwards and colleagues [2,3] describing patients with erosive rheumatoid arthritis (RA) successfully treated with B cell depletion have provided strong supporting evidence for this notion.

A number of markers, including CD19 and CD20, appear early in the process of B cell development (at the pro-B or

pre-B cell stage). They remain present until the stage of the mature B cell in the periphery, where conversion to a plasma cell is associated with loss of CD20, although the CD19 marker is still detectable. Much interest has focused on the role of CD20 in B cell physiology but it remains uncertain. Possible roles include its functioning as a calcium channel subunit [4].

In this brief review we focus on results to date of attempts to utilize B cell depletion based on the use of a chimeric mAb that is specific for human CD20, namely rituximab (MabThera®/Rituxan®; Roche Pharmaceuticals, Basel, Switzerland; Genentech, South San Francisco, USA; IDEC Pharmaceuticals, San Diego, USA), for the treatment of patients with autoimmune diseases.

**Rituximab as therapy for B cell lymphoma**

The potential of mAbs as therapeutic agents has long been postulated. In November 1997, rituximab was the first mAb to be approved for the treatment of any malignancy, with the US Food and Drug Administration granting it a license for treatment of relapsed or refractory, low-grade B cell follicular non-Hodgkin's lymphoma (NHL) [5]. High rates of B cell depletion are observed in patients receiving the stan-

ACR = American College of Rheumatology; cANCA = cytoplasmic antineutrophil cytoplasmic antibody; ds = double-stranded; ITP = idiopathic thrombocytopenia; mAb = monoclonal antibody; NHL = non-Hodgkin's lymphoma; RA = rheumatoid arthritis; RF = rheumatoid factor; SLE = systemic lupus erythematosus.

dard four weekly treatments of 375 mg/m<sup>2</sup>, with response rates of around 60% [6–8]. This depletion is usually sustained for 6–9 months and does not seem to be associated with a higher rate of infectious complications. In addition, molecular remission (i.e. remission of genetic mutations that are often associated with haematological malignancies such as B cell lymphomas) can occur and appears to be correlated with clinical response [9]. The use of rituximab in B cell lymphoma therapy has now been broadened; some groups are using it to 'purge' B cells before stem cell collection in peripheral blood stem cell transplantation [10]. It is also being investigated as an adjuvant to more conventional chemotherapy in more aggressive lymphoma and other B cell malignancies [9], and as an adjuvant following bone marrow transplantation [11].

### Rituximab in autoimmune diseases

Following these encouraging results in patients with B cell lymphoma, rituximab was used experimentally in other diseases presumed to be due to B cell pathology. The first autoimmune disease in which success was demonstrated was chronic idiopathic thrombocytopenia (ITP). In ITP, platelets are opsonized by autoantibodies (usually platelet-associated IgG) and prematurely destroyed by the reticuloendothelial system [12]. Approximately 25–30% of patients develop a chronic disease that becomes refractory to conventional therapy (including corticosteroids, intravenous immunoglobulin and splenectomy) [13]. Rituximab, used as a single agent at the doses suggested in NHL, has been observed to produce overall response rates of 30–50% (i.e. significant elevations in platelet counts sustained for 6 months or longer) [13–15]. Depletion of peripheral blood B cells occurred rapidly, as expected. In addition, rises in platelet counts were observed very quickly, generally within 1 week of the first rituximab infusion [13,14,16–18]. In the group of patients described in the literature, clinical responses were not associated with significant falls in levels of platelet-associated IgG, with only a minority of patients reaching levels found in normal individuals [13,14]. This early rise in platelets is thus unlikely to be secondary to removal of antiplatelet antibodies. One alternative suggestion is that opsonized B cells can inhibit Fc receptors on macrophages and removal of IgG-coated platelets [13].

Similar success has been observed in patients with autoimmune haemolytic anaemia refractory to conventional therapy [18–21] and in cold agglutinin disease [22–24]. Restoration of erythropoiesis has also been observed in refractory cases of pure red cell aplasia after treatment with rituximab [20,25,26]. Observed responses were of a similar rapidity of onset to that seen in the patients with ITP.

### Rheumatoid arthritis and rituximab

RA has classically been thought of as a predominantly T cell mediated disease [27]. However, interest in the role

of B cells in RA has recently been generated. The strength of this theory has been ratified by encouraging results with rituximab-induced B cell depletion in RA patients.

In an initial study, five patients resistant to at least five disease-modifying antirheumatic drugs received a B cell depletion protocol based on rituximab (with intravenous cyclophosphamide and oral prednisolone). All patients achieved at least an American College of Rheumatology (ACR) 50 response (three achieved an ACR 70 response). Immunoglobulin levels fell only modestly, chiefly in the first 10 weeks. Levels of IgM rheumatoid factor (RF) fell in all patients at varying rates [2]. This earlier open label study was extended to include 22 RA patients treated with five different protocols [3]. These protocols attempted to assess a possible dose–response relationship with rituximab and whether the inclusion or omission of cyclophosphamide was relevant. Major responses (i.e. ACR 50 or greater) were associated with higher doses of rituximab (i.e. equal to or greater than a total treatment dose of 600 mg/m<sup>2</sup>) in combination with cyclophosphamide. In these 22 RA patients, B cell depletion (with rituximab/cyclophosphamide/prednisolone) had a selective effect on autoantibody levels; IgA RF, IgG RF and IgG anti-cyclic citrullinated peptide antibodies fell more than their corresponding serum immunoglobulin classes. Clinical relapse was associated with rises in autoantibodies rather than only with return of B cells [28]. A multicentre randomized controlled trial has now shown that protocols based on rituximab are effective in treating patients with active RA [29]. Combination treatment with rituximab and cyclophosphamide, and with rituximab and methotrexate gave comparable results (i.e. ACR 50 responses of 45% and 50%, respectively) and were both superior to rituximab alone (ACR 50 of 32%) [30].

### Systemic lupus erythematosus and rituximab

B cell dysfunction has been thought to be critical in the pathogenesis of systemic lupus erythematosus (SLE), with evidence for direct pathogenic roles for at least some autoantibodies, most notably anti-double-stranded (ds)DNA [31]. Thus, the concept of B cell depletion as treatment for SLE seems rational.

Initial trials have shown promising results. Anolik and coworkers first reported their phase I/II trial of rituximab in 12 patients with SLE in 2001 [32], which was followed by a total group of 18 patients in 2002 [33]. Significant B cell depletion in the peripheral blood correlated with clinical improvement (assessed using the Systemic Lupus Activity Measure score). However, significant changes in anti-dsDNA titres and complement levels were not observed. In another open study, six SLE patients received a combination of two 500 mg rituximab infusions, two 750 mg cyclophosphamide infusions and high-dose oral corticosteroids over a 2 week period [34]. All five patients who

completed the study (one was lost to follow up at 3 months) improved clinically, from a median British Isles Lupus Assessment global score of 14 at the start of the study to a score of 6 after a period of 6 months. In four patients, haemoglobin levels and erythrocyte sedimentation rate improved and in all five patients C3 levels increased. In two patients, clinical remission lasted well beyond the period of B cell depletion, and the patients remained off further immunosuppressive therapy at 18 months of follow up. In the other patients, relapse occurred with B cell repopulation. The changes in anti-dsDNA levels varied between patients, with no consistent trends observed.

These open label studies in SLE suggest that B cell depletion based on rituximab is worthy of a randomized, double blind, controlled trial. The international validation efforts, conducted principally by the Systemic Lupus Erythematosus International Collaborating Clinics group, have produced both activity and damage indices for this disease. The British Isles Lupus Assessment index seems particularly suitable for use in drug trials given its ability to distinguish activity ('at a glance') in each of eight organs or systems. For a 'quick fix' the global score indices Systemic Lupus Activity Measure and Systemic Lupus Erythematosus Disease Activity Index are also available and widely used. This topic has been reviewed elsewhere [35].

### Use of rituximab in other autoimmune diseases

There have also been a number of recent open studies using rituximab in less common autoimmune diseases. Five patients with refractory dermatomyositis received rituximab as a single agent at four weekly doses of 100 mg/m<sup>2</sup> (two weekly doses for juvenile patients) [36]. Within 1–3 months, all patients experienced marked increases in muscle strength with improvements in quantitative scores between 20% and 60%. Dermatitis also improved dramatically. All improvements were sustained for at least 6 months.

Rituximab has also been used successfully in type II mixed cryoglobulinaemia. Fourteen refractory patients were treated with the standard 4 week NHL rituximab regimen [37]. Only low-dose oral steroids were allowed as concomitant immunosuppressive therapy. Clinical improvement was observed in the majority of patients at 6 months of follow up, although cryoglobulins only became negative in 30%. RF decreased in 61% and became negative in 23%, whereas C4 increased in 50% of patients.

One case report describes the successful use of rituximab in Wegener's granulomatosis [38]. The patient had failed to remain in remission at 5 years despite multiple immunosuppressive regimens. After the standard 4 week rituximab course (plus high-dose corticosteroids), full clinical remis-

sion with disappearance of cytoplasmic antineutrophil cytoplasmic antibodies (cANCA) was obtained. Nine months after the course, despite the maintenance of complete clinical remission, the cANCA titre started to rise again, along with a rise in B cell numbers. Rituximab treatment was thus repeated (without corticosteroids) and the patient remained in complete clinical remission, with a negative cANCA, at 8 months of further follow up.

Preliminary studies of patients with IgM antibody related polyneuropathies were among the first to suggest the benefit of rituximab in the treatment of autoantibody-associated diseases [39]. One case report has also described a successful outcome when rituximab was used therapeutically in a rare case of myasthenia gravis developing after a bone marrow transplant [40].

### The broader picture: combination therapy

The results of many of these trials report a variable effect on autoantibody levels despite effective B cell depletion. CD20 is lost from B cells in their terminal differentiation into plasma cells. Thus, many of the pathogenic plasma cells may not be effectively removed and may continue to produce antibodies. From these more recent trials of rituximab in RA and SLE, it seems highly likely that therapy with a combination of agents causing more widespread depletion of B cells, and possibly of plasma cells (i.e. high dose corticosteroids and cyclophosphamide), produces more effective and durable results.

However, most patients relapse despite full-dose rituximab, steroids and cyclophosphamide, and the question has been raised as to whether the regimens used are still not potent enough [34]. Clearly, further work must be done to determine optimal dosing schedules. There have been successful results after repeated treatment with rituximab [3,38], but again this requires further assessment. In one SLE patient, retreatment with rituximab alone was associated with failure to deplete B cells because of a specific immune response to rituximab itself (Leandro, Edwards, Cambridge and Isenberg, unpublished data). However, higher doses of rituximab, combination with other cytotoxic therapies and repeat treatment would inevitably raise the likelihood of increased toxicity in what thus far appears to be a relatively safe treatment. Furthermore, until we follow up the patients already treated for longer periods of time, we cannot know what the long-term consequences will be.

In the majority of studies conducted to date only minimal side effects were reported. These have mostly comprised infusion reactions (fevers, chills and rigors), which usually decrease with each subsequent reaction and can mostly be prevented by premedication with paracetamol, an antihistamine and corticosteroids. There does not appear to be a general trend toward excess infectious complica-

tions. This obviously compares very favourably with our conventional immunosuppressive treatments. However, all patients with autoimmune diseases who have been treated thus far with B cell depletion are patients who have received and failed (usually multiple) conventional immunosuppressive regimens. There is a need to compare B cell depletion directly in clinical trials with our 'standard of care' immunosuppressive regimens.

The exact mechanism of action of B cell depletion in autoimmune disease is still unclear. The clinical state improves over a number of months despite almost immediate B cell depletion [3]. Furthermore, this improvement appears to be sustained for longer than the period of B cell depletion. Work to elucidate this mechanism fully would be greatly beneficial. Rituximab is known to deplete B cells by antibody-dependent, cell-mediated cytotoxicity, by complement mediated cell lysis and/or by apoptosis [41]. Recent work conducted by Anolik and coworkers [32] in SLE patients indicates that polymorphisms of Fcγ receptors may contribute to the variability of B cell depletion, at least in patients treated with lower doses of rituximab. In patients with NHL, a correlation between polymorphisms of Fcγ receptors and response to rituximab has also been found [32].

Combination regimens with other biological agents are also being investigated. Interleukin-12 has been used with rituximab in patients with B cell NHL with some encouraging preliminary results [42]. It is likely that other mAbs will also be used in future studies.

### Conclusion

As discussed above, the open label trials of B cell depletion and the recently reported multicentre randomized controlled trial in patients with RA [29,30] now point to likely expansion in the use of this approach for patients with aggressive disease, not least because its cost is less than that of tumour necrosis factor-α blockers. B cell depletion, rather than rituximab (anti-CD20) therapy alone, offers the possibility of treating a range of autoimmune diseases. It is not a cure in most cases and its long-term side effects have not been fully elucidated. However, there now appears to be sufficiently encouraging data from open label studies in SLE, in particular, to encourage the undertaking of larger controlled clinical trials.

### Competing interests

None declared.

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