Repeated B lymphocyte depletion with rituximab in rheumatoid arthritis over 7 yrs

C. Popa, M. J. Leandro, G. Cambridge and J. C. W. Edwards

Introduction

B-lymphocyte depletion in patients with rheumatoid arthritis (RA), using the anti-CD20 monoclonal antibody, rituximab, either alone or in combination with cyclophosphamide (cy) and/or corticosteroid, was initiated in 1998 [1]. Following early encouraging clinical progress, a total of 37 patients were recruited over the subsequent 4 yrs to a programme of repeated cycles of B-lymphocyte depletion [2, 3]. The programme was carried out on an open-label basis on the grounds of clinical need, as judged in terms of moderate to severe uncontrolled disease and an inability to benefit from standard therapies.

The original objective of B-lymphocyte depletion was to investigate the possibility that it might induce long-term remission, such that repeated treatment might not be necessary [4]. Although improvement lasting up to 43 months has been seen from a single two-week cycle of therapy, experience to date is that all patients relapse [3]. In approximately half of the cases relapse occurs at the time of return of the circulating B cells, and in the other half it is delayed for up to a further 2½ yrs after B-cell return to the periphery (Fig. 1) [5]. As pilot studies of repeated cycles of therapy in the initial cohort of patients suggested comparable favourable clinical responses, it was decided to enrol further patients in a programme of repeat therapy to assess the long-term feasibility of such a strategy [1]. The drug combination and dose levels were modified during recruitment of the first 22 cases, as reported previously [2]. Subsequent to this, the protocol has remained largely unchanged and cy has been discontinued.

Experience with rituximab therapy in lymphoma patients had indicated that a single cycle of treatment would be associated with a period of B lymphopenia of ~6–9 months with little change in circulating immunoglobulin levels. Rates of infection appeared acceptable, but were difficult to interpret in the context of immunodeficiency associated with lymphoma and concomitant use of cytotoxic agents [6]. While a single episode of B lymphopenia was judged acceptable in the management of RA, the practicability of repeated episodes was less clear, especially if they were to run in rapid succession. The major objective of the programme described was to assess the viability of repeated cycles of therapy in a representative group of patients refractory to other therapies.

Methods

Subjects

Thirty-seven patients, satisfying the American College of Rheumatology (ACR) (formerly ARA) diagnostic criteria for RA [7], were treated on the basis of moderate to severe disease not adequately controlled by, intolerant of, or unsuitable for, methotrexate, intramuscular gold, sulphasalazine and, from the time of licensed availability, leflunomide and tumour necrosis factor (TNF)-neutralizing agents. Of all patients, 10 were males and 27 females, mean age 64 yrs, range 32–86 yrs, mean disease duration 18 yrs, range 5–40 years. Figure 2 shows the dates of first and subsequent treatment for the 37 patients described here.

The following patients had salient features that might have influenced outcome (numbers as per Fig. 2). Patients 11, 26 and 32 had secondary renal amyloidosis. Patients 18 and 26 were Steinbrocker functional grade IV, whereas all others were grade II or III. Subjects 8, 20, 28 and 30 were rheumatoid factor (RhF)
Repeated B lymphocyte depletion in rheumatoid arthritis

627

Fig. 1. The relationship between time (in months) to peripheral B-cell repopulation and relapse is shown for 24 patients following the first cycle of B-cell depletion therapy. Time to relapse and re-population were equal in 11 patients (the dotted line represents the line of best-fit). In the remaining 13 patients, the time to relapse can be seen to occur months to years after B-cell re-population (indicated by grey rectangle).

Fig. 2. Dates of administration of rituximab.

negative. Subject 8 (RhF−) was anti-nuclear antibody positive, but with erosive disease and no features of lupus. Subject 20 had psoriasis and subjects 28 and 30 a past and family history of psoriasis, respectively, but all had a symmetrical metacarpophalangeal- and proximal interphalangeal-dominant rheumatoid pattern of disease with no nail dystrophy and had been considered to have ‘seronegative RA’. Patient 36 had a past history of MALT-type lymphoma with no recent evidence of recurrence. Patient 14 had extensive skin vasculitis.

Treatment protocols

All treatment protocols were approved by the local hospital ethics committee and all patients gave informed consent for each cycle of treatment. The first 22 patients enrolled received protocols of varying intensity based on rituximab, corticosteroid and or cy as published previously [2], with previous disease modifying anti-rheumatic drugs (DMARDs) (excluding steroids) discontinued from day 0. In brief, the first five patients received rituximab, as four i.v. infusions on days 2, 8, 15 and 22, of 300, 600, 600 and 600 mg (200, 375, 375, 375 mg/m²), respectively, cy as i.v. infusions of 750 mg each on days 4 and 17 and oral prednisolone 60 mg on days 1–22, reducing to 30 mg on days 11–22 and then withdrawn over 3 weeks in subjects not previously taking steroids and, in the other cases, reduce to 5 mg daily over 6 weeks. Patients 6–22 received a similar protocol, but with either a reduced dose of rituximab, omission of cy or omission of corticosteroid [2]. Patients 23–37 received a simplified protocol of two i.v. infusions on days 1 and 8 of 1000 mg rituximab following 100 mg intravenous methylprednisolone premedication on each occasion (in a few courses, 1500 mg rituximab or 250 mg methylprednisolone were used). Patients receiving methotrexate continued on this agent at the same dose or, if stable at a dose reduced to 10 mg weekly. Patients not receiving methotrexate received no DMARD concomitant with the period of B-lymphocyte depletion with the exception of patient 17 who commenced intramuscular gold following her third cycle and leflunomide following her fourth cycle in an attempt to prolong the period of improvement, patient 32 who received azathioprine with both cycles, and patient 22 who continued her azathioprine with her second cycle. Patients on oral prednisolone continued on this agent with dose tapering and eventual withdrawal depending on their response to therapy. All patients used NSAIDs or analgesics as needed.

Decision to retreat

The decision to retreat was based on the clinical judgement of the investigators, which was very much informed by the patients’ own assessment of benefits/risk, which was influenced by a major element of caution during the early stages. The indications for re-treatment within this context were based on the satisfaction of the following four criteria: (i) B cells must have returned to the peripheral blood, indeed the decision to retreat was dependent on this having occurred; (ii) any return of symptoms of RA; (iii) rise in C-reactive protein (CRP) following an original fall of at least 50% in CRP during the previous course of B-cell depletion therapy; and (iv) adequate levels of circulating IgG.

Assessment

Patients were assessed prior to treatment and at least 3 months thereafter. This audit was not primarily intended to assess efficacy, and we have limited reporting of improvement to percentage changes in CRP levels from pre-treatment to nadir, for two reasons. First, over the 7-yr period clinical assessments were carried out by several different individuals at intervals that varied as the programme progressed. Second, all assessments were made unblinded to the type and timing of treatment. In view of the subjective nature of several of the elements of standard clinical grading systems these factors leave a wide margin of uncertainty in the reliability of clinical assessments. The primary objective of this report was to document feasibility and safety of the repeated therapy, and it was judged more appropriate to limit assessment of improvement to a simple objective measure. The use of the acute phase response as an index of improvement has limitations in circumstances, where there are reasons to think that it may be dissociated from clinical changes, for instance in the case of a therapy that acts directly on a cytokine. However, formal controlled trials of B-cell depletion suggest that CRP levels mirror clinical changes well, as might be expected from an indirect effect on cytokine-mediated events.

Results

The total follow-up duration at December 2005 was 180 patient-ys. Patient 25 declined follow-up from the outset. Follow-up in patients 14, 24 and 27 was intermittent but current status was established. Of the patients, 9 received one cycle of therapy, 13 patients two cycles, eight patients three cycles, five patients four cycles and two patients received five cycles (Fig. 2).
All patients achieved depletion of circulating B lymphocytes to levels below the limit of detection for all cycles of treatment (<0.005 x 10^9/l), except for one case (patient 8) following her second cycle of treatment. This patient had received a reduced protocol on her first treatment. In one patient (patient 3) treatment was aborted because of an urticarial reaction on the third cycle. Peripheral blood CD19 counts were available in most patients, and it was found that the period of B-cell depletion (CD19+ cells <0.005 x 10^9/l) was similar following successive cycles of treatment (Fig. 3).

The average duration of benefit from a single cycle of treatment was 15 months (range 6–43 months) and time to re-treatment averaged 20 months (range 5–60 months), the discrepancy largely reflecting the logistics of planning and executing re-treatment and in two cases temporary retrial of standard agents.

The level of improvement as measured by the CRP percentage drop after each cycle did not diminish significantly with subsequent cycles (Fig. 4). Twenty-two patients have now reached 5yr follow-up. Nineteen patients (51%) remain on the programme, including 10 reaching 5yr follow-up. Patients were withdrawn for lack of efficacy (n = 5), including all four rheumatoid factor negative cases), hypersensitivity infusion reaction (n = 1), brevity of response (n = 8), occurrence of respiratory complications (n = 1), or adequate control on standard therapy (n = 2).

Immediate adverse reactions to therapy were infrequent. The only immediate hypersensitivity reaction requiring withdrawal from the programme was an urticarial eruption during a third-cycle infusion. Four patients had febrile episodes within 5 days of an infusion, raising the possibility of some form of late hypersensitivity reaction, possibly mediated by immune complexes.

Two patients have died, but neither during a period of B-lymphocyte depletion. One was transferred to a TNF-neutralizing agent because of a relatively brief response to rituximab therapy. Three months after commencing the TNF-neutralizing agent he died suddenly with evidence of a respiratory infection. The second patient, who had been warfarinized for some years for a past history of cerebral thrombosis, suffered a cerebral haemorrhage.

Of 16 lower respiratory events in 10 patients, 12 were considered infective (fever, cough, sputum production) and the other four (aforementioned) had similar symptoms but occurred within 5 days of the second infusion, and therefore may have had a hypersensitivity rather than infective component. Infection was not formally proven in any single case. All patients responded to broad-spectrum antibiotics despite this lack of positive cultures. Only one of the apparently infective episodes occurred in association with low immunoglobulin levels.

Three patients receiving cy and rituximab developed carcinoma of the breast, all three being apparently disease-free following treatment at least 3 yrs later. One patient developed carcinoma of the ovary, currently in complete clinical and biochemical remission 2 yrs following cisplatin therapy, and one transitional cell carcinoma, treated locally. One patient who received rituximab without cy developed renal cell carcinoma, apparently successfully cleared by nephrectomy.

In most cases immunoglobulin levels remained within the normal range, even after repeated cycles of treatment. However, in a minority, progressive falls in all classes of immunoglobulins (IgA, IgM, IgG) were observed with repeated cycles (Fig. 5). IgM levels, in particular, tended to fall, in 12 cases to below the normal range and in three of these to undetectable levels. IgG levels fell to below the normal range in seven cases and IgA in one case. Table 1 shows the patients who had low levels of immunoglobulin isotypes after each cycle, and those who had more than one low isotype of immunoglobulins. Based on the aforementioned, it does not seem possible to identify a pattern which predicts which patients will develop hypogammaglobulinaemia in subsequent cycles. Hypogammaglobulinaemia was not associated with adverse clinical events except in one patient, with exacerbation of cough and sputum production in the context of bronchiectasis. Co-existent psoriasis in one rheumatoid factor positive and one rheumatoid factor negative case showed no improvement.

Discussion

An increasing number of biological agents that target specific immunological pathways are showing promise as potential therapies for RA. The ultimate measure of the usefulness of these agents is the ability to produce benefit over an extended period, without undue risk from immunodeficiency. B-lymphocyte depletion therapy based on rituximab has been shown to have a good level of efficacy in RA over a 1–2yr period [1–3, 8]. Three questions now require consideration. First, do hypersensitivity reactions or blocking immune responses to the drug limit repeated usage? Secondly, will experience with larger numbers reveal an important increase in infection risk during even a single cycle of therapy? Thirdly, and perhaps the issue of greatest uncertainty, will repeated cycles of depletion lead to progressive humoral immunodeficiency and risk of infection?

![Fig. 3](image1.png)

**Fig. 3.** Time in months to B-cell return to the periphery *(defined as CD19+ positive cells <0.005 x 10^9/l)* plotted for individual patients following successive cycles of B-cell depletion therapy (BCDT).

![Fig. 4](image2.png)

**Fig. 4.** Percentage decrease in C-reactive protein level from pre-treatment to nadir for repeated cycles of B-lymphocyte depletion with rituximab. Numbers of patients are given above each box plot. Median, range, 25th and 75th percentile charted.
Repeated B lymphocyte depletion in rheumatoid arthritis

B-lymphocyte depletion in RA was initiated on the basis that, if successful, it might be expected to induce sustained remission such that their immune systems are fully reconstituted most of the time, B-lymphocyte depletion is an extremely attractive form of therapy. The single-most significant drawback is that patients have to accept that relapse will eventually occur, and that at present there is no clear strategy for preventing this with pre-emptive re-treatment. The dilemma faced is that pre-emptive treatment will by definition reduce the period during which immunological competence is restored.

For patients who relapse at the time of B-lymphocyte repopulation, B-lymphocyte depletion is theoretically less attractive since continued immunosuppression is involved. Continued B-lymphocyte depletion may be unsuitable for such cases, but where no alternative therapy is available it may still be justified.

The experience with repeated cycles of B-lymphocyte depletion with rituximab is encouraging, in that efficacy appears to be maintained with repeated cycles, drop out from hypersensitivity appears uncommon, and no clear evidence for increased rates of infection due to hypogammaglobulinaemia has been found (only in one episode was the patient hypogammaglobulinaemic). There was little evidence of increased susceptibility to infection, in general, during periods of B-lymphocyte depletion. However, two caveats remain; a suggestion that lower respiratory tract problems may be more common and evidence for cumulative effects on total immunoglobulin levels, in particular IgM, following repeated cycles. The issue of lower respiratory tract problems requires continued surveillance.

In the cohort of patients described, B-lymphocyte depletion remained a satisfactory form of therapy in 45% (10/22) of patients recruited early and followed for 5 yrs. The overall continuation rate was 51% (19 patients remain on the programme). This may reflect in part a shift in the type of patient included as recruitment widened and also the changing availability of other biological agents. These rates cannot yet be compared directly with rates for TNF-neutralizing agents, but may be inappropriately low for several possible reasons. First, when faced with a choice of continuing rituximab or converting to a licensed biological agent, both the physician and the patient included in the judgement the fact that rituximab was an experimental therapy with relatively little safety documentation in RA, despite its extensive use in lymphoma. Secondly, discontinuation of rituximab in cases with relatively brief responses was also influenced by theoretical concern about inadequate periods of immunological reconstitution which may in future prove clinically unfounded. Thirdly, at least one patient decided to transfer to another agent because of the disappointment associated with relapse. The identification of biomarkers for relapse or other means will hopefully allow us to develop a strategy for pre-emptive treatment, and therefore avoid this issue in the future.

It seems likely in retrospect that three of the four rheumatoid factor seronegative cases, who did not respond clinically, would be better classified as ‘rheumatoid-type’ psoriatic arthropathy, and that neither psoriasis nor associated arthropathy is likely to respond to B-lymphocyte depletion. However, at the time of treatment there was no reason to think that these patients were other than seronegative RA. The REFLEX and DANCER studies suggest that there is a positive clinical response in some RhF negative patients [9, 10]. Detailed data on methodology of RhF detection and magnitude of clinical responses in these patients have not been published at the time of writing.

In summary, experience with repeated B-cell depletion therapy in RA suggests that ~80% of seropositive patients may respond, and 50–60% become susceptible to continuing control of disease. Secondary resistance appears not to be a problem over 2–5 yrs. Susceptibility to chest infection may be increased and requires further surveillance. Cumulative effects on immunoglobulin levels may occur with frequently repeated usage.

Conflicts of interest statement: J. E. has received honoraria for lectures and infrastructural clinical support from Roche
of £80,000. M. L. has received a research grant from GlaxoSmithKline Research and Development and honoraria from Roche Farmacêutica Química Lda.

References


| Table 1. Patients who had levels of immunoglobulin classes below the lower limit of the normal range are given after each cycle of B-cell depletion therapy |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                | 1st cycle       | 2nd cycle       | 3rd cycle       | 4th cycle       | 5th cycle       |
| IgM             | 5, 7, 10, 11, 12, 13, 17, 24, 25°, 29 | 5°, 7°, 10, 11°, 12°, 13°, 17, 30 | 7, 12, 17°, 19 | 12°, 17° | 17° |
| IgA             | 3°, 11°, 12°, 25° | 3°, 11°, 12° | 7, 12° | 12° | 23° |
| IgG             | 2, 3, 5, 11°, 23°, 25°, 30 | 3, 5, 7, 11°, 23°, 30° | 2, 6, 7°, 12, 23° | 12°, 23° | 23° |
| IgG + IgA       | 3, 11 | 3, 11 | 7, 12 | 12 | 23 |
| IgG + IgM       | 5, 11 | 5, 7, 11, 30 | 7, 12 | 12 |
| IgM + IgA       | 11, 12 | 11, 12 | 7, 12 | 12 |
| IgM + IgA + IgG | 11 | 11 | 7, 12 | 12 |

Patient numbers are those shown in Fig. 2. Patients denoted with an asterisk (*) had a low level of the immunoglobulin class prior to that respective cycle of treatment.