CONCISE REPORT

Effects of rituximab on resistant SLE disease including lung involvement

JA Reynolds1, V Toescu1,2, CS Yee1,2, A Prabu1,2, D Situnayake1 and C Gordon1,2

1Department of Rheumatology, City Hospital, Birmingham, West Midlands, UK; and 2Department of Rheumatology, Division of Immunity and Infection, University of Birmingham, Birmingham, UK

We present a retrospective review of 11 patients with refractory systemic lupus erythematosus (SLE) treated with rituximab after failing corticosteroids and at least one other immunosuppressive drug. We measured clinical response using the Classic British Isles Lupus Assessment Group (BILAG) index, serum complement and reduction in maintenance prednisolone dose. B cells were measured using flow cytometry, and lung function testing was used to assess severe pulmonary disease (three patients). The median patient age was 42 years (range, 25–64) with median disease duration 6 years (range, 2–12). In all, 10 of 11 patients responded initially, with median global BILAG reduction of 7.5 at 6 months ($P = 0.007$), with loss of all A and B scores by 7 months. Rituximab treatment was associated with normalisation of complement ($C_3 P = 0.008$, $C_4 P = 0.018$) and reduction in steroid requirement, median reduction 15 mg/day ($P = 0.036$). In 9 of 10 patients who responded, all other immunosuppressants were stopped. There was no significant difference in anti-dsDNA antibody titres in these responders, but they were negative or had low titres at baseline. B-cell depletion continued for median 4 months (range, 2–9), and disease flare occurred at a median 6.6 months (range, 1.5–23) and was preceded by B-cell recovery in all but two patients. Rituximab was beneficial in refractory SLE including severe neurological and cardiorespiratory disease by inducing disease remission, allowing withdrawal of other agents and reduction in steroid requirement. Rituximab appeared to stabilise and possibly improve progressive lung disease. *Lupus* (2009) 18, 67–73.

Key words: B cells; lung disease; rituximab; SLE

Introduction

Systemic lupus erythematosus (SLE) is a multi-system, inflammatory, autoimmune disorder characterised by the formation of anti-nuclear antibodies. The clinical manifestations of lupus range from mild symptoms to life-threatening multi-organ failure.1 SLE is responsible for considerable morbidity and premature death in these patients.2

Conventional treatment for SLE currently comprises a combination of corticosteroids and immunosuppressants, augmented by antimalarial agents. Although many patients can be well controlled with low-dose steroids and milder agents alone, a significant number requires increasingly potent agents in order to induce and maintain clinical remission. Although newer agents, such as mycophenolate mofetil, offer some hope to those who do not respond well to cyclophosphamide, a number of patients continue to suffer devastating disease.

Lupus is primarily a disease of B-cell origin.3 The B cell is involved in the development of the autoimmune state, in the proliferation and progress of the immune response and directly in tissue inflammation.4 Inhibiting or removing autoreactive B cells while leaving the rest of the immune system intact offers an attractive potential treatment strategy. Rituximab is a chimeric mouse-human monoclonal antibody directed against the CD20 found on all human B cells with the exception of very early (B-cell progenitor) and terminally differentiated (plasma) cells.5 CD20 provides an ideal target as it is neither internalised nor soluble, has no known natural ligand or analogue, and anti-CD20 immunoglobulin initiates B-cell death.6
To date, no randomised control trials of rituximab in lupus have been published although multiple case reports and small open-label studies described the clinical benefit of B-cell depletion. The largest open study of 24 patients with SLE (mean follow-up of 23 months) showed reduced disease activity, normalisation of complement and reduced steroid requirement in patients unresponsive to conventional immunosuppressants.7 The majority of these patients had lupus nephritis and had been treated with azathioprine and cyclophosphamide. Neuropsychiatric manifestations of lupus, predominantly acute confusional state and mood disorder also responded rapidly to rituximab.8

In this study, we report our experience of rituximab in patients with less common features of lupus including those with neurological (transverse myelitis, peripheral neuropathy, seizures, psychosis) and severe cardiorespiratory disease (interstitial fibrosis, pericardial pain).

**Methodology**

We identified 11 patients treated with rituximab, under regular follow-up in dedicated lupus clinics in Birmingham. Nine patients met the revised American College of Rheumatology (ACR) classification criteria for SLE.9 Two patients only satisfied three criteria. All the patients had a clinical diagnosis of lupus that was confirmed by a rheumatologist. Disease duration was defined as the period of time from development of the fourth (or third in those with less than four) criteria. All patients were treated with rituximab according to clinical need after failing to respond to one or more conventional immunosuppressants. All patients provided written consent to take part in a study with approval from the local research ethics committee.

Eight patients were treated with standardised doses of either 750 mg or 1 g of rituximab on two occasions, 2 weeks apart.7 Seven patients had concomitant intravenous cyclophosphamide (500–750 mg), and six patients had intravenous methylprednisolone (250–500 mg). Two patients were treated according to local protocols for treatment of B-cell lymphoma with two rituximab infusions of 375 mg/m² with no concomitant cyclophosphamide,10 and one patient received two infusions of 500 mg rituximab with 750 mg cyclophosphamide. Following treatment, patients were allowed to continue with hydroxychloroquine and prednisolone.

Disease activity was measured at routine follow-up clinic appointments using the Classic British Isles Lupus Assessment Group (BILAG) index.11 Global BILAG score was calculated by attributing numerical scores correlating to the categorical score for each individual system (A = 9, B = 3, C = 1, D/E = 0), which was then summated.7 Reduction in global BILAG score and loss of individual A or B scores provided objective assessment of disease response to treatment. A flare of disease was defined as a major (new A) or minor (new B) flare.12

The B lymphocyte count following rituximab was measured using flow cytometry. B-cell recovery was defined as B-cell count $\geq 0.01 \times 10^9/L$. We aimed to measure B cells every 4 weeks following rituximab although this was not always possible due to patients not attending appointments and other practical problems. Disease biomarkers especially anti-dsDNA antibody titres and serum complement were measured routinely at follow-up clinic visits. When there was no 6 months sample available, the next sample taken was used. All pulmonary function measurements were performed according to Association for Respiratory Technology and Physiology/British Thoracic Society (ATRP/BTS) guidelines for the measurement of respiratory function13 and were requested on clinical grounds.

Data were analysed using the Wilcoxon signed-rank test for non-parametric paired data.

**Results**

**Baseline characteristics**

We identified 11 lupus patients who had been treated with rituximab. In all, 9 of 11 patients fulfilled four ACR criteria and the remaining two patients fulfilled three. All 11 patients were positive for ANA. The demographic variables are summarised in Table 1. The mean patient age was 42 years (range, 25–64), and median disease duration was 6 years (range, 2–16).

At the initiation of rituximab treatment, four patients had no damage (SLICC/ACR damage index). The median damage index score was 1 (range, 0–5).

**Previous treatment**

Before rituximab therapy, all patients had failed one or more immunosuppressant agents (median number failed = 2). In all, 8 of 11 patients had previously been treated with intravenous cyclophosphamide. In addition to oral prednisolone, patients had previously been treated with azathioprine ($n = 3$), methotrexate ($n = 3$), cyclosporine A ($n = 4$) and mycophenolate mofetil ($n = 6$).
Immediately before rituximab therapy, most patients were receiving intravenous cyclophosphamide \((n = 2)\) or mycophenolate \((n = 5)\). Two patients were being treated with either cyclosporine A or azathioprine (Table 2).

**Disease activity at baseline**

In all, 8 of the 11 patients had active disease (A or B) scores on the BILAG index. These were predominantly neurological \((n = 4)\) and haematological \((n = 3)\), also general \((n = 2)\), renal \((n = 2)\), cardiorespiratory \((n = 2)\), mucocutaneous \((n = 2)\) and musculoskeletal \((n = 1)\). The three remaining patients had severe active cardiosrespiratory disease including interstitial lung disease and pericardial pain with accumulated damage. Neurological disease comprised of transverse myelitis, peripheral neuropathy, seizures and neuropsychiatric disorders. As these patients did not have the requisite investigations (e.g., chest radiograph, lung function tests) carried out in the 4 weeks before starting therapy, the Classic BILAG index score for these systems did not achieve disease activity scores of A or B despite the patients being symptomatic.

**Analysis of response following first dose of rituximab treatment**

Initial follow-up was censured at time of analysis or at re-treatment. At 3 months, there were 11 patients available for analysis, and at 6 months, there were 8 patients. The median follow-up after first treatment was 10 months (range, 3–13).

**B-cell counts**

All patients achieved full B-cell depletion \((CD19 < 0.005 \times 10^9/L)\) with rituximab regardless of the dose of rituximab used (and/or cyclophosphamide). Two patients had insufficient monitoring of B cell markers post-rituximab and were therefore excluded from the analysis of B-cell response. Seven of the nine patients analysed for B cells showed B-cell recovery of \(\geq 0.01 \times 10^9/L\) during the first year after treatment (median time to B-cell recovery = 4 months,

### Table 1  Profile of the 11 patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age</th>
<th>Ethnic origin</th>
<th>ACR criteria*</th>
<th>Disease duration (y)</th>
<th>SLICC/ACR damage index</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>30</td>
<td>South Asian</td>
<td>Arthritis, haem, immunol, ANA</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>47</td>
<td>Afro-Caribbean</td>
<td>Oral ulcers, arthritis, serositis, renal, haem, immunol, ANA</td>
<td>16</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>29</td>
<td>Caucasian</td>
<td>Photosensitivity, oral ulcers, arthritis, serositis, neuro, haem, ANA</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>32</td>
<td>Afro-Caribbean</td>
<td>Malar rash, oral ulcers, arthritis, renal, neuro, haem, immunol, ANA</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>25</td>
<td>Afro-Caribbean</td>
<td>Malar rash, oral ulcers, arthritis, renal, haem, immunol, ANA</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>42</td>
<td>Afro-Caribbean</td>
<td>Arthritis, haem, ANA</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>46</td>
<td>Caucasian</td>
<td>Renal, neuro, haem, immunol, ANA</td>
<td>11</td>
<td>5</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>58</td>
<td>Caucasian</td>
<td>Photosensitivity, serositis, immunol, ANA</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>64</td>
<td>Caucasian</td>
<td>Photosensitivity, arthritis, ANA</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>54</td>
<td>South Asian</td>
<td>Malar rash, discoid rash, arthritis, renal, haem, immunol, ANA</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>11</td>
<td>F</td>
<td>36</td>
<td>South Asian</td>
<td>Malar rash, oral ulcers, arthritis, immunol, ANA</td>
<td>6</td>
<td>1</td>
</tr>
</tbody>
</table>

Disease duration calculated from time of onset of the fourth ACR criteria\(^9\) (or third if <four).

### Table 2  Drug therapy before rituximab, immediately before and 3 months after rituximab

<table>
<thead>
<tr>
<th>Patient</th>
<th>Previous drug therapy</th>
<th>Drug therapy immediately before rituximab</th>
<th>Drug therapy after rituximab (3 months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>OHCHQ, AZT, CYP</td>
<td>CYP</td>
<td>OHCHQ (restarted)</td>
</tr>
<tr>
<td>2</td>
<td>OHCHQ, AZT, MTX, CYP, MMF</td>
<td>OHCHQ, MMF</td>
<td>OHCHQ</td>
</tr>
<tr>
<td>3</td>
<td>OHCHQ, MTX, MMF, CYA</td>
<td>OHCHQ, MMF</td>
<td>OHCHQ</td>
</tr>
<tr>
<td>4</td>
<td>OHCHQ, AZT, MTX, CYP, CYA</td>
<td>CYP</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>CYP, MMF, CYA</td>
<td>MMF</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>OHCHQ, CYP, MMF</td>
<td>CYA</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>CYA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>OHCHQ, CYP</td>
<td>OHCHQ</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>CYP, MMF</td>
<td>MMF</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>AZT</td>
<td>AZT</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>CYP, MMF</td>
<td>MMF</td>
<td>Excluded – non Responder</td>
</tr>
</tbody>
</table>

Abbreviations: OHCHQ: hydroxychloroquine; AZT: azathioprine; CYP: cyclophosphamide; MTX: methotrexate; MMF: mycophenolate mofetil; CYA: cyclosporine A.
range, 2–9). In two cases, clinical relapse occurred before B-cell recovery. These were both minor flares with B scores in general, mucocutaneous and renal systems and occurred 2.5 and 6 months before B cells were detected. The two patients with no B-cell recovery during follow-up have not yet had disease flare at 4 and 16 months.

Clinical response to first treatment
In all, 10 of 11 patients responded clinically to rituximab. One patient had minimal response to first treatment with rituximab despite B-cell depletion (B cell return at 3 months; re-treatment at 5 months).

Before treatment, the responders collectively had 5 A scores and 11 B scores. Six months after treatment, all A scores had resolved and only one B score remained (Table 3). This remaining B score was persistent in a patient with autoimmune haemolytic anaemia and reduced to a C score by 7 months. There was a reduction in the total number of C scores from 16 to 9. In all 10 responders, the median global BILAG index score reduction was 2 (range, −5–18; Wilcoxon signed-rank test \( P = 0.036 \)) at 3 months and 7.5 (range, 0–19; \( P = 0.018 \)) at 6 months (Figure 1).

During the follow-up period, only 4 of 10 responders had a flare of disease. The median time-to-flare, defined by new onset of A score (major flare) or B score (minor flare), was 6.6 months (range, 1.5–23).

Immunology
Rituximab showed no significant effect on anti-dsDNA antibody titres at 3 months or 6 months, but the responders were negative or only low titre positive at baseline (all <80 ku/L; borderline levels for our laboratory are 25–75 ku/L). At baseline complement, C3 level was low in 3 of 10 patients (<0.74 g/L) and C4 was low in 5 of 10 patients (<0.14 g/L). One patient has no results for C3/C4 levels measured after rituximab. By 6 months post-rituximab, the number of responders with normalisation of complement levels was eight of nine for C3 and seven of nine for C4. Increase in the complement levels showed statistical significance at 6 months; C3: baseline median = 0.89 g/L (Interquartile range [IQR], 0.76), 6 months median = 1.25 g/L (IQR, 0.72; \( P = 0.008 \)); C4: baseline median = 0.15 g/L (IQR, 0.19), 6 months median = 0.26 g/L (IQR, 0.175; \( P = 0.018 \)).

Immunosuppressants
The median steroid dose at baseline was 30 mg (IQR, 26.25). The median reduction at 3 and 6 months was 7.5 mg (IQR, 8.62; \( P = 0.03 \)) and 15 mg (IQR, 8.75; \( P = 0.036 \)), respectively. During this time, all other immunosuppressive agents were stopped in 9 of 10 patients. One patient continued with mycophenolate mofetil after rituximab at a constant dose. Hydroxychloroquine was continued in four patients and did not appear to reduce the incidence of disease flare.

During the initial 6 months after rituximab, no new immunosuppressants were started. Reintroduction of immunosuppressants was necessary in six of nine patients (excluding one patient who continued with mycophenolate). Immunosuppressants were restarted at least 6 months after rituximab-induced response. Mycophenolate (\( n = 3 \)) and azathioprine (\( n = 1 \)) were restarted at median 11 months (range, 10–29) in response to disease flare. Two patients were retreated with rituximab at 9 and 10 months.

Lung function
Three patients had severe lung disease, with significant morbidity. One patient responded well to rituximab,
with improvement in lung function. Following rituximab, there was an increase in forced vital capacity (FVC; Figure 2) and total lung capacity (data not shown). In the remaining two patients, respiratory function stopped deteriorating; one patient had insufficient lung function tests to identify any trend but clinically was stable and therefore was not retested. Improvement in FVC did not achieve statistical significance ($P = 0.11$).

### Response to rituximab re-treatment

At the time of analysis, three patients, including the non-responder, had received multiple infusions of rituximab. The initial non-responder received a total of two treatments, whereas the remaining two patients received three. Re-treatment was with 750 mg–1 g of rituximab and up to 750 mg cyclophosphamide, two doses of each 2 weeks apart. The non-responder had previously failed cyclophosphamide and mycophenolate and therefore a second attempt was considered appropriate.

The median time to re-treatment following previous rituximab therapy was 9 months (range, 4–10). In all cases, repeat treatment was prompted by a major flare (new A score) in one or more systems. The median global BILAG at re-treatment was 17 (range, 15–33).

Similarly to first rituximab treatment, the B-cell response to repeat treatments was highly variable. There was no correlation between the number of treatments given and the subsequent period of B-cell depletion. The B-cell depletion in these three patients, excluding that following the first treatment, was mean 5 months (range, 2–7). No relationship between the time for B-cell repopulation in the first and the subsequent treatments could be shown. The maximum reduction in global BILAG index score following rituximab was a median of 14 (range, 11–29) at 6 months after re-treatment. The initial non-responder had a very high titre of anti-dsDNA antibodies that only improved after re-treatment in parallel with clinical response. The median time-to-flare (major and minor) was 8 months (range, 5–10), whereas the median time-to-flare (major) was 9 months (range, 6–11) as shown in Table 5.

No immunosuppressants were restarted in two patients. One patient was restarted on mycophenolate briefly following the second dose, but this was stopped before the third dose as disease activity persisted. Median steroid reduction was 2.5 mg (IQR, 2.5) within 6 months of treatment. Improvement in disease activity as measured by the BILAG index and reduction in steroid dose are shown in Table 5.

### Adverse reactions

There were no serious infusion reactions. One patient experienced diffuse muscular and joint pain while...
receiving the second infusion rituximab. This was relieved by slowing the speed of the infusion and did not prevent the full dose being given. This patient is, however, reluctant to receive further rituximab. One patient who was paraplegic, and who had received three doses of rituximab, required admission for abscesses secondary to infected pressure sores and infected vasculitic leg ulcers. These required treatment with intravenous antibiotics but healed successfully.

Discussion

The results of this study lend further support to the use of rituximab in the management of severe SLE which has failed to respond to conventional immunosuppressants. This series of patients includes two patients who do not fulfil four ACR criteria for the classification of SLE but who had a firm clinical diagnosis of SLE. Much of the previous work has focused on renal, musculoskeletal and skin disease. In this study, our heterogeneous group comprises more patients with severe cardiorespiratory and neurological disease.

The magnitude of disease activity at baseline, as measured by the BILAG index, was variable within the group. This was particularly noticeable in the patients with severe cardiorespiratory disease and renal disease as there was significant under-scoring of activity in patients who had not had recent imaging or biopsies. These patients were severely symptomatic but achieved only low to modest global BILAG index scores at baseline. The observed minor reduction in global BILAG index score in these patients belies major symptomatic improvement. The new BILAG 2004 index would not have suffered from these initial assessment problems but was not available when this study started.

The treatment protocols evolved over the time of the study. Conservative doses of 500 mg 2 weeks apart were initially used; following the work of other groups; this was increased to 1 g with 750 mg cyclophosphamide 2 weeks apart. Smaller doses of rituximab did not correlate with a shorter duration of clinical remission.

There was complete B-cell depletion in all patients irrespective of the dose of rituximab used or the concomitant use of cyclophosphamide. Recovery of the B-cell population occurred at around 5 months, similar to that reported by other groups. In one patient, B-cell depletion occurred without any significant clinical response. Rituximab failure and clinical deterioration in the context of B-cell depletion have been reported previously, but he or she responded to a second course of rituximab, raising the possibility of incomplete tissue depletion of B cells after the first course.

In our study, B-cell repletion always preceded a major flare of disease (new A score). The significance of minor flares (new B score) is more difficult to define as they can be scored more easily, with little serious disease progression but symptoms that concern the patient. In our group, three of four of the minor flares occurred in one system only and only two of four prompted an increase in steroid treatment. Conversely, persistent renal disease may score only a C (i.e., no worse) following a B score. These problems are inherent to the scoring system of classic BILAG index but have been changed in the BILAG 2004 index.

Table 5 Changes in disease activity and steroid dose over 6 months following rituximab

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Second rituximab</th>
<th>Third rituximab</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time from previous RTX</td>
<td>A Scores</td>
</tr>
<tr>
<td>3</td>
<td>9 months</td>
<td>2A 1B 2C</td>
</tr>
<tr>
<td></td>
<td>1A 2B 1C</td>
<td>2C</td>
</tr>
<tr>
<td></td>
<td>1B 1C</td>
<td>17.5–15</td>
</tr>
<tr>
<td>4</td>
<td>10 months</td>
<td>1A 1B 3C</td>
</tr>
<tr>
<td></td>
<td>1B 1C</td>
<td>1B 1C</td>
</tr>
<tr>
<td>11 (non-responder)</td>
<td>4 months</td>
<td>Renal</td>
</tr>
<tr>
<td></td>
<td>1A 2B 1C</td>
<td>1B 1C</td>
</tr>
<tr>
<td></td>
<td>20–20</td>
<td>17.5–15</td>
</tr>
</tbody>
</table>

Abbreviations: RTX: rituximab; BILAG: British Isles Lupus Assessment Group.
Rituximab therapy eliminated major disease activity in all active systems by 6 months. Clinical improvement was confirmed by reduction in global BILAG index score, with concomitant normalisation of serum complement titres and reduction in daily prednisolone requirement. The duration of response was highly variable between individuals. In all, 6 of 11 subjects have remained in remission following one treatment and despite all other immunosuppressants being stopped. Most of these have, however, shown signs of B-cell recovery and may be expected to flare in the future. We were unable to show any significant change in dsDNA autoantibody titres although this has been shown by others recently.17 This may be due to many of our patients having low anti-dsDNA antibody titres at baseline and thus removing the potential for any significant reduction.

Repeated doses appear to be well-tolerated and of moderate efficacy as previously shown.18 Failure of B-cell depletion is postulated to be related to development of human antichimeric antibodies, which may be more prevalent in Afro-Caribbean patients.19 The main complications of rituximab appear to be mild infusion reactions and recurrent infections, particularly herpes virus.7,18,19

Very little is known about the role of rituximab in the management of lupus lung disease. There have been isolated case reports of improvement in lung disease particularly pneumonitis.20,21 We have shown that rituximab halted respiratory disease progression in our three patients. In one patient, there was objective improvement in lung function. This improvement can be attributed to rituximab alone as mycophenolate mofetil was stopped and prednisolone reduced. For the other patients with severe lung disease, pulmonary function testing was infrequent. Despite this, we suggest that rituximab halted deterioration in these patients, whereas disease progression had continued in previous immunosuppressants.

In summary, we provide further evidence for the use of rituximab in difficult to manage lupus, especially patients with predominantly lung and neurological disease. The treatment is safe, results in disease remission and allows the clinician to stop all other immunosuppressant agents. We feel that the effect on lung function warrants further investigation in a prospective trial.

Acknowledgements

We would like to thank Lupus UK, Arthritis Research Campaign and the Wellcome Trust Clinical Research Facility for their support. We are grateful to Dr Nicky Erb, Dr Jo Adu and Dr Mark Drayson for their assistance in obtaining clinical and laboratory data.

References
