CONCISE REPORT

Repeated B cell depletion in treatment of refractory systemic lupus erythematosus

K P Ng, M J Leandro, J C Edwards, M R Ehrenstein, G Cambridge, D A Isenberg

Ann Rheum Dis 2006;65:942-945. doi: 10.1136/ard.2005.044487

Objectives: To report the clinical outcome and safety profile of repeated B cell depletion in seven patients with refractory systemic lupus erythematosus (SLE).

Methods: Since June 2000, seven patients with refractory SLE had repeated cycles of B cell depletion (18 cycles in total, up to three cycles per patient) because of disease relapse. The clinical response (assessed by the British Isles Lupus Activity Guide (BILAG) activity index), duration of B cell depletion, and adverse events in these patients was reviewed.

Results: Four patients (Nos 1, 2, 3, 6) had three cycles of treatment and three (Nos 4, 5, 7) had two cycles. Four of the seven patients (Nos 1, 3, 5, 6) improved. The mean global BILAG scores dropped from 15 to 6 at 5-7 months. The median duration of clinical response and B cell depletion was 13 months and 6 months, respectively. After the third cycle, 2/4 patients (Nos 1 and 2) improved. The median duration of clinical benefit was 12 months. Most patients tolerate re-treatment very well.

Conclusion: Re-treatment with B cell depletion of patients with severe SLE is safe and may be effective for 6-12 months on average.

Systemic lupus erythematosus (SLE) is an autoimmune rheumatic disease characterised serologically by the production of numerous autoantibodies. Rituximab is a chimeric monoclonal antibody specific for human CD20 with variable regions of a murine antihuman CD20 B cell hybridoma fused to human IgG antibody. Recently, there have been encouraging preliminary clinical outcome reports about the use of B cell depletion therapy in patients with SLE.

Our centre reported significant clinical improvement in six patients with active, refractory SLE with a combination protocol of rituximab and cyclophosphamide.¹ Subsequently, we have reported 6 month follow up data on 24 patients using a combination protocol.² Looney *et al* showed that rituximab alone reduced global lupus activity measured by the Systemic Lupus Activity Measure (SLAM) index at 3 months in patients with relatively mild lupus.³

There may be a role for repeated B cell depletion in patients with severe SLE who relapse after one cycle of rituximab. However, the safety and clinical efficacy of this is unknown. This study reports the clinical outcome in seven lupus patients treated with repeated B cell depletion at our centre.

PATIENTS AND METHODS

Patients

Since June 2000, seven of 24 patients with refractory SLE receiving B cell depletion therapy in our centre have had repeated cycles of treatment. All patients fulfilled at least four

of the revised American College of Rheumatology criteria⁴ for the classification of SLE and gave informed consent to re-treatment. Patients were re-treated if a relapse of disease occurred. Relapse was defined as the appearance of a new British Isles Lupus Activity Guide (BILAG) "A" or two new "B"s (except for one patient who had a single new B) from a previous record of BILAG "C", "D" or "E" in any organ system. Conventional immunosuppressive treatment, including intravenous cyclophosphamide, had already failed for these patients. Clinical response was defined as a loss of BILAG "A" or "B" after treatment.

Assessment

Patients were assessed at 1-3 monthly intervals. At each visit, activity of disease was measured using the BILAG index. Antibodies to double stranded DNA (anti-dsDNA) were measured by enzyme linked immunosorbent assay (ELISA; Shield Diagnostics, Dundee, UK) (normal <50 IU/ml) and serum C3 by laser nephelometry (normal 0.90-1.80 g/l) at each assessment. Patients with lupus nephritis also had the protein/creatinine ratio (normal <13 mg/mmol) measured from a random urine sample.

Serum immunoglobulins levels were measured by immunoturbidometry (IgA normal 0.7-4.0 g/l, IgG normal 7.0-16.0 g/l, IgM normal 0.4-2.3 g/l) and B cell depletion monitored by circulating CD19+ cell count ($<0.005 \times 10^9/l$ in peripheral blood).

Treatment regimen

The treatment regimen for each cycle was two infusions of rituximab and intravenous cyclophosphamide, each given 2 weeks apart (table 1). Patient 4 had no cyclophosphamide owing to a previous allergy. Steroid cover was given with each cycle.

Routine immunosuppressive drugs were stopped before the first cycle except for hydroxychloroquine and oral steroids. However, mycophenolate was added in three patients (Nos 2, 3, and 6) after the third treatment cycle. Mycophenolate was introduced in patient 5 seven months after the second cycle. Patient 7 had methotrexate added 7 months after the initial cycle.

RESULTS

A total of 18 cycles of treatment and up to three treatment cycles per patient were given. Table 1 shows the patient demographics and clinical indication for re-treatment. Four patients (Nos 1, 2, 3, 6) had three cycles of treatment, whereas three patients (Nos 4, 5, 7) had two cycles. The mean time to re-treatment was 13 months (range 3-31).

Abbreviations: BILAG, British Isles Lupus Activity Guide; SLE, systemic lupus erythematosus

Table 1 Patients' demographics, treatment regimens, and duration of B cell depletion

Patient No	Sex/age (years)/ethnicity	Disease duration (years)	Regimen for each cycle	Duration since previous cycle (months)	Main clinical indication for each cycle	Previous treatments	Duration B cell depletion (months)
1	F/45/E	16	2×500 mg RTX 2×750 mg CYC 2×60 mg Pred		Arthritis, pleuritis, shrinking lung	Steroids, HCQ, AZA, CYC, MMF, MTX, CyA	4
			2×1000 mg RTX 2×750 mg CYC 2×60 mg Pred	7	Arthritis, fever, rash		6
			2×1000 mg RTX 2×750 mg CYC 2×100 mg MP	31	Arthritis, pleuritis		7
2	F/21/A	8	2×500 mg RTX 2×750 mg CYC 2×60 mg Pred		Nephritis (IV), arthritis	Steroids, HCQ, AZA, CYC, MMF	4
			2×1000 mg RTX 2×250 mg MP	10	Nephritis, arthritis, pleuritis		Nil
			3×hA20 (375 mg/m ²) 1×500 mg CYC 3×100 mg MP	25	Nephritis, haemolytic anaemia, thrombocytopenia		Partial depletion at 2 months
3	F/52/A	15	2×1000 mg RTX 2×750 mg CYC 2×60 mg Pred		Nephritis (IV), arthritis	Steroids, HCQ, AZA, CYC, MTX, CyA	6
			2×1000 mg RTX 2×250 mg MP	7	Arthritis		9
			1×1000 mg RTX 1×750 mg CYC 1×100 mg MP	9	Nephritis		2
4	F/41/A	11	2×1000 mg RTX 2×250 mg MP		Nephritis (IV)	Steroids, HCQ, CYC, AZA, MMF, MTX	9
			2×1000 mg RTX 2×100 mg MP	13	Arthritis		Depleted at 2 months, then lost to follow up
5	F/33/E	14	2×1000 mg RTX 1×750 mg CYC 2×100 mg MP		Nephritis (IV)	Steroids, HCQ, AZA, CYC, MMF	3
			2×1000 mg RTX 2×750 mg CYC 2×100 mg MP	3	Arthritis, rash, fever		6
6	F/33/A	18	2×1000 mg RTX 2×750 mg CYC 2×250 mg MP		Nephritis (V)	Steroids, HCQ, AZA, MMF, CYC	4
			2×1000 mg RTX 2×750 mg CYC 2×100 mg MP	11	Nephritis		Depleted at 2 months
			2×1000 mg RTX 2×750 mg CYC 2×250 mg MP	12	Nephritis (IV)		Partial depletion at 3 months
7	F/42/A	5	2×1000 mg RTX 2×750 mg CYC 2×100 mg MP		Arthritis	Steroids, HCQ, AZA, CYC, MTX, MMF	3
			2×1000 mg RTX 2×100 mg MP	12	Arthritis		Partial depletion at 6 months

E, European; A, Afro-Caribbean; RTX, rituximab; CYC, cyclophosphamide; Pred, oral prednisolone; MP, methylprednisolone; hA20, humanised anti-CD20 monoclonal antibody; HCQ, hydroxychloroquine; AZA, azathioprine; MMF, mycophenolate; MTX, methotrexate; CyA, ciclosporin A.

Clinical outcome after second treatment cycle

At 4–6 months, four of seven patients (Nos 1, 3, 5, 6) improved clinically. The mean BILAG global scores for all patients dropped from 15 to 6. The mean duration of response after this cycle was 13 months, with patient 3 relapsing at

9 months (fig 1). Patient 6 with nephritis improved with a decrease of the urinary protein/creatinine ratio from 1058 to 214 mg/mmol at 6 months. Patient 2 developed human chimeric antibodies but improved at 6 months with azathioprine and one pulse dose of cyclophosphamide.

Figure 1 Global BILAG scores after cycle 2 rituximab.

Patient 4 was lost to follow up at 3 months and patient 7 with severe polyarthritis did not improve with evidence of partial B cell depletion ($CD19^+$ count $0.02 \times 10^9/l$) at 6 months.

Laboratory data after second treatment cycle

In those patients who improved, there was a corresponding decrease of median anti-dsDNA from 567 IU/l to 466 IU/l and increase of C3 from 0.65 g/l to 0.78 g/l at 6–8 months. From the available data of two patients who responded, serum immunoglobulins decreased but remained above the lower limit of normal at 6–8 months (mean IgG from 39 g/l to 36 g/l, mean IgA from 5.15 g/l to 4.7 g/l and mean IgM from 1.55 g/l to 1.15 g/l).

The mean duration of B cell depletion was 6 months (range 5–7) from available data in three patients.

Clinical outcome after third treatment cycle

Two of four patients (Nos 1 and 2) improved, with global BILAG scores dropping from 10 to 2 and 27 to 6, respectively, at 7 months (fig 2). Patient 2 with human chimeric antibodies after the second cycle responded to the third cycle of a humanised anti CD20 antibody³ but relapsed a year later. Patient 1 has remained well without any immunosuppressive drugs 13 months later. Patient 3, with biopsy evidence of renal damage, progressed to renal failure shortly after her

third cycle. Patient 6 with lupus nephritis improved with a decrease of the protein/creatinine ratio from 589 to 287 mg/mmol after mycophenolate at the last follow up at 4 months.

Laboratory data after third treatment cycle

Data were available in one patient (No 1) at 4 and 12 months. At 4 months, anti-dsDNA titres decreased from baseline 280 IU/l to 115 IU/l and C3 increased from 0.15 g/l to 0.5 g/l. Baseline serum IgA and IgG dropped from 2.5 g/l to 2.22 g/l and 12.1 g/l to 8.09 g/l, respectively. Serum IgM decreased below the normal range to 0.13 g/l at 4 months and remained low at 0.15 g/l at 12 months. This patient repopulated her B cells at 7 months.

Adverse events

Most patients tolerated re-treatment very well. Patient 1 had a urinary tract infection after the second cycle, which resolved with antibiotics. Patient 2 had thoracic shingles during her third cycle (humanised anti-CD20), which settled with intravenous aciclovir. Only one patient (No 3) had a mild serum sickness-like reaction at the third cycle and refused to proceed with the second infusion.

DISCUSSION

This study has demonstrated that repeated cycles of rituximab in combination with cyclophosphamide may provide a viable therapeutic option in managing lupus patients who are difficult to treat. Previous studies using rituximab with or without cyclophosphamide have provided very encouraging short term clinical improvement.^{1,3} These data have supported the use of rituximab in randomised controlled lupus trials which have started recently.

Clinical benefit was seen in at least 50% of patients after re-treatment in this study. The lupus features that improved were heterogeneous with a corresponding serological benefit of decrease in anti-dsDNA titres and rise of C3 levels. Serum immunoglobulins levels were well preserved except for a mild reduction of IgM with the third cycle in patient 1.

The duration of clinical benefit after each cycle was frequently longer than the period of B cell depletion. With the second cycle of rituximab, the mean duration of clinical response and B cell depletion was 13 months and 6 months, respectively. This response is also seen in patient 1 after the third cycle. This

Figure 2 Global BILAG scores after cycle 3 rituximab.

prolonged period of improvement has been seen in several patients with rheumatoid arthritis after one cycle of rituximab.⁶

Interestingly, of the four patients who improved after cycle 2 in our study, the mean duration of response was 13 months compared with an initial response of 7 months, suggesting that re-treatment may have additional benefit.

Re-treatment may be undertaken on relapse or as maintenance therapy. Maintenance therapy with full dose rituximab every 3 months was beneficial in two lupus patients with cerebral involvement as described in a case report.⁷ The long term effects of B cell depletion in lupus are unknown with particular concern of immunosuppression. Furthermore, a maintenance regimen may not be necessary if there is an extended period of benefit with each cycle.

The effects of rituximab in SLE appear to differ from those in patients with lymphoma, with a notably shorter period of B cell depletion. B cell repopulation is usually seen between 6 and 9 months in patients with lymphoma⁸ and in lupus, this can be as early as 3 months.¹ This effect may be due to inherited or acquired deficiencies of complement^{9,10} and different pharmacokinetics of rituximab in patients with SLE.

Re-treatment with B cell depletion in patients with lupus appears to be safe, with no significant infections seen in this study. A mild serum sickness-like reaction was seen in patient 3. This occurrence has been reported in patients with primary Sjögren's syndrome treated with rituximab.¹¹

B cell depletion re-treatment may be an attractive option for patients with refractory lupus who relapse. This possibility will need to be clarified in future studies.

ACKNOWLEDGEMENTS

Dr Kristine Ng is a recipient of the Rose Hellaby scholarship.

Authors' affiliations

K P Ng, M J Leandro, J C Edwards, M R Ehrenstein, G Cambridge, D A Isenberg, Centre for Rheumatology, University College London, London, UK

Competing interest: None.

Correspondence to: Professor D Isenberg, Centre for Rheumatology, Arthur Stanley House, 40–50 Tottenham Street, London W1T 4NU, UK; D.Isenberg@ucl.ac.uk

Accepted 26 October 2005

Published Online First 3 November 2005

REFERENCES

- 1 Leandro MJ, Edwards JC, Cambridge G, Ehrenstein MR, Isenberg DA. An open study of B lymphocyte depletion in systemic lupus erythematosus. *Arthritis Rheum* 2002;**46**:2673–7.
- 2 Leandro MJ, Cambridge G, Edwards JC, Ehrenstein MR, Isenberg DA. B cell depletion in the treatment of patients with systemic lupus erythematosus - a longitudinal analysis of 24 patients. *Rheumatology (Oxford)* 2005;**44**:1542–5.
- 3 Looney RJ, Anolik JH, Campbell D, Felgar RE, Young F, Arend LJ, *et al.* B cell depletion as a novel treatment for systemic lupus erythematosus: a phase I/II dose-escalation trial of rituximab. *Arthritis Rheum* 2004;**50**:2580–9.
- 4 Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF, *et al.* The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1982;**25**:1271–7.
- 5 Tahir H, Rohrer J, Bhatia A, Wegener WA, Isenberg DA. Humanized anti-CD20 monoclonal antibody in the treatment of severe resistant systemic lupus erythematosus in a patient with antibodies against rituximab. *Rheumatology (Oxford)*, 2005;**44**:561–2.
- 6 Leandro MJ, Edwards JC, Cambridge G. Clinical outcome in 22 patients with rheumatoid arthritis treated with B lymphocyte depletion. *Ann Rheum Dis* 2002;**61**:883–8.
- 7 Weide R, Heymanns J, Pandorf A, Koppler H. Successful long-term treatment of systemic lupus erythematosus with rituximab maintenance therapy. *Lupus* 2003;**12**:779–82.
- 8 McLaughlin P, Grillo-Lopez AJ, Link BK, Levy R, Czuczman MS, Williams ME. Rituximab chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: half of patients respond to a four-dose treatment program. *J Clin Oncol* 1998;**16**:2825–33.
- 9 Cartron G, Watier H, Golay J, Solal-Celigny P. From the bench to the bedside: ways to improve rituximab. *Blood* 2004;**104**:2635–42.
- 10 Di Gaetano N, Cittera E, Nota R, Vecchi A, Grieco V, Scanziani E, *et al.* Complement activation determines the therapeutic activity of rituximab in vivo. *J Immunol* 2003;**171**:1581–7.
- 11 Piipe J, van Imhoff GW, Spijkervet FK, Roodenburg JL, Wolbink GJ, Mansour K, *et al.* Rituximab treatment in patients with primary Sjögren's syndrome: an open-label phase II study. *Arthritis Rheum* 2005;**52**:2740–50.