CONCISE REPORT

B cell depletion therapy in systemic lupus erythematosus: long-term follow-up and predictors of response

Kristine P Ng, Geraldine Cambridge, Maria J Leandro, Jonathan C W Edwards, Michael Ehrenstein, David A Isenberg

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Objectives: To describe the long-term clinical outcome and safety profile of B cell depletion therapy (BCDT) in patients with systemic lupus erythematosus (SLE). It was also determined whether baseline parameters can predict the likelihood of disease flare.

Methods: 32 patients with refractory SLE were treated with BCDT using a combination protocol (rituximab and cyclophosphamide). Patients were assessed with the British Isles Lupus Assessment Group (BILAG) activity index, and baseline serology was measured. Flare was defined as a new BILAG 'A' or two new subsequent 'B's in any organ system.

Results: Of the 32 patients, 12 have remained well after one cycle of BCDT (median follow-up 39 months). BCDT was followed by a decrease of median global BILAG scores from 13 to 5 at 6 months ($p=0.006$). Baseline anti-extractable nuclear antigen (ENA) was the only identified independent predictor of flare post-BCDT ($p=0.034$, odds ratio = 8, 95% CI 1.2 to 55) from multivariable analysis. Patients with low baseline serum C3 had a shorter time to flare post-BCDT ($p=0.008$). Four serious adverse events were observed.

Conclusion: Autoantibody profiling may help identify patients who will have a more sustained response. Although the long-term safety profile of BCDT is favourable, ongoing vigilance is recommended.

B cells are thought to play an important role in the pathogenesis of systemic lupus erythematosus (SLE).[1] B cell depletion therapy (BCDT), based on rituximab, a chimeric monoclonal antibody specific for CD20, has shown considerable promise in the treatment of patients with SLE.[2] More than 100 reported cases were identified in a recent review.[3] We have previously published the short-term outcome of 24 patients with SLE treated with BCDT.[4] BCDT is generally well tolerated, but its long-term safety profile is unknown as most studies have follow-up data of less than 1 year. We now report the long-term outcome of 32 patients with SLE (the original cohort and an additional eight patients) treated with BCDT at our centre. Given the somewhat variable response to BCDT in our patients, we sought to determine if there were any particular clinical or serological profiles which might predict a better response. As part of this ongoing analysis, we determined whether the baseline autoantibody profile with reference to extractable nuclear antigens (ENAs) and complement C3 levels could be used to predict the likelihood of flare after BCDT.

METHODS

Since June 2000, 32 patients with SLE (minimum follow-up of 9 months) have been treated with BCDT after failing standard immunosuppressive therapy. This included 24/32 who had had intravenous cyclophosphamide (CyC). All patients gave informed consent and fulfilled four or more of the revised American College of Rheumatology criteria for SLE.[5] We used a combination protocol of rituximab and CyC with steroid cover given 2 weeks apart. Patients 1–6 (table 1) received two infusions of 500 mg rituximab plus CyC, with a 2-week tapering course of oral prednisolone starting at 60 mg. The remaining patients received 1 g of rituximab plus 750 mg of CyC with 100 or 250 mg of intravenous methylprednisolone. Three patients (Patients 9, 12 and 27) did not receive CyC (one refused, another had an allergy and the other patient remained on methotrexate). Hydroxychloroquine was continued in 24 patients, but all immunosuppressive drugs were stopped at the start of BCDT except in four patients (Patient 15 was started on azathioprine, Patients 27 and 16 remained on methotrexate and azathioprine, respectively, and Patient 32 continued on combination azathioprine/methotrexate).

Patients were followed-up regularly with 1–3 monthly visits. At each visit, clinical activity was assessed using the BILAG (British Isles Lupus Assessment Group) activity index.[6] Bloods were tested by ELISA for baseline anti-dsDNA (anti-double-stranded DNA) antibody titres (normal <50 IU/ml) and anti-ENA (Shield Diagnostics, Dundee, UK) prior to BCDT. Serum C3 levels were measured using laser nephelometry (normal 0.90–1.80 g/l). The urinary protein creatinine ratio (PCR) was measured for patients with renal involvement. Patients were considered to have achieved B cell depletion if absolute CD19 counts were $<0.005\times10^9$/litre. A flare was defined as a new 'A' or a new 'B' present on two consecutive occasions in any organ system of the BILAG activity index.

Statistics

Results were analysed with the Prism and SPSS software programs. Wilcoxon matched-pairs signed-rank test was used to compare global BILAG scores and laboratory results before and after BCDT. Baseline anti-ENA profiles of patients who flared versus those who did not post-BCDT were compared with Fisher's exact analysis.

Multivariable logistic regression analysis was used to identify any independent predictors of flare. Kaplan–Meier curves were generated to compare baseline serum C3 levels with time to flare post-BCDT.

RESULTS

The mean age was 34 years (range 21–54) and all but two subjects were females. The mean duration of disease and follow-up after BCDT was 11 years (range 0.7–27 years) and 39 months (range 9–78 months), respectively. Two patients were excluded from analysis (Patient 1 was lost to follow-up

Abbreviations: BCDT, B cell depletion therapy; BILAG, British Isles Lupus Assessment Group; CyC, cyclophosphamide; dsDNA, double-stranded DNA; ENA, extractable nuclear antigen; PCR, protein creatinine ratio; SLE, systemic lupus erythematosus

Table 1 Clinical and serological details of systemic lupus erythematosus (SLE) patients treated with B cell depletion therapy

Patient	Ethnicity	Follow-up duration (months)	Previous therapies	Clinical indication for BCDT	Baseline global BILAG score	C3 (g/litre)	Anti ds-DNA (IU/ml)	Anti-ENA	Time to flare post-BCDT (months)
1*	W	3	pred, hcq, aza, cyc, mmf	Fever, organic brain syndrome, arthritis	13	0.89	295	–	
2	W	78	pred, hcq, mtx, cyA	Arthritis, serositis, skin vasculitis	15	0.88	244	–	10
3	W	73	pred, hcq, aza, cyA, mtx	Lymphadenopathy, arthritis, serositis	14	0.95	570	Ro, RNP	No flare
4	W	73	pred, hcq, aza, cyc, mmf	Skin vasculitis, arthritis, serositis, nephritis (IV)	12	0.15	352	Sm, RNP	7
5	AC	70	pred, hcq, aza, cyc, mmf	Skin rash/vasculitis, arthritis,serositis,nephritis (IV)	26	0.5	2866	RNP	8
6	W	65	pred, hcq, aza, cyc	Skin rash,arthritis,nephritis (IV)	13	0.64	2560	RNP	No flare
7	AC	63	pred, hcq, aza, cyc, mtx, cyA	Arthritis, serositis, skin vasculitis, nephritis (IV)	24	0.83	3879	Ro, La, RNP	7
8	W	60.5	pred, hcq, aza, mmf, IVIG, cyc	Autoimmune thrombocytopenia, haemolytic anaemia, nephritis (IV)	16	0.4	510	–	No flare
9	O	54.5	pred, aza, cyc	Arthritis, serositis, nephritis (IV)	11	0.52	218	Ro, La	22
10	AC	50.6	pred, hcq, aza, cyc, mmf	Skin rash, arthritis, nephritis (IV)	14	0.53	39	Sm, RNP	12
11	O	50	pred, cyc, aza, cyA, mmf	Fever, skin rash, serositis, nephritis (IV)	12	0.44	13	Ro	16
12	AC	16.2	pred, hcq, cyc	Arthritis, nephritis (IV)	12	0.88	118	Ro, Sm, RNP	8
13	W	46.6	pred, hcq, aza, cyc	Fever, arthritis, nephritis (IV)	20	0.65	10	–	No flare
14	W	47.2	pred, hcq, aza	Autoimmune thrombocytopenia	3	1.34	21	–	No flare
15	W	46.2	pred, aza, cyc, mmf	Fever, skin rash, CNS vasculitis, arthritis, nephritis (IV)	19	0.21	1364	–	28
16	AC	38.8	pred, cyc, aza	Nephritis (IV), haemolytic anaemia	12	0.81	15	–	No flare
17	AC	41.6	pred, aza, cyc, mmf	Nephritis (IV)	6	0.89	203	–	No flare
18†	W	38.5	pred, aza, cyc, mmf	Arthritis, nephritis (IV)	17	0.35	522	Ro, Sm, RNP	
19	W	38.2	pred, hcq, aza, cyc, mmf	Nephritis (IV),arthritis, rash,fever	8	0.68	267	Ro	3
20	AC	24.5	pred, hcq, aza, cyc, mtx	Arthritis	12	1.39	10	Ro	7
21	W	35	pred, hcq, aza, cyc	Arthritis, nephritis (IV)	12	0.61	1058	Sm,RNP	7
22	AC	5	pred, hcq, aza, cyc	Fever, lymphadenopathy, organic brain syndrome, arthritis, nephritis (IV)	45	0.42	4386	Ro, Sm, RNP	3
23	A	27.1	pred, cyc, aza, mtx, hcq	Fever, skin rash, serositis, skin vasculitis, arthritis	13	1.5	31	RNP	No flare
24	A	29.6	pred, hcq, aza, mtx, cyc	Fever, lymphadenopathy, skin rash, arthritis, anaemia	16	0.51	36	Ro, La, Sm, RNP	13
25	AC	23.8	pred, warfarin, mmf, aza, hcq, cyc	Nephritis (IV,V), alopecia, serositis, arthritis	6	1.15	124	Ro, La, Sm, RNP	5
26	O	23.6	pred, hcq, mmf	Nephritis (IV), skin rash	8	0.37	1825	Ro, La, Sm, RNP	4
27	W	15.9	pred, mtx, hcq, cyc, aza	Raynauds, arthritis, serositis, photosensitivity	15	1.07	21	RNP	5
28	A	12.9	pred, hcq, etanercept, ssa, mtx, infliximab	Arthritis, serositis, alopecia, mouth ulcers, Raynauds	17	1.45	10	RNP	10
29	W	12	pred, aza, ssa, hcq, mtx, mmf, IVIG	Nephritis (IV), serositis, Raynauds, arthritis, myositis	16	0.34	497	Ro, Sm, RNP	No flare
30	W	18.8	pred, cyc, mepacrine	Skin rash, arthritis, nephritis (IV), gut vasculitis	12	0.83	986	Sm, RNP	No flare
31	W	9.4	pred, hcq	Thrombocytopenia	5	0.88	60	Ro	No flare
32	W	9	pred, aza, mtx	Arthritis, rash, Raynauds	8	1	106	Ro, Sm, RNP	No flare

A, Asian; AC, AfroCaribbean; aza, azathioprine; anti-dsDNA, anti-double-stranded DNA antibodies; anti-ENA, anti-extractable nuclear antigen antibodies; BCDT, B cell depletion therapy; BILAG, British Isles Lupus Assessment Group; CNS, central nervous system; cyA, ciclosporin; cyc, cyclophosphamide; hcq, hydroxychroloquine; mmf, mycophenolate mofetil; mtx, methotrexate; O, other; pred, prednisolone; RNP, ribonucleoprotein; ssa, sulphasalazine; IVIG, intravenous immunoglobulin; W, white.
*Lost to follow-up at 3 months
†Did not respond to BCDT.

at 3 months and Patient 18 did not deplete after BCDT). Twenty-eight of 30 patients (93%) had follow-up for at least a year.

BCDT resulted in a decrease of median global BILAG scores from 13 (95% CI 11 to 15) to 5 (95% CI 4 to 6) at 6 months ($n = 29$, $p<0.0001$). We observed improvements of a wide spectrum of clinical features, but no identifiable features predicted a better response in this heterogenous cohort. There was corresponding serological improvement with a decrease of median anti-dsDNA antibody levels from 164 (95% CI 246 to 1022) to 58 (95% CI 87 to 509) IU/ml ($p = 0.006$) and an increase of median serum C3 from 0.73 (95% CI 0.60 to 0.88) to 0.90 (95% CI 0.80 to1.1) g/litre ($p = 0.007$). Patients with renal involvement ($n = 21$) had a decrease of median urinary PCR from 446 to 190 mg/mmol at 6 months, although this was not statistically significant ($p = 0.06$).

Of the 30 patients, 12 have remained well after one cycle of BCDT, with no flare. The mean time to flare after BCDT ($n = 18$) was 10 months (range 3–28). Five patients flared <6 months post-BCDT, half (9/18) had a flare between 6 and12 months post-BCDT and the remaining four patients relapsed after 12 months. The mean follow-up duration for patients who flared versus those who did not was 39 and 35 months, respectively. The median duration of B cell depletion was 4 months ($n = 24$, range 2–10). One patient (Patient 3) remains depleted and well on low-dose prednisolone

6 years after BCDT, although her serum IgM levels remain low, with normal IgA and IgG levels.

We have re-treated 10 patients who relapsed with at least another cycle of rituximab. The outcome of re-treatment is described in detail elsewhere.[7]

Minor adverse events noted were CyC-related microscopic haematuria (n = 1) and mild neutropenia (n = 1) which was self-limiting. Four serious adverse events were observed. Patient 29 developed pneumococcal pneumonia and septicaemia requiring ventilatory support 5 months after BCDT. This patient remained B cell depleted at the time of the event. Another patient (Patient 9) had a severe serum sickness-like reaction shortly after the second rituximab infusion, which resolved with intravenous steroids. One patient (Patient 22) died from pancarditis related to active SLE 5 months post-BCDT, with repopulation of B cells at 4 months. Patient 31 had a grand mal seizure related to hyponatraemia 8 h after the first infusion of CyC.

Two patients with follow-up duration less than 12 months were excluded from the following flare analysis. Patients with low baseline serum C3 (n = 21) had a shorter time to flare post-BCDT (p = 0.008) (fig 1). Patients with anti-ENA at baseline (21/28) were more likely to flare at any time after BCDT compared with patients without anti-ENA (p = 0.007, Fisher's exact test). There was no significant association of flare post-BCDT in patients with baseline individual anti-ENA of anti-Sm (n = 10), anti-La (n = 5), anti-Ro (n = 12) or anti-ribonucleoprotein (n = 17) antibodies. Baseline anti-ENA was the only identified independent predictor of flare post-BCDT (p = 0.034, odds ratio = 8, 95% CI 1.2 to 55).

DISCUSSION

The results of our study suggest that combination BCDT can be a therapeutic option for refractory SLE patients, with a favourable long-term safety profile. One-third of patients remain well after BCDT without introduction of further standard immunosuppressive drugs. Of the patients who relapse after BCDT, most flares occur between 6 and 12 months post-BCDT, usually several months after repopulation of B cells. One patient in our study developed severe pneumococcal sepsis following BCDT, and we recommend that, where possible, anti-pneumococcal vaccination should be carried out prior to BCDT treatment in all patients. Anti-pneumococcal responses are preserved after one cycle of BCDT in patients with SLE for at least 1 year.[8] Another patient had a provoked seizure related to hyponatraemia after the first infusion of CyC. Hyponatraemia and water intoxication have been reported after administration of low-dose CyC.[9] Although fluctuations do occur, anti-ENA levels as a whole generally remain unchanged after BCDT,[8] suggesting their production by longer lived plasma cells. We found that positive baseline anti-ENA was an independent predictor of relapse. This suggests that although disease activity was reduced by BCDT, the association of an expanded autoantibody repertoire, indicative of a more established autoimmune response, may result in a more protracted clinical outcome. It was intriguing that BCDT, like conventional immunosuppression, tends to lower pathogenic antibodies such as anti-dsDNA[10] but has little effect on anti-ENA.[11] This may imply that certain B cell populations are more susceptible to these therapeutic approaches than others. In this study, the statistical analysis was limited by small patient numbers and the heterogeneity of the cohort; however, we observed that patients with low baseline C3 levels tended to have an earlier flare. Although it has been shown that the efficiency of depletion of lymphoma cells may be influenced by the integrity of the complement system,[12] we did not observe any relationship between the duration of peripheral B cell depletion and flare.

In conclusion, baseline autoantibody profiling with reference to anti-ENA may help identify patients who will have a more sustained response from BCDT. This study also provides some reassurance that BCDT in lupus is safe on a long-term basis.

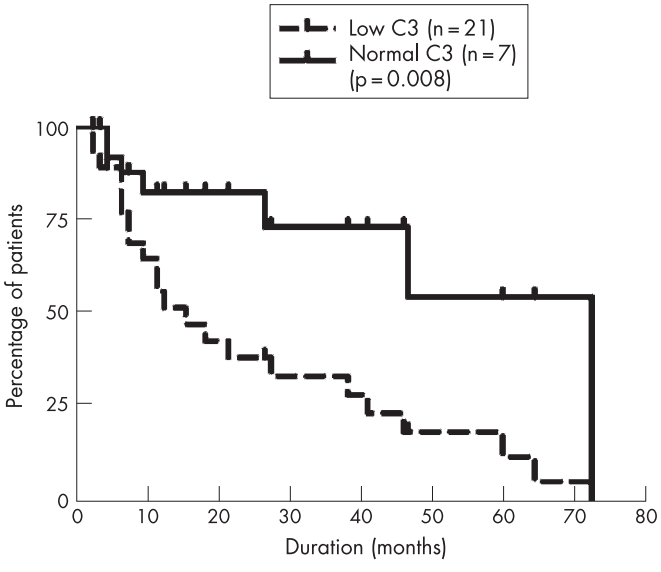

Figure 1 Baseline serum C3 and time to flare after B cell depletion therapy.

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Authors' affiliations

Kristine P Ng, Geraldine Cambridge, Maria J Leandro, Jonathan C W Edwards, Michael Ehrenstein, David A Isenberg, Centre for Rheumatology, University College London, London, UK

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Correspondence to: Professor D A Isenberg, Centre for Rheumatology, Room 331, 3rd floor, Windeyer Building, 46 Cleveland Street, London W1T 4JF, UK; D.Isenberg@ucl.ac.uk

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REFERENCES

1 **Mason LJ**, Isenberg DA. The pathogenesis of systemic lupus erythematosus. In: Davison AM, Cameron JS, Grunfeld JP, Ponticelli C, Ritz E, Winearls CG, van Ypersele, eds. *Oxford textbook of clinical nephrology*.3rd edn. Oxford: Oxford University Press, 2005:809–24.
2 **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.
3 **Sfikakis P**, Boletis J, Tsokos G. Rituximab anti-B-cell therapy in systemic lupus erythematosus: pointing to the future. *Curr Opin Rheumatol* 2005;**17**:550–57.
4 **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* 2005;**44**:1542–5.
5 **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.
6 **Hay EM**, Bacon PA, Gordon C, Isenberg DA, Maddison P, Snaith ML, *et al*. The BILAG index: a reliable and valid instrument for measuring clinical disease activity in systemic lupus erythematosus. *Q J Med* 1993;**86**:447–58.
7 **Ng KP**, Leandro MJ, Edwards JCW, Ehrenstein M, Cambridge G, Isenberg DA. Repeated B cell depletion in treatment of refractory systemic lupus erythematosus. *Ann Rheum Dis* 2006;**65**:942–5.
8 **Cambridge G**, Leandro MJ, Teodorescu M, Manson J, Rahman A, Isenberg DA, *et al*. B cell depletion therapy in systemic lupus erythematosus: effect on autoantibody and anti-microbial antibody profiles. *Arthritis Rheum* 2006;**54**:3612–3622.

9 **Salido M**, Macarron P, Hernandez-Garcia C, D'Cruz DP, Khamashtha MA, Hughes GR. Water intoxication induced by low-dose cyclophosphamide in two patients with systemic lupus erythematosus. *Lupus* 2003;**12**:636–9.
10 **McCune WJ**, Miskella M. Immunosuppression drug therapy. In: Wallace DJ, Hahn BH, eds. *Dubois lupus erythematosus*.6th edn. Philadelphia, PA: Lippincott Williams & Wilkins, 2002:1195–2018.
11 **Tench CM**, Isenberg DA. The variation in anti-ENA characteristics between different ethnic populations with systemic lupus erythematosus over a 10-year period. *Lupus* 2000;**9**:374–6.
12 **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.