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Sustained improvement in rheumatoid arthritis following a protocol designed to deplete B lymphocytes

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Abstract

Objectives. An open study of B-lymphocyte depletion was undertaken in rheumatoid arthritis (RA) patients to test the hypothesis that B lymphocytes may be essential to disease perpetuation.

Methods. Five patients with refractory RA were treated with a monoclonal anti-CD20 antibody, cyclophosphamide and prednisolone and followed for 12–17 months. Patient 2 received further treatments at 8 and 12 months and patient 4 at 11 months.

Results. At 26 weeks all patients satisfied the American College of Rheumatology ACR50 and patients 1–3 the ACR70 criteria of improvement, without further therapy. Patients 1, 3 and 5 achieved ACR70 at 1 yr and rheumatoid factor (RF) levels fell to normal. In patients 3 and 5, B lymphocytes returned without relapse. Patient 2 relapsed at 28 weeks and patient 4 at 38 weeks, coincident with the return of B lymphocytes in the presence of raised RF levels. Both achieved ACR70 on retreatment. Adverse events were limited to respiratory episodes (two patients) and marginal thrombocytopenia (one patient).

Conclusions. These findings are consistent with the concept that RA is critically dependent on B lymphocytes and suggest that B-lymphocyte depletion may be a safe and effective therapy.

KEY WORDS: B lymphocyte, Anti-CD20, Rituximab, Autoimmunity, Rheumatoid arthritis.

There is a consensus that the pathogenesis of rheumatoid arthritis (RA) includes roles for the T lymphocyte (interaction with antigen and major histocompatibility class II molecules), the macrophage (secretion of pro-inflammatory cytokines) and the synovial fibroblast (cartilage damage) [1–3]. The role of B lymphocytes is less well established, despite the most consistent immunological finding being production of autoantibodies directed against the Fc domain of IgG (rheumatoid factor, RF) [4, 5]. Histologically, the most specific features of rheumatoid synovium (also bone marrow) are follicles containing B lymphocytes and heavy infiltration with plasma cells [6].

Interest in the role of B lymphocytes has been rekindled by new information on the genetics, roles in cell signalling and crystal structure of RF [7–9]. Carson and co-workers [8] have argued that the mechanisms governing the survival of RF-committed B lymphocytes may differ from those for other autoreactive B lymphocytes. RF-committed B lymphocytes may obtain 'bypass' help from T lymphocytes recognizing foreign antigens [10]. This potentially resolves the paradox of a T-dependent anti-IgG response in the apparent absence of relevant autoreactive T lymphocytes.

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In 1998, we extended these ideas to construct a pathogenic hypothesis [5, 11, 12], which formed the rationale for the present study. The hypothesis comprises two key postulates: (i) that B lymphocyte clones, committed to certain species of IgG RF, may perpetuate their own existence, and that of other RF-specific clones, both through 'bypass' T-cell help and by providing themselves with their own antigen complexed with the survival-promoting complement fragment C3d,g [12, 13]; and (ii) that subsets of IgG1 RF can be predicted to induce both articular and extra-articular features of RA by generating tumour necrosis factor α (TNF-α) through interaction with the IgG Fc receptor FcγRIIIa [14–18]. Synovial and bone marrow involvement may be compounded by the readiness with which stromal cells from these tissues support the survival of B lymphocytes and plasma cells [19, 20].

This hypothesis led to the prediction that subtotal B-lymphocyte depletion might remove sufficient autoreactive B lymphocytes to induce the collapse of a pathogenic autoantibody-generating cycle, leading to long-term remission. An important caveat was that, in addition to B-lymphocyte depletion, it might be necessary to remove the autoantibodies implicated in driving their own production, in order to break the cycle.

B lymphocytes might also play a more general role in autoimmune disease, as antigen-presenting cells. B-lymphocyte depletion had already been considered

as a therapeutic strategy for autoimmune disease, but the prospect of requiring long-term B lymphopenia was discouraging. However, the idea of a 'vicious cycle', with the possibility that a short period of B lymphopenia might produce sustained improvement, appeared to justify a phase I clinical trial.

Clinical investigation of this concept was made possible by the availability of a specific B-lymphocyte-depleting agent of low toxicity. Rituximab (Mabthera; Roche Products, Welwyn, UK) is the clinical formulation of a murine IgG1 monoclonal anti-CD20 antibody [21, 22]. An open study of B-lymphocyte depletion in subjects with refractory RA was undertaken. The primary outcome measure was defined as the achievement of complete remission, as indicated by absence of clinical, biochemical or serological evidence of immunological/inflammatory activity 6 months after a single course of treatment.

The chosen B-lymphocyte-depleting protocol combined rituximab with corticosteroid and cyclophosphamide in a single 3-week course. The following factors contributed to this decision: (i) there is anecdotal evidence, from patients coincidentally receiving both low- and high-dose cytotoxic regimens, that RA is about as difficult to cure as non-Hodgkin lymphoma and that monotherapy with a pan-lymphocyte-depleting anti-CD52 antibody has not been effective [23]; and (ii) our hypothesis [12] indicates that a threshold of B-lymphocyte depletion would need to be reached. The protocol was, therefore, based on the type of combination therapy used in B-cell lymphoma. However, doses of prednisolone and cyclophosphamide were limited to those considered unlikely to increase morbidity significantly, within ranges not found to produce prolonged clinical effects on their own [24–26]. A single short course was used on the basis that, if a vicious cycle were broken, continued therapy would be unnecessary.

Methods

Subjects

Five subjects, detailed in Table 1, satisfying the American College of Rheumatology (ACR) criteria for classical RA, were selected on the basis of severe inflammatory disease that had not been adequately controlled despite trials of at least five disease-modifying anti-rheumatic drugs (DMARDs) as single agents (Table 1). DMARDs (excluding steroids; see below) were discontinued from day 0. All patients used diclofenac and/or coproxamol as needed.

Treatment protocol

The study was approved by the local hospital ethics committee. All patients received the following treatment. (i) Monoclonal chimaeric anti-CD20 antibody, rituximab (Mabthera) as four i.v. infusions (over 3 h) on days 2, 8, 15 and 22, of 300, 600, 600 and 600 mg respectively. Patient 5 omitted dose 4 because of an episode of fever and pleuritic pain. (ii) Oral prednisolone 60 mg on days 1–22, reducing in the three older subjects (perceived to be at higher risk of toxicity) to 30 mg on days 11–22 and then withdrawn over 3 weeks in subjects not previously taking steroids and, in the other cases, to 5 mg daily over 6 weeks. (iii) Cyclophosphamide as i.v. infusions on days 4 and 17 of 750 mg.

Assessment

Patients were assessed at recruitment, immediately prior to treatment, fortnightly for 3 months and then monthly after treatment for duration of early morning stiffness in minutes, pain on a 100-mm visual analogue scale, number of swollen joints (out of 28 joints), number of tender joints (out of 28 joints), physician's and patient's global scores on a 100-mm scale, health status by a health assessment questionnaire, erythrocyte sedimentation rate (ESR), serum C-reactive protein concentration (CRP), full blood count, renal and liver function tests, serum immunoglobulins, RF by latex fixation (positive or negative) and the titre of rheumatoid arthritis particle agglutination (RAPA), and antinuclear antibody (ANA) titre. Circulating B ($CD19^+$ by flow cytometry) and total lymphocytes were measured before, immediately after and monthly from 3 months after therapy until normal. B-lymphocyte data were incomplete for logistic reasons but the period of B-lymphocyte repopulation was covered in all cases.

Two levels of response were defined: (i) an improvement score of ACR(*n*), where *n* is the minimum percentage improvement in five measures, as defined by the ACR [27], and (ii) a state of complete remission.

Table 1. Details of patients at entry to the study

Patient	Age (yr)	Gender	Duration of RA (yr)	Erosive	Functional class	RAPA (ever +ve)	ANA (ever +ve)	Extra-articular features	DMARD failure[b]	DMARD/steroid at entry[b]
1	57	F	25	+	III	Yes	No	Anaemia[a]	GASMDP	P 7.5 mg/day
2	42	F	21	+	III	Yes	Yes	Anaemia[a]	GASMD	A 100 mg/day
3	69	F	40	+	III	Yes	Yes	–	GASMD	G 50 mg/month
4	50	F	11	+	III	Yes	Yes	Anaemia[a]	GASMP	P 10 mg/day
5	59	F	17	+	III	Yes	Yes	Subcutaneous nodules	GASMHD	M 12.5 mg/week

[a]Haemoglobin < 12 g/dl.
[b]G, gold; A, azathioprine; S, sulphasalazine; M, methotrexate; H, hydroxychloroquine; D, D-penicillamine; P, prednisolone.

Remission was defined as the absence of clinical or laboratory evidence of ongoing immunological activity. The ACR criteria for remission were not appropriate in the context of patients with disease of long duration, with pain and joint thickening due to irreversible joint damage. Remission was defined as early morning stiffness lasting < 10 min, pain score < 10 mm, fewer than four swollen joints (including knee effusions), fewer than two tender joints, ESR < 20 mm/h, CRP < 10 mg/l, RF negative by latex fixation and RAPA titre < 40.

Results

Clinical response

Clinical and laboratory indices with scales adjusted for comparability are shown in Fig. 1. Baseline, 6-month and 1-yr measurements, together with percentage improvement and ACR scores, are given in Table 2. All patients showed rapid improvement in synovitis. Anaemia resolved in all patients and nodules disappeared in patient 5. At 6 months, all patients achieved ACR50 and patients 1–3 achieved ACR70 without introduction of further therapy. The patients previously taking prednisolone had no difficulty in reducing their daily dose to 5 mg. All patients reduced their analgesic intake.

At 1 yr, patients 1 and 3 maintained improvement and patient 5 improved further, all achieving ACR70. ACR70 was maintained by patients 1 and 3 at the last assessment at 17 months. Patient 5 reports remaining well but declines further assessment visits. Despite a clinical impression of complete remission in patients 1–3 at 6 months and in patients 1, 3 and 5 at 1yr, the predefined criteria were not formally met because of residual pain and persisting, if reduced, RF levels.

Patient 2 developed a relapse of joint pain and swelling over a few days at week 28. Acute-phase measures rose rapidly. At 34 weeks she was retreated with a single 500-mg dose of rituximab and started on prednisolone 10 mg daily. The prednisolone was withdrawn over 3 months. ACR50 status was regained and was technically maintained at 52 weeks, but the CRP level had climbed again to 66.8 mg/l and the patient was again retreated with two cycles of 500 mg rituximab and 750 mg cyclophosphamide, each under cover of 5 days of 60 mg prednisolone, and regained ACR70 at 17 months. Patient 4 relapsed from 38 weeks and was retreated at 49 weeks with two cycles of 500 mg rituximab and 750 mg cyclophosphamide, each under cover of 5 days of 60 mg prednisolone and achieved ACR70 at 17 months.

Immunology

B-lymphocyte counts fell to undetectable levels in all cases and remained below normal for at least 6 months (Fig. 1). Normal levels were regained in all but patient 1. In patients 2 and 4, the return of B lymphocytes coincided with clinical relapse on all three occasions. Total lymphocyte counts showed no consistent trend (Fig. 2). Levels of RF immunoglobulin (Ig) M fell in all patients (Fig. 3) at varying rates. RAPA titres for patients 1, 3 and 5 fell to 1/40, 1/40, 1/20, with a variably negative or equivocal latex test. RF levels were raised in patients 2 and 4 but not in patients 3 and 5, at the time of B-lymphocyte repopulation (and relapse). ANA titres fluctuated with no clear trend, mostly below a significant level of 1/20. Levels of Igs showed a modest fall, chiefly in the first 10 weeks. Baseline, 6-month and 1-yr levels (g/l) were as follows: IgG, 11.4, 8.8 and 9.7 respectively; IgA, 2.4, 1.6, and 2.0; IgM, 1.6, 1.1 and 1.1.

Adverse events

No major adverse events attributable to the therapy were observed. No infusion-related toxicity was observed (in contrast to lymphoma patients). Patient 1 had a lower respiratory tract infection at week 27, which improved rapidly on antibiotic treatment. She had a history of asthma and a similar infection 10 yr before. Patient 2 developed transient thrombocytopenia, down to $110 \times 10^3/\text{mm}^3$, with minor bruising and also transient acne rosacea. Patient 5 had fever and chest pain on day 19, which settled on antibiotic treatment. She had similar episodes in the past year, with a differential diagnosis of infection or rheumatoid pleuritis.

Discussion

Irrespective of the mechanism by which they were achieved, the results obtained in this study suggest that the protocol used, or a modification thereof, may be of major benefit to subjects with RA. The original primary outcome measure was immunological rather than clinical and proved too stringent. Any statistical analysis of clinical benefit must, therefore, be *post hoc.* Nevertheless, despite small numbers of patients, a useful estimate of benefit can be made without the use of historical controls. A simple binomial analysis indicates that further, similar cases treated in the same way can be expected with 95% confidence to have a minimum chance of 47.8% of achieving ACR50 6 months after B-lymphocyte depletion. If the current status of subjects is taken as the end-point and the option of repeat treatment is included, the same percentage figures can be applied to ACR70 at 18 months. This worst-case estimate still compares well with other therapies [28, 29].

Use of historic controls is problematic, but ACR20/50/70 rates for placebo-treated subjects at 6 months are available from a range of DMARD studies. In a recent trial of etanercept [29], 11, 5 and 1% of 80 placebo-treated subjects achieved ACR20, 50 and 70 respectively at 6 months. Using these figures and a χ^2 analysis, the probability of the results of the present study being due to placebo response is estimated at $P < 0.001$ ($\chi^2 = 47.37$). Conceding a 10% placebo ACR50 rate, which has been observed in some trials, in the same size population the figure is still $P < 0.001$ ($\chi^2 = 22.89$).

Cyclophosphamide and steroid have a significant short-term effect on RA, and exclusion of the possibility

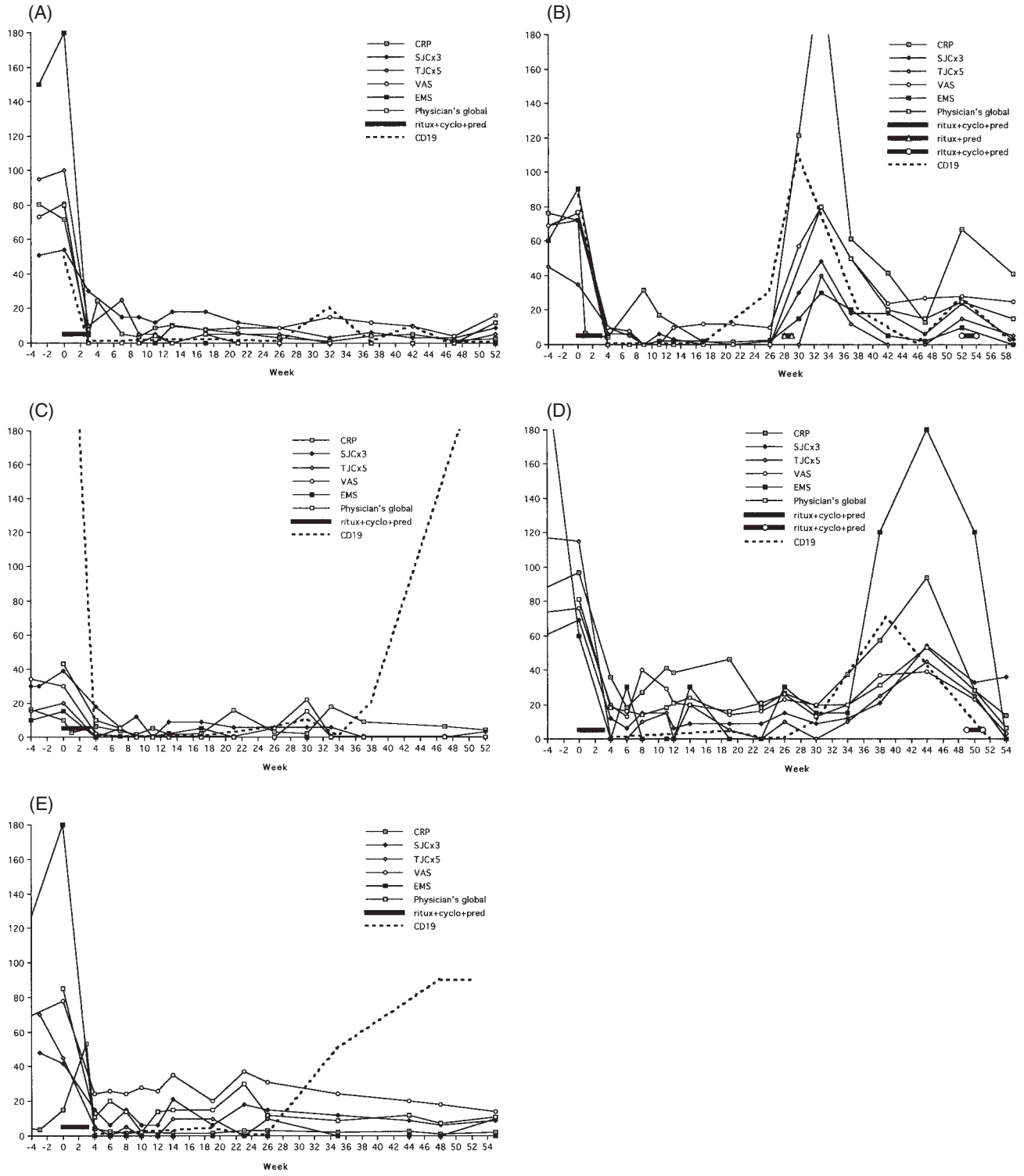

FIG. 1. Panels A–E show clinical and acute-phase indices and $CD19^+$ cell counts (per mm^3) for the first year following B-lymphocyte depletion in patients 1–5, respectively. Scales are adjusted to aid comparability.

that the results were due entirely to these drugs is an important issue. Data on the use of i.v. cyclophosphamide are sparse. However, recent studies confirm the accepted view that benefit 6 months after cyclophosphamide in this dose range is minimal [24–26], only one of eight subjects who received comparable or rather larger doses achieving ACR20 and none ACR50. On this basis, the probability of the present results being due to cyclophosphamide is estimated, by Fisher's exact test, as $P = 0.0008$. Analysis on the basis of any response

TABLE 2. Baseline, 6-month, 12-month and final outcome measures for patients 1–5 with percentage improvement and ACR category

Patient	Time point	Swollen joint count	Tender joint count	CRP (mg/l)	ESR (mm/h)	Pain VAS[a]	Physician's global assessment	Patient's global assessment	HAQ[b]	Early morning stiffness	ACR grade of improvement[c]
1	Baseline	18	20	71.4	65	81	80	82	1.75	1	
	26 weeks	3	1	3	10	9	0	7	0.75	0	
	% improved	83	95	96	85	89	100	91	57	100	70
	52 weeks	3	1	2.6	12	16	12	14	ND	0	
	% improved	83	95	96	82	80	85	83		100	70
	76 weeks	1	0	19.4	16	10	0	9	ND	0	
	% improved	94	100	73	75	88	100	89		100	70
2	Baseline	24	7	72	38	76	77	69	1.875	90	
	26 weeks	0	0	2.8	10	10	0	0	1.25	2	
	% improved	100	100	96	74	87	100	100	33	98	70
	52 weeks	8	3	66.8	29	28	25	28	ND	10	
	% improved	66	57	7	34	63	68	59		89	(50)
	76 weeks	2	0	3	11	20	0	0	ND	5	
	% improved	92	100	96	71	74	100	100		94	(70)
3	Baseline	13	4	9.8	36	30	43	34	0.75	15	
	26 weeks	2	0	3.4	23	5	0	7	0.25	0	
	% improved	85	100	65	36	83	100	79	66	100	70
	52 weeks	1	0	4.1	15	0	0	0	ND	0	
	% improved	92	100	58	58	100	100	100		100	70
	71 weeks	0	0	5.3	21	7	0	7	ND	0	
	% improved	100	100	66	42	77	100	79		100	70
4	Baseline	23	23	96.8	92	76	81	72	2	60	
	26 weeks	5	2	26.7	58	23	26	22	–[d]	30	
	% improved	78	91	72	37	70	68	69	–[d]	50	50
	52 weeks	11.5[e]	2.5	20.8	32	13	17	17	ND	60	
	% improved	50	89	79	65	83	79	76		0	(50)
	72 weeks	4	1	10.3	24	23	17	21	ND	30	
	% improved	83	96	89	74	70	79	71		50	(70)
5	Baseline	14	9	14.7	24	78	85	77	1	180	
	26 weeks	5	2	3.1	11	31	12	9	0.375	0	
	% improved	64	78	79	54	60	86	88	63	100	50
	55 weeks	3	2	2.1	8	14	11	14	ND	0	
	% improved	79	78	86	67	82	87	82		100	70
	Declined further follow-up										

[a]Visual analogue score.
[b]Health assessment questionnaire.
[c]ACR grades in parentheses are after retreatment.
[d]Abandoned because of language difficulty.
[e]Mean of 50- and 54-week data.

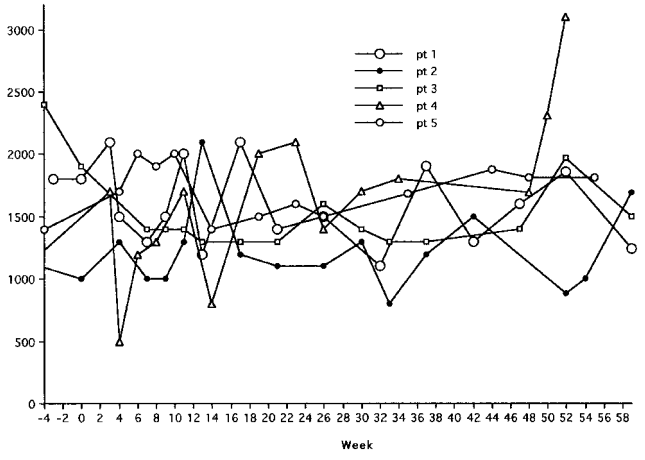

FIG. 2. Total lymphocyte counts (per mm^3) for patients 1–5 following B-lymphocyte depletion.

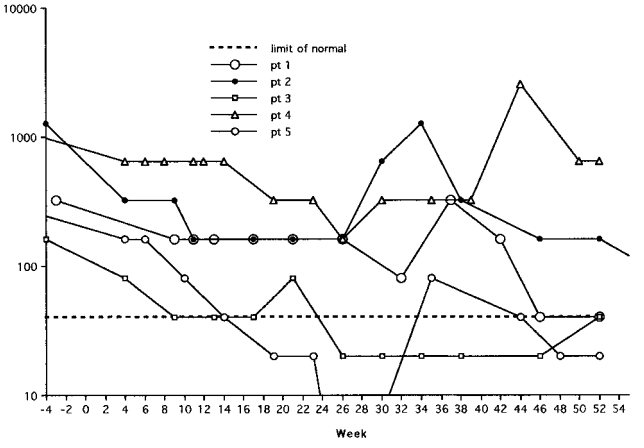

FIG. 3. RF RAPA titres for patients 1–5, following B-lymphocyte depletion.

at 6 months gives $P = 0.0047$. There remains a remote possibility that the present study has identified a protocol for steroid and cyclophosphamide with benefits way beyond all previous experience.

The further possibility, that steroid and cyclophosphamide contributed to the results, at least in part, through actions other than on B lymphocytes, seems very plausible. The original hypothesis did not predict that clinical benefit would follow immediately after B-lymphocyte depletion, but only when IgG RF levels had fallen significantly. The rapid improvement seen can be attributed to an anti-inflammatory 'kick start' from steroid and cyclophosphamide.

Cyclophosphamide and steroid may also contribute to B-lymphocyte death by either encouraging apoptosis or direct cytolysis. Rituximab monotherapy induces sustained remission in lymphoma less often than combination therapy. B lymphocytes in certain environments may be protected from biological killing by complement-inhibitory proteins, but may succumb if also exposed to cyclophosphamide.

Accepting all these possibilities, there remains a strong indication that B-lymphocyte depletion was a necessary component of the therapeutic action. Apart from the superior response to that reported with cyclophosphamide alone [24–26], when relapse occurred, on three occasions in two patients, it was in each case coincident with B-lymphocyte repopulation.

Although none of the patients fulfilled all predefined criteria at 6 months, the level and persistence of improvement in patients 1, 3 and 5 suggests that the immunological process had genuinely become inactive, at least at a point significantly upstream in the pathogenic pathway. The resistance of improvement to B-lymphocyte repopulation in patients 3 and 5 suggests that some sort of cycle can be broken if the conditions are favourable, as originally postulated. However, at least two possible forms of cycle need to be considered.

In the original hypothesis [12], disease perpetuation was ascribed to IgG RF-committed B-cell clones. It was argued that clones of this type should not return in the short term, following adequate depletion, unless continued IgG RF production provides sufficient stimulus to low-affinity RF clones to affinity-mature and/or class-switch to replace the original clones. The relapse of the two subjects with persistently high IgM RF levels would fit with this, but the evidence is so far circumstantial. Moreover, IgM RF may be a poor surrogate for IgG RF. Existing IgG RF assays were considered to be unreliable for use on unfractionated serum.

The results are also consistent with the alternative possibility that RA is driven by autoreactive T lymphocytes, which depend on antigen presentation by B lymphocytes for maintaining their activation. This would imply a failure of T-cell tolerance, which ceases to generate disease, either temporarily or in the long term, following interruption of T–B lymphocyte interaction. It would accommodate interactions with RF-specific B lymphocytes, as presenters of complexed antigen, but with RF secretion being epiphenomenal.

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