Clinical outcome following B cell depletion therapy in eight patients with refractory idiopathic inflammatory myopathy

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Abstract

Objective

To assess the efficacy of B lymphocyte depletion therapy (BCDT) in patients with refractive idiopathic inflammatory myopathy (IIM).

Method

Eight patients thought to have IIM were treated with BCDT utilising rituximab. Five were treated as part of an open label trial and three on the basis of perceived clinical need. Rituximab (1gram) and methylprednisolone (100mg) were given as intravenous infusions on days 0 and 14. The primary efficacy outcome at 6 months was 15% improvement in muscle strength and 30% reduction in CPK.

Results

Two patients with Jo-1 antibody positive dermatomyositis (DM) demonstrated a clinical response. Both achieved >30% improvement in CPK. In one, the CPK remained within the normal range for 10 months, the other had a normalised CPK and stabilisation of lung function tests for 36 months. Muscle strength by myometry, however, did not achieve the primary outcome, although, patient 1, demonstrated an improvement of 20% at 8 months (the patient had elective surgery of the hand during the study period). Jo-1 antibody levels fell modestly in both patients but remained detectable. Re-evaluation of three patients revealed that one had inclusion body myositis, one had sporadic muscular dystrophy and one subsequently developed nodular sclerosing lymphoma. All except one patient showed adequate B cell depletion with re-population occurring 3- >42 months after BCDT. One patient did not deplete and died of an unrelated cause.

Conclusion

This study emphasizes the importance of identifying and selecting the appropriate sub-group of patients with IIM most likely to respond to BCDT.

Key words

Myositis, B cells, rituximab, B cell depletion therapy

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Introduction

Idiopathic inflammatory myopathies (IIM) are chronic debilitating disorders primarily of the skeletal muscles although involvement of the skin, lungs, gastrointestinal tract and heart is common. Most patients with IIM have at least a partial response to corticosteroid therapy alone, but invariably other agents are needed to control the disease (1). However, a significant proportion of patients have chronic progressive disease despite conventional immunosuppressive therapy.

Although, PM and DM may differ immunologically, B cells may play a role supporting the underlying pathological process in both diseases. As part of the abnormal immune response, autoantibodies to nuclear and cytoplasmic antigens are found in up to 89% of patients with IIM (2). Myositis-specific autoantibodies (MSA) are found in approximately 30-40% of the patients with IIM. It has been demonstrated that levels of Jo-1 antibodies correlate with disease activity and may disappear with successful therapy (3, 4). Although this does not confirm a direct pathogenic role for these antibodies, it is suggestive that the regulation of MSA is closely linked to factors responsible for the disease process. Recent evidence suggests that it may relate to the ability of the RNA-associated proteins recognised by MSA to form immune complexes with these autoantibodies resulting in interferon-a (IFNα) production following internalisation by plasmacytoid dendritic cells (4, 5). Evidence for a pathological over-production of IFNα has been found in patients with SLE, Sjögren's and DM (5). Type 1 interferons can directly stimulate key players in the autoimmune response in particular dendritic, T and B cells which may also result in increasing autoantibody production (6, 7).

Over the last decade there has been a substantial growing interest in the role of B cells in autoimmune disease especially after the success of B cell depletion therapy (BCDT), based on the monoclonal anti-CD20 antibody, rituximab, in patients with rheumatoid arthritis (RA) (8). The efficacy of BCDT in patients with systemic lupus erythematosus (SLE) (9) and in other systemic autoimmune diseases (10), including small open label studies in rarer conditions such as IIM have been previously reported (10-15). We report here the results of an open label study of BCDT in five patients and report a further 3 patients treated on a clinical need basis due to refractory disease.

Patients and methods

Patient selection

Eight patients thought to have PM/DM were treated with BCDT utilising rituximab. Five were treated as part of an open label trial and three on the basis of perceived clinical need. All patients met the following inclusion criteria:

1. Adult patients with definite PM or DM by the criteria of Bohan and Peter (16).
2. Active disease as assessed by the treating physician (DAI) with muscle weakness of at least two muscle groups of at least grade 4/5 as measured by the standard six point MRC scale (17) plus elevation of creatine kinase
3. Refractory disease as defined by inadequate response to conventional treatment (Table I).

Patients were excluded if:

a) age <18yrs

b) women of childbearing potential

c) inclusion body myositis, muscular dystrophies or drug induced myositis or other major organ diseases.

Muscle biopsies taken at initial diagnosis were reviewed to ensure that they were diagnostic and therefore no biopsy was repeated immediately prior to therapy.

The open-label study was approved by the local hospital ethics committee and all patients gave informed consent.

Treatment protocol

Rituximab (Mabthera) was given as 1g intravenous infusions on day 0 and day 14. Corticosteroids were administered as intravenous methylprednisolone 100mg on day 0 and 14 and then patients were given a tapering dose of oral prednisolone 30mg, 20mg and then 10mg over 3 days following infusion of rituximab. Patients were then maintained on the pre-treatment dose of prednisolone (if any) or it was discontinued. Previous

immunosuppressives were continued if doses had been stable for 4 months prior to BCDT. Exceptions were Patient 1 whose Enbrel was discontinued, and Patients 4 and 7 where azathioprine was discontinued due to concerns that the combination with methotrexate would increase the susceptibility to infections (18).

Assessment

After initial screening, a complete physical examination was undertaken and muscle strength was evaluated by manual muscle testing (MMT) and myometry (assessment using a CITEC hand-held dynamometer [CIT Technics, Groningen, The Netherlands] for quantitatively measuring the specific strength of a muscle group) (19). Myometry is a validated tool for measuring very small changes in muscle strength. The technique chosen for this study was the "break test," which is most accurate when maximum strength is allowed to build up over 2-5 seconds before the "break" and is completed by a single assessor (20). Standard MMT of 17 muscle groups was performed at baseline and two monthly intervals using a 0-10 scale (with 0 being the lowest score and 10 the highest/normal- a total score of 170) (17). The muscles tested by MMT were the right and left gluteus medius, gluteus maximus, iliopsoas, quadriceps, deltoid, trapezius, biceps, triceps and neck flexors (all but the trapezius and neck flexors were tested by myometry-a total score is not available-percentage change from baseline is used). The same evaluator (SS) performed the assessment throughout the study period. Disease activity was also assessed using the Myositis Intention To Treat Index-MITAX (performed monthly for 6 months) and damage was assessed using the Muscle Damage Index-MDI (at baseline and 6 months) (21). The MITAX index assesses myositis activity in 7 different organ systems using the principle of the physician's intention to treat. Patients are distinguished from the most active, Grade A, to never active, Grade E. A MITAX Grade B indicates active disease requiring moderate steroid or immunosuppressive use. A numeric scoring system has been used to obtain the global score. The equivalent numeric scores for A, B, C, D, and E used were 9, 3, 1, 0 and 0. A global score for the MITAX, is the sum of the scores obtained for each organ system (a maximum global score of would be a grade A score in all 7 organ systems ie 63) (21). Other measures to assess disease activity included; physicians global activity assessment as assessed by a 10cm VAS and HAQ (recorded monthly for six months). The use of disease activity measures in patients with IIM has been reviewed (22).

Full blood count, creatine phosphokinase (CPK) levels, quantitative immunoglobulins and B cell count (CD19+ cells; normal range 0.03-0.4 x 10^9/L) were measured on a monthly basis for six months and then 2-3 monthly. Lung function tests were performed at baseline and 6 months on Patients 1 and 6, as both had evidence of interstitial lung disease. Sera from patients were tested for anti-Jo-1 antibodies by ELISA (Shield Diagnostics, Dundee) and were also sent to Dr Oddis's laboratory in Pittsburgh for further ENA analysis. The other Myositis Specific Antibodies (MSA) tested for by immunoprecipitation were PM-1, Scl 70, PL-12, EJ, OJ, KS, SRP, Mi-2 and Ku.

The primary outcome parameter in this study was an improvement of at least 15% in muscle strength by myometry and 30% reduction in CPK at 6 months (22). Secondary outcome measures: 15% improvement in total MMT, 20% improvement in physicians global activity assessment as assessed by a 10cm VAS, 15% improvement in HAQ and a 20% improvement in the MITAX score (22). However, patients 6-8 did not have the complete data set recorded as they were treated prior to the start of the open label study.

Results

Clinical findings

Demographic data and treatment at the start of study is summarized in Table I. Two patients were diagnosed as having polymyositis, 5 dermatomyositis and 1 patient juvenile onset dermatomyositis at the time of BCDT. Average disease duration was 14.9 years (range 4-40 years). Average DMARD use was 2.9 years prior to treatment with rituximab. Three patients were anti-Jo-1 antibody positive (all DM). Two of the eight patients (Patients 1 and 6) demonstrated a clinical response, both of whom had DM and were Jo-1 positive.

Depletion of B cells

Peripheral B cell counts (CD19+) fell to below the level of detection in our Pathology Department (<0.005 x 10^9/L) in all except one patient (Patient 8) (Fig. 1). Depletion lasted from 3 to more than 42 months after therapy.

Serum immunoglobulins

Total immunoglobulins showed a modest fall at 6 months in most patients but remained within the normal range except in one patient (Patient 2; Fig. 2). All classes of immunoglobulins were below the normal range in this patient before treatment and have remained low throughout her disease.

Adverse events

Rituximab was well tolerated in all patients except on retreatment of patient 4-an allergic response developed associated with hypotension, which required treatment with intravenous fluids and intravenous corticosteroids (no HACA results are available). One death occurred in a Jo-1 negative DM patient (Patient 8) within 5 weeks of treatment; this was the only patient not to demonstrate peripheral B cell depletion (Fig. 1). The patient had deterioration in muscle strength with respiratory muscle weakness and required high dose iv methylprednisolone as an inpatient with subsequent diverticular perforation and massive gastrointestinal haemorrhage with resultant multiorgan failure. The death was felt to be unrelated to rituximab as the patient had failed to deplete and also the concomitant disease process was felt to be a sufficient cause of death.

Patients with a positive clinical response

Patient 1

This patient had interstitial lung disease, in association with her muscle disease, which was stable at study entry (FVC, DLCO and kCO remained

Table I. Summary of patients at entry into the study and subsequent outcome.

Patient	Age/Sex	Diagnosis	Disease duration (yrs)	Auto-antibodies	Previous treatment	Treatment at entry	Duration of B cell depletion (months)	CPK at entry (24-173iu/l)	Outcome at 6 months post BCDT
1	56F	DM	14	Jo-1 +ve Rh F -ve	MTX, L, P, iv Cy, Cs IVIg, Pred	Pred 7.5mg Pred 7.5mg Enbrel 25mg	18	610	Responder Improvement muscle strength Normalisation of CPK for 10 months
2	56F	DM	13	MSA +ve Immuno-ppt 140 and 155kD antigens [17]	Pred 60 mg AZA, HCQ, Cs, Thalidomide, IVIg	Pred 15mg MTX 25mg	8	292	Non responder
3	50F	JDM	40	MSA -ve RhF 1:320 ANA 1:80 speckled diffuse	MTX, AZA, IVIg, Pred 29yrs	Nil	6	264	Non responder
4	57F	DM ATP	7	Jo-1 +ve RhF 1:640 ANA 1:40 speckled diffuse	MTX, Cs, Pred	MTX 10mg AZA 50mg	3	1571	Non Responder Improvement in thrombocytopenia, Subsequently diagnosed with nodular sclerosing lymphoma
5	M60	PM	4	MSA – ANA 1:160 homogeneous	MTX, Cs, IVIg Pred	Pred 15	8	460	Non responder Subsequently diagnosed with inclusion body myositis
6	F63	DM	6	Jo-1 +v e ANA 1:40 speckled	AZA pred	Pred 10mg AZA 150mg	>42	400	Responder Improvement in muscle strength to normal Stabilisation of DLCO
7	F31	PM	15	MSA –ve ANA 1:160 speckled diffuse	Pred, Aza, MTX (PO and S/C), IVIg	Pred 10 AZA 150mg MTX 10mg	6	3641	Non responder Subsequently diagnosed as sporadic muscular dystrophy
8	M 58	DM	20	MSA –ve ANA 1:80	MTX, Cs, IVIg	Pred 20mg	Did not deplete	1475	Non responder Died 1 month after BCDT

Δ: diagnosis; CPK: creatinine phosphokinase; ATP: Autoimmune Thrombocytopaenic Purpura; RhF: rheumatoid factor; Pred: prednisolone; M: methotrexate; L: leflunomide; P: penicillamine; iv: intravenous; cy: cyclophosphamide; Cy: cyclosporin; IVIg: intravenous immunoglobulin; A: azathioprine; HCQ: hydroxychloroquine; -ve: negative; +ve: positive.

stable throughout the study). At study entry enbrel was discontinued, but prednisolone 7.5mg was continued (Table I). She depleted well with some evidence of repopulation at 8 months but levels did not return to within the normal range until 18 months after therapy. There was a significant decrease in CPK within 1 month of BCDT from 610 IU/ml to 216 IU/ml (range 24-173). Her CPK normalized after three months and remained within the normal range until 10 months after initial treatment, although the average improvement in myometry at 6 months was only 6% (any change is defined as percentage change from baseline) (absolute figures; 36 newtons baseline, 38.1N six months, 43.1N 8 months). The patient had elective surgery (replacement MCP joints of the right hand) as she felt substantially better and wished to undertake the surgery whilst she remained well. This may have affected her muscle strength scores. However, at 8 months she demonstrated a 20% improvement in mean muscle strength as defined by myometry and this was sustained for a further two months (on analysis of individual muscle groups, she had a 27% improvement on mean MMT in the strength of hip flexion over baseline [127.5 to 161.5] at 8 months and a 48% improvement on myometry [44N to 65.3N]). There was no significant change in upper limb strength (due to previous shoulder replacement resulting from joint damage). She had a 20% improvement in disease activity scores as defined by the MITAX (Fig. 4). The global MITAX score reduced from 13 to 6 ie with a change in the muscle domain from a B score to C (data not shown). HAQ score did not change significantly until 3 months after therapy and maximized at 6 months (Fig. 4), an improvement of 13%. After 10 months the CPK had risen to 510 IU/ml associated with increasing myalgia and weakness. This coincided with the repopulation of peripheral B cells although levels did not return to normal

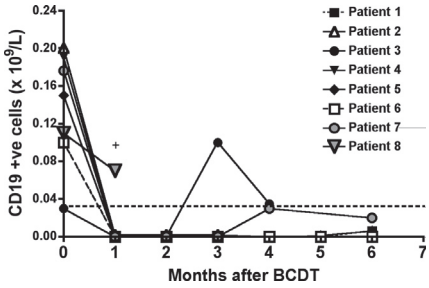

Fig. 1. Peripheral blood CD19+ cell counts following BCDT in the 8 patients in the study.

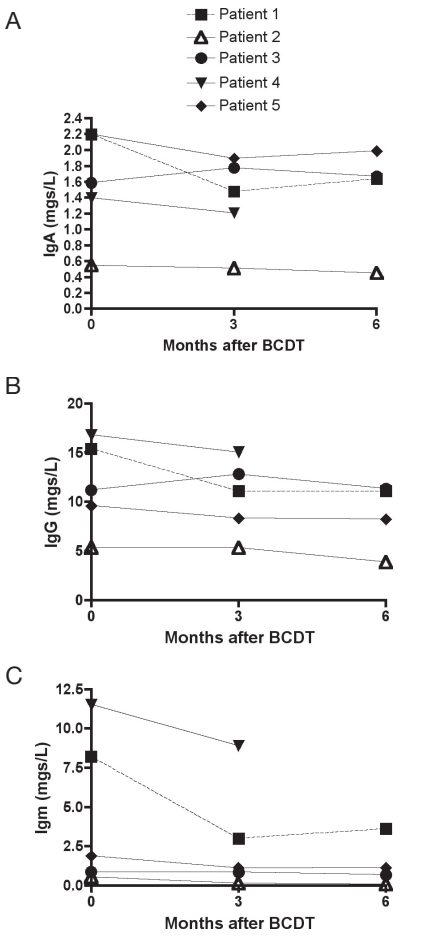

Fig. 2. Serum total immunoglobulin levels (IgA shown in Fig. 2A, IgG in Fig. 2B, IgM in Fig. 2C) for Patients 1-5 up to 6 months following BCDT.

for 18 months. This patient has subsequently been retreated with a second cycle of rituximab (2 x 1g rituximab and 2 x 750mg of cyclophosphamide 2 weeks apart) 18 months after her initial cycle of treatment with BCDT. Prior to retreatment she had been taking 15mg prednisolone, her CPK had risen to 452 IU/ml (26-140) and she was unable to walk unaided. Her CPK has remained within normal limits, 12 months after the second cycle of BCDT. This was accompanied by a 15% improvement in her MMT.

Patient 6

At study entry, this patient with Jo-1 positive dermatomyositis had evidence of widespread ground glass shadowing on HRCT chest scan despite prednisolone 20mg a day and azathioprine 150mg a day (both were continued during BCDT). Immediately prior to therapy she was unable to manage a flight of stairs. She had proximal myopathy grade 4/5. Baseline CPK was 400 which normalised one month following BCDT and remains within the normal range 36 months later. Muscle strength returned to normal at 6 months. However, the full dataset for MMT, MITAX, HAQ and MDI are not available as she was treated prior to the open label study. Her lung function tests have remained stable over a period of 3 years. Her pre-treatment FVC, DLCO and kCO were 50, 41 and 69% of predicted, 24 months-2, 39, 60%, and 36 months after therapy 64, 43, and 81%, with some resolution of ground glass findings. She now manages to walk a quarter of a mile unaided. This patient also depleted well and showed signs of repopulation after 28 months but 3 years after BCDT her CD19+ count is still low at 0.01 x 10^9/L.

Jo-1 antibody titers also fell modestly in both patients but remained detectable (Fig. 3).

Patients without a clinical response

Patient 2

This patient had severe skin disease which had been resistant to many previous therapies. Due to progressive skin ulceration she was given IV methylprednisolone, 4 months after initial B cell depletion but was not retreated with rituximab as she still demonstrated peripheral B cell depletion. She remained depleted up to 14 months after her initial therapy. She has remained refractory to all treatment. Serum from this patient immunoprecipitated 2 unidentified proteins approximately 155 and 140 kD. It is likely that this patient has a novel DM associated antibody.

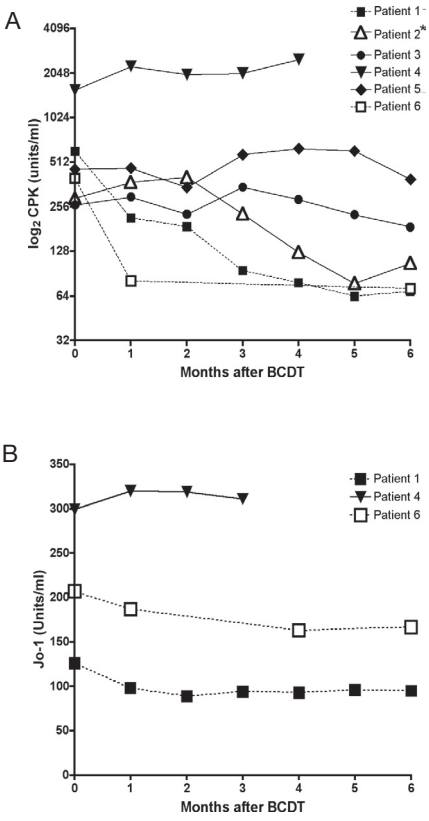

Fig. 3. Changes in CPK levels (Fig. 3A) for 6 of the patients and Jo-1 autoantibodies in the 3 Jo-1 positive patients (Fig. 3B) following BCDT.

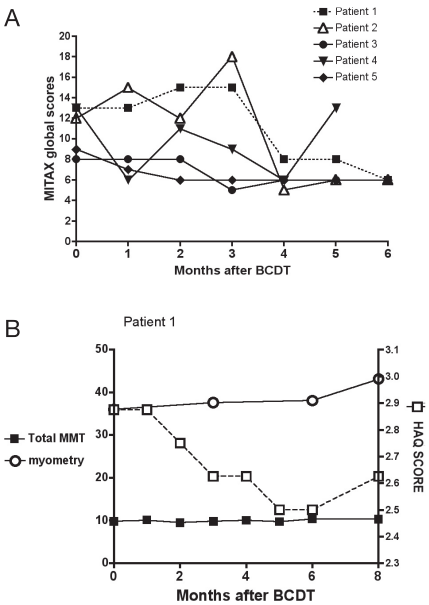

Fig. 4. MITAX Global scores for Patients 1-5 following BCDT (Fig. 4A) and HAQ, MMT and myometry for Patient 1 (Fig. 4B).

Patient 3

Had juvenile dermatomyositis for 40 years. She remained B cell depleted for 8 months after initial therapy with no significant improvement in strength or

CPK. This patient was again negative for all MSA, but immunoprecipitated an unidentified 110kD protein very weakly (but not the PL-12 RNA).

Patient 4

Although B cells were depleted in this patient, there was an early return 3 months after BCDT. Due to deterioration in muscle strength this patient was retreated with Rituximab and intravenous cyclophosphamide (750mg x2) with initial rapid B cell depletion but again, an early return of B cells after 4 months was noted. There was no significant improvement in muscle strength or CPK. Interestingly, this patient also had an autoimmune thrombocytopenia which did not respond to rituximab monotherapy but one month after retreatment (with addition of cyclophosphamide), her platelets returned to the normal range and have remained so. This patient had a polyclonal rise in her immunoglobulins. She has been subsequently diagnosed with nodular sclerosing Hodgkin's lymphoma.

Patient 5

With polymyositis developed distal limb weakness 6 months after initial B cell depletion. At study entry ankle dorsiflexion scored grade 5 on the MRC scale, and remained so on repeated examination. The patient complained of a short history of being unable to lift his feet to clear steps. Muscle strength was tested two monthly, with normal strength at ankle dorsiflexion at 4 months, and so the weakness was only picked up at the month 6 visit, with grade 3+muscle weakness on ankle and hallux dorsiflexion. He also had detectable weakness-rade 4 at his wrist flexors and thumb abductor. His weakness rapidly progressed over the ensuing months. A further biopsy demonstrated rimmed vacuoles consistent with inclusion body myositis although these were not present on the initial biopsy.

Patient 6

Also failed to show any improvement in muscle strength. She had a repeat muscle biopsy 24 months after initial B cell depletion. This showed increased variation in fibre size with some fibre hypertrophy, and although immunohistochemical staining for spectrin, merosin, dysferlin, dystrophin and alpha-sarcoglycan were comparable with normal controls, there was a marked increased in utrophin expression. The patient and the biopsy have subsequently been reviewed at The National Hospital for Nervous diseases at Queens Square Hospital, London, where her diagnosis has been revised to that of a sporadic muscular dystrophy. Re-review of her initial muscle biopsy did not, however, show any evidence of fibre hypertrophy.

Discussion

This study suggests that B cell depletion therapy may be effective in a subgroup of patients with refractory disease. Along with previous published case reports this appears to be in those with myositis specific antibodies, notably Jo-1 autoantibodies. Of the 23 cases of patients with IIM treated with rituximab (10-15) (both published and patients from the current study), 16 patients (12 DM) showed significant clinical response to B lymphocyte depletion therapy. Of these 16 patients who showed improvement, 9 patients were Jo-1 positive. Of the 4 patients with polymyositis reported, the 3 patients that demonstrated a response to rituximab were also Jo-1 positive. Unfortunately, information regarding the presence of other MSA is often not provided. The possible association of a clinical response to rituximab-based B cell depletion therapy with Jo-1 positivity is of interest. In myositis, the association of a positive response with Jo-1 rather than other MSA, could possibly reflect the different roles of individual autoantibody specificities in disease expression. This could perhaps be due to their ability to be deposited in vessels in the form of immune complexes or as 'drivers' of the pro-inflammatory process underlying the disease. In myositis, a number of MSA are RNA-binding proteins which could potentially activate the innate immune system through TLR on dendritic cells or macrophages when in the form of RNA/protein/antibody complexes (23). Others, such as Jo-1 could perhaps contribute to the deposits of immune complexes commonly found in small blood vessels in skin, muscle and lungs from Jo-1 positive DM (24). Therefore B cells/plasma cells may contribute antibodies directly to the inflammatory process in the muscle and other tissues, and BCDT may therefore give direct clinical benefit. The level of response will also be governed by the accessibility of such B cells to rituximab-mediated killing. However, the treatment regimes in the published reports vary widely and clearly the optimal regime and retreatment interval with rituximab remains to be established. Failure to respond to conventional therapy also necessitates a careful review of the diagnosis and a repeat biopsy should be considered.

In this study, in 2/8 patients, BCDT resulted in an improvement in symptoms, muscle strength, muscle enzymes and stabilisation in lung function. Two out of three of our anti-Jo-1 antibody positive patients responded to BCDT; the third patient failed to respond probably as a result of the underlying malignancy which was driving the disease. Certainly, Patient 1 responded well both to initial therapy and retreatment with normalisation of CPK. This patient was retreated with a combination regime of rituximab and cyclophosphamide with the aim of achieving a longer period of B cell depletion. The addition of cyclophosphamide may provide a higher level of B cell depletion by directly inducing plasma cell death. Although there was no improvement in overall muscle strength due to limitation by joint damage, she had improvement in the strength of the hip flexor muscles. This led to a significant improvement in HAQ score as she was now able to walk without assistance. There was no significant change in the damage score (MDI) during the follow-up period. Rituximab was well tolerated by all patients which is consistent with the experience in other autoimmune diseases (25).

In a similar open-label study of 7 patients with dermatomyositis (12), patients achieved maximal improvement in muscle strength as early as 12 weeks ranging from 36-113% increase over baseline. However, no data were

available on one patient. Of the two patients with sustained improvement in strength to 36 months, one patient was newly diagnosed and previously untreated. It was not clear how long the other patients had been on a stable dose of immunosuppressive therapy prior to BCDT.

In summary, B cells may play an important role in at least some cases of PM/DM but current depletion techniques may not necessarily address such a role, particularly if it involves autoantibodies produced by long lived plasma cells or memory B cells residing in an established protective niche in secondary lymph nodes or inflamed tissues. This may in part contribute to the lack of response seen in some patients. However results are encouraging in patients with Jo-1 antibodies. Further randomised controlled trials are underway to assess the role of B lymphocyte depletion in IIM.

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