Clin. exp. Immunol. (1983) **54**, 327–336.

Monoclonal antibodies to human leucocyte antigens in polymyositis and muscular dystrophy

DEBORAH ROWE,* D. A. ISENBERG† & P. C. L. BEVERLEY* **I.C.R.F. Human Tumour Immunology Group, School of Medicine, University College London, Faculty of Clinical Sciences, London, UK and †Cancer Research Centre, Tufts University, School of Medicine, Boston, Massachusetts, USA*

(Accepted for publication 24 June 1983)

SUMMARY

Biopsy specimens from patients with treated or untreated polymyositis and muscular dystrophy controls were examined by indirect immunoperoxidase staining with a panel of monoclonal antibodies to human leucocyte antigens. In untreated polymyositis, helper/inducer T cells were the predominant T cell subset. In treated cases few T cells were seen. Overall, few T cells were seen in dystrophic cases, most infiltrating cells being dendritic and lacking T cell antigens. Staining of sarcolemma with anti-HLA class 1 antibody is weak or negative except in areas adjacent to infiltrating leucocytes or where muscle fibre damage is apparent.

Keywords monoclonal antibodies leucocyte antigens polymyositis muscular dystrophy

INTRODUCTION

Polymyositis is a disease of unknown aetiology. It is frequently associated with other diseases, particularly malignancies, and a viral aetiology has been suggested (Mastaglia & Walton, 1970; Ben Bassat & Machtey, 1972; Sato *et al.*, 1971; Chou & Gutman, 1970). The hallmark of the pathological process is an inflammatory infiltrate in the affected muscles but the role of the infiltrating cells and the relative importance of humoral and cellular mechanisms remains obscure. Furthermore, a number of other muscle diseases, including facioscapulohumeral dystrophy also show inflammatory infiltrates and may be difficult to distinguish from polymyositis both clinically and histologically (Walton & Adams, 1958; Dubowitz & Brooke, 1973). Thus a better definition of the nature of the infiltrating leucocytes in this group of diseases might provide additional diagnostic or prognostic information as well as contributing to understanding of the disease processes.

In a preliminary immunohistological study of a small sample of polymyositis muscle biopsies (Rowe *et al.*, 1981), we have shown the presence of T lymphocytes which may be activated. In this follow up study we have examined a further series of biopsies taken at initial presentation as well as after therapy. We have also compared polymyositis and dystrophic biopsies and have used an extended panel of monoclonal antibodies (MoAbs) to examine the nature of infiltrating cells in more detail.

Correspondence: Dr P. C. L. Beverley, I.C.R.F. Human Tumour Immunology Group, School of Medicine, University College London, Faculty of Clinical Sciences, University Street, London WC1E 6JJ, UK.

MATERIALS AND METHODS

Patients. Twelve patients with polymyositis and one with dermatomyositis were studied. The polymyositis patients each met three or four of the criteria for the disease suggested by Bohan & Peter (1975). The patients with dermatomyositis met all five of the criteria suggested by these authors for this disease. The clinical details of all the polymyositis patients at the time of their muscle biopsy are recorded in Tables 1 & 2. Patients 1, 2 and 3 had biopsies taken both before and during treatment with corticosteroids. Patient 1 was re-biopsied a month after starting treatment. Patients 2 and 3 were both difficult to control on conventional doses of prednisolone and were eventually re-biopsied during flares of disease activity. Patients 4–7 were started on prednisolone following biopsy and have been reasonably well controlled on moderate doses of prednisolone prescribed for between 1 and 4 years. Patient 8 was given topical corticosteroids for her rash and as her proximal weakness was relatively mild, she has been followed for 1 year without systemic steroid treatment.

In the 'treated' patient group, patients 9, 11 and 12 were biopsied to try to decide whether their persisting proximal muscle weakness was due to steroid myopathy or continuing disease activity. Patients 10 and 13 were of interest in that they developed polymyositis whilst on treatment with prednisolone, given for other co-incident diseases. Seven patients with various different forms of muscular dystrophy were used as controls.

Collection of specimens. The specimens obtained by open or needle biopsy were snap frozen in isopentane using a liquid nitrogen coolant. Serial sections of 6 μm thick were cut, air dried and stored at $-20°C$ prior to examination.

Antisera. UCHT1 (T28) is an IgG1 mouse MoAb, derived from an immunization of BALB/c mice with human thymocytes followed by Sezary T cells. It identifies a determinant present on mature T lymphocytes and some thymocytes (Beverley & Callard, 1981).

DA2 is a MoAb of IgG1 class with specificity for a non-polymorphic determinant of HLA-Dr (Brodsky *et al.*, 1979). It was a gift of Dr M. Crumpton.

Anti-HLe-1 (2D1) is an IgG1 mouse MoAb derived from a mouse immunized with human peripheral blood mononuclear cells and identifies a determinant present on human T cells, B cells, monocytes and granulocytes (Beverley, 1980).

2A1 is an IgG1 mouse MoAb. It recognizes a non-polymorphic determinant of human HLA class I antigens.

Leu 2a (Becton Dickinson) is an IgG mouse anti-human MoAb recognizing the suppressor/cytotoxic T cell subset (Ledbetter *et al.*, 1981).

Table 1. Polymyositis patients untreated: clinical details

Patient number	Associated disease	Age	Sex	Duration of symptoms	Serum creatine kinase (iu/l)	EMG
1	SLE	21	F	3 months	1,263 ($n<120$)	Myopathic
2	—	61	F	6 months	1,117 ($n<45$)	N.D.
3	Myasthenia gravis	46	F	4 years	717 ($n<45$)	Myopathic
4	—	35	F	4 months	4,000 ($n<80$)	Myopathic
5	—	69	F	$2\frac{1}{2}$ years	172 ($n<150$)	Normal
6	Polyarthralgia	65	F	1 year	37 ($n<120$)	Myopathic
7	Scleroderma	55	F	9 months	546 ($n<140$)	Myopathic
8	(Dermatomyositis)	20	F	6 months	195 ($n<120$)	Myopathic

All biopsies were taken from patients prior to treatment. Patients 1, 2 and 3 were re-biopsied after varying periods of treatment and are designated patients 1a, 2a and 3a in Table 2.

Table 2. Polymyositis patients treated: clinical details

Patient number	Associated disease	Age	Sex	Duration of symptoms	Serum creatine kinase (iu/l)	EMG	Dose of corticosteroids
1a	SLE	21	F	4 months	720 ($n < 120$)	N.D.	1 month on pred. 25 mg/dy.
2a	—	68	F	7½ years	1,145 ($n < 70$)	Myopathic	On 5→25 mg/dy pred. for several years ± azathiop. or cyclophosphamide
3a	Myasthenia	48	F	6 years	53 ($n < 45$)	Myopathic	2 years on pred. 10→20 mg/dy
9	—	35	F	1½ years	437 ($n < 130$)	Myopathic	1 year on pred. 15 mg/dy
10	Fibrosing Alveolitis	45	M	1 year	3,600 ($n < 80$)	Myopathic	4 months on 20 mg/dy pred.
11	MCTD*	29	M	9 months	1,128 ($n < 80$)	Myopathic	6 months on 4→10 mg/dy pred.
12	MCTD*	56	F	2½ years	437 ($n < 130$)	Myopathic	2 years on 15→60 mg/dy pred.
13	Myasthenia	68	F	2 months	930 ($n < 50$)	Myopathic	1 month on 30 mg/dy pred.

*Mixed connective tissue disease.
Treated patients 1a, 2a and 3a are identical to untreated patients 1, 2 and 3, Table 1.
Pred. = prednisolone; azathiop. = azathioprine.

UCHT4 (C3) is an IgG2 mouse MoAb derived from a similar immunization schedule to UCHT1. It reacts with an antigenic determinant closely related to that detected by Leu 2a and gives similar staining patterns in tissue section (Beverley, 1982).

Leu 3a (Becton Dickinson) is an IgG1 mouse MoAb recognizing the helper/inducer T cell subset (Ledbetter *et al.*, 1981).

Peroxidase conjugated rabbit anti-mouse immunoglobulin antiserum was purchased from Dako. It was absorbed by passage through a human immunoglobulin sepharose column and titrated on tonsil sections using UCHT1 to ascertain the optimal working concentration.

Staining by indirect immunoperoxidase. Sections were washed for a minute in Tris-buffered saline (TBS), drained and incubated in 25 μl of the monoclonal first layer for 30 min at room temperature. The excess antibody was drained and the sections washed twice in TBS. They were then incubated with 25 μl of rabbit anti-mouse immunoglobulin conjugated to horseradish peroxidase, at a concentration of 1/50, in TBS for 30 min at room temperature.

Sections were washed twice in TBS and incubated with 3,3′ diaminobenzidine at a concentration of 6 mg in 10 ml. Three microlitres of hydrogen peroxide were added to this just before adding to the sections. After 7 min incubation the sections were washed in three changes of TBS and then for an hour in running tap water. Counterstaining was for 12 seconds in Mayer's haematoxylin. Sections were blued in tap water and dehydrated through a series of graded alcohols (50%, 70%, 90%, one minute each, and 100% alcohol, two changes of 5 min). They were then cleared in xylene (two changes for 5 min each). Permanent mounts were made in DPX. Results were crudely quantified on a 0→+++, according to the numbers of positive cells seen.

RESULTS

Staining with antibody to HLA class 1 antigens indicated a variation in both the quantity of antigen and its anatomic localization in all the muscle biopsies examined. Muscle fibre membranes

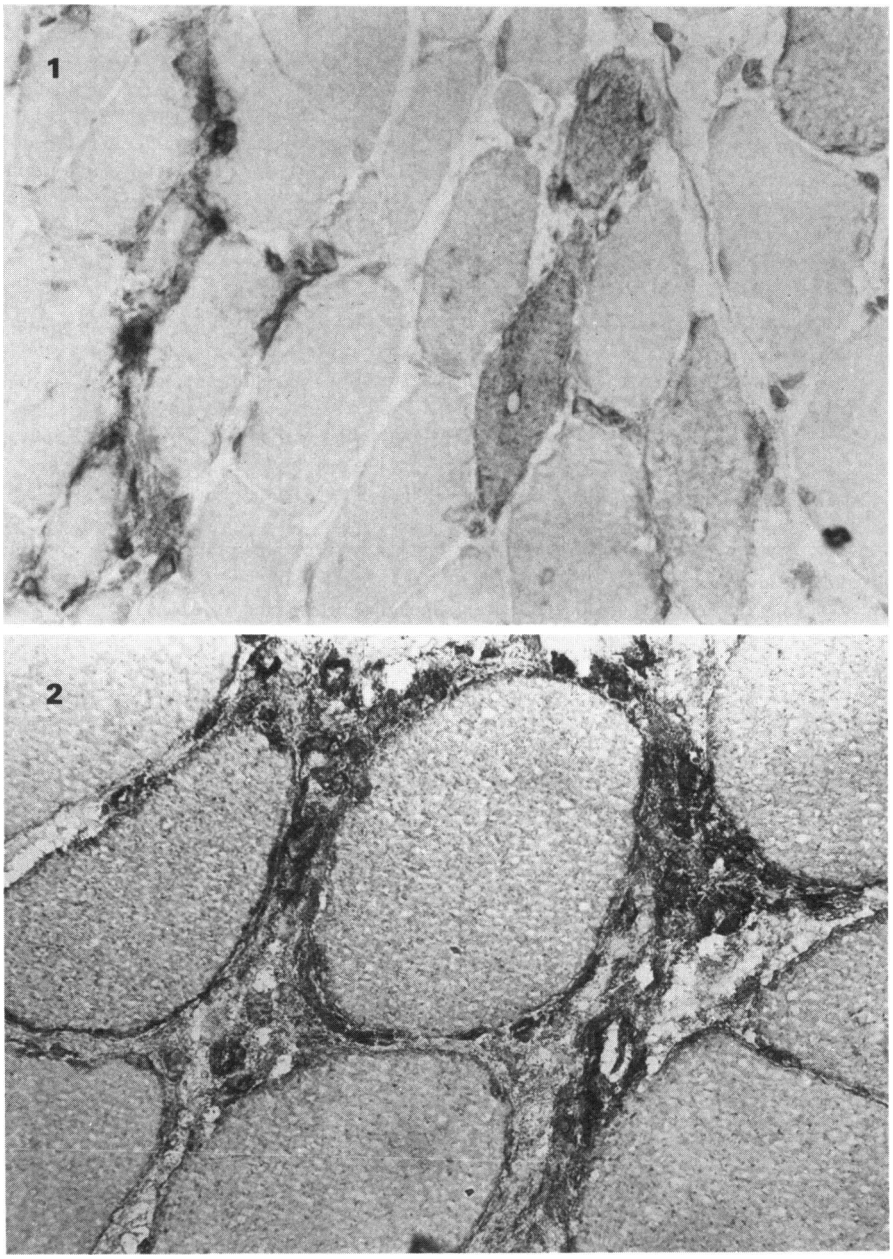


Fig. 1. HLA class I staining in the cytoplasm of damaged muscle cells with central nuclei. Patient 4. Indirect immunoperoxidase. Counterstained haematoxylin × 320.

Fig. 2. HLA class I staining of leucocyte infiltrated area. The sarcolemma (muscle membrane) is stained. Patient 15. Indirect immunoperoxidase. Counterstained haematoxylin × 320.

Table 3. Polymyositis untreated patients

Patient No.	2A1	HLe-1	DA2	UCHT1	UCHT4/Leu 2a	Leu 3a
1	+→++	+	+	−	−	−
2	+++	+++	+→++	++	+	+→++
3	+++	++→+++	++	+→++	+	+→++
4	+++	+++	+++	++	+→++	++
5	+++	+++	+++	++	++	+→++
6	ND	+→++	+→++	0→+	0→+	++
7	+++	+++	ND	+→+++	++	++→+++
8	+++	+→++	0→+	+→++	+	+→++

The number of cells stained was approximately quantitated using a 0→+++ scale.

2A1 is specific for HLA class I; HLe-1 is specific for all human leucocytes; DA2 is specific for HLA class I; UCHT1 is specific for T cells; UCHT4 is specific for suppressor/cytotoxic T cells.

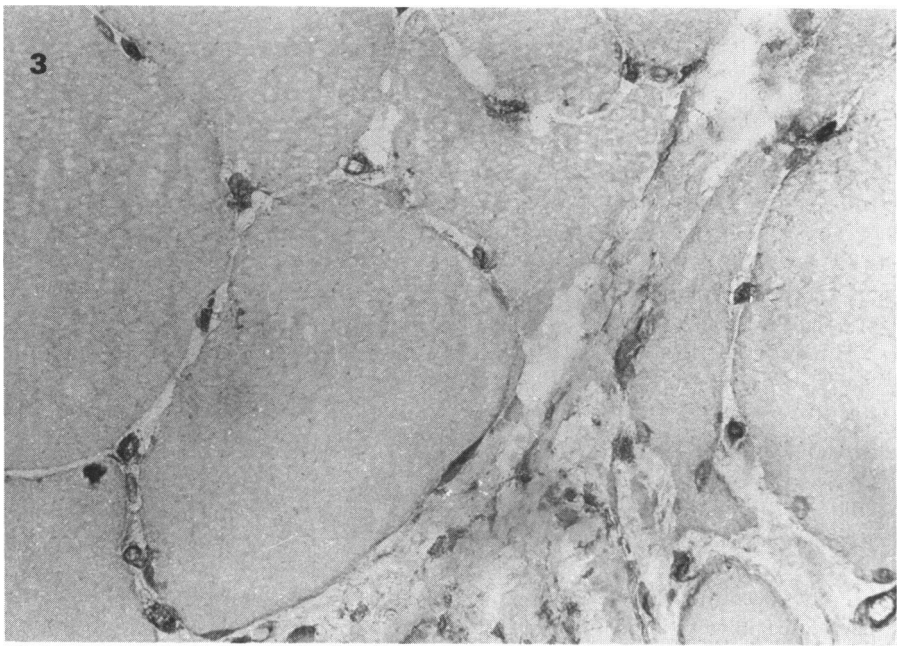
(sarcolemma) were usually negative, but badly damaged fibres, especially those with central nuclei frequently showed both intracytoplasmic and sarcolemmal staining (Fig. 1). Where there was a heavy infiltrate without obvious fibre damage, staining was usually confined to the sarcolemma. Adjacent areas of the same section with less infiltrating cells might show little or no sarcolemmal staining (Figs 2 & 3).

HLe-1 (anti-leucocyte) showed considerable leucocyte infiltration (greater than +) in seven out of eight untreated polymyositis patients (Table 3). Staining of both rounded and dendritic cells was seen. By contrast, three of the eight treated patients had very few infiltrating cells (patients 1a, 2a and 3a, Table 4). However, in some treated patients the level of leucocyte infiltration was as high as in the untreated patients (patients 9, 10, 11, 12, 13, Table 4). In the three patients who were re-biopsied during treatment low levels of infiltration were seen whereas before treatment two out of three showed a heavy infiltrate (patients 2 and 3, Table 3, 2a and 3a, Table 4). HLe-1 also showed both positive and negative cells in an infiltrate in a patient which appeared histologically homogenous (patient 7, Table 3, Fig. 4). These cells were also negative with the MHC antibodies and T cell antibodies, and may therefore represent damaged or regenerating muscle cells. In the dystrophic patients, the overall level of infiltration, as indicated by HLe-1, was lower than in untreated polymyositis, three of the patients having barely detectable infiltration. The muscle biopsy from patient 15 (Table 5) was the only one showing a +++ infiltrate. This patient had facioscapulo humeral dystrophy.

In untreated polymyositis patients the majority of leucocytes were stained both by HLe-1 and DA2 (anti-HLA class II). Many of these cells also stained with the anti-T reagents. In contrast, in dystrophic patients although staining with HLe-1 and DA2 was seen, there were fewer T cells and

Table 4. Polymyositis treated patients

Patient No.	2A1	HLe-1	DA2	UCHT1	UCHT4/Leu 2a	Leu 3a
1a	+→++	0→+	ND	0→+	ND	0→+
2a	+→++	+	+→++	0→+	−	−
3a	++	0→+	+	0→+	−	0→+
9	+++	+→++	+→++	+	0→+	0→+
10	+++	++	+	+	+	+
11	+++	++→+++	+++	+	+	0→+
12	++	++	−	0→+	ND	0→+
13	+++	+→++	+	0→+	0→+	+→++



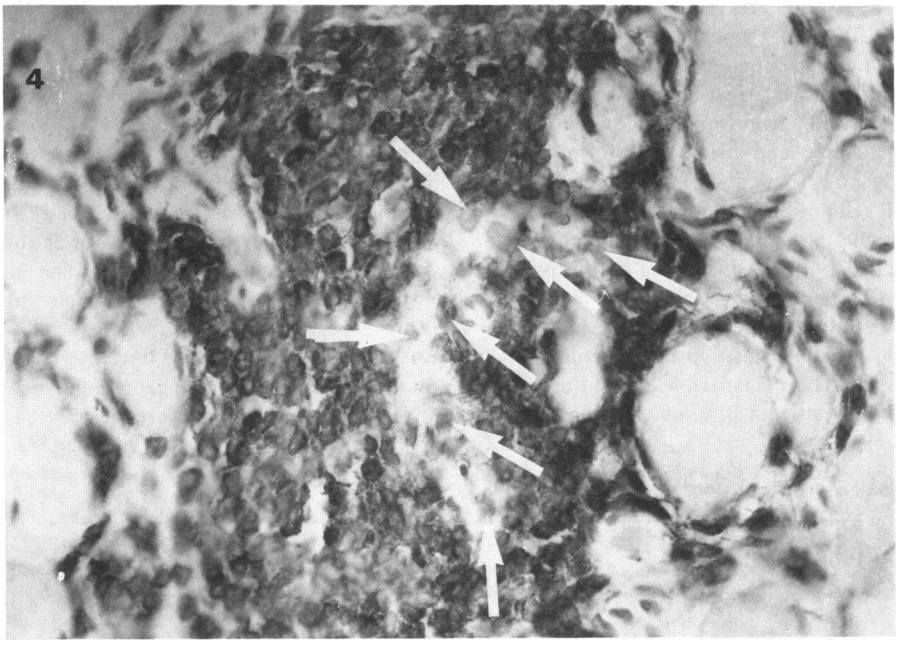


Fig. 3. HLA class I staining in uninfiltrated area of the same patient as Fig. 2. The sarcolemma (muscle membrane) is not stained. Patient 15. Indirect immunoperoxidase. Counterstained haematoxylin ×320.

Fig. 4. Infiltrating cells stained with anti-leucocyte antibody (HLe-1). Some of the cells (arrowed) are unstained. Patient 4. Indirect immunoperoxidase. Counterstained haematoxylin ×320.

many of the leucocytes were dendritic in shape. These cells were most probably monocytes. Staining was also mainly dendritic in character in polymyositis patients in whom the level of infiltration was low. In some patients more staining was seen with DA2 than HLe-1 (patients 2a, 3a, and 11, Table 4 and 14, 15 and 19, Table 5) and in some untreated patients hazy staining with DA2 was seen within muscle cells.

UCHT1 showed considerable numbers of T cells in the larger infiltrates of untreated patients (Table 3), exceeding + in all but one patient. In the treated patients many fewer T cells were seen, the numbers not exceeding + in any of the biopsies examined (Table 4). In the control dystrophic muscle the numbers of T cells ranged from 0→++. In untreated polymyositis (Table 3), more labelling was seen with Leu 3a (helper/inducer), than UCHT4 (suppressor/cytotoxic), the labelling usually being confined to the UCHT1 positive areas. Fig. 5 shows a section in which a high proportion of the infiltrating cells show membrane staining with Leu 3a. In the treated patients, far fewer T cells were present, this being reflected in the results with the T cell subset antibodies. There were not obviously greater numbers of Leu 3a positive cells than UCHT4 positive cells in the treated patients, but the number of cells stained by either antibody was small so that this conclusion is tentative.

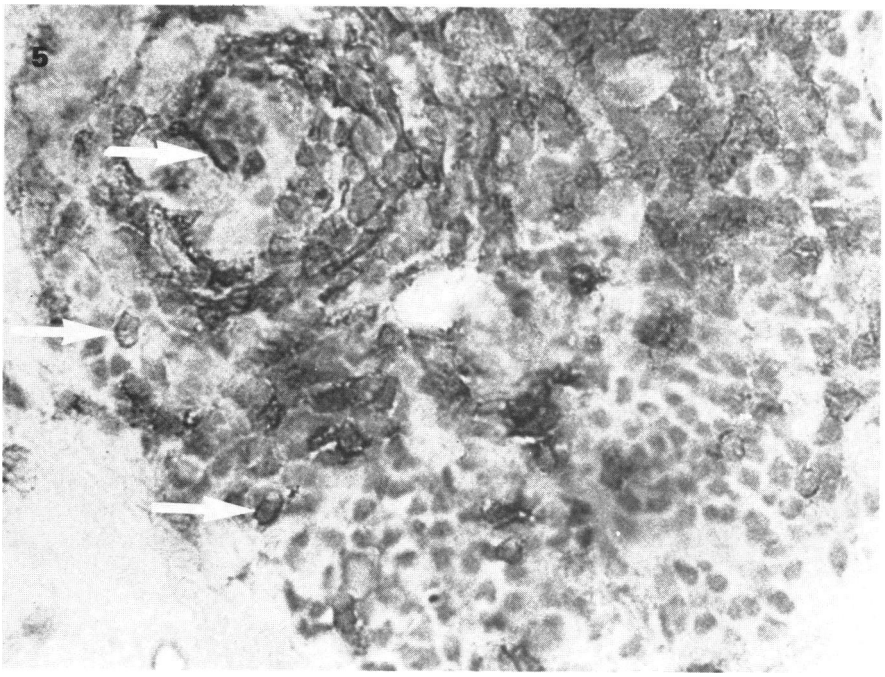
In the dystrophic biopsies very little staining was seen with the two T cell subset antibodies, the level only exceeding + in two cases (patients 15 and 20, Table 5). There were no greater numbers of helper/inducer T cells than suppressor/cytotoxic T cells as compared to the untreated polymyositis cases, but because there were few cells in the dystrophic muscles, this subjective estimate may be unreliable. In one case (patient 15, Table 5) dendritic type cells were stained cytoplasmically with Leu 3a. These cells were located intramuscularly, stained positively with DA2 and HLe-1 and most were not stained by the other T cell antibodies (Fig. 6). This pattern is suggestive of macrophages and was not seen in any of the polymyositis cases.

Table 5. Control patients

Patient No.	Disease	2A1	HLe-1	DA2	UCHT1	UCHT4/Leu 2a	Leu 3a
14	fascioscapulo humeral dystrophy	++	0→+	+→++	0→+	–	–
15	fascioscapulo humeral dystrophy	+++	+++	+++	++	+	+
16	limb girdle dystrophy	+→++	0→+	0→+	–	–	0→
17	dystrophia myotonica	+→++	+	+	0→+	0→+	0→+
18	scapuloperoneal dystrophy	ND	+	0→+	ND	–	0→+
19	chronic, non-specific dystrophy	+	0→+	+→++	0	ND	ND
20	dystrophia myotonica	+	+→++	0→+	+→++	+	+→++

DISCUSSION

Polymyositis is a potentially fatal disease generally considered to have a cell-mediated origin (Hudgson & Walton, 1979). Corticosteroids remain the mainstay of treatment although doubts have been expressed as to their efficacy (Medsger *et al.*, 1971; Bohan & Peter, 1975). It has not yet proved possible to predict in individual cases, whether the polymyositis will respond to corticosteroids. In particular the creatine kinase (CK) at presentation (De Vere & Bradley, 1975) and the biopsy picture using routine histological and histochemical stains (Riddoch & Morgan-



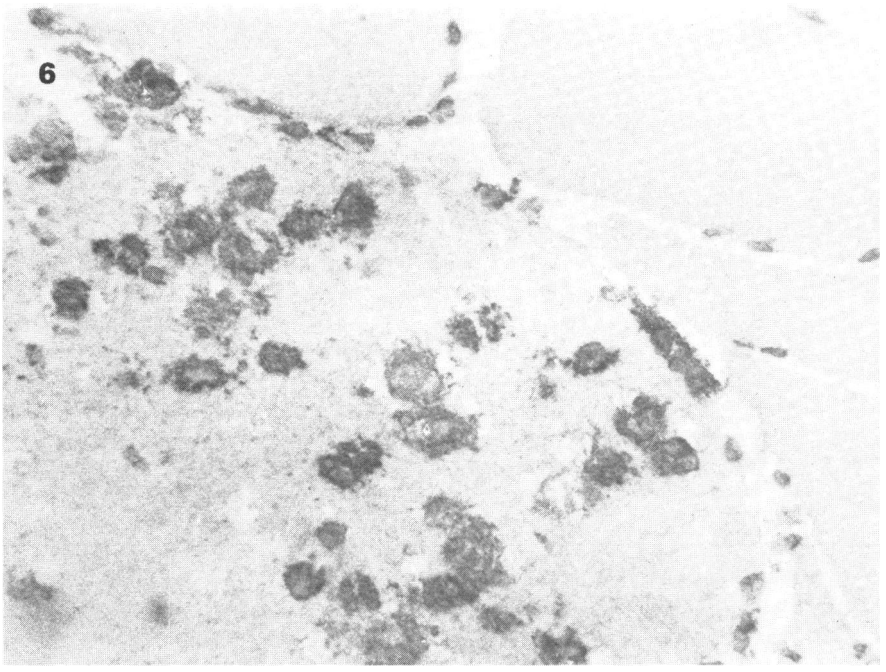


Fig. 5. Same infiltrate as Fig. 4 stained for helper/inducer T cells (Leu 3a). A large proportion of the cells are membrane stained (some membrane stained cells are arrowed). Patient 3. Indirect immunoperoxidase. Counterstain haematoxylin × 320.

Fig. 6. Infiltrating cells within muscle fibre stained for HLA class II (DA2). The cytoplasm of the infiltrating cells is heavily stained. Patient 15. Indirect immunoperoxidase. Counterstained haematoxylin × 320.

Hughes, 1975) have little prognostic significance. However, it has been claimed that in cases where the CK level is raised, serial measurements may be useful in assessing the effects of treatment with corticosteroids (De Vere & Bradley, 1975; Henriksson, 1980). The local effect of steroids on immune infiltrates is still a matter for conjecture. Corticosteroids in large doses (60 mg/day) have previously been shown to effect some reduction in the amount of inflammatory infiltrate (Bunch *et al.*, 1980).

Our results have shown reduction in the number of infiltrating leucocytes stained with HLe-1 in treated patients, this being particularly reflected in a reduction in the numbers of UCHT1 positive, T cells. HLe-1 also clarified the numbers of infiltrating immune cells in cell masses which appeared histologically homogenous, probably actually consisting of mixtures of leucocytes clearly stained with HLe-1 and displaced sarcolemma nuclei, which are HLe-1 negative (Fig. 4). After treatment, the ratio of T helper/inducer to suppressor/cytotoxic cells was reduced, suggesting a selective effect on the helper/inducer population, although the number of cells stained in the treated patients is small, making results difficult to interpret. Whether these changes are due to actual reduction in numbers of circulating lymphocytes or difference in numbers sequestered, and the relevance of this to the progress of the disease, is not obvious.

The larger numbers of Leu-3a (helper/inducer) T cells relative to suppressor/cytotoxic cells seen in untreated polymyositis muscle are the proportions seen in normal peripheral blood (Morimoto *et al.*, 1982). Infiltrates consisting predominantly of helper/inducer cells have also been described in synovium from rheumatoid arthritis (Janossy *et al.*, 1981) and labial biopsy tissue from patients with Sjögren's syndrome (Fox *et al.*, 1982). This situation is the reverse of that seen in some breast cancer infiltrates where the suppressor/cytotoxic T cell may predominate, suggesting fundamental differences in T cell roles in local autoimmune and malignant conditions.

Dystrophic muscle, in contrast to polymyositis, shows very few cells as indicated by HLe-1 staining. Most of the cells which were present were dendritic in appearance, DA2 positive, and thus probably macrophages. Very few T cells were seen indicating a different immunopathology for this disease. Intramuscular cells of dendritic appearance were seen in one case of dystrophic muscle. These cells were HLe-1 and DA2 positive. A small proportion stained with UCHT1 but the majority were negative. All showed cytoplasmic staining with Leu 3a and were negative for UCHT4. Their shape and phenotype is strongly suggestive of macrophages especially since Leu 3a has been observed to stain the cytoplasm of some cells of the macrophage/monocyte series in tissue sections of lymph node (D.Y. Mason & R. Warnke, personal communication). This population was not seen in any of the polymyositis cases.

It has been suggested that viruses may be implicated in the aetiology of polymyositis. Evidence for this comes from several studies demonstrating virus particles by electron microscopy (Chou & Gutman, 1970; Mastaglia & Walton, 1970; Sato *et al.*, 1971; Ben-Bassat & Machtey, 1972) as well as the successful culture of viruses from biopsy material (Kessler *et al.*, 1981). Since major histocompatibility complex antigens are important in T cell responses to viruses (McMichael, 1978), it is intriguing that HLA class I antigen is only detected in the cytoplasm and sarcolemma of damaged muscles or adjacent to areas of infiltration. Whether the altered expression of HLA is secondary to the lymphocyte infiltration is not known, but lymphokines, particularly interferons, are known to alter expression of HLA (Wallach, Fellows & Nevel, 1982).

Anti-HLA class II (DA2) showed as much staining as HLe-1 in infiltrates containing T cells indicating that the T cells were probably activated. Diffuse staining suggesting the possibility of an HLA class II positive factor within damaged muscle was also seen in three patients, also confirming our previous findings (Rowe *et al.*, 1981).

This follow up study has provided further information on the nature of the lesion in polymyositis, subject to the sampling problems of examining biopsy material. It has proved very difficult to relate this to prognosis particularly since patients have been biopsied at random times from presentation, and the taking of sequential biopsies is rare. In cases where the patients were re-biopsied, the patients had either not improved or had deteriorated, but in two out of three patients considerable reduction of infiltrating leucocytes was seen. Three patients were re-biopsied because they had failed to respond to steroid therapy. Steroids are known to cause muscle weakness so that means of distinguishing continuing disease activity from steroid effects are required. In two out of three re-biopsied patients there was a considerable reduction in the level of infiltration

suggesting that in these patients the continuing muscle weakness might have been directly attributable to the steroid therapy.

In conclusion, this study suggests that immunohistology may be helpful in the differential diagnosis of polymyositis and muscular dystrophies. Examination of infiltrates may also aid in monitoring the effects of therapy. Finally, a number of features of infiltrates have been revealed which may contribute to understanding of the disease process in polymyositis.

The authors thank Professor Richard Edwards and Dr J. Morgan-Hughes for allowing us to study their patients.

REFERENCES

Ben Bassat, M. & Machtey, L. (1972) Picorna-like structures in acute dermatomyositis. *Am. J. Clin. Path.* **58a,** 245.

Beverley, P.C.L. (1980) Production and use of monoclonal antibodies in transplantation immunology. In *Proceedings of the XI International Course in Transplantation and Clinical Immunology.* p. 87. Excerpta Medica, Amsterdam.

Beverley, P.C.L. (1982) The application of monoclonal antibodies to the typing and isolation of lymphoreticular cells. *Proc. R. Soc. Edinburgh.* **81B,** 221.

Beverley, P.C.L. & Callard, R.E. (1981) Distinctive functional characteristics of human T-lymphocytes defined by E-rosetting or a monoclonal anti-T cell antibody. *Eur. J. Immunol.* **11,** 329.

Bohan, A. & Peter, J.B. (1975) Polymyositis and dermatomyositis. *N. Engl. J. Med.* **292,** 403.

Brodsky, F.M., Parham, P., Barnstaple, C.J., Crumpton, M.J. & Bodmer, W.F. (1979) Monoclonal antibodies for analysis of the HLA system. *Immunol. Rev.* **47,** 3.

Bunch, T.W., Worthington, J.W., Combs, J.J., Duane, M., Ilstrup, M.S. & Engel, A.G. (1980) Azathioprine with prednisone for polymyositis. A controlled clinical trial. *Ann. Int. Med.* **92,** 365.

Chou, S.M. & Gutman, L. (1970) Picorna-like crystals in subacute polymyositis. A controlled clinical trial. *Neurology,* **20,** 205.

De Vere, R. & Broadley, W.G. (1975) Polymyositis, its presentation, morbidity and mortality. *Brain,* **98,** 637.

Dubowitz, V. & Brooke, M. (1973) *Muscle biopsy. A modern approach.* Second edition. W. B. Saunders, London.

Duke, O., Panayi, G.S., Janossy, G. & Poulter, L.W. (1982) An immunohistochemical analysis of lymphocyte subpopulations and their microenvironment in the synovial membranes of patients with rheumatoid arthritis using monoclonal antibodies. *Clin. Exp. Immunol.* **49,** 22.

Fox, R.I., Carstens, S.A., Forg, S., Robinson, C.A., Howell, F. & Vaughan, J.W. (1982) Use of monoclonal antibodies to analyse peripheral blood and salivary gland lymphocyte subsets in Sjögrens syndrome. *Arthrit. Rheum.* **25,** 419.

Henriksson, K.G. (1980) Polymyositis diagnosis, prognosis and treatment. *Linkoping Univ. Med. School.* Dissertations, Linkoping.

Hudgson, P. & Walton, J.N. (1979) Polymyositis and other inflammatory myopathies. In *Handbook of Clinical Neurology* (ed. by P. J. Vinker & G. W. Bruyn) pp. 41–51. North Holland Publishing, Oxford.

Janossy, G., Panayi, G., Duke, O., Bofil, M., Poulter, L.W. & Goldstein, G. (1981) Rheumatoid arthritis: a disease of T lymphocyte/macrophage immunoregulation. *Lancet,* **ii,** 839.

Kessler, W.A., Trenholme, G.M., Harris, A.A. & Levin, S. (1981) Acute myopathy associated with influenza A/Texas 1/77 infection. Isolation of a virus from a muscle biopsy specimen. *J.A.M.A.* **243,** 461.

Ledbetter, J.A., Evans, R.L., Lipinski, M., Cunningham-Rundles, C., Conrad, R.A. & Herzenberg, L.A. (1981) Evolutionary conservation of surface molecules that distinguish T lymphocyte helper/inducer and cytotoxic/suppressor subpopulations in mouse and man. *J. exp. Med.* **153,** 310.

Mastaglia, F.L. & Walton, J.N. (1970) Coxsackie virus-like particles in skeletal muscle from a case of polymyositis. *J. Neurol. Sci.* **II,** 593.

McMichael, A.J. (1978) HLA restriction of human cytotoxic lymphocytes specific for influenza virus. Poor recognition of virus associated with HLA-A2. *J. exp. Med.* **148,** 1458.

Medsger, T.A., Robinson, H. & Masi, A.T. (1971) Factors affecting survivorship in polymyositis. A life-table study of 124 patients. *Arthrit. Rheum.* **14,** 249.

Morimoto, C., Reinherz, E.L., Nadler, L.M., Distaso, J.A., Steinberg, A.D. & Schlossman, S.F. (1982) Comparison in T and B cell markers in patients with Sjögrens syndrome and systemic lupus erythematosus. *Clinical Immunol. Immunopathol.* **22,** 270.

Riddoch, D. & Morgan-Hughes, J.D. (1975) Prognosis in adult polymyositis. *J. Neurol. Sci.* **26,** 71.

Rowe, D.J., Isenberg, D.A., McDougall, J. & Beverley, P.C.L. (1981) Characterization of polymyositis infiltrates using monoclonal antibodies to human leucocyte antigens. *Clin. exp. Immunol.* **45,** 290.

Sato, T., Walker, D.L., Peters, H.A., Rees, H.H. & Chew, S.M. (1971) Chronic polymyositis and myxovirus-like inclusions. *Arch. Neurol.* **24,** 409.

Wallach, D., Fellows, M. & Revel, M. (1982) Preferential effect of γ interferon on the synthesis of HLA antigens and their mRNAs in human cells. *Nature,* **299,** 833.

Walton, J.N. & Adams, R.D. (1958) *Polymyositis.* Livingstone, Edinburgh.