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# Characterization of polymyositis infiltrates using monoclonal antibodies to human leucocyte antigens

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## SUMMARY

Frozen serial sections of muscle from 15 patients with polymyositis and three normal controls were studied by indirect immunofluorescence with a panel of mouse monoclonal antibodies to various human leucocyte components. The results showed good correlation with conventional histology. In addition, large numbers of T lymphocytes were identified in those cases with a marked inflammatory infiltrate. Many of the T cells probably bear HLA-DR antigen as the anti-HLA-DR antibody stained as many cells as the anti-leucocyte antiserum. This strongly suggests that the T cells present are 'activated'. In two patients HLA-DR-positive material was identified apparently diffusing from the infiltrates into muscle fibres suggesting its release as a soluble factor. In one case, structures with the appearance of giant cells were seen. The method promises to provide new information on the nature of infiltrating leucocytes which may provide more accurate diagnostic and prognostic information than conventional histology alone.

## INTRODUCTION

Polymyositis is generally considered to be a heterogeneous disorder characterized histologically by an inflammatory cell infiltrate and muscle fibre necrosis with accompanying regeneration. A considerable literature exists discussing the aetiology of polymyositis (Johnson, Fink & Ziff, 1972; Dawkins & Mastaglia, 1973; Pearson, 1976; Smith *et al.*, 1979). Most authors favour a cell-mediated mechanism, although much of the published data refers to work done on animal models or using *in vitro* techniques, which may not accurately reflect the disease in man.

Until now muscle biopsies have been assessed by routine histological and histochemical methods. With the development of the somatic cell hybridization technique for production of monoclonal antibodies (Kohler & Milstein, 1976) it has become possible to raise specific antisera against cell surface components of the lymphoid and myeloid cell series and a new technique has thus become available for the study of cellular infiltration in pathological conditions. These markers offer a more precise identification of the cells involved and may therefore help in the diagnosis of human polymyositis and provide clues to its pathogenesis.

In this study we have assessed muscle biopsies from three healthy volunteers, obtained by needle biopsy (Edwards, Wiles & Young, 1980), and 15 patients with polymyositis, obtained by open or needle biopsy. The results of conventional histology are compared with studies using monoclonal antibodies to human T lymphocytes, polymorphs, leucocytes and an HLA-DR marker.

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## MATERIALS AND METHODS

*Patients.* Each of the 15 patients met three or four of the Bohan & Peter (1975) criteria for polymyositis: namely, proximal muscle weakness, muscle biopsy changes, elevated muscle enzymes in serum and myopathic changes on electromyography. Eight of the patients (Nos II, III, VII, IX, XI, XII, XIV, XV: Table 1) were considered to have 'primary' disease, with no evidence of occult neoplasm, connective tissue disorder or relevant drug therapy. The other seven patients were considered to have 'secondary' polymyositis (Table 2). Patients I, V and VIII had systemic lupus erythematosus, with four or more of the American Rheumatism Association's criteria for the classification of this disease (Cohen *et al.*, 1971). Patients IV and XIII had definite rheumatoid arthritis, diagnosed by the criteria of Ropes *et al.* (1958). Both these diseases are known to be associated with polymyositis (Bohan & Peter, 1975). Patient VI had had an episode of erythema nodosum and was found to have a positive Kveim test. It was considered that she had sarcoidosis. This too may be associated with a polymyositis (Gardner-Thorpe, 1972). Patient X developed polymyositis whilst being treated with D-penicillamine for fibrosing alveolitis. This complication of therapy has been previously recorded (Lane & Mastaglia, 1978).

The muscle biopsies (open or needle, as specified in Tables 1 and 2) were performed on each of these patients as part of routine investigations for diagnostic purposes. Needle biopsies from three healthy volunteers were used as controls.

*Methods.* The muscle biopsy specimens whether obtained by needle or open biopsy were snap-frozen in isopentane using liquid nitrogen as a coolant and stored at $-70°C$. Serial sections 6 $\mu$m thick were cut prior to processing. Some were stained with haematoxylin & eosin, some for acid phosphatase while others were examined for the presence of the various human leucocyte surface markers. All sections were numbered and labelled independently and assessed 'blind'.

*Antisera.* UCHT1 (T28) is an IgG1 mouse monoclonal antibody derived from an immunization of BALB/c mice with human thymocytes followed by Sézary T cells. It identifies a determinant present only on mature T lymphocytes and some thymocytes (Beverley, Linch & Callard, 1981; Beverley & Callard, 1981). DA-2 is a monoclonal antibody 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. TG-1 is an IgM antibody derived from a mouse immunized with thymus membrane glycoprotein fraction eluted from a Con A column (Sullivan & Beverley, unpublished data). The antiserum stains peripheral blood neutrophils and eosinophils but not T and B lymphocytes or thymocytes. It also stains mature cells of the granulocyte lineage in the bone marrow (Beverley, Linch & Delia, 1980). Anti-HLe-1 (2D1) is an IgG1 mouse monoclonal antibody 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).

*Indirect immunofluorescence.* Frozen sections were dried for 1 hr before use and then washed for 20 min in phosphate-buffered saline (PBS) in a rocking water bath. Sections were incubated with 25 $\mu$l of the monoclonal first layer in saturating amounts (previously determined by titration) for 30 min at room temperature in a humidified chamber. They were then washed for 30 min in PBS, and excess fluid removed. Twenty-five microlitres of the second layer, immunoabsorbent-purified and human immunoglobulin-absorbed FITC sheep anti-mouse immunoglobulin antiserum was added at a dilution of 1/80 and incubated for 30 min in a humidified chamber. The diluent was buffered saline solution containing 20% heat-inactivated sheep serum. The sections were then finally washed for 20 min in PBS.

## RESULTS

The results of our conventional histological studies are broadly in line with those of others (Adams, 1973; Bohan & Peter, 1975). The pattern of muscle fibre damage and inflammation was either focal or diffuse but in all cases mononuclear cells predominated in the inflammatory infiltrate. A comparison of sections of the same area stained by haematoxylin & eosin and acid phosphatase

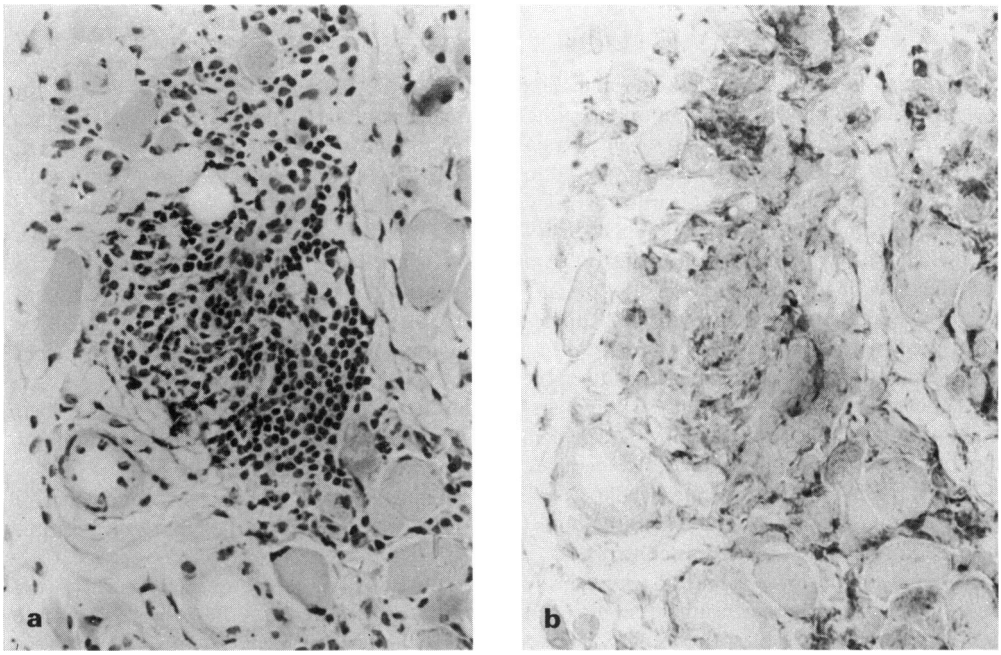



**Fig. 1.** (a) An area of inflammation composed of mononuclear cells to compare with (b) (patient XV). (H & E, original × 400.) (b) In this serial section stained for the reaction of acid phosphatase, only some of the infiltrating cells are positive indicating that they are macrophages (patient XV). (Acid phosphatase, original × 400.)

(Fig. 1a & b) shows that only a proportion of the infiltrating cells are macrophages. The exact nature and significance of the small mononuclear cells is less clear.

We therefore examined the nature of the infiltrating cells using a panel of monoclonal antibodies. Significant staining with the T cell marker UCHT1 was observed in several of our patients (Nos IV, VI, XIII, XV; Tables 1 and 2) providing clear evidence for the presence of T cells in the inflammatory infiltrates (Fig. 2). T lymphocytes were seen predominantly in sections showing more severe inflammation and when present showed either scattered distribution or localized aggregates of cells. While anti-HLA-DR and HLe-1 generally stained all cells within an area of infiltration, UCHT1 only stained a proportion (compare Fig. 2 with Fig. 3a & b).

The results obtained with the anti-HLA-DR monoclonal antibody (DA2) are revealing. In most cases the extent of the infiltrate delineated by this serum equals that seen with HLe-1 which has been shown to stain all leucocytes (Beverley, 1980). Even in cases in which most of the infiltrating mononuclear cells were T cells (Fig. 2), all the cells also stained with anti-HLA-DR. It seems likely, therefore, that at least some of the T cells must carry DR antigens. This suggests that some T lymphocytes are activated since HLA-DR is found on few resting T cells (Greaves *et al.*, 1979) but appears on activation with mitogens or alloantigens *in vitro* (Evans *et al.*, 1978).

In patient No. VI, although the predominant cell of the infiltrate was lymphocytic between muscle fibres, some of which showed regeneration and degeneration, there were a few focal collections of macrophages and epithelioid cells including a few typical giant cells which were not of muscle origin (Fig. 4). These stained strongly with anti-HLA-DR (Fig. 3a). The membrane of these cells was also outlined by anti-HLe-1 (Fig. 3b). The same cells were stained with acid phosphatase.

A third feature of these inflammatory infiltrates revealed by staining with anti-HLA-DR in two patients (Nos XIV and II) was the presence of DR-positive material seen as a hazy deposit apparently diffusing into muscle fibres from interstitial immune infiltrates (Fig. 5). This pattern of staining was seen only with the anti-HLA-DR although both the anti-HLe-1 and UCHT1 sera are of the same mouse immunoglobulin class (IgG1). It was not found in control biopsies.

Biopsy material from three normal individuals was stained with the same panel of antibodies.

**Table 1.** Clinical details and summary of results from patients with 'primary' polymyositis

| Patient number | Age | Sex | Dose of prednisolone at time of biopsy | Lymphocytes | Macrophages | Polymorphs | HLe-1* | DA-2* | UCHT1* | TG-1* |
|---|---|---|---|---|---|---|---|---|---|---|
| II | 50 | M | On prednisolone for 13 years. 5 mg daily at time of biopsy | + | + | − | + | + | 0/+ | − |
| III | 51 | M | 0 | ++ | + | − | +/++ | +/++ | − | 0/+ |
| VII | 33 | F | 60 mg: had been treated for several weeks | + | ++ | − | + | +/++ | − | + |
| IX | 39 | M | 10 mg: had been treated for several months | 0/+ | + | − | 0/+ | 0/+ | − | − |
| XI | 73 | M | 0 | + | + | − | 0/+ | 0/+ | − | 0/+ |
| XII | 60 | M | 0 | + | ++ | 0/+ | +/++ | +/++ | 0/+ | − |
| XIV | 49 | F | 0 | + | + | − | +/++ | +/++ | − | ++ |
| XV | 59 | F | 40 mg: had been treated for several weeks | ++ | ++ | − | ++/+++ | +++ | ++/+++ | 0/+ |

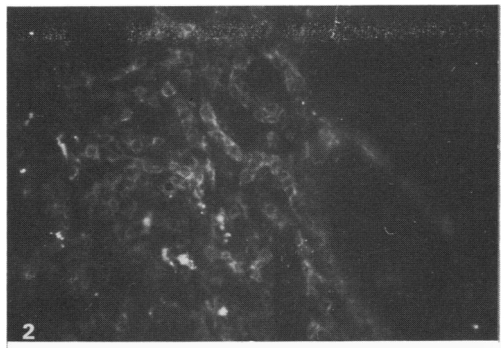
* HLe-1 is specific for all human leucocytes, DA-2 has specificity for HLA-DR, UCHT1 is specific for all mature human T lymphocytes, TG-1 is specific for human granulocytes.

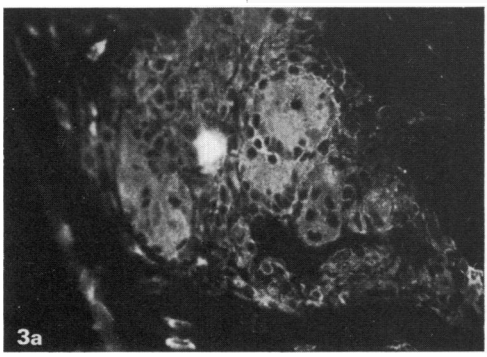
Patients IX, XI and XIV had quadriceps needle biopsies, patients XII and XV had quadriceps open biopsies and patients II, III and VII had biceps open biopsies.

**Table 2.** Clinical details and summary of results from patients with 'secondary' polymyositis

| Patient number | Age | Sex | Disease association | Dose of prednisolone at time of biopsy | Lymphocytes | Macrophages | Polymorphs | HLe-1 | DA-2 | UCHT1 | TG-1 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 35 | F | SLE | 45 mg: had been treated for several weeks | 0/+ | ++ | − | ++/++ | ++/+++ | + | + |
| IV | 48 | M | RA | 0 | ++ | +++ | − | +++ | +++ | +/++ | − |
| V | 25 | F | SLE | 10 mg: had been treated with steroids intermittently for 10 years | 0/+ | 0/+ | − | − | 0/+ | − | − |
| VI | 58 | F | Sarcoidosis | 0 | +++ | ++ | − | +++ | +++ | +/++ | − |
| VIII | 34 | F | SLE | ? | − | + | 0/+ | 0/+ | 0/+ | − | 0/+ |
| X | 60 | F | Fibrosing alveolitis, penicillamine therapy | 0 | − | + | − | 0/+ | 0/+ | − | 0/+ |
| XIII | 45 | M | RA | 120 mg: for 1 week | + | ++ | − | ++ | ++ | + | 0/+ |

Patients V and XIII had quadriceps needle biopsies, patients I and IV had quadriceps open biopsies, patients VI and X had triceps open biopsies and patient VIII had a biceps open biopsy.



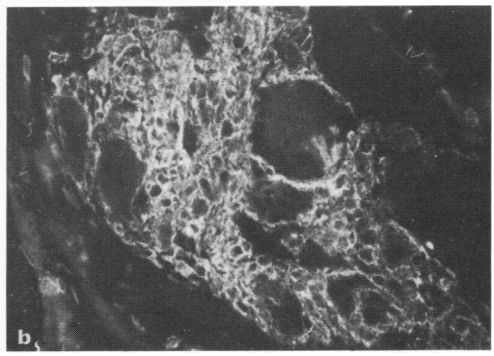
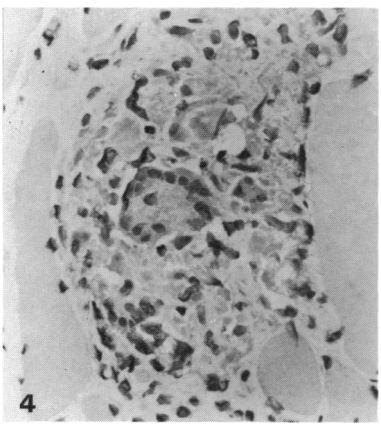




**Fig. 2.** Frozen section from patient VI stained with UCHT1 by indirect immunofluorescence. Numerous membrane-stained T cells can be seen between the unstained muscle fibres. (Original × 400, enlarged × 5.)

**Fig. 3.** (a) Frozen section from patient VI stained with anti-HLA-DR by indirect immunofluorescence showing large multinucleate stained cells with peripheral unstained nuclei. (Original × 400, enlarged × 5.) (b) Same area as (a) stained with HLe-1 by indirect immunofluorescence. The outer membrane only of the multinucleate cells has stained. (Original × 400, enlarged × 5.)



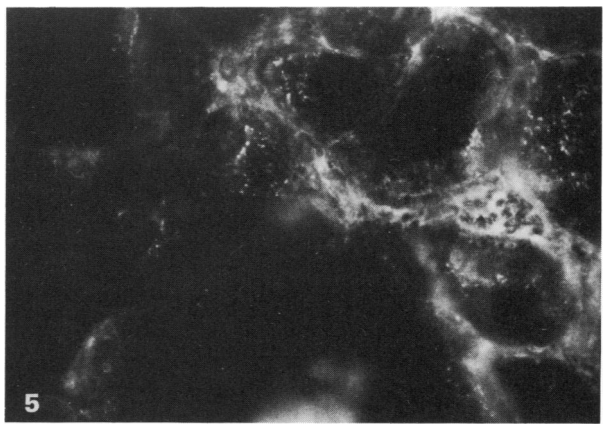




**Fig. 4.** A multinucleate basophilic cell which histologically might be interpreted as regenerating muscle. It is acid phosphatase-positive. It appears to correspond to the giant cells seen in Fig. 3a & b. (patient VI). (H & E, original × 400.)

**Fig. 5.** Frozen section from patient XIV stained with anti-HLA-DR. A hazy deposit apparently diffusing into the muscle fibres is visible. (Original × 400, enlarged × 5.)

Few leucocytes and no T lymphocytes were seen. In two individuals anti-HLA-DR stained small numbers of cells between the muscle fibres. These are presumed to be macrophages. A few isolated polymorphs stained with TG-1 were found in all three controls and similar numbers were also seen in all disease cases. In all three controls the overall level of staining with all the markers was very low and cells were never seen in clumps. Routine histological staining of the control biopsies showed normal appearances.

In both normal and polymyositis cases the background staining when only the second-layer anti-immunoglobulin fluorescein was applied was minimal. In some cases an IgG1 monoclonal antibody of irrelevant specificity was used as first layer and also gave no staining. Autofluorescence of unstained sections was insignificant.

## DISCUSSION

Polymyositis is an idiopathic inflammatory myopathy of unknown aetiology. It may be associated with connective tissue disorders or malignancy. Several lines of evidence suggest that the muscle damage may be due to an autoimmune response. The inflammatory infiltrate characteristic of the disease is mononuclear in type consisting of small and medium lymphocytes, plasma cells and monocytes while a variety of immunological disturbances have been demonstrated in association with the disease.

Antibodies to a variety of muscle components have been demonstrated in polymyositis patients (Caspary, Gubbay & Stern, 1964; Stern, Rose & Jacobs, 1967) but these are also seen in muscular dystrophies and neurogenic diseases of muscle. The amount of tissue damage caused by antibody is hard to assess although IgG, IgM and C3 have been noted as focal deposits in some patients with polymyositis (Whitaker & Engel, 1972; Mittelbach & Pongratz, 1969; Oxenhandler, Adelstein & Hart, 1977). Direct demonstration of infiltrating B cells has been difficult because of the extent of extracellular Ig deposits.

Investigations of the role of cellular immunity in polymyositis have been more extensive both in man and experimental animals. In man peripheral blood lymphocytes from patients have been shown to respond by proliferation to autologous muscle extracts and to be cytotoxic for fetal muscle cell cultures (Currie *et al.*, 1971; Cambridge & Stern, 1981), but the nature of the cytotoxic cells in this type of reaction is unclear since the genetic restriction of classical T cell cytotoxicity is not seen (McMichael, 1978). In animals, experimental myositis can be induced by injections of muscle in Freund's complete adjuvant (Dawkins, 1965; Smith *et al.*, 1979) and can be passively transferred by circulating peripheral lymphocytes, suggesting a role for T lymphocytes (Takayanagi, 1967).

Because of the animal data and the lack of convincing evidence for an important role for antibody in the pathology of polymyositis it seems likely that T lymphocytes play a major part in the inflammatory process. Direct evidence of their *in vivo* role has, however, been lacking. The finding of significant numbers of lymphocytes identified by the anti-T cell serum UCHT1 in several of our patients provides such evidence of T cell involvement in the lesions of the disease. In addition, many of the T cells probably bear HLA-DR antigen, since anti-HLA-DR stained as many cells as anti-leucocyte antiserum. This strongly suggests that these are activated T cells since HLA-DR-bearing T cells make up a minor fraction of normal peripheral blood or tissue T cells (Greaves *et al.*, 1979), but following activation *in vitro* or during the acute phase of certain viral infections, a high proportion express this antigen (Evans *et al.*, 1978). Double-label experiments will be required to confirm that individual cells do indeed carry both the T and HLA-DR antigens.

Staining with anti-HLA-DR has revealed an additional unexpected finding; while in most cases the staining was clearly associated with cell membranes or within the cytoplasm, in two patients a hazy deposit was seen apparently diffusing into muscle fibres (Figs 3a and 5). It seems extremely unlikely that this is a staining artefact since other monoclonal antibodies of the same immunoglobulin class (UCHT1 and HLe-1) showed no staining of this type. Possible explanations for the presence of the material would be the release of HLA-DR from dead or dying mononuclear cells but a more interesting although highly speculative explanation would be the secretion of HLA-DR-positive factors by the activated infiltrating cells. In the mouse there is extensive

immunochemical evidence for the presence of Ia-like antigens on secreted products of both macrophages (Erb, Feldmann & Hogg, 1976) and T lymphocytes (Howie *et al.*, 1979) and data in man suggest the existence of similar HLA-DR-positive factors (Mudawwar, Yunis & Geha, 1978). It is possible therefore that the diffuse material seen in some of our patients represents mediators secreted by immune cells which may play an important role in the maintenance of the inflammatory process within the muscle. In case VI the staining with anti-HLA-DR indicated that the giant cells were macrophage polykaryons rather than muscle cells with which they could be confused (Figs 3 and 4) (Neerunjun & Dubowitz, 1977). This concept was strengthened by staining of the periphery of the cells with anti-HLe-1. It is difficult to classify this case as it had more extensive lymphocyte infiltration than would be expected with sarcoid myopathy but the presence of the giant cell granulomas point to this diagnosis.

In this preliminary study it is difficult to assess satisfactorily the potential of this methodology. Many of the patients had received therapy, notably steroids, which would be likely to affect the immune response and therefore the spectrum of immunologically competent cells to be found in the infiltrates. There is in addition the well known problem of sampling errors in muscle biopsies so that we are not yet able to assess the prognostic significance of the different types of cells found in our material. In the future it will be important to study material from untreated patients and to apply additional markers capable of distinguishing subsets of T cells (Reinherz & Schlossman, 1980) and identifying monocytes (Breard *et al.*, 1980) and B cells (Brooks *et al.*, 1980). Even so, from our limited experience in this preliminary study it is possible to identify several features which may be prognostic indicators or markers for more accurately classifying categories of disease. We would suggest the presence or absence of B cells, T cell subsets, the state of activation of the cells (HLA-DR) and the extent of extra lymphocytic HLA-DR$^+$ material.

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