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Immunohistochemical analysis of mononuclear cell subsets in inflammatory and non-inflammatory myopathies

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SUMMARY Immunohistochemical procedures were used to analyse the subpopulations of mononuclear cells in muscle biopsies from 24 patients with polymyositis. The character of the cellular infiltrate was similar at the perivascular, perimysial, and endomysial sites, with cytotoxic-suppressor T lymphocytes (T_8^+) and macrophages being the dominant elements. Helper T lymphocytes (T_4^+) and B lymphocytes were present in smaller numbers. A control series of 17 muscle biopsies from normal subjects and patients with non-inflammatory myopathies and neurogenic conditions was also studied: the numbers of mononuclear cells present were much smaller than in polymyositis, but the ratio of T_4:T_8 lymphocytes was similar to that found in biopsies affected by polymyositis. We conclude that both cytotoxic-suppressor T lymphocytes and macrophages are important in the pathogenesis of inflammatory myopathy.

Polymyositis is an inflammatory disorder affecting muscle, with a characteristic constellation of clinical and laboratory findings.[1] In polymyositis as in other immunologically mediated conditions there is a disturbance of lymphocyte subpopulations in peripheral blood, with a considerable decrease in the numbers of cytotoxic-suppressor T cells.[2]

The nature of the subpopulations of mononuclear cells that actually infiltrate the muscle in polymyositis has only recently been explored. In some studies of these cellular infiltrates[3 4] the proportion of T-helper cells has been shown to be increased compared with that found in the peripheral blood of normal patients. In others[5] the cytotoxic-suppressor T cell subset predominates. Infiltration of muscle by mononuclear cells occurs in many different conditions and is not restricted to the so called inflammatory myopathies. It has been described in some muscular dystrophies,[6–8] metabolic myopathies,[9] and normal subjects.[8] To study this question further we characterised the mononuclear cells by using five different markers (monoclonal antibodies against T_1, T_4, T_8, pan B surface antigens on lymphocytes, and acid phosphatase staining) in cellular infiltrates of adjacent sections of muscle biopsies of patients with polymyositis.

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We also used the same techniques to investigate the T cell subsets in various non-inflammatory myopathies. We also investigated any possible correlation between serum creatine kinase concentrations and the intensity of inflammatory infiltrate at different sites in the muscle biopsy.

Material and methods

Cases of polymyositis during the years 1978–1984 were taken from the surgical pathology files of the London Hospital, and 24 cases fulfilled the diagnostic criteria of Bohan and Peter.[1] There were five cases of dermatomyositis, and two cases of polymyositis associated with neoplasia (one thymoma and one gastric carcinoma).

Seventeen biopsies from patients with non-inflammatory myopathies were chosen at random, including seven cases that showed no histological abnormality. Table 1 summarises the patient details. All the biopsies were originally reported histologically by one of us (MS).

The biopsy specimens had been stored at −160°C in liquid nitrogen; cryostat sections were cut at 5 microns and left overnight at room temperature. The sections were fixed in acetone for 20 minutes at room temperature, transferred to Tris buffered saline (TBS)

Table 1 *Clinical material*

Diagnosis	*No of cases*	*Sex*		*Age (years)*		*Serum creatine kinase *(U/l)*	
		M	*F*	*Mean*	*Range*	*Mean*	*Range*
Normal	7	2	5	35	(1–72)	68	(30–154)
Myopathic	6	4	2	36·5	(6–74)	71	(10–150)
Neuropathic	4	3	1	36	(22–57)	212	(15–454)
Inflammatory myopathy	24	13	11	54	(23–80)	1231	(24–5242)
Polymyositis	19	11	8	57	(28–80)	1074	(24–5242)
Dermatomyositis	5	2	3	41	(23–66)	1827	(44–3984)

* Normal value of creatine kinase < 160 U/l.

Table 2 *Primary antisera*

Surface antigen	*Antibody specificity*	*Monoclonal antibody Ig*	
		Subclass	*Source*
T_1	All peripheral T cells	IgG1	Dako Ltd
T_4	T helper and T cytotoxic cells	IgG2b	Ortho Diagnostics
T_8	T suppressor-cytotoxic cells	IgG2	Ortho Diagnostic
B	All peripheral B cells	IgG2b	Dako Ltd

at room temperature (pH 7·6), and washed thoroughly. The primary antisera were used at a dilution of 1/30 and incubated with the sections for 30 minutes (Table 2). This was followed by a static wash in TBS for two minutes. The end product was developed using diaminobenzidine incubation for eight minutes. After washing the sections were counterstained with haematoxylin. All staining runs included sections of normal tonsil as positive controls.

All biopsies from cases of inflammatory myopathy were stained by Gomori's acid phosphatase technique as a marker for macrophages.

The biopsies from patients with non-inflammatory myopathies and the "normal" cases were studied only with the antisera for T_4 and T_8 lymphocytes.

Each section was assessed blindly by two of us (NRL, JFR), using a semiquantitative scale for the number of each cell type at each of the three chosen sites (perivascular, perimysial, and endomysial), by grading the cellular aggregate from 0 to 3: 0 (no positive cells seen in section); 1 (aggregate of 1–25 cells); 2 (aggregate of 26–50 cells); and 3 (aggregate of > 50 cells).

In each case the section stained with haematoxylin and eosin was first examined to define the areas with cellular infiltrates, and then the number of cells bearing each surface antigen or showing acid phosphatase activity in the serial sections was assessed.

Mononuclear cells were considered to show a positive reaction with a surface marker when there was a definite rim of reaction product around the cell border. The acid phosphatase method showed macrophages as cells with a diffuse cytoplasmic staining.

The term "inflammatory score" refers to a mean

Table 3 *Mononuclear cell subpopulations in inflammatory and non-inflammatory myopathies*

	Inflammatory myopathy mean (SEM) inflammatory score	*Non-inflammatory myopathies mean (SEM) inflammatory score*
Perivascular location:		
T_4 lymphocytes	0·38 (0·15)	0·20 (0·09)
T_8 lymphocytes	0·75 (0·15)	0·35 (0·11)
B lymphocytes	0·38 (0·16)	
Macrophages	0·63 (0·13)	
Perimysial location:		
T_4 lymphocytes	0·33 (0·10)	0·35 (0·11)
T_8 lymphocytes	0·79 (0·12)	0·50 (0·12)
B lymphocytes	0·46 (0·10)	
Macrophages	0·79 (0·13)	
Endomysial location:		
T_4 lymphocytes	0·75 (0·12)	0·20 (0·12)
T_8 lymphocytes	1·08 (0·17)	0·25 (0·12)
B lymphocytes	0·67 (0·10)	
Macrophages	1·13 (0·14)	

Table 4 *Overall distribution of mononuclear cells within muscle biopsies of polymyositis*

	Percentage of total mononuclear cell infiltrate
Perivascular	26
Perimysial	28
Endomysial	46
Total	100

value calculated from the sum of the individual case scores for a particular cell type divided by the number of cases assessed. For example, if T_4 scores at the endomysial site for three cases of dystrophy were 0, 1, and 1 then the T_4 inflammatory score would be $(0 + 1 + 1)/3 = 0{\cdot}67$.

Results

The results of immunohistochemical analysis of mononuclear cell subsets are presented in Tables 3 and 4.

POLYMYOSITIS AND DERMATOMYOSITIS CASES

The character of the inflammatory infiltrate was similar at each of the three chosen sites (18% T_4^+ cells, 32% T_8^+ cells, 18% B cells, and 32% macrophages). There was, however, an appreciable positive gradient from vessel to muscle fibre, so that the highest numbers of mononuclear cells were located at the endomysial site and the lowest at the perivascular zone (Table 4). The T_4:T_8 ratio for these cases was calculated as 0·56.

The serum concentration of creatine kinase at the time of biopsy showed no correlation with the density or any component of the inflammatory infiltrate.

Four patients were receiving therapeutic doses of corticosteroids at the time of biopsy, but there was no apparent difference in the density or character of the cellular infiltrate in these cases.

NON-INFLAMMATORY CONDITIONS

The mean score for the T_4 and T_8 cells in the inflammatory infiltrate was lower than in the cases of polymyositis in all zones of non-inflamed muscle and showed no consistent gradient. The T_4:T_8 ratio (0·61) was reversed compared with that found in normal serum and so was similar to that found in the cases of polymyositis.

Discussion

Our observations confirm that T lymphocytes are the dominant component of the inflammatory infiltrate in polymyositis with a positive gradient towards the endomysium. The T cell subpopulations identified in this series show a T helper:cytotoxic-suppressor ratio of less than 1. Arahata and Engel[8] showed a similar overall positive gradient for T cells, with most cells concentrated at the endomysium. In their study of 15 cases of polymyositis, however, the T helper (T_4^+) cells were more numerous than T cytotoxic-suppressor (T_8^+) cells, except at the endomysial site. Rowe *et al*[10] found larger numbers of helper-inducer cells (leu $3a^+$) than cytotoxic-suppressor (UCHT4/leu2a$^+$) in untreated polymyositis.

We did not investigate the T lymphocyte subpopulations in the peripheral blood of these patients. Behan and Behan[2] found a selective depression of the number of cytotoxic-suppressor T cells in five of nine patients with acute disease and four of 14 patients with chronic active disease. These results, taken together with those of this study, suggest that cytotoxic-suppressor lymphocytes may be selectively sequestered in the muscles of patients with polymyositis, and this may explain the relative deficiency of these cells in the peripheral blood. Other investigators, however,[11] found no difference in the T cell subpopulations in the peripheral blood of patients with polymyositis compared with that of controls.

The contribution of macrophages to cellular infiltrates in polymyositis has been underestimated in previous studies. In our patients macrophages formed a principal component of the mononuclear cell infiltrate, not only at the endomysial site, but also around vessels and at the perimysium. Arahata and Engel[8] also recognised macrophages as an important element in polymyositis, particularly when they were found together with cytotoxic-suppressor T cells invading non-necrotic muscle fibres. This suggests that macrophages may have a role in the earliest stages of this condition, rather than merely being responsible for clearing necrotic fibres.

We found no correlation between the serum creatine kinase concentration and the inflammatory scores noted in the biopsies. It has been shown that muscle cultures challenged with lymphocytes from polymyositis cases may release creatine kinase without morphological evidence of cytodestruction,[12] and this may also occur in vivo.

Our work shows that apparently normal, myopathic, and neuropathic muscle biopsies may contain small numbers of lymphocytes of both T_4^+ and T_8^+ subsets, with T_8^+ being slightly more numerous. The gradient of inflammatory infiltrate towards the endomysium seen in polymyositis did not occur in the myopathic and neurogenic biopsies used as controls, in which occasional mononuclear cells were scattered throughout the biopsy in an apparently random fashion. Arahata and Engel[8] noted occasional mononuclear cells in muscle biopsies from patients with no

evidence of muscle disease, and the importance of this finding is still undetermined. Without the aid of specific immunoperoxidase techniques for their identification, these cells are very difficult to differentiate from muscle nuclei, particularly in a diseased muscle.

Polymyositis is a disease of unknown aetiology, and although viruses have been proposed as inciting agents,[1 3] the underlying immune mechanism remains undefined. Our observations support the hypothesis that polymyositis is produced by an effector response mediated by T cells, and imply that macrophages have an important role, perhaps in the presentation of muscle antigen to cytotoxic-suppressor T cells.

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