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Characterization of mononuclear exudates in idiopathic inflammatory myopathies

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Summary. Percentages of B-cells, T-cells and subsets Th, Ts, T activated, and macrophages were analyzed by using monoclonal antibodies in a series of 24 patients [19 dermatomyosis (DM) and 5 polymyositis (PM)]. Specific site of deposition of these cells was also identified (endomysial, perimysial and perivascular). We were able to find a greater number of endomysial T-cells in PM than in DM. However, B-cells were more frequent at perivascular sites in DM than in PM. These findings support the previous reported hypothesis that humorally-mediated immune damage in both vascular and muscle cells predominates in DM while cellular cytotoxic mediated damage is more marked in PM patients.

Key words: Inflammatory myopathy – Monoclonal antibodies – Inflammatory cells

Introduction

The idiopathic inflammatory myopathies (IIM) are a group of related entities of obscure causes. Increasing recognition of characteristic findings in different subgroups of patients with IIM has assisted in more comprehensive examination of muscle biopsy by light and electron microscopy (Carpenter et al. 1976; Bohan et al. 1977). Since the hallmark of the pathological process is an inflammatory infiltration of the affected muscles, the immunocytochemical characterization of mononuclear cell subsets could be of great interest in pathogenetic evaluation of these patients. Recent studies have employed monoclonal antibodies in the analysis of infiltrating mononuclear cells in muscle (Rowe et al. 1981; Bresnan et al. 1981; Giorno et al. 1984). According to one of these studies, most of the lymphocytes in the infiltrates were activated T cells (Rowe et al. 1981). Another study found no significant differences in mononuclear cell distributions in the subgroups of IIM (Giorno et al. 1984). Although more recently some authors (Arahata and Engel 1984; Engel and Arahata 1984, 1986) have clarified the subject classifying the infiltrates following its localization on muscle preparations, the lack of uniform data led us to design a prospective study using monoclonal antibodies in order to determine in a large group of patients with polymyositis (PM) or dermatomyositis (DM) their potential diagnostic value for better characterization of different subgroups of IIM.

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Materials and methods

Twenty-four patients with clinically different types of IIM were studied. Five patients were subclassified as PM, and nineteen as DM. In the DM group, 10 were of the adult idiopathic type, 3 of childhood type, and 6 paraneoplastic. All of them fulfilled all criteria for definite PM-DM. Group V of Bohan and Peter were specially excluded. All biopsies were obtained when clinical diagnosis was made, and no patients were on corticosteroid therapy. Routine standard histological and histochemical techniques were used in studying muscle biopsies, as well as glutaraldehyde fixed, araldite-embedded samples for ultrastructural study.

Control muscles were obtained through diagnostic biopsies of 3 alcoholic patients and 3 normal volunteers.

Frozen (−70° C) banked muscle biopsies were the tissue source. Four-micrometer serial cryostat sections were prepared, air dried, and fixed in acetone 10 min at 4° C before immunostaining.

Monoclonal antibodies employed were: Leu 4, Leu 3a, Leu 2a, Leu 12 and HLA-DR. All of them were from commercial sources (Becton Dickinson, CA, USA). Macrophages were identified by non specific esterase. Normal horse serum, affinity-purified biotinylated horse antimouse IgG, avidin, and bio-

tinylated peroxidase were obtained from Vector Laboratories, CA, USA (Vectastain ABC kit).

Immunoreagents were dissolved in phosphate-buffered saline, pH 7.2 (PBS), which also contained 3% bovine serum albumin (BSA), and 5% heat-inactivated horse serum.

Acetone-fixed cryostat sections were rehydrated in cold PBS for 5 min., and were sequentially treated at room temperature with H_2O_2 0.3%-methanol for 10 min; washed in cold PBS, three times, 10 min each; blotted with normal horse serum, and *floated,* 15 min. After rinsing slides individually, 1 quick dip in cold PBS was made. Monoclonal antibody solution in a humidity chamber (1 h at room temperature) was applied; followed by cold PBS rinses 3 times, 10 min each. Biotinylated affinity-purified horse antimouse solution was applied for 45 min at room temperature, with washes in cold PBS, 3 times each. Avidin-biotin peroxidase complex (prepared by mixing 10 ml each of avidin and biotinperoxidase in 1 ml of PBS, at least 30 min each) with later washing in 0.05 M TRIS, pH 7.72, for 10 min was made. Subsequently, the sections were treated with the diaminobenzidine-hydrogen peroxide medium for 15 min, rinsed with distilled water, lightly counterstained with Mayer's haematoxylin, blued briefly in ammonia water, dehydrated and mounted under DPX.

For control purposes additional sections were treated with nonimmune mouse immunoglobulins of the same subclass and concentration as the monoclonal antibodies employed in the series. Monoclonal antibody staining was checked each instance by using lymphoid tissues.

For each case, perivascular, perimysial, and endomysial sites were analyzed quantitatively. For each specimen and for each defined site, three of four cell accumulations were analyzed. Tissue sections were scored independently by two of the authors (J.C. Botet and J.M.[a] Grau) with no prior knowledge of routine interpretation. Counts determined by the two scorers were averaged (differences between individual scorers were 15% in all cases). The total number of mononuclear cell nuclei (excluding muscle and endothelial cell nuclei) was counted in defined areas of serial sections. The number of positive cells was determined and the percentages were expressed relative to the total number of cells counted. In each specimen and for each analyzed site, B cells, T cells, and macrophages accounted for 97% to 102% of the mononuclear cells. The number of HLA-DR$^+$ T cells in a given accumulation was estimated using the formula:

HLA-DR$^+$ T cells = all HLA-DR$^+$ cells − Leu 12$^+$ cells − macrophages

The Student t test was employed for the analysis of total cell count, chi square test with standardized deviates for comparing the homogenicity of infiltrates, and Mann-Whitney U test for the analysis of mononuclear cell subsets.

Results

In normal controls and alcoholic patients only a few extravascularly situated mononuclear cells were observed in otherwise normal muscle. Single macrophages were observed in the endomysium.

In all inflammatory myopathy patients a mixed exudate of T-cells, B-cells and macrophages was present. Total cell count in DM and PM groups (mean ± SD) are reflected in Table 1. When comparing these values for different sites and for each disease, we were only able to find a statistical difference between PM and DM for an endomysial localization of total T-cells ($p < 0.03$).

Table 1. Quantitative analysis of mononuclear cells in IIM. Total cell count (mean ± SD)

		DM	PM
Perivascular site	T: 2323	(43.02 ± 24.45)	356 (29.67 ± 11.51)
	B: 2185	(40.59 ± 22.34)	129 (10.75 ± 5.14)
	Macr.: 752	(14.11 ± 8.17)	115 (9.58 ± 4.67)
Perimysial site	T: 806	(23.60 ± 13.19)	175 (25 ± 12.19)
	B: 278	(7.97 ± 7.25)	33 (4.71 ± 4.39)
	Macr.: 305	(8.71 ± 5.91)	66 (9.43 ± 8.06)
Endomysial site	T: 547	(16.09 ± 7.56)	349 (43.62 ± 20.11)
	B: 117	(3.44 ± 2.63)	30 (3.75 ± 2.55)
	Macr.: 208	(6.12 ± 3.58)	129 (16.12 ± 8.06)

Table 2. Quantitative analysis of mononuclear cells in IIM

Cell type or marker	Perivascular cells (%)	Perimysial cells (%)	Endomysial cells (%)
Dermatomyositis			
B cells (Leu 12)	41.53 ± 7.69*	20.01 ± 6.95*	13.41 ± 5.35*
T cells (Leu 4)	44.16 ± 7.33*	58.02 ± 5.64	62.72 ± 5.71*
Leu 3a/T cells	60.8 ± 8.16	47.63 ± 5.55	39.87 ± 6.44*
Leu 2a/T cells	39.20 ± 8.16	52.37 ± 5.55	60.13 ± 6.44*
HLA-DR + /T cells	18.60 ± 3.31	18.66 ± 5.91	23.61 ± 5.02
Macrophages	14.29 ± 1.97	21.95 ± 3.62	23.85 ± 2.60
Polymyositis			
B cells (Leu 12)	21.58 ± 4.67*	12.12 ± 4.30*	5.90 ± 3.32*
T cells (Leu 4)	59.12 ± 2.91*	59.12 ± 4.42	68.85 ± 4.01*
Leu 3a/T cells	56.95 ± 5.29	42.07 ± 5.52	28.20 ± 5.01*
Leu 2a/T cells	43.05 ± 5.29	57.93 ± 5.52	71.80 ± 5.01*
HLA-DR + /T cells	21.61 ± 1.69	22.40 ± 3.44	28.90 ± 1.67
Macrophages	19.14 ± 2.61	24.11 ± 2.76	25.45 ± 1.10

All values are expressed as grand mean ± SD of the mean in each patient

* Statistical significance (between PM and DM group) $p < 0.05$

When studying the homogenicity of the infiltrates for each site and for each disease, we found that distribution of different cells subsets was non-uniform in both the DM and the PM group. B-cells showed a tendency to accumulate in perivascular space in both PM and DM patients. While there were no statistical differences concerning T cells and their Ts and Th subsets in DM, Ts cells showed a tendency to accumulate in endomysial localization ($p < 0.04$) and Th in perimysial localization ($p < 0.04$) in the PM group.

Percent values of B, T, Th, Ts, T activated, and macrophages for each site and disease are reflected in Table 2. B cell percentage was greater

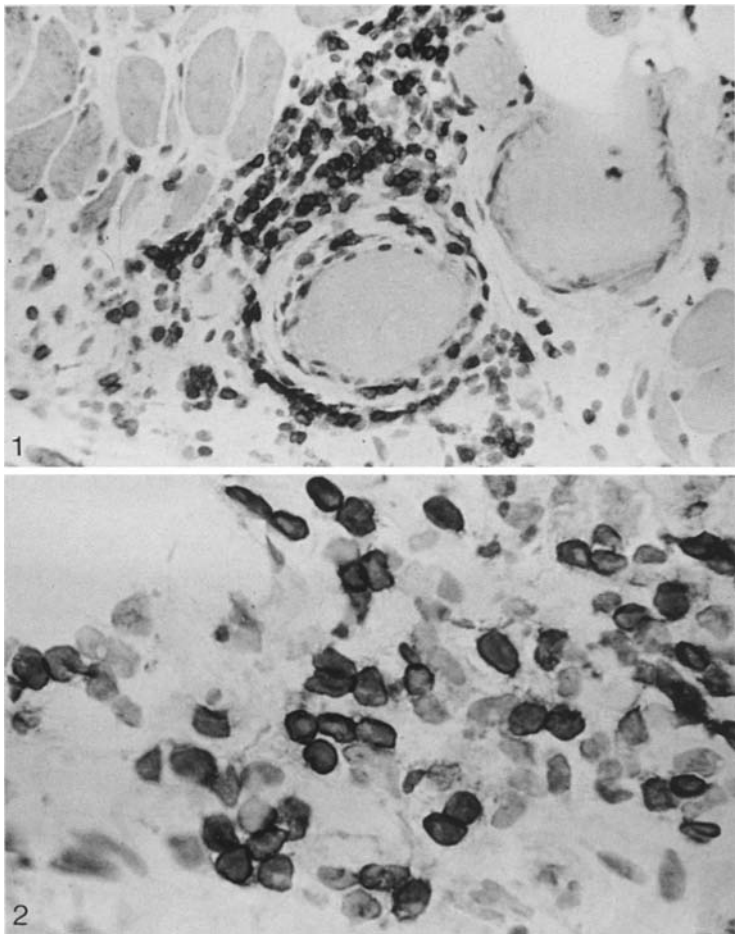

Fig. 1. Stained B-cells (Leu 12) at perivascular site in a DM case. ×182

Fig. 2. Stained T-cells (Leu 4) at endomysial site in a case of PM. ×252

in DM than in PM in all three considered localizations with a statistical significance (Fig. 1). Although the T-cell percentage was greater in PM than in DM for all three localizations, statistically significant values were obtained ($p < 0.05$) only in perivascular and endomysial sites (Fig. 2).

When analyzing T-cell subsets, DM and PM were significantly different, with a greater proportion for Th at endomysial sites for the former and a greater proportion of Ts for the latter in the same localization.

Although HLA-DR+cells were more abundant in PM than in DM, at all three localizations analyzed, there were no significant statistical differences.

The percentage of macrophages was also greater in PM than in DM in all three localizations, but again without significant statistical differences.

Discussion

In this study we have been able to demonstrate some differences between perivascular, perimysial and endomysial cellular infiltrates when comparing PM and DM.

Our results coincide closely with these reported by Arahata and Engel in 1984. We have found a high percentage of B-cells in all three localizations in DM. This presence of B-cells, in all degrees of maturity as identified by Leu 12 monoclonal antibody, should not be interpreted as a simple extravasation phenomenon since at the ultrastructural level we were able to find few mature plasma cells in the majority of DM cases (Fig. 3). The B-cell predominance in DM suggests that the immunopathogenesis in this disease is mainly humorally mediated and that a local humoral response probably occurs in the muscle itself. It seems currently well established that blood vessels are severely affected in DM and may represent a primary target of the autoimmune response (Whitaker and Engel 1972; Kissel et al. 1986). In this regard, two hypotheses may be proposed to explain the muscle fiber injury. First, muscle injury could be secondary to ischaemia (Carpenter et al. 1976; Kissel et al. 1986; Banker and Engel 1986). The second

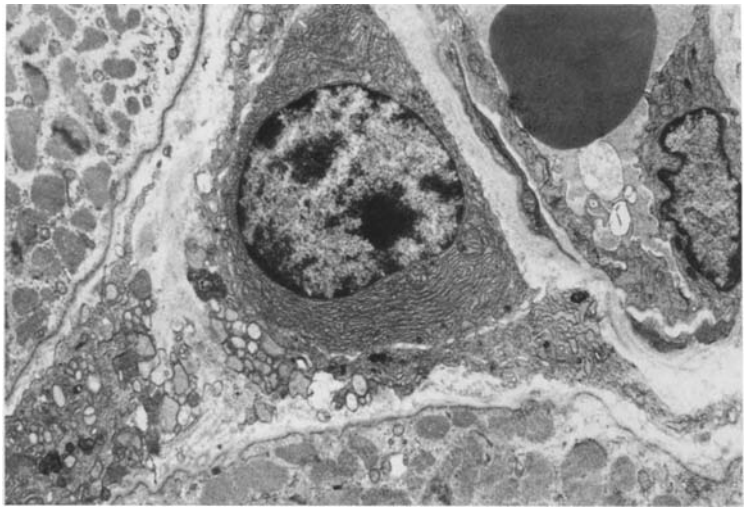

Fig. 3. Mature plasma cell adjacent to two muscle fibers and to a capillary in a case of DM. Original magnification. × 2800

hypothesis could be that both, endothelial cells and muscle fibers share common antigenic determinants (Banker and Engel 1986) resulting in primary damage.

However, the predominance of T-cells for all localizations in PM strongly suggests a direct cytotoxic mechanism. This is also supported by both the immunocytochemical (Engel and Arahata 1984) and ultrastructural observations (Urbano et al. 1986), in which the close opposition of cytotoxic or activated T lymphocytes to muscle fibers is well demonstrated.

The uniform macrophage response in each localization and disease seems to be a non-specific reaction. Its identification probably has no value in differentiating PM from DM.

We conclude, in view of our results, that characterization of exudative mononuclear cells in inflammatory myopathies may be helpful in evaluating the pathogenetic mechanism in these diseases.

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