Muscle Pathology in Polymyalgia Rheumatica: Histochemical and Immunohistochemical Study

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Immunohistochemical studies were performed on muscle biopsy specimens of polymyalgia rheumatica (PMR) to evaluate the extent of muscle and peripheral nerve involvement. The routine histochemistry revealed a mild variation of fiber size, type 2 fiber atrophy and type 2A or 2B fiber deficiency. In 63% of the PMR cases small angular fibers, pyknotic nuclear clumps or target-targetoid fibers were observed, suggesting neurogenic changes, although abnormalities were mild in degree. In immunocytochemical studies, neither major histocompatibility complex (MHC) class 1 nor class 2 products were expressed on the muscle surface membrane of PMR. But regarding intramuscular vessels, MHC class 2 products were distinctly visualized. On serial sections, combined deposits of IgG and C1q in perimysial arteries were seen in 38% of PMR.

These results suggest that arteries of small caliber might be involved in immunopathological processes, causing muscle and peripheral nerve damage.

Key words: Temporal arteritis, Vasculitis, Major histocompatibility complex, Immune complexes, Neuropathy, Immunohistochemistry

Polymyalgia rheumatica (PMR) is a clinical syndrome which was originally described by Barber in 1957 (1). Although myalgia is the cardinal feature of PMR, little is known about the muscle pathology. The close relationship between PMR and temporal arteritis (TA) has been documented (2, 3). The precise nature of this association remains unknown, but some investigators have suggested that PMR is a manifestation of arteritis (4). The evidence of vasculitis is lacking in muscle biopsy specimens of PMR (5, 6). To date there seem to be few immunological analyses on affected muscle tissue of PMR.

Recently, the association of TA/PMR and neuropathy has been reported (7, 8). Ultrastructural examination of the skeletal muscle in PMR suggests that a neurogenic mechanism play a pathogenetic role (9). But histological and histochemical examinations have revealed little evidence of neurogenic changes (5, 10).

In the present study, we examined muscle biopsy

specimens of PMR histochemically and immunocytochemically to determine the mechanism of muscle and nerve involvement.

MATERIALS AND METHODS

Muscle specimens were obtained from 11 cases of PMR, 12 polymyositis (PM), 4 dermatomyositis (DM), 1 polyarteritis nodosa (PN), and 9 cases of disease control (4 cases of motor neuron disease, 3 entrapment neuropathy, 2 myotonic muscular dystrophy) and 2 normal controls. In each instance, biopsy was performed after informed consent was ascertained. Diagnosis of PMR was made according to the criteria of Bird et al (11), and also the new criteria proposed in Japan (12). Only 2 of 11 patients with PMR complained of headache. None of the patients with PMR had visual symptoms, tenderness over the temporal artery or jaw claudication. A biopsy of the temporal artery was not performed in any case. All patients with PM

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and DM met 3 or 4 of the criteria of Bohan and Peter (13). None of the PMR patients had received steroid therapy prior to biopsy. Clinical data of patients with PMR are summarized in Table 1. Data of blood analyses and electrophysiological studies are described in the results section.

Biopsied muscle specimens were mounted and frozen in isopentane cooled by liquid nitrogen. Muscle sections were 10 μ m thick for routine histochemistry and 6 μ m thick for immunohistochemistry. For the immunoperoxidase method, muscle sections were dried in air, washed three times in phosphate-buffered saline (PBS, pH 7.2) for 10 min, fixed with cooled acetone for 5 min, and incubated in a moist chamber for 30 to 60 min with

various primary antibodies listed on Table 2. Antisera to human IgG, IgM, IgA or albumin were peroxidase conjugated. Products of these reactions were visualized with diaminobenzidine (DAB) and 0.01% hydrogen peroxide in PBS. For other antibodies, we used Biotin-Streptoavidin kit (BioGenex Laboratories, San Ramon) to visualize final products. To test specificity of reactions, control rabbit or mouse serum or PBS was used in place of primary antibodies. To avoid nonspecific binding of secondary antibodies to Fc receptors, we used the F(ab') fragment of antibodies conjugated to peroxidase.

Statistical analysis was carried out according to χ^2 test or Fisher's exact probability test.

Table 1. Clinical Data of Patients with Polymyalgia Rheumatica

Positive No. of Bird's criteria			ESR (mm/h)	CRP	RF(Rose) (U/ml)	ANF	anti-DNA ENA,RNP,Sm	CK (IU/l)	IgG (mg/dl)	IgA	IgM	IgE	CH50 (U)	C3/C4 (mg/dl)	IC μ g/ml	T4/T8
1*	M/61	4/7	103	9+	+(<16)	—	—	121	1,565	<u>275</u>	139	<u>398</u>	<u>80</u>	<u>140/49</u>	nd	nd
2*	M/79	6/7	80	9+	6	—	—	75	<u>2,313</u>	<u>453</u>	<u>498</u>	42	nd	<u>94/28</u>	nd	1.69
3	F/70	4/7	113	5+	<u>15</u>	—	—	32	<u>2,472</u>	<u>724</u>	123	72	<u>72</u>	<u>99/36</u>	1.8	nd
4	F/61	5/7	85	2+	<u>12</u>	—	—	45	nd	nd	nd	nd	nd	nd/nd	<u>3.5</u>	<u>2.53</u>
5	F/73	6/7	55	9+	<u>11</u>	<u>1:80 (H)</u>	—	<u>385</u>	921	274	38	124	nd	nd/nd	nd	nd
6	F/69	5/7	113	6+	8	<u>1:320(H)</u>	—	35	1,530	222	222	66	<u>68</u>	69/31	<1.5	nd
7	M/71	4/7	62	4+	8	nd	nd	43	1,690	266	105	290	<u>76</u>	<u>101/39</u>	nd	1.18
8	F/51	5/7	70	—	9	—	—	47	1,750	<u>407</u>	68	nd	45	66/9	<u>3.7</u>	2.10
9	F/66	6/7	52	3+	<u>11</u>	—	—	38	1,902	290	159	<u>732</u>	<u>61</u>	<u>76/43</u>	nd	<u>0.94</u>
10	M/71	6/7	52	7+	9	—	—	41	1,880	275	149	312	<u>64</u>	<u>105/43</u>	nd	<u>4.50</u>
11	F/50	5/7	64	3+	9	<u>1:40 (H)</u>	—	90	nd	nd	nd	nd	<u>60</u>	<u>90/28</u>	nd	2.10
(Normal)			(0–10)			(<150)			(820–2,290)	(130–380)	(60–380)	(<350)	(40 $\pm$ 10)	(49–81)(9–35)	(<3.0)	(1.8 $\pm$ 0.7)

* Immunocytochemistry was not analyzed

M, male; F, female; No., number; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; RF, rheumatoid factor; ANF, anti-nuclear factor; ENA, extractable nuclear antigen; RNP, ribo-nucleoprotein; IC, immune complexes; nd, not determined

Table 2. Monoclonal and Polyclonal Antibodies Used in this Study

Cell type or antigen	Antibody	Source
HLA-A,B,C.	W6/32 (monoclonal)	Sera-lab
HLA-DR	L243 (monoclonal)	Becton Dickinson
T cytotoxic/suppressor cell (CD8)	anti-Leu-2A (monoclonal)	Becton Dickinson
T helper/inducer cell (CD4)	anti-Leu-3a (monoclonal)	Becton Dickinson
B cell (CD 19)	anti-Leu-12 (monoclonal)	Becton Dickinson
C9	anti-C9 (polyclonal)	Wako
Clq	anti-Clq (polyclonal)	Dakopatts
Ig G	anti-IgG γ chain (polyclonal; F(ab'))	Dakopatts
Ig M	anti-IgM μ chain (polyclonal; F(ab'))	Dakopatts
Ig A	anti-IgA α chain (polyclonal; F(ab'))	Dakopatts

RESULTS

Histological and histochemical findings (Fig. 1)

In PMR, there were mild myogenic changes: a mild variation of fiber size with a few necrotic or regenerating fibers, and fibers with internal nuclei (Fig. 2). Intramuscular vessels appeared normal on hematoxylin and eosin (HE) stain. In 63% of PMR cases, neuropathic features such as small angular fibers (6/11), target or targetoid fibers (1/11), pyknotic nuclear clumps (3/11), and small group atrophy (1/11) were observed.

Regarding the muscle fiber type abnormalities, type 2 fiber atrophy was observed in 81% (9/11) [type 2A atrophy in 27% (3/11), type 2B atrophy in 45% (5/11), both type 2A and 2B atrophy in 9% (1/11)] and fiber type deficiency was seen in 45% (5/11) [type 2A deficiency in 18% (2/11), 2B deficiency in 36% (3/11)]. The fiber type grouping was seen only in two patients (18%) and to a very

mild in degree.

Immunocytochemical findings

Neither MHC class 1 nor class 2 products were expressed on the sarcolemma of PMR and controls. In contrast, MHC class 1 and class 2 products on the sarcolemma were observed in 63% and 56% of PM-DM, respectively (Fig. 3). In the blood vessel walls (presumably in endothelial cells), MHC class 2 products were stained more strongly in PMR than in controls and PM-DM (Fig. 4).

Deposition of IgG, IgM and IgA, alone or in combination, was detected on the sarcolemma and in the intramuscular vessel wall of PMR, PM-DM, and controls. Although the specimens were washed extensively with PBS before fixation with cooled acetone, there were a few deposits of immunoglobulin, complement and albumin even in normal control specimens. Thus, we considered it abnormal when staining was clearly more than in controls. Deposition of IgM was more frequent in the sarcolemma of PMR than in that of PM-DM and disease controls ($p < 0.05$).

On serial muscle sections, the association of IgG and Clq in the intramuscular vessel wall was seen in three out of eight cases of PMR (Fig. 5), three out of 11 of PM-DM, and one case of PN. There was no such association in controls. In one case of PM-DM, association of IgG, IgM and Clq was detected. In another case of PM-DM, combination of IgM and Clq was seen. In PMR, vessels with immunoglobulin and complement staining were 50–200 μm in diameter, and they were usually located in the perimysial area. No vessels in the endomysial area, many of which were considered to be capillaries, were stained with immunoglobulin or complement in PMR. The staining of C9 was significantly more frequent in PM-DM than in PMR and disease controls ($p < 0.05$). Clq was also stained more frequently in vessels of both PMR and PM-DM than in those of disease controls ($p < 0.05$). Sections incubated with control mouse or rabbit serum or PBS were negative for staining of immunoglobulin or complements.

A few mononuclear cells were seen in the endomysium or perivascular area of PMR. The majority of those cells were acid phosphatase positive and were classified as macrophages.

Fig. 1. Summary of histological and histochemical findings of polymyalgia rheumatica.

Fig. 2. Polymyalgia rheumatica showing a slight variation of fiber size with a few necrotic (A, B) or regenerating (basophilic) fibers (C; asterisk). Fibers with internal nuclei (A) are few. Nuclear clumps (D; arrows) are seen amongst normal looking fibers. Hematoxylin and eosin (HE) stain; $\times 200$.

Endomysial Leu 2a (cytotoxic/suppressor), Leu 3a (helper/inducer) positive T cells and perivascular Leu 12 positive (B) cells were rarely observed.

Other clinical data of PMR (Table 1)

Erythrocyte sediment rate (ESR) and C reactive protein (CRP) were increased in each case of PMR. Increased IgG, IgA, IgM and IgE was seen in 22 (2/9), 33 (3/9), 11 (1/9) and in 25% (2/8) of PMR patients, respectively. Creatine kinase (CK) activities in serum were normal except in one case. The concentration of CK in this case (case 5) had returned to normal before the start of steroid therapy.

Study of T lymphocyte subsets showed an increased ratio of T4/T8 in 50% (2/4) and a decreased ratio in 25% (1/4). Immune complexes were detected in 50% (2/4). Increased level of CH50, C3 or C4 was observed in 83% of cases (5/6), 67% (6/9) or 56% (5/9), respectively. Rheumatoid factors were raised in 45% (5/11) of cases, but to a very mild

degree. Antinuclear antibodies were also weakly positive in 30% (3/10), but none of the cases had antibodies to deoxyribonucleic acid (DNA) or to extractable nuclear antigens (ENA).

Sensory or motor nerve conduction velocity was measured in nine cases of PMR. In seven out of nine cases (78%), the nerve conduction velocity was slightly reduced. Electromyography was also abnormal in eight out of 11 cases (73%): mild myopathic changes 4/11 (36%), mild neuropathic 2/11 (18%), both mild myopathic and neuropathic 2/11 (18%).

DISCUSSION

Recent immunohistochemical studies have shown that class 1 or class 2 products of major histocompatibility complex (MHC) are expressed on muscle fibers in inflammatory myopathy or in other neuromuscular disorders (14–16). If the skeletal muscle of PMR is a target of the immunopatho-

Fig. 3. Serial sections from a case of dermatomyositis. (A) There are prominent mononuclear cells in perimysial areas. Necrotic and phagocytic fibers can also be seen. (B) MHC class I products are expressed on almost all muscle fibers; some muscle fibers also show cytoplasmic staining. (C) MHC class II staining is limited to the fibers near the perimysium and to those in the vicinity of mononuclear cells. $\times 200$.

logical process, those products might be expressed on the sarcolemma. We demonstrated this was not the case in PMR, although MHC class I and class II products were observed on the muscle of PM-DM by the same procedure.

Fig. 4. Immunohistochemistry of HLA-DR. HLA-DR expression is more marked in intramuscular vessels of polymyalgia rheumatica (A, B) than in those of motor neuron disease (C). $\times 200$.

Cell-mediated cytotoxicity has been considered to play a major role in the pathogenesis of PM-DM (17, 18). The present results suggested that the muscle cell itself is not a target of MHC restricted T cells in PMR. Many previous studies and the present results showed that there are abnormal subpopulations of T cells in the peripheral blood of PMR (19, 20). This abnormality might be related

Fig. 5. Serial sections from a case of polymyalgia rheumatica stained with HE (A), immunostaining for IgG (B), IgM (C), IgA (D) or Clq (E). Arrows indicate the deposition of IgG (B) and Clq (E). $\times 200$.

to vascular pathology rather than to muscle injury, since more distinct deposition of MHC class 2 products and combined deposits of IgG and Clq were occasionally detected in the intramuscular vessels.

The IgG and Clq deposition in intramuscular vessels of PMR could indicate the presence of immune complexes, since sera from patients with TA or PMR contained increased levels of immune complexes (21). And in some cases of temporal arteritis, immunoglobulin and complement deposits have been demonstrated in biopsied temporal arteries (22, 23). An electron microscopic study of synovium in PMR also revealed microvascular changes, suggesting the existence of immune com-

plexes (24). These reports are consistent with present results, implicating a pathogenetic role of immune complexes in PMR, although some authors indicated a lack of specificity of these deposition, which suggests the possibility of a non-specific reaction to muscle injury (25–28). In TA, involved arteries were mainly large ones that directly branched from the aortic arch. The diameter of vessels, where the immunoglobulin and complement deposition was demonstrated, was small and ranged between 50 and 200 μm . If the caliber of involved arteries differs in TA and PMR, the difference might be attributed to the size of immune complexes (29).

Histochemical and electrophysiological findings have indicated involvement of peripheral nerve in

PMR. However, the clinical significance of neuropathy was obscure. The vascular involvement, which was demonstrated in the skeletal muscle of PMR, could also cause neuropathy. On the other hand, neuropathy in PMR could be coincidental, since patients with PMR are generally of old age. Further pathologic study of the peripheral nerve is necessary on this point.

Every case of PMR in this study fulfilled the two different diagnostic criteria (11, 12), and none showed symptoms which suggest other collagen diseases. All patients with PMR also showed prompt and favorable early response to the steroid therapy. Thirty percent of our cases, however, indicated weakly positive antinuclear antibodies and 45% of the cases had mildly raised rheumatoid factors. In PMR, antinuclear antibodies and rheumatoid factors have been considered to be generally negative (12). The pathogenetic significance of antinuclear antibodies or rheumatoid factors in PMR would be a matter of further study.

In conclusion, the present study suggests that muscle and peripheral nerve manifestations in PMR is caused by vascular involvement of small vessels, which is induced by immune complexes.

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