Case Report

Chronic Polymyositis: Presence of Coxsackievirus A9 Antigen in Muscle

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We report a case of chronic recurrent polymyositis associated with increasing antibody titers of coxsackievirus A9 in serum during clinical exacerbations. Muscle biopsy specimens showed pathologic changes consistent with chronic myositis, including perivascular mononuclear cell infiltration and hyalinization of muscle fibers with cytoplasmic vacuolations. The specific fluorescence was observed in the muscle fibers stained with antiserum for coxsackievirus A9. These findings indicate that this viral subtype as the etiologic agent in this case and virus plays a pathogenic role in some cases of chronic polymyositis.

Key Words: Polymyositis, Coxsackievirus A9, Viral myositis.

Although a good deal of evidence has been accumulated implicating an autoimmune mechanism in the pathogenesis of polymyositis,^{1, 2)} the viral etiology has also been suggested.³⁻⁷⁾ We here report a case of chronic polymyositis associated with increase in antibody titer of coxsackievirus A9 during clinical exacerbations. In the present case, positive fluorescence was observed in the muscle fibers stained with antiserum for coxsackievirus A9. The evidence of viral presence in this case indicates that virus plays a pathogenic role in some cases of chronic polymyositis.

CASE REPORT

A 56-year-old man began to experience stiffness of shoulders and thighs, and difficulty in ascending stairs and holding the arms overhead since April 1983. These symptoms became progressively worse and he was admitted in July 1983. There was no past history of arthralgia and dermatitis. Results of physical examination were normal. The patient was alert and oriented. Mild dysphagia was noted, but the speech and phonation was normal. The anterior neck muscles and shoulder-

and pelvic-girdle muscles were moderately weak. Moderate symmetrical weakness was also present in all extremities, predominantly involving proximal muscles. Muscle tenderness, atrophy or fasciculations were not observed. Deep tendon reflexes, sensation, and coordination were normal.

Examination of the urine and blood was normal. The sedimentation rate was 5 mm in one hour. Antinuclear factor and RAHA were positive at a titer of 1:320 and 1:60, respectively. But the LE test, anti-DNA antibody, RA and CRP were negative. Serum immunoglobulin levels were normal. Among serum enzyme studies, GOT was 171 IU/L (normal, 10-35), GPT 184 IU/L (0-40), LDH 144 IU/L (120-320), and CPK 2581 IU/L (28-170). Peripheral blood T-cell subsets were analyzed with an immunofluorescent technique using an OK series of monoclonal antibodies:⁸⁾ the proportion of T3-cells (total T-cells) was 35% (normal, 62 ± 7%), T4-cells (helper/inducer T-cells) 35% (38 ± 7), and T8-cells (suppressor/cytotoxic T-cells) 3% (23 ± 5). Repeated T-cell studies showed similar results. The barium swallowing examination showed mild reflux of barium into

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the trachea. ECG was normal.

Electromyography: EMG was performed on the right deltoid, rectus femoris, hamstrings and anterior tibial muscles. Insertion activity was increased and spontaneous fibrillation potentials were present in these muscles. On minimum voluntary contraction large numbers of polyphasic potentials of low amplitude and short duration were observed. The interference pattern in these muscles was increased in density. The findings were considered to be indicative of a florid myopathic process.

Histologic studies of muscle: Biopsies of the right quadriceps femoris were performed in July and September 1983. Histopathology of the first biopsy specimens were normal. Light microscopic examination of the second biopsy specimens showed increase in variation in muscle fiber diameter and hyalinization of muscle fibers, with cytoplasmic vacuolations (Fig 1). Perivascular and interstitial mononuclear cell infiltration, however, was sparse.

Virological studies: Serum antibody titers of 31 viruses (poliovirus type 1–3, coxsackievirus type A7, 9 and 16, and type B1–6, echovirus type 1, 4, 6, 7, 9, 17 and 25, adenovirus type 1–8 and 19, and mumps virus) were measured by the microneutralization test using microplates containing monolayers of monkey kidney cells. Antibody of echovirus type 11 and 12 was measured by the hemagglutination-inhibition test. The results showed a titer of 1:512 for coxsackievirus A9,

1:32 for echovirus 11, 1:32 for coxsackievirus B4 and 1:32 for poliovirus 1, while those of other viruses were negative. Subsequent antibody studies repeated once every two or three weeks showed decline of coxsackievirus A9 titer from 1:512 to 1:32 (Fig 2), without substantial changes in other viral titers.

Frozen muscle biopsy specimens were studied to detect coxsackievirus A9 antigen in the muscle with an indirect immunofluorescent technique. The sections were incubated at 37°C for 30 min with monkey coxsackievirus A9-specific antiserum (neutralization antibody titer: $\times 2,000$, supplied by the National Institute of Health of Japan). After a wash with phosphate buffered saline, the

Fig. 2. Temporal relationship between clinical exacerbations and levels of coxsackievirus A9-antibody titer as well as CPK value in serum. Open circles; antibody titers of coxsackievirus A9, solid circles; CPK values (IU/L).

Fig. 1. Perivascular cell infiltrations and hyalinization of muscle fibers with cytoplasmic vacuolations (HE stain, $\times 80$).

Fig. 3. Localized specific fluorescence in the muscle fibers stained with antiserum for coxsackievirus A9 ($\times 100$).

the sections were stained at 37°C for 30 min with fluorescein-conjugated goat anti-monkey IgG immunoglobulins (Cappel Labs., Cochranville, USA). Biopsy specimens were also studied with direct fluorescent antibody stains for IgG and complements (C3 and Clq) (Behring, Marburg, West Germany).

Localized specific fluorescence was demonstrated in the cytoplasm of the second-biopsied muscle fibers stained with antiserum for coxsackievirus A9 (Fig 3). Positive fluorescence was not observed in any sections incubated with a normal monkey serum. There was no deposition of IgG and complements in the sections. Electron microscopic observation of other fragments of the biopsy specimens failed to demonstrate virus in the tissue.

Clinical course: Treatment with prednisolone, 40 mg per day, was undertaken for two months, and then tapered to 5 mg per day. The patient improved gradually and returned to work in October 1983. Antibody titers of coxsackievirus A9 and values of serum CPK also gradually declined (Fig 2). In October 1984, however, weakness of lower limb muscles associated with an increase in CPK value developed (Fig 2). Among 33 viral antibody titers studied, an increase in titer was observed only for coxsackievirus A9 during the second exacerbation (Fig 2).

DISCUSSION

Although coxsackievirus A9 is known to possess a marked pathogenicity for skeletal muscle,⁹⁾ there have been only two reports of chronic myositis caused by that particular virus in humans; congenital myositis reported by Freudenberg et al¹⁰⁾ and neonatal myositis by Tang et al.¹¹⁾ To our knowledge, this is the first report of chronic polymyositis by coxsackievirus A9 in the adult.

Structures closely resembling coxsackieviruses have been demonstrated in muscle from patients with chronic polymyositis.^{4, 5, 7)} However, serovirological studies were not performed in any of these cases. We here demonstrated both increase in antibody titer of coxsackievirus A9 in serum and presence of its viral antigen in muscle in a patient with chronic polymyositis. The positive

fluorescence for coxsackievirus A9 in the muscle fibers was considered to be specific because any fluorescence was not demonstrated by normal histology. Concurrent increase in antibody titer of coxsackievirus A9 with clinical exacerbations and continuous decline in parallel with clinical and biochemical improvement pinpointed this viral subtype as the etiologic agent in our case. The normal histology of the first biopsy muscle might indicate multifocal myositis, which is also consistent with coxsackievirus A9-myositis.⁹⁾ Judging from the findings of paucity of inflammatory cell infiltration and focal fluorescence for that virus in the cytoplasm of the muscle fibers, the failure to detect the virus by electron microscopic study was probably due to low quantity of the virus in the muscle.

The present case seems to make several important points. First, the present case would have probably been labelled "autoimmune polymyositis" in the absence of virological studies: presence of autoreactive antibodies (antinuclear factor and RAHA), imbalances of peripheral blood T-cell subsets, and clinical response to corticosteroid treatment would support autoimmune polymyositis. The absence of IgG and complement deposition in or around the muscle fibers, however, did not support antibody-mediated myositis in our case. The temporal relationship between clinical exacerbation and the viral antibody titer was highly suggestive of chronic viral polymyositis or virus induced-autoimmune polymyositis, in which cell-mediated immunity to muscle was initiated by viral myositis.

Second, taking account of an increasing number of reports of acute viral myositis,¹²⁻¹⁵⁾ the present case indicates that viral titer determination should become a routine part of the patient evaluation in chronic polymyositis.

Finally, it is still not known whether the virus plays a pathogenic role in chronic polymyositis, but the evidence of viral presence in this patient suggests that could be the case.

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