

Muscle Regeneration and Cell-mediated Cytotoxicity in the Autologous Muscle Culture of Dermatomyositis

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Muscle culture from a dermatomyositis patient was performed to investigate the muscle regeneration and cell-mediated cytotoxicity. In the primary culture, spindle-shaped mononuclear myoblasts exponentially increased in number. In the secondary culture, the myotubes fused with the myoblasts and/or other myotubes into large thick syncytia. The cell morphology and growth pattern of the cultured muscle cells in dermatomyositis were identical to those of normal healthy controls. The autologous mononuclear cells added in the secondary culture of day 14 adhered to the surface membrane of the myotubes. Three days after this treatment, the myotubes underwent degenerative changes. On the other hand, in two series of sister cultures, each added with the autologous serum and control medium, no remarkable morphological changes were observed. The results of the present study suggest that dermatomyositis could be precipitated by the associated abnormal cell-mediated cytotoxicity, but not by abnormalities in muscle fibers per se.

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Dermatomyositis (DMs) is an inflammatory myopathy associated with skin rash in which degenerative and subsequent regenerative process occur in voluntary muscles. In our present study, autologous muscle culture was carried out to elucidate whether myopathological changes seen in DMs are elicited by abnormalities in muscle fibers per se or by the associated abnormal immune reaction.

MATERIAL AND METHODS

Case report

A 78-year-old Japanese male was hospitalized in August 1983, complaining of facial edema, hoarseness, dysphasia and proximal muscular weakness. He had heliotrope suffusion on the upper eyelids, diffuse erythema on the chest, Gottron's sign on the fingers and poikiloderma on the bilateral knees.

The histopathology of muscle bundles taken from the right biceps showed perifascicular atrophy, mixture of degenerative fibers and regenerative basophilic fibers containing internal nuclei and occasional perivascular mononuclear cell infiltrates. On adenosine triphosphatase staining, type 1, 2A and 2B fibers were adequately distributed with mildly increased number of type 2C fibers. The histochemical staining for nicotinamide adenine dinucleotide tetrazolium reductase revealed markedly disorganized intermyofibrillar networks showing numerous marks of moth-eaten appearance. No significant findings were obtained on the other histochemical stainings including acetylcholine esterase, alkaline phosphatase, acid phosphatase, periodic acid Schiff, oil red O and cytochrome C oxidase.

The laboratory data revealed elevated creatinine, s-GOT, LDH, creatine phosphokinase, aldolase and myoglobin in the serum. Electromyogram showed a myogenic pattern. Thorough investigation of the patient failed to reveal associated malignancy.

Prednisone therapy of 50 mg daily was initiated, followed by the improvement of the clinical symptoms and the laboratory data. The dose of prednisone is now tapered to 10 mg daily.

Muscle culture

One-half of the biopsied muscle was subjected to culture study. The muscle bundles depleted from connective tissue were dissected into small pieces, followed by digestion with 2000 units of dispase in 37°C water bath for one hour. The sediment obtained from this sample by centrifugation at 1000 rpm for 5 min was dispersed in Dulbecco's minimum essential medium (MEM) containing 10% heat-inactivated fetal bovine serum (FBS). This was placed in a 35 mm gelatin-coated plastic dish (Falcon) and incubated at 37°C in a 5% CO₂ incubator. Half of the medium was changed every other day. The muscle bundles from the right biceps of a 43-year-old healthy male was concomitantly cultured using the same technique for comparative study. On the 21st day of the primary culture, cell passage was performed in triplicate.

On the 14th day of the secondary culture, the following procedures were carried out. Mononuclear cells collected by the density gradient centrifugation method from the peripheral blood of the patient were added in one of the triplicate dishes at the concentration of 2×10^6 /ml. In the second sister culture, MEM containing 10% serum from the patient was totally replaced by 10% FBS-MEM. The third sister culture serving as a control was maintained with fresh 10% FBS-MEM.

The cultured muscle cells were consecutively observed with a phase-contrast microscope throughout the experimental period.



Fig. 1. High magnification view of a syncytium. Note a large thick myotube with centrally located multinuclei and fine fibrils in the cytoplasm.

RESULTS

During the first week of the primary culture, mitotic and migratory activities of myoblasts were prominent. Mononucleated spindle-shaped myoblasts increased exponentially in number to form clusters. In the second week of culture, they started to show cytoplasmic fusion eventuating in asynchronous formation of binucleated myotubes. In the third week of culture, a small number of multinucleated myotubes were recognized.

In the secondary culture, the myotubes repeated the cytoplasmic fusion with the neighboring myoblasts and/or other myotubes, and during the second week of culture they formed large thick syncytia with prominent branching. Aggregated multinuclei were centrally located in the abundant cytoplasm of the myotubes. The cytoplasm was filled with fine fibrils, but was still devoid of cross striation (Fig. 1). The morphologic aspect and differentiation process of the cultured muscle cells from DMs were identical to those obtained from the healthy normal subject.

When the secondary culture was treated with the autologous mononuclear cells on day 14, the mononuclear cells migrated to adhere to the surface of the myotubes. Three days after this treatment, these myotubes underwent vacuolar degeneration (Fig. 2). The degenerated myotubes finally detached from the dish and floated in the culture medium while the myoblasts remained unaffected. The myotubes and the myoblasts cultured on the autologous serum as well as the control medium showed no apparent morphological changes.



Fig. 2. On the third day of the treatment with the autologous mononuclear cells, a myotube undergoes vacuolar degeneration.

DISCUSSION

Morphological changes recognized in cultured muscles from various muscular disorders reflect the presence of similar changes taking place within the muscles *in vivo* (1, 2). The morphology and growth pattern of the cultured muscle cells from DMs were identical to those observed in the cultured normal muscle and to the previous description by Askanas (3). The present study confirmed no morphological abnormalities or aberrant events in maturation of muscle cells in DMs.

The cardinal histopathologic findings in polymyositis-dermatomyositis (PMs-DMs) are strongly reminiscent of cell-mediated cytotoxicity (4, 5). With the culture study several authors (6-9) tried to reincarnate the cell-mediated cytotoxicity in PMs-DMs. These culture studies confirmed that the mononuclear cells from PMs-DMs patients were cytotoxic to xenogeneic and allogeneic fetal muscles. In these culture studies utilizing heterologous muscle and mononuclear cells, the possible contribution of the proliferation of lymphocytes in response to foreign histocompatibility antigen on muscle cells and non-specific attack by K cells could not be denied (4, 10).

Most important to note in our study is that we dealt with the autologous materials from a DMs patient. A review of current literature on culture study utilizing autologous material disclosed only one abstract reported by Band et al. (11). However, detailed methodological information is lacking in their report. We observed, for the first time, the autologous mononuclear cells attacking the myotubes *in vitro* condi-

tion. The degeneration of muscle cells seen in this culture added with the autologous mononuclear cells was not due to the aging of culture because the sister culture with the control medium did not undergo degeneration. It is most probable that the similar immunological event is taking place *in vivo* in DMs.

It is considered that there is little involvement of humoral immunity in the pathogenesis of DMs although PMs-DMs sera contain various myositis-related autoantibodies (12). In the present study, after exposure to the autologous serum the muscle cells from the patient appeared morphologically unchanged. The production of the myositis-related autoantibodies most probably results from the antecedent muscle damage.

The membrane components of muscle cells are the target sites exposed to the immune system in PMs-DMs. Among those antigens, the acetylcholine receptor (13) and JO-1 antigen (14) are well elaborated. In the present study, myoblasts remained unchanged throughout the attack by the autologous mononuclear cells. It can be said, however, that the target antigens must be newly appeared surface membranous antigens on the myotubes in the process of muscle differentiation.

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