

Immunohistochemical Study of Rods in Nemaline Myopathy

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Summary. We used immunohistochemistry to study the expression of some proteins of the cytoskeleton (desmin and vimentin) and of the sarcomere (tropomyosin, myosin and actin), in muscle fibers of a patient with nemaline myopathy. Desmin, myosin and actin were present in the regions of rods but not vimentin. Tropomyosin was irregularly seen in these regions, and with less intensity. We concluded that it is possible that, in this disease, there is a derangement of the assembly of architectural and contractile proteins, which secondarily assume a particular subsarcolemmal disposition forming rods.

Key words—congenital myopathy, muscle, nemaline myopathy, intermediate filaments.

INTRODUCTION

In 1963, Conen et al.¹⁾ and Shy et al.²⁾ described a new and rare type of congenital myopathy characterized by the presence of rod-shaped structures in muscle fibers (nemaline myopathy). Since these two original reports, about 100 cases have been described, and hereditary pattern of transmission documented.³⁾

The clinical manifestations can appear at birth, during childhood or in adult life. Generalized muscle weakness, respiratory distress and poor suckling can be present in the birth-onset form. In the childhood form, muscle involvement is diffuse and generally symmetrical; speech and swallowing are often disturbed and the intellectual performance is normal. In the third group (adult-onset), there are many presentations. In some patients, there is a diffuse and slowly progressive weakness at childhood. Other patients

are asymptomatic until adult age. In some cases, a subacute proximal and distal weakness may be the first symptom.

There is not a clear relationship between the severity of the weakness and the number of rods in the muscle fibers, suggesting that there are other factors responsible for the weakness in this congenital myopathy. Nonaka et al.⁴⁾ suggested that there is a close relationship between the gravity of the disease and the increase of lysosomal enzymes in muscle.

Histologically the rods can be viewed by the Gomori trichrome technique. Generally, a Type 1 fiber predominance is present and the diameter of Type 1 fibers is disproportionately smaller. Rods are frequently placed in the subsarcolemmal regions. Fine analysis of the rods shows a clear relationship with the normal Z lines.⁵⁾ Rods exhibit a filamentary structure with a double striation, one parallel and the other perpendicular to the long axis. Thin filaments are contiguous with the edges of the rods. Sometimes one can confirm a streaming of Z discs in contact with the rods.

Immunofluorescence techniques have demonstrated the presence of 10S component of alpha-actinin⁶⁾ and actin,⁷⁾ in the rods. These proteins are normally present in Z lines.

In this study we used histochemical and immunohistochemical methods and antibodies to some proteins of the cytoskeleton and sarcomere to study the muscle biopsy of a childhood-onset case of nemaline myopathy in search for a possible mechanism involved in the rod formation.

MATERIALS AND METHODS

Muscle biopsy was obtained from the left biceps. For histochemistry the specimen was frozen in n-hexane

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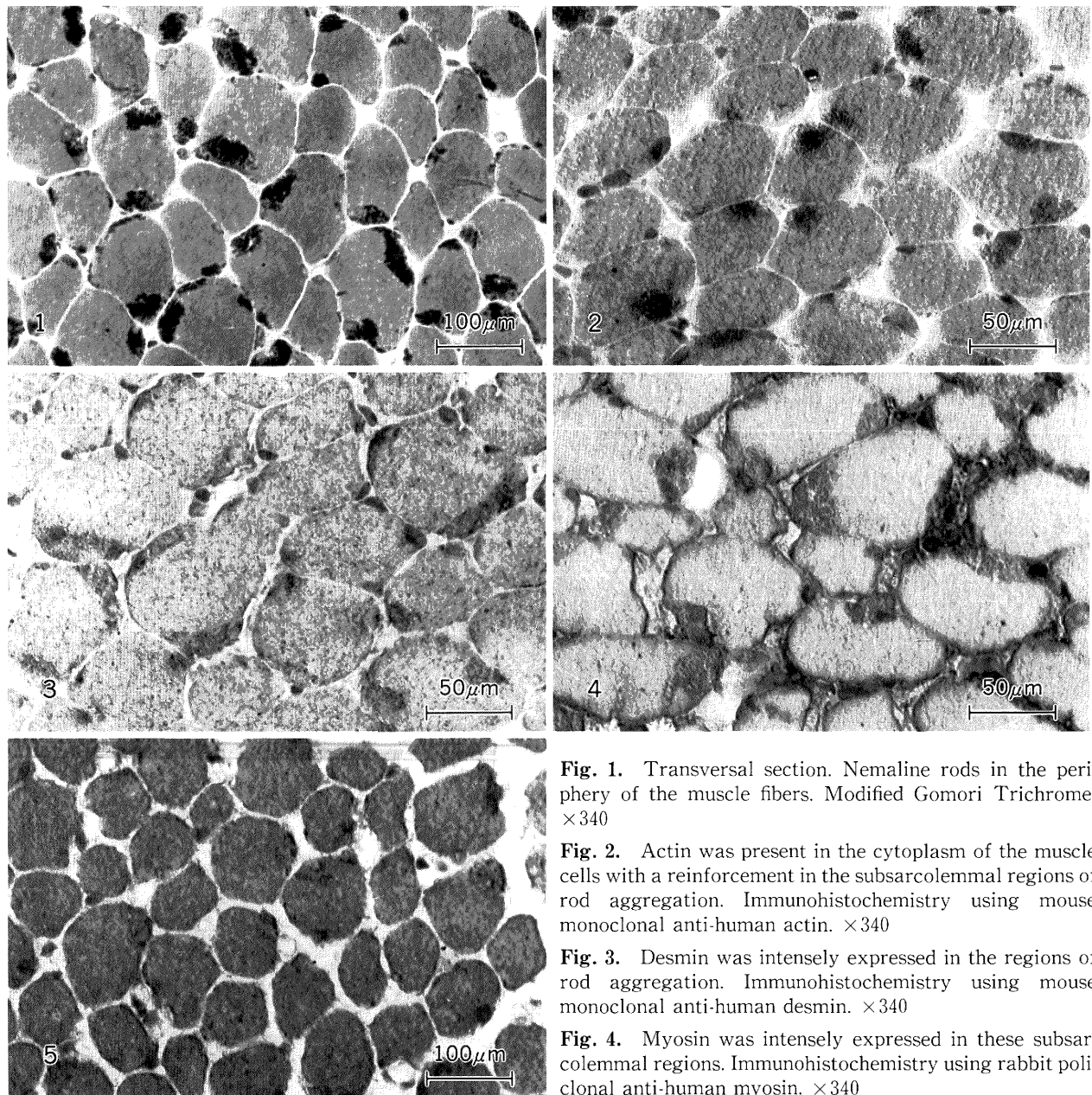


Fig. 1. Transversal section. Nemaline rods in the periphery of the muscle fibers. Modified Gomori Trichrome. $\times 340$

Fig. 2. Actin was present in the cytoplasm of the muscle cells with a reinforcement in the subsarcolemmal regions of rod aggregation. Immunohistochemistry using mouse monoclonal anti-human actin. $\times 340$

Fig. 3. Desmin was intensely expressed in the regions of rod aggregation. Immunohistochemistry using mouse monoclonal anti-human desmin. $\times 340$

Fig. 4. Myosin was intensely expressed in these subsarcolemmal regions. Immunohistochemistry using rabbit polyclonal anti-human myosin. $\times 340$

Fig. 5. Tropomyosin is expressed in all the cytoplasm of the muscle cells with variable intensity in the periphery (regions of rods). Immunohistochemistry using mouse monoclonal anti-human tropomyosin. $\times 340$

previously cooled in liquid nitrogen and cut in a cryostat. For electron microscopy a fragment was fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffered saline (PBS) at 4°C, post-fixed with 2% osmium tetroxide, dehydrated and embedded in araldite. Ultrathin sections were stained with uranyl acetate and lead citrate.

Histochemical stains and reactions were performed

according to Dubowitz.⁸⁾ Immunohistochemistry was performed using a streptavidin-biotin-peroxidase complex to detect the expression of proteins of the cytoskeleton (desmin and vimentin) and proteins of the sarcomere (myosin, tropomyosin and actin). Mouse monoclonal anti-human desmin (DAKO-desmin), mouse monoclonal anti-human vimentin (DAKO-vimentin), rabbit polyclonal anti-human myosin (Miles-Yeda),

mouse monoclonal anti-human tropomyosin (SIGMA) and mouse monoclonal anti-human actin (DAKO-HHF-35) were used.

RESULTS

Light microscopy

Muscle biopsy showed a generalized hypotrophy of fibers, hexagonal or rounded in shape. Endomysial tissue was slightly increased. ATPases reactions at distinct pHs showed an absolute predominance of Type 1 fibers (100%). These fibers presented homogeneous subsarcolemmal eosinophilic areas. The modified Gomori trichrome stain revealed nemaline rods in these areas (Fig. 1).

Immunohistochemistry

Actin was present in the cytoplasm of the muscle cells with a reinforcement in the subsarcolemmal regions of the aggregation of rods (Fig. 2). Desmin was poorly expressed in the cytoplasm of the muscle fibers, except in the regions of rods aggregation, where its expression was intense (Fig. 3). Myosin was expressed in the cytoplasm with a great reinforcement in these subsarcolemmal regions (Fig. 4). Tropomyosin was observed in the cytoplasm including these subsarcolemmal regions, but with variable intensity (Fig. 5). Vimentin expression was not observed in the cytoplasm of muscle cells nor in the regions of the rods.

Electron microscopy

All the examined fibers showed clusters of dense structures, with parallel disposition, similar to normal Z lines, attaching to thinner filaments and forming a fuse. These clusters were localized in the subsarcolemmal areas, juxtaposed with fiber nuclei.

DISCUSSION

Composition of the nemaline bodies

Immunofluorescence techniques have demonstrated the presence of the 10S component of alpha-actinin⁽⁶⁾ and actin⁽⁷⁾ in the rods. These proteins are present in the Z lines of normal muscle fibers. It was also demonstrated that alpha-actinin isoforms in nemaline myopathy were not different from those of control muscles.⁽⁹⁾ It therefore was proposed that the rod formation is due to a derangement of the control mechanisms that restricts alpha-actinin to Z

disks.^(10,11)

In the present investigation, we were able to observe the expression not only of actin, but also of desmin, myosin and less intensely of tropomyosin but not of vimentin, in the region of nemaline bodies, using the sensible method of streptavidin-biotin-peroxidase. Vimentin is a protein of the cytoskeleton present normally only in regenerative fibers of adult muscle. Thus, the absence of the expression of this protein in muscle cells or rods was expected. Desmin is an intermediate filament 10 nm thickness, situated between the edges of the Z disks in normal muscle fibers, probably linked to the maintenance of the transversal order of the contractile filaments. This protein is expressed uniformly in normal adult muscle. In the present study we found an intense expression of desmin in the regions of rods. However, this does not mean that there is an increase in the total content of this intermediate filament in muscles of nemaline myopathy. Probably there is only a derangement of its distribution, since studies of the quantification of desmin in the muscle specimen of nemaline myopathy show it to be normal.⁽¹²⁾

Although rods are structurally similar to Z discs, the edges of the rods are contiguous with thin filaments, giving the idea that, in fact, different types of filaments compose their whole structure. This can explain why actinin,⁽⁶⁾ actin, desmin, myosin and tropomyosin (in the present study) were observed in rods. Based on these findings, it is conceivable that there is a congenital derangement of the assembly of structural and contractile proteins in nemaline myopathy. These proteins can be stored in subsarcolemmal places in the muscle fibers in nemaline myopathy. Secondly, they can cluster themselves and assume an abnormal disposition.

Relationship of nemaline myopathy with other congenital and acquired myopathies

Nemaline bodies are not exclusive of this congenital myopathy. These bodies can be seen in acquired myopathies such as hypothyroid myopathy,⁽¹³⁾ polymyositis,⁽¹⁴⁾ and HIV infection.⁽¹⁵⁾ This fact is additional evidence that the formation of nemaline bodies is a secondary phenomenon. Moreover, there are other situations in which a disturbance of the assembly of non-contractile proteins is involved in the pathogeny of congenital myopathies. In desmin storage myopathy there is an abnormal storage of this intermediate filament in muscle cells, but nemaline bodies are absent.^(16,17) It has also been demonstrated that in centronuclear myopathy there is an abnormal accumulation of desmin in the periphery of the central

placed nuclei.¹⁸⁾ Thus, derangements of the assembly of non-contractile filaments are not exclusive to nemaline myopathy.

We concluded that the rod formation is a complex phenomenon that probably involves a derangement of the assembly of muscle structural and contractile proteins, that secondarily assume an abnormal disposition in the subsarcolemmal regions. These are not only proteins of the sarcomere but also of an intermediate filament (desmin), responsible for the organization and maintenance of the alignment of Z disks and transversal disposition of the sarcomere. This derangement could be the primary phenomenon in the rod formation.

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