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Desmin and Vimentin as markers of regeneration in muscle diseases

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Summary. Localization and distribution of desmin and vimentin have been studied in different neuromuscular disorders using monoclonal antibodies. We have demonstrated that vimentin, although virtually absent in normal human muscle fibers, is expressed in regenerating fibers in different neuromuscular disorders. Moreover, these fibers showed a strong positivity with desmin antibodies. In normal muscle fibers desmin is only localized at Z-line level. These results suggest that desmin and vimentin may be over-expressed during muscle regeneration processes, probably because of their importance in the structural organization of the sarcomere.

Key words: Immunohistochemistry – Vimentin – Desmin – Muscle – Regeneration

Vimentin and desmin, the predominant subunit of fibroblastic intermediate filaments (IF), are found in most cells of mesenchymal origin [6]. The content and distribution of vimentin in differentiated muscle has been subject of some disagreement: Bennett et al. [1] observed that both desmin and vimentin were distributed in the form of longitudinal filaments in immature myotubes, but as they matured vimentin gradually disappeared. In contrast, Granger and Lazarides [8] reported the two proteins that coexist at the periphery of Z disk in isolated myofibrils of adult chicken skeletal muscle by immunofluorescence microscopy. Osborn et al. [11] have demonstrated, however, that adult muscle fibers of several mammalian species were not decorated by vimentin antibodies, thus supporting the observations of Bennett et al. [1].

Anti-desmin antibodies have been already used to study myopathic disorders: they stain intensely the immature regenerating fibers present in some myopathic disorders and the periphery of nemaline rods [15]. Osborn and Goebel [10] and Schroeder et al. [14] demonstrated that antibodies to desmin stain the cytoplasmic bodies, in a congenital myopathy and in two adult-onset myopathies, respectively. Desmin accumulation has been also reported [2] in intermediate filament (IF) myopathy. The presence and distribution of both desmin and vimentin in various muscle pathology has been recently described [3, 4, 13].

Extending this line of study we performed an immunohistochemical study using both monoclonal antibodies against desmin and vimentin to compare the expression of these IF in different myopathic disorders.

Materials and methods

Cryostatic sections (6 μm) were obtained from muscle biopsies of 15 patients previously studied at Neurological Institute of Milan University and affected by different neuromuscular disorders: two distal myopathies, two polymyositides, one Duchenne muscular dystrophy, two spinal muscular atrophy, one Type II fiber uniformity, one congenital fiber type disproportion, one minimal change myopathy, one central-core disease, one multiple core disease, one congenital dystrophy and one congenital myotonic dystrophy. As controls we used five normal muscle biopsies taken from the same location as the diseased muscle during orthopedic surgery.

A complete battery of histological (H&E., Gomori trichrome) and histochemical reactions (PAS, Oil red O, NADH, COX, SDH, ATPase pH 9.4, 4.6, 4.3, acid phosphatase, acridine orange) was carried out according to Dubowitz [5]. The diagnosis of Duchenne muscular dystrophy was made using the original polyclonal antibodies against the 60-kDa fragment of dystrophin [9]. Muscle regeneration was evaluated looking for basophilic fibers at H&E. and RNA-positive fibers at acridine orange [5].

Immunofluorescence studies

Sections were collected on coverslips treated with 1% polylysine. We used the following antibodies: (1) mouse anti-desmin monoclonal antibodies raised against desmin purified from porcine stomach (Amersham Int. code RPN 1101) diluted 1:5 in 1%

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BSA/PBS; and (2) mouse anti-vimentin monoclonal antibodies raised against vimentin purified from porcine eye lens (Amersham Int. code RPN 1102) diluted 1:5 in 1% BSA/PBS.

As second antibody we used a FITC-labelled sheep anti-mouse Ig G (Amersham Int. code N 1031) diluted 1:25 in 1% BSA/PBS. Sections were rinsed in PBS 0.1 M pH 7.4. Finally sections were mounted in glycerol/PBS 1:1 and observed with a Leitz fluorescence microscope.

Results

Desmin immunoreactivity in normal human muscle

In all muscle fibers desmin immunoreactivity corresponded to the intermyofibrillar network in cross-sections and to transverse striations in longitudinal sections (Fig. 1 a,b). Subsarcolemmal regions demonstrated a strong reactivity. Smooth muscle cells of blood vessels were also positive.

Vimentin immunoreactivity in normal human muscle

Vimentin immunoreactivity was absent in normal human muscle fibers both in longitudinal and transverse sections. A partial reactivity was observed in fibroblasts (Fig. 1c) and in vascular cells.

Desmin immunoreactivity in pathological muscle

Immunohistochemical localization of desmin showed a normal intermyofibrillar pattern of reactivity in patients affected by distal myopathies, Duchenne muscular dystrophy, polymyositis and spinal muscular atrophy. In patients affected by congenital myopathies a normal pattern of desmin reactivity was present in type 2 fiber uniformity, congenital fiber type disproportion and minimal change myopathy, while in central-core disease and multiple core disease immunoreactivity was lacking in the areas corresponding to the cores and very strong around them and at the sarcolemmal level (Fig. 2a).

Strong desmin immunoreactivity was observed in scattered fibers in polymyositis and in Duchenne muscular dystrophy. In spinal muscular atrophy some of the small angulated fibers were also positive (Fig. 2b–d). Strongly Desmin-positive fibers were basophilic at H&E. and stained by acridine orange. Denervated fibers were also basophilic at H&E. but not recognized by acridine orange.

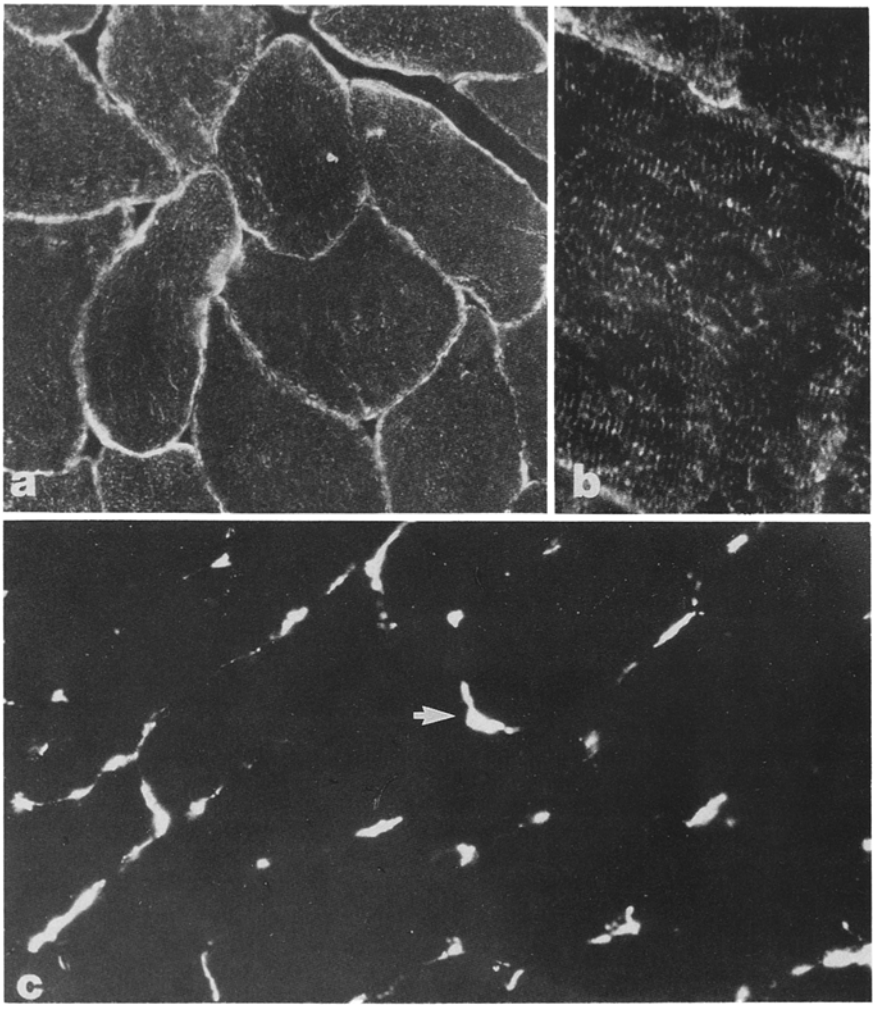

Fig. 1.a, b Desmin localization in normal human muscle; **a** cross-section, **b** longitudinal section. Desmin immunoreactivity corresponds to the intermyofibrillar network in **a** and to transverse striations in **b**. Strong sarcolemmal binding is evident in **a**. **c** Vimentin localization in normal human muscle, cross-section. Vimentin immunoreactivity is present in fibroblasts (*arrow*) and absent in muscle fibers. **a,c** × 250; **b** × 400

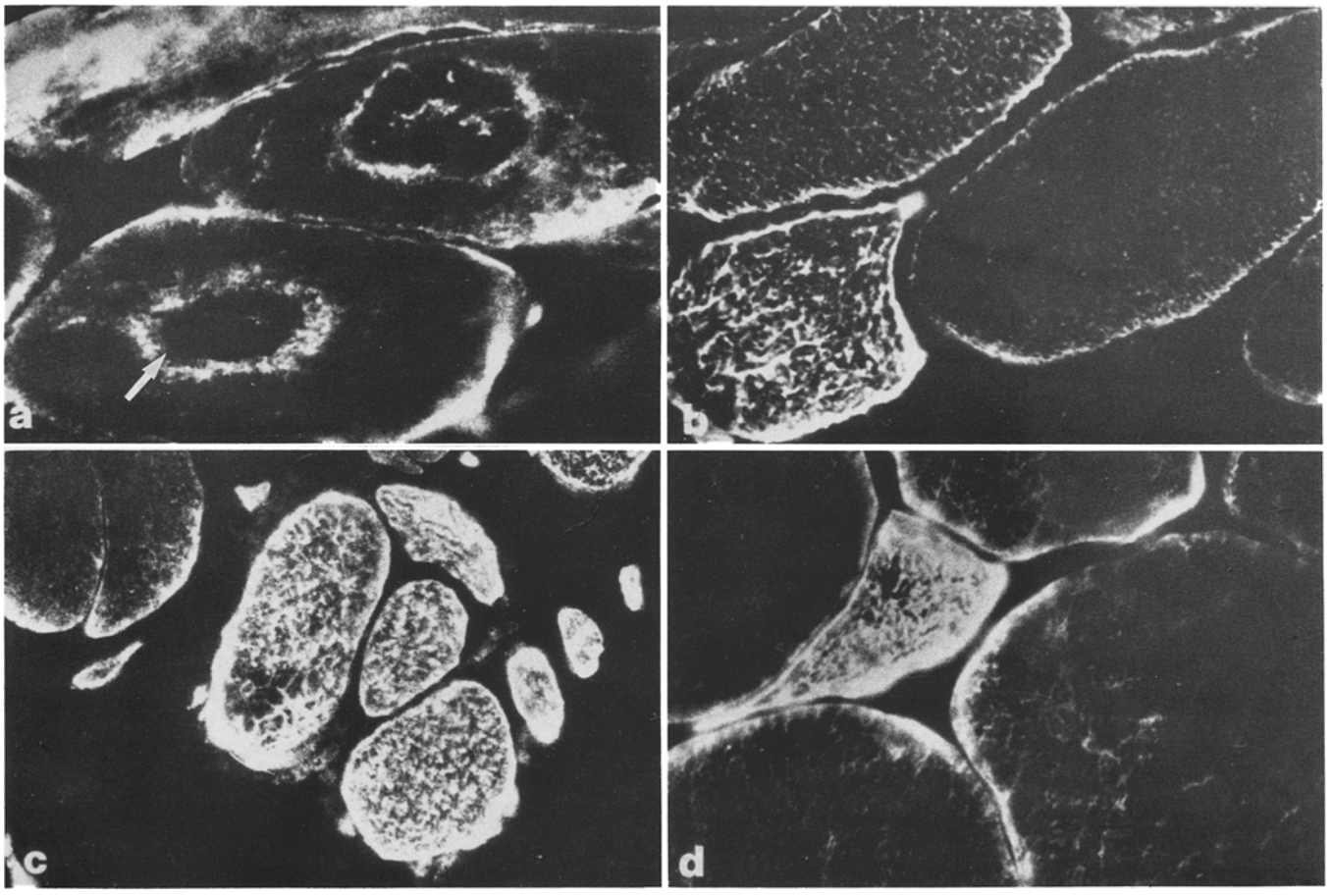

Fig. 2a–d. Desmin in muscle diseases. **a** Cross-section central core disease: desmin is lacking in the areas corresponding to the cores (*arrow*) and very strong around them and at the sarcolemmal level. **b–d** Cross-sections showing abnormally strong desmin immunoreactivity in scattered fibers in polymyositis (**b**), in Duchenne muscular distrophy (**c**) and in spinal muscular atrophy (**d**). **a–d** × 400

Vimentin immunoreactivity in pathological muscle

Strong and diffuse Vimentin immunoreactivity was observed in scattered fibers in distal myopathies, Duchenne muscular dystrophy, congenital dystrophy, polymyositis, and in some angulated fibers of spinal muscular atrophy (Fig. 3b–d). Vimentin-positive fibers were basophilic at H&E. and stained by acridine orange. The small angulated fibers in spinal muscular atrophy were also basophilic at H&E, but not recognized by acridine orange.

No immunoreactivity was present in muscle tissue of congenital myopathies (Fig. 3a).

Discussion

We studied the localization and distribution of desmin and vimentin in different neuromuscular disorders using monoclonal antibodies.

Our results show that strong desmin immunoreactivity is present in scattered fibers in polymyositis and in Duchenne muscular dystrophy. Moreover vimentin, although virtually absent in normal human muscle, as previously described [11], is also expressed in the same fibers. The acridine orange positivity and basophilic appearance show they are regenerating fibers. In spinal muscular atrophy we found that some of the small angulated fibers were also desmin and vimentin positive, however, it is not clear whether they are denervated fibers. Such data have already been described but their interpretation is still uncertain [3].

It has recently been reported that the immunolocalization of desmin and vimentin seems a good marker of muscle fiber regeneration [3]. Moreover, desmin and vimentin are expressed in myotubular myopathy showing a pattern similar to fetal muscle and indicating an arrest in morphogenesis in this congenital myopathy [12].

Several studies, performed in developing myotubes and in chicken muscle, have shown that Vimentin surrounds each myofibril Z disc and forms honeycomb-like networks within each Z plane. This distribution is complementary to that of alpha-actinin within a given Z plane [8]. Desmin and vimentin distribution is coincidental throughout the development of myotubes, but

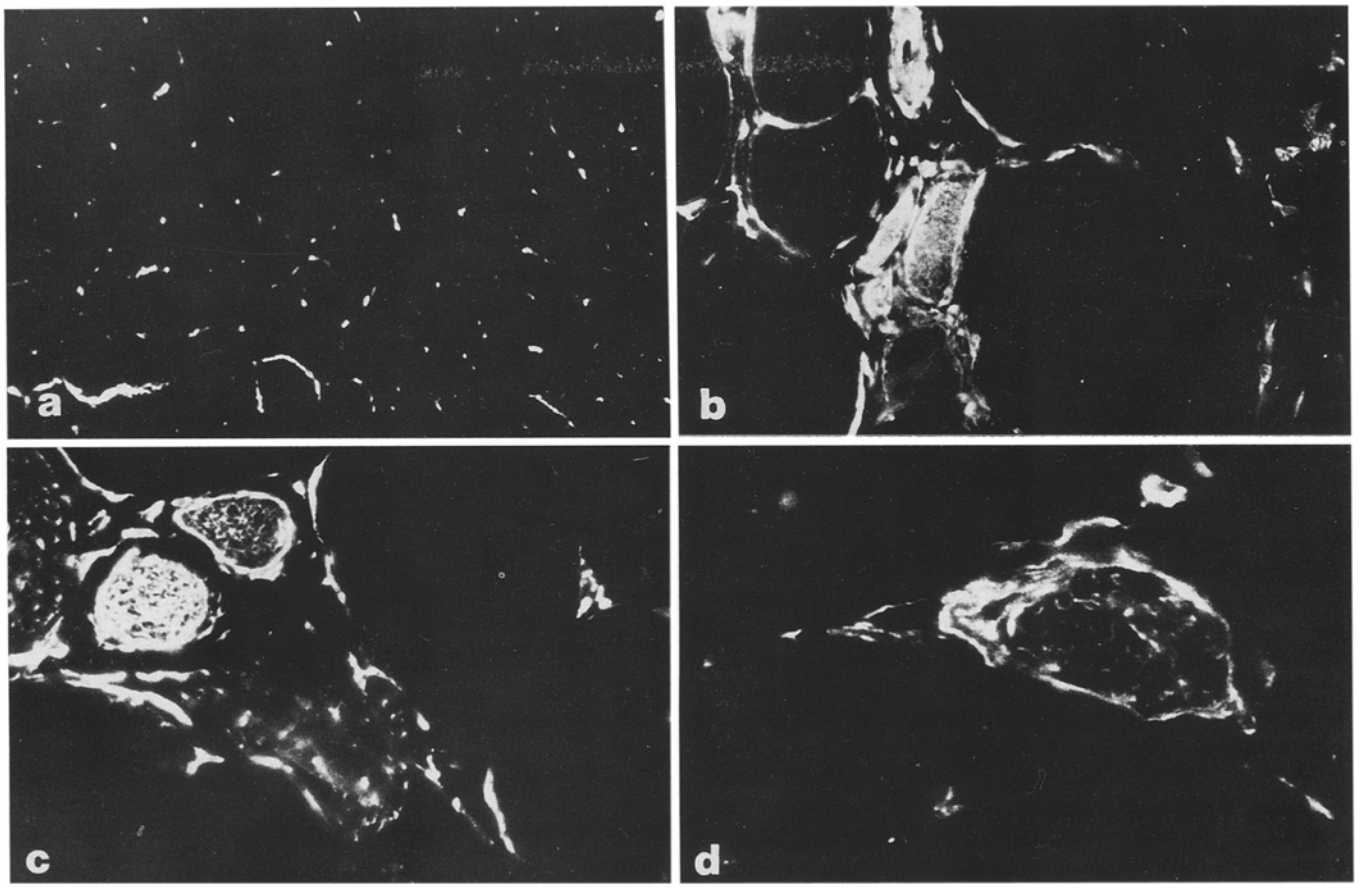

Fig. 3a–d. Vimentin in muscle diseases. **a** Cross section showing no reactivity in a case of congenital myopathy. **b–d** Cross sections showing that vimentin is strongly expressed in scattered fibers in polymyositis (**b**), in Duchenne muscular dystrophy (**c**) and in spinal muscular atrophy (**d**). **a** × 250; **b–d** × 400

the concentration of vimentin is gradually reduced as the myotubes mature, and becomes largely undetectable at the time of hatching [17]. Nevertheless, Granger and Lazarides [8] and Gard and Lazarides [7] presented strong evidence that in the chicken an antigen with molecular weight identical to that of vimentin is found in adult muscle at the periphery of Z discs. Moreover, well-differentiated chick myotubes synthesize and acidic protein of molecular weight and isoelectric point identical to that of vimentin. It has been suggested that a protein with the same molecular weight as vimentin but with differing immunological properties and primary sequence exists in mature skeletal muscle and that anti-vimentin sera may cross-react to varying degrees with desmin, possibly explaining the disparate immunofluorescence observations made in different laboratories [16, 17]. However, in our studies we do not observe any reactivity in normal and pathological muscle cells using monoclonal antibodies against vimentin with the exception of the regenerating fibers and some of the denervated ones.

Our results suggest that vimentin and desmin may be expressed during muscle regeneration processes, probably because of their importance in the structural organization of the sarcomere, and are, therefore, a reliable marker of muscle fiber regeneration.

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