Immunofluorescent Localization of Vimentin and Desmin Intermediate Filaments in Regenerating Rat Skeletal Muscle

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Abstract

Immumofluorescence staining for vimentin and desmin was investigated in Bupivacaine - induced skeletal muscle regeneration in rat. The results indicate that desmin is required for intermediate filaments formation at the myoblast stage of development, whereas both desmin and vimentin are expressed in regenerating myotubes, and immature muscle fibers. Vimentin and desmin provide an useful method for evaluation of muscle fiber regeneration in experimental conditions.

Key words: skeletal muscle, rat, bupivacaine, regeneration, desmin, vimentin.

BAM 2 (1): 27-31, 1992

The existence of abundant, morphologically distinct intermediate filaments was first demonstrated in embryonic skeletal muscles by Ishikawa et al. in 1968 [10]. Their diameter of 8-12 nm is intermediate to those of actin filaments (6 nm) and myosin filaments (15 nm). Although quite homogeneous in morphology they are heterogeneous in biochemical and immunological criteria [12]. Intermediate filaments have been observed in many cell types of diverse origin including skeletal muscle [3, 10]. Their synthesis, composition and localization appear different in embryonic and mature skeletal muscle cell. The protein subunit of the filaments of premitotic myoblasts is vimentin, whereas both vimentin and desmin are present in postmitotic myoblasts and developing myotubes in culture [1]. When the muscle cell matures vimentin gradually disappeares [1, 15].

The protein subunit of the adult skeletal muscle is desmin [15], although some authors claim that vimentin is codistributed with desmin [6, 7]. Intermediate filaments have been shown to surround the myofibrils of skeletal muscle fibre at the level of the Z-line, linking myofibrils to the nucleus, the plasma membrane, the sarcotubular system and to each other [6, 7, 15]. Thornell in 1983 [14] first described increased synthesis of desmin in regenerating fibers in human myopathic muscle and Helliwel in 1988 [9] found increased desmin staining at an early stage of experimental regeneration in rat. Therefore, we studied vimentin and desmin distribution during Bupivacaine (BPVC) induced regeneration in rat skeletal muscle.

Materials and Methods

Sixteen male Wistar albino rats (body weight 250 g approximately) received intra-muscular injection of 0,6 ml of 0,75% BPVC into the right anterior tibial muscle.

Eight control animals were injected with 0,6 ml of 0,9% sodium chloride solution at the same site. Four BPVC injected and 2 control animals were killed by ether overdose 3, 5, 7 and 14 days after injection. The anterior tibial muscles were immediately removed and frozen in isopentane cooled in liquid nitrogen.

Eight µm cryostat sections were stained with hematoxylin and eosin to assess the overall morphological changes in the muscle. Five µm cryostat sections were stained by an indirect immunofluorescence technique using monoclonal antibodies against vimentin (DAKO-VIMENTIN, V9) and desmin (DAKO-DESMIN, D33). In brief, anti-vimentin (diluted 1:10 with phosphate buffer saline - PBS) and anti-desmin (diluted 1:100 with PBS) were applied to tissue sections for 30 min. Sections were then rinsed with PBS and incubated for another 30 min with appropriate fluorescein-conjugated secondary antibody (diluted 1:50 with PBS). After washing with PBS sections were mounted in glycerol and viewed with an Opton Zeiss standard Lab 16 microscope equipped with epifluorescence optics.

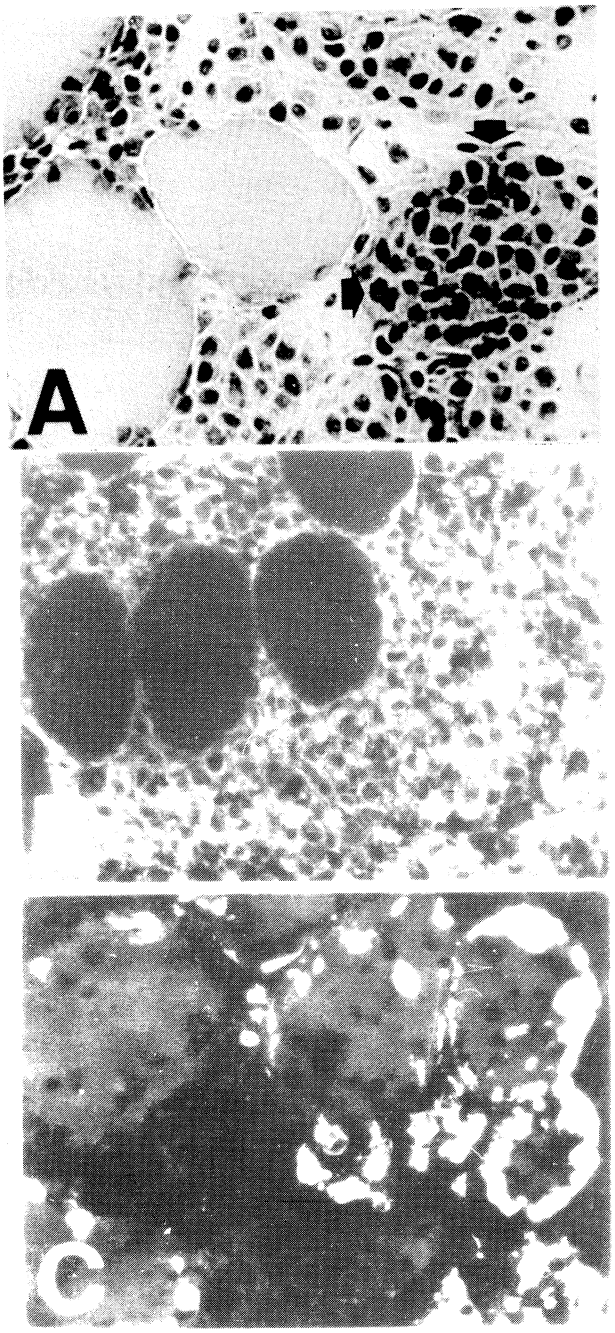

Figure 1. Three days after BPVC injection. A, Necrotic area filled with mononucleated cells, some of them with dark basophilic cytoplasm (arrows). HE x 500. B, Weak fluorescence in the area of mononucleated cells invading the necrotic zone. Anti-vimentin x 400. C, Myoblasts located peripherally under the BM of necrotic muscle fiber show strong cytoplasmic fluorescence. Anti-desmin x 400.

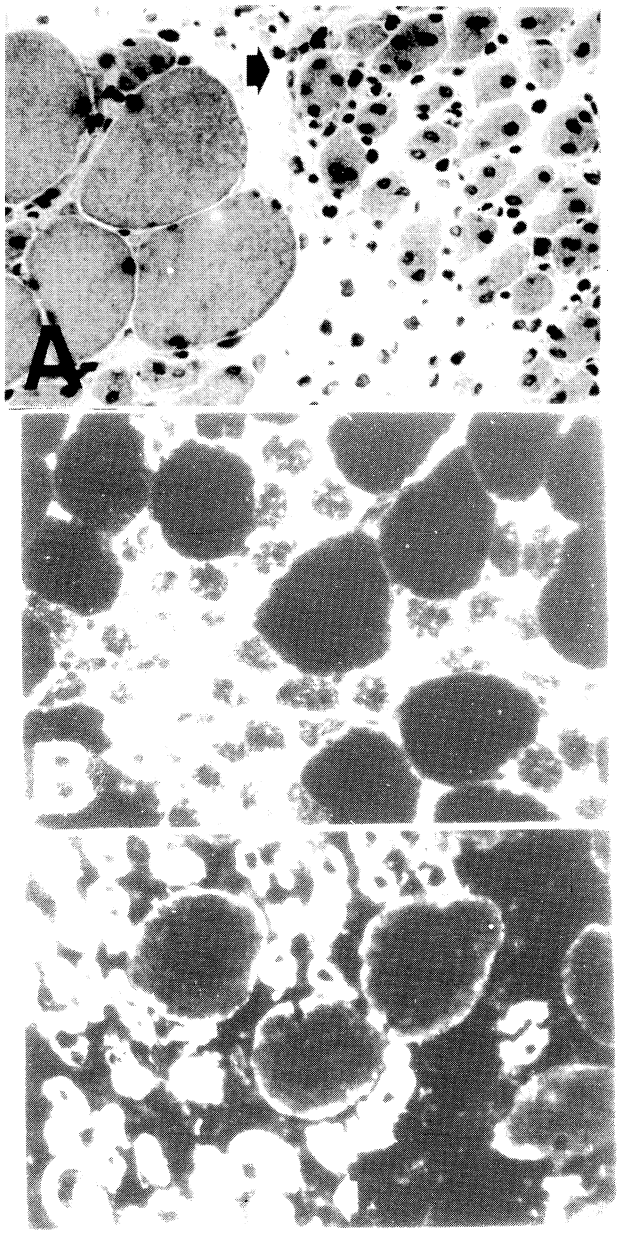

Figure 2. Five days after BPVC injection. A, Area of newly formed myotubes (arrows). HE x 500. B, Uneven weak cytoplasmic distribution and stronger extracellular fluorescence in the area of newly formed myotubes. Anti-vimentin x 400. C, Myotubes with strong cytoplasmic fluorescence (arrow). Anti-desmin x 400.

Controls consisted of sections incubated with nonimmune goat serum as the first incubating solution, neither of which yielded any fluorescence.

Results

Three days after BPVC injection

Numerous rounded clusters of macrophages and mononuclear basophilic cells were observed in the area of BPVC injection in HE staining (Fig. 1a) Weak disperse fluorescence in the area filled with macrophages was seen in vimentin staining (Fig. 1b). In desmin staining, macrophages were unstained, whereas strong cytoplasmic fluorescence was seen in numerous round and spindle-shaped cell, presumably myoblasts. These cells were often located at the periphery of the necrotic fibers (Fig. 1c).

Five days after BPVC injection

Areas of previous damage got filled with small myotubes with cytoplasmic basophilia and central often multiple nuclei (Fig. 2a). A weak fluorescence with uneven distribution in the sarcoplasm of the newly formed fibers was seen in vimentin staining (Fig. 2b). The intensity of staining was higher at the periphery of fibers. Desmin staining revealed multiple positive regenerating myotubes with strong cytoplasmic fluorescence and lack of the fluorescence in the center (Fig. 2c).

Seven days after BPVC injection

The new fibers enlarged and became less basophilic. Their nuclei were still centrally located (Fig. 3a). Positive diffuse cytoplasmic fluorescence of regenerating fibers was much weaker in vimentin (Fig. 3b) than in desmin (Fig. 3c). In vimentin staining bright fluorescence between regenerating fibers, probably related to the presence of blood vessels and fibroblasts was observed. Desmin fluorescence was confined to the muscle fibers, its distribution was uneven with accumulations in the center and under the sarcolemma.

Fourteen days after BPVC injection

Newly regenerating muscle fibers increased in size. Central location of many regenerating myofiber nuclei was still observed. Vimentin and desmin staining intensity decreased and was barely detectable.

Discussion

BPVC induced model of muscle fiber degeneration and subsequent regeneration has been widely used in studies of skeletal muscle regeneration. Regeneration is apparent within 3 days after BPVC injection and results in complete muscle recovery within 4 weeks.

Our morphological observations agree satisfactorily with the other light microscopic studies [2, 8]. In our experiments myoblasts were intensely labeled with desmin. Strong desmin fluorescence was also seen in the cytoplasm of myotubes. The intensity of desmin staining decreased as the myotubes matured. Vimentin failed to

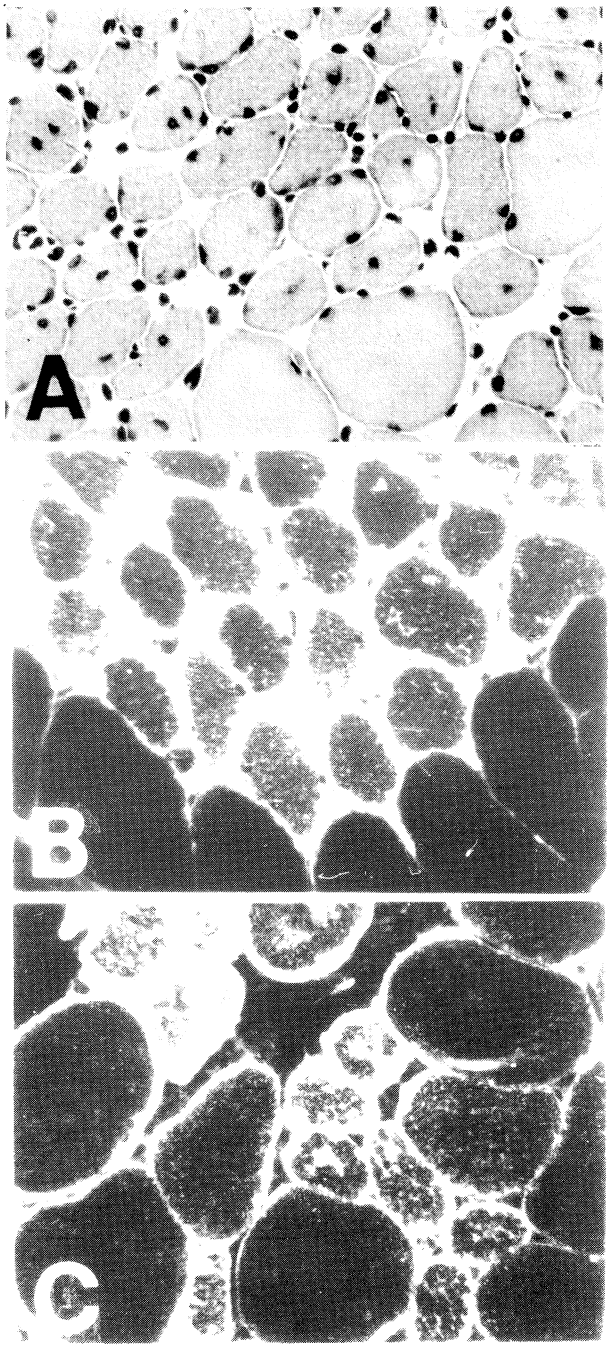

Figure 3. Seven days after BPVC injection. A. New young muscle fibers with central nuclei. HE x 400. B. Diffuse cytoplasmic fluorescence with accumulation under sarcolemma in immature muscle fibers. Weak fluorescence between fibers is observed. Anti-vimentin x 400 ℃. Diffuse cytoplasmic fluorescence with accumulation in the center and under sarcolemma. Note lack of fluorescence between fibers. Anti-desmin x 400.

identify myoblasts in BPVC treated rat muscle in our experiment. The presence of high density of desmin in myoblasts indicates that desmin not vimentin is synthetized by myoblasts and that desmin is required for intermediate filaments formation at this stage of development. Increased synthesis of vimentin was observed, however, in myotubes and immature muscle fibers.

It is known from the literature [1, 3, 6, 7, 10, 13] that both vimentin and desmin are present in the cells of myogenic origin. Vimentin has also been demontrated in a variety of mesenchymal cells including fibroblasts, endothelial cell and monocytes [4, 5, 12]. Some cells are known to contain two types of intermediate filaments. In all such cases studied vimentin is coexpressed with the cell-type-specific intermediate filaments. For example vimentin and desmin are reported to coexist in smooth vascular muscle [15] and skeletal muscle [6, 7]. Shifts in the expression of intermediate filaments in myoblastic cell lineage from vimentin to desmin are known to occur during embryonic development in vivo [13] as well as during the cell maturation in culture [1]. Benett et al. [1] studying myogenic cell in culture and Tokayasu et al. [13] studying developing chick muscle in vivo found that the distribution of vimentin and desmin was coincidental throughout the development of myotubes but vimentin was gradually reduced in amount and became undetectable. In contrast Lazarides [11] and Gard and Lazarides [6] reported the coexistance of the two proteins in developing myotubes in culture. To our knowledge vimentin has not been studied in experimental models of muscle disease. Although regeneration of muscle is thought to pass through the same sequential events as maturation of fetal muscle, our observations of vimentin expression during BPVC induced muscle fiber regeneration are not concordant with previous studies of vimentin synthesis during chick myogenesis [13].

Most authors [1, 6, 7, 13] describe high vimentin concentration in premitotic myoblasts and a gradual decrease with the maturation of myotubes.

In the present study, however, vimentin was not observed in myoblastic cells in the earlier stages of regeneration, contrary to desmin. Temporal relationship between appearance of vimentin and desmin in BPVC induced regeneration is also different from that previously observed by Benett et al. [1] in cultured muscle and Tokayasu et al. [13] in developing chick muscle in vivo. In our experiments vimentin and desmin are both expressed in regenerating myotubes. Our studies support the observations of Gard and Lazarides [6] who reported that, in developing myotubes in culture, the distribution of desmin and vimentin is similar.

Recently Helliwell [9] described increased desmin staining at an early stage of regeneration in BPVC (injected subcutaneously over the anterior tibial muscle) model. He applied immunohistochemical staining for desmin using an anti-desmin antibody known to react with rat desmin (from Dakopatts Ltd) and the peroxidase-antiperoxidase method and observed strong positive diffuse cytoplasmic staining most intense at days 3 and 4 and progressively decreasing up to normal within 7 days. Slight differences in time-course between his results and ours might be related to different site (subcutaneous versus intramuscular) of BPVC injection. He concluded that the desmin-positive cells, clustered within the basal lamina of necrotic fibers are probably activated satellite cells. In our observations however increased synthesis of desmin lasts longer and is seen up to 7 days, when regenerating fibers reach the stage of myotubes and immature muscle fibers.

Taking into account conflicting results from the literature on vimentin synthesis during muscle fiber development [1, 6, 11, 13] further detailed studies are required.

Acknowledgments

The authors are grateful to Professor Irena Hausmanowa-Petrusewicz for helpful discussions and advice.

This work was supported by grant from Polish Academy of Sciences No: 06.02.II-5.1.

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References

[1] Benett B, Fellini SA, Toyama Y, Holtzer H: Redistribution of intermediate filaments subunits during skeletal myogenesis and maturation in vitro. *J Cell Biol* 1979; 82: 577-584.

[2] Benoit PW and Belt WD: Destruction and regeneration of skeletal muscle after treatment with a local anaesthetic, bupivacaine (Marcaine). *J Anat* 1970; 107: 3, 547-556.

[3] Campbell GR, Chamley-Campbell J, Gröshel-Stewart U, Small JV, Anderson P: Antibody staining of 10 nm (100 A) filaments in cultured smooth, cardiac and skeletal muscle. *J Cell Sci* 1970; 37: 303-322.

[4] Franke WW, Schmid E, Osborn M, Weber K: Different intermediate sized filaments distinguished by immunofluorescence microscopy. *Proc Natl Acad Sci USA* 1978; 75: 5034-5038.

[5] Franke WW, Schmid E, Osborn M, Weber K: Intermediate sized filaments of human endothelial cells. *J Cell Biol* 1979; 81: 570-580.

[6] Gard DL and Lazarides E: The synthesis and distribution of desmin and vimentin during myogenesis in vitro. *Cell* 1980; 19: 263-275.

[7] Granger BL and Lazarides E: Desmin and vimentin coexist at the periphery of the myofibril. *Cell* 1979; 18: 1053-1063.

[8] Hall-Craggs ECB: Rapid degeneration and regeneration of a whole skeletal muscle following

treatment with bupivacaine (Marcaine). *Exp Neurol* 1974; 43: 349-358.

[9] Helliwell TR: Lectin binding and desmin staining during Bupivacaine - induced necrosis and regeneration in rat skeletal muscle. *J Pathol* 1988; 155: 317-326.

[10] Ishikawa H, Bischoff R, Holtzer H: Mitosis and intermediate-sized filaments in developing skeletal muscle. *J Cell Biol* 1968; 38: 538-558.

[11] Lazarides E: Intermediate filaments - mechanical integrators of cellular space. *Nature* 1980; 249-256.

[12] Lehte V-P, Virtanen I, Kurki P: Intermediate filaments anchor the nuclei in nuclear monolayers in cultured human fibroblasts. *Nature* 1978; 272: 175-177.

[13] Tokayasu KT, Naher PA, Singer SJ: Distribution of vimentin and desmin in developing chick myotubes in vivo. I. Immunofluorescence study. *J Cell Biol* 1984; 98: 1961-1972.

[14] Thornell LE, Eriksson A, Eström L: Intermediate filaments in human myopathies, in Dowben RM and Skay JW (eds.): *Cell and Muscle Motility.* Plenum Publishing Corporation, 1983; pp 85-136.

[15] Osborn M, Caselitz J, Weber K: Heterogeneity of intermediate filament expression in vascular smooth muscle. A gradient in desmin positive cells from the rat aortic arch to the level of the arteria iliaca communis. *Differentiation* 1981; 20: 196-202.