MYOFIBROBLASTS IN A ZONE OF MYOCARDIAL INFARCTION

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UDC 616.127-005.8-018.63

KEY WORDS: myocardial infarction; myofibroblasts; ultrastructure; immunomorphology.

The term "myofibroblasts" is applied to cells which combine the morphological and functional features of fibroblasts and of smooth-muscle cells. Myofibroblasts are found in granulation tissue [5, 6], the capsule of the kidney [9], regenerating tendon [11], damaged cartilage [7], in cirrhosis of the liver [8], in atherosclerotic plaques [4], and in carcinoma of the breast [10]. The study of myofibroblasts in the zone of the myocardial infarction is of great interest because the problem of the myogenic cells in this case have not yet been solved. A previous investigation using immunomorphologic methods [1] showed that in the region of an infarct cells resembling myoblasts did not contain striated muscle myosin. The nature of these cells has not yet been explained. The present investigation was devoted to a further study of these cells.

EXPERIMENTAL METHOD

A myocardial infarct was produced in 43 noninbred male albino rats weighing 300-350 g by ligation of branches of the left coronary artery. The operation was performed under ether anesthesia. Three intact rats served as the control. The animals were killed by decapitation under ether anesthesia 2, 3, 4, 5, 6, 7, 9, 11, 30, 45, 60, and 120 days after the beginning of the experiment. Frozen sections through the rat heart were fixed in 96% ethanol for 10 min and incubated with rabbit antisera against smooth- and striated-muscle myosin. The method of obtaining the antisera and their immunomorphologic characteristics were described previously [2]. After washing in buffered physiological saline the sections were incubated with goat antibodies against rabbit IgG labeled with fluorescein isothiocyanate. Some frozen sections were counterstained with hematoxylin-eosin and picrofuchsin. Material for electron-microscopic investigation was treated in the usual manner.

EXPERIMENTAL RESULTS

On treatment of the heart sections with antiserum against striated-muscle myosin intensive luminescence of the myocardial muscle fibers was observed. The boundaries of the focus of the infarct and individual cardiomyocytes preserved in the focus of injury were clearly outlined.

A weak reaction for smooth-muscle myosin was detected immunomorphologically 3 days after the operation in some elongated cells in the zone of the infarct. Electron-microscopic investigation revealed activated fibroblasts, lying singly or in small groups with a well-developed rough endoplasmic reticulum, lamellar complex, and many ribosomes and polysomes. Often in the peripheral regions of the cytoplasm of the fibroblasts there were small concentrations of myofibrils, oriented along the long axis of the cell (Fig. 1a, b). Dense bodies also were found (Fig. 1b). These cells according to data in the literature [3, 6-11], can be classed as myofibroblasts, although at this stage of the investigation they still showed predominantly the characteristic structural features of fibroblasts, and the ultrastructural features of the smooth-muscle cells were not so clearly expressed. In particular, by contrast with the smooth-muscle cell, they had no continuous basement membrane. Only in a few places, bordering on bundles of myofilaments, were segments of the newly formed base-

Laboratory of Immunomorphology, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Kraevskii.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 92, No. 12, pp. 740-743, December, 1981. Original article submitted May 15, 1981.

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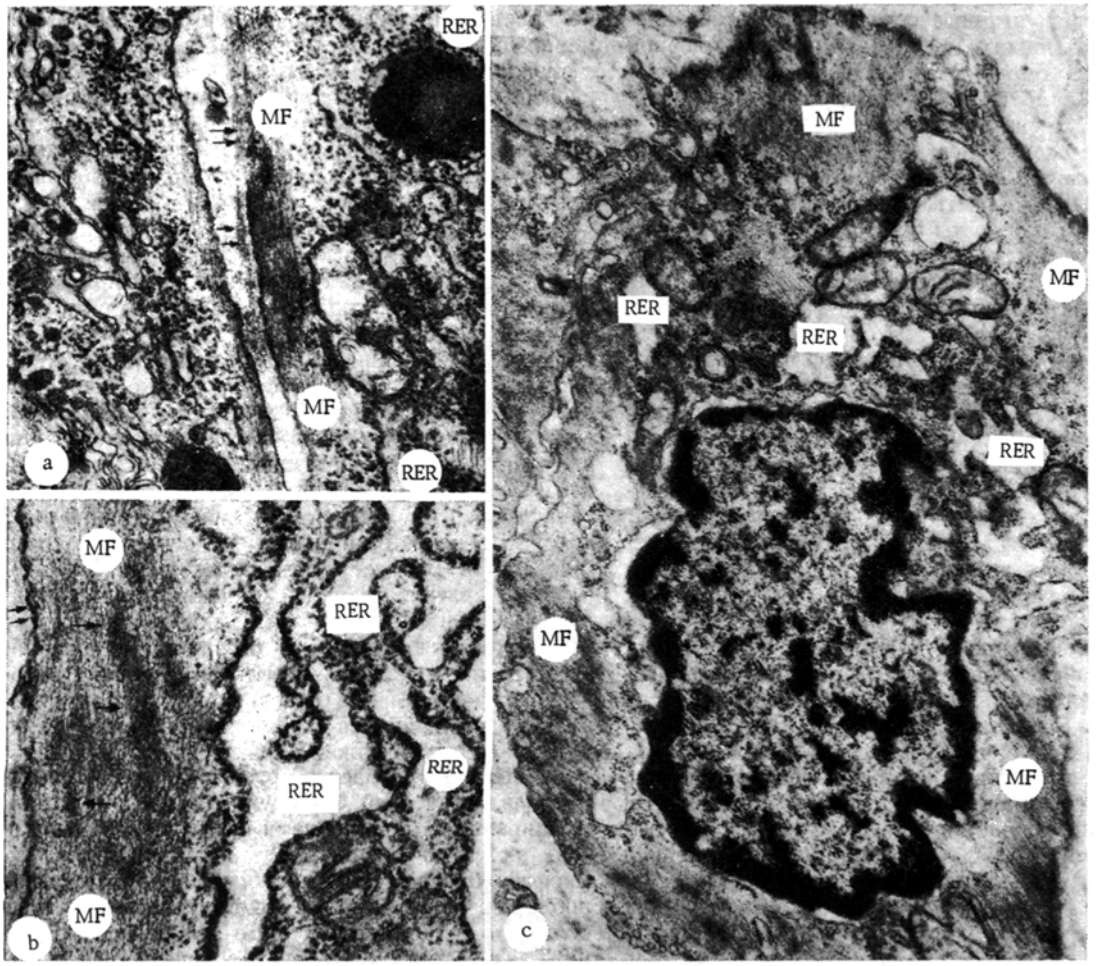

Fig. 1. Ultrastructure of myofibroblasts in zone of postinfarct scar. a) Myofibroblasts 3 days after myocardial infarction, myofilaments only in peripheral part of cytoplasm, fragmented basement membrane, 40,000 ×; b) myofibroblasts 5 days after infarction, well-developed rough endoplasmic reticulum, accumulation of myofilaments at periphery of cell cytoplasm, electron-dense bodies, 50,000 ×; c) myofibroblast 11 days after infarction, much of its cytoplasm occupied by myofilaments, dilated cisterns of rough endoplasmic reticulum in perinuclear zone of cytoplasm, 30,000 ×. MF) Myofilaments; RER) rough endoplasmic reticulum; one arrow — dense bodies; two arrows — basement membrane.

ment membrane visible (Fig. 1a). These cells had no developed contractile apparatus and no evidence of active micropinocytosis, which are so marked in smooth-muscle cells.

On luminescence microscopy 7 and in particular, 11 days after the operation bright luminescence was found in some cells of the postinfarct scar after treatment of the sections with antiserum against smooth-muscle myosin, evidence that these cells had a high myosin content (Fig. 2a). Accumulations of brightly luminescent cells, lying close together, were seen (Fig. 2b). Electron microscopy at this time showed cells with a well-developed contractile apparatus. A wide band around the periphery of these cells was occupied by myofilaments, with many dense bodies in them (Fig. 1c). In the more central areas of cytoplasm there were accumulations of intermediate filaments and polysomes. These cells could be completely surrounded by a basement membrane, but the basement membrane of some cells was fragmented. In certain areas of the cytoplasm micropinocytotic vesicles could be seen. The cells contained a well-developed rough endoplasmic reticulum, characteristic of activated fibroblasts, and consisting of dilated, irregularly shaped cisterns (Fig. 1c). These cells can also be classed as myofibroblasts, but unlike the myofibroblasts in the earlier stages of the investigation, they had the ultrastructural features characteristic of smooth-muscle cells. Evidence of their origin from fibroblasts is given by the incomplete development of

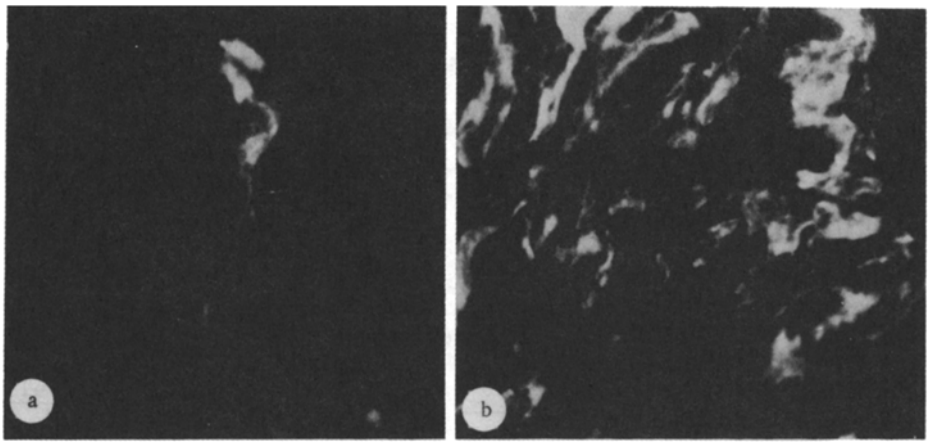

Fig. 2. Detection of smooth-muscle myosin in myofibroblasts by immunofluorescence: a) 7 days after myocardial infarction, singly lying cell giving reaction for myosin; b) cluster of cells reacting to antiserum against smooth-muscle myosin, 1100 ×.

the basement membrane and the presence of intermediate forms of cells, in which the features of the smooth-muscle cell gradually became more pronounced.

Myofibroblasts were more numerous in the postinfarct scar 30 and 45 days after the operation. Structural features of smooth-muscle cells predominated in them. After 120 days, no smooth-muscle myosin could be found in the cells of the scar on immunomorphologic investigation. Electron-microscopic investigation at this time revealed myofibroblasts with very small peripheral concentrations of myofilaments. Fibroblasts with a relatively well-developed rough endoplasmic reticulum, not containing concentrations of myofilaments, were the predominant cell forms.

According to one view, myofibroblasts may arise from fibroblasts [3, 7-9, 11]. It is also asserted that smooth-muscle cells can change into myofibroblasts and, through them, into fibroblasts [9]. The results of the present investigation support the hypothesis that fibroblasts can convert into myofibroblasts. However, strict proof of this hypothesis has not yet been obtained. The results also suggest that cells in the postinfarct zone of the myocardium, which have been taken for myoblasts, may in fact by myofibroblasts. The functions of myofibroblasts have not been adequately explained. Their contractility is the best known [5, 6, 8]. They also produce collagen and elastin [12]. There is evidence that myofibroblasts may play the role of fibroblasts [3]. All these three aspects of myofibroblast function are evidently manifested in the postinfarct scar, and this is a matter of great importance for restoration of normal cardiac activity after infarction.

LITERATURE CITED

1. V. S. Rukosuev, Kardiologiya, No. 9, 106 (1969).
2. B. V. Shekhonin, I. S. Alkadarskii, V. S. Rukosuev, et al., Byull. Éksp. Biol. Med., No. 8, 233 (1981).
3. P. Buntrock, Zbl. Allg. Pathol., 124, 48 (1980).
4. F. Flora, E. Dahl, and E. Nelson, Arch. Neurol., 17, 162 (1967).
5. G. Gabbiani and G. Majno, Am. J. Pathol., 66, 131 (1972).
6. G. Gabbiani, M. Le Lous, A. J. Bailey, et al., Arch. Pathol. Anat. Abt. B, Zellpathol., 21, 133 (1976).
7. F. N. Ghadially, J. M. A. Lalonde, and N. K. Yong, J. Submicrosc. Cytol., 12, 447 (1980).
8. C. Irle, O. Kocher, and G. Gabbiani, J. Submicrosc. Cytol., 12, 209 (1980).
9. K. Kobayashi, Cell Tissue Res., 195, 381 (1978).
10. H. Ohtani and N. Sasano, Arch. Pathol. Anat. Abt. A, 385, 247 (1980).
11. F. Postacchini, L. Accini, P. G. Natali, et al., Cell. Tissue Res., 195, 81 (1978).
12. R. Ross, J. Cell Biol., 32, 155 (1967).