CELLULAR POLYMORPHISM IN THE MYOINTIMAL THICKENING OF THE RABBIT AORTA

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The subendothelial intima of the adult human aorta is recognized by typical and modified smooth-muscle cells, and also by blood-borne cells [2, 5]. Among the intimal cells, stellate cells are sharply distinguished by their shape [3, 7]. It was shown previously that the number of stellate cells is increased in a zone of atherosclerosis [3]. The origin and role of these cells remain unclear. No attempt has been made to model and investigate the principles governing cellular polymorphism by means of experiments on animals. Damage to the endothelium of arteries by a balloon catheter, leading to the formation of myointimal thickening (MIT), is widely used to study the pathogenesis of atherosclerosis [1, 6].

The aim of this investigation was to study the shape of cells in MIT of the rabbit aorta.

EXPERIMENTAL METHOD

Chinchilla rabbits weighing 2-3 kg were kept under ordinary animal house conditions on a standard diet. By means of a balloon catheter, the abdominal aorta of seven rabbits was de-endothelized under pentobarbital anesthesia (40 mg/kg). The animals were killed 21 days after the operation. One hour previously, a solution of Evans' blue was injected intravenously in a dose of 1 ml/kg (20 mg/ml) to mark out the zone of damage. Next, under pentobarbital anesthesia the iliac artery was washed out with medium 199 and heparin for 1 min under a pressure of 100 mm Hg, and then fixed under the same pressure in 2.5% glutaraldehyde solution, made up in medium 199, for 5 min. The segment of the aorta containing the "blue" zone was excised and subjected to immersion fixation in glutaraldehyde for not less than 24 h. The thickened area of the intima of the aorta was mechanically separated from the media. Small pieces of each layer were incubated in a mixture of 30% KOH and 96% ethanol (1:1) at a temperature of 37°C, and carefully shaken. After 1.5-2 h the tissue fragments were completely dissolved, and the cell suspension studied in the phase contrast microscope. To study the shape of the cells in situ, film preparations were obtained by the method in [8] in our own modification. Unlike in the original method, we obtained serial film preparations. Strips of aorta were dehydrated in a series of alcohols of increasing concentration. The dehydrated specimens were immersed for a few seconds in a 20% solution of celloidin, then placed on slides, previously covered with celloidin (with the endothelial side toward the glass). After the celloidin had solidified the fragments were separated from the glass with forceps. As a result, a celloidin film with a cell monolayer adherent to it remained on the slide. The remaining fragment of intima was again immersed in celloidin and the procedure was repeated once again. A further repetition of the procedure enabled the next serial film to be obtained. The film preparations were stained with hematoxylin. For scanning electron microscopy the specimens were postfixed in 1% OsO_4 solution for 1 h, dehydrated, dried by the critical point method, sprayed with platinum, and examined in the S-570 microscope ("Hitachi," Japan). Semithin sections and ultrathin sections for transmission electron microscopy were obtained by the usual method.

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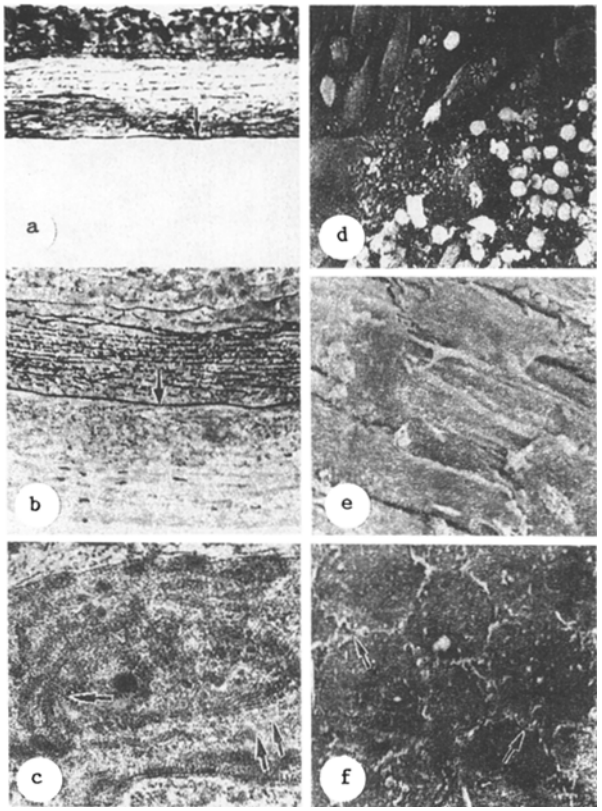

Fig. 1. Structure of MIT of rabbit aorta: a) intact aorta (control). Arrow indicates inner elastic membrane. Semithin section. 90×; b-f) MIT: b) nonendothelized zone of MIT. Arrow indicates inner elastic membrane, star indicates MIT. Semithin section, 90×; c) smooth-muscle cell of synthetic phenotype. Well developed rough endoplasmic reticulum (arrow) and Golgi apparatus (double arrow). Transmission electron microscopy, 15,000×; d) boundary of endothelial layer (star), adhesion of leukocytes (arrow) to surface of smooth-muscle cells. Scanning electron microscopy, 500×; e) nonendothelized surface of MIT. Elongated smooth-muscle cells with even surface. Scanning electron microscopy, 2500×; f) nonendothelized surface of MIT. Flat polygonal cells with high concentration of microvilli (arrows) in region of junctions. Scanning electron microscopy, 2000×.

EXPERIMENTAL RESULTS

Under normal conditions the intima of the rabbit aorta consists only of endothelium, the inner elastic membrane, and a thin connective-tissue layer between them (Fig. 1a). A neointima was formed 3 weeks after de-endothelization (Fig. 1b). It consisted of modified smooth-muscle cells, in whose cytoplasm the contractile apparatus was reduced, and a rough endoplasmic reticulum, Golgi complex, and mitochondria predominated (Fig. 1c). The endothelium, in the form of pannus, spread from the "wound" edges for a distance of 3-5 mm, after which re-endothelization ceased. As a result, a large part of the defect facing the lumen of the aorta was lined with smooth myocytes. The central part of MIT, deprived of endothelium, stained with Evans' blue, and accordingly it could be identified macroscopically.

The endothelium at the edges of the defect consisted of a continuous monolayer of spindle-shaped cells, stretched along the axis of the vessel. Actually on the boundary of the "white" zone the endotheliocytes became irregularly polygonal in shape. The surface of the "blue" zone appeared polymorphic. Precisely at the site of contact with the endothelium, the cells were arranged chaotically. Wide spaces were found between them. These cells were covered by numerous microvilli.

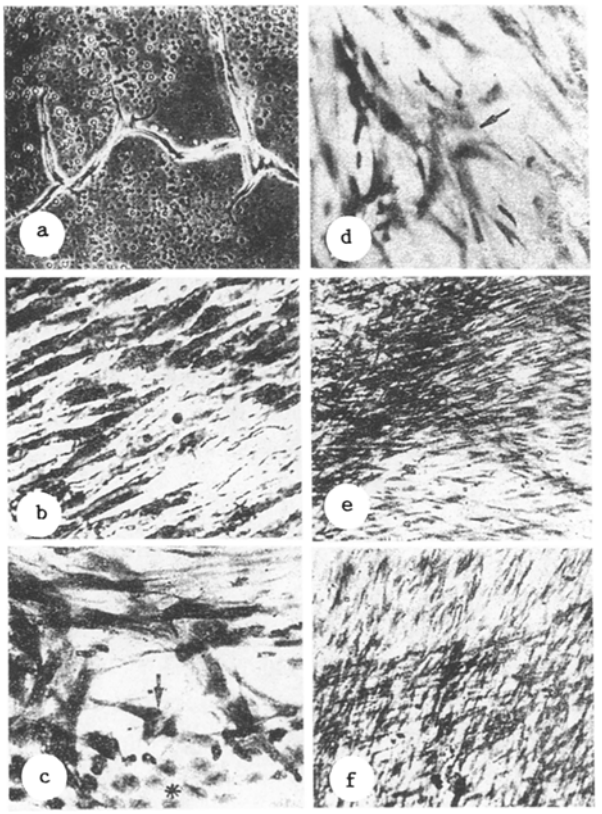

Fig. 2. Shape of cells in MIT of rabbit aorta. a) Stellate cells, isolated by alcoholic-alkaline dissociation. Phase-contrast microscopy, 400×; b-f) film preparations, Weigert's hematoxylin: b) spindle-shaped cells in central part of MIT. Surface film. 400×; c) Cells with outgrowths (arrows), in contact with endothelial cells (star) in horizontal plane. Surface film, 400×; d) stellate cell (arrow), located beneath endothelium. Second film. 400×; e) Spindle-shaped cells in deep layers of nonendothelized part of MIT. Second film. 70×; f) Spindle-shaped cells in deep layers of endothelized part of MIT. Third film. 70×.

TABLE 1. Ratio between Cells of Different Shapes in Myointimal Thickening and Media of Rabbit Aorta (in %, $\bar{X} \pm \bar{x}$)

Layer	Stellate cells	Irregularly shaped cells with outgrowths	Y-shaped cells	Spindle-shaped cells
Myointimal thickening	4,8±0,2	2,2±0,4	7,5±0,6	85,8±1,2
Media	0±0	0±0	6,4±0,5	93,6±0,5

In the region of the intercellular spaces many leukocytes were adherent (Fig. 1d). Nearer to the center of the "blue" zone the cells were more often spindle-shaped in appearance, more densely packed, and arranged at an angle to the axis of aorta. Their surface became more even (Fig. 1e). In some areas, patterned polygonal cells with a high concentration of microvilli in the region of the junctions faced the lumen of the aorta (Fig. 1f).

Cells of MIT in suspension differed in shape. Spindle-shaped, Y-shaped, and irregularly shaped cells with outgrowths, and stellate cells could be distinguished (Fig. 2a). The relative numbers of cells of different shapes are given in Table 1. Cells of the media beneath MIT were spindle-shaped or Y-shaped (Table 1).

Analysis of the film preparations layer by layer revealed the following general rule. Cells in the center of the thickening, irrespective of the level at which they were situated, were spindle-shaped (Fig 2d). In the very first film, containing the cell layer facing the lumen of the aorta, Y-shaped cells appeared nearer to the periphery of the "blue" zone, with stellate cells on the boundary with the endothelium (Fig. 2c). On the 2nd film, containing the next layer of cells of the neointima, stellate cells were found only in the zone located under the endothelium (Fig. 2d). All film preparations obtained from deeper layers of the neointima contained predominantly spindle-shaped cells (Fig. 2e, f).

Thus, in the neointima of the rabbit aorta formed after de-endothelization, stellate cells were found and were located in the immediate vicinity of the endothelium. Stellate cells were considered to belong solely to the intima of human arteries [3, 7]. In the present investigation we found stellate cells in MIT of the rabbit aorta, formed as a result of de-endothelization.

In the present experiments, just as in those of Reidy [4], MIT was only partially endothelized. Consequently, with the aid of this model it is possible to study the morphology of cells of MIT depending on their location relative to the endothelium, in both horizontal and vertical planes. Analysis of serial film preparations showed that stellate cells as a rule are located in the immediate vicinity of the endothelium. The possibility therefore cannot be ruled out that it is the endothelium which influences the shape of cells of MIT.

LITERATURE CITED

1. G. R. Campbell, J. H. Campbell, J. A. Manderson, et al., Arch. Pathol. Lab. Med., **112**, No. 10, 977 (1988).
2. E. E. Emeson and A. L. Robertson, Am. J. Pathol., **130**, No. 2, 369 (1988).
3. A. N. Orekhov, E. R. Andreeva, and V. V. Tertov, Soc. Med. Rev. A. Cardiol., **1**, 75 (1987).
4. M. A. Reidy, Lab. Invest., **59**, No. 1, 36 (1988).
5. R. Ross, T. M. Wight, E. Strandness, and B. Thiele, Am. J. Pathol., **114**, 79 (1984).
6. R. Ross, New Engl. J. Med., **314**, 488 (1986).
7. M. Schonfelder, Pathol. Microbiol., **33**, 129 (1969).
8. S. M. Schwartz and E. P. Benditt, Circulat. Res., **41**, No. 2, 248 (1977).