ROLE OF SMOOTH-MUSCLE CELLS IN THE FORMATION OF THE ATHEROSCLEROTIC PLAQUE

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The role of smooth muscle cells in the formation of atherosclerotic plaques was studied by the fluorescent antibody method on autopsy material with the aid of antiserum against smooth-muscle actomyosin. The principal forms of atherosclerotic lesion (lipid stain, fibrous plaque, atheromatous plaque) were investigated in the aorta, the brain vessels, and the coronary arteries. Smooth-muscle cells were found in the intima along with atherosclerotic foci, in the lipid stain and fibrous tissue of the plaque, but not in the atheromatous masses. Proliferation and migration of smooth-muscle cells are regarded as an essential factor in the morphogenesis of atherosclerosis.

KEY WORDS: atherosclerotic plaque; smooth-muscle cells, myosin; Coons' method.

Investigations of atherosclerosis carried out mainly on experimental material have shown the leading role of smooth-muscle cells in the morphogenesis of the atherosclerotic plaque [6, 7, 14, 20]. However, only three papers on the discovery of smooth-muscle cells in the atherosclerotic plaque by the fluorescent antibody method could be found in the literature [4, 10, 11]. Moreover, the data described by Knieriem et al. [10, 11] are open to question because these workers did not take into account differences in the antigenic composition of actomyosin from smooth and striated muscles.

The object of the present investigation was to study the role of smooth muscle cells in the formation of atherosclerotic lesions by Coons' method, using specific antiserum against smooth muscle actomyosin.

EXPERIMENTAL METHOD

Autopsy material (obtained from the cadavers of eight persons with atherosclerosis) was used. Various types of atherosclerotic lesions (lipid stains, fibrous and atheromatous plaques) were investigated in the aorta, the brain vessels, and the coronary arteries. Sections were cut in a cryostat and fixed for 7 min in 96% ethanol. Smooth-muscle cells were identified by Coons' indirect method using antiserum against smooth-muscle actomyosin [2] and labeled antibodies against rabbit γ-globulin. Serial control sections for morphological investigation were stained with hematoxylin–eosin and Sudan III.

EXPERIMENTAL RESULTS

Investigation of the aorta revealed numerous smooth-muscle cells between the elastic membranes of the media (Fig. 1). In areas of the intima, a few smooth-muscle cells, smaller than in the media, could also be seen side by side with atherosclerotic foci.

Examination of the lipid stains in the aorta always revealed bright specific fluorescence of the small smooth-muscle cells contained in them (Fig. 2a). The number of smooth-muscle cells in the fibrous plaques in the aorta varied, and small elongated smooth-muscle cells were distributed parallel to the lumen of the aorta among the fibrous elements (Fig. 2b). Specific fluorescence of smooth-muscle cells was absent in the interior of the plaques among the atheromatous masses. Smooth-muscle cells were detected only in the

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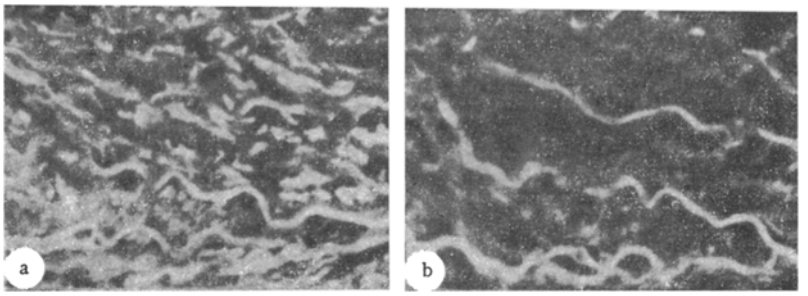

Fig. 1. Smooth-muscle cells in media of the aorta: a) specific fluorescence of elongated smooth-muscle cells and autofluorescence of elastic membranes; b) serial control section: autofluorescence of elastic membrane. Here and in Figs. 2 and 3: treatment with antiserum against smooth-muscle actomyosin by Coons' indirect method. In the control, antiserum absorbed beforehand with actomyosin (120x).

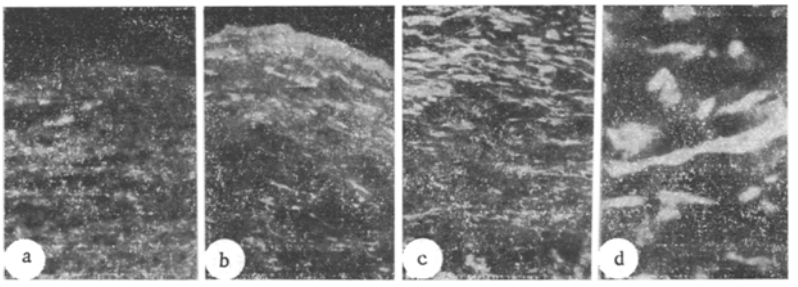

Fig. 2. Smooth-muscle cells in atherosclerotic lesion of the human aorta: a) specific fluorescence of smooth-muscle cells in lipid stain (120 ×); b) small, elongated smooth-muscle cells in fibrous plaque (120x); c) smooth-muscle cells in surface layer of plaque (120x); d) cells of smooth-muscle origin in surface layer of atherosclerotic plaque (270 x).

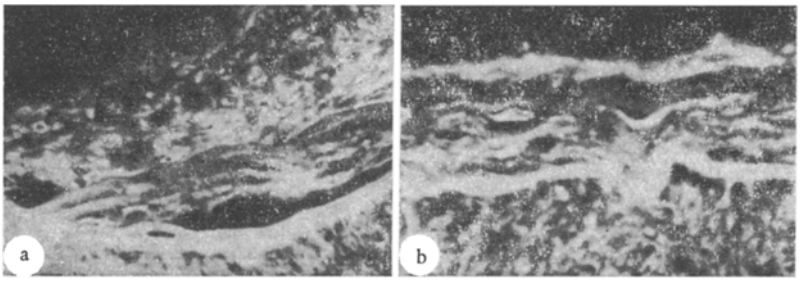

Fig. 3. Smooth-muscle cells in atherosclerotic foci in cerebral vessels: a) middle cerebral artery – numerous smooth-muscle cells at border of plaque (240x); b) smooth-muscle cells in thickened intima of middle cerebral artery (240x).

surface layer and base of the plaque (Fig. 2c, d). In both fibrous and atheromatous plaques in the aorta particularly large collections of smooth-muscle cells could be seen at the edges of the plaque.

Investigation of the coronary and cerebral arteries also showed that smooth-muscle cells constantly participate in the formation of atherosclerotic lesions of the vessel wall. Smooth-muscle cells were invariable morphological components of atherosclerotic plaques and lipid stains in the coronary arteries and large branches of the internal carotid artery. Just as in the aorta, no smooth-muscle cells could be seen in the atheromatous plaques, but they were found in lipid stains, in their fibrous tissue of plaques of varied structure, and, in particular, they were very numerous in the intima at the edges of the plaque (Fig. 3a).

Elongated smooth-muscle cells were constantly visible in the thickened intima of the coronary arteries and arteries at the base of the skull (Fig. 3b).

Cells of smooth-muscle origin were thus found in all forms of atherosclerotic lesion of the blood-vessel walls investigated: in lipid stains and in fibrous and atheromatous plaques. The following points for discussion arise in the analysis of the data: 1) the possible role of smooth-muscle cells in the formation of the atherosclerotic plaque, 2) the sources of cells of smooth-muscle origin appearing in the plaque, and 3) the factors causing proliferation of the smooth-muscle cells.

Many workers have stated that in atherosclerotic foci not only macrophages of hematogenous origin, but also smooth-muscle cells can accumulate lipids [14, 18, 19]. Some workers consider that in atherosclerosis the synthesis of lipids by smooth-muscle cells of the vessel wall is intensified [7]. The hypothetical ability of smooth-muscle cells in atherosclerotic plaques to synthesize the fibrous elements of the connective tissue was postulated originally on the basis of investigations with the light microscope [19]. Direct proof of the formation of elastic and collagen fibers by smooth-muscle cells has been obtained in tissue culture [15, 16]. So-called modified smooth-muscle cells with all the features of a well-developed synthetic function appear in the intima in atherosclerosis.

Some workers consider that smooth-muscle cells in the atherosclerotic plaque develop from precursor cells brought by the blood stream [9]; endothelial cells, it is suggested, can be modified into smooth-muscle cells [12]. However, the most widely held view is that cells with the characteristics of smooth muscle migrate into the intima from the media in a wide range of pathological states, including atherosclerosis [1, 3, 14, 17].

Some workers consider that smooth-muscle cells are the only type of functionally active cells in the media of blood vessels [6]. Proliferation of the smooth-muscle cells of the media and their migration into the intima are possibly the stereotyped response of the vessel wall to injury. Meanwhile injury to the intima is evidently the most important pathogenetic factor in the mechanisms of formation of atherosclerotic lesions [5, 8, 13, 17]. It has been suggested that the stimulus to proliferation and migration of the smooth-muscle cells may be the action of a hypothetical blood factor [17, 18], lipoproteins [17, 20], or components of the thrombus and, in particular, platelets [9, 18].

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