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POLYMORPHISM OF SMOOTH-MUSCLE CELLS OF AN ATHEROMATOUS PLAQUE OF THE HUMAN AORTA

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Some investigators consider [7] that the smooth-muscle cells (SMC) of blood vessels play the key role in the pathogenesis of atherosclerosis. The overwhelming majority of intimal cells of human blood vessels are known to be SMC [11]. During the development of atherosclerosis, the relative numbers and structure of the cells change [9]. The SMC actively produce collagen, which leads to the formation of a vascular fibrous plaque [4]. It has been suggested [5] that two phenotypes of SMC exists in the wall of a blood vessel affected by atherosclerosis, one consisting of cells with an abundance of myofilaments, the other of cells transformed from the usual "contractile" state into a "synthetic" state [7]. Indirect evidence in support of this possibility is given by the increase in the proportion of altered or "modified" SMC in the human atherosclerotic plaque [14]. The cytoplasm of such cells contains few myofilaments and is packed with endoplasmic reticulum and a lamellar apparatus, evidence of active synthesis and secretion of protein [9, 14]. Meanwhile the absence of definite morphological criteria or markers of the modified SMC makes it difficult to study these cells and to determine their precise localization.

In this paper we described the results of analysis of expression of a number of cytoskeletal proteins in cells of an atheromatous plaque of the human aorta.

EXPERIMENTAL METHOD

A double immunofluorescence method, enabling the localization of two proteins to be studied in the same cells, was used. Autopsy material was obtained from 12 persons dying at the age of 28-72 years as a result of trauma, and was used 3-6 h after death. Unchanged segments of the vessel and atheromatous plaques, with a finely granular amorphous mass in their central part, were excised from the thoracic aorta. Frozen sections of the vessel 5 μ thick were cut and kept at −20°C for not more than 1 week. Antiserum to smooth-muscle myosin [3], monoclonal antibodies to vimentin [15] and desmin [6], and also monoclonal antibodies to the surface of human SMC, not reacting with macrophages and endothelial cells, but specifically bound with SMC and fibroblasts in culture [10], and with SMC and pericytes from human tissue sections, were used in the investigation. The sections were immersed for 5 min in phosphate-buffered saline (PBS), containing 10 mM phosphate buffer (pH 7.4) and 2 mg/ml bovine serum albumin, and subsequently incubated with monoclonal antibodies to vimentin, desmin, or SMC (diluted 1:20, 1:100, and 1:50 with PBS respectively), and then with antiserum to myosin (dilution 1:20) for 1 h at 25°C. Antibodies bound with the tissue were revealed by subsequent

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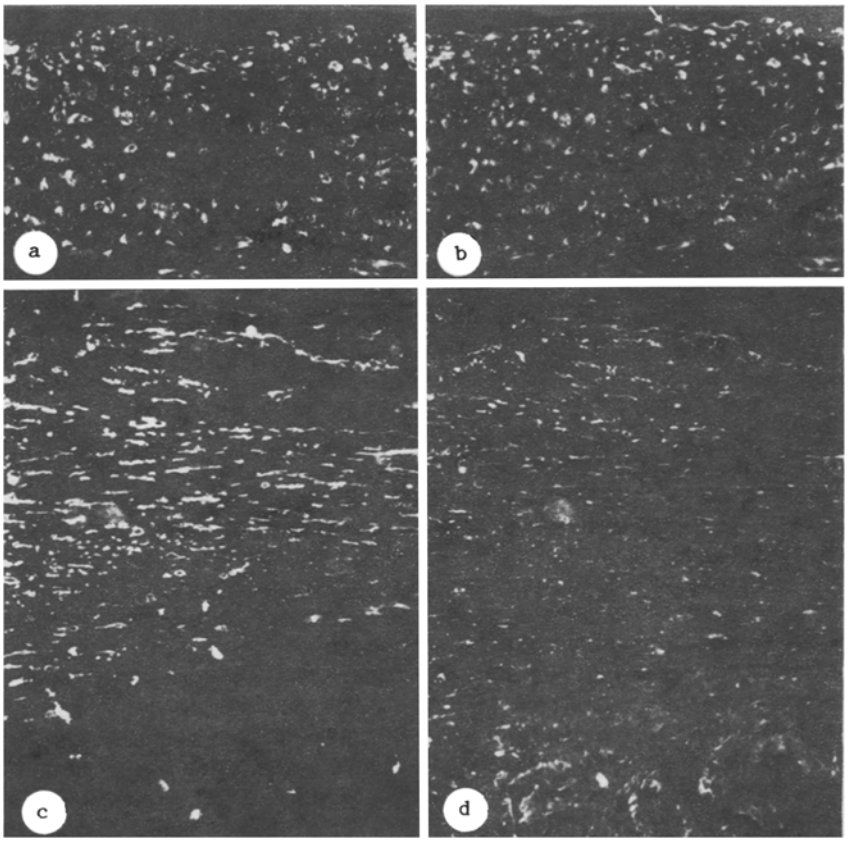

Fig. 1. Demonstration of myosin (a, c) and vimentin (b, d) in visually unchanged part of vessel (a, b) and in atheromatous plaque (c, d) of human aorta. Transverse (a, b) and longitudinal sections through aortic wall. Lumen of vessel above. Residual endothelium (arrow) stains for vimentin (b), but not for myosin (a). Double immunofluorescence method. a, b) 200 ×; c, d) 125 ×.

treatment of the sections with goat antibodies to light and heavy chains of mouse IgG, labeled with fluorescein isothiocyanate, and with sheep's antibodies to rabbit IgG, labeled with tetramethyl isothiocyanate. The concentration of labeled antibodies was 0.05-0.01 mg/ml PBS. In experiments to verify the specificity of staining, the stage of treatment with antibodies or antiserum was omitted or IgG of unimmunized animals was used. The preparations were studied under an Opton microscope through a standard set of filters for fluorescein and rhodamine fluorescence. After recording of the staining, the sections were fixed in formalin in PBS and nonspecific esterase by staining with Mayer's hematoxylin or with Oil Red O.

EXPERIMENTAL RESULTS

In all sections of the aorta without exception cells containing smooth-muscle myosin were identified in the intima and media of the vessel, and these were considered to be SMC. In the visually unchanged intima of the vessel SMC were distributed relatively densely, in close contact with each other (Fig. 1a). Vimentin, a protein of intermediate filaments, characteristic of mesenchymal tissue cells, was found in virtually all SMC (Fig. 1b). In the atherosclerotic plaque SMC were more widely separated and were localized on the surface of the plaque and around the atheromatous mass (Fig. 1c). Analysis of the distribution of vimentin in these same sections through the plaque showed that some cells staining positively for vimentin did not contain myosin. They were particularly numerous in the immediate vicinity of the atheromatous masses (Fig. 1d). Like SMC these cells were elongated, they had one nucleus, and they were distributed mainly along the long axis of the vessel. These cells stained more intensively with monoclonal antibodies to the surface of SMC (Fig. 2b) than the neighboring cells which contained myosin (Fig. 1a). In serial sections through the same areas of the plaque, solitary macrophages were found and were clearly visible after the histochemical reaction for

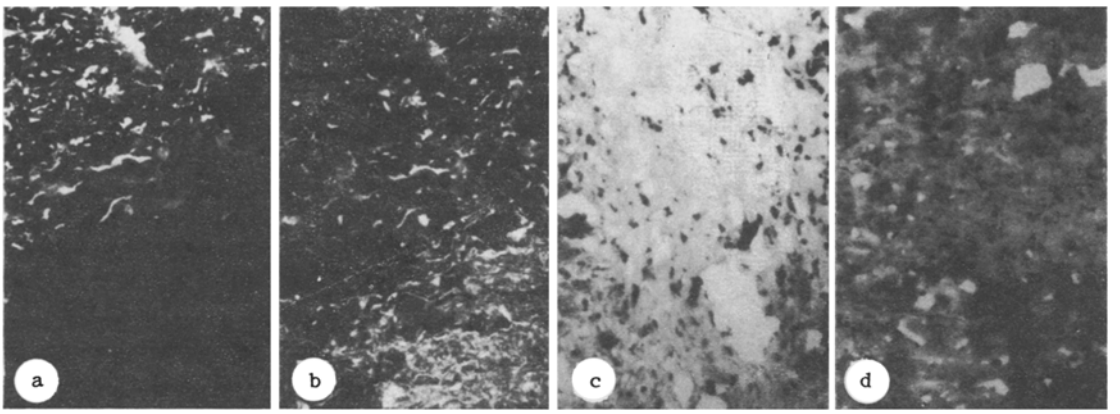

Fig. 2. Localization of myosin (a) and staining with antibodies to surface of SMC (b), demonstration of nonspecific esterase (c) and lipids, stained with Oil Red O (d), in cells of human atheromatous plaque. Some sections (c, d) counterstained with hematoxylin. Double immunofluorescent method (a, b) and serial sections through same part of plaque (c, d). 200 ×.

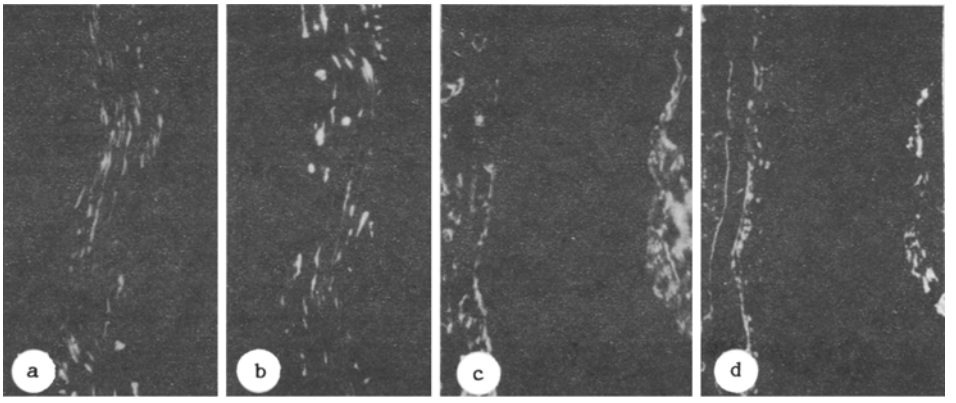

Fig. 3. Demonstration of myosin (a, c) and desmin (b, c) in cells of atheromatous plaque (a, b) and subjacent media and adventitia (c, d) of human aorta. Desmin-positive cells in surface of plaque (b) and in adventitial vessel (d) always contained myosin (a, c). Double immunofluorescence method. 200 ×.

nonspecific esterase (Fig. 2c). Cells with abundant lipid inclusions, staining with Oil Red O, were found comparatively rarely in this zone (Fig. 2d). Only in two of twelve plaques could small vessels growing in from the adjacent media be detected. In all other cases, no connection was found between cells of the plaque and adventitial blood vessels.

Combined staining with antiserum to myosin and with antibodies to desmin revealed SMC containing desmin in four of the 12 atheromatous plaques (Fig. 3a, b). Such cells were never found in the unchanged intima of the aorta, but were found only in single cells of the media adjacent to the adventitia and in small adventitial vessels (Fig. 3c, d).

These data are evidence of polymorphism of the SMC of the human atheromatous plaque. This is true in particular of SMC containing desmin. Desmin is a protein of intermediate filaments, characteristic of smooth muscles and never found in cells of the normal intima of the human aorta [8]. Desmin has been found in intimal SMC of the aorta only in experimental animals, but de-endothelization of the vessel and subsequent proliferation of the intimal cells led to complete replacement of desmin by vimentin [12]. Desmin-containing cells in an atherosclerotic plaque of the human aorta were first found comparatively recently [13]. However, the investigation in question used polyclonal antibodies to desmin, which does not rule out the possibility of a cross reaction with vimentin [6]. The use of monoclonal antibodies was able

to resolve these doubts, but some problems remained unsolved. In particular, it is not clear what functional changes in SMC are associated with expression of a protein which, although characteristic of cells of this type, does not appear in the unchanged vessel.

Besides desmin-positive cells and ordinary SMC containing vimentin, cells not stained with antiserum to myosin also were present in the human atheromatous plaque. The appearance of such cells in the plaque may be the result of a change of phenotype of some SMC in the wall of the vessel similar to that taking place spontaneously in a culture of vascular SMC on the 7th-8th day of its maintenance in vitro [5]. Evidence in support of this explanation is given also by the demonstration of cells not staining with antiserum to smooth muscle myosin as early as during the first day of culture of subendothelial cells from the adult human aorta [1]. Meanwhile, considering the character of localization of cells not containing myosin, the possibility of their formation from proliferating pericytes, penetrating into the plaque together with invading adventitial vessles, as has been shown to take place in nonspecific aorto-arteritis [2], cannot be ruled out. Whatever the case, the myosin-negative cells discovered in the aorta may have an ultrastructure characteristic of modified SMC [9, 14] or of fibroblast-like intimal cells, distinguished by high protein synthesizing activity [2].

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