DISTRIBUTION OF MYOSIN, DESMIN, AND VIMENTIN IN SMOOTH-MUSCLE CELLS OF HUMAN EMBRYONIC VESSELS

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Besides microfilaments and microtubules, the list of principal fibrillar components of the cell cytoskeleton also includes intermediate filaments, from 7 to 11 nm in diameter. According to their immunologic and biochemical properties, intermediate filaments are subdivided into five types: keratin, vimentin, desmin, glial fibrillar acid protein, and neurofilaments [10, 13, 18]. Some workers [16, 19] have demonstrated heterogeneity of smooth muscle cells (SMC) in vitro and in situ in adult animals by an immunofluorescence method using antibodies to vimentin (V) and desmin (D), and have distinguished three types of SMC depending on the ratio between V and D: V-positive/D-positive, V-positive/D-negative, and V-negative/D-positive. The ratio between these phenotypes evidently depends on the species of mammal studied, the type of blood vessel [2, 3, 9], and the severity of the pathological process in the vessels, especially arteriosclerosis [4, 9], and it is also connected with certain factors influencing differentiation of SMC [17]. Heterogeneity of SMC also has been demonstrated in various parts of the rat aorta [8]. The aim of this investigation was to study phenotypes of SMC in human fetal blood vessels by an immunofluorescence method using antibodies to cytoskeletal (vimentin, desmin) and contractile (myosin) proteins of SMC.

EXPERIMENTAL METHOD

An immunofluorescence study was made of five human fetuses obtained after spontaneous abortions because of isthmicocervical uterine insufficiency. The age of the fetuses was 18-20 weeks and was determined with the aid of a special correlation table. To prepare frozen sections pieces were taken from three parts of the aorta (arch of the aorta, thoracic and abdominal aorta), and from the femoral and brachial arteries and veins, and the ureter. Immediately after removal the pieces of tissue on filter paper were flooded in Tissue Tek (Miles) and frozen in liquid nitrogen. Serial sections 5 μ thick were fixed for 3 min in cold acetone and treated with antibodies by the indirect immunofluorescence method [14]. Monoclonal antibodies to V and D were generously provided by Professor K. Weber (Max Planck Institute for Biophysical Chemistry, Gottingen, West Germany), and polyclonal rabbit antibodies to smooth muscle tissue myosin (M) were obtained by the method described below. Purified smooth-muscle M from hens' stomachs [1] in Freund's complete adjuvant was injected in a dose of 1 mg subcutaneously into rabbits at several points. At the 3rd and 5th weeks booster injections of antigen in Freund's incomplete adjuvant were given in a dose of 0.5 mg per animal. The antibody titer was monitored by enzyme immunoassay. Blood was taken from the animals 3 days after the last injection, every 2 days. To abolish cross reaction of the antiserum thus obtained with nonmuscle M, it was passed through an affinity column with immobilized M from human platelets [12]. Affinity sorbents were obtained by conjugation of purified proteins with CNBr-activated sepharose-4B (Pharmacia, Sweden) by the method recommended by the manufacturer. The specificity of the antibodies thus obtained was tested by the immunoblotting method [15]. Polyacryamide gel electrophoresis in the presence of SDS was carried out as in [5] (Fig. 1).

As the second antibodies we used FITC-labeled conjugates to rabbit and mouse IgG in working dilutions.

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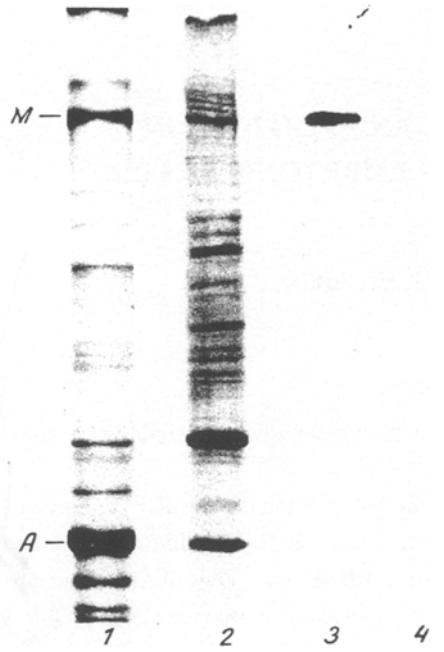

Fig. 1. Control of specificity of antibodies to smooth-muscle M. Homogenate of media of rabbit aorta (1) and of cultured endothelial cells from human umbilical vein (2) was analyzed by electrophoresis in 7.5% polyacrylamide gel. The corresponding bands of nitrocellulose with applied proteins (3, 4) were treated with the antibodies obtained to smooth-muscle M and with the second antibodies, conjugated with peroxidase. M) Myosin heavy chains, A) actin.

In all cases serial sections were stained with hematoxylin and eosin, examined, and photographed on RF-3 film in an Orthoplan fluorescent microscope (Leitz).

EXPERIMENTAL RESULTS

In the fetal aorta M was detected in SMC of the intima and media (Fig. 2, Ia). Monoclonal antibodies to D did not reveal D-positive cells in all layers of the aorta (Fig. 2, Ib). Conversely, monoclonal antibodies to V revealed specific fluorescence both in SMC of the intima and media and in endothelial cells and fibroblasts of the adventitial layer (Fig. 2, Ic). The picture described above was similar in all three parts of the aorta of all the fetuses studied.

In the femoral artery SMC were detected in the media and the intima after incubation with antibodies to M (Fig. 2, IIa). After treatment with antibodies to D, isolated D-positive SMC, located on the outer side of the media, were found among the main mass of D-negative cells (Fig. 2, IIb). Meanwhile V-positive cells were distributed over the whole wall of the vessel, including endothelial cells (Fig. 2, IIc).

The distribution of SMC myosin in the femoral vein was the same as in the aorta and femoral artery (Fig. 2, IIIa). Monoclonal antibodies to D revealed a large number of D-positive SMC, located mainly in the media of the femoral vein (Fig. 2, IIIb). The pattern of localization of the D-positive cells was similar to that observed in the aorta and femoral artery (Fig. 2, IIIc). Incidentally, in the brachial artery and vein antibodies to M, D, and V revealed the same character of specific fluorescence of SMC as in the femoral artery and vein.

M- and D-positive SMC with the same pattern of localization in the muscle layer were found in the fetal ureter (Fig. 2, IVa, b). V-positive cells were present in smaller numbers than M- and D-positive SMC, in the muscular layer of the ureter, and also in the adventitial layer and adjacent loose connective tissue (Fig. 2, IVc).

M was detected in all the fetal vessels and ureter studied, i.e., in both vascular and nonvascular SMC, so that it can be regarded as a marker of SMC. According to our data, D-positive SMC were absent in the fetal aorta, in general agreement with results published in [7]. Although the authors cited sometimes found isolated D-positive cells in the human fetal aorta, they were

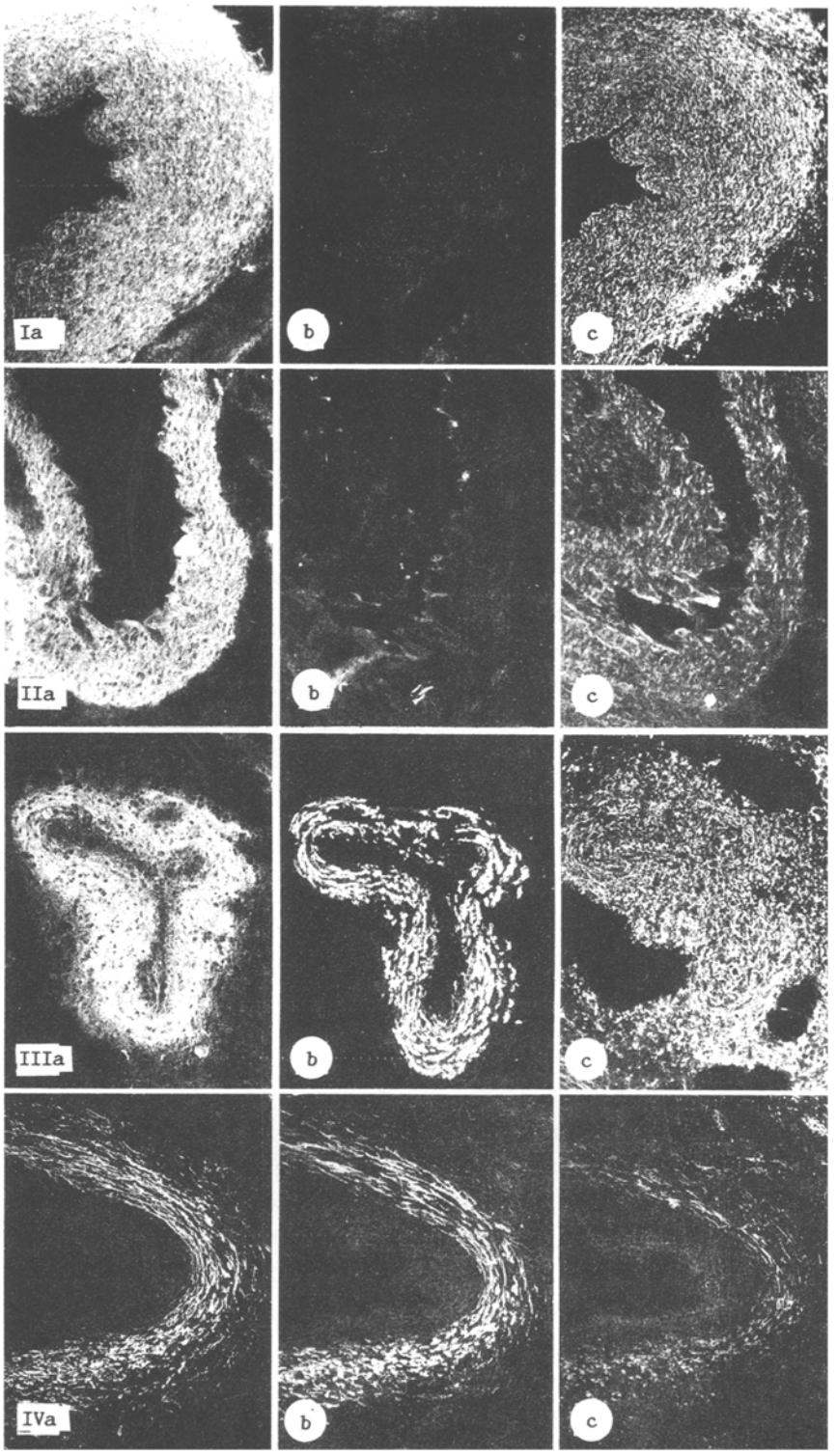

Fig. 2. Localization of M, D, and V in SMC of aorta (I), femoral artery (II) and vein (III), and ureter of human fetus (IV) by immunofluorescence method. Serial frozen sections. I: M-positive (a), D-negative (b), V-positive (c) SMC. V also was found in adventitial fibroblasts. Magnification 220. II: Distribution of M in SMC of intima and media of femoral artery (a), only isolated D-positive SMC are present (b), V in SMC of endothelial cells and in fibroblasts of adventitial layer. Magnification 270. III: Large quantity of M (a), D (b), and V (c)-positive SMC. Magnification 270. IV: Antibodies to M (a), D (b), and V (c) demonstrate identical phenotype of SMC from femoral vein. Magnification 220.

unable to confirm this finding by the results of their SDS-gel electrophoresis and immunoblotting. Nevertheless, it can be tentatively suggested that D-positive SMC may have already begun to appear at this stage of fetal development (20 weeks), with a gradual increase in their total number. Many workers have thus constantly noted the presence of D-positive SMC in the wall of the adult human aorta [11, 19].

Isolated D-positive SMC were sometimes found in the fetal femoral and brachial arteries against a general background of D-negative cells, unlike in the fetal aorta. Later their number increased sharply, and in the adult, D-positive SMC predominated in arteries of muscular type, compared with the aorta [8, 9].

The discovery of a large number of D-positive SMC in the media of the fetal femoral and brachial veins was somewhat unexpected. We could find no description of this fact in the accessible literature. Moreover, the results of the investigation showed that the phenotype of SMC of the veins was identical with the phenotype of nonvascular SMC, especially the ureter, i.e., in organs differing sharply in their functions.

The use of antibodies to myosin, desmin, and vimentin thus reveals heterogeneity of SMC, manifested at the early stages of ontogenetic development and depending on the type of vessels. In human fetal vessels, depending on expression of proteins of the intermediate filaments, two phenotypes of SMC can be distinguished: D^-, V^+, and D^+, V^+. The use of desmin as a marker of differentiation of SMC [4, 11] permits a deeper understanding of the role of heterogeneous SMC in the development of the pathological process in the vessel wall in, for example, arteriosclerosis, and it may also be used in the diagnosis and classification of tumors [6]. The mechanism of modification of these SMC phenotypes at different stages of pre- and postnatal development and their morphological and functional features are undoubtedly interesting.

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