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IMMUNOMORPHOLOGICAL INVESTIGATION OF DISTRIBUTION OF COLLAGEN OF TYPES I, III, IV, AND V IN PRIMARY CULTURE OF HUMAN AORTIC CELLS

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UDC 616.132-004.6-02:547.962.9/-092

KEY WORDS: collagen; immunofluorescence; fibrosis; intracellular antigens.

Fibrosis is the most important manifestation of atherosclerosis of human arteries. This process is associated with the accumulation of connective-tissue proteins, namely collagen, in the vessel wall. The main part of the connective-tissue matrix in the intact vessel wall consists of type III collagen, whereas in the atherosclerotic intima it consists of type I collagen [5]. Collagens of types IV and V also are present in blood vessels [2, 6, 14] as components of basement membranes. The principal cells which produce collagen in blood vessels are smooth-muscle cells. It has been shown that these cells can synthesize collagen both in the blood vessel *(in vivo)* and also in culture *(in vitro)* [1, 11].

Cultures of vascular smooth-muscle cells are widely used at the present time to study mechanisms of atherogenesis and, in particular, mechanisms of fibrosis [9]. A particularly promising model for such investigations is evidently a primary culture, in which the particular features of vascular cells are preserved to the greatest degree [1]. A primary culture of human aortic cells has recently been obtained and characterized [4, 8] and it can be hoped that it will serve as a useful tool with which to study mechanisms of fibrosis in atherosclerosis in man.

The object of this investigation was the immunocytochemical detection of different types of collagen in such a culture.

EXPERIMENTAL METHOD

Experiments were carried out on 7-day cultures of cells isolated with the aid of enzymes from human aorta. The cells were isolated and cultured as described previously [4, 8]. The morphological characteristics of the cultures were described in [4]. The cells were seeded in Lab Tek chambers (Miles Laboratories, USA) with a density of 10^4 cells/cm^2. On the 7th day of culture the cells were washed with isotonic phosphate buffer, pH 7.4, and fixed with

Institute of Experimental Cardiology, 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 E. I. Chazov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 96, No. 10, pp. 110-111, October, 1983. Original article submitted January 19, 1983.

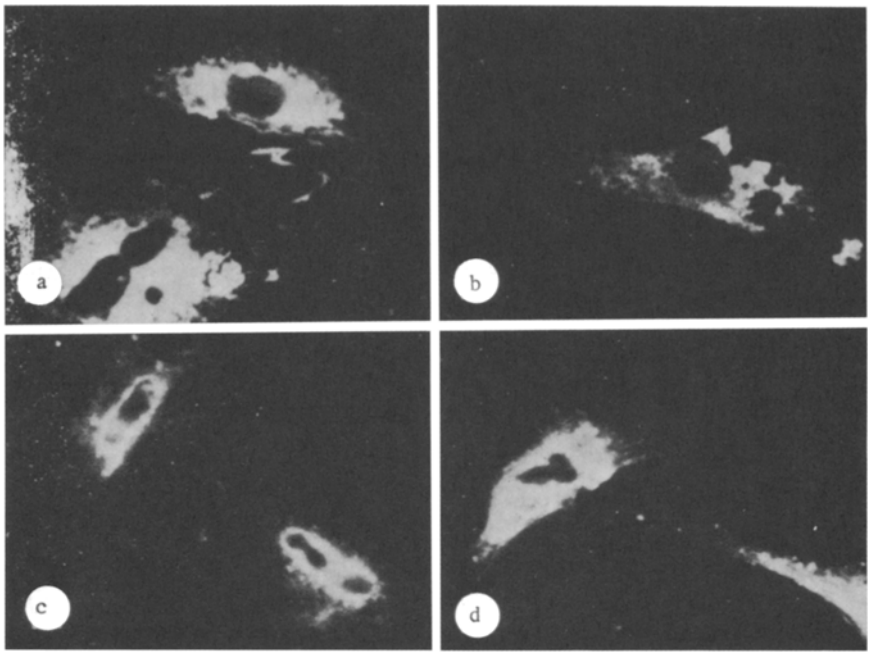

Fig. 1. Immunocytochemical detection of collagens of types I, III, IV, and V inside cells in a primary culture of human aorta (7 days in culture). Collagen of: a) type I, b) type III, c) type IV, d) type V. Fixation with formaldehyde followed by treatment with acetone. 400×.

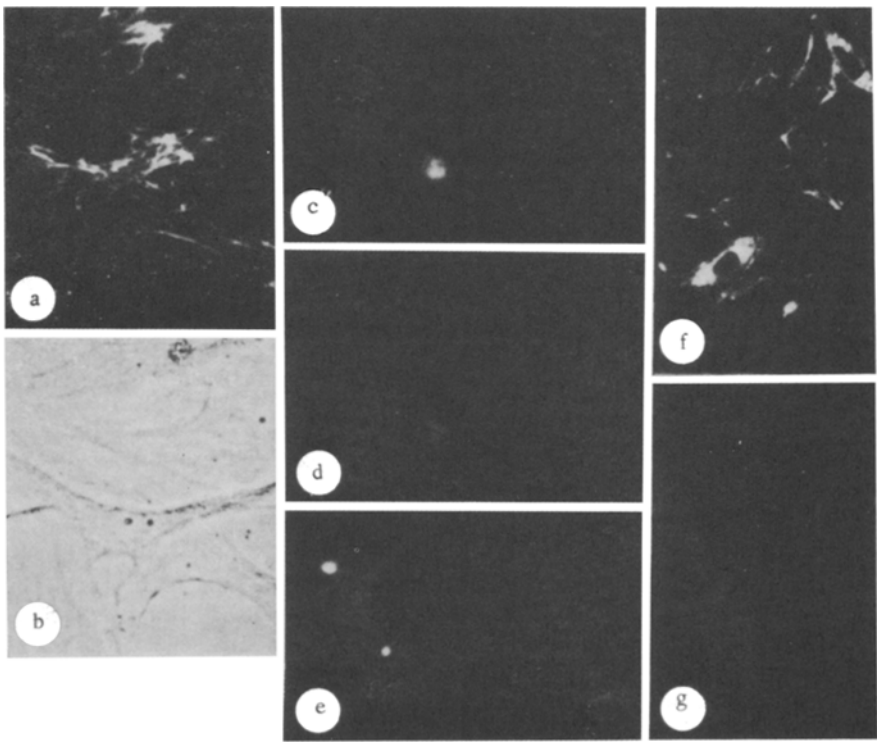

Fig. 2. Immunocytochemical detection of collagens of types I, III, IV, and V on surface of human aortic cells in primary culture (7 days in culture). Collagen of: a) type I, b) the same, c) type III, d) type IV, e) type V, f) type I (treated with acetone); g) nonimmune globulins. a, c-g) immunofluorescence, b) phase contrast. Fixation with formaldehyde. 400×.

4% formaldehyde. Indirect immunofluorescence staining was carried out by the scheme described in [15]. To investigate the intracellular localization of antigens, cells fixed with formaldehyde were treated with cold acetone.

Preparations of collagen of types I, III, IV, and V were purified by differential salt precipitation in neutral and acid solutions from a pepsin extract of human placenta [3, 7, 13]. Antisera were obtained after repeated immunization of rabbits with collagen solutions emulsified in Freund's adjuvant. Antibodies, consisting mainly of IgG, were isolated by affinity chromatography of the antisera on immunosorbents obtained by cross-linking the corresponding type of collagen to BrCN-activated Sepharose 4B [3, 7]. Immunoglobulins of nonimmune rabbits (IgG) were isolated from serum and purified by chromatography on DEAE-cellulose [3]. Goat antibodies against rabbit IgG, labeled with fluorescein isothiocyanate (FITC), were generously provided by Professor V. S. Rukosuev (Laboratory of Immunomorphology, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR).

Fluorescence was investigated with an Opton-III photomicroscope (West Germany), optimized for observation of the fluorescence of fluorescein.

EXPERIMENTAL RESULTS

The location of four types of collagen in a primary culture of human aortic cells was studied on the 7th day of culture, for at that time the cells seeded in the culture have succeeded in spreading out into a monolayer and have preserved their differentiated state [1]. To detect collagen in the culture, cells fixed with formaldehyde were treated with acetone, to make the intracellular antigens accessible for antibodies against collagen. Antibodies against all four types of collagen revealed antigens inside the smooth-muscle cells in culture. These intracellular antigens were evidently procollagens of the corresponding types. They were distributed diffusely in the cells in the ectoplasm (Fig. 1). When nonimmune γ-globulins were used instead of antibody preparations no fluorescence was observed, evidence that the staining was specific (Fig. 2g). These observations suggest that the smooth-muscle cells of the human aorta can synthesize all four types of collagen in culture.

For immunocytochemical staining of extracellular collagen the reaction with antibodies was carried out on preparations of cells fixed with formaldehyde but not treated with acetone. In this way only type I collagen could be found (Fig. 2a, b). The extracellular matrix consisted of fibrils forming a network on the surface of the cells and between them (Fig. 2a, f). Antibodies against the other three types of collagen did not reveal corresponding antigens in the extracellular matrix.

An attempt was made to discover whether a change in the conditions of culture leads to expression of collagen of types III, IV, and V on the cell surface. With this aim the seeding density of the cells was increased to $2 \cdot 10^4$ and $5 \cdot 10^4$ cells/cm^2; the duration of culture was increased to 14 and 21 days, and sodium ascorbate (50 µg/ml daily) also was added to the culture medium. These procedures are known to stimulate collagen synthesis in culture [12]. However, a change in the conditions of culture did not lead to the appearance of collagen of these types in the composition of the extracellular matrix. The cultured cells contained only type I collagen on their surface, and the quantity of it was not appreciably different from that found after culture under ordinary conditions.

Human aortic cells can thus evidently synthesize in primary culture all four types of collagen present in the aorta *in vivo*. The extracellular matrix on the surface of the cells is formed from collagen of type I only, in harmony with its situation in the vessel *in vivo*, where smooth-muscle cells are surrounded by type I collagen whereas other types of collagen are bound with noncellular structures. Collagens of types III, IV, and V, synthesized in culture, are evidently present in the culture medium, for we know that most of the collagen synthesized by smooth-muscle cells in culture is soluble in the medium [10].

It can be concluded from these results that the ability of human aortic cells to synthesize different types of collagen in primary culture, and also the charcter of formation of the collagen matrix are very similar to what takes place in the blood vessel *in vivo*. Primary culture of human aortic cells can accordingly be regarded as a model suitable for the study of mechanisms of formation of the connective-tissue matrix during the development of fibrosis.

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MONOAMINE OXIDASE ACTIVITY IN SYMPATHETIC GANGLIA OF RABBITS DIFFERING IN RESISTANCE OF THEIR CARDIOVASCULAR FUNCTIONS TO EMOTIONAL STRESS

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UDC 616.834-008.931:577.152.143/-02:613.863-092.9

KEY WORDS: catecholamines; hypothalamus; rabbits.

The study of catecholamines and the enzymes of their metabolism in structures of the peripheral nervous system during stress is of great interest. It was shown previously [3] that changes in activity of tyrosine hydroxylase, a key enzyme of catecholamine biosynthesis, in the ganglion nodosum and stellate ganglion differ in rabbits "predisposed" and "resistant" to emotional stress. In rabbits "predisposed" to stress a marked decrease in tyrosine hydroxylase activity was observed in the stellate ganglion and ganglion nodosum, whereas in rabbits "resistant" to stress tyrosine hydroxylase activity in the stellate ganglion and ganglion nodosum did not differ from the control level.

The object of this investigation was to study activity of monoamine oxidase (MAO), an enzyme inactivating catecholamines in neurons of the superior cervical and stellate ganglia and the ganglion nodosum in rabbits differing in the resistance of their cardiovascular functions to emotional stress.

EXPERIMENTAL METHOD

Emotional stress was induced in immobilized mature Chinchilla rabbits by simultaneous aperiodic stimulation of the negative emotiogenic centers of the hypothalamus (ventromedial nuclei) and electrodermal stimulation according to a specially devised stochastic scheme. The ventromedial hypothalamic nuclei were stimulated by bipolar nichrome electrodes, and electrodermal stimulation was applied through steel needles, implanted subcutaneously into one of the animal's hind limbs. The parameters of the electric current (square pulses: frequency 50 Hz, pulse duration 1 msec, voltage 5-10 V, duration of stimulation 1-2 min) were

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(Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Val'dman.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 96, No. 10, pp. 111-113, October, 1983. Original article submitted March 25, 1983.

 0007/4888/83/9610-1476$07.50