EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

Localization of Collagen-Producing Cells in Normal and Atherosclerotic Intima of Human Aorta

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Cells containing type I procollagen (PC1-cells) are identified immunocytochemically in grossly normal and atherosclerotic human aortic intima. Lipid streaks have the greatest content of these cells. PC1-cells are unevenly distributed over the intimal sublayers. They concentrate predominantly in the proteoglycan sublayer adjacent to the lumen. It is hypothesized that lipid accumulation by intimal cells stimulates collagen synthesis.

Key Words: *atherosclerosis, type I procollagen; human aortic intima*

Accumulation of the extracellular matrix or fibrin is a major manifestation of atherosclerosis. Type I interstitial collagen is the main component of the atherosclerotic plaque matrix in human aorta [6,10]. Collagen accumulated in atherosclerotic lesions is synthesized by vascular smooth muscle cells [4]. Cells producing type I procollagen (PC1-cells) were identified in atherosclerotic plaques of human arteries [9]. However, little is known about collagen synthesis in early atherosclerotic lesions (initial lesions and lipid streaks). In the present study we identified PC1-cells and examined their location in grossly normal human aortic intima and atherosclerotic lesions: initial lesions, lipid streaks, and lipofibrous and fibrous plaques.

MATERIALS AND METHODS

Aortas were obtained at autopsy of sudden death victims (age 30-60 years) within 1.5-3 h after death. Grossly normal and atherosclerotic areas of the aortic intima were categorized according to the classification of the American Heart Association [11]. The following types of atherosclerotic lesions were specified: initial lesions, lipid streaks, and lipofibrous and fibrous plaques. Aortic specimens were fixed in methanol:chloroform:acetic acid (6:3:1), embedded in paraplast, and cut into 5-μ thick sections. Collagen-producing cells were identified immunocytochemically using PSI.D8 monoclonal antibodies to type I procollagen. The lipoidosis areas in atherosclerotic lesions were characterized with the use of anti-apoB monoclonal antibodies. The antigen-antibody binding sites were identified by the biotin-streptavidin-peroxidase method.

Statistical analysis of the results was performed with the use of Student's *t* test.

RESULTS

Cells synthesizing type I procollagen were not found in grossly normal area of human aortic intima and in the aortic media. They were identified in atherosclerotic intima, being most abundant in lipid streaks (Fig. 1).

The distribution of PC1-cells over the intimal sublayers was uneven. The majority of these cells were located in the proteoglycan sublayer under the endothelium (Fig. 2).

PC1-cells were morphologically different. We have distinguished two types of these cells: flat elon-

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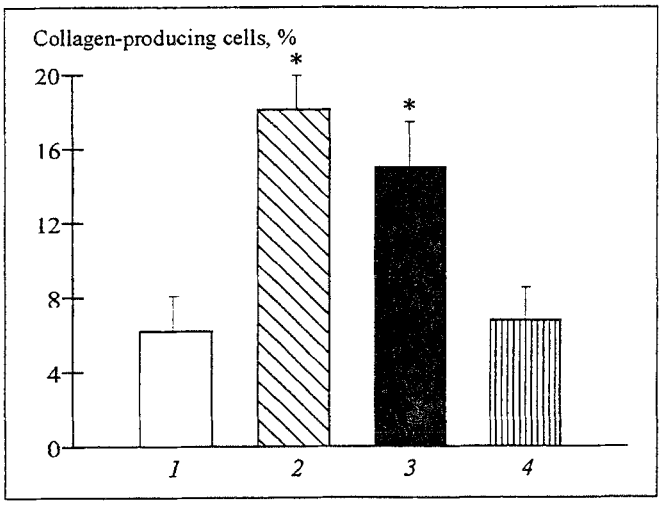

Fig. 1. Content of PC1-cells in atherosclerotic intima of human aorta. *1*) initial lesion (n=6); 2) lipid streaks (n=13); 3) lipofibrous plaques (n=8); *4*) fibrous plaques (n=5); *n*) number of specimens. Asterisk indicates statistically significant difference in the content of PC1-cells compared with that in initial atherosclerotic lesions and fibrous plaques.

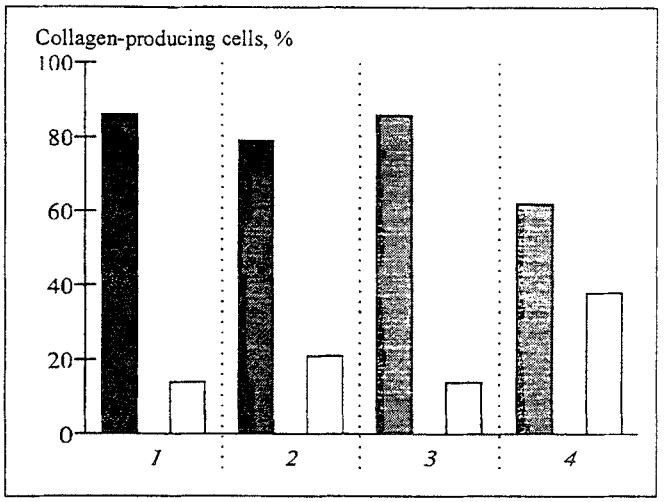

Fig. 2. Distribution of PC1-cells over the sublayers of human aortic intima. *1*) initial lesion (n=15): 2) lipid streaks (n=25); 3) lipofibrous plaques (n=16); *4*) fibrous plaques (n=11). Black bars: the number of PC1-cells in the proteoglycan layer; white bars: the number of PC1-cells in the muscular-elastic layer.

gated cells located in the cap of fibrous or lipofibrous plaque between the fibers of the connective tissue matrix (Fig. 3, *a*) and round cells reacting with anti-apoB antibodies; their cytoplasm resembled that of foam cells (Fig. 3, *b*).

Our findings indicate that PC1-cells are located not only in atherosclerotic plaques but also in earlier atherosclerotic lesions: initial lesions and lipid streaks, where the processes of intra- and extracellular lipid accumulation predominate [1,2]. It should be noted that the number of collagen-synthesizing cells in initial lesions is comparable to that in fibrous plaques. Previously, we showed that the main manifestations of atherosclerosis (lipid and collagen accumulation, intimal thickening, and increased number of cells in the intima) are confined to the proteoglycan sublayer of the intima [7]. The majority of PC1-cells were located in the proteoglycan sublayer, which is consistent with the results of immunocytochemical identification of various collagen types in atherosclerotic

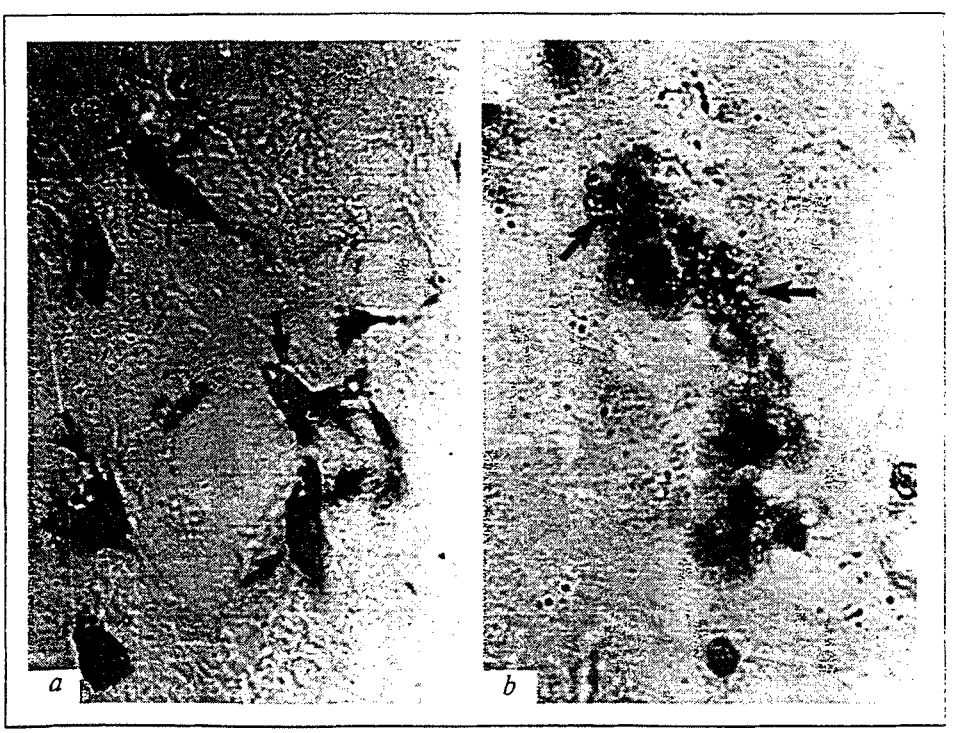

Fig. 3. Immunocytochemical identification of PC1-cells in atherosclerotic intima of human aorta. Magnification 850. Nuclei are counterstained with methyl green. *a*) PC1-cells in the fibrous cap of a lipofibrous plaque (arrows); *b*) PC1-cells in lipid streak area. Foam cells are indicated with arrows. Small empty spaces appeared in the cytoplasm after lipid extraction.

lesions [5,7,10]. Experiments with primary cultures of human aortic intima cells showed that intracellular lipid accumulation coincides with increased production of extracellular proteins [8]. The results of the present study suggest that intracellular lipid accumulation in vascular cells stimulates collagen synthesis by intimal cells, which leads to the progression of atherosclerotic lesions.

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