Immunocytochemical Study of the Localization of Scavenger Receptor in Human Aortic Smooth-Muscle Cells

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Scavenger receptor was sought *in situ* in human aortic smooth-muscle cells and in a primary culture of intact human aortic intima using antibodies to scavenger receptor. For identification of smooth-muscle cells, double staining making use of antibodies to murine α-actin was used. The presence of scavenger receptor in smooth-muscle cells of the intima and media of human aorta was demonstrated on aortic slices. In cultured smooth-muscle cells from normal human aortic intima scavenger receptor was distributed over the entire surface of the cell membrane, forming clusters in some places. These results suggest that human aortic smooth-muscle cells express scavenger receptor.

Key Words: *human aorta; smooth-muscle cells; scavenger receptor*

Accumulation of neutral lipids, and specifically of cholesterol esters, in vascular wall cells, is one of the principal manifestations of atherosclerosis. Studies carried out on cultures of macrophages and smooth-muscle cells (SMC) of intact human aortic intima showed that B,E-receptor-mediated capture of native low density lipoproteins (LDL) did not lead to lipid accumulation in cultured cells [4,13]. Incubation of macrophages with modified forms of LDL was found to result in an increase of intracellular lipids [3,4]. Endocytosis of modified LDL is considered to be mediated by scavenger receptor (SR), whose activity, in contrast to that of B,E-receptor, is not regulated by the intracellular lipid content [4,6]. Human blood monocytes [5,6], bovine endothelial cells [2,12], rabbit fibroblasts [7], etc., have been shown to be capable of expressing SR. Immunocytochemical methods have demonstrated that macrophages and endothelial cells express SR in human aortic wall [9], but SMC, which are the principal cellular component of the intimal and medial layers of the human aorta, possess no such ability [1,11]. Studies on cultures of intact human aortic intima carried out in our laboratory showed that aortic intimal SMC capture and destroy the modified LDL, the activity of capturing modified LDL being at least twice as high as the capture of native LDL [14]. We proposed that human aortic intimal SMC express SR, and in order to confirm this by immunocytochemical methods, we studied the presence and specific features of SR in human aortic cells.

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MATERIALS AND METHODS

Aortas of males aged 40 to 60 years who had died suddenly of coronary disease were used in the study. The aorta was removed 1.5 to 3 h postmortem. Apparently intact portions of the aorta were fixed in methacarn (ethanol:chloroform:acetic acid - 6:3:1) for studies *in situ*. For *in vitro* experiments, the cells were isolated by enzymatic methods from human aortic intima as described previously [11] and placed on slides at a density of $10^4/cm^2$. The cells were incubated in a CO_2 incubator at 100% humidity and 37°C. On day 7 of culturing the cells were washed

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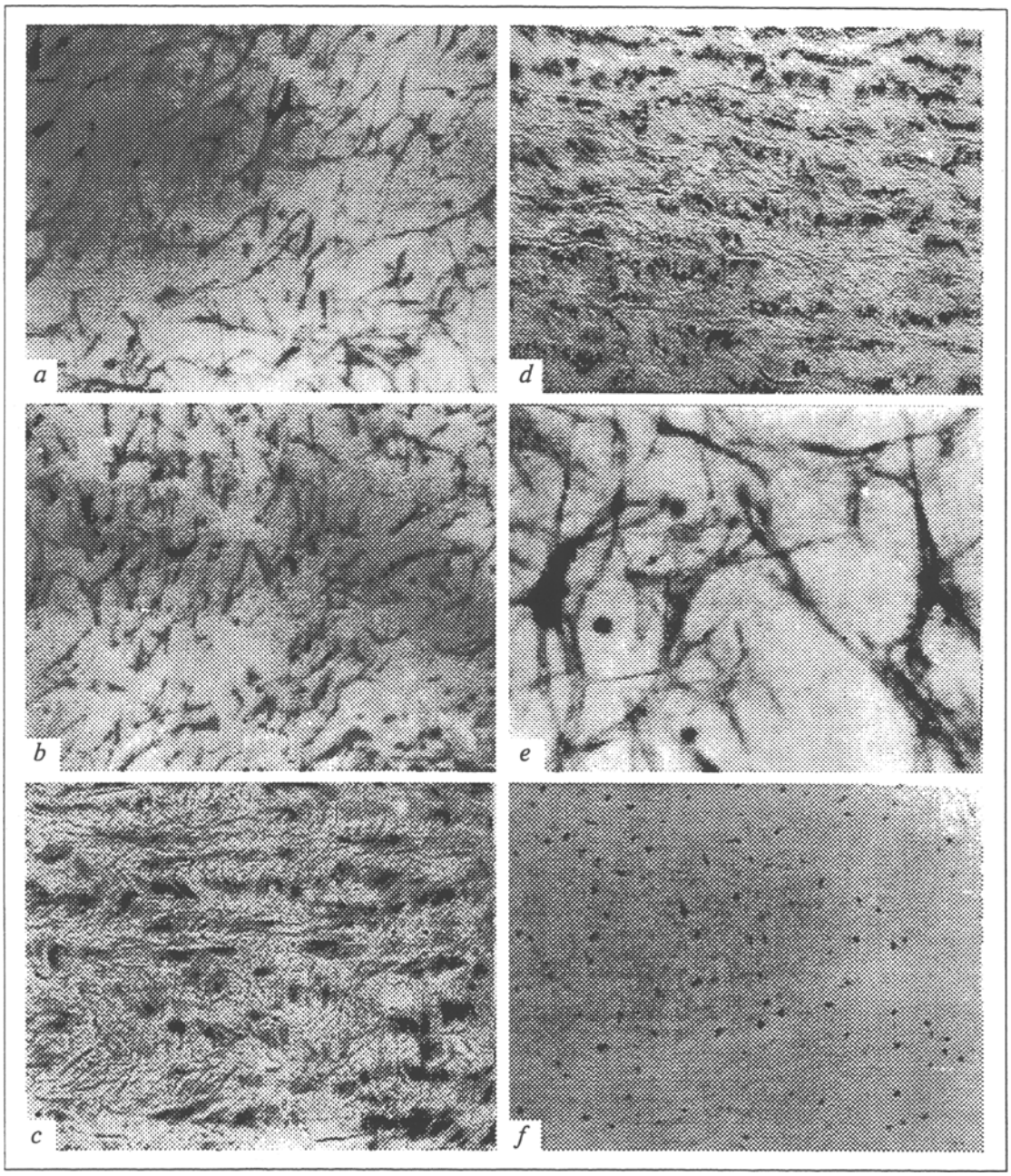

Fig. 1. Immunocytochemical detection of SR and α−actin on serial slices of human aorta, ×250. *a, b, e, f*) human aortic intima; *c, d*) media; antibodies to SR (*a, c, e*), and to α−actin (*b, d*), nonimmune rabbit serum (*f*).

in isotonic phosphate buffer at pH 7.4 and fixed with 4% formaldehyde in isotonic phosphate buffer. Polyclonal rabbit antibodies to SR were kindly provided by Dr. D. P. Via (Baylor Medical College, Houston, Texas). Antibodies were used in a concentration of 25 μg/ml. Nonimmune rabbit serum was used as the negative control. Double staining with antibodies to SR and to the SMC marker α-actin (Boehringer Mannheim) was used for identification of cells stained for SR in a cell culture. To visualize the binding sites of antigen-specific antibodies, we used species-specific antibodies labeled with fluorescein isothiocyanate (FITC) (Sigma) and Texas red (Amersham) in the cell culture and antibodies labeled with horseradish peroxidase (Amersham) on slices. 3,3'-diaminobenzidine tetrahydrochloride (Sigma) was used as the substrate for peroxidase. The preparations were examined under a Carl Zeiss photomicroscope III.

RESULTS

The presence of SR in human aortic intima cells was examined on tangential slices permitting study

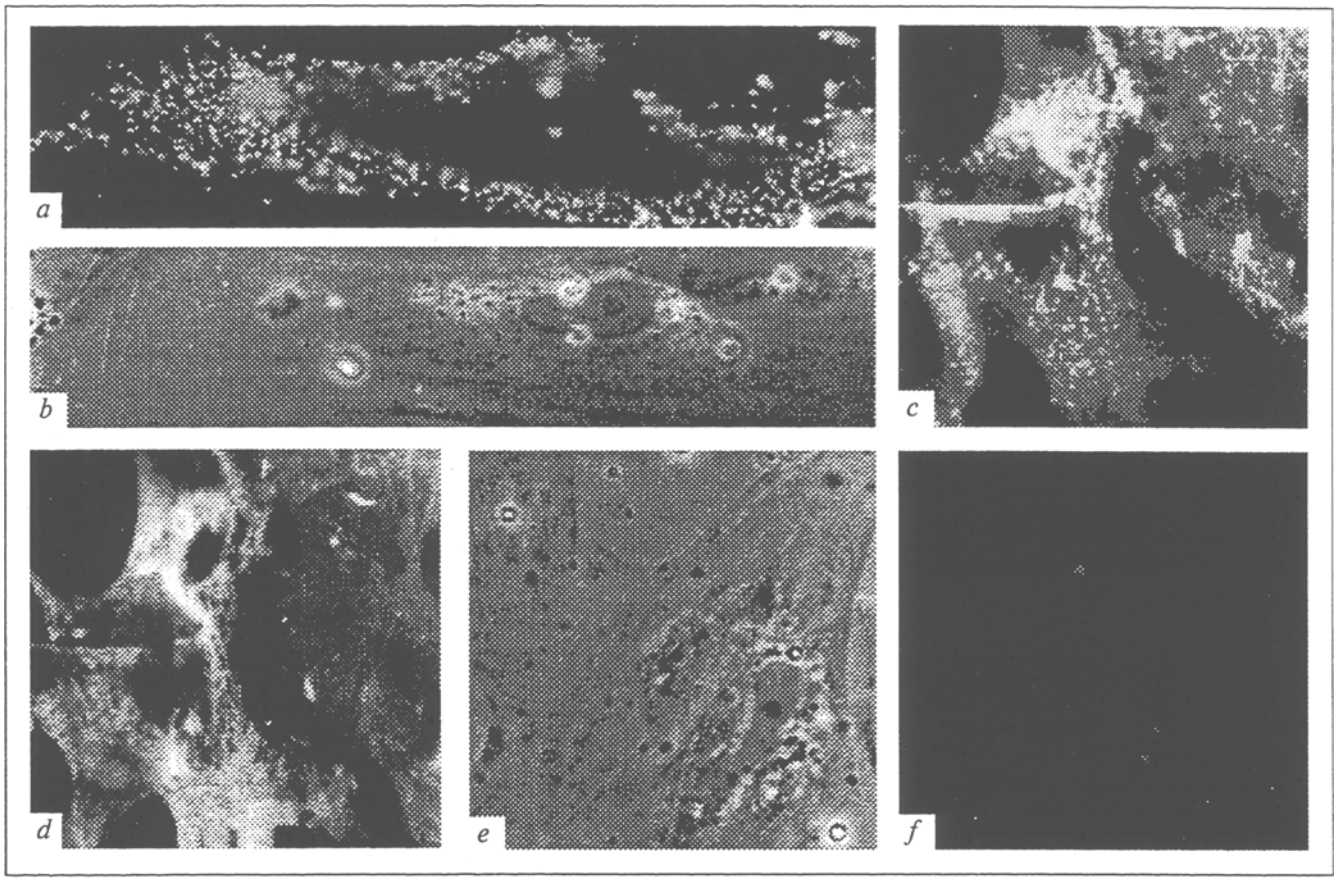

Fig. 2. Immunocytochemical detection of SR and α−actin in a primary culture of human aortic intimal cells. *a, b*) cell of a primary culture of human aortic intima; antibodies to SR. Immunofluorescence (*a*), phase contrast (*b*), ×800. *c, d*) double staining with antibodies to SR (*c*) and antibodies to α−actin (*d*), ×500. *e, f*) cell of a primary culture of human aortic intima; nonimmune rabbit serum. Phase contrast (*e*), immunofluorescence (*f*), ×800.

of the cell arrangement in the intimal layer. The majority of the intimal cells were stellate cells forming a unified cellular network [10]. In the media, where spindle SMC are densely packed and form a solid cellular layer with small intercellular spaces [10], SR was searched for on vertical slices.

Figure 1, *a* shows a portion of intact human aortic intima with numerous processes intensively stained for SR. Figure 1, *b* depicts a portion of aortic intima stained for murine α-actin, corresponding to the site on Fig. 1, *a*. Comparison of these sites showed that cells containing α-actin (Fig. 1, *b*) and possessing numerous processes correspond to cells stained for SR (Fig. 1, *a*) and that both cell populations are similar in localization and shape. A higher magnification (Fig. 1, *e*) shows that SR-stained cells represent stellate SMC characteristic of human aortic intima [11].

Examination of the medial layer of the aorta revealed numerous SR-stained cells (Fig. 1, *c*), to which cells similar in shape and localization on serial slices stained with antibodies to α-actin (Fig. 1, *d*) corresponded.

No cell staining was observed on serial slices incubated with nonimmune rabbit serum (Fig. 1, *f*).

The localization of antibodies to SR was studied in primary cell cultures of intact human aortic intima using FITC-labeled species-specific antibodies.

Bright fluorescent spots were observed on the cell surface at sites of antibodies to SR. These fluorescent spots were unevenly distributed on the surface of the cell membrane, forming clusters chaotically distributed on the cell surface (Fig. 2, *a, b*).

Previous studies [1] demonstrated that more than 87% of cells in a culture isolated from intact human aorta contain α-actin. Double staining for SR and α-actin was carried out to identify the cells stained for SR and showed that cells interacting with antibodies to SR (Fig. 2, *c*) contain α-actin (Fig. 2, *d*); hence, they are SMC.

Our research showed that the intimal and medial SMC on human aorta slices and the aortic intima cells in a primary culture stain with antibodies to SR. Hence, not only macrophages and endotheliocytes of the aorta, but human aortic SMC are capable of expressing SR. It may be assumed that the previously revealed ability of

human aortic intimal SMC to capture and destroy modified LDL [14] is due to the presence of SR in the SMC of human aortic intima.

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