American Journal of Pathology, Vol. 136, No. 5, May 1990

Heterogeneity of Smooth Muscle Cells in Atheromatous Plaque of Human Aorta

Vladimir R. Babaev, Yurii V. Bobryshev, Olga V. Stenina, Edvard M. Tararak, and Giulio Gabbiani*

From the Institute of Experimental Cardiology, USSR Cardiology Research Center, Academy of Medical Sciences, Moscow, USSR; and the Department of Pathology, University of Geneva, Geneva, Switzerland*

This study was undertaken to investigate the expression of cytoskeletal proteins and the ultrastructure of cells in normal intima and atheromatous plaque of human aorta. It has been established, using double-labeling immunofluorescence, that smooth muscle cells (SMC) in normal aortic intima contain myosin, vimentin, and alpha-actin but do not react with antibodies against desmin. In contrast, 7 of 28 atherosclerotic plaques contained many cells expressing desmin in addition to the other cytoskeletal proteins characteristic of normal intima SMC. These cells were localized predominantly in the plaque cap and had the ultrastructural features of modulated SMC, ie, well-developed endoplasmic reticulum and Golgi apparatus. Besides, some cells in the 13 atherosclerotic plaques proved to be myosin, alpha actin, and desmin negative but contained vimentin and actin as revealed by fluorescent phalloidin. These cells were found in the immediate proximity of atheromatous material and reacted with a monoclonal antibody specific to SMC surface protein (11G10) but not with monoclonal anti-muscle actin (HHF35) and anti-macrophage (HAM56) antibodies. Electron microscopy of this plaque zone revealed that the cytoplasm of these cells was filled with rough endoplasmic reticulum and a developed Golgi complex. At the same time, a certain proportion of cells in this region retained morphologic features of differentiated SMC such as the presence of a basal lamina and myofilament bundles. The revealed peculiarities of cytoskeletal protein expression and the ultrastructure of cells in human aortic atherosclerotic plaques may be explained by a phenotypic modulation of vascular SMC. (Am J Pathol 136, 1031–1042)

According to a number of researchers, smooth muscle cells (SMC) play a key role in the pathogenesis of atherosclerosis because SMC proliferation and secretion of extracellular matrix components are the earliest and most important manifestations of the disease.[1,2] Therefore, the attempts to reveal specific characteristics of atherosclerotic plaque cells are quite understandable. Early electron microscopic investigations pointed to the heterogeneity of atherosclerotic plaque cells in human arteries (for review see Geer and Haust[3]). Current reports describe variants of vascular SMCs in atherosclerotic plaque,[4] as well as differences between ultrastructural features of cells located in the intimal thickening *versus* those located in the underlying media.[5] It has been established that the SMC population in human atherosclerotic plaques is heterogeneous with respect to monoclonal antibody binding,[6,7] expression of class 2 transplantation antigen,[8] and cytoskeletal proteins.[9,10] Thus cells containing desmin were detected in atherosclerotic plaques of human aorta, whereas they have not been found in normal vascular intima.[9,10] The ultrastructure of these cells is presently not known; however in experiments of rat aortic arterial denudation, SMCs present in intimal thickening exclusively express vimentin, despite the fact that the normal rat aortic media is characterized by many (60%) SMCs containing desmin.[11]

Cell culture studies have shown that vascular SMCs can express two phenotypes, one called 'contractile' and the other 'synthetic' state.[12] Modulation between these phenotypes is accompanied by changes in ultrastructure and myosin expression.[12,13] It has been suggested that the process termed SMC 'modulation' develops most actively at the site of atherosclerotic lesions.[14] Recently we have shown that primary culture of human aortic subendothelial cells contain 6% to 33% of myosin-negative cells, their number being especially high in cultures derived from advanced atherosclerotic lesions.[15] However, it still remains obscure whether myosin-negative SMCs are

Supported in part by the Swiss National Science Foundation, grant 3.108-0.88.

Accepted for publication December 15, 1989.

Address reprint requests to Vladimir R. Babaev, Institute of Experimental Cardiology, Cardiology Research Center of USSR, 3rd Cherepkovskaya Str. 15A, 121552, Moscow, USSR.

Table 1. *Summary of Study Cases and Immunoreactivity of the Cells in Normal Intima (N) and Atheromatous Plaque (P) of Human Aorta*

Case	Sex	Age (yrs.)	Cause of death	Postmortem interval (hrs.)	Vessel area	Cell-staining pattern		DAL
						Desmin+	Myosin−	
1	F	14	Trauma	4	N	−	−	ND
2	M	18	Trauma	4	N	−	−	0–1
3	M	21	Trauma	3.5	N	−	−	0–1
4	F	34	Trauma	3.5	N	−	−	1–2
					P	−	−	
5	M	34	Asphyxia	4	N	−	−	1–2
					P	−	+	
6	M	37	Congestion heart failure	4	N	−	−	2–3
					P	−	−	
7	M	37	Acute myocardial infarction	4.5	N	−	−	2–3
					P	−	−	
8	M	41	Trauma	3	N	−	−	1–2
					P	−	−	
9	F	42	Trauma	3.5	N	−	−	0–1
10	M	45	Trauma	4	N	−	−	1–2
					P	−	−	
11	M	47	Acute myocardial infarction	3	N	−	−	2–3
					P	−	−	
12	F	50	Traumatic shock	4	N	−	−	1–2
13	M	50	Myocardial infarction	5	N	−	−	3
					P	+	+	
14	M	56	Myocardial infarction	2.5	N	−	−	2–3
					P	−	−	
15	M	58	Trauma	3	N	−	−	2–3
					P	−	−	
16	M	59	Homicide	2.5	N	−	−	2–3
					P	−	+	
					P	−	−	
17	F	59	Myocardial infarction	4.5	N	ND	−	3–4
					P	+	+	
18	M	60	Myocardial infarction	4	N	−	−	ND
					P	ND	+	
19	M	62	Myocardial infarction	2	N	−	−	3–4
					P	−	−	
20	M	64	Trauma	4.5	N	−	−	2–3
					P	+	+	
21	F	65	Trauma	3	N	−	−	3
					P	−	−	
22	M	65	Myocardial infarction	3	N	−	−	3–4
					P	−	+	
23	F	66	Myocardial infarction	3.5	N	−	−	ND
					P	+	+	
24	M	69	Trauma	3.5	N	−	−	2–3
					P	−	−	
25	M	70	Cardiogenic shock	2.5	N	−	−	3–4
					P	−	−	
26	M	70	Cradiogenic shock	4	N	−	−	3–4
					P	−	+	
27	F	72	Acute myocardial infarction	3	P	+	+	3–4
					P	−	−	
28	F	75	Myocardial infarction	3.5	N	−	−	3–4
					P	+	−	
29	F	78	Acute myocardial infarction	3.5	N	−	−	3–4
					P	+	+	
					P	−	+	
30	F	83	Poisoning	3	N	−	−	2–3
					P	−	+	

Desmin+, desmin − positive cells.
Myosin−, myosin − negative cells.
DAL, degree of atherosclerotic lesion.[17]

present in the normal human arterial wall and how frequently they are seen in atherosclerotic plaques.

In this study, we have used double-labeling immunofluorescence and electron microscopy to investigate the expression of cytoskeletal proteins and the ultrastructure of grossly normal intima and atheromatous plaque of human aorta. Our results indicate that some atherosclerotic plaque cells express desmin, the other are myosin and

Table 2. *Antisera and Antibodies Used in the Study for Characterization of Cell Phenotypes in the Atherosclerotic Plaque*

Designation	Type	Specificity	Cells identified	Reference/source	Working dil
V9	MC	Vimentin	Fibroblasts SMC, macrophages, endothelial cells	17	1:20
DE-R-II	MC	Desmin	SMC	18	1:100*
αsm-I	MC	alpha-actin	SMC	19	1:20
IIG-10k	MC	SMC protein	SMC, fibroblasts,	20, 21	1:100*
HHF35	MC	Muscle actins	SMC	22	1:300*
HAM56	MC	Undetermined	Macrophages, endothelial cells	6	1:500*
ASMM	PC	SMC myosin	SMC	15	1:50
	PC	Desmin	SMC	9	1:10
	PC	Desmin	SMC	Sigma Corp.	1:30
	PC	Factor YIII-Rag	Endothelial cells	Behring Diagnostics	1:50
	PC	Human lysozyme	Macrophages	Behring Diagnostics	1:100

SMC, smooth muscle cells.
MC, monoclonal.
PC, polyclonal.
dil, dilution.
* As ascites fluids.

alpha-actin negative, but all these cells have the ultrastructural features of modulated SMCs. A preliminary report of part of this study has been published.[16]

Materials and Methods

Tissue Samples

Segments of human thoracic aorta were obtained at autopsy from 29 individuals whose ages ranged from 14 to 83 years. Relevant characteristics of the donors and postmortem intervals are summarized in Table 1. Types of atherosclerotic lesion were visually classified according to the scheme of Smith[17]; both the atherosclerotic plaque and grossly normal portion of aorta were taken. Specimens were dissected transversely. One half was embedded in OCT compound (RA Lab), frozen in isopentane cooled in liquid nitrogen, and stored at −70°C until use. The other half was used for electron microscopy or fixed in formalin, embedded in paraffin, and sectioned for hematoxylin and eosin staining.

Antisera and Antibodies

The sources and specificities of all antibodies used in this study are summarized in Table 2. Briefly, monoclonal anti-vimentin (V9)[18] and anti-desmin (DE-R-11)[19] antibodies were presented by Prof. K. Weber and M. Osborn (Max Planck Institute Biophys. Chem., Gottingen, FRG). Monoclonal antibodies (MAbs) against alpha-smooth muscle actin were characterized elsewhere.[20] Monoclonal antibodies to a surface protein of SMCs (IIG10K) were donated by M. Glukhova (Cardiology Res. Center USSR). The latter antibodies specifically stain SMCs and fibroblasts, but not endothelial cells in culture[21] or macrophages in cryostat sections of human aorta.[22] Monoclonal antibodies to muscle actins (HHF35)[23] and to macrophages (HAM56)[6] were given by Dr. Allen M Gown (Department of Pathology, University of Washington, Seattle, WA). Rabbit antisera against human smooth muscle myosin (ASMM)[15] and against chicken gizard desmin[9] were characterized previously. Rabbit antisera to human factor VIII-related antigen and human lysozyme were obtained from Behring Diagnostics Corp. Both tetramethylrhodamineisothiocyanate (TRITC)-labeled goat anti-rabbit IgG (GAR) and fluorescein isothiocyanate-labeled goat antibodies to mouse heavy and light chains of immunoglobulin (FITC-GAM) were purchased from Calbiochem-Behring, La Jolla, CA.

Immunofluorescent Staining

Serial 5-μ thick sections were cut on a cryostat and dried for 20 minutes at room temperature. Sequential sections were incubated for 1 hour at 37°C with the various MAbs diluted in phosphate-buffered saline (PBS). After washing with PBS, the sections were treated with ASMM or other polyclonal antisera for 30 to 60 minutes. The sections were washed again and incubated with FITC-GAM and TRITC-GAR added simultaneously. Labeled antibodies were used at final concentration of approximately 0.01 to 0.05 mg/ml in PBS. In some cases for demonstration of actin filaments, the sections were incubated with TRITC-labeled phalloidin (Sigma, West Germany). For negative control staining specificity we omitted the primary anti-

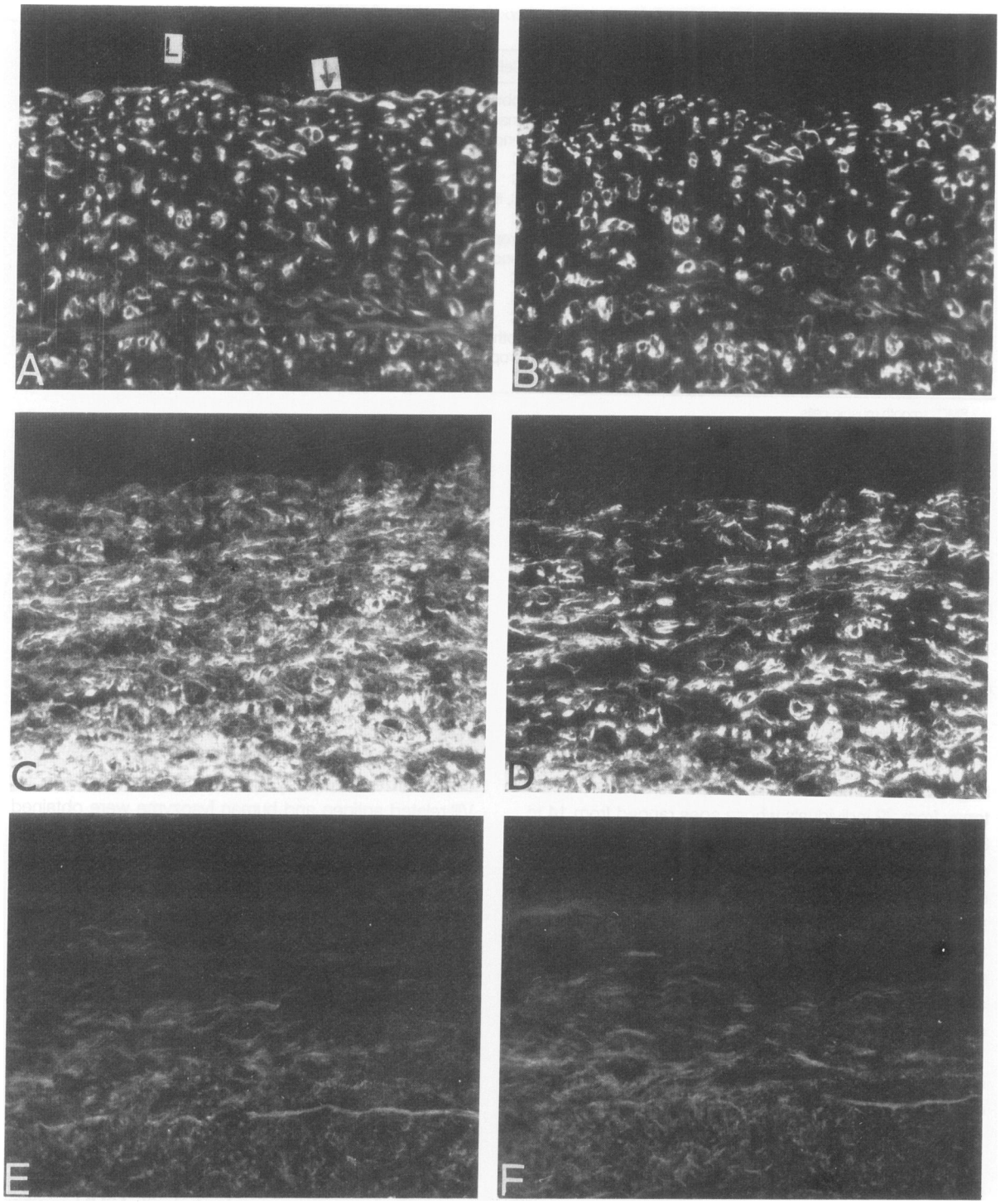

Figure 1. *Double-immunofluorescent staining of normal aortic intima for vimentin* (A), *myosin* (B), *alpha-actin* (C), *F-actin revealed by labeled phalloidin* (D), *desmin* (E), *and negative-control staining* (F). *Note endothelial cells (arrow) are positive with antibodies to vimentin but not myosin. Original magnification, ×200. L, lumen of aorta.*

body, or incubated with normal serum of the respective species. For positive controls we used the remaining slides of each set. Photographs were taken with an Opton photomicroscope equipped with a standard filter set.

Histochemistry

After fluorescent analysis, some preparations were fixed in formalin and stained for acid alpha-naphtyl esterase activity[24] or with Oil Red 0.[25]

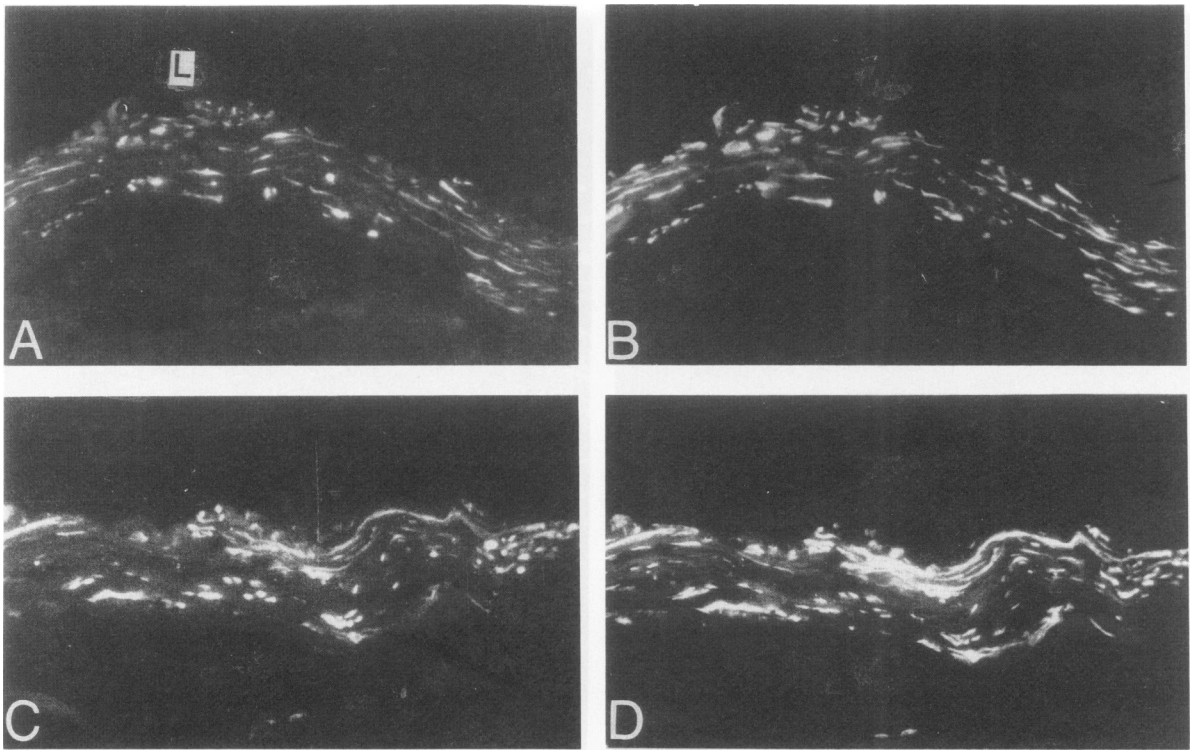

Figure 2. *Double-immunofluorescent staining of atherosclerotic plaque cap for desmin* (A), *myosin* (B), *alpha-actin* (C), *and F-actin revealed by phalloidin* (D). *Original magnification* ×200. *L, lumen of aorta.*

Transmission Electron Microscopy

Samples of normal aortic intima and plaques were washed with PBS and fixed with 2.5% glutaraldehyde in 0.1 mol/l (molar) cacodylate buffer. They were postfixed in 1% osmium tetroxide, dehydrated in ethanol and propylene oxide, and embedded in araldite resin. Special attention was paid to the plaques containing the clusters of desmin-positive and myosin-negative cells seen under immunofluorescent microscope. Selection of the plaque zone was done on the semithin sections stained with toluidine blue. Thin sections were cut on an LKB Ultratome III, stained with 2% uranyl acetate in 0.5 mol/l NaCl, and examined with a JEM 100-B electron microscope at 80 kV.

Results

Immunofluorescent staining of cryostat sections of normal human aorta revealed the presence of many vimentin-positive cells in the intima (Figure 1A). Nearly all cells, with the exception of the endothelium, stained with the antibodies to smooth muscle myosin (Figure 1B). Subendothelial cells had an elongated shape on the sections cut along the long axis of the vessel and were rather densely packed but without contacting with each other. Nearly all intimal SMCs contained alpha-actin (Figure 1C) and total actin (Figure 1D). The cells of normal intima treated with antibodies to desmin (Figure 1E) were no different from the control (Figure 1F), ie, they were desmin negative.

In all atherosclerotic plaques, cells were more dispersed then in unaffected aortic intima. Most of the plaque cells had a cytoskeletal protein expression similar to that of the SMCs of normal aortic intima. Only 7 of 28 plaques contained desmin-positive cells (Figure 2A). These cells were localized predominantly in the plaque cap and only in two cases were they found in deeper plaque layers. On longitudinal sections of the vessel, the cells appeared to be elongated, containing smooth muscle myosin (Figure 2B) and intensively stained with the antibodies to vimentin (not shown), alpha-actin (Figure 2C), and F-actin, as revealed by fluorescent phalloidin binding (Figure 2D).

At the same time, myosin-negative cells were found in 13 atherosclerotic plaques of human aorta. These cells were revealed in sections after double staining with a monoclonal antibody to vimentin (Figure 3A) and rabbit antisera to smooth muscle myosin (Figure 3B) that allowed us to identify clusters of such cells localized in the immediate proximity of an atheromatous core adjacent to the normal intima. These cells had an elongated shape, were rather thin and often densely packed, contained phalloidin-reactive actin (Figure 3C), but neither the alpha-actin isoform (Figure 3D) nor desmin (not shown).

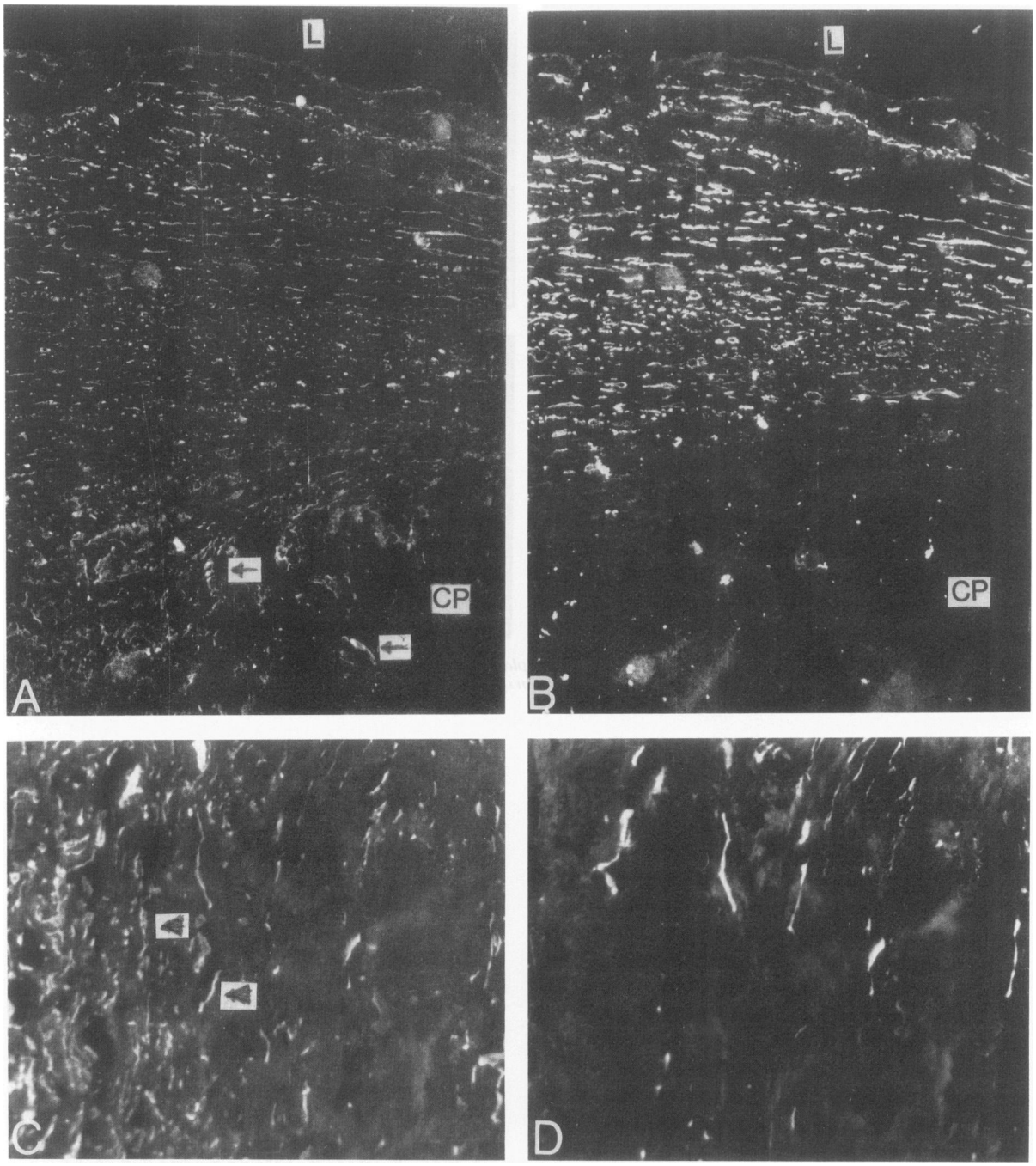

Figure 3. *Double-immunofluorescent staining of atheromatous plaque for vimentin* (A), *myosin* (B), *F-actin revealed by phalloidin* (C), *and alpha-actin* (D). *Note some cells are positive with antibody to vimentin (arrows) and contain F-actin (arrowheads) but negative with antibodies to myosin and alpha-actin, respectively. Original magnification,* A, B: ×*125,* C, D: ×*200. L, lumen of aorta; CP, core of plaque.*

Localization of myosin-negative cells in the form of clusters makes it possible to study their specific characteristics. Analysis of serial sections of the same plaques showed that the cells reacted with a monoclonal antibody specific to SMC surface protein, 11G10 (Figure 4A) that can be seen in double-staining experiment with antisera to smooth muscle myosin (Figure 4B). The antibody 11G10, however, also bound to pericytes in vasa vasorum of aortic adventitia (Figures 4C and D). At the same time, myosin-negative cells are not stained with a monoclonal antibody to muscle-actins, HHF35 (Figure 4E), and a monoclonal anti-macrophages antibody, HAM56 (Figure 4G), that were noted in double labeling with phalloidin (Figure 4F) and antisera to smooth muscle myosin (Figure

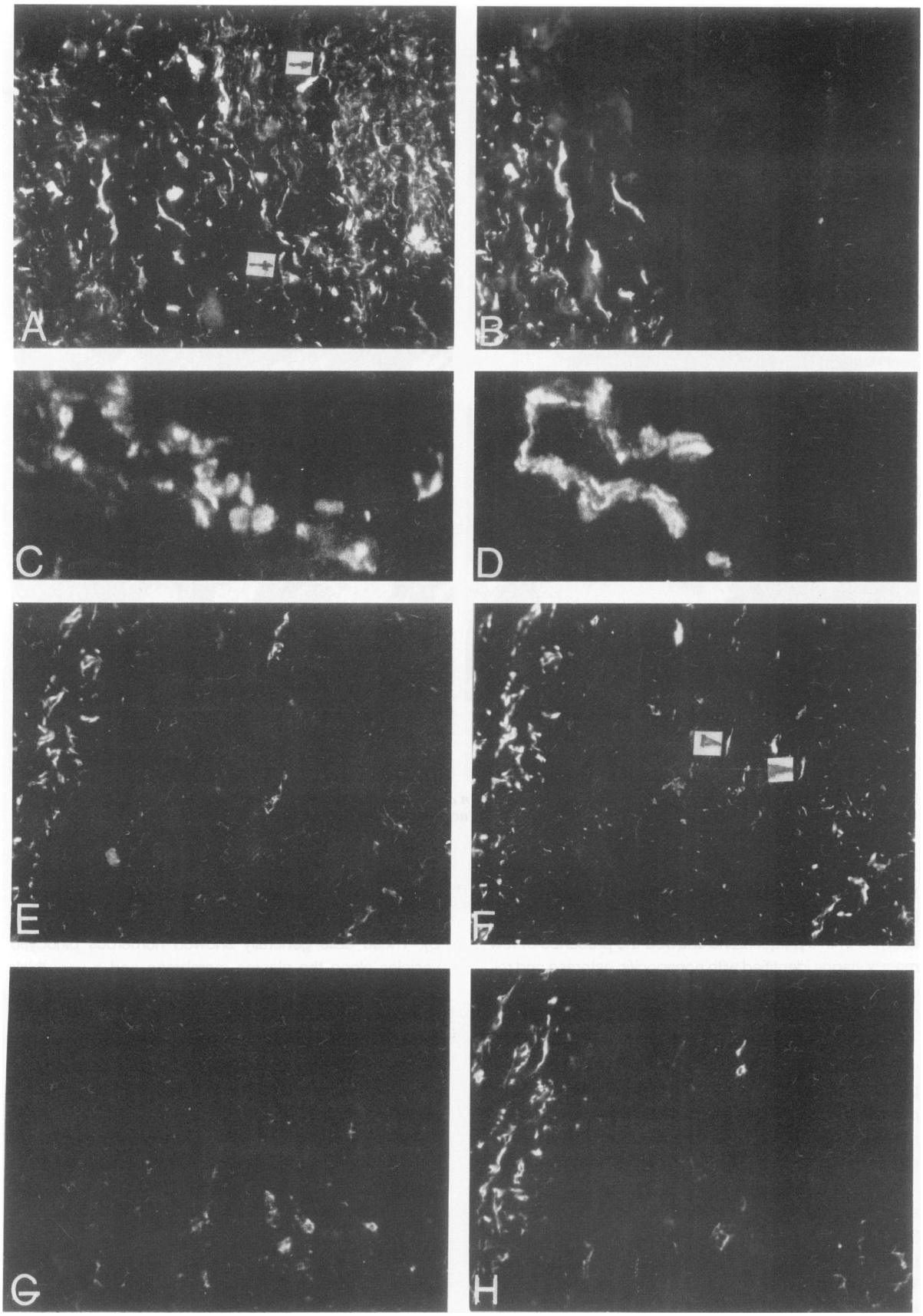

Figure 4. *Double-immunofluorescent staining of atheromatous plaque with monoclonal antibodies to SMC surface protein, IIG1O* (**A, C**), *to muscle-actin, HHF35* (**E**) *and macrophages, HAM56* (**G**) *in combination with antisera to smooth muscle myosin* (**B, D, H**) *and F-actin revealed by phalloidin* (**F**). *Note some cells are positive with antibody to SMC surface protein (arrows) and with labeled phalloidin (arrowheads) but negative with antibodies to smooth muscle myosin and muscle-actin, respectively. Original magnification,* **A, B:** ×*200;* **C, D:** ×*400; and* **E–H:** ×*125.*

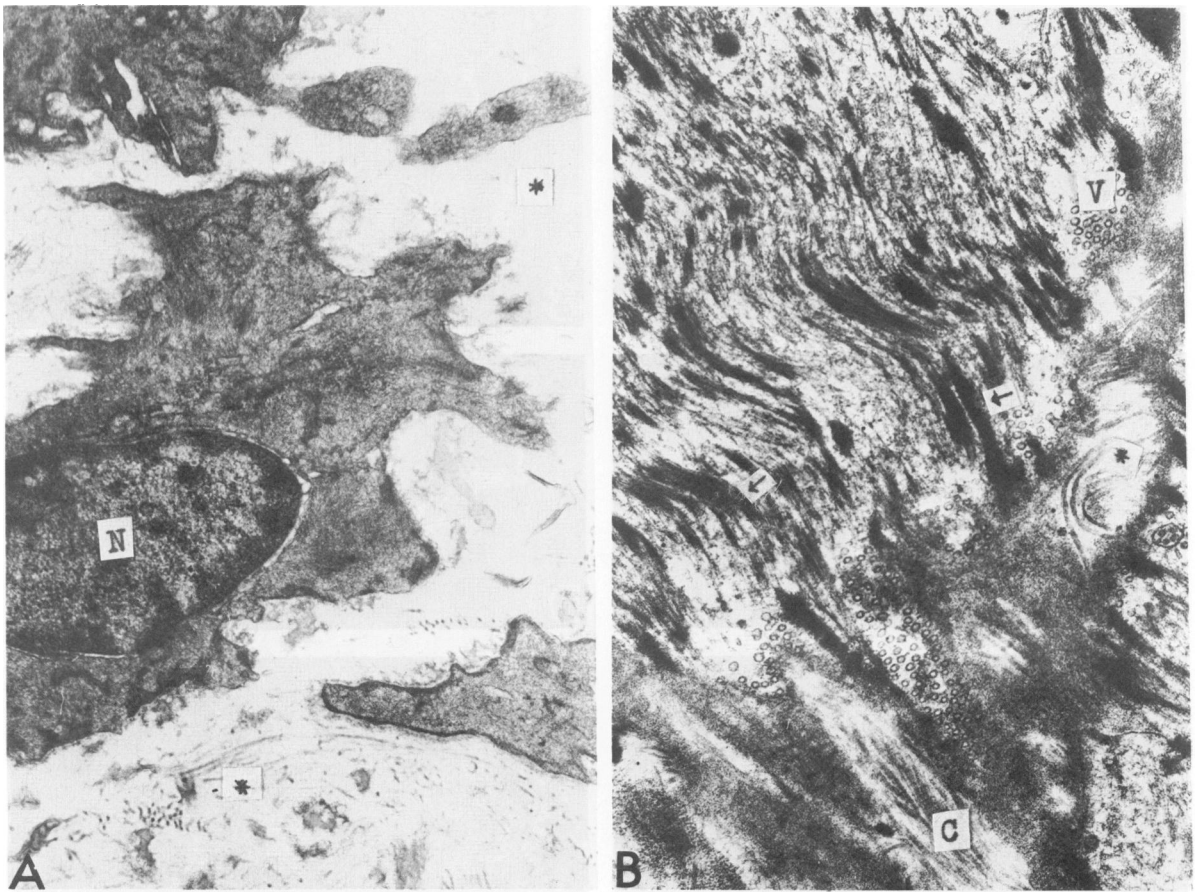

Figure 5. *Electron micrograph of SMC in normal intima of human aorta. Note tangential section of myofilament bundles (arrows). Original magnification,* A: *×12,100;* B: *×21,000. N, nucleus; V, pinocytic vesicles; C, collagens; *, extracellular matrix.*

4H), respectively. In addition, the cells did not react with the antisera to factor VIII-related antigen or with human lysozyme (data not shown). Furthermore, histochemical analysis of this plaque zone showed the presence of few macrophages identified with nonspecific esterase and relatively few cells rich in lipid inclusions, stained with oil red 0 (data not shown).

It is noteworthy that cells with these cytoskeletal changes were often found in large atherosclerotic plaques of elderly individuals (Table 1). Only in two plaques were small vessels growing in from the adjacent media detected. In all other cases no connection between plaque cells and adventitial blood vessels was found.

Electron microscopic analysis of normal aortic intima showed the cells with distinguishing characteristics of SMC. The cytoplasm of the cells was filled with myofilaments that converge into dense area (Figures 5A and B). The cells also contained very few profiles of endoplasmic reticulum in perinuclear region, number of pinocytic vesicles, and a limiting basement lamina.

In all plaque caps containing desmin-positive cells, we have found cells with elongated shape and a large size. The cells contained a number of profiles of endoplasmic reticulum and Golgi apparatus localized around the nuclei and irregularly distributed myofilament bundles concentrated mostly near the outer membrane (Figures 6A and B). These cells generally showed numerous pinocytic vesicles along the plasma membrane and a very thick basal lamina, which often consisted of several layers and merged with surrounding connective tissue fibers.

The investigation of the plaques containing myosin-negative cells revealed that the cells with cytoplasm were partially or fully filled with rough endoplasmic reticulum and a well-developed Golgi apparatus (Figures 7A to D). These cells were close to others that were characterized by the presence of few myofilament bundles, indistinct interrupted basal lamina, and multiple pinocytotic vesicles.

Discussion

Our data indicate that a subset of human atherosclerotic plaque cells exhibit a specific pattern of cytoskeletal protein expression. This particularly concerns desmin-positive cells. It is accepted that desmin is a good marker of

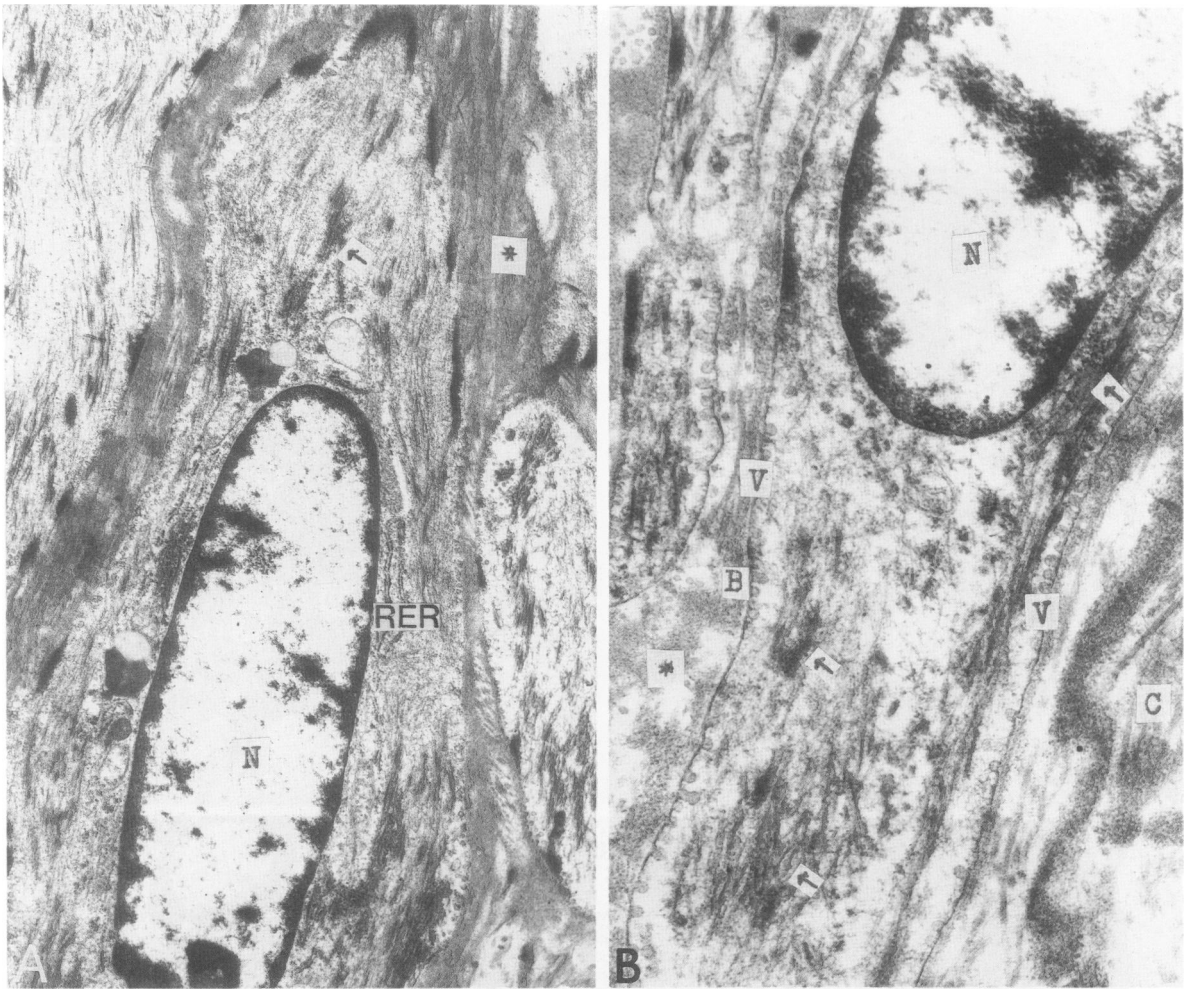

Figure 6. *Ultrastructural profiles of the cells in aortic atheromatous plaque cap. Note transparent intracellular matrix, rough endoplasmic reticulum (RER), myofilaments bundles (arrows), and basal lamina (B). Original magnification,* **A:** *×20,500;* **B:** *×31,200. Abbreviations same as in Figure 5.*

muscular differentiation,[18] but one of the characteristic features of intimal SMCs in human aorta is the absence of this protein.[26,27] Therefore it is intriguing to find desmin-positive cells in complicated[9] and developing[10] human atherosclerotic plaques. In our samples, about 25% of the examined plaques contained desmin-positive cells and all were without complications.

With respect to the expression of other cytoskeletal proteins, these desmin-positive plaque cells were no different from those of normal aortic intima. They contained vimentin, myosin, alpha-actin, and cytoplasmic actins. We consider these cells modified or modulated SMCs because they possessed an extensive ultrastructural endoplasmic reticulum along with multiple myofilament bundles. Further immunocytochemical studies at the electron microscopic level will define the structure of the cells more precisely.

In addition to the desmin-positive cells, some human atherosclerotic plaques contained cells that did not stain with the antiserum to smooth muscle myosin. Myosin-negative cells expressed vimentin and F-actin but neither desmin nor alpha-actin. In a previous study, a decrease in the content of alpha-actin was registered in atherosclerotic plaque of human aorta compared to medial SMC.[28] Our results indicate that this finding may be explained by a decreased level of this protein in a certain proportion of the plaque cells.

Ultrastructurally, myosin-negative cells may be distinguished by hypertrophic synthetic apparatus: their cytoplasm is filled with rough endoplasmic reticulum and Golgi complex. Cells with a similar ultrastructure previously found in human atheromatous plaque and were termed 'fibroblastlike' or 'nonidentified' cells.[3]

Thus, along with SMCs of normal intima and most atherosclerotic plaque cells, the human aorta may contain cells characterized by specific expression of desmin, myosin, and alpha-actin, as well as ultrastructural changes. The origin of these cells is disputable, and while the

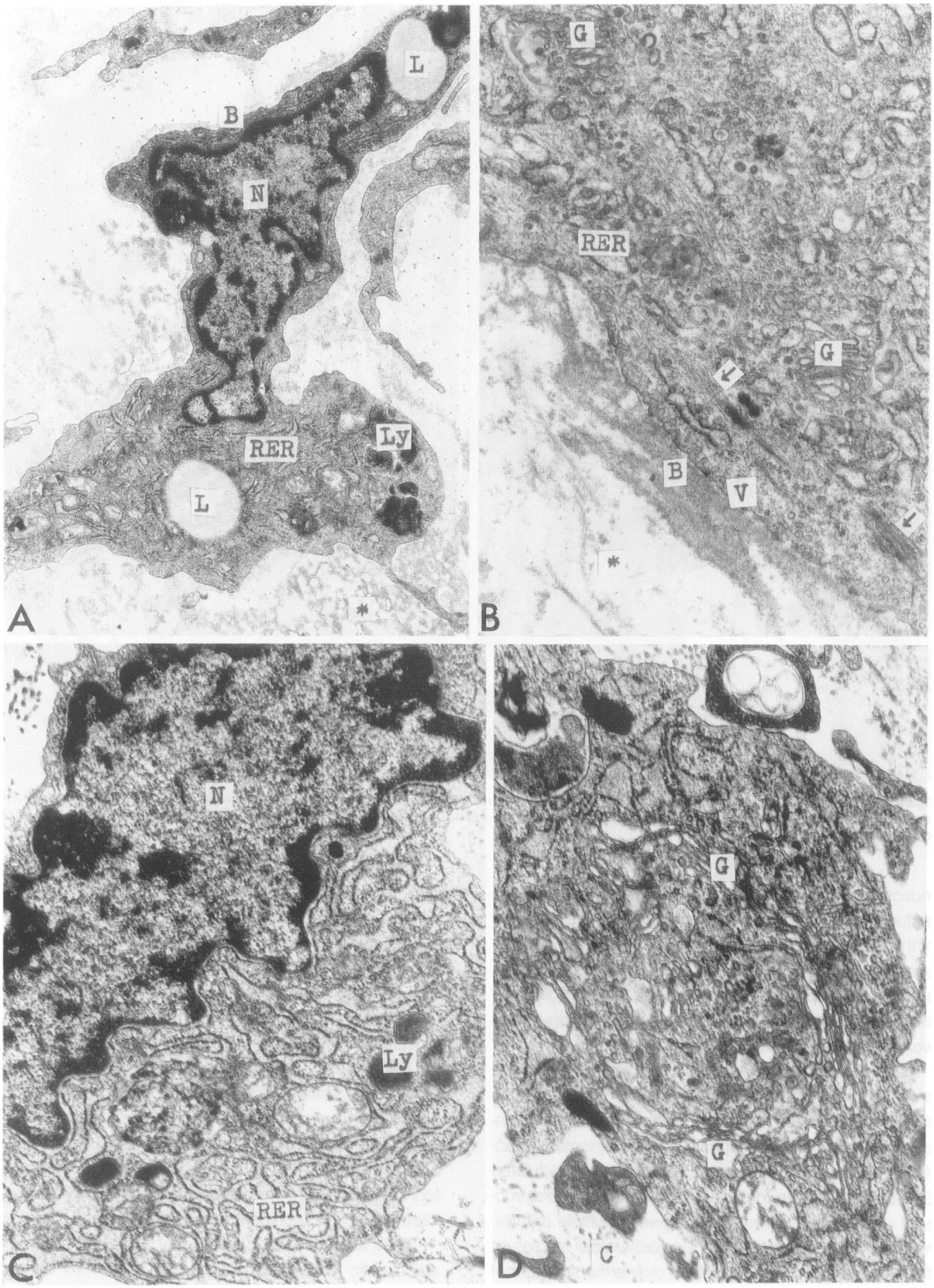

Figure 7. *Electron micrograph of human aortic athromatous plaque containing myosin-negative cells. Note well-developed rough endoplasmic reticulum (RER) and Golgi complex (G), as well as myofilaments bundles (arrows) and basal lamina (B). Original magnification,* **A**: ×*8700;* **B**: ×*29,900;* **C**: ×*25,200;* **D**: ×*24,100. Abbreviations same as in Figure 5.*

smooth muscle nature of desmin-positive cells is beyond doubt, the nature of myosin-negative cells remains unknown. The myosin-negative cells are stained with a monoclonal antibody to SMC surface protein (11G10) but did not react with anti-muscle actin antibody (HHF35) that was used as a SMC marker.[6] The HHF35 antibody has been shown to recognize the alpha and gamma actin from smooth muscle sources, but not the beta (nonmuscle) actin that is expressed by endothelial cells and fibroblasts.[23] Because myosin-negative cells have no alpha-actin, it is possible that the absence of staining of the cells by HHF35 antibody may reflect a low level of gamma actin expression that cannot be detected by indirect immunofluorescence.

At the same time, myosin-negative cells failed to react with any known specific markers of endothelial cells and macrophages. In addition, some of them retained characteristic ultrastructural features of SMC, specifically basal lamina, myofilament bundles, and abundant pinocytotic vesicles. These findings suggest that both desmin-positive and myosin-negative cells may be phenotypic variants of vascular SMCs. Similar phenotypic changes of vascular SMCs take place in primary culture when the cells modulate from a usual 'contractile' to a 'synthetic' state.[12] This process is accompanied by alterations of ultrastructure[29] and a switch in smooth muscle myosin[13,30] and alpha-actin[31,32] expression.

The factors responsible for initiation of SMC phenotypic modulation are unclear.[33] It is well known that, besides the vascular cells themselves, platelets and macrophages produce growth factors for SMCs *in vitro*[33,34] and macrophages also act to stimulate vascular SMC phenotypic change to the synthetic state.[35] The other factors playing an important role in determining the SMC phenotype appear to be local or the surrounding extracellular matrix. Recently it has been demonstrated that the adhesive glycoprotein fibronectin and laminin may control the differentiation properties of cultured arterial SMCs.[36] In our view, desmin-positive plaque cells described here differ phenotypically from the myosin-negative cells in a way that may be mediated by the presence of massive connective tissue capsules similar to those described earlier.[4,5] It has been demonstrated by immunocytochemical analysis that these capsules contain several concentric layers of fibronectin and collagen of different types.[37]

Thus alterations in the cytoskeletal protein expression and ultrastructure observed in a certain portion of atherosclerotic plaque cells of human aorta may result from a phenotypic modulation of vascular SMCs, and this process may play an important role in development of the atherosclerotic lesion.[14]

References

1. Ross R: The pathogenesis of atherosclerosis—an update. N Engl J Med 1986, 314:488–500
2. Schwartz SM, Campbell GR, Campbell JH: Replication of smooth muscle cells in vascular disease. Circ Res 1986, 58: 427–444
3. Geer JC, Haust MD: Smooth Muscle Cells in Atherosclerosis. *In* Pollak OJ, Simms HS, Kirk SE, eds. Monographs on Atherosclerosis, Vol. 2, Karger, Basel, 1972.
4. Ross R, Wight TN, Strandness E, Thiele B: Human atherosclerosis. I. Cell constitution and characteristics of advanced lesions of the superficial femoral artery. Am J Pathol, 1984, 144:79–93
5. Mosse PRL, Campbell GR, Wang ZL, Campbell JH: Smooth muscle phenotypic expression in human carotid arteries. I. Comparison of cells from diffuse intimal thickenings adjacent to atheromatous plaques with those of the media. Lab Invest 1985, 53:556–652
6. Gown AM, Tsukada T, Ross R: Human atherosclerosis. II. Immunocytochemical analysis of the cellular composition of human atherosclerotic lesions. Am J Pathol 1986, 125:191–207
7. Orekhov AN, Kalantarov GF, Andreeva ER, Prokazova NN, Trakht IN, Bergelson LD, Smirnov VN: Monoclonal antibody reveals heterogeneity in human aortic intima: Detection of a ganglioside antigen associated with a subpopulation of intimal cells. Am J Pathol 1986, 122:379–385
8. Jonasson L, Holm J, Skalli O, Gabbiani G, Hausson GK: Expression of class II transplantation antigen on vascular smooth muscle cells in human atherosclerosis. J Clin Invest 1985, 76:125–131
9. Kocher O, Gabbiani G: Cytoskeletal features of normal and atheromatous human arterial smooth muscle cells. Human Pathol 1986, 17:875–880
10. Osborn M, Gaselitz J, Puschel K, Weber K: Intermediate filament expression in human vascular smooth muscle and in arteriosclerotic plaques. Virch Archiv 1987, 411:449–458
11. Kocher O, Skalli O, Bloom WD, Gabbiani G: The cytoskeleton of rat aortic smooth muscle cells. Normal conditions and experimental initial thickening. Lab Invest 1984, 150:645–652
12. Chamley-Campbell JH, Campbell GR, Ross R: The smooth muscle cell in culture. Physiol Rev 1979, 59:1–61
13. Larson DM, Fujiwara K, Alexander RW, Gimbrone MA: Myosin in cultured vascular smooth muscle cells: Immunofluorescence and immunochemical studies of alterations in antigenic expression. J Cell Biol 1984, 99:1582–1589
14. Campbell GR, Campbell JH: Smooth muscle phenotype changes in arterial wall homeostasis: Implications for the pathogenesis of atherosclerosis. Exp Mol Path 1985, 42: 139–147
15. Babaev VR, Antonov AS, Zacharova OS, Romanov Yu A, Krushinsky AV, Tsibulsky VP, Shirinsky VP, Repin VS, Smirnov VN: Identification of intimal subendothelial cells from human aorta in primary culture. Atherosclerosis 1988, 71:45–56
16. Babaev VR, Tararak EM: Polymorphism of smooth-muscle cells of an atheromatous plaque of human aorta. Bull Exp Biol Med 1988, 106:1053–1056
17. Smith EB: The relationship between plasma and tissue lipids in human atherosclerosis. Adv Lipid Res 1974, 12:1–49

18. Osborn M, Debus E, Weber K: Monoclonal antibodies specific for vimentin. Europ J Cell Biol 1984, 34:137–143
19. Debus E, Weber K, Osborn M: Monoclonal antibodies to desmin, the muscle-specific intermediate filament protein. EMBO J 1983, 2:2305–2312
20. Skalli O, Ropraz P, Trzeciak A, Benzonana G, Gillessen D, Gabbiani G. A monoclonal antibody against—smooth muscle actin: A new probe for smooth muscle differentiation. J Cell Biol 1986, 103:2787–2796
21. Glukhova MA, Kabakov AY, Ornatsky OI, Frid MG, Smirnov VN: Monoclonal antibodies that distinguish between human aorta smooth muscle and endothelial cells. FEBS Lett 1985, 189:291–295
22. Glukhova MA, Ornatsky OI, Frid MG, Kabakov AE, Adany RR, Muszek L, Smirnov VN: Identification of smooth muscle-derived foam cells in the atherosclerotic plaque of human aorta with monoclonal antibody 11G10. Tissue Cell Res 1987, 19:657–663
23. Tsukada T, Tippens D, Gordon D, Ross R, Gown AM: HHF35, a muscle-actin specific monoclonal antibody: I. Immunocytochemical and biochemical characterization. Am J Pathol 1987, 126:51–60
24. Ennist DL, Jones KH: Rapid method for identification of macrophages in suspension by acid alpha-naphthyl esterase activity. J Histochem Cytochem 1983, 31:960–963
25. Gomori G: Organic Substances in Microscopic Histochemistry. Chicago, Chicago University Press, 1952. p. 97
26. Gabbiani G, Schmid E, Winter S, Chaponnier C, De Chastonay C, Vandekerckhove J, Weber K, Franke WW: Vascular smooth muscle cells differ from other smooth muscle cells. Predominance of vimentin filaments and a specific alpha-type actin. Proc Natl Acad Sci USA, 1981, 78:298–302
27. Frank ED, Warren L: Aortic smooth muscle cells contain vimentin instead of desmin. Proc Natl Acad Sci USA 1981, 78: 3020–3024
28. Gabbiani G, Kocher O, Bloom WS, Vandekerckhove J, Weber K: Actin expression in smooth muscle cells of rat aortic intimal thickening, human atheromatous plaque and cultured rat aortic media. J Clin Invest 1984, 73:148–152
29. Thyberg J, Nilsson J, Palmberg L, Sjolund M: Adult human arterial smooth muscle cells in primary culture. Modulation from contractile to synthetic phenotype, Cell Tissue Res 1985, 239:69–74
30. Rovner AS, Murphy RA, Owens GK: Expression of smooth muscle and nonmuscle myosin heavy chains in cultured vascular smooth muscle cells. J Biol Chem 1986, 261:14740–14745
31. Fatigati V, Murphy RA: Actin and tropomyosin variants in smooth muscles. Dependence on tissue type. J Biol Chem 1984, 259:14383–14388
32. Owens GK, Loeb A, Gordon D, Thompson MM: Expression of smooth muscle-specific alpha-isoactin in cultured vescular smooth muscle cells: Relationship between growth and cytodifferentiation. J Cell Biol 1986, 102:343–352
33. Nilsson J: Growth factors and the pathogenesis of atherosclerosis. Atherosclerosis 1986, 62:185–199
34. Ross R, Raines EW, Bowen-Pope DF: The biology of platelet-derived growth factor. Cell 1986, 46:155–169
35. Rennick RE, Campbell JH, Campbell GR: Vascular smooth muscle phenotype and growth behaviour can be influenced by macrophages in vitro. Atherosclerosis 1988, 71:35–43
36. Hedin U, Bottger BA, Forsberg E, Johansson S, Thyberg J. Diverse effects of fibronectin and laminin on phenotypic properties of cultured arterial smooth muscle cells. J Cell Biol 1988, 107:307–319
37. Krushinsky AV, Babaev VR, Romanova TG, Domogatsky SP: Organization of pericellular matrix in fibrous plaque of human aorta. Abstracts of Fifth Munster international arteriosclerosis simposium. 1988, 46(Abstr)

Acknowledgments

The authors thank Dr. Allen M. Gown from the Department of Pathology, University of Washington, for his gift of antibodies and valuable discussion of the manuscript; Drs. Klaus Weber and M. Osborn, Max-Planck Institute Biophys. Chem., Gottingen, and Dr. M. Glukhova, Cardiology Research Center, USSR, for providing us with monoclonal antibodies; and Vitaly Sirotkin for excellent technical assistance.