Virchows Arch A (1987) 411:449–458

Virchows Archiv A

Intermediate filament expression in human vascular smooth muscle and in arteriosclerotic plaques

Mary Osborn[1], **Jörg Caselitz**[2], **Klaus Püschel**[3], **and Klaus Weber**[1]

[1] Max Planck Institute for Biophysical Chemistry, D-3400 Göttingen, Federal Republic of Germany
[2] Institute of Pathology, University of Hamburg, Martinistrasse 52, D-2000 Hamburg, Federal Republic of Germany
[3] Institute of Forensic Medicine, University of Hamburg, D-2000 Hamburg, Federal Republic of Germany

Summary. Different regions of human aorta and of other human arteries obtained at autopsy were analyzed with regard to their topography and to the different stages of arteriosclerosis. Material was studied by immunocytochemical techniques with antibodies specific for either desmin (D) or for vimentin (V), the two types of intermediate filament proteins present in vascular smooth muscle cells. In normal arteries endothelial cells as well as the adjacent intimal cells were D−V+. In the media D+V+ as well as D−V+ cells were present, with the relative numbers of each cell type dependent on the particular blood vessel. When cells in arteriosclerotic plaques at different stages of development were examined an occasional plaque showed cells of the D+V+ type. In the majority of plaques however the cells were V− D+. In plaques where severe ulceration and necrotic material was present D−V+ cells were found at the border of the lesion: foam cells when they could be identified appeared to be D−V+.

Key words: Vascular smooth muscle – Arteriosclerosis – Atherosclerosis – Vimentin – Desmin – Foam cells

Introduction

In blood vessels in general three coats surrounding the lumen are visible: the internal tunica intima, the intermediate tunica media, and the external tunica adventitia. The tunicae have different functions and are each associated with different major cell types. Thus the intima, responsible for exchange with the blood, is characterized by the presence of endothelial cells and of a small layer of subendothelial "stromal cells", the media which is involved in the control of the diameter of medium and small vessels and the mechanical integrity of large ones, contains large numbers of smooth muscle cells and the adventitia, responsible for nourishing and protecting the vessel, contains connective tissue cells, nerve cells and smaller blood vessels (vasa privata).

The different cell types present in blood vessels can be distinguished by morphological, functional and immunocytochemical assays. Morphological studies have shown that the relative thickness of the different tunicae varies with the type of blood vessel and its location, while functional studies have distinguished contractile cells from the so-called metabolic cells (Ross and Glomset 1973; Simionescu and Simionescu 1977; Chamley-Campbell et al. 1979). Immunohistochemical studies using antibodies to intermediate filaments have shown that while human endothelial cells contain only the vimentin type of intermediate filaments (Franke et al. 1979), vascular smooth muscle cells both in situ and in culture can be either desmin positive/vimentin positive (D+V+) or desmin negative/vimentin positive (D−V+) (Osborn et al. 1981; Schmid et al. 1982; Frank and Warren 1981). The relative ratio of these two vascular smooth muscle cell types seems to depend both on the mammalian species and on the blood vessel under study (e.g. Frank and Warren 1981; Gabbiani et al. 1981). Thus in rat aorta the ratio of D+V+ to D−V+ cells in aorta was low distal to the brachiocephalic artery but high in the distal parts of the aorta, the common iliac artery and in certain other vessels (Osborn et al. 1981). In rabbit vascular smooth muscle, cells of the abdominal aorta and the main pulmonary artery were D− V+, while in some other vessels D+V+ cells were found (Berner et al. 1981). It was further suggested

Offprint requests to: M. Osborn

that low numbers of the D+V+ type might be typical of elastic arteries while muscular arteries and some other blood vessels might be characterized by large numbers of the same cell type. Other proteins have also been shown to vary along the aorta. Thus for instance gradients of collagen and of elastin gene expression have been noted in porcine aorta (Davidson et al. 1985). Again such studies suggest that not all vascular smooth muscle cells are phenotypically equivalent.

The current study was initiated several years ago to try to clarify two questions. First, is there a different distribution pattern of intermediate filament proteins in the cells of different human blood vessels? Second does the composition of intermediate filaments change during development of a human arteriosclerotic plaque? After this study was completed, a study by Kocher and Gabbiani (1986) directed to answering similar questions has appeared.

Materials and methods

Normal human arterial tissue and arteriosclerotic plaques were collected at autopsy from four individuals (male 34 years, male 50 years, male 65 years, male 67 years). Autopsies were performed as soon as possible and not later than ten hours after death. Additionally, blood vessels from an amputated leg from a female 70 years were analysed, since this case represented an advanced stage of arteriosclerosis. Different regions of the aorta as well as specimens from several larger arteries were selected for study. These included the ascending aorta, thoracic aorta, abdominal aorta, femoral, popliteal, carotid, renal and pulmonary arteries.

Some arteries showed slight, moderate or severe arteriosclerotic lesions which were identified macroscopically. Since we were interested in basic features of arteriosclerosis a relatively simple classification scheme was used. Plaques were classified according to Hudson (1965) as: Type 1. fatty streaks, dots or patches. Type 2. pale, raised, smoother fibrous plaques containing a variable amount of fat. Type 3. Plaques showing ulceration; calcification, haemorrhage or thrombosis (complicated lesions). The type 3 plaques thus belong to the "second" type of arteriosclerosis according to Doerr (1978). Some 15 individual plaques were examined during this study. Figures 1–3 (with the exception of Fig. 2a–c) are from vessels of a 34 year old male, Fig. 2a–c and Fig. 4 from the amputated leg.

The blood vessels were cut into short segments and frozen in isopentane cooled in liquid nitrogen to −140° C. They were then stored at −70° C until use. Sections 5 µm in thickness were cut on a cryostat at −15° C and after transfer to glass slides were dried for 30–90 min at room temperature. Sections were then either stained with toluidine blue, or were processed for immunofluorescence microscopy with antibodies to desmin and vimentin (see below). Specimens were often additionally stained with Hoechst dye 33242 to reveal the position and number of the nuclei. Using this method the number of cells in a given region of the aorta could be evaluated. Toluidine blue staining was used to select arterial plaque material for the immunocytochemical studies.

Primary antibodies were antigen-affinity-purified and used at final concentrations of 20–50 µg/ml.

1. Rabbit desmin antibody elicited with desmin purified from chicken gizzard was purified on chicken desmin coupled to Sepharose 4B. This antibody recognizes desmin in an immune blot (Osborn et al. 1981).
2. Guinea pig vimentin antibody elicited with vimentin purified from mouse 3T3 cells was affinity-purified on vimentin purified from rabbit chondrocytes. This antibody has been shown to recognize vimentin but not desmin in an immune blot.

Murine monoclonal antibodies used in a few experiments included

1. Desmin antibody (DEB5) raised against desmin purified from porcine stomach. This antibody recognizes desmin and not vimentin in immunoblotting experiments (Debus et al. 1983).
2. Vimentin antibody (V9) raised against vimentin purified from porcine eye lens. This antibody recognizes vimentin and not desmin in immunoblotting experiments (Osborn et al. 1984).

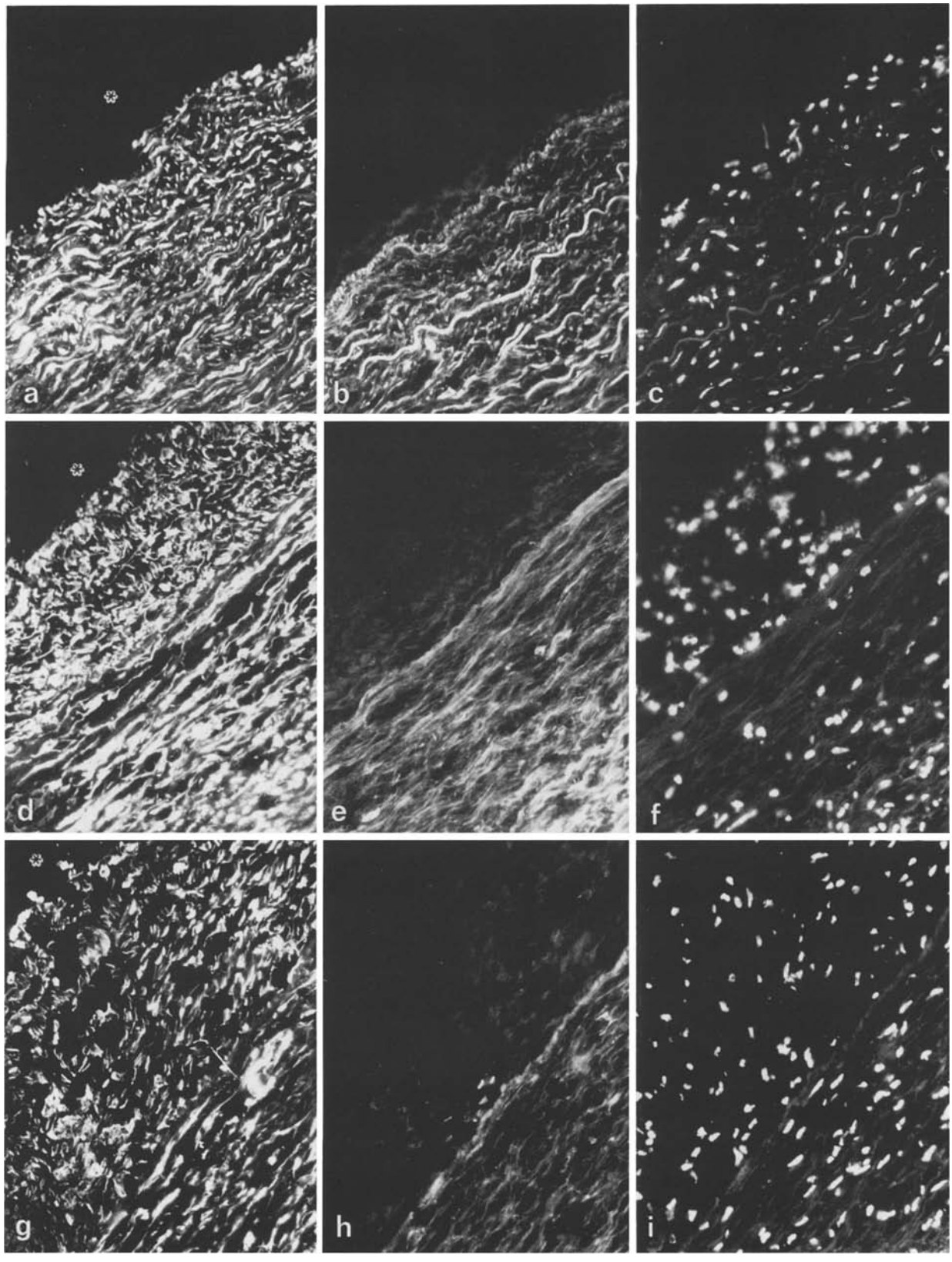
V9 and DEB5 can be purchased from commercial sources (Amersham, Little Chalfont, Bucks, UK, Boehringer Mannheim, FRG, Dako, Glostrup, Denmark, ICN Biochemicals, Lisle, IL, USA, Oncogene Science, Manhasset, NY, USA).

Fluorescently labeled second antibodies were used at final concentrations of approximately 0.4 mg/ml in phosphate-buffered saline. They were FITC-labeled goat anti-rabbit IgGs and rhodamine-labeled goat anti-guinea pig IgGs (Cappell Laboratories, Cochranville, PA, USA).

Indirect immunofluorescence microscopy was carried out on sections prepared as above, fixed in acetone at −10° C for 6–10 min and then air-dried. 5–10 µl of the desmin or vimentin antibodies was added to each section. The sections were incubated at 37° C for 45–60 min and then washed with phosphate-buffered saline. 5–10 µl of the second antibody carrying the fluorescent tag was then added. The sections were incubated at 37° C for 45–60 min and again washed with phosphate-buffered saline before being mounted in Mowiol 4-88 (Hoechst, Frankfurt, FRG). For double label immunofluorescence microscopy used in the illustrations shown in this manuscript procedure C from Osborn et al. (1980) was used. Vimentin and desmin antibodies were added simultaneously. After the wash step rhodamine-labeled goat anti-guinea pig IgGs and fluorescein-labeled goat anti-rabbit IgGs were also added simultaneously. Controls in which the first antibody was omitted showed that the level of non-specific staining was low and was limited to the elastic fibers of some arteries. This staining can be clearly distinguished from the positive staining of cells of the intima and media with the intermediate filament antibodies.

In DNA staining with Hoechst 33242 the stock solution contained 1 mM Hoechst 33242 in 25% ethanol, 75% phosphate buffered saline. After the fluorescence procedure given above was completed, a drop of this stock solution was added to the specimens. After a further incubation for 10 min at room temperature, excess Hoechst solution was removed by draining

Fig. 1. Sections from different parts of human aorta stained in double immunofluorescence microscopy both with antibodies to vimentin (**a, d, g**) antibodies to desmin (**b, e, h**) and with Hoechst dye to reveal the nuclei (**c, f, i**). (**a–c**) Ascending aorta, (**d–f**) thoracic aorta, (**g–i**) abdominal aorta. Thus for example (**a–c**) show the same section of ascending aorta stained with vimentin antibodies (**a**), desmin antibodies (**b**), and Hoechst (**c**). Note that the intima indicated by I on the left of the figure is thickest



in the abdominal aorta. Note also that in the media regions shown many cells appear to be D+V+, but in the intima the cells are D−V+. The elastic fibers are often autofluorescent and can be used to align the different staining patterns. The lumen (*) is in the upper left corner of each micrograph. All micrographs, ×150

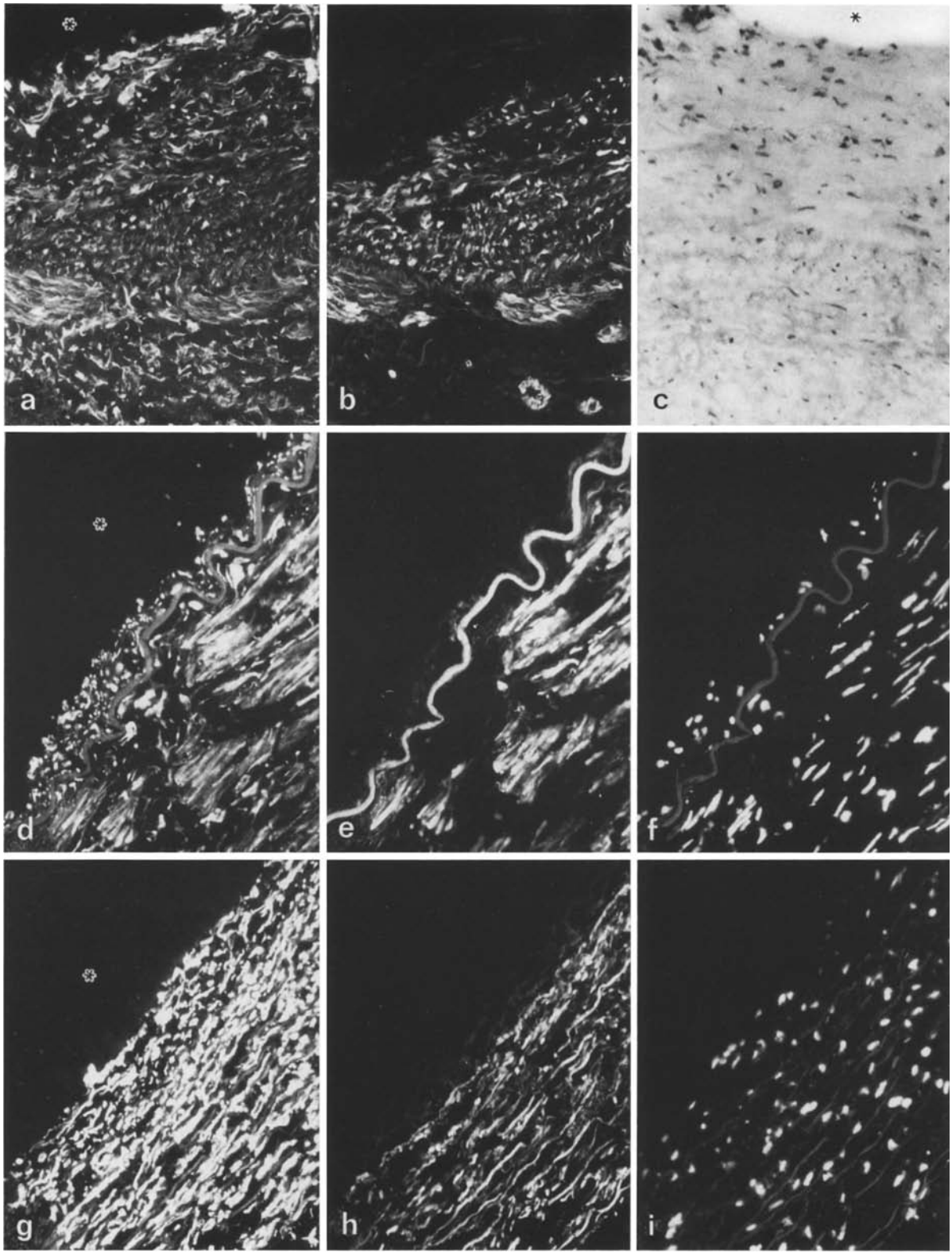


Fig. 2. Sections of different human arteries stained in double immunofluorescence microscopy with antibodies to vimentin (**a, d, g**) and antibodies to desmin (**b, e, h**) and with Hoechst (**f, i**), **c** Shows a parallel section to **a, b** stained with toluidine blue. (**a–c**) Femoral artery, (**d–f**) renal artery, (**g–i**) pulmonary artery. Thus for example **d–f** shows the same section of renal artery stained with vimentin antibodies (**d**), desmin antibodies (**e**) and Hoechst (**f**). Note the relatively thin lining of D−V+ cells near the lumen (*) in (**a**), and in the intima in (**d**) and (**g**). Note also that many cells in the media region shown are desmin-positive, and that the number of desmin-positive cells in this region differs depending on the artery selected for study. Autofluorescent elastic fibers are visible in some micrographs. All micrographs, × 150

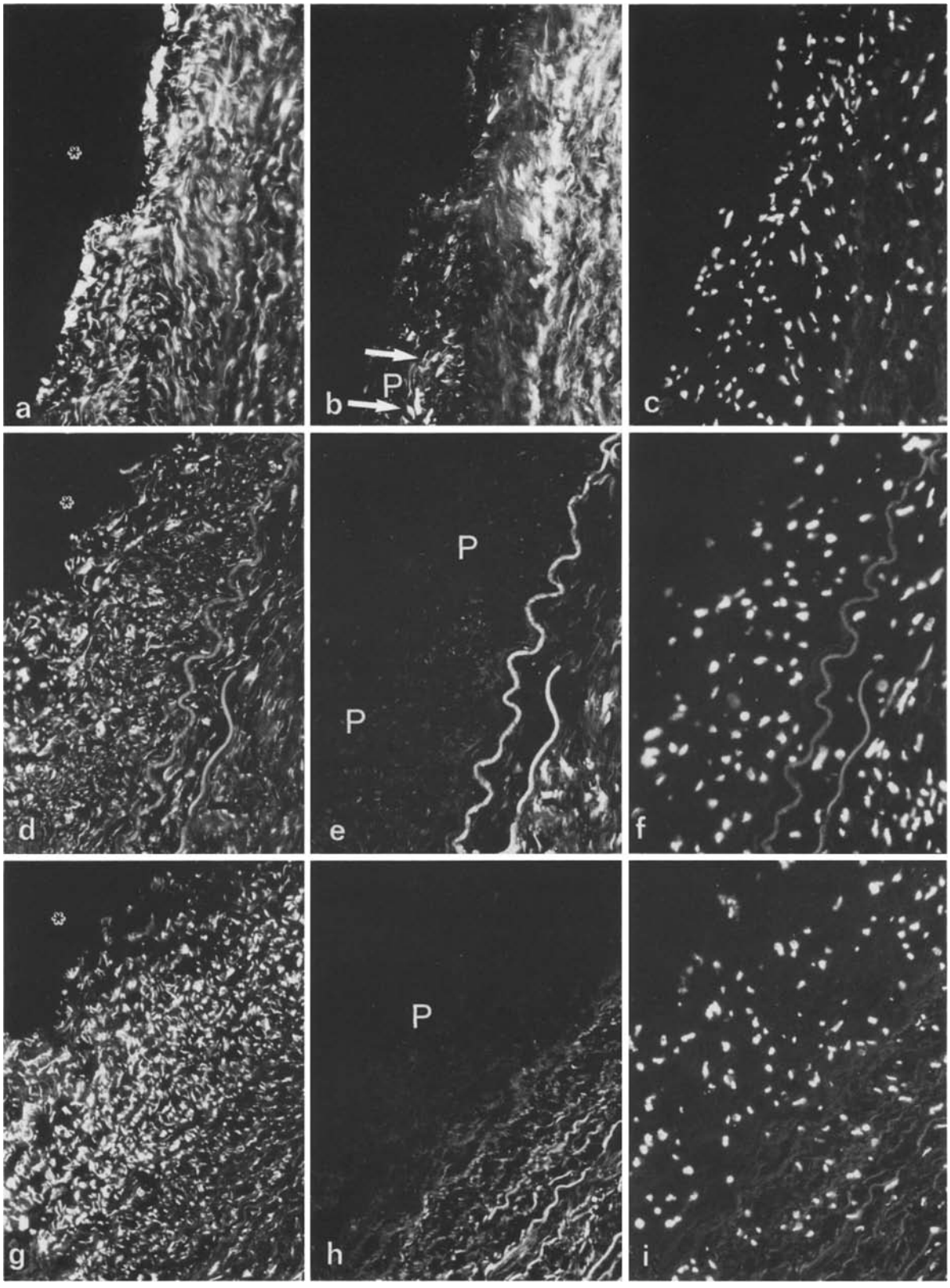


Fig. 3. Sectioned type I and type 2 human arteriosclerotic plaques. (**a–c**) Shows an early arteriosclerotic plaque in the aorta abdominalis stained for vimentin (**a**), desmin (**b**), and with Hoechst (**c**). Note the thickening of the intima associated with the development of the plaque shown by the Hoechst stain. Note that this type I plaque contains some cells that are positive for desmin and vimentin (arrows in **b**) as well as other cells that are only vimentin-positive. (**d–i**) Shows two type 2 arteriosclerotic plaques stained for vimentin (**d, g**), desmin (**e, h**) and with Hoechst (**f, i**). (**d–f**) Is a plaque in the femoral artery, and (**g–i**) a plaque in the ascending aorta. Note the increase in thickness associated with the plaque region, and that in both plaques almost all cells are D−V+. P plaque region. All micrographs, × 150

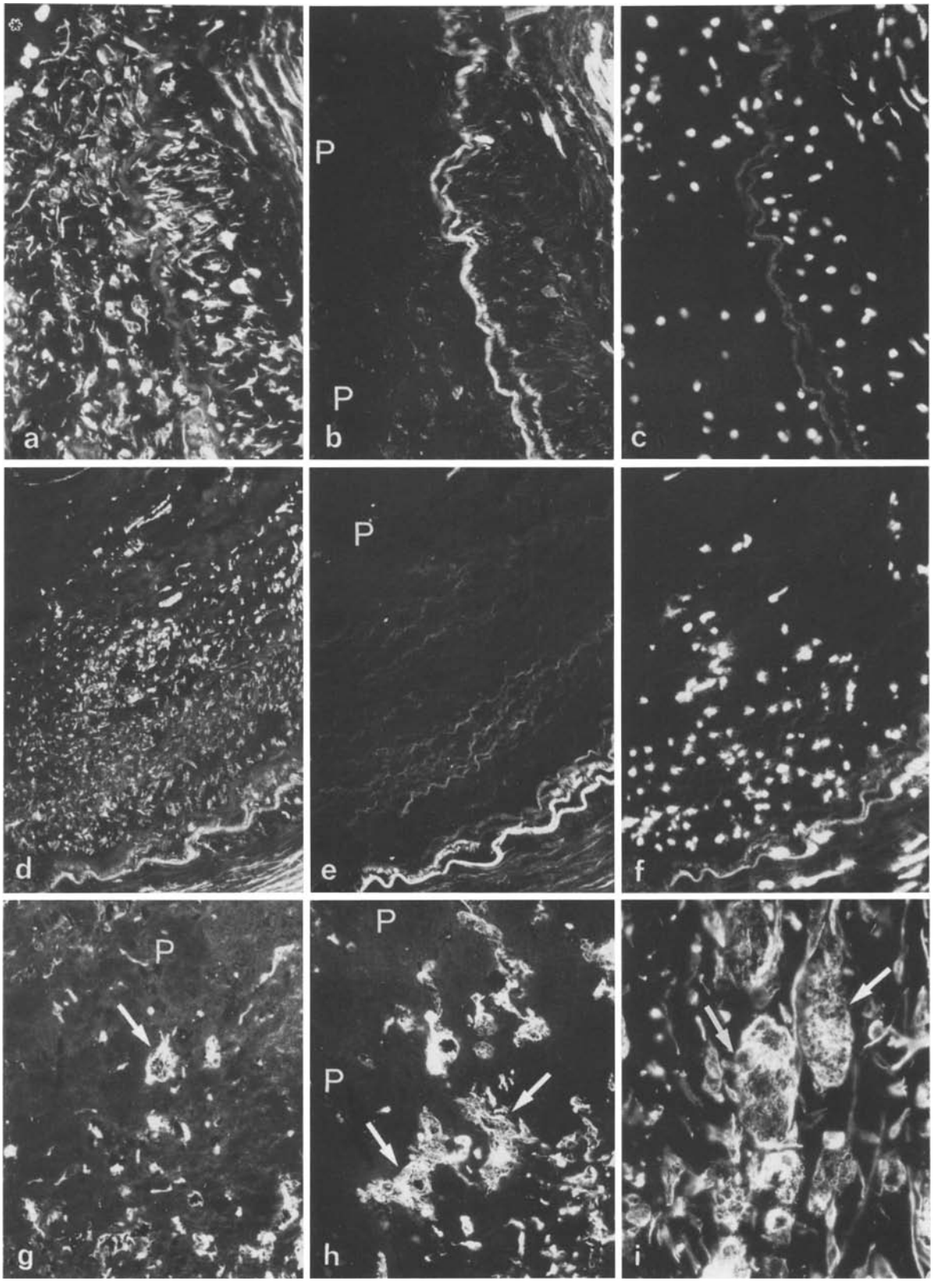


Fig. 4. Sectioned material from advanced stage human arteriosclerotic plaques. **(a–c)** and **(d–f)** Show two type 3 plaques which occluded the popliteal artery stained for vimentin **(a, d)**, desmin **(b, e)** and with Hoechst **(c, f)**. Note the decreased density of cells in the plaques at this advanced stage compared to the type I and type 2 plaques in Fig. 3, and the focal augmentation

the slide on filter paper, and the specimen was mounted directly in Mowiol 4-88 without an intervening wash. Specimens were examined with Planapo Zeiss lenses and the appropriate filters to allow separation of Hoechst, fluorescein and rhodamine fluorescence. Most photographs were made with a 16× lens to try to obtain an overview.

Results

Human arteries are often subdivided into two types: elastic and muscular arteries (for review see Simionescu and Simionescu 1977). Here we have used the aorta as a representative of the elastic type and the renal and femoral arteries as representatives of the muscular type. However, this distinction between the two artery types is not absolute and the transition between them is often gradual. In addition the pulmonary artery was selected as an example of an artery of the low pressure system.

Different regions of normal human aorta are illustrated in Fig. 1. The ascending aorta is shown in Fig. 1 a–c, the thoracic in Fig. 1 d–f, and abdominal in Fig. 1 g–i. These sections were made from the aorta of a single individual. In each instance the section was stained in double label fluorescence microscopy with antibodies to vimentin and antibodies to desmin and the position of the nuclei was revealed using Hoechst 33242. In the section of the ascending aorta, taken above the aortic valves, the endothelial cell layer can be easily distinguished and is vimentin positive and desmin negative i.e. D−V+. A narrow zone of intimal stromal cells is visible beneath the endothelial cells and these are D+V+ (Fig. 1 a). The border to the media is not distinct but within the media the network of elastic fibers and smooth muscle cells can be distinguished. Most of the smooth muscle cells appear both vimentin (Fig. 1 a) and desmin (Fig. 1 b) positive i.e. D+V+. In the aorta thoracalis (Fig. 1 d–f) and aorta abdominalis (Fig. 1 g–i) the tunica intima is thicker than in the region of the aorta close to the heart (compare for instance Fig. 1 a, d). The media is more distinct in these regions and is separated from the intima by the lamina elastica interna. The smooth muscle cells in the portion of the media shown are again mostly of the D+V+ type.

The vimentin and desmin distribution in human arteries other than the aorta are illustrated in Fig. 2 a–i. The femoral artery is shown in Fig. 2 a–c, and the renal artery in Fig. d–f. Again the endothelial cells retained in the section and the subendothelial intimal cells are D−V+. The relative ratios of cells in the media that are D+V+ to those that are D−V+ appears to be different in the two arteries. Thus a higher proportion of cells seem to coexpress desmin and vimentin in the femoral artery than in the renal artery.

The pulmonary artery belongs to the low pressure system and at its beginning is an elastic artery. The cells of the intimal layer are D−V+, whereas in the media cells that are D+V+ were found intermingled with elastic fibers which were autofluorescent in this specimen.

Arteriosclerotic plaques were located by macroscopical examination and classified according to Hudson (1965). The existence of a plaque suitable for further study was confirmed later at the light microscopical level by toluidine blue staining. Sections through the plaques were cut and stained in double immunofluorescence microscopy with both vimentin and desmin antibodies and additionally treated with the Hoechst dye 33242 which is specific for DNA and therefore reveals the position and number of nuclei (Figs. 3 and 4).

Fatty streaks (type 1 plaques) are characterized by an increase in the thickness of the intima giving rise to the so-called intimal oedema. This is followed by an increase in the number of subendothelial or stromal cells present in the intima. This situation is clearly revealed by the use of the Hoechst DNA stain. In most plaques examined in this study almost all such cells were of the D−V+ type. However a few early plaques were noted in which intimal cells of the D+V+ type were clearly present. One such plaque is documented in Fig. 3 a–c, where coexpression of desmin and vimentin in some of the cells can clearly be seen (arrows Fig. 3 b).

The arteriosclerotic plaque of type 2 (Hudson 1965) is increased in thickness when compared with the early plaque and the number of cells present in the plaque is increased (cf. the Hoechst stains shown in Fig. 3 c, f). In such plaques (Fig. 3 d–f, g–i) almost all the cells seem to contain only vimentin i.e. they are of the D−V+ type.

The complicated arteriosclerotic plaque (type 3 according to Hudson 1965) is characterized by large lipid accumulations and ulceration. This

of vimentin-positive, desmin-negative cells in **f** at the border of these plaques. (**g–i**) Show the focal augmentation of vimentin-positive cells at the border of an arteriosclerotic plaque. Note the networks of vimentin filaments in the foam cells identified in all three micrographs by arrows; (**g**), (**h**) from plaques in the aortic arch. (**i**) From a plaque in the abdominal aorta. P plaque region. Micrographs **a–f** ×150, **g, h** ×240, **i** ×380

property sometimes makes immunofluorescence microscopy difficult as autofluorescence is often encountered in the calcified plaque. In the plaques shown in Fig. 4a–c and 4d–f which occluded the femoral artery the number of cells in the plaque is reduced as shown by the Hoechst stain (Fig. 4c, f). At the border of such plaques, a similar cellular distribution of vimentin and desmin is seen as in the type 2 plaques i.e. the cells are of the D−V+ type.

In some plaques foam cells were found. These were always very strongly vimentin positive (cf. Fig. 4g–i). When examined in double label immunofluorescence microscopy they appeared to be desmin negative. Thus, for example, in the desmin channel the cells in Fig. 4g and h showed no staining (data not shown).

Discussion

The results on different regions of the human aorta documented in Fig. 1 show for the media that the ratio of D+V+ cells to D−V+ is different in different regions of the aorta. In our study the D+V+ cells in the media often appeared more numerous in the region of the media closest to the lumen; a finding which was also seen in an earlier study of different regions of rat aorta (Osborn et al. 1981). The artery walls in man are, however, much thicker than those of the rat; for instance reconstructions over the whole width of the artery wall for human the ascending aorta or for human abdominal aorta would occupy more than the page length when printed at the same magnification as Fig. 1. Thus when only the regions closest to the intima are visualized as in the figures selected to illustrate this manuscript, the longitudinal gradient of D+V+ cells is not so apparent. Our results also seem to show that muscular arteries such as the femoral artery and the renal artery have a higher percentage of medial smooth muscle cells that are of the D+V+ type than do arteries of the elastic type e.g. the thoracic aorta. In this connection we note the recent data of Kocher and Gabbiani (1986) showing that after dissection and enzymatic digestion 4%, 11% and 37% of medial smooth muscle cells are D+V+ in samples of human thoracic aorta, coronary artery and femoral artery respectively. Further systematic studies are needed to compare the extent of the longitudinal gradient in the human relative to the rat and to determine whether there is a correlation with the different pressure exerted on different vessels.

Arteriosclerosis is, as others have stressed, a complicated disease process, where the clinical manifestations result from the interplay of a large number of different processes (Constantinides 1986; Ross and Glomset 1973, 1976; Ross 1986; Schwartz et al. 1986; Doerr 1963; Lindner 1985). In its early stages it is characterized by cells which appear to proliferate abnormally leading to an increase in the number of cells in the subintimal layer and in consequence an elevation of the intimal layer, visible on the inside of the artery as a white streak (Ross and Glomset 1976; Schwartz et al. 1986). Investigation of the cells in such plaques shows that while in the normal arteries we studied endothelial cells, and almost all cells of the subintimal layer were D−V+, in a few type I plaques some D+V+ cells were found (e.g. Fig. 3a–c). In the type 2 and type 3 plaques, we studied almost all cells appeared of the D−V+ type (e.g. Figs. 3d–i, 4a–f). Finally in complicated lesions huge masses of collagen and lipid with calcium deposits are present. The few cells found in or adjacent to such plaques seem to have only vimentin. When foam cells could be identified, almost all appared to be D−V+. This IF content is consistent with a monocyte derived macrophage origin for foam cells since macrophage IFs are of the vimentin type. The IF content is also consistent with an adaptation of a smooth muscle cell of the D−V+ type for the specific lipid scavenging and storage functions usually attributed to foam cells. However results from other groups in which monoclonal antibodies specific for antigens of either macrophages or smooth muscle cells have been used on foam cells would seem to support an origin from macrophages for the vast majority of foam cells (Aqel et al. 1985; Klurfeld 1985; Roessner et al. 1986). In the case of the adipose differentiation of 3T3 cells, dramatic rearrangements of the vimentin filaments have been documented and it has been demonstrated that the vimentin filaments encircle and contain the lipid droplets (Franke et al. 1987). It will be interesting to see if, as suggested by figures such as 4g–i the same is true for foam cells. Our results on IF typing of cells in arteriosclerotic plaques complement and extend those of others. In intimal thickenings induced by balloon catheterization of rat aorta a high content of vimentin but not desmin was demonstrated by gel electrophoresis (Gabbiani et al. 1982; Kocher et al. 1984). More recently the same laboratory has suggested on grounds of immunofluorescence microscopy that some cells in plaques may also coexpress desmin (Kocher and Gabbiani 1986).

Interpretation of the results is complicated by the fact that although it has been known for some years that vascular smooth muscle cells can be ei-

ther D−V+ or D+V+ (<1% are +V−D+), the relation between these two cell types and the factors that influence a possible transition between them are not understood. It is not known for instance whether the D−V+ cells can differentiate into D+V− cells under appropriate in vivo or in vitro stimulation, or alternatively whether the two cell types represent two separate and unrelated cell populations. It is also not clear, in spite of many in vitro studies of animal model systems, whether one or both vascular smooth muscle cell types can contract. To reconcile our results and those of others on normal arteries, where no D+V− cells are found in the intima, with the intermediate filament protein content of cells in the occasional arteriosclerotic plaque where some D+V− cells are found two origins for such cells can be considered (Ross 1986; Schwartz et al. 1986). First D+V− cells could migrate from the media through holes in the elastic interna and could then begin to replicate within the subintimal layer. Alternatively the change could occur initially in some cells of the intimal layer causing them to switch on desmin expression. Other plaques may originate directly from the D−V+ type of vascular smooth muscle cells. It might be expected that plaques containing desmin might be found more frequently in regions of the aorta with a high percentage of D+V+ smooth muscle cells in the media. Indeed Molony et al. (1986) have quantitated the changes that occur in vimentin and desmin expression in the intima in different blood vessels using rabbits fed cholesterol. In the thoracic aorta the vimentin content was preferentially increased, while in the abdominal aorta the relative content of both desmin and vimentin was increased. Such results are consistent with the monoclonal hypothesis for the development of atherosclerosis (Benditt and Benditt 1973) which has recently received some support by the demonstration that human atherosclerotic plaques contain active oncogenes and that growth factors may play an additional role (Penn et al. 1986; Scott 1987). Regardless of the origin of the D+V+ cells, as the plaque develops the prediction is that cells of the D−V+ type replicate preferentially, a hypothesis for which there is some limited experimental support from in vitro studies (Travo et al. 1982). The preferential replication of D−V+ cells is also in line with recent experiments showing that while in the media α smooth muscle actin is the predominant actin isoform present, in the fibrous plaque and in replicating smooth muscle cells in vitro the β isoform predominates (Gabbiani et al. 1981, 1984; Kocher and Gabbiani 1986b). These results again therefore stress that the palette of cytoskeletal proteins may be very different in the plaque and in the normal aortic media, and therefore that studies on such proteins may help understand some of the processes associated with the initiation and development of arteriosclerosis.

Acknowledgement. We thank Sabine Schiller and Suzanne Isenberg for expert technical assistance.

References

Aqel NM, Ball RY, Waldmann H, Mitchison M (1985) Identification of macrophage and smooth muscle cells in human atherosclerosis using monoclonal antibodies. J Pathology 146:197–204

Benditt EP, Benditt JM (1973) Evidence for a monoclonal origin of human atherosclerotic plaques. Proc Natl Acad Sci USA 70:1753–1756

Berner PF, Frank E, Holtzer H, Somlyo AP (1981) The intermediate filament proteins of rabbit vascular smooth muscle: immunofluorescent studies of desmin and vimentin. J Muscle Res Cell Motility 2:439–452

Chamley-Campbell J, Campbell GR, Ross R (1979) The smooth muscle cell in culture. Psysiol Rev 59:1–61

Constantinides P (1984) Ultrastructural Pathobiology. Elsevier, N.Y.

Davidson JM, Hill KE, Mason ML, Giro MG (1985) Longitudinal gradients of collagen and elastin gene expression in the porcine aorta. J Biol Chem 260:1901–1908

Debus E, Weber K, Osborn M (1983) Monoclonal antibodies to desmin, the muscle-specific intermediate filament protein. EMBO J 2:2305–2312

Doerr W (1963) Perfusionstheorie der Arteriosklerose. Georg Thieme, Stuttgart

Frank ED, Warren L (1981) Aortic smooth muscle cells contain vimentin instead of desmin. Proc Natl Acad Sci USA 78:3020–3024

Franke WW, Hergt M, Grund C (1987) Rearrangement of the vimentin cytoskeleton during adipose conversion: Formation of an intermediate filament cage around lipid globules. Cell 49:131–141

Franke WW, Schmid E, Osborn M, Weber K (1979) The intermediate sized filaments of human endothelial cells. J Cell Biol 81:570–580

Gabbiani G, Schmid E, Winter S, Chaponnier C, de Chastonay C, Vandekerckhove J, Weber K, Franke WW (1981) Vascular smooth muscle cells differ from other smooth muscle cells: predominance of vimentin filaments and a specific α-type actin. Proc Natl Acad Sci USA 78:298–302

Gabbiani G, Rungger Brandle E, de Chastonay C, Franke WW (1982) Vimentin containing smooth muscle cells in aortic intimal thickening after endothelial injury. Lab Invest 47:265–269

Gabbiani G, Kocher O, Bloom WS, Vandekerckhove J, Weber K (1984) Actin expression in smooth muscle cells of rat aortic intimal thickening, human atheromatous plaque and cultured rat aortic media. J Clin Invest 73:148–152

Hudson REB (1965) Cardiovascular pathology. Vol. 1. Edward Arnold, London

Klurfeld DM (1985) Identification of foam cells in human atherosclerotic lesions as macrophages using monoclonal antibodies. Arch Pathol Lab Med 109:405–449

Kocher O, Gabbiani G (1986a) Expression of actin mRNAs in rat aortic smooth muscle cells during development, exper-

imental intimal thickening, and culture. Differentiation 32:245–251

Kocher O, Gabbiani G (1986b) Cytoskeletal features of normal and atheromatous human arterial smooth muscle cells. Hum Pathol 17:875–880

Kocher O, Skalli O, Bloom WS, Gabbiani G (1984) Cytoskeleton of rat aortic smooth muscle cells. Normal conditions and experimental thickening. Lab Invest 50:645–652

Lindner J (1985) New morphological and biochemical aspects of atherosclerosis. Pathol Res Pract 180:329–335

Molony L, Hagen P-O, Schachat FH (1986) Intermediate filament heterogeneity in normal and hypercholesterolemic rabbit vascular smooth muscle cells. Exp Cell Res 163:78–86

Osborn M, Caselitz J, Weber K (1981) Heterogeneity of intermediate filament expression in vascular smooth muscle: A gradient in desmin positive cells from the rat aortic arch to the level of the arteria iliaca communis. Differentiation 20:196–202

Osborn M, Debus E, Weber K (1984) Monoclonal antibodies specific for vimentin. Eur J Cell Biol 34:137–143

Osborn M, Franke WW, Weber K (1980) Direct demonstration of the presence of two immunologically distinct intermediate-sized filament systems in the same cell by double immunofluorescence microscopy: vimentin and cytokeratin fibers in cultured epithelial cells. Exp Cell Res 125:37–46

Penn A, Garte SJ, Warren L, Nesta D, Mindich B (1986) Transforming gene in human atherosclerotic plaque DNA. Proc Natl Acad Sci USA 83:7951–7955

Roessner A, Vollmer E, Zwadlo G, Sorg C, Greve H, Grundmann E (1986) Differentiation of macrophages and smooth muscle cells with monoclonal antibodies in the arteriosclerotic plaque of the human aorta. Verh Dtsch Ges Pathol 70:365–370

Ross R (1986) The pathogenesis of atherosclerosis – an update. N Engl J Med 314:488–500

Ross R, Glomset JA (1973) Atherosclerosis and the arterial smooth muscle cell. Science 180:1332–1339

Ross R, Glomset JA (1976) The pathogenesis of atherosclerosis. N Engl J Med 295:369–377

Schmid E, Osborn M, Rungger-Brändle E, Gabbiani G, Weber K, Franke WW (1982) Distribution of vimentin and desmin filaments in smooth muscle tissue of mammalian and avian aorta. Exp Cell Res 137:329

Schwartz S, Campbell GR, Campbell JH (1986) Replication of smooth muscle cells in vascular disease. Circulation Research 58:427–444

Scott J (1987) Oncogenes in arteriosclerosis. Nature 325:574–575

Simionescu N, Simionescu M (1977) In: Weiss L, Gneep RO (eds) Histology McGraw Hill, NY, p 373

Travo P, Weber K, Osborn M (1982) Coexistence of vimentin and desmin type intermediate filaments in a subpopulation of adult rat vascular smooth muscle cells growing in primary culture. Exp Cell Res 139:87–94

Accepted June 22, 1987