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Distribution patterns of apolipoproteins A_1, A_2, and B in the wall of atherosclerotic vessels*

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Summary. Atherosclerotic vessels were analysed histochemically for distribution, quantity, and composition of apolipoprotein (Apo) types in the vascular wall. The specimens comprised all stages of atherosclerosis, from very discrete intimal changes to complicated lesions. The vessel specimens were marked with antibodies against human Apo A_1, A_2, and B. Apo A_1 can be demonstrated in even the earliest stage of atherosclerosis, and increases with the progression of the disease. In the initial stage, Apo A_1 is found first in lumen-adjacent layers of the intima, and is evident in deeper layers of the wall as the disease progresses. Arteries of muscular type show accumulation of Apo in an earlier stage (or in greater quantity at the same stage) than arteries of elastic type. At all stages, the amount of Apo A_1 always exceeds that of A_2 and B. In the intima, Apo B is higher than Apo A_2, the media contains hardly any Apo B, and the adventitia has less B than A_2. Within the intimal layer, Apo A_1 and A_2 are found in an intracellular (mainly in foam cells) or in an extracellular location, according to the stage of atherosclerosis. Apo B is almost exclusively extracellular; only cases of advanced atherosclerosis show some intracellular localization (mostly in foam cells), visualized as electron dense lamellar organelles, probably of lysosomal origin. In the media, Apo A_1 and A_2 are accumulated in intracellular deposits, whereas the extracellular storage of Apo A_1, A_2 and B is observed only in cases with the most severe damage. Our investigations suggest that the accumulation of apolipoproteins in the vascular wall is effected not only by insudation from the plasma, but also by neosynthesis and/or metabolism by locally derived cells or cells immigrating in the process of atherosclerosis. The presence of Apo A_1 and A_2 in the vessel wall is now documented, and their role at this site apparently differs from that in the plasma.

Key words: Atherosclerosis – Apolipoprotein A_1, A_2, B – Immunohistochemistry

Introduction

That cholesterol plays a leading role in the genesis and progression of atherosclerosis is undisputed. Epidemiological studies have defined hypercholesterolaemia as an essential risk factor for the ischaemic sequelae of atherosclerosis (Lipid Research Clinics Program 1984; Patsch 1987). In coronary heart disease hypercholesterolaemia is seen as a risk factor of the first order, with hypertension and nicotine abuse (Assmann 1982). From numerous experimental data and data from patients with familial hypercholesterolaemia, the increase of cholesterol levels in the blood alone is known to provoke atherosclerotic changes in the absence of any other risk factors.

Cholesterol and other plasma lipids, being insoluble in water, are bound to proteins in the liver or bowel to form lipoprotein complexes. These are transported to the recipient organs via the blood. Such lipoprotein complexes differ with regard to their composition and proportion of apoproteins (Apo) and lipids. The lipoprotein fractions most relevant for cholesterol metabolism and atherogenesis are the low-density and high-density lipoproteins (LDL and HDL). About two-thirds of the transport of serum cholesterol is effected by LDL and one third by HDL (Patsch 1987). LDL carries the highest cholesterol content (about 50%) among the lipoproteins; it is seen as the main supplier of cholesterol to cells. The intake of cholesterol by cells is effected by linking LDL to specific receptors on their surface (Goldstein and Brown 1977), which recognize LDL by its protein component, Apo B. HDL, in comparison, has a distinctly lower cholesterol content but the highest level of Apos, and so has the capacity to bind surplus cholesterol. HDL is thought to act as an effective cholesterol

* Dedicated to Professor E. Grundmann on the occasion of his 70th birthday

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acceptor capable of bringing back excess cholesterol from the periphery to the liver (reverse cholesterol transport) (Mahley 1981; Brown and Goldstein 1983), where it can be eliminated as bile acid. Cholesterol uptake by HDL is actually regulated by Apo A_1: it activates the enzyme lecithin-cholesterol-acyl-transferase, which is capable of turning free cholesterol into an ester taken in by the HDL particle.

According to modern lipid theories, the genesis and progression of atherosclerotic lesions depends not only on the absolute amount of cholesterol-bearing lipoproteins circulating in the plasma, but also on the ratio of their components (Gotto and Jackson 1978; Avogaro et al. 1979; Stössel 1980). HDL linked to Apo A_1 has an inhibiting effect while LDL linked to Apo B has a promoting effect on atherosclerosis, as has been confirmed by many investigations (Gordon et al. 1977; Vergani et al. 1978; Avogaro et al. 1978; Miller 1980; Maciejko et al. 1983; van Stiphout et al. 1986). These studies, however, refer only to the plasma levels of HDL and LDL, whereas their actual distribution in the tissues of atherosclerotic lesions is still poorly understood and has seldom been investigated.

The following study was designed to identify the exact location of Apo A_1, A_2 and B in the arterial wall during different stages of atherosclerosis. Since these three proteins are known to be the main structural protein components of HDL and LDL, morphological and morphometric data should permit conclusions on the local metabolic pathways of the two lipoproteins responsible for cholesterol transport.

Table 1. Criteria for semiquantitative classification of the apolipoprotein content in different layers of the arterial wall

a) Intima and adventitia:		
−	No deposits	
+	Few deposits	(<75)
++	Moderate deposits	(≥75)
+++	Many deposits	(≥150)
++++	Excessive deposits	(≥225)
b) Media:		
−	No deposits	
+	Few deposits	(<25)
++	Moderate deposits	(≥25)
+++	Many deposits	(≥50)
+++	Excessive deposits	(≥75)

(+), in both layer categories, few samples were in class +; the majority were in class −

Table 2. Morphological features of atherosclerosis in consecutive stages

Stage I
Intimal oedema and/or fibrous thickening Fatty streaks with macrophage foam cells Thinly dispersed extracellular lipid particles
Stage II
Preatheroma with increased numbers of smooth muscle cells in confluent extracellular lipid pools
Stage III
All changes as in stage II, with additional signs of the complicated lesions, such as ulceration, thrombosis, calcification

Materials and methods

Our investigation comprised 55 arteries of different types: 32 samples of aorta (elastic type), and 23 of coronary arteries (muscular type). The specimens originated from autopsy material excised immediately post mortem. Aortic tissue was taken at diaphragmatic level, segments of coronaries from the proximal 2 cm of the anterior descending branch of the left coronary artery. Immunohistochemical and light-microscopical work-up was applied to formol-fixed, paraffin-embedded material using the double indirect alkaline phosphatase techniques. Ultrastructural examination used Lowicryl-K4M-embedded material and a protein-A-gold technique.

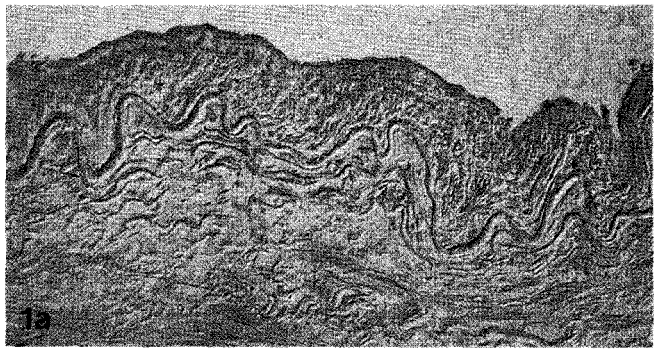
For investigation at the light-microscopical level, primary polyclonal antibodies against human Apo A_1 (dilution 1:30000), A_2 (dilution 1:20000) or B (dilution 1:20000) were raised from sheep (Boehringer, Mannheim, FRG). For detection of "mature" tissue macrophages the monoclonal antibody 25-F-9 was applied (Zwadlo et al. 1985). For immunoelectron microscopy we used a polyclonal rabbit antibody against prefixed Apo A_1 (Harrach and Robenek 1990) and a polyclonal antibody against Apo B (Sigma, Munich, FRG).

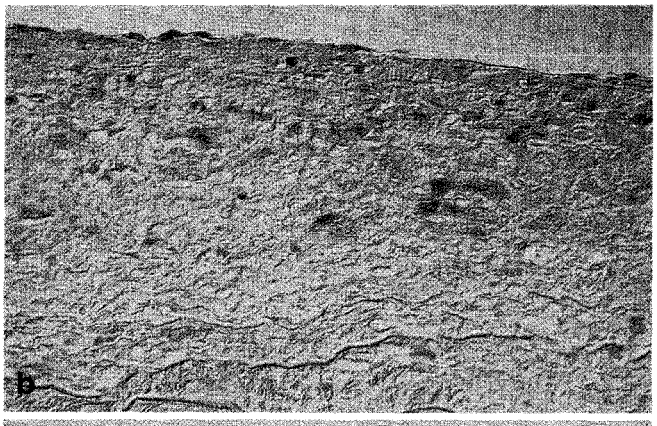
The detection system for light microscopy consisted of rabbit-antisheep-immunoglobulin coupled to alkaline phosphatase 1:25, and goat-antirabbit coupled to alkaline phosphatase 1:100 (Dianova, Hamburg, FRG) with admixture of neofuchsin solution and naphthol AS-BI phosphoric acid by simultaneous suppression of endogenous alkaline phosphate by levamisole.

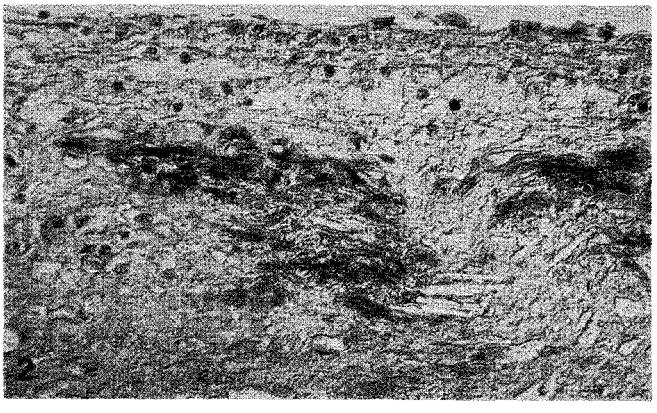
For immunohistochemical double labelling, we first employed an indirect peroxidase technique (rabbit-antimouse peroxidase labelled 1:150; Dianova, Hamburg, FRG), then the alkaline phosphatase method.

Semiquantitative evaluation started with a light microscopical survey for the selection of representative areas comprising all three layers of the arterial wall. Apo deposits labelled by immunohistochemical staining were counted fieldwise in 250-fold magnification, with separate documentation for intima, media, and adventitia. In intima and adventitia, the Apo content of the individual samples ranged from 0 to 300 deposits per field; the following classification therefore appeared reasonable for semiquantitative assessment of A_1, A_2 and B (Table 1a). In the media, the apolipoprotein contents of preparations was in a distinctly lower range, between 0 and 100 deposits per field, so that we chose the classification outlined in Table 1b for semiquantitative evaluation. We wish to emphasize that the same "class" or "symbol" would describe an Apo content in the media that is about three times lower than in the intima or adventitia. A common classification adaptable to all three wall layers would imply a confusingly large number of classes preventing the clear presentation of results.

The criteria for histological staging of the samples according to the degree of atherosclerotic vascular changes are listed in Table 2. Since none of the proposed terminologies of atherosclerotic vascular lesions has been universally accepted (Stary 1990a), we looked for a more manageable and reliable system. In view of our number of cases, we did not want to adopt the descriptive classification of Stary (1987, 1990b) and attempted a simple three-step system using pathoanatomical and clinical aspects (Haimovici 1977). The proportion of cases (aortic/coronary lesions) in the stages was found as follows: Stage I, 17 (10/7), Stage II, 19 (11/8), Stage III, 19 (10/9). More detailed substaging would provide too much division of the data base in semiquantitative analysis.







Results

In carrying out the *qualitative* analysis we found that with the first signs of atherosclerosis, in contrast to unaffected vessels (Fig. 1a), a few deposits of Apo are observed in the intima, localized extracellularly in the connective tissue matrix of subendothelial areas near the lumen (Fig. 1b). Any involvement of unequivocally subendothelial foam cells or other local cells at such an early stage is excluded.

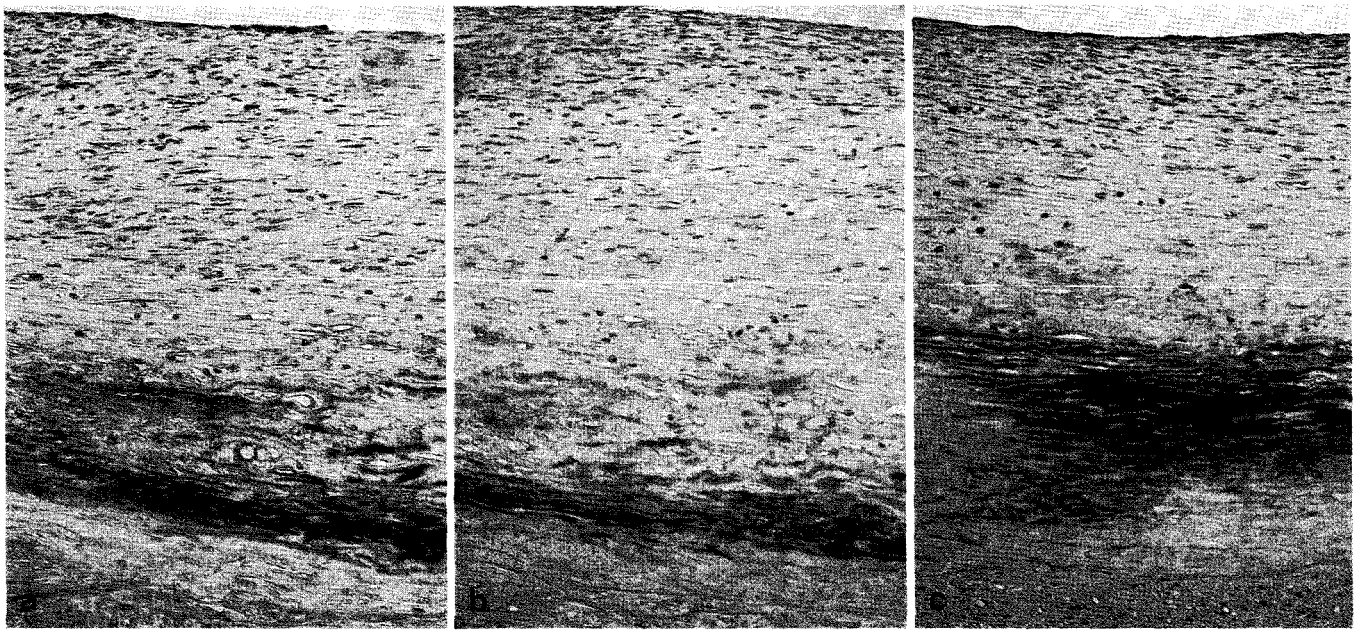
With increasing intimal thickening and swelling of connective matrix, Apo deposits spread to the deeper parts of the intima (Fig. 2). At this stage, the media is still uninvolved, but with the progression of atherosclerotic changes, the localization pattern of the three Apos undergoes a distinct alteration in the distribution of Apo A_1 and A_2 and that of Apo B.

In the intima of our stage II, Apos accumulate preferentially in the basal layers of the plaque near the internal elastic lamina (Fig. 3a–c). Apart from extracellular Apo deposits, Apo A_1 and A_2 may also be found intracellularly, in the spindle-shaped cells of the fibrous cap and in the foam cells (Figs. 3a, b, 4a). Occasionally deposits are also seen in the smooth muscle cells of the inner

Fig. 1a, b. Labelling with anti-apolipoprotein (Apo) A_1 antibody: **a** no atherosclerosis; **b** atherosclerosis stage I, with a few deposits in the intima. a, b, ×400

Fig. 2. Stage I of atherosclerosis: abundant deposits of Apo A_2 are seen in distinctly thickened intima, also involving the deeper intimal strata. ×400

Fig. 3a–c. Stage II of atherosclerosis in a muscular-type artery with distinct accumulations of Apos in the deeper intimal strata adjacent to the internal elastic lamina, with partial formation of foam cells. Apo B is found only in extracellular localization (**c**), Apo A_1(**a**) and A_2(**b**) are found additionally in intracellular deposits, predominantly in foam cells, but also in spindle-shaped elements. a, b, c, ×300



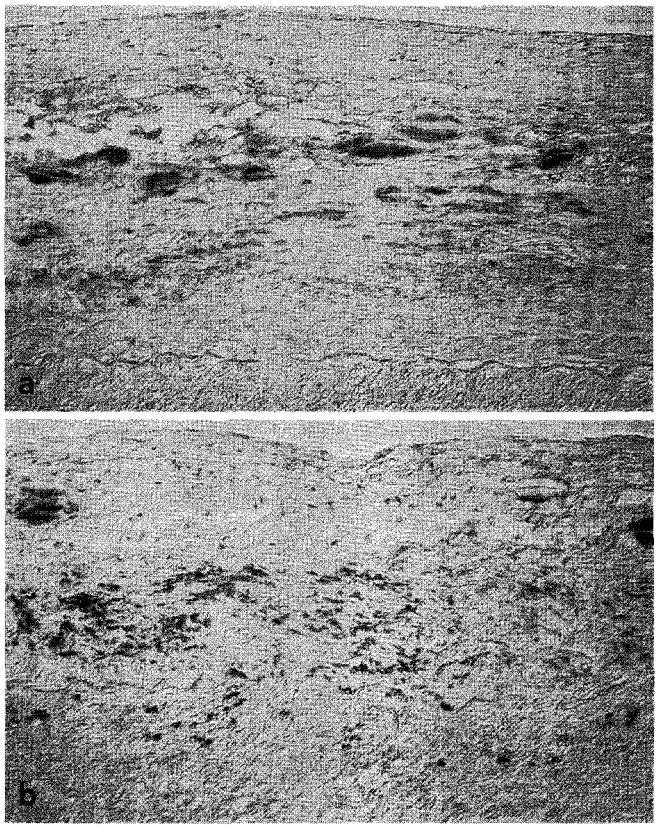


Fig. 4a, b. Apo A_1 in numerous foam cells (**a**) in contrast to exclusively extracellular accumulations of Apo B (**b**) in a distinctly thickened intima of atherosclerosis stage II. a, b, ×400

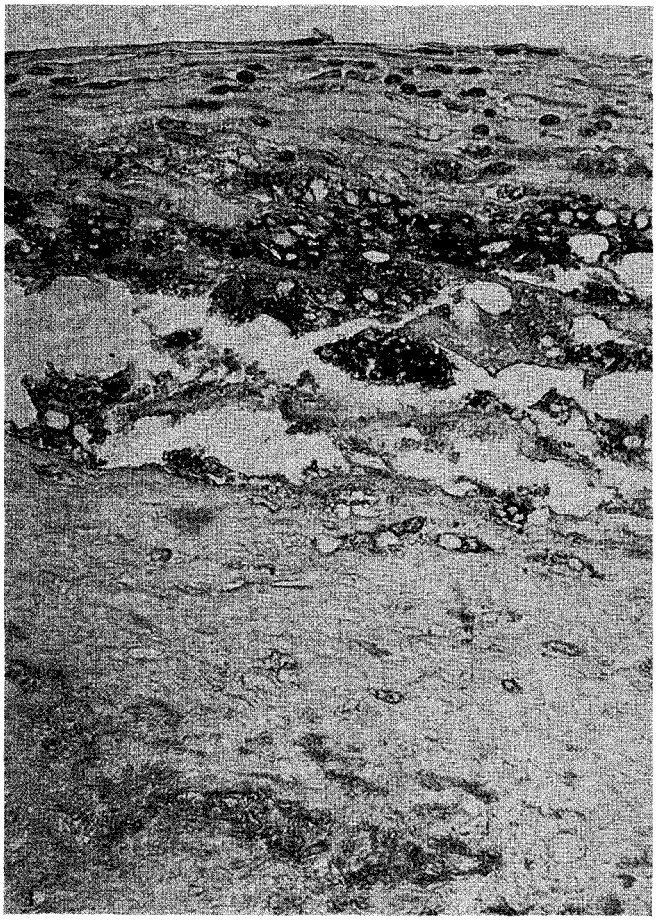


Fig. 5. Immunohistochemical double labelling: antibody 25-F-9 against mature tissue macrophages (*brown*) and antibody against Apo A_1 (*red*, intra- and extracellular location). Mixture of red and brown colouring in foam cells. ×400

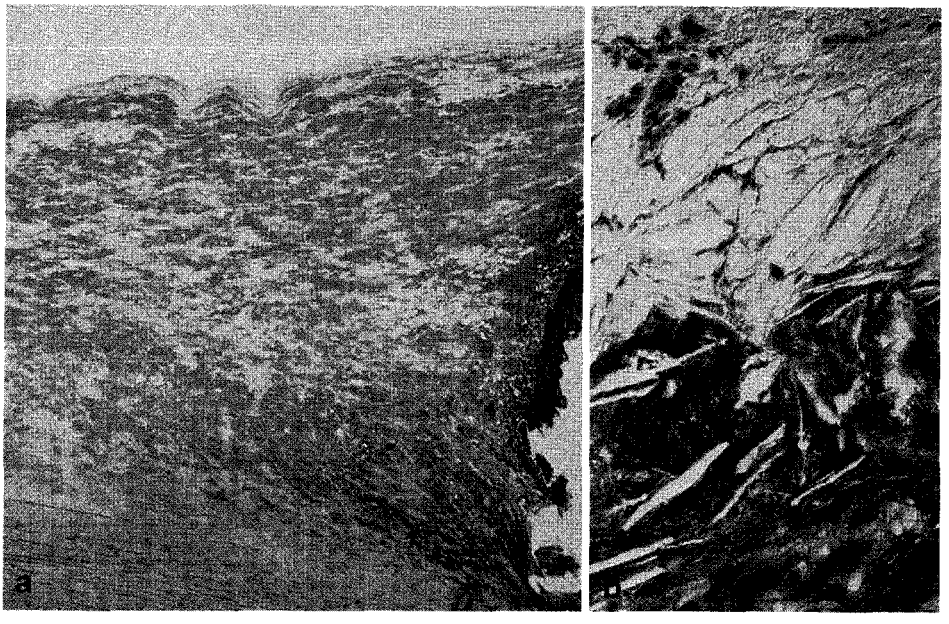


Fig. 6a, b. Advanced stage III of atherosclerosis with **a** calcification and largely even distribution of Apo A_2 over the whole vascular wall and **b** mainly extracellular deposits of Apo B and formation of cholesterol crystals in the medial area. a, ×50; b, ×400

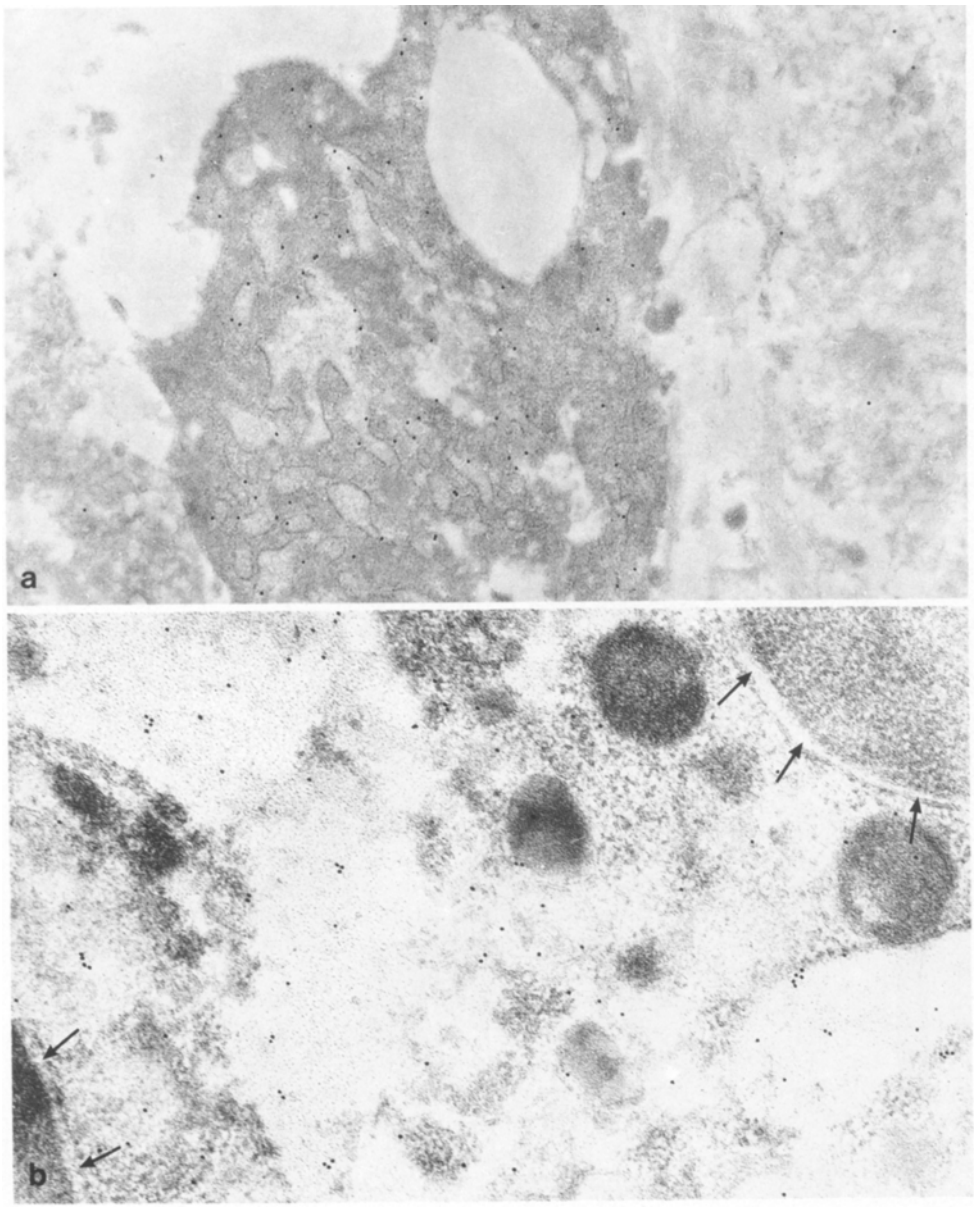


Fig. 7a, b. Intra- and extracellular labelling of Apo A_1 in an advanced stage of atherosclerosis. **a** Foam cell of smooth muscle cell origin. ×35000 **b** macrophages (*arrows* indicate nuclei). ×40000

media. Apo B deposits, in contrast, are found almost exclusively in an extracellular localization (Figs. 3c, 4b).

That the majority of foam cells should be classified as macrophages is illustrated by Fig. 5, with Apos staining red and macrophages staining brown. The majority of foam cells distinctly show a mixed, reddish-brown colouring.

With advanced atherosclerosis, the basal accumulation of Apos is increased. Occasionally, a zone of necrosis will form at the base of the plaque, with formation of cholesterol crystals, scarcity of nuclei, and abundance of apolipoproteins. The process continues during our stage III until nearly all strata of the intima are occupied by Apo lipoprotein deposits. If the plaque eventually penetrates the internal elastic lamina, extracellular apolipoproteins can also be found in the media (Fig. 6a). This holds for all three Apos. Apo A_1 and A_2 are also found in an intracellular deposition, especially in foam cells, whereas Apo B is seldom intracellular (Fig. 6b), except in advanced atherosclerotic lesions, where it may sometimes appear in foam cells. Evidence of its presence can be found at the ultrastructural level (Figs. 7a, b and 8a, b).

The adventitia, however, shows no marked alterations of deposit patterns during the whole progress of atherosclerosis: Higher magnification reveals vivid luminal staining of the vasa vasorum and evidence of erythrocytes outside these vessels. This may suggest that the Apo content of the adventitia may be the result of tearing vasa vasorum during preparation, or of diffusion from it; both factors would be independent of the stage of atherosclerotic change. In fact, the rather loose tissue of the adventitia, unlike the tighter strata of the media, permits unimpeded spreading of Apos from the serum.

In a *semiquantitative* analysis of Apo A_1 (Fig. 9a, b) evidence of antigens can be recorded in the early stage

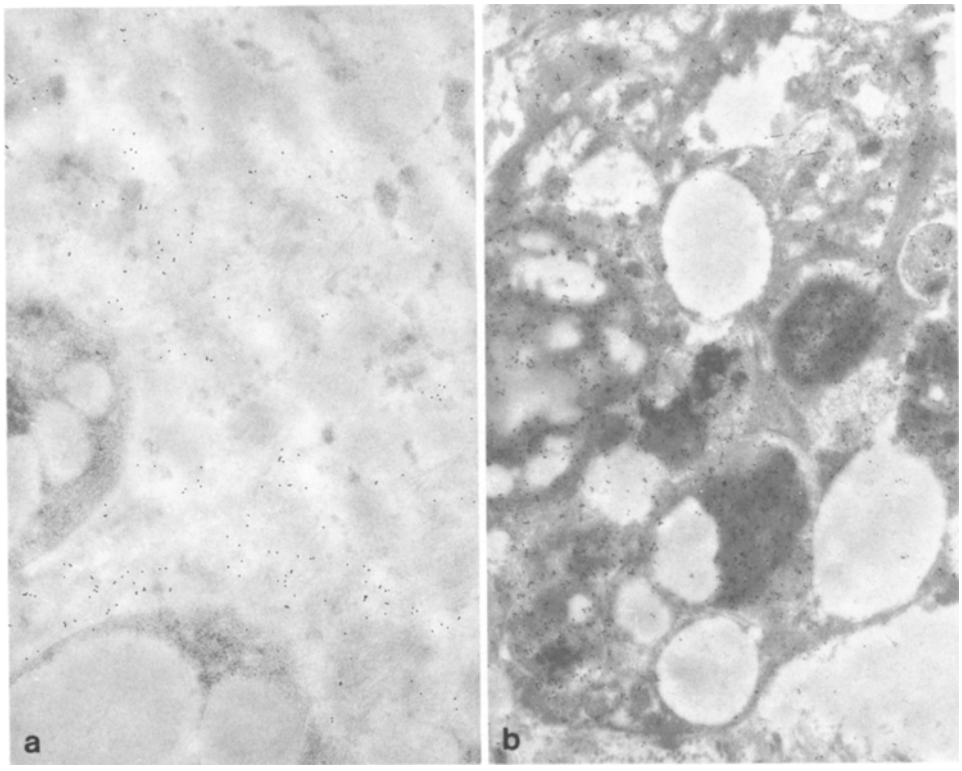


Fig. 8a, b. Labelling of Apo B with **a** exclusively extracellular deposits. ×22000 **b** intracellular deposits mainly in electron-dense lamellar organelles of foam cells. ×18000

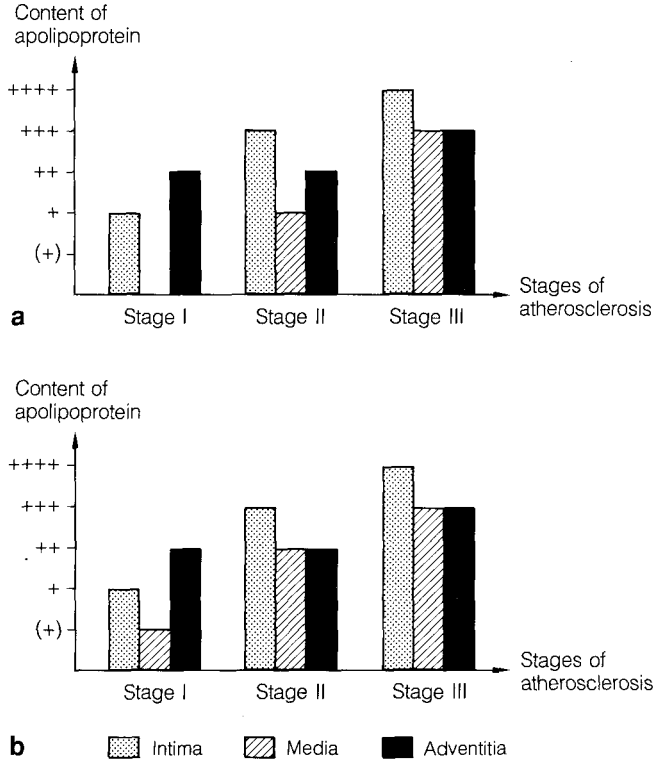


Fig. 9a, b. Distribution of Apo A_1 in the wall of **a** aortas and **b** coronary arteries in atherosclerosis

of atherosclerosis. All arteries of our stage I carry granular deposits of Apo A_1; its presence and amount increase with progression of the stages. These deposits, however, are not evenly distributed over the layers of the arterial wall, but show differing amounts in the intima, media and adventitia.

Apo A_1 content rises considerably in the intima and media (up to three steps of the classification), while the adventitia shows only a moderate rise (by one step). It has to be noted that in stage I, the intima is carrying less deposit than the adventitia – a pattern confirmed in the majority of samples from this stage. Moreover, some samples with a very discrete intimal thickening and adjacent normal areas were found to carry no Apos at all in the intima and media, while they were always present in the adventitia of all samples. These relative proportions are greatly altered during the progression of atherosclerosis: the intimal deposits show a marked increase, while the adventitial content of Apo A_1 remains at the moderate or low initial level.

The Apo content in the media shows a distinct rise with advancing stages, but the total number of medial deposits always remains lower than that in the intima and adventitia. There are also differences between the two vascular types with regard to the medial pattern: coronary arteries carry their Apos earlier and in greater abundance than aortas. In stage I, there are no Apo A_1 deposits in the aortic media, and they are still rather few in stage II. In contrast, muscular arteries show some deposits in stage I in some cases, and in stage II a moderate number of medial deposits is seen in nearly all cases. Later on, stage III displays approximately similar patterns in both vessel types.

For Apo A_2 (Fig. 10a, b) the behavioural pattern resembles that of Apo A_1: the difference in the two distribution patterns is manifest in the mostly lower amounts of Apo A_2 found in the same sample or, comparatively in lesions of the same stage. The difference to Apo A_1

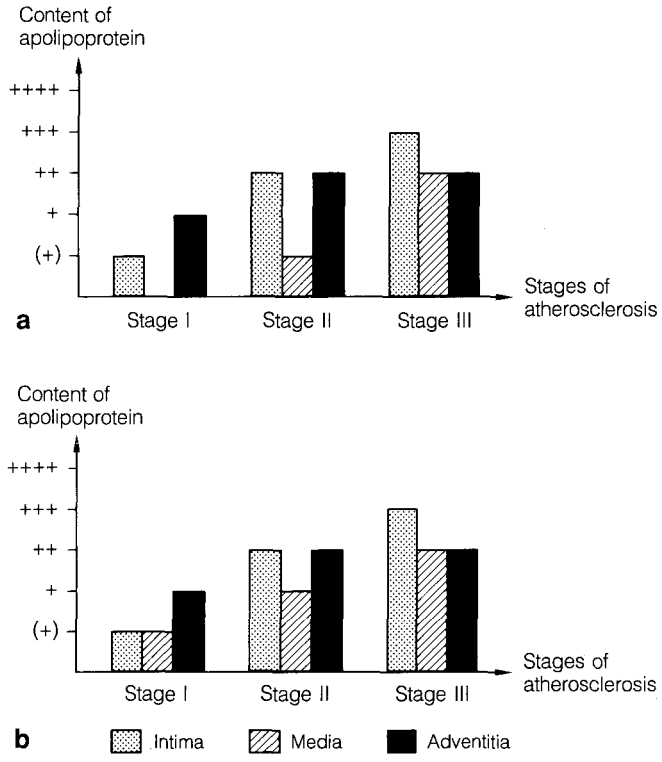


Fig. 10a, b. Distribution of Apo A_2 in the wall layers of **a** aortas and **b** coronary arteries in atherosclerosis

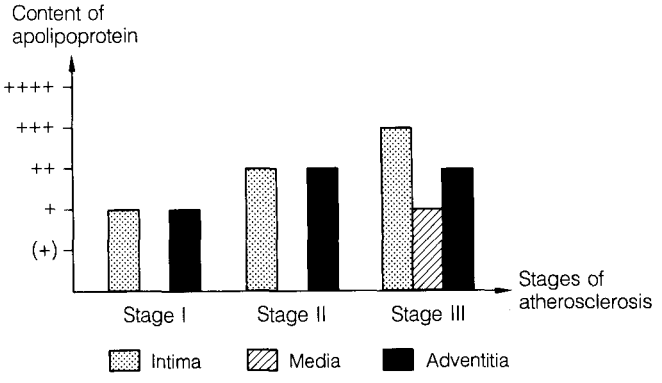


Fig. 11. Distribution of Apo B in the wall layers of aortas and coronary arteries in atherosclerosis

is about one step. For Apo B (Fig. 11) the distribution pattern is distinctly different from that of Apo A_1 and A_2. There was a strong congruence in the arteries of elastic type and those of muscular type. Although the overall amount of Apo B increases with progression through the stages, the proportion among the layers of the wall is slightly shifted: the mean content of Apo B in the intima does not increase so markedly (only by 2 steps) as that of Apo A_1 and A_2 (3 steps; cf. Figs. 9, 10). The rise, however, is more marked in the intima than in the adventitia. The media shows only a few deposits in stage III lesions.

Discussion

In the genesis of atherosclerosis a crucial role is attributed to the LDL and HDL, which are responsible for the transport of cholesterol (Gordon et al. 1977; Avogaro et al. 1979; Assmann 1982). The present investigation is concerned with the in situ localization and distribution of their main structural proteins. Apo A_1, A_2 and B were present in all stages of the atherosclerotic lesion, and the number of deposits rises in the different vascular layers in parallel with the progression of atherosclerosis.

The increase is most conspicuous in the intimal and medial layers of the arterial wall, where the morphological changes of atherosclerosis are most visible, and where the metabolic supply is effected by diffusion from the vascular lumen. In view of the effects of different plasma levels of Apo A_1 and A_2 and their contrast to those of Apo B, the coincident increase of all three within the vascular wall in the progression of atherosclerosis was a rather unexpected observation. Although a rise in Apo B content with advancing atherosclerosis would be plausible according to current knowledge (Niendorf et al. 1990), quantities of the other two proteins, Apo A_1 and A_2, would be expected to drop, since HDL, of which they are the main structural components, is thought to exert a protective effect. Our results, however, agree with those of Carter et al. (1987), who found that the role of Apo A_1 within the lesion is not as protective in the wall as in the plasma.

Two principal mechanisms appear possible for the accumulation of Apos in the arterial wall: the insudation of lipoproteins from the plasma into the vascular wall, and the neosynthesis of lipoproteins or apolipoproteins alone in the vascular wall, from local cells or from cells migrating to the wall during the development of atherosclerosis.

The insudation mechanism is widely accepted; it was demonstrated recently in a hyperlipidaemic rabbit model for Apo B by Mora et al. (1989). Morphological evidence for the other pathway, however, is still scarce. The most severe structural changes occurring during atherosclerotic progression always affect the vascular intima, and this is also true of apolipoprotein distribution. The normal intimal areas next to the lesions never signalled the presence of LDL and HDL, a result that agrees with those of other immunohistochemical studies (Hoff et al. 1975a, b; Yomantas et al. 1984; Carter et al. 1987) and is confirmed by electron microscopic data (Hoff and Gaubatz 1975). However, Feldman et al. (1984) described the presence of Apo B in the arterial intima before any development of a definite lesion. Their findings, however, were recorded only in areas of intimal thickening or oedematous swelling, a status that would be classified as an "early lesion" of stage I of our system.

A particularly important role is attributed to the intact vascular endothelium which is the guardian of homeostasis in the arterial wall (Ross 1986). Vasile et al. (1983) have shown that intimal cholesterol uptake is regulated by vesicular transcytosis. Only a minor role is attributed to intercellular transport (Simionescu 1979).

Obviously, the normal intima effects a physiological turnover of lipoproteins that is not demonstrable by immunohistological techniques.

With the onset of the atherosclerotic process, these conditions seem to undergo a pathologic alteration (Hüttner et al. 1982; de Chastony et al. 1983; Yost and Herman 1988). The lipoprotein level rises to a degree that permits immunohistological demonstration. According to Jellinek and Detre (1986), the different risk factors of atherosclerosis concur to increase permeability for macromolecules, among them cholesterol-bearing lipoproteins. This may explain the uniform morphological pattern of the arterial wall's reaction to different atherogenic noxae.

Our findings of LDL and HDL in the intimal layers immediately beneath the endothelium is suggestive of insudation from the vascular lumen into the subendothelial space. Moreover, the exclusively extracellular localization of lipoproteins in early lesions leads to the inference of an insudation from the lumen by trans- or intercellular routes.

With the progression of atherosclerosis, lipoproteins are fixed in the lesion area by matrix elements that are synthesized in increasing amounts, preventing re-diffusion into the vascular lumen (Srinivasan et al. 1986). Proteoglycans are the components responsible for lipoprotein retention (Mawhinney et al. 1978; Nakashima et al. 1985), and the joint localization of Apo B and sulphated glycosaminoglycans in atherosclerotic lesions was successfully demonstrated using immunohistological techniques by Yutani et al. (1987). In contrast, HDL is not thought to form complexes with proteoglycans (Vijayagopal et al. 1981). However, Ylä-Herttuala et al. (1987) suggested that HDL is retained in the intimal layer by another mechanism, since their investigations confirmed that the amount not only of Apo B, but also of Apo A_1 depended closely on the actual quantity of sulphated glycosaminoglycans. As a possible mechanism for HDL retention they proposed a kind of molecular sieve which would prevent the passage of macromolecules through the 5-nm pores of proteoglycans.

If severe fibrosis and an increasing number of smooth muscle cells are manifest in the progression of atherosclerosis, lipoproteins show a preferential localization in the basal regions of the plaque. In this "intermediate" lesion (type III according to Stary, stage II in our classification), Apos are obviously unable to join the constant flux of liquids from the vascular lumen towards the periphery or adventitia. Being thus unable to enter the media, they will accumulate preferably in the deeper strata of the intima. In agreement with Smith (1990) we conclude from these observations that the internal elastic lamina acts as an effective barrier against the deeper intrusion of lipoproteins. In addition, the fibrotic area above basal deposits of lipoproteins is continuously thickened by smooth muscle cells synthesizing extracellular matrix during the progression of the atheromatous plaque. Consequently, the efflux of lipoproteins towards the vascular lumen is made increasingly difficult by longer pathways of diffusion. Thus, insudating lipoproteins are more and more rigidly locked in the basal strata of developing atheromas; their massive accumulation is illustrated by the numerous Apo deposits observed in our samples of advanced lesions. The cytotoxicity of high cholesterol levels and the hypoxia enhanced by delayed diffusion due to increasing fibrosis may together cause the eventual necrosis occurring in the plaque base. Many cases of our stage III will show not only numerous deposits of Apos in these areas, but also precipitation of calcium salts, reflecting the further failure of metabolic homeostasis in the arterial wall and indicating the metabolic peculiarities of cholesterol and cholesterol-bearing lipoproteins.

Intracellular accumulation of HDL Apos does not begin with the first signs of atherosclerotic change, such as the extracellular deposits, but only towards the end of our stage I, when some cells carrying Apo A_1 and A_2 were seen, mostly situated in the area of extracellular deposits. These initial manifestations of vascular change are known in the literature as "fatty streaks" or as type II lesions according to Stary (1987, 1990a, b). The actual number of HDL-storing cells increases continuously with the progression of atherosclerosis, whereas intracellular deposits of Apo B were seen in foam cells during the advanced stage of atherosclerosis (mainly stage III of our classification, and Stary's lesion types following his intermediate type III). Our preliminary ultrastructural studies suggest that these cells are metabolizing, or perhaps synthesizing, Apos.

Cells of the intima bearing HDL deposits were mainly classified as foam cells derived from macrophages. This cell type is the first to accumulate lipid droplets and plays a very important role in the uptake and degradation of lipoproteins (Stary 1990a). Additionally, Apos were found in endothelial and smooth muscle cells, but only occasionally and in few of our arterial samples. The cholesterol esters involved are undergoing a continuous cycle of hydrolysis and re-esterification, according to the concept of LDL receptor pathways proposed by Brown and Goldstein (1983). For the maintenance of cellular cholesterol homeostasis, macrophages have to rely on active cholesterol acceptors (e.g. HDL) in their immediate surroundings, as otherwise they will be transformed into foam cells. Here, too, would be a possibility of intervention via "reverse cholesterol transport", when macrophages within the plaque are secreting Apo E (Vollmer et al. 1991). Our investigations at the ultrastructural level strongly suggest such metabolic pathways.

According to Gerrity (1981) and Watanabe et al. (1985), part of the macrophage population is able to remigrate into the vascular lumen and to return the lipids previously ingested into the blood. Thus, they may involve a kind of lipid clearance system. Up to a certain step in the sequence of atherosclerosis development, macrophages may play a protective role possibly capable of retarding the progression of atherosclerosis.

However, the increasing amount of lipid cores also represents the "graveyards of macrophage-derived foam cells" (Stary 1990a). They form the main bulk of necrotic material in the base of atherosclerotic plaques (Stary 1983), as shown in Stary's studies of coronaries and aor-

tas from more than 400 patients. Whenever macrophages die, atherogenic cholesterols previously ingested will be re-released, and lysosomal enzymes may damage the surrounding tissue. Further mechanisms include the secretion of factors promoting the migration of smooth muscle cells from the media into the intima, and their subsequent proliferation in this area (Rogers et al. 1986; Glenn and Ross 1981).

In view of their known secretory capacity, the potential of the macrophages for synthesizing HDL Apos must also be considered. They do produce and secrete a large variety of proteins, peptides and other substances, thereby co-regulating the "milieu" of their extracellular environment (Schmitz et al. 1987). Functional morphological in situ-investigations are expected to offer a new approach to the understanding of local lipid metabolism.

In the same stage of the lesion, coronary arteries (arteries of muscular type) always show more deposits of Apo than the elastic-type vessels represented by the aorta. Apparently, the lipoproteins are able to proceed from intima to media only after having accumulated in great quantity in the deeper strata of the intima during advancing atherosclerosis. Only then will the concentration gradient on both sides of the internal elastic lamina be steep enough to permit part of the intimal proteins to surmount or pervade this barrier. The pervasion obviously occurs more readily in muscular arteries than in the elastic aorta. This agrees with the results of Smith and Staples (1980), although these authors used different methodology (extract analysis).

Like the intima, the media carries Apo A_1 and Apo A_2 in intracellular deposits, and by analogy, LDL entering the cells may be immediately degraded via lysosomes; Apo B is destroyed and only cholesterol will be accumulated. This is supported by our preliminary in situ studies at the ultrastructural level and by the observation of isolated fatty smooth muscle cells in the medial area bordering the intima. Whether the fat storage in smooth muscle cells is actually effected this way is questionable; rising intracellular cholesterol content is known to suppress the formation of specific LDL receptors and, consequently, the uptake of native LDL (Goldstein and Brown 1977). Even modified LDL is unable to produce an overload of cholesterol in smooth muscle cells, which, in contrast to macrophages, do not possess the so-called scavenger receptor (Brown and Goldstein 1983). Logically, this system would ensure the cholesterol homeostasis of smooth muscle cells and preclude excess lipidization. Nevertheless, Wolfbauer et al. (1986) proposed a possible mechanism for an excess uptake of fat by smooth muscle cells, but such processes seem to involve only a proportion of the cells in vivo.

The adventitial mantle of the artery plays a separate role in the distribution of Apos. We believe that the tearing of vasa vasorum due to preparative handling and/or the immediate diffusion through loose adventitial tissue will effect an almost even distribution of Apos throughout all stages of atherosclerosis, thereby preventing an adequately differentiated morphological evaluation.

Current knowledge and our own results at both light and electron microscopical levels confirm the occurrence of insudation, but open up a concept of Apos synthesis in the vascular wall following atherosclerotic alteration, in addition to synthesis by immigrating cells such as macrophages. In this context, the detailed functional and morphological assessment of local metabolic pathways and their possible deviations will need further investigation at the ultrastructural level, with the help of appropriate immune markers.

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References

Assmann G (1982) Lipidstoffwechsel und Atherosklerose. Schattauer, Stuttgart

Avogaro P, Bittolo-Bon G, Cazzolato G (1978) Plasma levels of apolipoprotein A_1 and apolipoprotein B in human atherosclerosis. Artery 4:385–394

Avogaro P, Bittolo-bon G, Quici GB (1979) Are apolipoproteins better discriminators than lipids for atherosclerosis? Lancet I:901–903

Brown MS, Goldstein JL (1983) Lipoprotein metabolism in the macrophage: implications for cholesterol deposition in atherosclerosis. Annu Rev Biochem 52:223–261

Carter RS, Siegel RJ, Chai AU, Fishbein MC (1987) Immunhistochemical localization of apolipoproteins A_1 and B in human carotid arteries. J Pathol (Lond) 153:31–36

de Chastonay G, Gabbiani G, Elemer G, Hüttner I (1983) Remodeling of the rat aortic endothelial layer during experimental hypertension. Lab Invest 48:45–52

Feldman DL, Hoff HF, Gerrity RG (1984) Immunhistochemical localization of apoprotein B in aortas from hyperlipemic swine. Arch Pathol Lab Med 108:817–822

Gerrity RG (1981) The role of the monocyte in atherogenesis. I. Transition of blood-borne monocytes into foam cells II. Migration of foam cells from atherosclerotic lesions. Am J Pathol 103:181–200

Glenn KC, Ross R (1981) Human monocyte-derived growth factor(s) for mesenchymal cells: activation of secretion of endotoxin and concavalin A. Cell 25:603–615

Goldstein JL, Brown MS (1977) The low-density lipoprotein pathway and its relation to atherosclerosis. Annu Rev Biochem 46:897–930

Gordon T, Castelli WP, Hjortland MC, Kannel WB, Dawber TR (1977) High density lipoproteins as a protective factor against coronary heart disease: Framingham study. Am J Med 62:707–714

Gotto AM, Jackson RL (1978) Plasma lipoproteins and atherosclerosis. Atherosclerosis Rev 3:231–242

Harrach B, Robenek H (1990) Polyclonal antibodies against prefixed apolipoprotein A-1: an approach to circumventing fixation-induced loss of antigenicity in immunocytochemistry. Arteriosclerosis 10:564–576

Haimovici H (1977) Atherogenesis. Recent biological concepts and clinical implications. Am J Surg 134:174–178

Hoff HF, Gaubatz JW (1975) Ultrastructural localization of plasma lipoproteins in human intracranial arteries. Virchows Arch [A] 369:111–121

Hoff HF, Heideman CL, Jackson BL, Bayardo RJ, Kim HS, Gotto

AM (1975a) Localization patterns of plasma apolipoproteins in human atherosclerotic lesions. Circ Res 37:72–79
Hoff HF, Lie JT, Titus JL, Bajardo RJ, Jackson RL, De Bakey ME, Gotto AM (1975b) Lipoproteins in atherosclerotic lesions. Arch Pathol Lab Med 99:253–258
Hüttner I, Costabella P, de Chastomay C, Gabbiani G (1982) Volume, surface and junctions of rat aortic endothelium during experimental hypertension: a morphometric and freeze fracture study. Lab Invest 46:489–504
Jellinek H, Detre Z (1986) Role of the altered transmural permeability in the pathomechanism of arteriosclerosis. Pathol Res Pract 181:693–712
Lipid Research Clinics Program (1984) The lipid research clinics coronary primary prevention trial results. II. The relationship of reduction in incidence of coronary heart disease to cholesterol lowering. JAMA 251:365–374
Maciejko JJ, Holmed DR, Kottke BA, Zinsmeister AR, Dinh DM, Mao SJT (1983) Apolipoprotein A_1 as a marker of angiographically assessed coronary artery disease. N Engl J Med 309:385–389
Mahley RW (1981) Cellular and molecular biology of lipoprotein metabolism in atherosclerosis. Diabetes 30:60–65
Mawhinney TP, Augustyn JM, Fritz KE (1978) Glycosaminoglycanlipoprotein complexes from aortas of hypercholesterolemic rabbits. I. Isolation and characterization. Atherosclerosis 31:155–167
Miller GJ (1980) High density lipoproteins and atherosclerosis. Annu Rev Med 31:97–108
Mora R, Lupu F, Simionescu N (1989) Cytochemical localization of β-lipoproteins and their components in successive stages of lyperlipidemic atherogenesis of rabbit aortas. Atherosclerosis 79:183–195
Nakashima Y, Matsushima T, Takahara K, Kuroiwa A, Nakamura M (1985) The analysis of lipids and glycosaminoglycans of low-density-lipoprotein-glycosaminoglycan complexes isolated from normal, fatty streaks, and fibrous plaque of human aortic intima. Int Angiol 4:487–493
Niendorf A, Rath M, Wolf K, Peters S, Arps H, Beisiegel U, Dietel M (1990) Morphological detection and quantification of lipoprotein (a) deposition in atheromatous lesions of human aorta and coronary arteries. Virchows Arch [A] 417:105–111
Patsch JR (1987) Lipide. In: Wick G, Schwarz S, Förster O, Peterlik (Eds) Funktionelle Pathologie. Fischer, Stuttgart, pp 430–445
Rogers KA, Hoover RL, Castellot JJ, Robinson JM, Karnovsky MJ (1986) Dietary cholesterol-induced changes in macrophage characteristics. Relationship to atherosclerosis. Am J Pathol 125:284–291
Ross R (1986) The pathogenesis of atherosclerosis – an update. N Engl J Med 314:488–500
Schmitz G, Robenek H, Assmann G (1987) Role of high-density lipoprotein receptor cycle in macrophage cholesterol metabolism. Atherosclerosis Rev 16:95–107
Simionescu N (1979) Enzymatic tracers in the study of vascular permeability. J Histochem Cytochem 27:1120–1130
Smith EB (1990) Transport, interactions and rotation of plasma proteins in the intima: the barrier function of the internal elastic lamina. Eur Heart J 11 [Suppl E]:72–81
Smith EB, Staples EM (1980) Distribution of plasma proteins across the human aortic wall. Atherosclerosis 27:578–590
Srinivasan SR, Vijayagopal P, Dalfers ER, Abbate B, Radhakrishnamurty B, Berenson GS (1986) Low density lipoprotein retention by aortic tissue. Atherosclerosis 62:201–208
Stary HC (1983) Macrophages in coronary artery, in aortic intima, and in atherosclerotic lesions of children and young adults up to age 29. In: Schettler FG, Gotto AM, Middelhoff G, Habenicht ARJ, Jurutka KR (eds) Sixth International Symposium on Atherosclerosis. Springer Publishing, New York, pp 462–466
Stary HC (1987) Evolution and progression of atherosclerosis in the coronary arteries of children and adults. In: Bates SR, Gangloff ED (eds) Atherogenesis and aging. Springer, New York Berlin Heidelberg, pp 20–36
Stary HC (1990a) Changes in the cells of atherosclerotic lesions as advanced lesions evolve in coronary arteries of children and young adults. In: Glagov S, Newman WP, Schaffer SA (eds) Pathobiology of the human atherosclerotic plaque. Springer, Berlin Heidelberg New York, pp 93–106
Stary HC (1990b) The sequence of cell and matrix changes in atherosclerotic lesions in the first forty years of life. Eur Heart J 11 [Suppl E]:3–19
Stössel JP (1980) Wie entsteht Arteriosklerose? Med Klin 75:348–357
van Stiphout WAHJ, Hofman A, Kruijssen HACM, Vermeeren R, Groot PHE (1986) Is the ratio of apo B/apo A_1 an early predictor of coronary atherosclerosis? Atherosclerosis 62:179–182
Vasile E, Simionescu M, Simionescu N (1983) Visualization of the binding endocytosis and transcytosis of low density lipoprotein in the arterial endothelium in situ. J Cell Biol 96:1677–1689
Vergani C, Trovato G, Dioguardi N (1978) Serum total lipids, lipoproteins, cholesterol, apoproteins A and B in cardiovascular disease. Clin Chim Acta 87:127–133
Vijayagopal P, Srinivasan SR, Radhakrishnamurty B, Berenson GS (1981) Interaction of serum lipoproteins and a proteoglycan from bovine aorta. J Biol Chem 256:8234–8241
Vollmer E, Roessner A, Bosse A, Käsberg B, Robenek H, Sorg C, Winde G, Böcker W (1991) Immunohistochemical double labelling of macrophages, smooth muscle cells, and apolipoprotein E in the atherosclerotic plaque. Pathol Res Pract 187:184–188
Watanabe T, Hirata M, Yoshikawa Y, Nagafuchi Y, Toyoshima H, Watanabe T (1985) Role of macrophages in atherosclerosis. Lab Invest 53:80–90
Wolfbauer G, Glick JM, Minor LK, Rothblat GH (1986) Development of the smooth muscle foam cell: Uptake of macrophage lipid inclusions. Proc Natl Acad Sci USA 83:7760–7764
Ylä-Herttuala S, Solakivi T, Hirvonen J, Laaksonen H, Möttönen M, Pesonen E, Raekallio J, Akerblom HK, Nikkari T (1987) Glycosaminoglycans and apolipoproteins B and A_1 in human aortas. Arteriosclerosis 7:333–340
Yomantas S, Elner VM, Schaffner T, Wissler RW (1984) Immunhistochemical localization of apilopiprotein B in human atherosclerotic lesions. Arch Pathol Lab Med 108:374–378
Yost C, Herman IM (1988) Age-related and site-specific adaptation of the arterial endothelial cytoskeleton during atherogenesis. Am J Pathol 130:595–604
Yutani C, Go S, Imakita M, Ishibashi-Ueda H, Hatanaka K, Yamamoto A (1987) Autopsy findings in two patients with homozygous familial hypercholesterolemia. Special reference to apolipoprotein B localization and internalization defect of low density lipoprotein. Acta Pathol Jpn 37:1489–1504
Zwadlo G, Bröcker EB, Bassewitz DB von, Feige U, Sorg C (1985) A monoclonal antibody to a differentiation antigen present on mature human macrophages and absent from monocytes. J Immunol 134:1487–1492