Immunostaining of Human Autopsy Aortas With Antibodies to Modified Apolipoprotein B and Apoprotein(a)

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A systematic immunohistochemical study of different stages of atherosclerosis in human aortas was performed using several antibodies. Because oxidation of lipoproteins could be a key event in atherogenesis, an antibody against apolipoprotein B (apoB) from low-density lipoprotein (LDL) modified with the lipid peroxidation-specific aldehyde, 4-hydroxynonenal (4-HNE) (anti-4-HNE-apoB), was raised in rabbits. This antibody recognizing 4-HNE protein adducts was used in concert with an antibody to apo(a) from lipoprotein(a), considered also potentially atherogenic, as well as with an antibody and a monoclonal antibody (mAb) to apoB. Autopsy material from 12 corpses was investigated. The immunohistochemical investigation by the alkaline-phosphatase technique included control specimens regarding postmortem artifacts by autolysis and oxidation. The results from six specimens from five corpses are presented. A positive staining with the antibody to apoB but not with anti-4-HNE-apoB was seen in the normal intima. The thickened intima of early, transitional, and advanced atherosclerotic lesions and atheromata showed a predominantly extracellular staining with all antibodies and the applied mAb. To test the specificity of the staining, antibodies preadsorbed by the appropriate antigens and nonimmune sera were used, giving negative results. These findings indicated a colocalization of epitopes derived from lipid peroxidation of polyunsaturated fatty acids and epitopes specific for apoB and apo(a) during atherogenesis in humans. (Arterioscler Thromb. 1993;13:1689-1699.)

KEY WORDS • modified LDL • lipoprotein(a) • lipid peroxidation • 4-hydroxynonenal • immunohistochemistry • atherosclerosis

There is increasing evidence that oxidative modification of low-density lipoprotein (LDL) might strongly enhance the atherogenicity of this class of lipoproteins (see References 1 to 4 for review). Oxidative modification makes LDL cytotoxic5 and converts it to a form readily recognized by the scavenger receptor(s) on macrophages as proved in in vitro studies.6,7 By accumulating lipids these cells become foam cells, a cell type typical of atherosclerotic lesions.8 During lipid peroxidation in LDL a variety of aldehydic products is formed by fragmentation of polyunsaturated fatty acids such as linoleic acid or arachidonic acid.9 Malondialdehyde (MDA), hexanal, and 4-hydroxynonenal (4-HNE) are formed to the greatest extent.10 The latter aldehyde shows the strongest reactivity with certain amino acid residues of apolipoprotein B (apoB).11 Antibodies raised against LDL, modified by either 4-HNE or MDA, and against copper-oxidized LDL immunostain atherosclerotic lesions of Watanabe heritable hyperlipidemic (WHHL) rabbits.12-15 An oxidatively modified LDL has been extracted from athero-otic lesions of rabbits and humans,16 and colocalization of mRNA of the acetyl-LDL receptor and 15-lipoxygenase with oxidation-specific lipid-protein adducts in human atherosclerotic lesions is reported.17 Additional support for the hypothesis that oxidation of lipoproteins is involved in atherogenesis comes from observations of autoantibodies against oxidized LDL in the serum of atherosclerotic patients.13,18,19 Lipoprotein(a) [Lp(a)] is also considered as an independent risk factor for atherosclerosis. Lp(a) represents an LDL particle to which apo(a), a glycoprotein, is bound. Raised serum concentrations of Lp(a) correlate positively with angiographically proved coronary heart disease20 and with the risk of myocardial infarction at younger age21 and have been found to predict vein graft restenosis in coronary bypass patients.22 Lp(a) serum levels also correlated well with carotid atherosclerosis.23 Lp(a) and apo(a) have been detected immunohistochemically in the artery wall.24,25 A link between thrombosis and atherosclerosis is believed possible with respect to the atherogenicity of Lp(a).26,27 However, oxidative modifications render Lp(a) into a lipoprotein that is recognized by the scavenger receptors28-30 and thus might be considered to contribute to its atherogenicity. To show oxidized LDL-, MDA-, or 4-HNE-altered protein in atherosclerotic lesions, immunohistochemical studies have been performed, mainly in WHHL rabbits.12-15 The WHHL rabbit is doubtlessly a valuable and
important model for studying familial hypercholesterol-
emia. However, an animal model is not able to reflect the 
hetereogeneity of human atherosclerosis. Since oxided/modiﬁed LDL and Lp(a) are probably the two 
most atherogenic lipoproteins, a relevant immunohisto-
chemical study is of special importance. A possible 
co-localization of oxidation-derived lipid-protein ad-
ducts and apo(a) in human lesions has not yet been 
studied. As to the role of Lp(a) in atherogenesis in 
transgenic mice, it is suggested that immobilized apo(a) 
might enhance the deposition of apoB-containing lipoproteins in the vessel wall.31

Thus, the aim of this work was to investigate the 
different stages of atherosclerosis with antibodies to 
apoB from LDL modiﬁed by 4-HNE (4-HNE-apoB), to 
apo(a), and to apoB as well as to a monoclonal antibody 
(mAb) to apoB to systematically study the presence and 
distribution of the corresponding antigens in atheroscle-
rotic lesions in humans.

Methods
Preparation of Aortic Tissue
Human aortal specimens were collected 3 to 8 hours 
postmortem from a total of 12 autopsy cases at the 
Institute of Pathology, University of Graz. Before the 
study we tested for possible artifacts caused by autolysis 
in the corpses. Samples were taken at several time 
points (3 to 10, 20, 30, and 40 hours) after death and 
were analyzed immunohistochemically with the anti-
bodies and nonimmune sera used in this study. Since 
these preliminary immunohistochemical experiments 
showed that the results from 3 to 10 hours did not differ, 
a period between 3 to 8 hours after death was consid-
ered as safe. Four to 8 samples of the distal thoracic or 
abdominal aorta were excised from each autopsy case. 
These aortal specimens showed either no, slight, mod-
erate, or severe atherosclerotic lesions by visual inspec-
tion. To avoid artifacts by oxidation the vessels were 
rinsed with phosphate-buffered saline (PBS) containing 
EDTA (1 mg/mL) immediately after opening. Addition-
ally, the entire time that the samples were exposed to 
the air did not exceed 4 minutes until they were 
freezed in methylpentane, precooled in liquid ni-
trogen, and then stored in liquid nitrogen for further 
processing. Analogous aortal specimens were ﬁxed in 
10% neutral buffered formalin and were then embed-
ed in parafﬁn. Data from 5 corpses (Table) repre-
sentative of the samples investigated were documented.

Preparation and Modiﬁcation of Lipoproteins
Human LDL (1.020 to 1.050 g/mL), Lp(a) (1.090 to 
1.210 g/mL), and high-density lipoprotein (HDL3) 
(1.125 to 1.210 g/mL) were obtained by potassium 

![Graph](http://atvb.ahajournals.org/)

**Fig 1.** Line graph showing results of solid-phase com-
petitive ﬂuorescence immunoassay of anti-4-hydroxy-
nonenal (4-HNE)-apolipoprotein B with native low-den-
sity lipoprotein (LDL) (–○–); 4-HNE-LDL (–●–); 
oxidized LDL (–□–); oxidized lipoprotein(a) (–×–); 
high-density lipoprotein (–□–); and human serum al-
bumin (–▲–). The wells were coated with 4-HNE-LDL (1 
μg/mL protein concentration), and a 10 000-fold dilution 
of the antisera was added in the absence and presence of 
increasing amounts of the competitors. Each point 
represents the mean value of triplicate determinations. 
Results are expressed as described under "Methods." 
B/Bo indicates the amount of antibody bound to the 
coated antigen in the presence (B) and the absence (Bo) 
of the competitor.
FIG 2. Alkaline phosphatase-anti-alkaline phosphatase immunostaining of serial cryosections showing normal intima and an atheroma in a deeper intimal region (donor A). Primary antibodies were a, anti-apolipoprotein B (apoB) and b, anti-4-hydroxynonenal-apoB (original magnification ×40; bar=0.6 mm).

Preparation and Characterization of the Antibodies

An antibody against human LDL modified by 4-HNE was raised in the rabbit and purified by affinity chromatography as described. Before the immunohistochemical study this antibody was characterized in competition tests based on a solid-phase fluorescence immunoassay by using a 1234 Delfia research fluorometer (Wallac Oy, Turku, Finland). The common assay procedure has been described recently. Briefly, microtiteration plates (A/S Nunc, Roskilde, Denmark) were coated with 4-HNE-LDL (1 μg/mL). After washing, a constant dilution of the antibody to 4-HNE-apoB was added together with an equal volume of assay buffer containing increasing amounts of competitive lipoproteins. Incubation was performed for 2 hours at room temperature. After washing, the amount of rabbit immunoglobulin G (IgG) bound was detected by adding 50 ng/well of a goat anti-rabbit IgG (Sigma) labeled with europium (Delfia, Eu³⁺-labeling kit 1244-302, Pharmacia, Uppsala, Sweden) according to the manufacturer's description. After a 1-hour incubation and washing, the enhancement solution (Pharmacia) was added to measure the fluorescence counts in the wells with the fluorometer. The results were expressed as B/Bo, where B is the amount of antibody bound to the coated antigen in the presence of the competitor and Bo is the amount of antibody bound in the absence of the competitor.

The strongest competitor preventing the antibody to 4-HNE-apoB from binding to the antigen used for coating (4-HNE-LDL) was 4-HNE-LDL itself, followed by oxidized LDL and oxidized Lp(a), whereas native LDL, HDL, or human serum albumin did not reveal any cross-reactivity with 4-HNE-LDL over a wide range of concentrations tested (Fig 1).

The polyclonal antibody to apo(a) was from rabbits. It was the same as used in previous clinical studies and its characteristics are carefully described in a recent report. As checked by Western blot analysis it did not react with apoB, the other protein of Lp(a). The mAb to apoB was generated by mouse hybridoma cells. BALB/c mice were immunized with native human LDL, and the immunized mouse spleen cells were fused with mouse sp2/0.AG 14 myeloma cells in the presence of polyethylene glycol. It was shown by a radiolabeling ligand-binding assay that the epitopes recognized by this antibody on apoB overlap or are near the binding site of the LDL receptor. The polyclonal antisera to apoB (anti-apoB) was purchased from Behring (Ch.-B./lot 15 3627 E; Behringwerke AG, Marburg, FRG). The mAb KP1, specific for macrophage antigens, was from Dakopatts, Glostrup, Denmark.

The dilutions of the antibodies used as primary antibody were as follows. Polyclonal anti-apoB was used at a 1:1000 dilution for all sections shown in the figures. Polyclonal anti-4-HNE-apoB was used at a dilution of 1:250 in all experiments except those shown in Fig 4, in which a 1:125 dilution was used. Polyclonal anti-apo(a) was used in all sections at a dilution of 1:150. The mAb to apoB was used at a dilution of 1:1000, and the mAb KP1 was diluted at 1:1000.

Immunohistochemical Analysis

Serial cryosections of the autopsy material were performed. Frozen aortal sections (4 μm thick) were air dried and then fixed with cold (−20°C) acetone for 5 minutes. Immunohistochemical analysis was performed according to the alkaline phosphatase-anti-alkaline phosphatase (APAAP) complex technique. The primary antibody was diluted in 1% bovine serum albumin and was incubated for 1 hour in a humid chamber at room temperature. For the primary mAb a rabbit anti-mouse (pan) immunoglobulin (Dakopatts) was used as the second antibody in a dilution of 1:30 and incubated for 30 minutes. For the antibodies generated in the rabbit, an affinity-purified mouse anti-rabbit IgG (1:500; Dakopatts) was used as the linking antibody. Before the addition of APAAP (Dakopatts), the sections were washed twice in PBS. APAAP (diluted 1:100 in PBS) was applied for 30 minutes, and the color reaction was performed with fast-red working solution (10 mg naphthol-AS-MX-phosphate [Serva Feinbiochemika, Heidelberg, FRG] was dissolved in 0.5 mL...
Fig 3. Alkaline phosphatase–anti-alkaline phosphatase immunostaining of consecutive cryosections of an early atherosclerotic aortic lesion (donor B). Primary antibodies were a, anti–apolipoprotein B (apoB); b, monoclonal antibody to apoB; c, anti-apoB+low-density lipoprotein (LDL); d, anti–4-hydroxynonenal (4-HNE)–apoB; e, anti–4-HNE–apoB+LDL; f, anti-apo(a); g, anti-apo(a)+lipoprotein(a); h, nonimmune rabbit serum; i, nonimmune mouse serum; and j, phosphate-buffered saline (original magnification ×40).

N,N-dimethylformamide [Serva] and 50 mL veronal acetate buffer [pH 9.2] to which 50 mg fast red TR [Serva] and 10 mg levamisol [Sigma] were added. After filtration and adjustment of the pH to 9.2 to 9.8 the solution was applied for 30 minutes at room temperature. The immunohistochemical analysis was also performed with paraffin-embedded samples by using the same dilutions of the primary antibody as given for the cryosections but without any pretreatment of the sections with protease.

The specificity of the immunohistochemical reactions was tested with the following experiments. The antibody-
ies were mixed with their respective antigens added in excess (LDL, 4-HNE-LDL, and Lp(a) at 1 mg/mL protein concentration). This antibody-antigen solution was used as a replacement for the primary antibody.

LDL was also added in excess (1 mg/mL protein concentration) to anti-4-HNE-apoB to replace 4-HNE-LDL for specificity control. For further controls the sections were treated identically, but the antibodies used in each step were replaced by the respective nonimmune animal sera and PBS.

Microscopy and photography were performed by using instrumentation from Zeiss, Oberkochen, FRG.

Results

Grossly normal tissues and visible lesions in samples from the thoracic and abdominal aortas of 12 corpses were studied by immunohistochemical analysis. Six samples from 5 specimens representative of different stages of atherosclerotic lesions were selected for presentation in this article. An important issue was to prove that no oxidation of LDL occurred during preparation of the samples until they were deep frozen in liquid nitrogen or during the staining procedure. To verify this issue, consecutive sections containing both normal intima and intima with an atheroma in the deeper region prepared 8 hours after death from donor A were studied. With anti-apoB a positive staining distributed in both the normal intima and the core of the atheroma was obtained (Fig 2a). However, using anti-4-HNE-apoB no reaction could be observed in the normal intimal region, since this antiserum does not distinguish apoB from native LDL (Fig 1), and only the atheroma displayed a positive reaction (Fig 2b). The immunoreactive product of this atheroma shown at the depth of this plaque represents an accumulation of 4-HNE-protein adducts most probably derived from oxidized LDL. This material could have been oxidized by resident macrophages and after their degeneration surrounded and encapsulated by more tightly textured cell-poor fibrous tissue. If oxidation had occurred, all the normal intimal region should also have shown a positive reaction to anti-4-HNE-apoB, and a staining pattern similar to that obtained with anti-apoB should have been formed. From the results it can clearly be deduced that no 4-HNE epitopes were generated as in vitro artifacts during manipulation of the sections of the aorta. This observation was verified by other samples (data not shown).

An early atherosclerotic lesion of the aorta of donor B was investigated (Fig 3). The staining for apoB detected by anti-apoB (Fig 3a) as well as by the apoB-specific mAb (Fig 3b) predominated in the intima. Specificity controls are a necessity when studying atherosclerotic lesions and the deposition of lipoproteins.
by means of immunohistochemical analysis. Therefore, anti-apoB was incubated with LDL before application, and the staining disappeared (Fig 3c). The pattern of staining with anti-4-HNE-apoB was similar to that with anti-apoB (Fig 3d) regardless of preincubation of this antiserum with nonoxidized LDL (Fig 3e). This was an immunohistochemical control in that anti-4-HNE-apoB did not recognize any native LDL or apoB. Anti-apo(a) produced a similar immunohistochemical staining (Fig 3f). This reaction was abolished after addition of Lp(a) to the antiserum (Fig 3g). Further controls to make the immunohistochemical staining
Early macrophage-rich fatty streaks were also investigated in three corpses. The results obtained with one of them (donor C) are presented. The presence of macrophages was verified by means of the mAb KP1 (Fig 4a). Anti-4-HNE-apoB together with anti-apo(a) were used to identify corresponding lipoprotein-related epitopes in this kind of lesion. The 4-HNE epitopes (Fig 4b) and apo(a) (Fig 4c) were found to be predominantly...

Fig 5. Alkaline phosphatase-anti-alkaline phosphatase immunostaining of consecutive cryosections of a transitional atherosclerotic aortic lesion (donor D; left column) and an advanced atherosclerotic aortic lesion with intimal thickening and calcification (donor E; right column). Primary antibodies were a and d, anti-apolipoprotein (apo) B; b and e, anti-4-hydroxynonenal-apoB; c, anti-apo(a); and f, monoclonal antibody to apoB (original magnification ×40 [left], ×80 [right]).

valid included nonimmune rabbit serum (Fig 3h), nonimmune mouse serum (Fig 3i), and PBS (Fig 3j).
deposited extracellularly and only a very faint intracytoplasmic staining of some macrophages could be detected.

A transitional atherosclerotic lesion with a pronounced thickening of the intima of donor D and an advanced lesion with thickening of the intima and calcification of donor E were investigated (Fig 5). The lesion of donor D displayed a broad staining with anti-apoB (Fig 5a). A deposition of 4-HNE-derived epitopes (Fig 5b) and apo(a) (Fig 5c) also occurred in the thickened intima, but their distribution varied topographically. In the advanced lesion of donor E a colocalization of apoB as assessed with anti-apoB (Fig 5d) or with the mAb to apoB with 4-HNE-derived epitopes (Fig 5e) was observed in the upper part of the intima. Only a weak positive reaction of the antiserum to apo(a) could be observed (results not shown). The staining obtained was specific in each sample, since it was abolished after preincubation of the antibodies with the corresponding antigens, and additional controls were
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FIG 6. This page and opposite page. Hematoxylin-eosin and alkaline phosphatase-anti-alkaline phosphatase immunostaining of consecutive sections of paraffin-embedded specimens from an aortic atheroma with cholesterol clefts in the core surrounded by fibrotic strands (donor B). a, Hematoxylin-eosin staining; primary antibodies were b, anti-apolipoprotein (apo) B; c, anti-apoB+low-density lipoprotein (LDL); d, anti-4-hydroxynonenal (4-HNE)-apoB; e, anti-4-HNE-apoB+4-HNE-LDL; f, anti-apo(a); g, anti-apo(a)+lipoprotein(a); h, nonimmune rabbit serum; and i, phosphate-buffered saline (original magnification x40).

made with nonimmune rabbit serum, nonimmune mouse serum, and PBS, revealing a negative background reaction (results not shown).

To test an advanced stage of an atherosclerotic process, an aortal atheroma was investigated in detail. In contrast to the samples shown in Figs 2 through 5, which were cryosections, this investigation was performed on paraffin-embedded material. The autopsy material was taken from donor B. The hematoxylin-eosin-stained section showed an atheroma with its cholesterol clefts containing pulp surrounded and encapsulated by fibrotic strands (Fig 6a). The atheromatous material and fibrous capsule (noncellular) displayed a positive staining with anti-apoB, whereas the uppermost subendothelial layer did not react (Fig 6b). After preincubation of anti-apoB with LDL the reaction was abolished (Fig 6c). A similar staining was observed after incubation of the sample with anti-4-HNE-apoB (Fig 6d) and anti-apo(a) (Fig 6f). Preincubation of the two antibodies with their appropriate antigens [4-HNE-LDL and Lp(a)] could prevent the staining (Fig 6c and 6g, respectively). Nonimmune rabbit serum used instead of the primary antibody revealed a weak background staining (Fig 6h), but PBS was negative (Fig 6i). The reaction pattern on paraffin sections was nearly identical to that of the cryocuts except for a faint background staining.

Discussion
In this study the histological distribution of lipid peroxidation–specific epitopes concomitantly with the presence of apoB and apo(a) was systematically investigated in human atherosclerotic lesions. Antibodies to 4-HNE–apoB and apo(a) were used for immunohistochemical studies in this combination for the first time. As shown in Fig 1, anti–4-HNE–apoB did not recognize native LDL, HDL, or serum albumin, even with a sensitive, competitive, solid-phase fluorescence immunoassay, but had strong cross-reactivity with oxidized LDL and oxidized Lp(a). This antibody also recognized human serum albumin modified with 4-HNE.33

In addition to the results obtained by the competition immunoassay that showed a strong cross-reactivity of anti–4-HNE–apoB for oxidized LDL, the performance of relevant control specimens in an immunohistochemical analysis was crucial. The experiments shown in Fig 3 proved the clear-cut specificity of this antibody for lipid peroxidation–derived epitopes, since preincubation of the antibody with native human LDL could not abolish the staining of an early atherosclerotic lesion (Fig 3e). However, in all sections investigated the staining of anti–4-HNE–apoB was completely abolished when preincubated with 4-HNE–LDL. This clearly showed that this antibody did not attach to the tissue components of the lesions in any nonspecific way.
An important point was stressed in a previous study that used antibodies or mAbs against LDL, either modified by MDA or 4-HNE or oxidized, for immunostaining atherosclerotic lesions from WHHL rabbits: Lipid peroxidation-specific epitopes do not necessarily have to be present on oxidized LDL. They might also be expressed on membrane or connective tissue proteins and/or their breakdown products. Of course, this might also be true for our study. However, as demonstrated, the staining with anti-4-HNE-apoB always reached only those parts of the intima also stained by the antibodies to apoB. This fact would indicate that the 4-HNE-derived epitopes in atherosclerotic lesions probably originated from lipid peroxidation in apoB-containing lipoproteins. This assumption is further supported by the finding of local LDL modifications in the atherosclerotic rabbit aorta.

The penetration of the endothelial layer of a normal intima or an intima in the early stages of atherosclerosis by apoB from oxidized LDL and/or Lp(a) is shown in Fig 2a and in Fig 3a and 3b. Whereas in the normal intima we could not find staining with anti-4-HNE-apoB (Fig 2b), in early lesions with thickening of the intima a strong immunoreaction with anti-4-HNE-apoB and anti-apo(a) was found (Fig 3d and 3f, respectively) that displayed a more diffuse extracellular distribution pattern. The type of early lesion investigated in Fig 3 contained few fat-filled cells but revealed high LDL and Lp(a) concentrations, which agrees with previous reports. The macrophage-rich area of a fatty streak (Fig 4) also showed predominantly extracellular staining with anti-4-HNE-apoB and anti-apo(a). Only some of the macrophages revealed a faint intracellular staining. This observation deserves further investigation, since it is generally accepted that the uptake of oxidized LDL by macrophages results in the formation of foam cells. Support for this assumption is given in a study by Rosenfeld et al, who demonstrated intracellular staining of macrophages in fatty streaks and transitional lesions of WHHL rabbits by antibodies against oxidized or modified LDL. Only in advanced lesions, in addition to a cell-associated staining, is a more diffuse extracellular staining reported. All sections were paraffin embedded. In that study, before the animals were killed the aortic tree was perfusion-fixed with paraformaldehyde containing sucrose solution supplemented by antioxidants to prevent autolysis and oxidation of tissue. In our study such a treatment was not possible. However, in another study that used cryosections, as we did here, no intracellular deposition of MDA-protein adducts could be detected in foam cell–rich lesions from WHHL rabbits. It is unclear whether the origin of the samples or the preparation techniques influence the results obtained.

Another explanation for the observed discrepancies might be that different stages of development of foam cells exist. In some of them modified apoB of the incorporated LDL might already have been degraded, whereas in others this protein could still be present intracytoplasmically. Moreover, a constant overloading of macrophages with oxidized LDL could take place in WHHL rabbits, whereas in atherosclerotic humans, who normally have much lower serum cholesterol levels, foam cell formation and progression of lesions is a much slower process. This might in part also account for the differences between the results reported here with human specimens and those obtained with WHHL rabbits.

An advantage of an investigation of atherosclerotic lesions of human origin over studies performed in the WHHL rabbit is that the occurrence and distribution of Lp(a) and apo(a) can be studied. We included an antisera specific for apo(a) in this investigation, although Lp(a) serum levels were not available. However, a correlation between apo(a) in plasma and tissue had been described. Apart from an overall distribution of apo(a) in the atherosclerotic lesions, we repeatedly saw a favorable association of this glycoprotein with laminar and fibrous structures, which agrees with other studies. A coexistence among apoB, 4-HNE–derived epitopes, and apo(a) was found immunohistochemically (Figs 3, 5, and 6). The fibrous tissue surrounding the core of advanced lesions (representing the final product of the stimulating effects of cytokines and growth factors produced by macrophages and myofibroblasts) sometimes was also penetrated by substances reacting with anti-4-HNE-apoB and anti-apo(a). These substances probably derived from the atheromatous pulp and/or from degenerating histolytic foam cells. Thus, the atheroma examined revealed a strong staining with anti-4-HNE-apoB and anti-apo(a) (Fig 6d and 6f, respectively). This coexistence might also be explained as an oxidative modification of Lp(a). A relevant immunohistochemical proof of a colocalization of 4-HNE epitopes and apo(a) in the region between the cholesterol clefts in the atheroma is also provided in Fig 6. The distribution of the immunoreactive products may indicate a diffusion of apoB of oxidized LDL and apo(a) from the core of the atheromatous pulp into the surrounding fibrous tissue. The uppermost layer, which displays no deposition of these substances, is sharply demarcated from the altered region, representing a newly formed intima.

The results of this immunohistochemical investigation of human atherosclerotic lesions in different stages showed both the predominant extracellular deposition of epitopes specific for lipid peroxidation and apoB and that a considerable portion of the regions revealing those epitopes contains apo(a).

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