Comparative localization of apolipoproteins (apo) B and A in small, raised fibrolipid lesions was performed to determine whether low density lipoproteins (LDL) and high density lipoproteins (HDL) may be involved in the formation of the earliest identifiable lipid-rich core regions found in atherosclerotic fibrous plaques. Apo B was observed associated with the superficial layer of foam cells in collagensous areas of the lesion cap and within the lipid-rich core region. The lipid-rich core region was well-circumscribed by an intense band of apo B staining along both luminal and medial aspects. Apo A staining was confined to the noncellular elements of the fibrolipid lesion. Collagensous areas were speckled with fine, punctate granules of immunoreactive apo A. A homogeneous, granular apo A staining pattern was characteristic throughout the core region. With these data from a relatively early lesion in the development of the atherosclerotic fibrous plaque, we conclude that: 1) both LDL and HDL can accumulate in the lipid-rich core region since no preferential staining for apo B over apo A was observed in this region, and 2) the paucity of cell-associated apo A staining in small fibrolipid lesions suggests that HDL interacts with the arterial wall differently than does LDL.

(Arteriosclerosis 8:499–508, September/October 1988)
methanol-peroxide or other caustic agents which may also destroy tissue antigens.

Thus, the present study aims to examine the location of apo B and apo A within a well-defined lesion type that is considered to be a precursor or early form of the atherosclerotic fibrous plaque. We have previously described the fibrolipid lesion as a potential intermediate lesion between fatty streak and fibrous plaque. The lesions covered less than 16 square millimeters of surface area and were characterized by a superficial layer of foam cells, a core of noncrystalline or crystalline lipid, and a developed or developing collagenous cap. Total cholesterol concentration in the fibrolipid lesions was similar to that in fatty streaks; however, the ratio of unesterified to total cholesterol was similar to that found in fibrous plaques. These small fibrolipid lesions may provide the opportunity to examine processes that are not secondary effects of lesion development. In addition, by comparative localization of LDL and HDL in this fibrous plaque precursor, we were able to determine whether LDL only, or both LDL and HDL, are selectively retained in the early core region.

Methods

Selection of Aortic Tissue

Aortas obtained at autopsy were examined for the presence of small raised lesions, termed fibrolipid lesions. The aortas were from persons ages 10 to 87 years, 74% of whom were 45 years old or younger. Trauma was the major cause of death in about 90% of the cases. All distinct raised lesions covering less than 16 square millimeters were dissected from the descending thoracic and abdominal aorta. The fibrolipid lesions, when viewed from above, displayed either a cluster of yellow dots, a homogeneous yellowish cover, fingerlike projections of a white fibrous material, or a complete pearly-white cap. A total of 34 fibrolipid lesions from 30 aortas were sliced transversely with the aid of a McIlwain Mechanical Tissue Chopper (Mickle Laboratory, Brinkmann Instruments, Westbury, NY); these were flash-frozen and stored at -70°C until sectioned. Slices of normal intima and larger fibrous plaques were also frozen.

Cytochemical Methods

Sections contiguous to those used for LDL and HDL localization were stained with oil red O in 60% isopropyl alcohol to demonstrate the distribution of lipid. The a-naphthyl acetate method was used to stain selectively for macrophage-like cells. In addition, tissue sections were stained with hematoxylin and eosin (H&E) to aid in identification of the lipid-rich core, which was found to be distinctly basophilic.

To assess the nature of the lipid present in the lesions, sections were viewed under polarized light and the melting points were determined with the aid of a Bailey TS-2ER heating stage element (Bailey Instruments Incorporated, Saddle Brook, NJ). This method entailed heating the sections (20 degrees per minute) to 43°C to 100°C. Because arterial cholesterol esters melt to an isotropic state at or before 43°C, any remaining bright birefringence was probably due to phospholipid-rich lamellar crystals or to free cholesterol monohydrate crystals.

Antibodies

Rabbit polyclonal antibodies directed against human apo B, the major apoprotein of LDL; human apo A (A-I and A-IV), the major apoprotein of human HDL; and human albumin and fibrinogen were obtained from Calbiochem (San Diego, CA). Affinity-purified biotinylated goat antirabbit IgG (heavy and light chain) was obtained from Vector Laboratories (Burlingame, CA).

Rabbit antibodies to human apo B and apo A were affinity-purified using the method of DiFerrante et al.

This entailed making an affinity column by linking 21 mg of LDL or 11 mg of HDL to 1 gram of cyanogen bromide-activated sepharose 4B. The LDL and HDL used on the column was from pooled plasma of normolipemic volunteers. The LDL fraction used was between 1.023 and 1.055 g/ml and the HDL fraction was between 1.063 and 1.21 g/ml. The column was washed with 0.1 M NaHCO3, 0.5 M NaCl buffer (pH 8.5) at 4°C before application of antiserum. Upon application of antiserum, the serum was recycled for 1 hour at 4°C. The column was again washed so as to remove all unbound material. The optical density at 260 nm was monitored and when a zero optical density reading was obtained, the polyclonal anti-apo B or anti-apo A was eluted with 0.2 M glycine-HCl (pH 2.8). Fractions were collected in 1.0 M Tris buffer (pH 8.0). The protein content of the fractions was monitored at 260 nm, and fractions with the highest optical density were pooled and dialyzed overnight at 4°C against 0.1 M NaHCO3, 0.5 M NaCl buffer (pH 8.5). Aliquots of the purified antiserum were frozen and stored at ~70°C until used.

Immunocytochemistry

The flash-frozen samples of the fibrolipid lesions were sectioned at 10 μm using an AO cryostat (American Optical, Buffalo, NY). The sections were flash-dried onto acid alcohol-cleaned (1% glacial acetic acid in 95% ethanol), polylysine-coated (1 mg of polylysine/ml distilled water) slides and these were allowed to dry overnight. The lesion sections were then fixed in cold absolute acetone (~20°C) for 30 seconds and delipidated in acetone for 1 minute at room temperature. Without delipidation, the intensity of staining assessed by visual inspection appeared to be reduced. Without delipidation, the cholesteryl ester crystal formation, which occurred as a result of tissue drying, disrupted the structural integrity of lesions. After delipidation, the sections were rinsed in phosphate-buffered saline (PBS, 10 mM phosphate, 0.9% NaCl, pH 7.2) made fresh daily in glass-distilled water. All incubations were performed in a humid chamber at 25°C. Sections were preincubated in 1% normal nonimmune goat serum for 1 hour. The serum was immediately blotted from around the tissue section, and the affinity-purified antibodies to apo B or apo A were applied. To determine that the patterns of apo B and apo A staining were unique and not a result of plasma perfusing through the vessel wall, the tissue distributions of two unrelated blood proteins, antihuman albumin and fibrinogen, were determined on several contiguous sections. Based on results of

500 ARTERIOSCLEROSIS VOL 8, NO 5, SEPTEMBER/OCTOBER 1988
preliminary experiments with atherosclerotic tissue sections, the primary antibodies were diluted in PBS to a final protein concentration of 5.7 µg/ml for anti-apo B, 3.1 µg/ml for anti-apo A, and 10 µg/ml for anti-albumin and anti-fibrinogen. Tissue sections were incubated in primary antibody for 6 hours, then repeatedly rinsed in PBS. Optimization of antibody dilution and incubation time was performed. Varying dilutions of both anti-apo B and anti-apo A were compared. The incubation time for the tissue sections varied from 2 to 48 hours. Comparable results were obtained regardless of the time period of incubation or antibody dilution. Affinity-purified biotinylated goat antirabbit IgG was diluted in PBS to a final protein concentration of 34 µg/ml and was applied to the tissue sections for 1 hour. Avidin DH and biotinylated glucose oxidase obtained from Vector Laboratories (Burlingame, CA) were mixed 30 minutes before incubation so that avidin-biotin-glucose-oxidase complexes (ABC-GO) were formed. Sections were repeatedly rinsed in PBS and immediately incubated in the ABC-GO complexes for 1 hour. The tissue was again rinsed in PBS, followed by several washes in 50 mM Tris-HCl buffer (pH 8.2) made fresh daily in glass-distilled water. The final color formation was precipitated using a Vectastain substrate kit (Vector Laboratories, Burlingame, CA), which contained glucose, phenazine methosulfate, and nitroblue tetrazolium. These three compounds were mixed before staining in a 50 mM Tris-HCl buffer (pH 8.2) in the concentrations prescribed by Vector Laboratories. The tissue sections were incubated in this disclosing reagent for 30 minutes in the dark or until a definite blue-purple precipitate was seen. The slides were thoroughly washed in distilled water to terminate the reaction and were stored without coverslips in the dark until viewed and photographed.

The following controls were performed to qualitatively assess the extent of nonspecific staining: 1) omission of primary antibody, 2) omission of primary and secondary antibodies, 3) incubation in disclosing reagent alone, 4) use of an unrelated antibody instead of the anti-apo B or anti-apo A antibody, and 5) use of an unrelated biotinylated antibody instead of the goat antirabbit IgG antibody.

A control for binding of the Fc portion of the antibodies to the Fc receptor of cells in the section was performed as described by Jonasson et al.31 This entailed making immune complexes by incubating human serum albumin with antihuman albumin antibodies (Calbiochem, San Diego, CA) at a molar ratio of 1:1 for 3 hours at 37°C. The immune complexes were diluted in PBS to a final protein concentration of 231 µg/ml (determined using the Lowry procedure for proteins) and were incubated on sections in a manner similar to that described for the anti-apo B and anti-apo A antibodies.

The specificity of the affinity-purified antibodies was assessed by radioligand binding. Crossreactivity of anti-apo B was examined by incubating radiiodinated HDL with the antibody. Anti-apo A was incubated with radiiodinated LDL. As a control, radiiodinated LDL and HDL were also incubated with anti-apo B and anti-apo A, respectively. The relative sensitivities of the apo B and apo A antibodies were also assessed. One to 10 nanograms of very low density lipoprotein (VLDL), LDL, HDL, albumin, and fibrinogen were blotted on nitrocellulose paper. The primary antibodies were diluted in PBS to a final protein concentration similar to that used for tissue sections (i.e., 5.7 µg/ml anti-apo B, 3.1 µg/ml anti-apo A, and 10 µg/ml for anti-albumin and anti-fibrinogen). The nitrocellulose paper that had blotted the lipoproteins and plasma proteins was then treated as previously described for tissue sections.

Results

Histologic Examination of Fibrolipid Lesion

The fibrolipid lesions evaluated in this study were similar in appearance to those previously reported.26 The intimal thickness of the lesions at their thickest ranged from 240 to 850 µm with a mean thickness of 644 ± 179 µm. The lesions covered between 2 and 16 square millimeters of surface area with a mean of 9.8 ± 6.7 mm². The fibrous plaques examined in the study were 1739 ± 490 µm in maximal intimal thickness and extended more than 1 square centimeter.

Several consistent histologic features characterized the fibrolipid lesions. Foam cells were commonly found (in 88% of the lesions) overlying the lipid-rich core, while in the larger fibrolipid lesions (12%) foam cells were found in the shoulders of the lesions. Esterase staining was confined to this superficial layer of foam cells. These foam cells were not seen beyond the edges of the raised lesion in the surrounding normal intima. Cells in the musculoelastic (deepest) intimal layer appeared to be predominantly smooth muscle cells, based on their spindle-shaped nuclei, scant perinuclear cytoplasm, and esterase negativity. Flat, basophilic, esterase-negative cells were also present above the lipid-rich core region embedded in bundles of collagen.

The basophilic lipid-rich core was found in several locations within the fibrolipid lesions. The lipid-rich core was present in the musculoelastic layer of the intima in 32% of the lesions. A few lesions (24%) contained the lipid-rich core above the inner limiting membrane, which marks the boundary between the musculoelastic and elastic-hyperplastic intimal layers. Other lesions (44%) contained an enlarged core region located in both the musculoelastic and elastic-hyperplastic layers. This region of basophilia also stained with oil red O. The lipid-rich core observed under polarized light was brightly birefringent at room temperature. A minority (29%) of fibrolipid lesions contained crystalline forms, which retained birefringence on heating to 100°C and thus were identified as cholesterol monohydrate.25

Apolipoprotein B Localization

Apo B was observed in several histologically distinct compartments of the human aortic fibrolipid lesion (Figures 1A, 1B, 1C, and Table 1). Collagenous areas in the lesion cap and in surrounding normal intima were stained. Foam cells, identified by hematoxylin and eosin, oil red O, and esterase-positive staining and found in the cap or shoulders of the lesion, exhibited cell-associated immuno-reactive apo B (Figure 2A). Our light microscopic observations did not distinguish whether the staining
was intracellular or pericellular. Apo B staining was also seen along bands of collagen and elastin as a fine granular material (Figure 2A). This type of staining pattern was most prominent in the normal intima adjacent to the fibrolipid lesion. The lipid-rich core regions of the lesions were stained intensely for apo B. In the smallest fibrolipid lesions, intense patches of apo B staining were observed within the lipid-rich core previously elucidated by oil red O and hematoxylin and eosin staining (Figure 3A). In the larger lesions, a well-circumscribed apo B-positive core region was seen (Figure 3B). An intense band of apo B staining was present at the boundaries of the core region on both luminal and medial aspects. Within this well-demarcated region, apo B staining was homogeneous, finely granular, and associated with connective tissue elements as observed in the more superficial region of the lesion. In few lesions (15%), the lipid-rich core did not stain for apo B. Instead, a wave of immunoreactive apo B was observed to extend from the lumen to about the level of the inner limiting membrane (Figure 3C). In these lesions, the musculoelastic layer of the intima and media were...
devoid of apo B staining. Medial apo B staining was absent except in larger lesions (29%) where a region of faint staining was observed beneath the fibrolipid lesion. The media underlying normal intima lateral to the lesion did not exhibit any immunoreactive apo B. In contrast, apo B staining was diffusely distributed in the adventitia underlying lesioned and nonlesioned regions.

Examination of apo B in fibrous plaques revealed a slightly different pattern of apo B staining. The basophilic lipid-rich core was seen as a well-demarcated region of apo B staining. At the boundaries of the core region, an intense rim of staining similar to that seen in the larger fibrolipid lesions was observed. The more central portion of the core region consisted of a faintly stained granular material. Unlike the fibrolipid lesion, the cap overlying the core did not contain appreciable amounts of apo B staining; however, some cell-associated apo B staining was present. Variable medial and adventitial staining was observed.

**Apolipoprotein A Localization**

Apo A exhibited a different staining pattern from that of apo B (Figures 1A, 1B, 1D, and Table 1). No intense apo A staining about foam cells was observed. Foam cell-associated apo A staining was comparable in intensity to the media underlying the lesion (Figure 1D). Pericellular apo A staining was occasionally apparent in large fibrolipid lesions; however, the majority (91%) of the lesions did not exhibit cell-associated apo A. As was the case with apo B, collagen bands, which are the major component of the superficial regions of the lesion, were speckled with fine, punctate granules of apo A staining (Figure 2B). In most cases (85%), normal intima adjacent to lesions contained apo A staining along collagen bundles and within deeper intimal layers. Apo A was consistently found within the lipid-rich core of the fibrolipid lesion (Figures 1D and 4). A homogeneous, granular apo A staining pattern characterized the core region. Medial apo A staining was observed beneath several of the lesions. A faint, homogeneous, finely granular staining pattern was observed between the elastin bands of the media (Figure 5). The adventitia beneath the lesioned and nonlesioned intima contained immunoreactive apo A.

---

**Table 1. Frequency of Observed Apolipoproteins B and A Staining Patterns**

<table>
<thead>
<tr>
<th>Stained regions</th>
<th>Apolipoproteins</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
</tr>
<tr>
<td>Lipid-rich core</td>
<td>85%*†</td>
</tr>
<tr>
<td>Connective tissue</td>
<td>100%</td>
</tr>
<tr>
<td>Cell-associated</td>
<td>100%</td>
</tr>
<tr>
<td>Media beneath lesion</td>
<td>29%</td>
</tr>
<tr>
<td>Adventitia</td>
<td>100%</td>
</tr>
<tr>
<td>Normal intima adjacent to fibrolipid lesion</td>
<td>79%</td>
</tr>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>100%*†</td>
</tr>
<tr>
<td></td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>9%</td>
</tr>
<tr>
<td></td>
<td>91%</td>
</tr>
<tr>
<td></td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>100%</td>
</tr>
</tbody>
</table>

*Percentage of lesions exhibiting the particular staining pattern.
† The lipid-rich core staining was distinctly more intense than the surrounding tissues.
Figure 3. Apo B staining pattern of the lipid-rich core within fibrolipid lesions. A. Intense well-defined patches of apo B staining (arrows) can be seen in the lipid-rich core. The collagenous cap is also stained. Internal elastic lamina (arrowhead), lumen (L). × 73. B. The core region is well-circumscribed by apo B staining. Note the intense band of staining on the luminal side (arrows) and at the level of the internal elastic lamina (arrowhead). The lesion cap is also stained. × 124. C. The core region is not well-defined, but instead a homogeneous band of apo B staining extends from the lumen (L) to about the inner limiting membrane (micron bar). Note lacuna-like areas of the cap which are devoid of staining (arrows). Fresh-frozen 10 μm sections. Stains were avidin-biotin-glucose oxidase and nitroblue tetrazolium. × 124. Bars = 100 μm.

Specificity and Sensitivity of Antibody Staining

No staining was seen when the primary antibody to apo B and apo A was omitted (Figure 6). An absence of staining was observed when tissue sections were incubated in the avidin-biotin-glucose oxidase complex plus disclosing reagent, or disclosing reagent alone. Incubation of the section with an unrelated primary antibody or biotinylated second antibody also gave similar findings to that seen in Figure 6. Sections incubated with the rabbit antihuman albumin or fibrinogen were diffusely stained. Intimal and medial staining was present, while no specific association to the lipid-rich core or foam cell regions was observed. Treatment of tissue sections with immune complexes to test for cellular Fc receptor binding of the primary and secondary antibodies also revealed an absence of staining. No crossreactivity between anti-apo B and HDL, or between anti-apo A and LDL was observed by radioligand binding. With respect to the sensitivity of
Figure 4. Apo A staining pattern of the lipid-rich core within fibrolipid lesions. This section was adjacent to that shown in Figure 3B. The lipid-rich core is well-delineated by the apo A staining. An intense band of staining is seen on the luminal edge (arrows). No staining is present at the level of the internal elastic lamina (arrowhead). This pattern is in contrast to that seen in 3B for apo B. The lesion cap (Cp) has reduced staining compared to 3B. L = lumen. Fresh-frozen 10 μm section. Stains were avidin-biotin-glucose oxidase and nitroblue tetrazolium. × 110. Bar = 100 μm.

Figure 5. Medial apo A staining. The region beneath the fibrolipid lesion contains immunoreactive apo A which is seen as a homogeneous, finely granular material between and along elastic fibers (arrows). Internal elastic lamina (arrowhead). Fresh-frozen 10 μm section. Stains were avidin-biotin-glucose oxidase and nitroblue tetrazolium. × 128. Bar = 100 μm.

Antibody staining, 5.7 μg protein/ml of anti-apo B was able to detect 1 ng of LDL blotted on nitrocellulose paper, and 3.1 μg protein/ml of anti-apo A detected 1 ng of HDL. No crossreactivity with albumin or fibrinogen was observed.

Discussion

In the present study, we have identified antigens associated with LDL and HDL within an early raised atherosclerotic lesion of the human aorta. The lesions examined in this study had all of the histologic characteristics previously described for the fibrolipid lesion, which we demonstrated to be a likely progenitor of the fibrous plaque.26'27 The major results of this study can be outlined as follows: 1) both apo A and apo B are found in the lipid-rich core of the human aortic fibrolipid lesions,28'27 suggesting that both HDL and LDL are retained in this location; and 2) the frequent observation of apo B, but not apo A, staining in the foam-cell region of small fibrolipid lesions suggests that LDL and HDL may interact differently with the arterial wall.

The immunologically detected apo A and apo B in this study may be present in the form of intact HDL and LDL particles or degraded particles. The presence of apo B confirms a retention of some form of LDL at a particular site, because apo B does not dissociate from the lipoprotein particle. Apo A can dissociate from HDL under some conditions, but it is almost entirely bound to lipoproteins in plasma.32 This fact, along with the small size of HDL which would allow it to penetrate arterial permeability barriers more easily than LDL, allow the inference that apo A localization likewise corresponds to HDL localization in the arterial wall.

Cellular interactions with LDL may play a role in the abundant deposition of lipid within the lipid-rich core. We observed apo B staining associated with foam cells of the lesion cap and shoulders. At the light microscopic level, it was unclear whether the apo B staining was intracellular or pericellular. Previous authors have disagreed on the question of whether immunostainable apo B is present within cells.20'21'22 The presence of LDL in the foam cell region and the consistent presence of foam cells in the fibrolipid lesion raise the possibility of an LDL-cellular interaction in lesion genesis. Our primary interest has been in the formation of the early lipid-rich core region, which is found below the foam cell region and is unlikely to result from foam cell necrosis.28 We speculate that LDL diffusing through the foam cell region and not internalized by cells may be modified, perhaps by oxidation of the lipoprotein particles33 or by transfer of free cholesterol from the cells.34 Such modified LDL may be more prone to deposit deeper in the lesion in the lipid-rich core region. The present study simply confirms that the opportunity for cellular interaction with LDL exists; testing of further hypotheses will await the development of antibodies specific for modified LDL and of appropriate in vitro systems.

The eventual association of native or modified LDL with extracellular matrix constituents is consistent with the morphologic evidence. In the lipid-rich core region, abundant extracellular immunoreactive apo B was observed. This result supports the hypothesis that lipoprotein interactions with connective tissue elements may play a role in
core formation. Furthermore, in the normal intima surrounding the fibrolipid lesion, bands of collagen and elastin were speckled with apo B staining. We have recently reported that three-fourths or more of deep extracellular lipid in normal intima was associated with elastic fibers. The source of this lipid was unclear; however, several investigators have found that LDL cholesterol can bind to elastin. Noma and colleagues extended this work by demonstrating immunologically the presence of apo B on the surface of LDL-incubated elastin. Others have reported an interaction of LDL with sulfated glycosaminoglycans and proteoglycans. It is interesting to note that Camejo and colleagues and Bihari-Varga have observed that when HDL is incubated with the lipoprotein-proteoglycan complexes, it tends to form small amounts of insoluble LDL-proteoglycan material. Thus, it seems plausible that LDL or modified-LDL binds to elastin or proteoglycans, and this interaction modulated by HDL may be an important mechanism for core region formation.

The hypothesis that lipoproteins interact with connective tissue elements is also supported by previously published data related to the quantity of apo B present in atherosclerotic lesions. Hoff and Smith have shown different degrees of extractability of apo B depending on lesion type and area sampled. They report that in the fibrous cap, an area consisting predominantly of collagen, most of the apo B is loosely bound and extractable with buffer, while in the necrotic core, the Triton-extractable (i.e., tightly bound) apo B predominates. The idea that apo B is present in different forms in the core region versus surrounding intima is consistent with our morphologic findings, which indicate that a gradient of apo B staining exists across the lipid-rich core. At the periphery of the core region, an intensely stained band is present, while more faint staining is seen in the center of the core. The morphologic data presented here in association with quantitative data reported by others would tend to suggest that, with lesion development, the lipid-rich core enlarges by accumulation of LDL apo B/lipid around the periphery of the core, which may become tightly bound to the connective tissue matrix as the lesion matures.

The presence of apo A in the fibrolipid lesion suggests that HDL plays a role in lesion development. HDL was homogeneously distributed within the lipid-rich core region, but unlike LDL was not found associated with the foam cell region. HDL was also present in the surrounding normal intima along collagen and elastin bands. The finding of specific localization of apo A to the core region is in contrast to that of Walton and Williamson, who reported a diffuse localization of HDL. Such a discrepancy may be explained by the difference in purity of the antibodies used and the sensitivity of the technique used to visualize the apo A. In this study, affinity-purified rabbit antihuman- apo A, which was determined not to crossreact with apo B, was used. It is possible that a cruder preparation may contain antibodies to other blood macromolecules that may also be found in the arterial wall (i.e., albumin or fibrinogen). We observed diffuse noncompartmental staining in lesions stained for albumin and fibrinogen. This was in stark contrast to the specificity of staining observed with anti-apo A. This data would tend to suggest that the observed immunoreactive apo A was not due to the presence of apo A in the core region. In this study, affinity-purified rabbit antihuman-apo A, which was determined not to crossreact with apo B, was used. It is possible that a cruder preparation may contain antibodies to other blood macromolecules that may also be found in the arterial wall (i.e., albumin or fibrinogen). We observed diffuse noncompartmental staining in lesions stained for albumin and fibrinogen. This was in stark contrast to the specificity of staining observed with anti-apo A. This data would tend to suggest that the observed immunoreactive apo A was not due to the presence of apo A in the core region.

The role of HDL in the arterial wall is unclear, but, as suggested by Camejo et al., HDL may be a significant modulator of LDL interactions. It is also possible that HDL may be involved in reverse cholesterol transport out of the lipid-rich core. Adams and Abdulla have shown that cholesterol crystals in suspension are progressively destroyed when incubated with HDL. Others have shown that in the plasma, lipid can transfer to HDL particles or between HDL and other lipoproteins and membranes. If HDL particles in the arterial wall maintain the capability to accept cholesterol, cholesteryl esters, and triglycerides, then the formation of the lipid-rich core region may be the result of complex interactions between LDL deposition and HDL resorption of lipids in the core region.

Figure 6. Specificity of antibody staining when the primary antibody is omitted. No staining was seen with the following controls: 1) omission of primary and secondary antibody; 2) incubation in disclosing reagent alone; 3) use of an unrelated primary or secondary antibody; and 4) incubation in immune complexes. L = lumen, internal elastic lamina (arrowhead). Fresh-frozen 10 μM section. Stains were avidin-biotin-glucose-oxidase and nitroblue tetrazolium. The photograph was taken with the condenser iris diaphragm one-half closed to provide greater section definition. × 128. Bar = 100 μM.
To explain the difference in distribution of apo B and apo A (i.e., apo B, but not apo A, localizing to the foam cell region) one might postulate that the thresholds of sensitivities of the antibodies for the antigen might differ. We evaluated the sensitivities of the antibodies by immunoblotting and radioisogand binding. In each case, the concentration of antibody used to assess the tissue distribution detected 1 ng of LDL or HDL. Previous investigators have reported that 6 to 7 µg of apo B/mg dry tissue is found in aortic lesions, ranging from fatty streaks to fibrous plaques. On the other hand, the apo A content of fibrous plaques was 0.64 µg/mg dry tissue. The lower apo A content makes it somewhat more likely that a threshold for staining effect in the foam cell region might not have been reached in this study. However, the sensitivities for both antigens were quite high and the characterizations of both apo A and apo B distribution benefits from internal controls (i.e., the presence or absence of staining in the core region, the surrounding normal intima, and the media). Thus, it seems appropriate to conclude that the antigen localization differs with respect to the foam cell region.

The most important new finding of this study with regard to atherogenesis is that LDL, as well as HDL, is localized to the lipid-rich core region. The precise roles of these lipoproteins and the mechanisms of their accumulation in arterial tissue remain to be determined.

References


Index Terms: lipoproteins • low density lipoproteins • high density lipoproteins • immunochemistry • human fibrolipid lesion
Human aortic fibrolipid lesions. Immunochemical localization of apolipoprotein B and apolipoprotein A.
T M Bocan, S A Brown and J R Guyton

Arterioscler Thromb Vasc Biol. 1988;8:499-508
doi: 10.1161/01.ATV.8.5.499
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1988 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/8/5/499