Apolipoprotein A-I and B Distribution in the Human Cornea

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Purpose. To determine the presence and localization of apolipoprotein A-I, a marker for high density lipoprotein, and apolipoprotein B, a marker for low density lipoprotein, in human cornea; to examine the relationship of these lipoprotein markers with areas of lipid accumulation in the cornea.

Methods. A-I and B apolipoproteins were localized in frozen sections of human corneas with specific monoclonal antibodies using avidin–biotin immunoperoxidase labelling. Corneal lipid was colocalized with apolipoproteins by oil red 0 staining of immunostained sections.

Results. Staining data showed that apolipoprotein B and lipid accumulated in the extracellular spaces of peripheral corneal stroma. However, their distributions were not coincident. The posterior region of peripheral corneal stroma (including Descemet’s membrane) often contained lipid without immunodetectable apolipoprotein B. Unexpectedly, apolipoprotein A-I was associated with many keratocytes throughout the cornea in addition to an extracellular distribution heaviest in peripheral cornea.

Conclusions. Lipid deposits lacking apolipoprotein B in peripheral cornea suggest that if accumulated corneal arcus lipid is derived from extracellular deposition of plasma low density lipoprotein, the low density lipoprotein is altered such that it loses its immunoreactive apolipoprotein B. The unexpected association of apolipoprotein A-I with keratocytes suggests that these cells are either taking up or synthesizing a protein sharing an immunoreactive epitope with apolipoprotein A-I. Invest Ophthalmol Vis Sci. 1993;34:3574–3578.

Two lipoproteins thought to be important in the turnover of normal and abnormal lipid deposits in the body are high density lipoprotein (HDL) and low density lipoprotein (LDL). These may be demonstrated histochemically by their respective contents of apolipoprotein A-I (apoA-I) and apolipoprotein B (apoB). Recent literature has discussed abnormal deposits of blood lipoproteins in blood vessels. These reports suggest that LDL is involved in the deposition of vessel lipids and HDL is involved in their removal.1

Little has been reported in the literature about the lipid deposition process in the cornea even though this tissue is a common and clinically important site for lipid deposits. Lipid deposition in the cornea occurs with age and is present in the majority of persons older than 50 years.2 Corneal arcus development is accelerated in persons with many dyslipoproteinemias.3-5 In these instances, corneal arcus may alert the clinician to an underlying disorder of plasma lipoprotein metabolism that may be associated with the development of premature cardiovascular disease.3-5 The current study was therefore undertaken to determine the presence and distribution in the human cornea of HDL and LDL by histologic identification of their respective major protein components, apoA-I and apoB.

To our knowledge, the only relevant observations on the distribution of corneal lipoproteins have been those of Walton et al.6,7 He demonstrated LDL in the peripheral cornea and suggested a pathogenetic correlation with age-related corneal arcus. We have partly confirmed Walton’s observation for the localization of LDL and have added new unexpected observations for the localization of HDL.

METHODS

Ten eyes from ten persons (age range, 72 to 92 years) were obtained through the assistance of the Lions Eye Bank (District 22-C). All but one eye had grossly visible
corneal arcus. The previously frozen eyes were allowed to thaw and the corneas were excised from each eye, 0.5 to 1.5 cm posterior to the limbus. Frozen sections were prepared as follows: corneas were bisected in the vertical meridian, embedded in OCT compound (Tissue-Tek, Mishawaka, IN), sectioned at 7 to 10 μm parallel to the vertical meridian, and mounted on slides coated with 3% gelatin. The tissue sections were stored at -70°C.

For immunohistochemistry, tissue sections were fixed in absolute acetone at room temperature for 7 minutes or fixed in 4% formalin for 5 minutes, rinsed three times (3 minutes each rinse) in 0.05 M Tris-saline buffer (pH 7.6), and then immersed in 10% horse serum in Tris-saline buffer for 10 minutes. The tissue sections were next incubated for 2 hours with mouse monoclonal antibodies diluted 1:100 with Tris-saline buffer. The monoclonal antibodies used were specific for human apolipoprotein B (Cappel #59409, Durham, NC; Medix Biotech #MIA1608, Foster City, CA) and human apolipoprotein A-I (Cappel #59403 and #59404, Medix Biotech #MIA1404). All monoclonal antibodies were immunoglobulin G and purified from ascites fluid. Negative controls were substitution of the apolipoprotein specific monoclonal antibody with a mouse monoclonal antibody (Cappel #36345) that has no known hapten- or antigen-binding activity. Frozen sections of human liver tissue were processed and immunostained similar to sections of cornea. The liver sections served as a positive tissue control because it is established that liver synthesizes both apoA-I and apoB.

After incubation, the slides were again rinsed with Tris-saline buffer three times. Tween-20 (1 μl/1 ml) (Fisher, Pittsburgh, PA) was added to the Tris-saline buffer for the third wash. The slides were then treated for 1 hour with biotin-labeled horse anti-mouse immunoglobulin G diluted 1:200, rinsed again, and treated for 45 minutes with avidin-biotin-peroxidase complex diluted 1:100 in accordance with the instructions supplied with the immunostaining kit ( Vectastain ABC kit, Vector Laboratories, Burlingame, CA). The slides were then rinsed three times with Tris-saline buffer. Next, tissue sections were developed in diaminobenzidine solution for 2 to 4 minutes, which yielded a black reaction product. Diaminobenzidine solution was prepared by adding 0.06% diaminobenzidine and 1 ml of an 8% nickel sulfate solution to 200 ml of Tris-saline buffer. The solution was filtered before addition of 200 μl of 30% hydrogen peroxide. The sections were counterstained with 1% methyl green in some cases to demonstrate cell nuclei, dehydrated in ascending alcohols and xylene, and mounted with Permount (Fisher).

To stain fat, frozen sections were thawed at room temperature for one half hour, fixed in 4% formalin for 5 minutes, immersed in absolute propylene glycol for 2 minutes, and then stained for 6 hours with 0.5% oil red 0 dissolved in propylene glycol. Oil red 0 stains fat a red color. The slides were then rinsed in distilled water, stained with Mayer's hematoxylin for 1 minute, rinsed again in distilled water and finally mounted with glicerin jelly. Two or more sections from four corneas were doubly stained with apoB monoclonal antibody followed by oil red 0. Sections that were doubly stained were fixed in 4% formalin for 5 minutes instead of acetone before antibody staining.

RESULTS

ApoB, the marker for LDL, was characteristically present in the periphery of all corneas. ApoB occurred predominantly in the anterior half of the stroma and, sometimes, in a short beginning segment of peripheral Bowman’s membrane. There was no staining of Descemet’s membrane. The staining extended no more than 2 to 3 mm axialward, superimposed on or between the collagenous fibers (Fig. 1). The distribution of apoB was entirely extracellular in the corneal stroma. Keratocytes and endothelium did not stain. The absence of apoB staining of keratocytes was confirmed by examining sections that had been counterstained with methyl green. Optical sectioning of these counterstained sections allowed us to determine that there was no apoB staining of keratocytes. Rather, apoB immunostaining occurred along the stromal collagen lamellae.

There was inconstant apoB staining of some peripheral corneal epithelial cells and their basement

![Figure 1. Immunostained apoB in peripheral human cornea. ApoB staining was extracellular and predominantly in the anterior stroma (lower arrow). Intense apoB staining occurred in the vascular limbus (arrowhead). There was an absence of apoB staining (and oil red 0 staining [not shown]) in the remaining limbal region (*). ApoB staining is present at the beginning of Bowman’s membrane (upper arrow). The cornea extends to the lower edge of the photomicrograph. Magnification X30.](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933172/)
membranes. The paralimbal conjunctiva, the vascular limbus, and other richly vascular tissue (e.g., ciliary processes) showed an intense and smudgy type of apoB staining. This was in contrast to the granular stain in the corneal stroma. ApoB staining was absent from the remaining limbal region (the clear interval, indicated by * in Fig. 1). There was occasional intense staining of conjunctival epithelial cells (Fig. 2a).

Oil red 0 staining also showed an exclusive localization in the extracellular compartment of the peripheral cornea (including the eye without obvious arcus). However, in contrast to apoB staining, oil red 0 staining extended through the full stromal thickness including Descemet's membrane (compare a and b in Fig. 2). Also, oil red 0 staining of peripheral Bowman's membrane extended more centrally than did apoB staining of this membrane. Although there was heavy apoB staining of the vascular limbus, there was no oil red 0 staining of the vascular limbus (compare a and b in Fig. 2). As was the case for apoB staining, there was no oil red 0 staining of the clear interval (data not shown). The contrasting distributions of apoB and oil red 0 staining were confirmed in sections that were doubly stained for both constituents (Fig. 3).

In contrast to apoB and oil red 0 staining, apoA-I was selectively present in many but not all keratocytes throughout the breadth of the stroma (Fig. 4), and in corneal endothelial cells (Fig. 5). Most keratocytes in the center of the cornea showed some degree of apoA-I staining. However, only some keratocytes were heavily stained. In the periphery of the cornea, many keratocytes showed no apoA-I staining. ApoA-I staining was present focally in the extracellular compartment in the center of the posterior corneal stroma (Fig. 5). Heavier diffuse extracellular staining occurred in the periphery of the cornea. There was focal apoA-I staining of Bowman's membrane and Descemet's membrane. Additionally, the vascular sclera and episcleral tissue showed diffuse extracellular apoA-I staining.

Liver tissue showed positive staining for both apoA-I and apoB. Monoclonal antibodies from different sources but of similar specificity (either anti-apoA-I or anti-apoB) gave similar patterns of staining in cornea or liver. There was no staining of cornea or liver with the mouse monoclonal antibody directed toward an irrelevant antigen.

DISCUSSION

ApoA-I, a marker for HDL, and apoB, a marker for LDL, demonstrated contrasting localization patterns in the cornea. ApoB was extracellular and limited to the peripheral cornea. ApoA-I was also extracellular in the peripheral cornea. However, apoA-I was additionally associated with keratocytes throughout the corneal stroma in a distribution similar to that of experimentally induced aberrant lipogenesis.10 Walton previously suggested a correlation of apoB with the deposition of lipid that can be stained with oil red 0 and that comprises corneal arcus.6 The pattern of apoB and oil red 0 staining in our study differed, however, from Walton's. In our study, apoB occurred predominantly in the anterior half of the corneal stroma and in the vascular regions of the limbus and conjunctiva, whereas oil red 0-stained lipid was present in the entire thickness of the cornea including Descemet's membrane. Also, oil red 0 staining showed a more extensive involvement of Bowman's membrane than was observed for apoB staining. Such a pattern of oil red 0-stained lipid is characteristic for corneal arcus.11 It is possible that our use of double staining of both lipid and apoB in the same section (Fig. 3) rather than in separate frozen sections as Walton did allowed us to observe the differences in their distribution.

The significance of the fact that lipid deposits lacking immunodetectable apoB accumulate in the region marked by corneal arcus remains to be deter-
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FIGURE 3. Dual staining of apoB and lipid in peripheral human cornea. The corneal tissue section was first immunostained to localize apoB and then oil red 0-stained to colocalize lipid. ApoB is present in the anterior region of the corneal stroma, whereas oil red 0-stained lipid is present throughout the entire thickness of the corneal stroma including Bowman’s and Descemet’s membranes. Singly stained parallel sections showed that the anteriorly located apoB staining was black and the anteriorly located oil red 0 staining was red. Overlap of the red oil red 0 staining and black apoB staining in the doubly stained section produced a brown color as shown in the region within the bracket. Magnification X90.

mined. This suggests that if accumulated corneal arcus lipid derives from extracellular deposition of plasma LDL, the LDL is altered by some process such that LDL loses immunoreactive apoB. Previously, Sheraidah et al also questioned if arcus lipoides-associated lipids are simply infiltrated LDL. When they isolated and analyzed arcus lipoides-associated lipid they could not account for the majority of this lipid as being identical with unaltered LDL. This was because of a relative lack of apoB in this lipid as compared to the amount of recovered cholesterol.

We would tentatively interpret the diffuse apolipoprotein staining of the vascular areas of the limbus as having resulted from diffusion of lipoproteins from the blood vessels. The localization of extracellular apoB and apoA-I in the peripheral cornea could be related to its proximity to the limbal vasculature. Similarly, the occasional staining of the epithelial cells may represent secondary lipoprotein uptake from the tear film.

To date, no observations have been recorded on the distribution of apoA-I in the cornea. A new, unexpected finding of this study is the association of apoA-I with keratocytes. Although keratocytes throughout the cornea had apoA-I staining, not all keratocytes showed staining. Association of apoA-I with keratocytes could represent local synthesis of apoA-I, or a related protein sharing an immunoreactive epitope with apoA-I. Currently, however, the liver and intestine are the only known sites of synthesis of apoA-I in humans.

Alternatively, association of apoA-I with keratocytes could reflect uptake of exogenously derived apoA-I by keratocytes. Maurice has suggested that the largest molecule capable of diffusing across unswollen human cornea would be less than 18 nm in diameter. Thus, HDL (≤10 nm) but not LDL (≥22 nm) would be theoretically capable of diffusion to the center of the cornea from the limbal vasculature.

Cholesterol accumulates in the central cornea in a...
FIGURE 5. Immunostained apoA-I in posterior human cornea. ApoA-I was associated with the corneal endothelium (arrowheads) and along the anterior aspect of Descemet's membrane. Besides some focal extracellular deposits of apoA-I (long arrows), a number of keratocytes show associated apoA-I (short arrows). Magnification ×150.

number of inherited metabolic disorders of HDL metabolism (e.g., Fish-Eye Disease and lecithin:cholesterol acyltransferase deficiency) as well as in Schnyder's corneal crystalline dystrophy. The finding of apoA-I in the central cornea suggests a link between the accumulation of cholesterol in these diseases and altered lipoprotein metabolism. Whatever the ultimate explanation of keratocyte-associated apoA-I, the cornea offers unique possibilities in the study of lipid turnover not only in the cornea, but also in the body as a whole.

Key Words
cornea, apolipoproteins, lipid, cholesterol, corneal arcus, immunohistochemistry, LDL, HDL

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