thematic review

Thematic Review Series: Lipids and Lipid Metabolism in the Eye

Apolipoprotein B-containing lipoproteins in retinal aging and age-related macular degeneration

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Abstract The largest risk factor for age-related macular degeneration (ARMD) is advanced age. With aging, there is a striking accumulation of neutral lipids in Bruch's membrane (BrM) of normal eye that continues through adulthood. This accumulation has the potential to significantly impact the physiology of the retinal pigment epithelium (RPE). It also ultimately leads to the creation of a lipid wall at the same locations where drusen and basal linear deposit, the pathognomonic extracellular, lipid-containing lesions of ARMD, subsequently form. Here, we summarize evidence obtained from light microscopy, ultrastructural studies, lipid histochemistry, assay of isolated lipoproteins, and gene expression analysis. These studies suggest that lipid deposition in BrM is at least partially due to accumulation of esterified cholesterol-rich, apolipoprotein B-containing lipoprotein particles produced by the RPE. Furthermore, we suggest that the formation of ARMD lesions and their aftermath may be a pathological response to the retention of a subendothelial apolipoprotein B lipoprotein, similar to a widely accepted model of atherosclerotic coronary artery disease (Tabas, I., K. J. Williams, and J. Borén. 2007. Subendothelial lipoprotein retention as the initiating process in atherosclerosis: update and therapeutic implications. *Circulation.* 116:1832–1844). This view provides a conceptual basis for the development of novel treatments that may benefit ARMD patients in the future.—Curcio, C. A., M. Johnson, J-D. Huang, and M. Rudolf. **Apolipoprotein B-containing lipoproteins in retinal aging and age-related macular degeneration. *J. Lipid Res.* 2010.** 51: **451–467.**

Supplementary key words retinal pigment epithelium • Bruch's membrane • drusen • basal deposits • cholesterol • retinyl ester

INTRODUCTION TO OUTER RETINA AND CHOROID, STATEMENT OF PURPOSE

Embryologically part of the central nervous system, the retina (**Fig. 1A**, B) converts light energy to an electrochemical signal for transmission to the brain through the optic nerve. The 100 million rod and cone photoreceptors, located at the outer surface of the retinal sheet, are supported by the retinal pigment epithelium (RPE). This polarized monolayer serves diverse functions essential for optimal photoreceptor health, including daily phagocytosis of photoreceptor outer segment tips, vitamin A metabolism, maintenance of retinal attachment, and coordination of cytokine-mediated immune protection. The photoreceptor distribution varies considerably across the 1,000-mm^2 retina, with cone density approaching 200,000/mm^2 in the fovea and rod density similarly high 2–4 mm away from the foveal center. Surrounding the fovea is a pile-up of inner retinal neurons that anatomically defines the 6 mm-diameter extent of the macula, subtending 21° of visual angle.

Two vascular systems supply the human retina. The inner retinal layers rely on the intrinsic retinal circulation and the photoreceptors and RPE depend on the choroid located external to them (Fig. 1C). About 200–300 μm thick, the choroid has the highest blood flow per unit volume in the body, with 7-fold greater flow in the macula relative to the periphery. The innermost 2–4 μm of the choroid, i.e., subjacent to the RPE, is Bruch's membrane (BrM, Fig. 1B).

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Abbreviations: apo, apolipoprotein; ARMD, age-related macular degeneration; BlamD, basal laminar deposit; BlinD, basal linear deposit; BrM, Bruch's membrane; CAD, coronary artery disease; CM, chylomicron; EC, esterified cholesterol; HBL, hypobetalipoproteinemia; MTP, microsomal triglyceride transfer protein; OTAP, osmium-tannic acid-paraphenylenediamine; QFDE, quick-freeze/deep-etch; RPE, retinal pigment epithelium; TG, triglyceride; UC, unesterified cholesterol.

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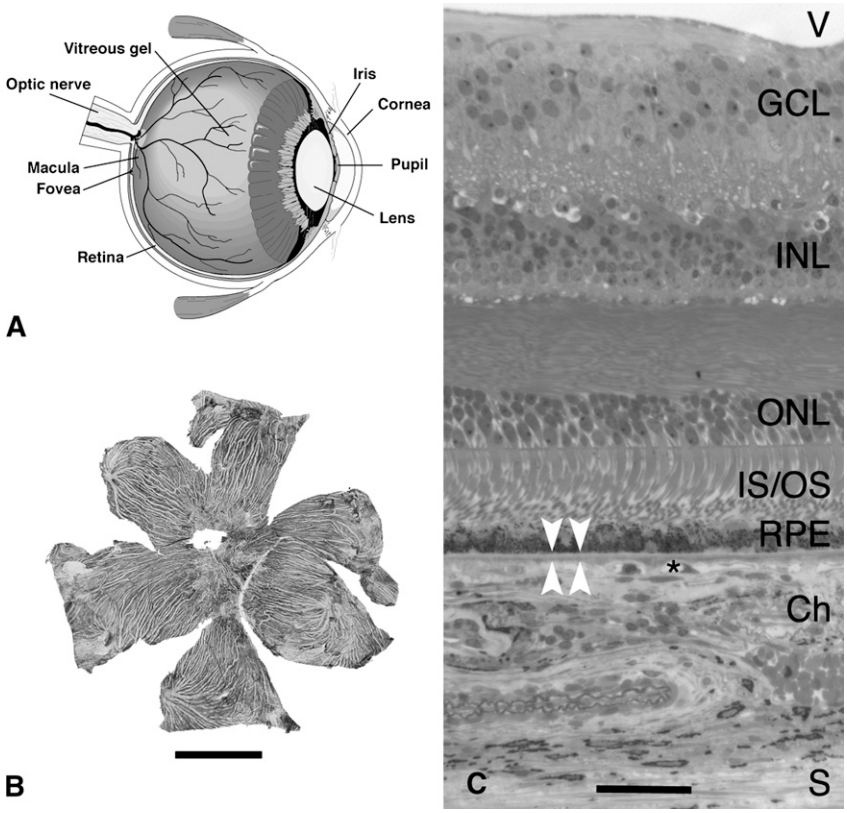

Fig. 1. Chorioretinal anatomy. A: Cross-section of human eye. B: Flattened choroid from a human eye. Choroidal melanocytes impart pigmentation. Pale stripes are empty vessels. Bar, 10 mm. C: Histological section of eye wall, including retina of the macula. V, vitreous; GCL, ganglion cell layer; IPL, inner plexiform layer; INL, inner nuclear layer; OPL, outer plexiform layer; ONL, outer nuclear layer; IS/OS, inner and outer segments of photoreceptors; RPE, retinal pigment epithelium; Ch, choroid; S, sclera. Bar, 50 μm. Arrowheads bracket BrM, and asterisk indicates choriocapillaris.

This five layer extracellular matrix is laid flat along one side of the choriocapillaris, a dense capillary bed (**Fig. 2A**). Unhindered transport across BrM of nutrients to and metabolites from the RPE is essential for normal vision by the photoreceptors (1, 2). It has been instructive for us to analogize BrM, a wall of a capillary bed, to the intima of a large artery, due to its position between diffusion barriers, thickening throughout adulthood, and extracellular matrix composition (3, 4) (see below).

Age-related macular degeneration (ARMD) is a major cause of vision loss in the elderly of the industrialized world. The largest risk factor for ARMD is advanced age. One of the most prominent age-related changes to the human retina is the accumulation of histochemically detectable neutral lipid in normal BrM throughout adulthood (5). This significant, universal, and poorly understood change to BrM has the potential to have a major impact on physiology of the RPE. Further, this lipid deposition occurs in the same BrM compartment as the pathognomonic extracellular, lipid-containing lesions of ARMD.

Our thinking about how lipids contribute to ARMD has been extensively influenced by the vast knowledge base available for atherosclerotic coronary artery disease (CAD), a condition for which lipoprotein deposition in vessel walls is a well-established causative agent (Fig. 2B,C). We have been educated about a lipoprotein-centered view of atherosclerosis by many different mentors whose work encompasses cell biology, biochemistry, physical chemistry, and descriptive and experimental pathology of human tissues and animal models (6–19). In this review, we summarize and contextualize recent work, primarily from our own laboratories, establishing that age-related lipid accumulation in BrM can be accounted for by cholesterol-rich lipoproteins of intraocular origin. Many other ARMD-relevant topics (e.g., cell death, genetics, neovascularization, visual function) have been recently reviewed (20–23). A version of this review tailored to an ophthalmic audience is available elsewhere (24).

INTRODUCTION TO ARMD

Epidemiology and risk factors

The macula, required for high-acuity vision, is vulnerable to ARMD (25). The disease has two forms. An underlying degenerative process ("dry" ARMD) includes pathognomonic extracellular lesions (see next section) and RPE cell death via apoptosis across the macula, resulting in a slow but devastating loss of vision as photoreceptors also die (26–28). Choroidal neovascularization ("wet" ARMD), in which choriocapillaries cross BrM and spread laterally within the plane of these lesions, is an urgent and sight-threatening complication of this process. Clinical ophthalmology has

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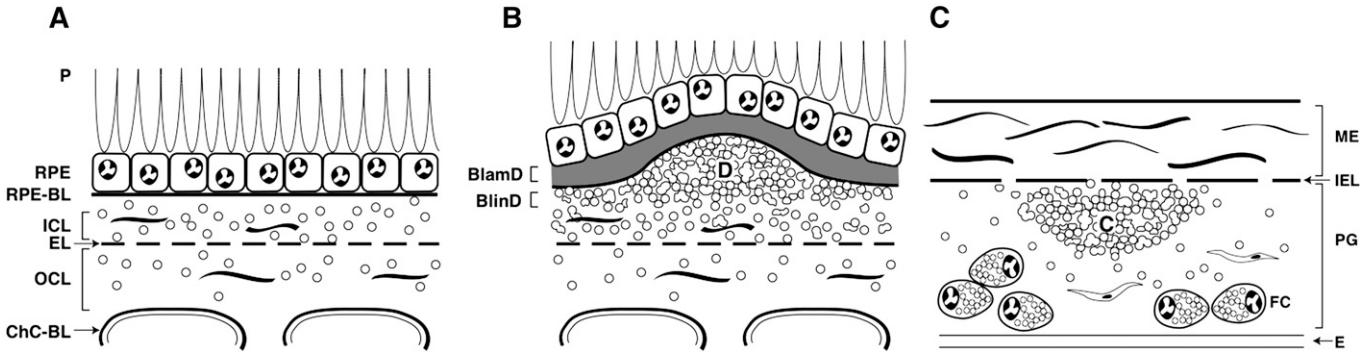

Fig. 2. ARMD lesions versus plaque. Schematic cross-sections of RPE/ BrM complex from a normal eye (A) and an eye with ARMD (B), compared with atherosclerotic arterial intima (C). Endothelium and vascular lumens (choriocapillary, A, B; artery, C) are at the bottom. Small circles indicate EC-rich lipoproteins, native and modified. Drawings are not at scale. The thickness of normal BrM and intima is 4–6 μm and 100–300 μm, respectively. A: P, photoreceptors; RPE, retinal pigment epithelium; RPE-BL, RPE basal lamina; ICL, inner collagenous layer; EL, elastic layer; OCL, outer collagenous layer; ChC-Bl, basal lamina of choriocapillary endothelium. B: BlamD, basal laminar deposit; BlinD, basal linear deposit; D, druse. C: ME, musculo-elastic layer; IEL, internal elastic layer; C, lipid-rich core; PG, proteoglycan layer; FC, foam cells.

been revolutionized by the recent advent of highly specific inhibitors of vascular endothelial growth factor that, when injected intravitreally, not only retard vision loss but also improve vision in many of the 15% of ARMD patients afflicted with choroidal neovascularization (29). Further, some patients at early to intermediate stages of dry ARMD can benefit from supplementation with micronutrient vitamins and zinc (30).

Of ARMD risk factors (31), advanced age and family history are strongly and consistently related to ARMD across multiple studies. Cigarette smoking, hypertension, and cataract surgery appear to increase the risk of progression to neovascular ARMD in most studies. Other risk factors like obesity and atherosclerotic vascular disease have less consistent findings and weaker associations, and no association exists between ARMD and diabetes or ARMD and plasma cholesterol levels. Linkage studies and genome-wide scans have identified polymorphisms in complement factors H and I, HTRA1, ARMS2, and mitochondrial DNA polymorphism A4917G as risk factors, and complement factor B, C3, and apolipoprotein (apo)E4 as protective factors (32–39).

Histopathology of extracellular lesions

ARMD's characteristic lesions are aggregations of lipid-containing extracellular debris in the RPE/BrM complex (drusen and basal deposits, **Fig. 3B**,C) that ultimately impact RPE and photoreceptor health (40, 41). Drusen are yellow-white deposits seen behind the RPE in a retinal fundus examination. They are typically classified as "hard" and "soft" on the basis of their borders and the level of risk conferred for advanced disease (higher risk for soft) (42–45). Drusen are defined histologically as focal, dome-shaped lesions between the RPE basal lamina and the inner collagenous layer of BrM. They are found, at least in small numbers, in most older adults (46, 47). Molecular constituents of drusen, virtually all identified during the last decade, include vitronectin, tissue inhibitor of metalloproteinase 3 (TIMP-3), complement factor H, fibrillar and nonfibrillar amyloid, complement component C3, and zinc (33, 48–53). The prominence of complement proteins in drusen in conjunction with associations with sequence variances in complement encoding-genes in ARMD (see previous section) has stimulated the current interest in understanding the alternate complement pathway in retinal disease.

Of importance to this review, drusen contain abundant cholesterol and apolipoproteins. Studies using sudanophilic dyes, filipin, or polarizing microscopy identified neutral lipids and polar lipids in age-related and ARMD-related drusen (3, 54–59) (Fig. 3D,E). All drusen, whether hard or soft, have abundant esterified cholesterol (EC) and unesterified cholesterol (UC). Druse cholesterol assumes different morphologies and distributions, including UC-rich cores and EC-rich lakes, which are thought to reflect different formative processes. The distinctive clefts signifying cholesterol crystals in tissue sections (60) have not been reported in these sub-RPE deposits. Immunoreactivity for multiple apolipoproteins, including B, A-I, C-I, C-II, and E, appears in drusen with frequencies ranging from 100% (apoE) to ~60% (A-I) (57, 61–64). ApoC-III, which is abundant in plasma, is present in fewer drusen (16.6%) than apoC-I (93.1%), which is sparse in plasma, indicating either a specific retention mechanism for plasma-derived apolipoproteins within drusen, or an intraocular source.

Basal deposits are two diffusely distributed lesions associated with ARMD that have distinctly different size, composition, and significance (65) (Fig. 3B,C). Basal laminar deposit (BlamD), between the RPE and its basement membrane, forms small pockets in many older normal eyes or a continuous layer as thick as 15 μm in eyes with ARMD (58, 65, 66). Ultrastructurally, BlamD resembles basement membrane material and contains laminin, fibronectin, and type IV and VI collagen (67–70). Thick BlamD, associated with risk for advanced ARMD (65), is more heterogeneous, containing vitronectin, MMP-7, TIMP-3, C3, and C5b-9 (66) and histochemically detectable EC and UC. These components are possibly in transit from the RPE to BrM and/or drusen (55, 57, 58).

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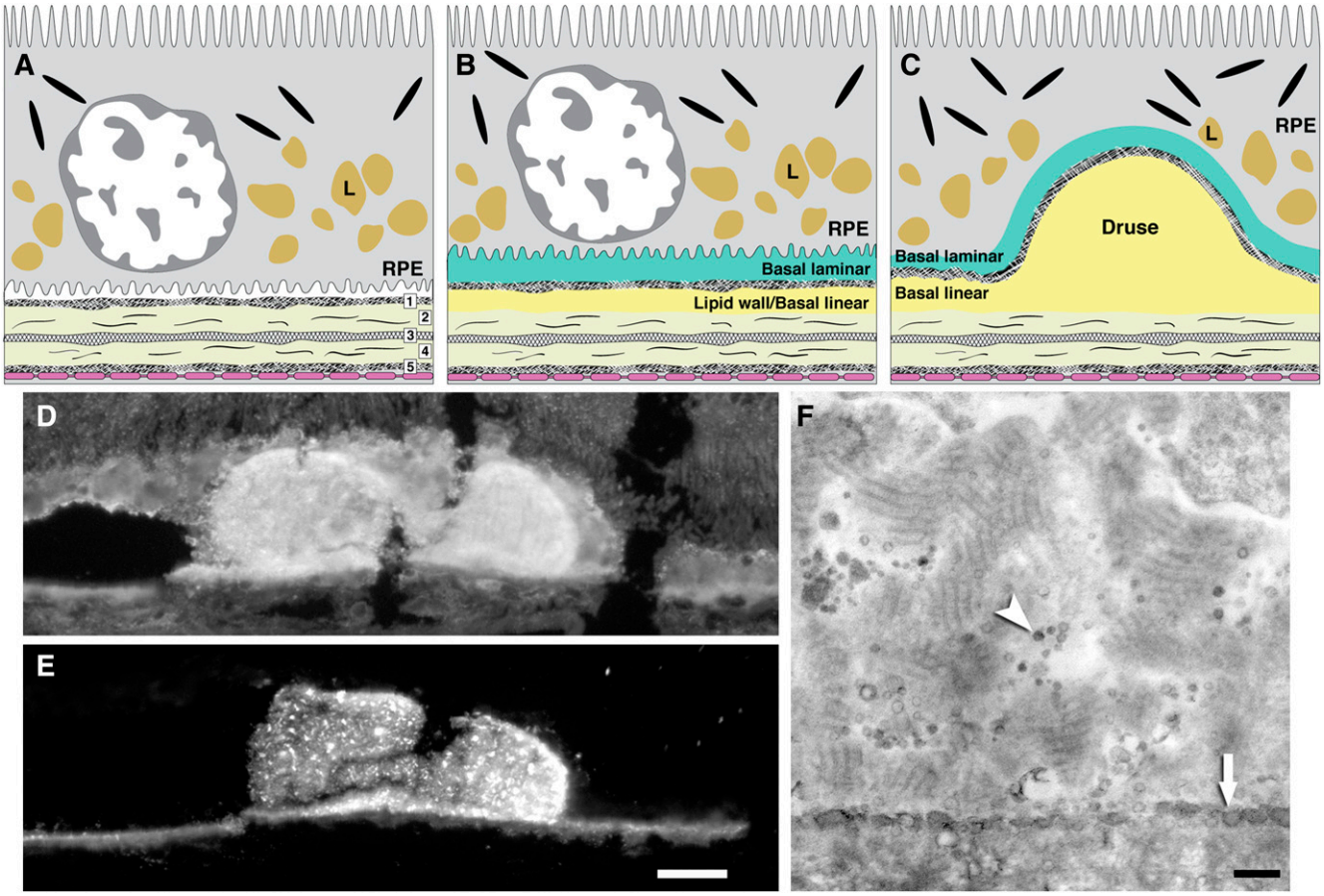

Fig. 3. Bruch's membrane and extracellular lesions associated with ARMD. A: BrM has 5 layers in a normal eye: 1, basal lamina of the RPE; 2, inner collagenous layer; 3, elastic layer; 4, outer collagenous layer; 5, basal lamina of the choriocapillary endothelium (fenestrated cells, pink). L, lipofuscin (an abundant, autofluorescent age-pigment resulting from photoreceptor outer segment phagocytosis). B: An ARMD eye has basal laminar deposit (BlamD) and basal linear deposit (BlinD) on the inner and outer aspect of the RPE basal lamina. The precursor to BlinD is the lipid wall. C: Drusen, BlinD, and the lipid wall occupy the same plane. D, E: Filipin fluorescence. Bars, 25 μm. Adapted from (57). D: Druse and surrounding chorioretinal tissue contain UC. E: Same druse as D and BrM contain EC. Speckles represent lakes of EC within druse. F: Detail of BlamD in an ARMD eye postfixed using OTAP (73). RPE is off the top edge, and BrM is at the bottom. BlamD has lipoprotein-like particles distributed in linear tracks (arrowhead), and BlinD has pooled neutral lipid (arrow). Adapted from (87).

Basal linear deposit (BlinD), between the RPE basement membrane and the inner collagenous layer of BrM, is a thin (0.4–2 μm) lipid-rich layer. Because BlinD is located in the same plane and contains the same material as soft drusen, these lesions are likely alternate forms (layer and lump) of the same entity (71). The principal component of these lesions contains lipid and has been known as membranous debris (42, 65, 72). As viewed by transmission electron microscopy following osmium tetroxide postfixation, membranous debris appears as variably sized, contiguous coils or whorls of uncoated membranes consisting of uni- or multi-lamellar electron dense lines surrounding an electron-lucent center. Although membranous debris is frequently interpreted as vesicles with aqueous interiors, recently evidence indicates that it is not vesicular (see below). Rather, ARMD tissues postfixed with osmium-tannic acid-paraphenylenediamine (OTAP) to preserve neutral lipid (73) contain linear tracks of solid particles across BlamD and lipid pools within the layer of BlinD (Fig. 3F). Light microscopic histochemical and ultrastructural studies together suggest that BlinD and soft drusen contents are much more highly enriched in UC than the membranes of surrounding cells (24, 55, 58). On the basis of these and other observations, we proposed to change the name of this major lipid-containing component of ARMD-specific lesions from "membranous debris" to "lipoprotein-derived debris" (24).

NEUTRAL LIPIDS ACCUMULATE WITH AGE IN BRM

Clinical observations on the natural history of fluid-filled RPE detachments in older adults led to the hypothesis by Bird and Marshall (74) that a lipophilic barrier in BrM blocked a normal, outwardly-directed fluid efflux from the RPE. This hypothesis motivated a pivotal laboratory study by Pauliekhoff et al. (5), who used three histochemical stains to identify lipids in eyes from donors aged 1 to 95 years with grossly normal maculas (**Fig. 4A–**D). Oil red O-binding material localized exclusively to BrM, whereas two other dyes (Bromine Sudan Black B and Bromine-Acetone-Sudan Black B) labeled cells throughout the choroid in addition to BrM. All stains indicated the

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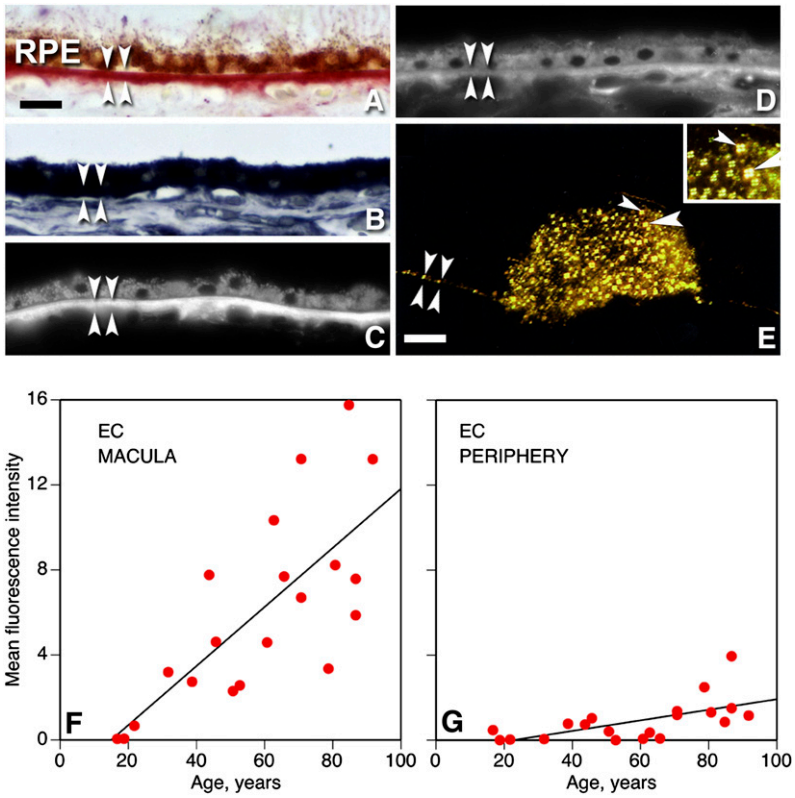

Fig. 4. Lipids in BrM: localization and increase with age. A–D: Histochemical localization of lipids in macular RPE/ BrM complex of an elderly donor. RPE is at the top and choroid at the bottom of each panel. BrM is bracketed by arrowheads. Bar in A, 20 μm. A: Oil red O stains BrM and lipofuscin within RPE. B: Sudan Bromine Black B stains BrM, RPE, and cells throughout choroid. C: Filipin for esterified cholesterol stains BrM. RPE lipofuscin autofluorescence is much less intense than filipin. D: Filipin for unesterified cholesterol stains BrM, RPE, and choroidal cells. E: EC localized in BrM (between brackets) and in a druse viewed with polarized light at 10°C. Birefringence shaped like Maltese crosses signify liquid crystals of EC (arrowheads, higher magnification in inset). Bar, 50 μm. From (56), with permission from the Association for Research in Vision and Ophthalmology. F,G: Filipin fluorescence due to EC increases markedly with age in normal eyes, more in the macula than in the periphery. Fluorescence intensity ($\times 10^{-6}$ arbitrary units) for each eye is corrected for background autofluorescence of BrM. From (3).

same trend with age: no eyes from donors <30 years old exhibited staining, eyes from donors 31–60 years old exhibited variable staining, and eyes from donors ⩾61 years old exhibited moderate to intense staining (Fig. 4A–D). These findings were repeated in other series of eyes (56, 75).

Lipids that bind the histochemical stain oil red O increase with age in normal human connective tissues, including the sclera (76), cornea (77), intima of large arteries (78), and BrM (5). In the intima, the oil red O-positive material comprises small (60–200 nm) extracellular droplets highly enriched in EC relative to UC [(69% EC, 22% UC, and 9% phospholipid (PL)] (73, 79–82). The source of EC in sclera, cornea, and arterial intima is LDL translocated from plasma into connective tissues. EC-enriched particles are thought to arise from smaller LDL particles by extracellular matrix-mediated trapping of LDL, degradation of LDL protein and/or PL components, and fusion of the remaining lipid components (9). Thus, it is important to determine whether lipid deposition in BrM is an ocular manifestation of this ongoing systemic process or a phenomenon unique to retina.

Two 2001 studies established that EC was a prominent component of this deposition in BrM. Curcio et al. (3) examined the EC and UC content of BrM using the fluorescent, polyene antibiotic filipin (Fig. 4C,D). This compound binds specifically to the 3-β-hydroxy group on cholesterol and other sterols. Filipin can be used in tissue sections to identify both UC and EC (79, 83, 84). EC is localized after native UC is extracted with ethanol, EC is hydrolyzed with the enzyme cholesterol esterase, and the newly released UC is bound to filipin. With this method, EC was localized and quantified in macula and temporal periphery of 20 normal eyes from age 17 to 92 years (3) (Fig. 4F,G). In the macula, EC was undetectable before age 22 years and then rose linearly throughout adulthood to reach high and variable levels in aged donors. EC was detectable in periphery at roughly 1/7th the level of macula but still increased significantly with age. In the same eyes, UC in macular BrM also increased throughout adulthood, although not as steeply as EC, and it did not increase significantly with age in peripheral retina (not shown).

Also in 2001, Haimovici et al. (56) used hot stage polarizing microscopy (85, 86) to examine the birefringence and melting characteristics of lipids in BrM and sclera (Fig. 4E). EC in tissue sections appears as liquid crystals ("Maltese crosses") when examined through a polarizing filter. When sections are heated and cooled slowly, liquid crystals melt and reform at characteristic temperatures dictated by the saturation level of the major ester. This study showed that BrM and drusen contained Maltese crosses (Fig. 4E) that melted at a higher temperature than those in sclera. Few birefringent crystals signifying triglyceride (TG) were found in either tissue, and the prominent age-related increase in BrM EC was again detected.

EVIDENCE FOR AN INTRAOCULAR APOB-CONTAINING LIPOPROTEIN

BrM lipoprotein morphology and distribution

Ultrastructural studies through the years revealed numerous small (<100 nm), round electron-lucent spaces

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in BrM of older eyes (for review, see ref. 24). Because these spaces occasionally had a single electron-dense line at their borders, they were frequently described as either membranous or vesicular (i.e., liposomes with aqueous interiors). However, conventional methods of tissue preparation for thin-section transmission electron microscopy extract tissue lipids. The OTAP technique (73) was used to show that BrM vesicles were actually solid and electron dense particles (3, 87). Because evidence presented above indicates that the particles are lipoproteins, this name will be used.

Particles were even more striking when demonstrated by quick-freeze/deep-etch (QFDE), an ultrastructural tissue preparation method that reveals lipids and extracellular matrix in exquisite detail. Similar to freeze fracture but with an etching step that removes frozen water from the tissue (88), QFDE was used to demonstrate lipoprotein accumulation in the aortic wall at the earliest stages of atherosclerosis (89–91). Used to examine BrM, QFDE revealed solid particles accumulating with age that also exhibited a shell and core structure (**Fig. 5A**) (92, 93). Particles typically varied in size from 60–100 nm but could be as large as 300 nm. Occasionally particles appeared to coalesce with one another. The appearance of particles by QFDE was consistent with their designation as lipoproteins (93). They appear solid and do not etch significantly during the QFDE process, indicating that they contain little water. Particles resembled LDL particles in the aortic intima previously seen by QFDE (89). Particles were found in the same locations in BrM as the lipid-containing particles identified using OTAP (3). Particles were extracted with the Folch reagent for lipid (94). The age-related accumulation of particles within BrM throughout adulthood is consistent with light microscopic and biochemical studies of lipid deposition in this tissue.

Composition of lipids in BrM/choroid and isolated lipoproteins

Determining the composition and morphology of lipids accumulating in BrM can be problematic due to the size and marked cellular heterogeneity of the choroid, which is much thicker than BrM itself. Nevertheless, seven studies assayed lipids from extracts of BrM/ choroid with varying degrees of choroid removal using techniques including thin-layer chromatography, gas chromatography, enzymatic fluorimetry, and electrospray ionization (summarized in ref. 24). To avoid potential tissue contamination problems and to provide direct evidence for particles with lipoprotein-like properties, two recent studies used a double, high-salt buffer (95) to release actual lipoproteins from BrM/ choroid homogenates (63, 96). From these nine studies total, a consensus for the composition and morphology of BrM neutral lipid-containing structure can be derived. Results obtained with comprehensive assays, results repeated with different assays and/or by different laboratories, consistency with histochemical findings, and consistency between tissue and lipoprotein studies were given greater weight in drawing these conclusions.

The lipid compositions of BrM/choroid and that of BrM lipoproteins isolated from that tissue (**Table 1**) are very similar, suggesting that appropriate lipoprotein isolation techniques can retain most particle properties. Particles released from BrM exhibit a density similar to plasma VLDL, and they have a spherical particle morphology indicating a neutral lipid core (mean diameter = 66 nm, Fig. 5B). Fractions containing lipoproteins also contain apoB, apoA-I, and apoE, all of which have been identified in BrM by immunohistochemistry. These features, as well as those described below, justify considering the particles seen in situ (see previous section) as genuine lipoproteins.

We note the following salient features of BrM lipid/ lipoproteins, and where appropriate, compare results to plasma lipoproteins: *1*) RPE/BrM/choroid is much more enriched in neutral lipid/ EC than neurosensory retina (3, 97, 98). *2*) Relative to macula, peripheral BrM/choroid has neutral lipid with similar fatty acid composition and less EC relative to UC (3, 97). *3*) Neutral lipids increase markedly with age relative to PLs, especially after age 60 years (99, 100). *4*) Within RPE/BrM/choroid, EC is the prominent neutral lipid, exceeding TG by 4- to 10-fold (tissues) or higher (lipoproteins) (63, 96, 98), a notable departure from plasma apoB lipoproteins in the same size class (Table 1). An early report (100) of high TG content in BrM was not replicated. *5*) EC represents 50–60% of the cholesterol detected (Table 1, **Table 2**) (3, 96, 98). *6*) The predominant fatty acids in the neutral lipid/EC fraction are linoleate (18:2, 45.1% for tissues, 41.3% for lipoproteins), oleate (18:1, 20.3%, 18.9%), palmitate (16:0, 13.9%, 17.4%), arachidonate (20:4n6, 6.8%, 6.5%), and stearate (18:0, 2.5%, 3.0%) (63, 96, 98). Together these compounds account for 88–89% of the EC detected.

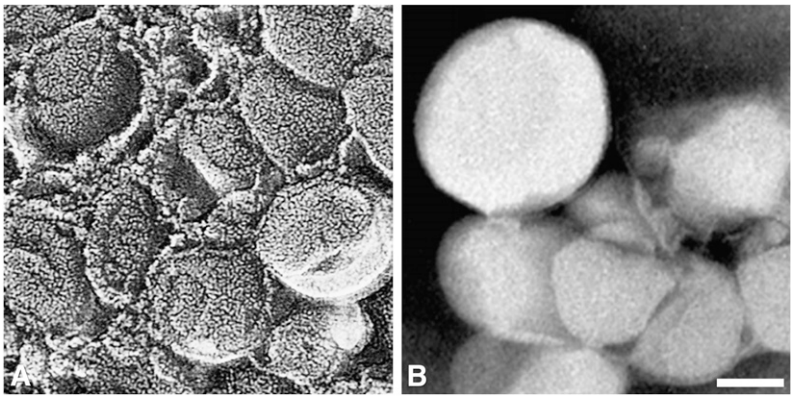

Fig. 5. BrM lipoproteins, in situ and isolated. A: Core and surface of tightly packed lipoproteins, by quick-freeze deep etch (93). B: Lipoprotein particles isolated from BrM are large, spherical, and electron-lucent (63). Bar = 50 nm.

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TABLE 1. Lipid profiles of BrM/ choroid, and lipoproteins isolated from BrM and plasma

Mole%	EC	DG	FA	TG	CL	LYPC	PC	PE	PS	UC
BrM/ choroid	29.8	0.7	3.6	3.0	1.5	0.6	15.4	12.6	6.0	26.7
BrM lipoproteins	32.4	1.7	6.3	3.3	3.2	1.8	14.2	9.5	4.6	22.9
Plasma CM	8.0	1.1	1.3	73.5	n.d.	0.6	8.6	1.4	n.d.	5.5
Plasma VLDL	8.2	1.2	0.9	62.0	n.d.	0.5	15.4	1.9	n.d.	9.9
Plasma LDL	49.6	0.2	1.1	5.8	n.d.	0.5	18.7	1.3	n.d.	22.8

Data modified from reference 96.

EC, esterified cholesterol; DG, diglyceride; FA, nonesterified fatty acid; TG, triglyceride; CL, cardiolipin; LYPC, lysophosphatidylcholine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; UC, unesterified cholesterol. Sphingomyelin and retinyl ester are also present in lipoproteins but could not be expressed as mole%. n.d., not determined.

Docosahexaenoate (22:n6, 0.5%, 0.5%), a major component of retinal membranes, is present in minute quantities in BrM (Tables 1, 2) (63, 96–98, 101). *7*) In isolated particles, phosphatidylcholine is more abundant than phosphatidylethanolamine by a factor of 1.29, and sphingomyelin, which binds tightly to cholesterol, is abundant relative to phosphatidylcholine (0.70). These ratios differ from plasma lipoproteins (Table 1). *8*) Isolated particles contain measurable retinyl ester, the transport form of dietary vitamin A (102). *9*) The distribution of the major lipid classes in BrM lipoproteins differ importantly from apoB-containing plasma lipoproteins (Table 1,2). These differences include less TG, less PC, and more UC, indicating that the particles in BrM are not simply a transudate from the circulation. *10*) Within each lipid class, however, BrM lipoprotein fatty acids were, overall, remarkably similar to those plasma lipoproteins, with the principal exception of ~20% lower linoleate (18:2n6) among all lipid classes. This finding raises the possibility that plasma lipoproteins are a source of individual lipid classes in BrM lipoproteins.

RPE lipid processing

Converging and repeatable evidence from light microscopic histochemistry, physical chemistry, ultrastructure, and lipid profiling of tissues and isolated lipoproteins has thus established EC as the primary lipid accumulating with age in BrM. Further, of the major lipid classes, only EC is exclusively localized to BrM, i.e., not also distributed throughout the choroid. High EC concentration within aged BrM is a critical clue to its source and mechanism of deposition. Similar considerations were raised decades ago for atherosclerotic plaques, when seminal studies showed that their EC composition included linoleate-rich EC from insudated plasma lipoproteins and oleate-rich EC within intimal cells (9, 81, 82, 103). The preceding section enumerated several distinct differences between BrM lipoproteins and plasma lipoproteins, suggesting that they are not simply derived from a plasma transudate. If they are instead generated locally, the lipoproteins could represent the oil red O-binding droplets that occasionally appear in RPE (104, 105), released by dying or otherwise stressed RPE cells into BrM. However, relative to the BrM particles, droplets in RPE are much larger (1–2 μm), and they have less EC (105) and more retinyl ester (106). Further, few RPE cells exhibit droplets in any one eye, and this lipoidal degeneration is not widely prevalent across eyes. Droplet release is unlikely to account for a large and universal phenomenon like age-related BrM lipid accumulation. Excluding the possibility of cell death leaves the best-understood mechanism to release EC from a healthy cell, namely, within the core of an apoB-containing lipoprotein. Available evidence, summarized below, now favors direct secretion of lipoproteins into BrM by RPE.

Expression of lipoprotein pathway genes. mRNA transcripts for both apoE and apoB have been confirmed in human RPE and RPE/choroid preparations (57, 107, 108), with apoE expression levels third behind brain and liver (62). RPE contains mRNA transcripts for apos A-I, C-I, and C-II, but not C-III (57, 64, 109). Full-length apoB protein was localized to native RPE using a specific monoclonal antibody (57, 108). Of significance to the apoB system, apoBEC-1 mRNA is not detectable in human RPE (108). However, it is present in rat RPE and the rat-derived RPE-J cell line (L. Wang and C. A. Curcio, unpublished observations), along with an apoB-48-like protein immunoprecipitable by anti-rat apoB (96), suggesting that rat RPE expresses both apoB-100 and apoB-48 as does liver in this species. Of major importance to the apoB system is the presence of both mRNA and protein for the large subunit of microsomal triglyceride transfer protein (MTP) within native human RPE. The latter was localized with apoB itself to punctate intracellular bodies, presumably endoplasmic reticulum, within both the RPE and, surprisingly, ganglion cells of the neurosensory retina (108). Mouse RPE expresses both MTP isoforms (110). Dual expression of apoB and MTP signifies that RPE has the capability of secreting lipoprotein particles. RPE also express mRNA transcripts for acyl cholesterol acyltransferase-2 (ACAT-2), one of two cellular cholesterol-esterifying enzymes and the

TABLE 2. BrM lipoproteins vs plasma apoB-lipoproteins

Lipoprotein	Diameter, nm	Apolipoproteins	EC/(EC+UC)	EC/TG	PC/PE	SPM/PC
BrM	66	A-I, B-100, E, C-I, -II	0.58	11.32	1.29	0.70
Chylomicron	>70	A-I, B-48, C-I, -II, -III	0.59	0.11	6.09	0.28
VLDL	25-70	B-100, C-I, -II, -II, E	0.45	0.13	8.30	0.28
LDL	19-23	B-100	0.69	8.52	14.90	0.37

EC, esterified cholesterol; TG, triglyceride; PC, phosphatidylcholine; PE, phosphatidylethanolamine; UC, unesterified cholesterol; SPM, sphingomyelin. All lipids from references 63 and 96. BrM apolipoproteins from references 63, 64, and 96. Plasma lipoprotein diameters and apolipoproteins from reference 200. Plasma lipoprotein SPM/PC calculated from reference 16.

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one more specifically associated with lipoprotein production (111, 112).

These RPE gene expression data provide a basis for redesignating the pigmentary retinopathies of abetalipoproteinemia and hypobetalipoproteinemia as intrinsic retinal degenerations, consistent with the recently expanded role of apoB in mammalian physiology [e.g., (14, 113)]. Mutations of the MTP gene cause a rare autosomal recessive disorder, abetalipoproteinemia (ABL, MIM 200100, Bassen-Kornzweig disease). In addition to absent plasma apoB-lipoprotein (114, 115), ABL features a retinopathy with pigmentary changes, reduced electro-oculogram and electroretinogram signals, and a predilection for angioid streaks (fractured BrM) (116, 117). Truncating mutations of the APOB gene cause hypobetalipoproteinemia (HBL, MIM 107730), a genetically heterogeneous autosomal trait primarily characterized by asymptomatic low plasma LDL (118) and a retinal degeneration (119, 120).[2] That the retinopathies associated with these mutations are only partly alleviated by dietary supplementation with lipophilic vitamins normally carried on plasma apoB-lipoproteins (117) is consistent with the interpretation of intrinsic retinal degenerations.

RPE lipid composition and apolipoprotein secretion. Study of neutral lipid homeostasis in outer retina has been highly focused on understanding the biosynthesis and metabolism of docosahexanoate, which constitutes 35% of the fatty acids in photoreceptor outer segment phospholipids (123). Classic studies established that this fatty acid is transiently stored in the RPE as newly synthesized, rapidly hydrolysable TG before being recycled across the interphotoreceptor matrix to the neurosensory retina (124, 125).

Although essential, excess cholesterol can be toxic, and pathways for UC and EC release that are well studied in other cells are now known to be operative in the RPE as well. Cultured RPE can secrete 37 kDa apoE into high-density fractions (d = 1.18–1.35 g/ml) (126). These investigators also demonstrated a transfer of radiolabeled docosahexaenoate from outer segment membranes to HDL or lipid-free apoA-I in the medium, presumably by ABCA1-mediated mechanisms (127), although this may represent a minute proportion of total fatty acids in HDL (Table 2). ARPE-19 cells (from human) and RPE-J cells (from rat) secrete EC into a lipoprotein-containing fraction following fatty acid supplementation (oleate for ARPE-19, palmitate for RPE-J) (96, 108). Similar in size (mean, 56 nm) to the particles found in native BrM (see above), they also contain little TG. Importantly, RPE-J cells and medium also contain immunoprecipitable [^{35}S]methionine-labeled apoB in a full-length (512 kDa) form and a lower molecular weight band that may be apoB-48 (96). This definitive assay for protein synthesis and secretion was also used to show secretion from human-derived ARPE-19 cells (128).

To summarize, the data reviewed so far support the hypothesis that the RPE constitutively secretes an apoB-containing lipoprotein particle from its basolateral aspect into BrM for eventual clearance into plasma. This model does not preclude other mechanisms of cholesterol release from RPE (129, 130). We envision a large lipoprotein (**Fig. 6**) in the VLDL size and density class that contains apoB-100, apoA-I, apoE, apoC-I, apoC-II, and possibly other proteins. It is secreted with an EC-rich neutral lipid core. The apoB-containing lipoproteins secreted by cultured RPE cell lines are unusual in several respects compared with plasma apoB-lipoproteins (Fig. 6). Particles are EC-rich despite being as large as TG-rich VLDL. Particles are EC-rich when newly secreted, unlike LDL and HDL, whose composition is achieved by enzymatic remodeling in plasma. Although it is possible that smaller lipoproteins secreted by the RPE (126) accumulate in BrM and fuse together to form large particles, as postulated for LDL in arterial intima (9), the size distribution of particles accumulating with age in BrM is inconsistent with a continuously evolving population.

Source of lipids found in RPE lipoproteins. A signature biological activity in the posterior eye is the renewal afforded by daily ingestion of photoreceptor outer segment tips by the RPE, which has the highest phagocytotic load in the body. Older literature speculated that the age-related accumulation of BrM neutral lipid is related to this activity (131, 132). An initially attractive hypothesis that an apoB-lipoprotein from the RPE could eliminate fatty acids released by lysosomal phospholipases after outer segment phagocytosis (108) is now considered unlikely for several reasons. Phagocytosis is not required for secretion of neutral lipids or apolipoproteins in RPE cell lines (96, 108, 126). Other cells store and transport excess fatty acids as TG, but BrM lipoproteins are not TG-rich (see above). BrM lipoproteins and drusen are highly enriched in EC, which photoreceptors lack (123). BrM lipoproteins and

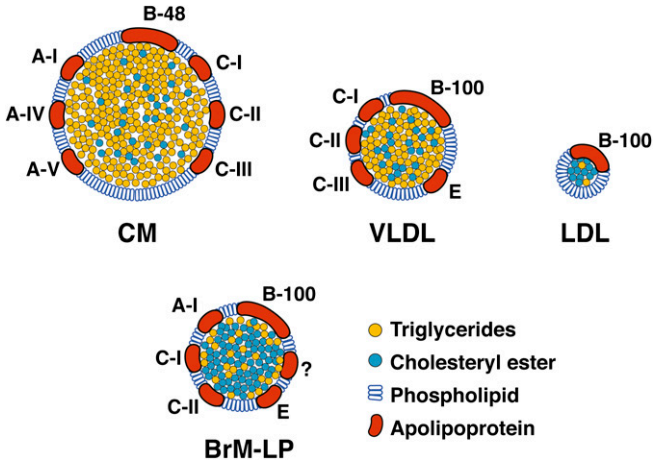

Fig. 6. ApoB-containing lipoproteins, including BrM lipoprotein (BrM-LP). Particle diameters are approximately to scale. Plasma lipoprotein diagrams from (17). BrM-LP composition data from direct assay (63, 96) and by inference from druse composition and RPE gene expression (57, 64). Not all surface proteins on the BrM-lipoprotein are known. Reproduced with permission from Progress in Retinal and Eye Research (PRER).

[2] A retinal degeneration with thick basal deposits in an HBL patient is now considered late onset retinal degeneration, which segregated separately from HBL (119, 121, 122).

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drusen are more highly enriched in UC than outer segments membranes (123, 133). No lipid class in BrM lipoproteins has a fatty acid composition like that of outer segments, which are rich in docosahexaenoate (22:6n3) (123, 134) (**Fig. 7**). Several potential explanations for the dissimilarity between BrM lipoproteins and outer segments have been offered elsewhere (24, 96). For example, among the fatty acids within photoreceptors, only docosahexaenoate (22:6n3) is efficiently recycled back to the retina (124), which would leave little for export. Perhaps the simplest explanation is that BrM lipoproteins are dominated by sources other than outer segments, specifically plasma lipoproteins, which they closely resemble with regard to fatty acid composition (Fig. 7).

RPE expresses functional receptors for both LDL (LDL-R) and HDL (scavenger receptor B-I, SRB-I) and takes up these plasma lipoproteins (109, 135–138), likely for resupply of essential lipophilic nutrients and cellular constituents. LDL-derived cholesterol taken up by RPE partitions remarkably fast (within hours) into membranes of the neurosensory retina (109, 139). In humans, LDL and HDL also carry the xanthophylls lutein and zeaxanthin, which are major components of macular pigment (140), as well as vitamin E (141). These pigments and vitamin E are all essential for photoreceptor health (138, 142–144).

BRM, LIPOPROTEINS, AND TRANSPORT

EC-rich barrier in aged BrM; lipid wall

QFDE analysis of normal eyes at different ages (92, 93, 145, 146) reveals that significant lipoprotein accumulation begins by the fourth decade of life in or near the elastic layer of BrM in the macula, and to a lesser degree, the periphery. This process is reminiscent of the preferential deposition of lipoprotein-derived EC near elastin in arterial intima (147). With advancing age, following a filling of the elastin layer with these particles and other debris, lipoproteins also appear in the inner collagenous layer, as if particles were backing up toward the RPE. The outer collagenous layer showed a more complicated pattern, with an early accumulation that decreased later in life, as if their source had diminished once the elastin layer was obstructed.

In eyes over 60 years of age, accumulated particles filled most of the inter-fibrillar space in the inner collagenous layer, and groups of particles began to appear between this layer and the RPE basal lamina. In eyes over 70 years of age, this process culminates in the formation of the lipid wall (92), a dense band 3–4 particles thick external to the RPE basal lamina (**Fig. 8**). This tightly packed layer of space-filling particles displaces the structural collagen fibers at the same location in younger eyes that help bind BrM to the RPE basal lamina. Taken together, these observations suggest that lipoprotein accumulation starts in the elastic layer, backs up into the inner collagenous layer, and eventually forms the lipid wall. This blocks the source of lipoproteins to the outer collagenous layer and explains why the concentration of particles in this layer drops in eyes >60 years of age. Importantly, this lipid wall forms at the same location where BlinD is later seen to develop.

The barrier hypothesis: lipid accumulation and transport through BrM

Unlike age-related accumulation of EC in cornea, sclera, and arterial intima, that which occurs in BrM is apparently unique in its potential impact on fluid and nutrient exchange. Deposition of lipoprotein-derived EC may render BrM increasingly hydrophobic with age and impede transport of hydrophilic moieties between the RPE and choroidal vessels. Two decades ago, Bird and Marshall (74) first introduced the hypothesis of a physical barrier, distinct from the physiological blood-retina barrier (148), that could predispose older individuals to multiple retinal conditions (74, 122, 149). Substantial experimental support for a barrier, obtained in BrM/choroid explants from donor eyes of different ages, demonstrates a reduced hydraulic

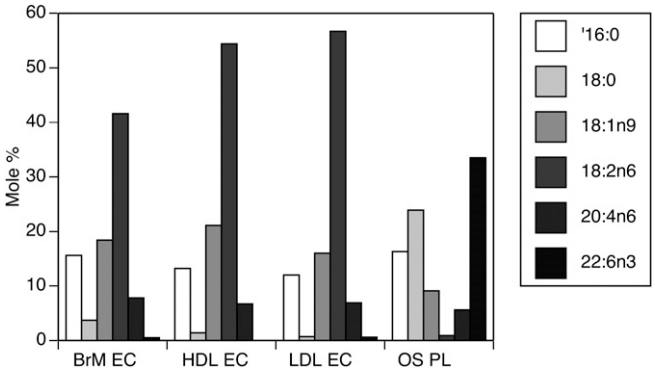

Fig. 7. Fatty acids in BrM EC resemble plasma lipoproteins, not photoreceptors. Fatty acid composition of BrM EC and LDL EC is taken from (96). HDL EC is reported for 20 normolipemic volunteers (199). Fatty acids in photoreceptor outer segment phospholipids taken from (134).

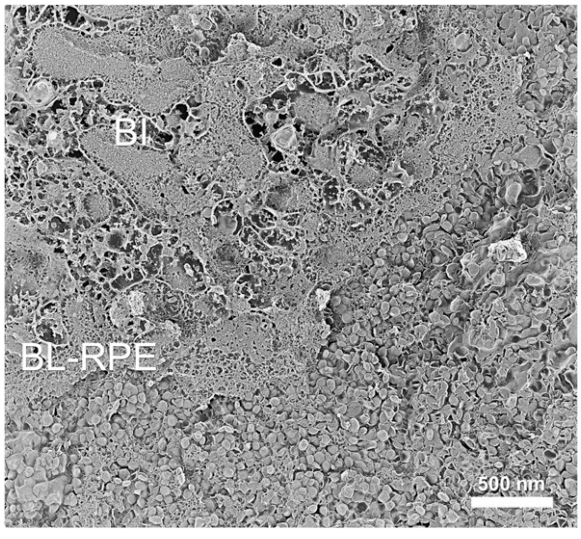

Fig. 8. Lipid wall. In this oblique fracture plane across BrM, lipoproteins are densely packed in the lipid wall (lower aspect of figure). Normal macula of an 82-year-old donor, prepared by QFDE. Taken from (93) BI, basal infolding of RPE cell; BL-RPE, basal lamina of the RPE. Reproduced with permission from PRER.

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conductivity and permeability to macromolecules of BrM both in aging and in ARMD (150–153). The hydraulic resistivity of BrM (inverse of conductivity) correlates strongly with its lipid content (2). Indeed, the age-related increase in hydraulic resistivity of BrM exactly mirrors that of the age-related increase of histochemically detected EC in BrM (1) (**Fig. 9**). Hydraulic resistances add when flow-limiting regions are in series. Lipid accumulation in BrM adds a new hydraulic resistance in series with an existing resistance. It cannot be bypassed, as the flow must pass through this layer.

This conclusion, based on correlative data in human tissues, is supported by experimental studies of lipid deposition in a model extracellular matrix, Matrigel™ (154). In this system, the hydraulic conductivity (inverse of resistivity) of Matrigel™ was lowered by >50% when 5% LDL-derived lipids (by weight) were added (**Fig. 10**). This effect was surprisingly large, much larger than if 5% latex spheres were added to the Matrigel™, and larger than predicted by theoretical considerations of interstitial fluid movement (154).

Transition from aging (lipid wall) to ARMD (BlinD)

The striking spatial correspondence of the lipid wall, located between the inner collagenous layer and RPE basal lamina in aged eyes, and BlinD, located in the same compartment in eyes with ARMD, makes the lipid wall a likely direct antecedent to BlinD. Ultrastructural examination of eyes at different ARMD stages (65, 71, 155) can identify transitional forms between lipid wall and BlinD components. Whereas the lipid wall consists of packed lipoproteins of similar size, BlinD in its earliest forms consisted of material resembling fused lipoproteins of irregular size. Throughout adulthood, then, RPE production of apoB lipoproteins for normal physiological transport to

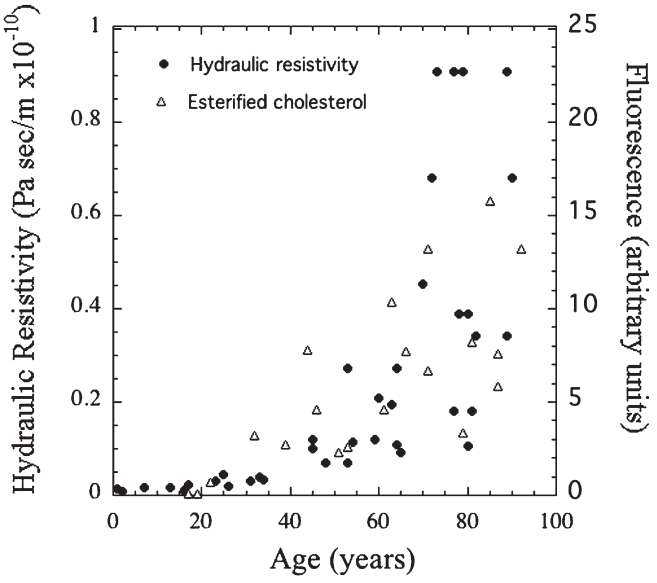

Fig. 9. Hydraulic resistivity and BrM EC in aging. Hydraulic resistivity (2) of excised BrM/choroid (closed symbols) and fluorescence due to histochemically detected esterified cholesterol in sections of normal BrM (3) (open symbols) as a function of age. From (1), with permission.

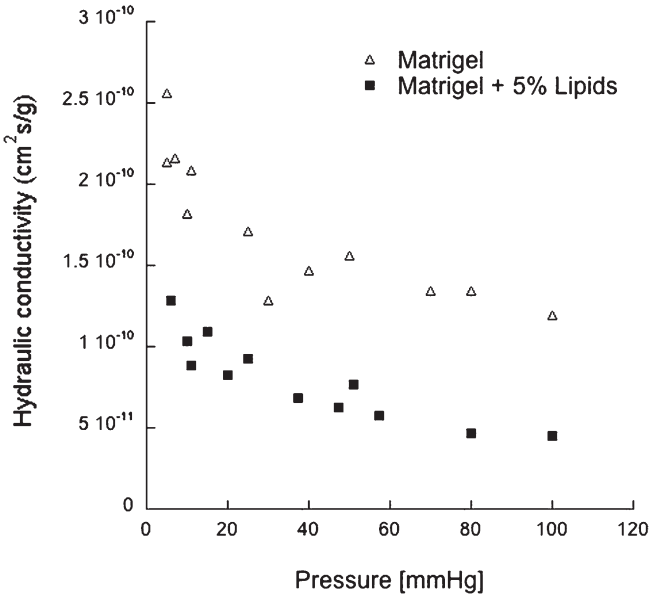

Fig. 10. Lipoprotein-derived lipids reduce fluid transport through an artificial matrix. The hydraulic conductivity, as a function of perfusion pressure, of 1% Matrigel (open triangle) or 1% Matrigel with 5% LDL added (closed squares). Modified from (154), with permission.

the choriocapillaris may be gradually blocked by their own age-related accumulation in BrM, eventually filling this tissue and resulting in a new layer caused by the backward accumulation of these particles toward the RPE. Once this lipid wall begins to form between the inner collagenous layer and the RPE basal lamina, more lipids preferentially fill this potential space, leading to the formation of BlinD, which is linear because of the geometry of the space containing it. Formation of BlinD may damage RPE cells via decreased transport of nutrients and waste products. The response of the RPE cells to this insult may include production of excessive basal lamina materials, contributing to the formation and local accumulation of BlamD (Fig. 3). Drusen formation likely involves the same processes, with participating nonRPE cells eliciting inflammation, further debris accumulation, and three-dimensional expansion of these deposits (156).

RESPONSE-TO-RETENTION OF AN INTRAOCULAR APOB LIPOPROTEIN

The pathology associated with atherosclerotic CAD is widely thought to be initiated by the physiological response-to-retention of lipoproteins in the blood vessel wall (19). Following retention in the inner arterial wall, lipoproteins are prone to oxidation and fusion into products that activate the complement system. Complement activation is chemotactic for monocytes, and complement deposition coincides with cholesterol accumulation (157). Trapped lipoproteins would also be susceptible to sphingomyelinase that generates ceramides, which in turn have untoward effects such as induction of nuclear factor kappa B, stimulation of apoptosis, and other pro-inflammatory events (19).

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Our current view of ARMD pathogenesis can be compared with the response-to-retention (19) model of CAD at multiple steps (**Fig. 11**). In a striking parallel with apoB-lipoprotein-instigated disease in arterial intima, the RPE/BrM complex in aging and ARMD also exhibits accumulation of oxidized lipoproteins, different forms of cholesterol, lipid-rich and structurally unstable lesions, and inflammation-driven downstream events. The antecedents of disease in normal physiology have been highlighted with new information and ideas about lipoprotein particles as a source of extracellular cholesterol, intraocular source(s) of lipoprotein particles, and biological processes that can drive lipoprotein production.

We propose that in younger eyes, lipoprotein particles cross BrM for egress to plasma. With advanced age, transit time across BrM increases due to changes in extracellular matrix, particle character, clearance mechanisms, either alone or in combination, resulting in the accumulation of either native or modified particles, especially at the site of the lipid wall. In conjunction with other cellular processes, lipoprotein accumulation and modification eventually causes the formation of BlinD and drusen and contributes to ongoing RPE stress and formation of BLamD. Our model does not indicate that deposit formation alone initiates ARMD. Instead, it is likely that the response-to-retention of lipoproteins in BrM (19) gives rise to, and may even be required for, other key events like complement activation, neovascularization, and RPE cell death. Lipoproteins retained in arterial intima undergo numerous modifications, including oxidation, aggregation, fusion, glycation, immune complex formation, proteoglycan complex formation, and conversion to cholesterol-rich liposomes (158). Potentially, all these processes also occur in the RPE/choroid complex (159), as oxygen levels are high (160), high levels of irradiation could promote lipid peroxide formation (161), BrM contains proteoglycans and advanced glycation end products (162–165), and the immediately adjacent RPE is a source of secreted enzymes [e.g., sphingomyelinase (166)] that can act on lipoproteins.

The lipid components of BrM lipoproteins are potentially subject to oxidative modification that leads to further deleterious consequences. Antibodies versus copper sulfate-oxidized LDL or oxidized phosphatidylcholine reveal immunoreactivity in BrM, drusen, basal deposits of eyes with ARMD, photoreceptor outer segments, some RPE cells, and in surgically excised neovascular membranes (111, 167, 168). ARPE-19 cells exposed to oxidized LDL undergo numerous phenotypic shifts including altered gene expression (111). Oxysterols, studied as toxic byproducts of LDL oxidation or as enzymatically generated metabolites of UC, can contribute to macrophage differentiation into foam cells and induce cell death in RPE cell lines (169–172). 7-Ketocholesterol present near BrM in adult monkey eyes is thought to originate from BrM lipoprotein deposits that oxidized in the high-oxygen choroidal environment (172). Such oxidation products can contribute to ARMD's progression to choroidal neovascularization and chorioretinal atrophy (172, 173).

The hypothesis that an RPE-derived apoB-lipoprotein retained in a vascular intima evokes downstream consequences (Fig. 11) also provides a new backdrop for the recently recognized roles of inflammatory proteins and regulators in ARMD (174–176). The alternative complement pathway has been a subject of intense inquiry, because proteins of the complement cascade localize to drusen, and variants in CFH, FB, and C3 genes modulate risk for ARMD (33, 36, 37, 177). For reference, atherosclerotic plaques contain many of the same molecules (157, 178, 179), and sub-endothelial LDL is considered an important activator of complement in incipient plaques.

Like atherosclerotic plaques, drusen and basal deposits feature cholesterol and apoB deposition, and BrM, like arterial intima and other connective tissues, accumulates lipoprotein-derived EC with advanced age. Although knowledge about CAD can provide a powerful conceptual framework for developing new ideas about ARMD pathobiology, RPE physiology, and therapeutic approaches (3, 4), these two complex multi-factorial diseases differ in important and ultimately informative ways, both at the level of the vessel wall and in patient populations. Here, we note key differences between events in BrM and those in arterial intima (4, 24). BrM and intima likely have different major sources of neutral lipid-bearing lipoproteins (RPE vs. liver and intestine via plasma). BrM is very thin and arterial intima is thick. Hemodynamics of the choriocapillaris are different from those of a large muscular artery. BrM is influenced by an endothelium and an epithelium, whereas intima supports an endothelium only. Foam cells congregate early in plaque formation, but macrophages are associated primarily with neovascularization in advanced

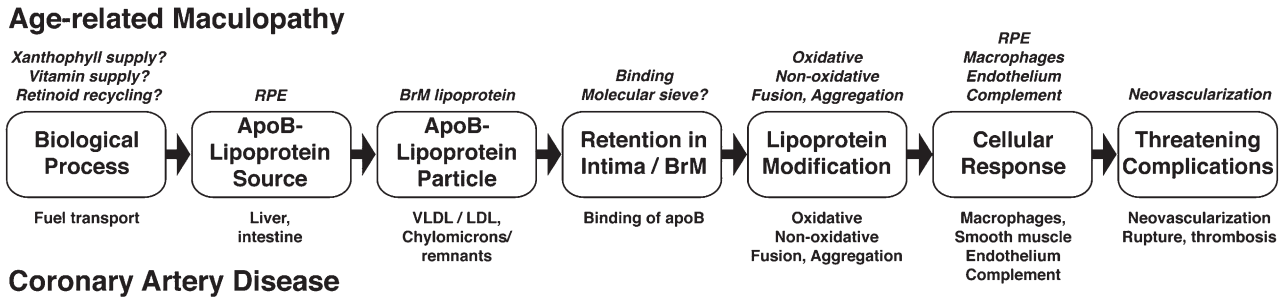

Fig. 11. Response-to-retention: ARMD, coronary artery disease. The hypothesized progression of ARMD has many parallels to the Response-to-Retention hypothesis of atherosclerotic coronary artery disease (19), beginning with apoB-lipoprotein deposition in a vessel wall. Reproduced with permission from Progress in Retinal and Eye Research.

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ARMD. Smooth muscle cells make up the fibrous cap of plaques but have no defined role in ARMD as yet. Fibrous collagens (types I and III) and elastin are major components of incipient lipid-rich plaque core, and they are present where lipoproteins accumulate in aging BrM but are absent from drusen and BlinD in ARMD. Cholesterol crystals do not occur in BrM or sub-RPE lesions unlike plaque. Capillaries participating in neovascularization invade from different directions relative to the elastic layer (choriocapillaris in ARMD, media in CAD, Fig. 2) (180, 181).

Further, despite the apparent commonality of apoB-initiated disease, key differences between ARMD and CAD also exist at the level of patients and populations. Results of multiple studies seeking associations between measures of elevated plasma cholesterol and ARMD, or HDL levels and ARMD, have been largely inconclusive (for review, see ref. 182). ARMD is not considered to be associated with type 2 diabetes (31, 183), a major CAD risk factor. Observational and retrospective studies of plasma lipid-lowering treatments (e.g., statins) in ARMD patients have had decidedly mixed effects (184). The apoE4 genotype is well established as a modest but consistent risk factor for CAD, conferring decreased longevity and increased mortality (185), yet it surprisingly protects against ARMD, reducing risk by 40% (61, 186). One model invokes the opposing effect of cellular cholesterol export from RPE into BrM and from macrophages into intima to explain the opposite direction of apoE4's influence in ARMD and CAD (24).

CONCLUSIONS AND FUTURE DIRECTIONS

Recent work has now strongly implicated the RPE as a secretor of EC-rich apoB lipoproteins that normally function to remove cholesterol from RPE cells, but when retained in BrM with age contributes importantly to impeding RPE and photoreceptor function and to forming the specific lesions of ARMD. The conceptual framework, borrowed heavily from decades of atherosclerosis research, provides a wide knowledge base and sophisticated clinical armamentarium that can be readily exploited for the ultimate benefit of ARMD patients. Tools, techniques, and experimental approaches borrowed from the study of CAD should be applied to the eye in order to address critical questions in the near term.

The goal of new therapeutic approaches for ARMD may be best served by a comprehensive understanding of the RPE as a polarized secretor of lipoproteins. The least understood portion of Fig. 11 is the biological function(s) of the postulated apoB system. Elsewhere, we speculated that plasma lipoproteins taken up by RPE are stripped of nutrients required by photoreceptors (e.g., xanthophylls, cholesterol, vitamin E) and repackaged for secretion in BrM as large, EC-rich apoB-lipoproteins (24). Lipoprotein secretion by RPE cells could dispose of excess UC and forestall toxicity in concert with recycling of EC and retinoids (187) back to plasma. Within the RPE, apically-directed recycling of docosahexaenoate to the retina (124) may thus be accompanied by an independent, basally-directed recycling of plasma lipoproteins repackaged for egress to the systemic circulation across BrM. Fortunately, ample work on cell culture systems developed for atherosclerosis and diabetes research can provide expert guidance. Essential questions include identifying the full range of input lipids to an RPE lipoprotein, identifying the full range of lipid egress pathways from the RPE, identifying how an RPE apoB-lipoprotein compares to those from other well-characterized apoB secretors, and untangling the relationship of apical and basal lipid transport systems (109, 124, 188). Recently developed high-fidelity polarized RPE cultures systems (189) will be essential for answering these questions.

The macula is unique to humans and other primates, and only macaque monkeys are known to accumulate neutral lipid in BrM (105) and apoE in drusen (190). A short-lived species that also displays these characteristics and permits in vivo tests of causation remains a high priority research goal. Eyes of established mouse models of atherosclerosis have already been examined in pursuit of that goal. As reviewed elsewhere (24), at least 10 studies using mice with genetic manipulation of apoB or apoE pathways, some in combination with LDL-receptor deficiency, have been published. Some of the most promising models exhibit histochemically and/or ultrastructurally detectable neutral lipid deposits in BrM, RPE degeneration, and upregulation of pro-angiogenic factors (110, 191–194). Interpretation of studies using transgenic mice expressing lipoprotein-related genes are hindered by RPE expression of the same genes, complicating definitive attribution of effects to plasma lipoproteins of hepatic/intestinal origin or to lipoproteins of RPE origin. Furthermore, the effects described in these mouse models occur in the setting of hyperlipidemia, which is not associated with ARMD pathogenesis. Future animal models that independently manipulate RPE- and plasma-derived lipoproteins, e.g., by using Cre-loxP technology to effect tissue-specific gene deletion (195, 196), will thus be highly informative.

We anticipate that the model we presented for BrM lipoprotein accumulation may facilitate the construction of improved in vitro model systems to study transport across natural and artificial matrices. Such systems could use readily available LDL as an acceptable surrogate for BrM lipoproteins because of its high EC content. Use of such systems have already demonstrated that LDL can pass through bovine BrM (197), although slowed in transit, and that LDL deposition significantly decreases the hydraulic conductivity of an extracellular matrix (154). Future studies focused on determining why lipid first begins to accumulate in BrM, the mechanism by which this occurs, and how this accumulation impedes transport will be guided by past work on factors influencing lipoprotein modification and aggregation in the arterial intima (e.g., ref. 198).

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