Basal Linear Deposit and Large Drusen Are Specific for Early Age-Related Maculopathy

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Objective: To determine the distributions of basal laminar and basal linear deposits in Bruch membrane (BM) with respect to age and early age-related maculopathy (ARM).

Methods: The foveas of 41 human eyes (<60 years [n = 9]; ≥60 years [n = 32]), preserved no later than 3.5 hours post mortem, were examined using light and electron microscopy. Ten eyes met histopathologic criteria of the Alabama Age-related Macular Degeneration Grading System for early ARM. We calculated the specificity, sensitivity, and odds ratios for the association of basal laminar and basal linear deposits with early ARM.

Results: Both deposits occurred only in eyes older than 60 years. The highest specificities and sensitivities for early ARM were attained for eyes that had basal linear deposits or large (>125 µm) drusen, followed by eyes with any quantity of basal laminar deposits that also contained membranous debris. Eyes with ARM were 24 times more likely than age-matched control eyes to have basal linear deposits or large drusen (P = .002).

Conclusions: Basal linear deposits and large drusen with membranous contents constitute different morphologic forms of the same ARM-associated lesion and may be significant for progression to late ARM.


AGE-RELATED maculopathy (ARM) is a major cause of vision loss in the elderly. Although its causes are poorly understood, it is agreed that the most prominent clinical and histopathologic features of ARM are lesions that involve retinal pigment epithelium (RPE) and Bruch membrane (BM). Bruch membrane is a 5-layer connective tissue sandwich interposed between the basal surface of the RPE and the choriocapillaris, the blood supply to the photoreceptors (Figure 1). Moving outward from the RPE, these layers are the RPE basal lamina (RPE-BL), the inner collagenous layer, the elastic layer, the outer collagenous layer, and the choriocapillaris BL. Debris in inner BM, variably called basal linear (BlinD)3-5 or basal laminar deposits (BlamD),6-10 have figured prominently in hypotheses of ARM pathogenesis for more than 2 decades. Green and Enger9 proposed that the terms BlamD and BlinD refer to 2 lesions with distinctive morphologic characteristics and positions relative to the RPE-BL (Figure 1). Basal laminar deposit consists primarily of fibrous long-spacing collagen (FLSC) and an amorphous material similar in electron density and texture to BL3,4,7 located between the RPE and the RPE-BL (ie, internal to the RPE-BL). In contrast, BlinD consists primarily of membranous material located between the RPE-BL and the inner collagenous layer (ie, external to the RPE-BL). Similar membranous debris is also found in soft drusen, ie, large focal deposits with sloping sides that are also external to the RPE-BL.6 Therefore, BlinD is sometimes referred to as diffuse drusen.9 We herein use the generic term “basal deposits” to denote BlamD or BlinD.

The role of basal deposits in the development of late ARM, characterized by choroidal neovascularization (CNV) and geographic atrophy of the RPE, remains controversial. That eyes with large, soft, or confluent drusen are at risk for CNV has been established by clinical studies using fundus photographs.11-13 In contrast, basal deposits are not visible in the fundus. As inferred through postmortem histopathologic examination, the presence of BlinD is thought to place an eye at risk for late ARM.14-15 Clinicopathological correlations have led to the following 3 related hypotheses about basal deposits: (1) Membranous debris in BM is associated with ARM;15 (2) BlinD and soft drusen are diffuse and focal deposits, respectively, of the same membranous material;16 and (3) BlamD is not specific for ARM, because its most prominent component, FLSC, is also found elsewhere. Determining the relative risk for BlamD and BlinD is important for
SUBJECTS AND METHODS

Our results are based on the analysis of 41 eyes from 18 women and 24 men aged 17 to 92 years. Eyes were obtained from 34 donors and 7 patients requiring orbital exenteration for the removal of craniofacial tumors. No donors or patients were diabetic. Clinical records were obtained through contact with donor families and follow-up with ophthalmologists and optometrists. Use of human tissues and clinical records was approved by the institutional review board at the University of Alabama at Birmingham.

After removal of the cornea and lens, globes were fixed by immersion for a minimum of 24 hours in 0.1 mol/L phosphate-buffered 1% paraformaldehyde and 2.5% glutaraldehyde (n = 35), 4% paraformaldehyde and 0.5% glutaraldehyde (n = 5), or 4% paraformaldehyde (n = 2). Different fixatives gave similar results. Median time to preservation was 2 hours 25 minutes (range, 1 hour 5 minutes to 4 hours 2 minutes) for 34 donor eyes and 23 minutes (range, 6 to 54 minutes) for the 7 surgical specimens. Eyes were inspected grossly and photographed as previously described.20 The macular retina, RPE, choroid, and sclera were divided horizontally with a razor just superior to the foveal depression. An approximately 2-mm-wide block containing the fovea was osmicated, dehydrated using ethanol and propylene oxide, and embedded in epoxy resin (Epon-Araldite; Electron Microscopy Sciences, Fort Washington, Pa) using standard procedures. Blocks were sectioned serially at 1 µm and stained with 2% toluidine blue in 2% sodium borate. Sectioning was terminated at the foveal center, recognized by the absence of ganglion cells and the presence of laterally traveling processes in the Henle fiber layer. Ultrathin (gold) sections were cut, stained with uranyl acetate and lead citrate, and examined using an electron microscope (JEOL1200 EXII; JEOL USA, Peabody, Mass). We scanned a single section (median length, 1.5 mm) through the foveal center of each eye along the entire RPE-BL at 5000× magnification with 10× binoculars. Representative photographs were taken at 5000× and printed at 15 000× magnification. Basal laminar deposit was considered present in an eye if FLSC or amorphous material was found internal to the RPE-BL. Basal linear deposit was considered present if irregular membranous debris was found just external to the RPE-BL.

Two to three 1-µm sections at least 60 µm apart through the foveal rod-free zone were evaluated for histopathologic features. We assigned semiquantitative grades to drusen size,21 RPE changes,13 and total basal deposits.3,8,9 Focal deposits that raised the RPE to half of its typical height were considered drusen. Cross-sectional diameter was measured across the druse base, and individual foci were measured where drusen were confluent. Sections of 1 µm through eyes with ARM had numerous drusen (median, 8; range, 1-10). Not all drusen could be examined using electron microscopy, however, because drusen contents were sometimes extracted by processing.

Our overall strategy was to determine the number of eyes with early ARM or with no ARM, with and without ultrastructurally identified BlamD and BlinD, then subject these raw data to 2 different analyses. Both analyses are critically dependent on our case definition of early ARM. Maculopathy status was determined for all eyes using the histopathologic criteria of the Alabama Age-related Macular Degeneration Grading System.20 To make our histopathologic case definition logically resemble those used by epidemiological studies for fundus appearance,13-14 we use as primary criteria only features that are typically visible in the fundus (ie, drusen and RPE change) and not those that are visible only microscopically (ie, photoreceptor degeneration9 and basal deposits20). The presence of basal deposits was used as a secondary criterion to distinguish ARM from other conditions with RPE change. Unlike epidemiological studies, we used drusen size rather than type (eg, hard or soft) as a primary criterion, because size is an objective measure, and most soft drusen are at least 63 µm.14 Early ARM20 was defined as the absence of late ARM (ie, CNV, geographic atrophy, disciform degeneration) and the presence of 1 druse greater than 1.25 µm or severe RPE change (ie, healing, migration, or atrophy) in any graded section. Eyes with RPE change also had to have 1 or more drusen of any size or a continuous layer of total basal deposits (ie, BlamD and BlinD combined). Ten eyes from donors or patients aged 60 to 90 years (mean age, 73.7 years) (Table 1) met these criteria.

Our first analysis was to test our 3 related hypotheses by calculating the specificity and sensitivity of BlamD and BlinD for early ARM.26 These measures allow us to determine if either basal deposit is a good marker for true ARM. Such a marker would be a lesion or combination of lesions that yielded high specificity, ie, absence from eyes without ARM, and high sensitivity, ie, presence in many eyes with ARM. We computed these values using the following formulas:

Specificity = d/(b + d)
Sensitivity = a/(a + c)

where a indicates the number of eyes with ARM and with deposits present; b, without ARM and with deposits present; c, with ARM and with deposits absent; and d, without ARM and with deposits absent. We did not compute these values for eyes with only large drusen, because these lesions were part of the case definition, and therefore specificity and sensitivity are expected to be high. The first hypothesis (that membranous debris is associated with ARM) predicts that the highest specificities will belong to lesions containing membranous debris. The second hypothesis (that BlinD and large drusen are 2 forms of the same lesion) predicts that the sensitivities calculated using the total number of eyes containing BlinD and large drusen should be greater than the values calculated using the number of eyes with either lesion alone, provided that large drusen contain membranous debris. The third hypothesis (that BlamD is not associated with ARM) predicts that the specificity for any quantity of BlamD will be low.

Our second analysis was to estimate the associations between histopathologically defined ARM and basal deposits and drusen, using odds ratios (OR) and 95% confidence intervals (CI).27-29 Odds ratios were calculated using the following equation:

(a × d)/(b × c)

The OR is a measure of comparative risk that compares the odds that ARM occurs among persons with a particular characteristic with the odds that ARM occurs among those lacking the characteristic. This analysis enabled us to calculate the strength and statistical significance of associations and compare them for individual or combined lesions. Because of the small sample size, exact methods were used to calculate CIs and P values. All statistical tests were conducted at the .05 level (2-sided).
identifying the fundus markers of the highest-risk lesions and for guiding development of animal models of ARM.

Testing hypotheses about the specificity of different basal deposits requires demonstrating their relative absence from eyes without ARM and their presence in eyes with ARM. Electron microscopy is required to distinguish BlamD from BlinD definitively,3,4,7-9,14,17 and unfortunately, studies that examined eyes without ARM using electron microscopy had few total specimens or few eyes with ARM or did not look for both basal deposits.3,4,18,19 In our study, we identified basal deposits using electron microscopy in eyes with ARM, age-matched eyes without ARM, and young donor eyes. All eyes were quickly preserved and specifically prepared for electron microscopy to reduce fixation and processing artifacts. Diagnosis of ARM was ascertained using criteria for fundus appearance and histopathologic characteristics. We confirmed the hypothesis that BlinD and soft drusen are 2 forms of the same ARM-associated lesion.

RESULTS

An electron micrograph from an eye lacking basal deposits (Figure 2, A) shows the basal surface of the RPE and the 3 inner layers of BM. The ultrastructure of BlamD is illustrated in Figure 2, parts B to F, and its component frequencies in the 41 eyes are summarized in the following tabulation:

<table>
<thead>
<tr>
<th>Component</th>
<th>No. of Eyes</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLSC</td>
<td>25</td>
</tr>
<tr>
<td>Amorphous light material</td>
<td>25</td>
</tr>
<tr>
<td>Amorphous dark material</td>
<td>25</td>
</tr>
<tr>
<td>Membranous debris</td>
<td>14</td>
</tr>
<tr>
<td>Pigmented debris</td>
<td>3</td>
</tr>
<tr>
<td>Space</td>
<td>13</td>
</tr>
<tr>
<td>Total with BlamD</td>
<td>26</td>
</tr>
</tbody>
</table>

The most frequently seen components of BlamD were FLSC (Figure 2, B) and an amorphous basement membranelike material that had 2 distinct electron densities. The less dense material (Figure 2, C) was identical to normal BL in electron density and texture, and it typically enveloped the denser material (Figure 2, C). Membranous debris identical to material accumulating external to the RPE-BL was also present within BlamD (Figure 2, D and F). Atypical BlamD at the sloping margin of 1 large druse16 contained mostly amorphous material and electron-lucent circular profiles (Figure 2, E). A narrow rim of electron-lucent space occasionally surrounded other BlamD components but never occupied more than a small fraction of the area between the RPE and the RPE-BL (Figure 2, B).

Of ultrastructurally detectable, diffuse abnormalities external to the RPE-BL, the 2 most common features were membranous debris and non–membrane-bounded electron-lucent droplets. These findings are illustrated in Figure 3 and Figure 4 and summarized for the 38 gradable eyes in the following tabulation:

Table 1. Eyes With Early ARM*

<table>
<thead>
<tr>
<th>Patient No./Sex/Age</th>
<th>Eye</th>
<th>OE</th>
<th>LED, mo</th>
<th>VAcc</th>
<th>Lens†</th>
<th>Phot/FA‡</th>
<th>Significant Macular History§</th>
<th>Status of Fellow Eye</th>
<th>Chart</th>
<th>ALARMDGS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/M/60 R</td>
<td>Yes</td>
<td>0.0</td>
<td>20/30</td>
<td>2</td>
<td></td>
<td>x/−</td>
<td>Drusen, mild ARM</td>
<td>Yes</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>2/F/63 L</td>
<td>No</td>
<td>9.7</td>
<td>20/25</td>
<td>2</td>
<td></td>
<td>x/−</td>
<td>Dry ARM</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>3/M/64 L</td>
<td>No</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>x/−</td>
<td>No visual complaint as per family</td>
<td>NA</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>4/M/74 R</td>
<td>No</td>
<td>23.1</td>
<td>20/40</td>
<td>3</td>
<td>x/x</td>
<td>x/−</td>
<td>Dry ARM and/or pigment changes</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>5/M/74 L</td>
<td>No</td>
<td>18.7</td>
<td>20/20</td>
<td>2</td>
<td>x/−</td>
<td>x/−</td>
<td>Probable ARM, pigment clumping</td>
<td>Yes</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>6/M/74 L</td>
<td>Yes</td>
<td>0.0</td>
<td>20/50</td>
<td>3</td>
<td>x/−</td>
<td>x/−</td>
<td>No drusen</td>
<td>Yes</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>7/M/75 L</td>
<td>Yes</td>
<td>0.6</td>
<td>20/60</td>
<td>2</td>
<td>NA</td>
<td>x/−</td>
<td>Drusen</td>
<td>Yes</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>8/M/80 L</td>
<td>Yes</td>
<td>0.3</td>
<td>20/40</td>
<td>0</td>
<td>x/x</td>
<td>x/−</td>
<td>Few drusen, areas RPE atrophy</td>
<td>Yes</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>9/F/83 R</td>
<td>No</td>
<td>7.0</td>
<td>20/60</td>
<td>0</td>
<td>NA</td>
<td>x/−</td>
<td>Giant drusen, ARM</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>10/F/90 R</td>
<td>No</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>x/−</td>
<td>NA</td>
<td>NA</td>
<td>Yes</td>
<td></td>
</tr>
</tbody>
</table>

*ARM indicates age-related maculopathy; OE, orbital exenteration; LED, last examination before death or surgery; VAcc, visual acuity, corrected; Phot/FA, color fundus photographs or fluorescein angiogram; ALARMDGS, postmortem fundus findings using Alabama Age-related Macular Degeneration Grading System20; R, right; L, left; NA, not available; and RPE, retinal pigment epithelium.
†0 indicates clear natural lens or posterior chamber intraocular lens; 2, moderate cataract; and 3, significant cataract.
‡x indicates yes; minus, no.
§Indicates quotes from chart.
Abnormalities    No. of Eyes
Membranous debris    9
Electron-lucent droplets    13
Heterogeneous debris    3
Pigmented debris    1
Cells    2
Other    6
Total eyes with changes    20

Closed membranous profiles with empty interiors (Figure 3, A and B), typical of BlinD, were large, irregular, and variable in size (mean diameter, 135 nm; range, 33-267 nm). They resembled the debris in some large drusen (Figure 3, B), but formed a thin sublamina external to the RPE-BL (Figure 3, A, and Figure 4, A and C) rather than a mound. Eyes with BlinD also had membranous debris within BlamD internal to the RPE-BL.

Figure 2. Ultrastructure of basal laminar deposit (BlamD). Retinal pigment epithelium (RPE) is at the top of all panels. Black arrows indicate RPE plasma membrane; black arrowheads in parts B to F, RPE basal lamina (RPE-BL); d, druse (superficial surface only); s, electron-lucent space; and bar, 1 µm. A, Normal Bruch membrane (See Figure 1 for explanation of abbreviations). B, Asterisk indicates fibrous long-spacing collagen (FLSC); white arrow, obliquely sectioned FLSC. C, Black and open wide arrows indicate amorphous material of 2 discrete electron densities. D, Long arrowhead indicates membranous debris. E, Sloping margin of a large druse contains flocculent material, presumably fluid. F, BlamD contains FLSC and membranous debris near the RPE-BL and amorphous material and coated vesicles near the RPE.
(n = 7) (Figure 4, C and E), sometimes forming distinctive linear tracks (Figure 4, E). Eyes without a separate layer of BlinD had isolated membranous profiles only within BlamD (n = 4). Retinal pigment epithelial somata also contained debris as individual profiles (Figure 4, C) or within large vacuoles (Figure 5) (n = 3).

In contrast to membranous debris, electron-lucent droplets lacked a distinct membrane, but were occasionally surrounded by a single thin electron-dense line (Figure 3, C). They were also smaller, rounder, and more uniform in size (mean diameter, 75 nm; range, 33-117 nm) than debris profiles. Droplets were rarely seen on the internal side of the RPE-BL (Figure 4, D), but they were scattered throughout both collagenous layers of most eyes older than 40 years. Droplets formed a distinct sublayer just external to the RPE-BL in 13 eyes (Figure 3, C, and Figure 4, D). Some eyes (n = 5) had 2 discrete sublayers of membranous debris and droplets, with the debris closer to the RPE-BL (Figure 3, A, and Figure 4, C). In 2 eyes, droplets were embedded in moderately electron-dense homogenous substance, forming a diffuse (Figure 4, B) or small focal deposit.

By comparing the appearance of basal deposits in ultrathin and adjacent 1-µm sections, we determined that BlamD can be detected reliably using light microscopy, but BlinD cannot (Figure 6 and Figure 7). In 1-µm sections (Figure 6, A), BlamD is a bushy light blue material between the base of the lavender RPE somata and the pale edge of RPE-BL, forming isolated patches or a continuous layer (Figure 6, B-D). In contrast, BlinD forms a burgundy or desaturated grayish pink layer external to the RPE-BL, tinctorially distinct from RPE cytoplasm, BlamD, and the inner collagenous layer (Figure 7). Basal linear deposit was most easily detectable when it was continuous with drusen (Figure 7, A and B) or obviously irregular (Figure 7, C and D). Basal linear deposit was difficult to detect in 1-µm sections when it formed a thin smooth layer that blended in with the RPE-BL (Figure 7, E and F). In no case, however, was an abnormality in the inner collagenous layer graded as BlamD or vice versa by light microscopy.

To justify combining eyes with BlinD and eyes with large drusen (>125 µm) in the calculation of lesion specificity and sensitivity for ARM, we first demonstrated that drusen in these eyes contain membranous debris (Figure 8). Large drusen in 6 eyes resembled descriptions of soft drusen. All drusen examined in these eyes contained heterogeneous vesicular profiles similar to those in BlinD but larger (Figure 8, A), as well as varying proportions of other components. These components included lakes of a homogeneous, moderately electron-dense material (Figure 8, B and C) and intermixed electron-dense granules and circular electron-lucent spaces (Figure 8, D). Large drusen in a seventh eye had progressed to almost complete calcification (not shown) and did not contain membranous debris.

Both basal deposits increased with age (Table 2), as they were ultrastructurally detectable only in eyes 60 years and older. Within the older group, however, their distributions were markedly different. Basal laminar deposit was present in 26 (81%) of eyes, and BlinD in only 9 (28%). There also was evidence of an age-related increase within the older eyes. Basal laminar deposit was present in 8 (73%) of seventh-decade eyes and 7 (88%) of ninth-decade eyes. Percentages of eyes with BlinD are less reliable due to smaller numbers, but 4 (50%) of ninth-decade eyes contained BlinD.

Specificities, sensitivities, and ORs for basal deposits in eyes older than 60 years are shown in Table 3. As stated earlier, our goal was to find a lesion or combination of lesions with high specificity and sensitivity for ARM. In 3 eyes with ARM, the inner collagenous zone split, and its contents washed out during processing. Although we could infer the presence of BlinD from indentations along the RPE-BL, we did not include these eyes in our calculations to ensure that hypothesis testing was conservative. Three of the 4 most specific lesions (0.68-0.73) contained membranous debris (BlinD, BlinD or large drusen, and BlamD with membranous debris). The fourth specific lesion (0.83) was a continu-
ous layer of BlamD. Of these 4, the highest sensitivity was found for eyes that had BlinD or large drusen (0.90). The second highest sensitivity was found for eyes with any quantity of BlamD that also contained membranous debris (0.70). Basal linear deposit alone or a continuous layer of BlamD, both of which were quite specific for early ARM, were found in only some eyes with ARM, and therefore had only moderate sensitivity (0.43 and 0.30, respectively). Finally, the specificity of 0.18 for any quantity of BlamD was the lowest observed, despite the fact the sensitivity of this lesion was high (0.80) due to its presence in many eyes with ARM.

The calculation of ORs (Table 3) emphasized the strong association between early ARM and BlinD or large drusen. Eyes with ARM were 24 times more likely than age-matched control eyes to have these lesions ($P = .002$). Although the 95% CI is wide due to the small number of eyes, its lower bound (3.52) is much larger than unity, indicating a reliable effect. Also consistent with the specificity calculations, eyes with ARM were 5 times more likely than controls to have BlamD that contained membranous debris, but this effect did not reach significance ($P = .06$). Odds ratios for other lesions did not achieve significance.

**COMMENT**

Our results support the hypothesis that BlinD and large drusen with membranous contents constitute different morphologic forms of the same ARM-associated lesion.9,14,15 Electron microscopy enabled us to detect small basal deposits, and until a marker molecule is found for BlinD, this specific lesion is best identified by this method. Because our case definition for early ARM used only features visible in the fundus as primary criteria, we conclude that large drusen and severe RPE changes are fundus markers for membranous debris. By establishing membranous debris as a salient feature of ARM, our results also have implications for assessing the validity of animal models, which to date have...
exhibited BM abnormalities resembling BlamD but not BlinD. To our knowledge, specificity and sensitivity have not been calculated in previous histopathologic studies of ARM, and therefore it is important to consider assumptions and limitations of our analysis. First, determining that membranous debris is strongly associated with early ARM does not prove causality. In fact, it is more likely that membranous debris is a specific manifestation signifying that the RPE has been sufficiently damaged by other processes to produce funduscopically visible lesions. Second, our conclusions regarding specificity are based on the relative, not complete, absence of BlinD from eyes without ARM. It is possible that the few eyes without ARM and with membranous debris actually had incipient ARM that did not meet the criteria of large drusen or severe RPE change. Third, ARM may be a group of genetically heterogeneous diseases. That 1 lesion is highly specific (absent from eyes without ARM) and highly sensitive (present in many eyes with ARM) can indicate that 1 ARM genotype dominated the sample or that basal deposits constitute a final common phenotype for multiple ARM genotypes. Our analysis cannot distinguish between these possibilities.

Our data are consistent with the idea that membranous debris in diffuse or focal forms places an eye at risk for visual loss due to late ARM. After early uncertainty about the plane invaded by choroidal blood vessels in ARM, it is now clear that they ramify in the plane of BlinD and drusen (ie, external to the RPE-BL) and not in the plane of BlamD. Previous histopathologic studies did not determine the relative risk associated with BlinD and BlamD, as these disagree on what proportion of eyes with ARM and CNV also have membranous debris. In 2 large series of surgically excised neovascular membranes, BlamD was present in virtually all ARM specimens, and BlinD was present in very few ARM specimens. However, the group without ARM was younger than patients with ARM, and therefore specificity could not be established for basal deposits in the absence of age matching. Furthermore, in intact eyes and surgically excised membranes, BlinD may be underestimated due to its low detectability using routine histological methods.

Figure 5. Vacuole of membranous debris in retinal pigment epithelium (RPE) cytoplasm. Arrowheads indicate RPE basal lamina; bar, 2 µm.

Figure 6. Bruch membrane (BM) and basal laminar deposit (BlamD) in toluidine-blue–stained 1-µm sections. Bar indicates 10 µm. A, Normal retinal pigment epithelium (RPE) and BM. White arrows indicate the RPE basal lamina; black arrows, the elastic lamina. B, Patch of BlamD (arrow). C, Thin continuous layer of BlamD (arrow). D, BlamD (arrow) overlying a druse (d) 150 µm in diameter.
nally, BlinD may be degraded by vessels growing in the plane external to the RPE-BL, lowering its detectability even further. The mechanisms by which membranous debris facilitates CNV are unknown, but may include providing a cleavage plane for opportunistic vascular buds or providing factors that stimulate and/or attract cells.

Our morphologic observations of BlinD are consistent with previous reports of a layer of membrane-bounded, round-to-oval, electron-lucent profiles external to RPE-BL in normal eyes and those with ARM. Membranous debris is reportedly confined to central macula, and its occasional presence in the subretinal space suggests that it is ultimately derived from photoreceptor outer segments. On the basis of our results, we suggest that the RPE produces membranous debris and deposits it in BM in the following 2 ways: a steady accumulation of individual vesicles resulting in diffuse deposits (BlinD) and an episodic delivery of large vacuoles resulting in focal deposits (soft drusen). The balance between both mechanisms presumably determines whether an eye has soft drusen, BlinD, or both. Sub-RPE material other than membranous debris may exist in focal and diffuse form, including an electron-dense, finely granular substance with numerous droplets (Figure 3, C, D) and debris seen in type II mesangiocapillary glomerulonephritis. The amorphous material accompanying membranous debris in large drusen is suggestive of yet another process involved in drusen formation. Irregular lakes of a moderately electron-dense material, previously interpreted as disintegrating hard drusen, appear to grow in situ by aggregation of debris or other components.

Our morphologic observations in well-preserved eyes confirmed that FLSC and amorphous basal lamina-like material in 2 electron densities are major components of BlamD. Electron-lucent space, a previously described component, was minimal, perhaps because our specimens were opened quickly after death and pre-

Figure 7. Basal linear deposit (BlinD; triple arrowheads) in toluidine-blue–stained 1-µm sections. Bar indicates 10 µm. A and B, Continuous with small drusen (d). C and D, Irregularly thickened layer. E and F, Blended with retinal pigment epithelium basal lamina.
Of the various forms of BlamD examined, only BlamD containing membranous debris had high sensitivity and specificity for early ARM. A continuous layer of BlamD, irrespective of debris content, was relatively specific but was poorly sensitive (ie, present in a few eyes with early ARM). Although having any amount of BlamD was nonspecific, it is important to note that 8 of 9 eyes with ARM and BlinD or large drusen also had at least some BlamD. The ninth eye, with calcified drusen, was at a more advanced disease stage, when BlamD disappears.

Our data are consistent with a 3-phase model of basal deposit processing that reflects the following escalating levels of RPE damage: secretion of the less dense BL-like material by the RPE; polymerization or condensation of smaller molecules to produce FLSC and the denser amorphous material; and finally, release of membranous debris, at which point lesions are visible in the fundus. The stimulus that initiates BlamD secretion is unknown, but may be related to changes in filtration caused by the age-related ac-
cumulation of debris in BM.15,19 Our data also support the idea that the significance of BlamD in ARM pathogenesis lies in predisposing inner BM to further abnormalities.32 We suggest that BlamD facilitates the passage of membranous debris into the inner collagenous zone by separating the RPE from its BL, which is normally closely apposed.

On the basis of our results, we make 3 suggestions regarding the nomenclature of basal deposits. First, we caution against using the term “BlamD” to denote a lesion in a specific location (ie, external to the RPE-BL) and its principal component, membranous debris. This component can be located elsewhere (ie, internal to the RPE-BL), and other material can occur in diffuse deposition in a specific location (ie, external to the RPE-BL). Second, we encourage the use of the term “diffuse drusen” for BlinD. This term, the meaning of which is generally accepted,65-67 A high proportion of lipids in membranous debris and droplets resemble the extracellular lipid hypothesis requires the improved morphologic examination that non–lipid-extracting ultrastructural methods could provide.64 Even in our conventionally processed material, however, membranous debris and droplets resemble the extracellular lipid-rich material in developing atherosclerotic plaques.65-67 A high proportion of lipids in membranous debris and droplets would explain the presence of empty space in BlamD and the inner collagenous layer, the fragility of soft drusen, and the poor correlation between ultrastructure and histochemically determined BM lipid content.4,16,17,56 A proper evaluation of the lipid hypothesis requires the improved morphologic examination that non–lipid-extracting ultrastructural methods could provide.64

### Table 3. Sensitivity and Specificity of Basal Deposits and Drusen for ARM

<table>
<thead>
<tr>
<th></th>
<th>BlinD† or Large Drusen†</th>
<th>BlamD With Membranous Debris</th>
<th>Continuous BlamD§</th>
<th>BlinD†</th>
<th>Any BlamD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ARM Non-ARM</td>
<td>ARM Non-ARM</td>
<td>ARM Non-ARM</td>
<td>ARM Non-ARM</td>
<td>ARM Non-ARM</td>
</tr>
<tr>
<td>Present, No.</td>
<td>9 6</td>
<td>7 7</td>
<td>3 4</td>
<td>3 6</td>
<td>8 18</td>
</tr>
<tr>
<td>Not present, No.</td>
<td>1 16</td>
<td>3 15</td>
<td>7 19</td>
<td>4 16</td>
<td>2 4</td>
</tr>
<tr>
<td>Specificity</td>
<td>0.73</td>
<td>0.68</td>
<td>0.83</td>
<td>0.73</td>
<td>0.18</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>0.90</td>
<td>0.70</td>
<td>0.30</td>
<td>0.43</td>
<td>0.80</td>
</tr>
<tr>
<td>Odds ratio</td>
<td>24.00</td>
<td>5.00</td>
<td>2.00</td>
<td>2.00</td>
<td>0.90</td>
</tr>
<tr>
<td>95% Confidence interval</td>
<td>3.52-163.75</td>
<td>1.02-24.47</td>
<td>0.36-11.57</td>
<td>0.33-11.87</td>
<td>0.03-6.07</td>
</tr>
<tr>
<td>P</td>
<td>.002</td>
<td>.06</td>
<td>.05</td>
<td>.04</td>
<td>.009</td>
</tr>
</tbody>
</table>

*ARM indicates eyes with age-related maculopathy; BlinD, basal linear deposit; and BlamD, basal laminar deposit.†Only 7 of 10 eyes with ARM gradable for BlinD.‡n = 7, including 1 with calcified drusen.§Using light microscopy.

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