Clinicopathologic Findings in Age-Related Macular Degeneration

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Age-Related Macular Degeneration (AMD)

- 9% of population
- 33% of autopsy eyes over 65 years old
- “non-exudative” type-areolar atrophy, drusen, basal deposits
- “exudative” type-choroidal neovascularization (CNV)
drusen

age-related macular degeneration

• morphologic changes at level of RPE associated with vision

• continuous layer of BlamD and membranous debris under the macula
“non-exudative” AMD
(geographic atrophy)
non-exudative AMD-areolar (geographic) atrophy
“non-exudative” AMD-areolar (geographic) atrophy
• drusen related
• drusen unrelated
Types of Drusen
- hard
- soft
- large
- confluent
Characteristics of Drusen and Bruch’s Membrane in Postmortem Eyes With Age-Related Macular Degeneration

Christoph W. Spraul, MD; Hans E. Grossniklaus, MD

We performed a histopathologic study to compare eyes with different stages of age-related macular degeneration (AMD) with age-matched eyes to identify characteristics associated with exudative vs nonexudative AMD. We analyzed 51 eyes, which were obtained from an eye bank, from 40 donors with different stages of AMD and compared them with 40 age-matched control eyes. The eyes were processed for light microscopy, and the degree of calcification of Bruch’s membrane, fragmentation of Bruch’s membrane, the number of different types of drusen, and the presence of basal laminar (linear) deposit were assessed in the macular and extramacular regions. In the macular area, a statistically significant difference was observed for the degree of calcification (P = .02) and fragmentation (P = .03) of Bruch’s membrane in eyes with exudative AMD (1.6 and 5 per eye, respectively) compared with eyes with nonexudative AMD (0.8 and 1 per eye, respectively) and control eyes (0.8 and 0 per eye, respectively). Eyes with AMD displayed notably softer, more confluent, and larger drusen and basal laminar (linear) deposit in the macular area compared with control eyes. Calcification and fragmentation of Bruch’s membrane, soft, confluent, and large drusen, and basal laminar (linear) deposit but not hard drusen correlate with the histological presence of AMD. The degree of calcification and fragmentation of Bruch’s membrane is greater in eyes with exudative compared with nonexudative AMD.

hard drusen

soft drusen

large drusen

confluent drusen
basal laminar deposit
Is Basal Laminar Deposit Unique for Age-Related Macular Degeneration?

Theo L. van der Schaft, MD; Wim C. de Bruijn, PhD; Cornelia M. Mooy, MD;
Diane A. M. Ketelaars, BSc; Paul T. V. M. de Jong, MD, PhD

- The ultrastructural nature and distribution of basal laminar deposit, considered to be a precursor of age-related macular degeneration, were studied in 42 human maculae. Basal laminar deposit was found from age 19 years on, not only between the retinal pigment epithelial cells and their basement membrane but also more often on the choriocapillary side of Bruch's membrane. No direct relationship was found with other aging changes, such as calcifications in Bruch's membrane, accumulation of lipofuscin granules, or drusen in the macular area. Material similar to basal laminar deposit can be found in the trabecular system, in the cornea, and also in many other organs and tissues. On a structural and morphometrical basis, we think that basal laminar deposit is similar to fibrous long-spacing collagen and thus does not seem to be a purely ocular abnormality.

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

Christine A. Cucio, PhD; C. Leigh Millican

**Objectives:** 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.

Green and Enger.
Ophthalmology 1993;100:1519-1535

-760 eyes with AMD from 450 patients
-nodular drusen 6.2%
-soft drusen 28.0%
-basal laminar deposit 54.7%
-basal linear deposit 27.6%
Relationship of Basal Laminar Deposit and Membranous Debris to the Clinical Presentation of Early Age-Related Macular Degeneration

Shirley Sarks, Svetlana Cherepanoff, Murray Killingsworth, and John Sarks

PURPOSE. To correlate basal laminar deposit (BLamD) and membranous debris, including basal linear deposit (BLinD), with the evolution of early age-related macular degeneration (AMD).

METHODS. A clinicopathologic collection of 132 eyes with a continuous layer of BLamD was reviewed. The thickness and type of BLamD and the sites of membranous debris deposition were correlated with the clinical progression of the disease.

RESULTS. Two types of BLamD, termed early and late, were identified based on light microscopic appearance by using the picro-Mallory stain. The progressive accumulation of late type BLamD correlated well with increasing BLamD thickness, advancing RPE degeneration, poorer vision, increasing age, and clinically evident pigment changes. Membranous debris initially accumulated diffusely as BLinD, most eyes with BLinD and early BLamD remaining funduscopically normal. However, membranous debris also formed focal collections as basal mounds internal to the RPE basement membrane and as soft drusen external to the basement membrane. Eyes in which membranous debris remained confined to basal mounds belonged to older patients with poorer vision, whereas patients with soft drusen were younger and had better vision.

CONCLUSIONS. The presence of BLinD and early BLamD define threshold AMD, which manifests clinically as a normal fundus. Although late BLamD correlates most closely with clinical pigment abnormalities, it is the quantity and sites of membranous debris accumulation that appear to determine whether the disease develops pigment changes only or follows the alternative pathway of soft drusen formation with its attendant greater risk of choroidal neovascularization (CNV). (Invest Ophthalmol Vis Sci. 2007;48:968–977) DOI:10.1167/iovs.06-0443
Early BlamD
<table>
<thead>
<tr>
<th>BlamD</th>
<th>Clinical</th>
<th>Pathology</th>
</tr>
</thead>
</table>
| Early BlamD | • normal | • fibrillar  
• amorphous  
• polymerized  
• $\leq 7 \mu m$  
• patchy/continuous |
| Late BlamD | • patchy: focal hyperpigmentation  
• continuous: pigment changes | • hyalinized clumps  
• excrescences  
• flocculent  
• $> 7 \mu m$  
• patchy/continuous |
Memb. debris - basal mounds
Memb. debris
-soft drusen
Membranous Debris

<table>
<thead>
<tr>
<th>Basal mounds</th>
<th>Clinical</th>
<th>Pathology</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>• pigment changes</td>
<td>• membranous internal to BlamD</td>
</tr>
<tr>
<td></td>
<td>• dot-like drusen</td>
<td>• &gt; 30μm</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soft drusen</td>
<td>• intermediate drusen ≤63μm-124μm ≥125μm</td>
<td>• membranous external to BlamD</td>
</tr>
<tr>
<td></td>
<td>• large drusen</td>
<td>• granular (regressed)</td>
</tr>
<tr>
<td></td>
<td>• small ≤63μm indistinguishable from hard drusen</td>
<td></td>
</tr>
</tbody>
</table>
None or patchy early BLamD

Cont thin early BLamD

Threshold early AMD
Fundus normal 74%

Normal Aging

Excess production MD (High risk CNV)

Intermediate or large drusen
-/+ Pigment changes

Intermediate or large drusen
Pigment changes (100%)

Continuous early BLamD
+/- Patchy late BLamD
Basal mounds

Continuous late BLamD
Basal mounds

BLinD + Soft drusen

BLinD + Granular drusen

DRUSEN-RELATED
Geographic atrophy

DRUSEN-UNRELATED
Geographic atrophy

1.74-2.79 mm²/yr or 1 MPS DA/yr
### International Classification and Grading System of AMD

<table>
<thead>
<tr>
<th>Stage</th>
<th>Definition</th>
</tr>
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<tbody>
<tr>
<td>0a</td>
<td>No signs of ARM</td>
</tr>
<tr>
<td>0b</td>
<td>Hard drusen (&lt;63um) only</td>
</tr>
<tr>
<td>1a</td>
<td>Soft Indistinct drusen (&gt;=63um)</td>
</tr>
<tr>
<td>1b</td>
<td>Pigmentary abnormalities only</td>
</tr>
<tr>
<td>2a</td>
<td>Soft indistinct drusen (&gt;=63um) or reticular drusen only</td>
</tr>
<tr>
<td>2b</td>
<td>Soft indistinct drusen (&gt;=63um) with pigmentary abnormalities</td>
</tr>
<tr>
<td>3</td>
<td>Soft indistinct drusen (&gt;125=um) or reticular drusen with pigmentary abnormalities</td>
</tr>
<tr>
<td>4</td>
<td>Geographical atrophy or CNV</td>
</tr>
</tbody>
</table>
Evidence of Inflammatory Component in Pathogenesis of AMD


Senile macular degeneration: the involvement of immunocompetent cells.

**Penfold PL, Killingsworth MC, Sarks SH.**


Senile macular degeneration. The involvement of giant cells in atrophy of the retinal pigment epithelium.

**Penfold PL, Killingsworth MC, Sarks SH.**


Macrophages related to Bruch's membrane in age-related macular degeneration.

**Killingsworth MC, Sarks JP, Sarks SH.**
RPE/cc damage

cellular elements

dendritic cells
Drusen including C5b-9 from cc

vitronectin, clusterin, apoE, membrane cofactor protein, complement receptor 1
IgG and C5b-9 deposition in drusen

Anderson et al.
AJO 2002;134:411-431
CD45+ microglia/dendritic cells

PCL

RPE

BlamD

Bruch’s

cc

BlamD and BlinD
CD45+ microglia/dendritic cells

PCL

RPE

MCP1/IL8

Bruch’s cc

macrophages/cytokines

BlamD

MCP1/IL8

macrophages/cytokines
*macrophages from cc*

*macrophages processing basal deposits*
? M2 macrophages
- complement factor H (Y402H)
- complement factor B/C2
- complement factor 3
- LOC387715/ARMS2
- HTRA1 gene ApoE2/E4
- toll-like receptor 4
- downregulatory intraocular environment (DIE)
- M1 and M2 macrophages
1. Genetic (CFH)/environmental susceptibility to damage
2. Accumulation of membranous debris
3. Low grade inflammatory response
4. Alteration in DIE
5. M1 to M2 (innate to acquired) response

Nusenblatt and Ferris; AJO 2007: 144:618-626
CNV in ARMD

- 13% clinically
- 20%-26% histologically
- subRPE, subretinal, combined growth patterns
- occult vs classic FA pattern
potential space for CNV between bld and Bruchs
CNV between BLD and Bruchs with ingrowth site
multiple ingrowth sites
multiple ingrowth sites

confluent growth subRPE growth pattern
type 1 CNV

subRPE CNV
subretinal growth pattern

subretinal CNV
subRPE growth pattern

combined growth pattern
combined growth pattern

subretinal component
subRPE component
Clinicopathologic Correlations of Surgically Excised Type 1 and Type 2 Submacular Choroidal Neovascular Membranes

HANS E. GROSSNIKLAUS, MD, AND J. DONALD M. GASS, MD

type 1 (subRPE)

(type 2 (subretinal))
- CNV contains cellular & extracellular constituents found in granulation tissue

- CNV represents a stereotypic, non-specific response to a specific stimulus

- CNV is a dynamic process with stimulatory, maintenance and involutional stages
Active inflammatory CNV

Dual labeled CK18/MCP

Dual labeled CD68/TF
## Cytokine/ECM molecules in CNV

<table>
<thead>
<tr>
<th>Component</th>
<th>Cytokines/ECM Molecules</th>
</tr>
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<tbody>
<tr>
<td>RPE</td>
<td>IgG, MCP, aFGF, bFGF, TGFβ, <strong>VEGF</strong>, IL8, TF, TIMP3, PEDF</td>
</tr>
<tr>
<td>macrophages</td>
<td><strong>VEGF</strong>, TF, MMP9</td>
</tr>
<tr>
<td>endothelium</td>
<td>aFGF, bFGF, TGFβ, MMP2, PDGF</td>
</tr>
<tr>
<td>pericytes HSCs</td>
<td>ang1</td>
</tr>
</tbody>
</table>
Macrophage and retinal pigment epithelium expression of angiogenic cytokines in choroidal neovascularization

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Departments of1 Ophthalmology and 2Pathology, Emory University School of Medicine, Atlanta, GA; 3Department of Ophthalmology, University of Heidelberg, Heidelberg, Germany; 4Department of Ophthalmology, University of Michigan School of Medicine, Ann Arbor, MI

Purpose: To determine the expression of angiogenic cytokines in macrophages and retinal pigment epithelium cells in choroidal neovascularization (CNV).

Methods: Ten surgically-excised subfoveal CNV specimens and ten eye bank eyes with subfoveal CNV were routinely processed, serially sectioned, and immunostained for factor VIII (F8), CD68 (KP1), cytokeratin 18 (CK18), vascular endothelial growth factor (VEGF), tissue factor (TF), and monocyte chemotactic protein (MCP). The CNV was classified as “inflammatory active” (more inflammation than fibrosis) or “inflammatory inactive” (more fibrosis than inflammation). The immunostaining was graded as none, mild (+), moderate (++), or heavy (+++). Five additional surgically-excised CNV specimens were dual labeled with CK18/MCP or CD68/TF and confocal scanning laser microscopy was performed.

Results: Vascular endothelium, macrophages, and RPE expressed F8, KP1, and CK18 respectively. Macrophages expressed + to ++ VEGF and ++ to +++ TF; RPE expressed ++ to +++ VEGF and ++ to +++ MCP. Staining for angiogenic cytokines was stronger in inflammatory active versus inflammatory inactive CNV. RPE dual labeled for CK18/MCP and macrophages dual labeled for CD68/TF.

Conclusions: This study shows that RPE cells express MCP, a cytokine involved with macrophage recruitment, and that macrophages express TF in CNV. Macrophages and RPE express VEGF, thus perpetuating angiogenesis. TF is involved with fibrin formation and provides a scaffold effect for growth of the CNV complex. CNV likely represents a dynamic process with inflammatory active and inflammatory inactive (involutional) stages.
1. Biochemical changes in microenvironment results in M1 response from choriocapillaris with destruction of elastic layer of Bruch membrane → M2 response/CNV. These changes include VEGF and MMP2 production.

2. Wound repair response (granulation tissue proliferation) → involutional changes. These changes may involve TGFβ, PEDF, and TIMP3.

*Initiation, active inflammatory, and involutional stages may repeat at edges and/or within CNV.
Non-exudative AMD

• early threshold AMD: fundus normal 74%
• limited membranous debris: older, poor vision
• excess sub-RPE membranous debris: younger, CNV
• genetic basis of disease
Exudative AMD

- type 1, type 2, combined growth patterns
- initiation, active inflammatory, involutional stages
- anti-angiogenic therapy contingent on stage of disease