Relationship between RPE and Choriocapillaris in Age-Related Macular Degeneration

D. Scott McLeod, Rhonda Grebe, Imran Bhatto, Carol Merges, Takayuki Baba, and Gerard A. Lutty

PURPOSE. The purpose of this study was to examine the relationships between choriocapillaris (CC) and retinal pigment epithelial changes in age-related macular degeneration (AMD). Morphologic changes in the retinal pigment epithelium (RPE)/choriocapillaris complex were quantified in dry and wet forms of AMD, and the results were compared with those in aged control eyes without maculopathy.

METHODS. Postmortem choroids from three aged control subjects, five subjects with geographic atrophy (GA), and three subjects with wet AMD were analyzed using a semiquantitative computer-assisted morphometric technique developed to measure the percentages of retinal pigment epithelial and CC areas in choroidal wholemounts incubated for alkaline phosphatase activity. The tissues were subsequently embedded in methacrylate and were sectioned so that structural changes could be examined.

RESULTS. There was a linear relationship between the loss of RPE and CC in GA. A 50% reduction in vascular area was found in regions of complete retinal pigment epithelial atrophy. Extreme constriction of remaining viable capillaries was found in areas devoid of RPE. Adjacent to active choroidal neovascularization (CNV) in wet AMD, CC dropout was evident in the absence of retinal pigment epithelial atrophy, resulting in a 50% decrease in vascular area. Lumenal diameters of the remaining capillaries in wet AMD eyes were similar to those in control eyes.

CONCLUSIONS. The primary insult in GA appears to be at the level of the RPE, and there is an intimate relationship between retinal pigment epithelial atrophy and secondary CC degeneration. CC degeneration occurs in the presence of viable RPE in wet AMD. The RPE in regions of vascular dropout are presumably hypoxic, which may result in an increase in VEGF production by the RPE and stimulation of CNV. (Invest Ophthalmol Vis Sci. 2009;50:4982–4991) DOI:10.1167/iobs.09-3639

Age-related macular degeneration (AMD) is the leading cause of severe vision loss in patients older than 50 in industrialized countries. AMD is defined by the presence of at least one geographic atrophy (GA). With the aging of the global population, the epidemiology of AMD remains largely unknown. Clinically and histologically, AMD is generally classified into two major subtypes: dry or nonexudative AMD, of which geographic atrophy (GA) is a severe form, and wet or exudative AMD. Dry AMD progresses more slowly and manifests with drusen, geographic or focal atrophy of the retinal pigment epithelium (RPE), and photoreceptor dysfunction and degeneration. Wet AMD, on the other hand, is more debilitating and often develops after early dry AMD. The key feature of wet AMD is choroidal neovascularization (CNV), the growth of new blood vessels from the choroid into the region underlying the RPE or extending into the subretinal space. In general, AMD pathology is characterized by degeneration involving the retinal photoreceptors, retinal pigment epithelium, Bruch’s membrane, and choriocapillaris.

Two hypotheses have evolved regarding the pathogenesis of AMD. One is that retinal pigment epithelial atrophy causes secondary choriocapillaris loss and photoreceptor degeneration. The other is that choroidal vascular insufficiency results in dysfunction of the RPE and photoreceptor degeneration. Both of these events could lead to severe vision loss. The RPE and choriocapillaris share a mutualistic relationship. If one of the components is pathologic or compromised, either or both may become dysfunctional or may degenerate (or both). Experimental studies of selective destruction of the RPE by the administration of sodium iodate or mechanical debridement showed that degeneration or removal of RPE caused atrophy of the choriocapillaris. These changes affect not only perfusion of the choriocapillaris, but also appear to compromise blood flow in the large choroidal vessels. Changes in choriocapillaris perfusion after RPE removal may involve loss of RPE-derived trophic factors that maintain the integrity of the endothelium, leading to choriocapillaris atrophy. Studies have shown that basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), endothelin-1, and insoluble molecules in the extracellular matrix produced by RPE act as survival factors for choroidal endothelial cells in particular and vascular endothelial cells in general.

The roles of hemodynamic changes, ischemia, and oxidative stress in AMD have long been recognized. Oxidative stress, has been implicated in the pathogenesis of AMD and vascular disease and may be the potential mechanism linking these two diseases. Systemic inflammation has also been identified as a risk factor for both AMD and cardiovascular mortality. A growing body of evidence links AMD with cardiovascular disease, suggesting that a dysfunctional circulatory system may be a common denominator for both conditions.
In this study, we quantified and correlated retinal pigment epithelial atrophy and choriocapillaris degeneration in aged non-AMD postmortem eyes and in eyes with GA and exudative AMD. Additionally, we examined lumenal diameters, structural changes in Bruch’s membrane, and loss of fenestrations in choriocapillaris endothelium.

**Materials and Methods**

**Donor Eyes**

Choroids were analyzed from three aged control subjects, five subjects with GA, and three subjects with wet AMD (Table 1). Donor eyes were obtained from the National Disease Research Interchange and through the help of Janet Sunness and Carol Applegate (Greater Baltimore Medical Center, Baltimore, MD). Mean ages were 78 (±7.2) years for controls, 83 (±9.7) years for subjects with GA, and 80.6 (±3.5) years for subjects with wet AMD. The eye banks provided each subject’s age, sex, cause of death, brief ocular history (if available), brief medical history, and postmortem interval. Table 1 presents the characteristics of each subject used in this analysis. All donors were Caucasian. The diagnosis of AMD was made by reviewing ocular medical history (if available) and postmortem gross examination of the posterior eye-cups. The protocol of the study adhered to the tenets of the Declaration of Helsinki regarding research involving human tissue and was approved by the Johns Hopkins Medicine Institutional Review Boards.

**Tissue Preparation**

Globes were opened at the limbus, anterior segments were removed, and eyecups were examined using a stereomicroscope (Stemi 2000; Carl Zeiss, Inc., Thornwood, NY). Gross images were obtained with a digital microscope camera (MicroPublisher; QImaging, Surrey, BC, Canada) and were imported by a plug-in into digital imaging software (Adobe Photoshop CS3; Adobe Systems Inc., San Jose, CA) on a workstation computer (MacPro; Apple, Cupertino, CA). The globes were examined and photographed using epi-illumination and retroillumination or transillumination. Epi-illumination was used to view pigmentary changes in the retina and scarring associated with subretinal CNV. Transillumination accentuated hyperpigmentation and hypopigmentation, allowing us to clearly define the area of retinal pigment epithelial atrophy. Two of the five GA eyes were clinically documented by Janet Sunness (Greater Baltimore Medical Center, Baltimore, MD), and the others were diagnosed with GA because each had a clearly defined area of retinal pigment epithelial atrophy that was centered on the fovea (Table 1). Wet AMD was diagnosed by the presence of scarring in the epi-illuminated globes or by the presence of CNV in the tissue after alkaline phosphatase incubation in the absence of a clearly demarcated area of retinal pigment epithelial atrophy. The retinas were then excised from the eyecups and processed for ADPase flat-embedding and will be the subject of a subsequent study. The choroids, with RPE intact, were imaged, dissected away from the sclera, fixed, and incubated for alkaline phosphatase (APase) activity, as described previously.

After incubation, the choroids were washed and postfixed in 2% paraformaldehyde in 0.1 M cacodylate buffer, pH 7.2, at 4°C before they were partially bleached in 30% hydrogen peroxide at 4°C. The tissue was inspected microscopically every 2 or 3 days during the bleaching process to ensure that some pigment remained visible. Bleaching was halted when the retinal pigment epithelial pigment turned light tan and permitted visualization of the underlying APase choroidal vasculature. The choroids were washed extensively and stored in 2% paraformaldehyde in 0.1 M cacodylate buffer, pH 7.2, at 4°C until they were processed further.

Wet choroids were placed on slides, and several radial cuts were made in the tissue to allow for flattening. Flat preparations were initially imaged on a stereomicroscope using the system described. The submacular choroid, including the entire posterior pole, was trimmed from the tissue after low-magnification transilluminated and epi-illuminated images were obtained.

**Morphometric Analysis**

The wet excised APase-incubated choroid was placed on a slide with the RPE closest to the objective, coverslipped under buffer, and imaged at higher magnification using the digital color microscope camera on a Zeiss photomicroscope (Carl Zeiss, Inc.). RGB images (2048 × 1536 pixels) depicting areas of choroid equal to 1 mm² were captured with a digital camera (MicroPublisher; QImaging) and were imported into digital imaging software (Adobe Photoshop CS3; Adobe Systems Inc.) by plug-in. Two different types of illumination were used for imaging each field. Transmitted light from the microscope base was used to image viable blood vessels because it provided excellent visualization of the blue APase reaction product. Fiberoptic cables were used to epi-illuminate and image viable RPE because they highlighted the partially bleached melanin granules within the cells. Images were captured and imported directly into digital imaging software (Adobe Photoshop CS3; Adobe Systems Inc.).

Through the color range command of the digital imaging select menu, blue APase staining was sampled using the eyedropper tool, and vessels were automatically selected in transmitted light images (Fig. 1). Segments of vessels not selected using the eyedropper were added with the magic wand tool while holding the shift key. This selection

### Table 1. Characteristics of Human Donor Eyes

<table>
<thead>
<tr>
<th>Case</th>
<th>PMT</th>
<th>DET</th>
<th>Age/Race/Sex</th>
<th>Primary Cause of Death</th>
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<td>4</td>
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<td>Cardiac arrhythmia</td>
<td>CAD</td>
<td>GA</td>
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<tr>
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<td>Lung cancer</td>
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<td>Pulmonary Fibrosis</td>
<td>GA</td>
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<td>9</td>
<td>6</td>
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<td>Congestive heart failure</td>
<td>CAD</td>
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<td>77/C/F</td>
<td>GI bleed</td>
<td>COPD, HTN</td>
<td>Wet AMD</td>
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</table>

DET, death to enucleation time; PMT, postmortem time (death to fixation); C, Caucasian; M, male; F, female; AMD, age-related macular degeneration; DM, diabetes mellitus; HTN, hypertension; COPD, chronic obstructive pulmonary disease; GA, geographic atrophy; CAD, coronary artery disease; MI, myocardial infarction; NA, not available.

* Patient followed up clinically at Wilmer by Janet Sunness.
technique allowed us to digitally isolate the blue choroidal vessels from other color features within the image. This method, therefore, sampled large and small blood vessels in a field. By selecting tan from the partially bleached pigment in reflected light images, the same process was used to digitally isolate the RPE (Fig. 1). Blood vessel selection and retinal pigment epithelial selection were then copied and pasted into new RGB documents and converted to grayscale. The image size of the new documents was reduced to 640 × 480 pixels, saved as TIFF formats, and imported into ImageJ software (developed by Wayne Rasband, National Institutes of Health, Bethesda, MD; available at http://rsb.info.nih.gov/ij/index.html). Thresholding was performed, and images were converted to binary. Percentage vascular area (VA) or percentage retinal pigment epithelial area was measured on binary images (black vessels or RPE on a white background) using the “compute percent black and white” command in the measurement macros. This process was repeated for each field to be analyzed within the excised choroidal tissue. Before thresholding, grayscale images were saved to disc and were used to make morphometric measurements of choriocapillaris diameters. For aged controls, this analysis was performed in the submacular region. In GA eyes, submacular areas with retinal pigment epithelial atrophy, areas at the border of atrophic and nonatrophic RPE (border zone), and areas with no retinal pigment epithelial atrophy were measured. There were a few GA eyes with CNV, but they were not included in the CNV analysis because they were very small and consisted of only several large blood vessels; in other words, they were not a neovascular network. In wet AMD

**FIGURE 1.** Flow diagram showing the processing of images for determination of percentage RPE or vascular area in a control subject (case 3). Color selection was used to isolate the RPE (left) or vasculature (right). Pixels were copied and pasted into new images and converted to grayscale, and thresholding was performed. The image was then converted to binary and imported into imaging software, in which measurements were made using the “compute percent black and white” command in the measurement macros.

**FIGURE 2.** Raw color images of RPE using epi-illumination (A) and APase-stained choroidal vessels using transillumination (B) and converted binary images used to make final percentage black and percentage white determinations from a flat preparation of an 80-year-old Caucasian male aged control subject (case 2). Scale bar, 100 μm.
FIGURE 3. Comparison of percentage RPE area and percentage vascular area in aged control subjects and in the three regions analyzed in GA choroids. There was no statistically significant difference between aged control and nonatrophic regions of GA eyes in terms of RPE area ($P = 0.292$) and vascular area ($P = 0.067$). However, in GA choroid, there was a statistically significant decrease in RPE ($P < 0.0001$) and vascular ($P = 0.0001$) areas in the border region compared with the nonatrophic region (asterisk). Similarly, there was a significant decrease in the RPE area ($P < 0.0001$) and the vascular area ($P = 0.0013$) in the atrophic region compared with the border region (asterisk).

![Graph](https://example.com/graph.png)

<table>
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<th>% RPE Area</th>
<th>% Vascular Area</th>
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<td></td>
<td></td>
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<tr>
<td>Non-Atrophic</td>
<td></td>
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</tr>
<tr>
<td>GA Border</td>
<td></td>
<td></td>
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<tr>
<td>GA Atrophic</td>
<td></td>
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FIGURE 4. APase choroid from an 88-year-old Caucasian male (case 7) with a well-defined area of GA (arrows) measuring 26.3 mm$^2$ is shown with epi-illumination (A) and transillumination (B). The optic nerve head (NH) is indicated. Scale bar, 2 mm.

![Image](https://example.com/image.png)

FIGURE 5. APase choroid from a 79-year-old Caucasian male with GA (case 8) showing nonatrophic (A, D, G, J), border (B, E, H, K), and atrophic (C, F, I, L) regions. Images captured with epi-illumination (A–C) highlight the RPE and were processed to make the binary images used to measure RPE area (D–F). The same three regions are shown with transillumination (G–I), along with the binary images used to measure percentage vascular area (J–L). Scale bar, 100 μm.

![Images](https://example.com/images.png)
specimens, regions outside CNV formations in the submacular choroid were analyzed.

Capillary diameters were measured with the measuring tool in ImageJ software (developed by Wayne Rasband, National Institutes of Health, Bethesda, MD; available at http://rsb.info.nih.gov/ij/index.html) on calibrated grayscale images. At least 15 capillaries were measured in at least three fields of each region of interest. Only the portion of the capillary distant from any branches, bifurcations, or arteriolar or venular connections was measured.

After the wet preparation analysis was completed, some pieces of the choroidal tissues containing regions of interest were flat embedded in glycol methacrylate (JB-4; Polysciences Inc., Warrington, PA), as described previously, and were sectioned for further histologic analysis.23,24 Sections were stained with periodic acid Schiff (PAS) and hematoxylin or hematoxylin and eosin.

Transmission Electronic Microscopy

Tissue from one aged control (case 1) and two GA subjects (cases 4, 5) was fixed overnight in 2.5% glutaraldehyde/2% paraformaldehyde in 0.1 M cacodylate buffer, pH 7.3, before washing in 0.05 M cacodylate buffer and were postfixed in 1% OsO4 in 0.05 M cacodylate buffer for 90 minutes. Tissue was dehydrated in a series of graded ethanol (E'TOH; 50%, 70%, 80%, 95%, 100%) and was stained with 1% uranyl acetate in 100% E'TOH. Tissue was then infiltrated in 100% resin (LX112; Ladd Research Industry, Burlington, VT) for 4 to 6 hours under vacuum and finally embedded in a final change of 100% resin (LX112; Ladd Research Industry) and polymerized at 60°C for 36 to 48 hours. Ultrathin sections were cut (Ultramicrotome UCT; Leica Microsystems, Wetzlar, Germany), stained with uranyl acetate and lead citrate, and analyzed under a transmission electron microscope (H7600; Hitachi, Tokyo, Japan).

Statistical Analysis

Data are reported as mean ± SEM. Statistical evaluation of the data involved calculating probability values using the Student’s t-test for two samples assuming unequal variances. P ≤ 0.05 was considered significant.

RESULTS

Percentage RPE and Percentage Vascular Area

In aged control eyes, the submacular RPE was relatively homogeneous, was uniform in size and pigmentation, and had a typical cobble-stone like morphology (Fig. 2). Retinal pigment epithelial coverage was 95.3% ± 4.2% (Fig. 3). The submacular choriocapillaris vasculature was intensely stained for APase and appeared normal morphologically, with regular branchings and freely interconnecting capillaries (Fig. 2B). Measurements demonstrated that the vascular area was 79.6% ± 3.8% (Fig. 3). In GA subjects (Fig. 4), there was a well-defined area of submacular retinal pigment epithelial atrophy and degenerative changes in choriocapillaris. Outside the area of atrophy (Figs. 5A, 5D, 5G, 5J), the RPE appeared mottled and, in some cases, had...
scattered small whitish drusen (Fig. 5A). The choriocapillaris had normal APase activity and appeared relatively normal morphologically in areas without retinal pigment epithelial atrophy (Fig. 5G). Measurements obtained from nonatrophic regions (approximately 1 mm from the border of atrophic and nonatrophic areas) revealed that the retinal pigment epithelial coverage was 92.52 ± 3.24% and the vascular area was 72.26 ± 4.8%, which were not significantly different from the percentages in control eyes (%RPE, P = 0.292; %VA, P = 0.067; Fig. 3). At the border of atrophy (Figs. 5B, 5E, 5H, 5K), the remaining intact retinal pigment epithelial cells were hypertrophic, and some cells appeared hyperpigmented. The choriocapillaris had reduced interconnecting segments and was attenuated in areas of retinal pigment epithelial atrophy. In these regions, the percentage of retinal pigment epithelial coverage was 38.1% ± 5.8%, and the vascular area was 52.3% ± 3.3%, both significantly lower than in nonatrophic regions in GA choroids (%RPE, P < 0.0001; %VA, P = 0.0001). Within the regions of atrophy (Figs. 5C, 5F, 5I, 5L), only a few scattered retinal pigment epithelial cells remained, and the retinal pigment epithelial coverage was 1.8% ± 3.9% (Fig. 3). The choriocapillaris was highly attenuated and had reduced branchings, with few interconnecting segments (Figs. 5I, 5L). The percentage of vascular area was 38% ± 5.7%, which was significantly lower than of border regions (P = 0.0013) in GA eyes (Fig. 3).

In the choroids of wet AMD eyes (Fig. 6), measurements were made from binary images 1 mm peripheral to the CNV (Fig. 7). The percentage of retinal pigment epithelial coverage in these regions was 95.9% ± 1.8%, and in the vascular area it was 59.6% ± 15.9%. The decrease in vascular area was evident well beyond the submacular region and in one case extended peripherally 10 mm from the CNV into the equatorial choroid (Fig. 6C). The greater the distance from the CNV, however, the greater was the percentage of vascular area. Compared with aged control eyes (Fig. 8), wet AMD eyes had no significant changes in retinal pigment epithelial coverage outside the CNV area (P = 0.85). However, the percentage vascular area in these regions was significantly reduced, reflecting the loss of interconnecting capillary segments in these regions immediately in advance of the CNV (P = 0.009).

**Capillary Diameters**

Mean capillary diameters (Fig. 9) were 14.6 ± 1.1 μm in aged control eyes. In GA eyes, capillary diameters were 13.9 ± 1.4 μm in nonatrophic regions and were not significantly reduced compared with aged controls (P = 0.4434). Capillary diameters were 10.34 ± 0.7 μm in border regions and 7.9 ± 1.2 μm in regions of atrophy. Compared with capillaries in nonatrophic regions, capillary diameters at the border were significantly reduced (P = 0.001). Similarly, capillary diameters in the atrophic region were significantly reduced compared with those at the border (P = 0.007). Capillary diameters in wet AMD eyes were 13.9 ± 0.4 μm. There was no significant difference in diameters between the aged control and the viable capillaries in wet AMD eyes 1 mm outside the CNV area (P = 0.348).

**Histology**

Cross-sections through the three regions analyzed in GA eyes confirmed the observations made in flat preparations (Fig. 10). In nonatrophic regions, the choriocapillaris displayed broad lumens with APase-positive endothelial cells. The lumens often contained serum APase. The RPE had a normal structure, with rounded nuclei and melanosomes concentrated in the apical cytoplasm. In border regions, many capillaries were withdrawn from the intercapillary pillars, RPE was increased in height, and basal laminar deposits were present (Fig. 10H, open arrow). Capillaries in regions devoid of RPE showed severe degenerative changes, and, in some cases, only remnants of basement membrane material remained. The remaining viable capillaries were extremely constricted, having been withdrawn from the surrounding intercapillary pillars, which had increased PAS staining. Bruch’s membrane was generally devoid of deposits or drusen in atrophic regions.

In wet AMD eyes (Fig. 11), sections from regions temporal to submacular CNV that showed good staining of vessels in flat preparations revealed broad capillary lumens with endothelial cells and pericytes. In some subjects, the lumen contained serum APase. Retinal pigment epithelia were of relatively uniform size, and Bruch’s membrane was unremarkable. In areas closer to the CNV, the capillary dropout that was observed in flat preparations was confirmed. Although some capillaries were obviously patent because of the presence of serum APase within their lumens, others were degenerated with only remnants of basement membrane material present. The adjacent retinal pigment epithelia were hypertrophic, had a scalloped morphology, and basal laminar deposits were present. Sections taken at the peripheral edge of CNV formations showed hypertrophic RPE overlying the growing tips of the blood vessels, basal laminar deposit, and choriocapillaris dropout. In addition to the changes associated with choriocapillaris, medium and large choroidal arteries in dry and wet AMD eyes showed
Pathologic changes consistent with both hyaline and hyperplastic arteriosclerosis (Fig. 12).

Ultrastructure

Examination of TEM sections of an aged control eye demonstrated a thin endothelium with numerous fenestrations with single-layered diaphragms along the inner aspect of the choriocapillaris (Fig. 13). Additionally, fenestrations were seen along the outer capillary wall, but to a lesser degree. In GA eyes where RPE was present, fewer fenestrations per capillary were observed compared with control choroid. In border regions, the number of fenestrations was reduced even more, and few, if any, were observed in regions of atrophy. In these areas, the inner aspect of the capillary endothelium was thickened, and cytoplasmic loops were observed projecting into the lumenal space (Fig. 14). Often these loops had fenestrations within them (Fig. 14C) but were considered abnormal because they were not in a position where they would be functional. Fenestration counts made from 2 GA eyes revealed that areas with RPE had statistically significant higher numbers of fenestrations.
per capillary \((6.53 \pm 0.996)\) than regions devoid of RPE \((0.55 \pm 0.235)\), where few, if any, were observed (case 4, \(P = 0.019\); case 5, \(P = 0.001\)).

***DISCUSSION***

Although limited in the number of eyes examined, this study demonstrates a linear relationship between the loss of RPE and choriocapillaris in GA. A 50% mean reduction in vascular area was found in regions of retinal pigment epithelial atrophy compared with regions in GA eyes with RPE and in aged control eyes without maculopathy. We did not observe complete loss of choriocapillaris in areas of total retinal pigment epithelial atrophy even though the atrophy had been documented clinically for 20 years before death in one patient. However, the surviving capillaries that remained in these regions were extremely constricted and were half the diameter of capillaries in which RPE was present. Moreover, we noted a loss of fenestrations in the endothelium of these constricted capillaries, suggesting functional changes. Experimental studies have shown that retinal pigment epithelial cell death precedes choriocapillaris atrophy\(^5\) and that destroying RPE causes loss of fenestrations.\(^2\)\(^,\)\(^4\) Presumably, this occurs because RPE constitutively produces factors such as VEGF\(^25\) that stimulate the formation of fenestrations.\(^26\) VEGF is also a potent vasodilator\(^27\) and endothelial cell survival factor\(^28\) and it induces angiogenesis.\(^27\) Retinal pigment epithelial cells in vitro have been shown to secrete VEGF basally (toward the choriocapillaris), and VEGF receptors in humans are expressed on the chorioidal endothelium facing the retinal pigment epithelial layer.\(^29\) Consistent with these observations and the known functions of VEGF, it is reasonable to conclude that in GA where the RPE has atrophied, the source of VEGF is removed, and capillaries either constrict and lose fenestrations or degenerate and eventually atrophy. The clinical impression that areas of retinal pigment epithelial atrophy in GA lack a choriocapillaris may be attributed to the extreme constriction of the surviving vascular segments and loss of fenestrations; hence, the dye filling of these segments is negligible, and leakage through fenestrations does not occur.

**FIGURE 12.** Arteriosclerotic changes shown in PAS- and hematoxylin-stained sections from GA (A, B) and wet AMD (C, D) eyes. (A) An artery in a GA eye (case 5) showing hyperplastic changes consisting of concentric laminations of smooth muscle cells and basement membranes. (B) An artery in a GA eye (case 4) showing the formation of nodules that replace the media during hyalinosis. (C) Hyperplastic arteriosclerotic changes in a wet AMD eye (case 10) and (D) hyaline arteriolosclerosis in a wet AMD eye (case 9). Arrows: outer vessel wall; asterisks: lumens. Scale bars: 20 \(\mu\)m (A, B, D); 30 \(\mu\)m (C).

**FIGURE 13.** Fenestrations (arrowheads) along the inner aspect of the choriocapillaris endothelium in an aged control eye (A) and in a GA eye where RPE was present (B), near the border region (C), and in the region of atrophy (D). Scale bar, 500 nm.

**FIGURE 14.** Ultrastructure of choriocapillaris in a GA eye in transmission electron microscopy sections from a region with RPE (A), border region (B, C), and area of atrophy (D). Capillaries from the region with RPE had fine endothelial cell cytoplasmic processes surrounded by a thin basement membrane. Endothelial cells in capillaries from the border region (B) were often vacuolated (arrow) and had thickened processes. Others were highly vacuolated and had extensive cytoplasmic infoldings (C) that had fenestrations (arrowhead). In atrophic regions (D), degenerative capillaries were collapsed and consisted almost entirely of basement membrane material (arrowheads). Scale bar, 1 \(\mu\)m.
The close association we observed between degenerating RPE and choriocapillaris suggests that, at least in GA, retinal pigment epithelial atrophy occurs first, followed by choriocapillaris degeneration. The mechanism of retinal pigment epithelial degeneration in AMD is likely to be multifactorial, with oxidative stress, environmental factors, intense light exposure, and genetics all potentially involved. Oxidative stress has been proposed in AMD through several mechanisms, including blue light-induced photochemical released oxidants that damage cells, cigarette smoke-related oxidants such as hydroquinone that alter the Bruch’s membrane, iron-induced oxidative damage to the outer retina, and possibly advanced glycation end products within the Bruch’s membrane. Lesions induced by oxidative injury may accumulate over time and trigger affected retinal pigment epithelial cells to undergo apoptosis. If oxidative stress is involved in the etiology of AMD, then the aging process of RPE and the development of AMD might be prevented or delayed by increasing the antioxidant capacity of RPE. In support of this hypothesis, the Age-Related Eye Disease Study has demonstrated that a combination of antioxidants and zinc supplements, beyond what can be achieved through diet alone, reduced the risk of severe AMD and vision loss in humans. In this study, choriocapillaris dropout occurred in the three wet AMD specimens, without retinal pigment epithelial atrophy in advance of CNV, resulting in a 50% decrease in vascular area compared with aged control eyes. The theory that the choroidal vasculature plays a driving role in AMD development is well established. Duke-Elder suggested that most cases of AMD resulted from sclerosis and obliteration of the choriocapillaris in the submacular area. Later Friedman proposed a hemodynamic model for AMD, which he later revised to a vascular model. This model suggests that AMD is a vascular disorder characterized by impairment of choriocapillary perfusion. It highlights the roles of the atherosclerotic process and blood pressure in the pathogenesis of the disorder. AMD shares common risk factors with cardiovascular disease, and recent large clinical studies support that vascular factors may contribute to its pathogenesis. Studies demonstrate that persons with hypertension and atherosclerosis are more likely to have AMD. Quantitative and qualitative ocular blood flow abnormalities have been consistently described in early and late AMD, and numerous studies have implicated ischemia as a primary stimulus for AMD. A vascular deficit in AMD is supported by previous histologic findings that showed a reduction in the cross-sectional area of the choriocapillaris in persons with AMD. The driving mechanism for the development of neovascularization is hypoxia/ischemia. The presence of choriocapillaris atrophy in the vicinity of CNV has been reported previously. Melrose et al. described a patient with choroidal nonperfusion underlying subretinal neovascularization. They suggested that chronic macular ischemia occurred secondary to choroidal blood flow impairment. Hayashi and de Lacy used indocyanine green angiography, demonstrated watershed zones or areas of choroidal circulatory disturbance associated with CNV in AMD. Our results support these previous findings and lend credence to the theory of a vascular basis for the development of at least the exudative form of AMD. The observed intimate association between CNV and viable retinal pigment epithelia suggests that their presence and the factors they produce are required for the growth and expansion of pathologic neovascularization.

In conclusion, despite the limited number of specimens analyzed, this study demonstrated that choriocapillaris degenerates in GA and exudative AMD, but the etiology of the two types of AMD may differ. Retinal pigment epithelial atrophy appears to be the initial insult in GA, whereas choriocapillaris degeneration precedes retinal pigment epithelial atrophy in wet AMD. The mutualistic relationship between choriocapillaris and RPE is ultimately lost in both forms of AMD.

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References


