3 Histopathology of Age-Related Macular Degeneration and Implications for Pathogenesis and Therapy

Ru-ik Chee, Abdallah Mahrous, Lisa Koenig, Lindsay Skye Mandel, Fahd Yazdanie, Chi-Chao Chan, and Mrinali P. Gupta

Abstract

Aging is associated with a number of histological changes in the choroid, Bruch's membrane, RPE, and neuroretina. Outside of the normal physiologic aging spectrum of changes, abnormal deposits such as basal laminar deposits, basal linear deposits, and soft drusen are known to be associated with AMD. Progression of AMD to advanced stages involving geographic atrophy, choroidal neovascularization, and/or disciform scars can result in debilitating vision loss. Knowledge of the angiogenic pathway and its components that stimulate neovascularization has led to the development of a new paradigm of intravitreal anti-VEGF pharmacotherapy in the management of neovascular AMD. Currently however, there are no available treatments for the modification of disease progression in non-neovascular AMD, or for the treatment of geographic atrophy. Further understanding of the histopathology of AMD and the molecular mechanisms that contribute to pathogenesis of the disease may reveal additional therapeutic targets.

Keywords

Age-related macular degeneration · Histopathology · Basal laminar deposits · Basal linear deposits · Drusen · Geographic atrophy · Choroidal neovascularization · Disciform scar

3.1 Introduction

Age-related macular degeneration (AMD) is an ocular disease characterized by pathologic changes in the posterior pole of the eye, in structures such as the outer neurosensory retina, retinal pigment epithelium (RPE), Bruch's membrane, and the choriocapillaris in elderly patients. These pathological abnormalities differ from a number of age-related histological changes considered to be part of the physiologic spectrum.

AMD is broadly classified into "dry" and "wet" subtypes. Histologically, accumulation of waste material resulting in basal deposits (i.e., basal laminar deposits (BlamD) and basal linear

R.-i. Chee · A. Mahrous · L. Koenig · L. S. Mandel · F. Yazdanie
Department of Ophthalmology, Weill Cornell Medical College, New York, NY, USA

C.-C. Chan
National Eye Institute, National Institutes of Health, Bethesda, MD, USA

M. P. Gupta (✉)
Department of Ophthalmology, Weill Cornell Medical College, New York, NY, USA

Retina Associates of Orange County, Laguna Hills, CA, USA
e-mail: mgupta@retinaorangecounty.com

E. Y. Chew, A. Swaroop (eds.), *Age-related Macular Degeneration*, Advances in Experimental Medicine and Biology 1256, https://doi.org/10.1007/978-3-030-66014-7_3

deposits (BlinD)) beneath the RPE and into Bruch's membrane characterizes early dry, non-exudative, or non-neovascular AMD (Fig. 3.1). Drusen are the clinical hallmark of AMD that can be noted on fundus ophthalmoscopic examination and represent deposits within Bruch's membrane. Wet, exudative, or neovascular AMD is mainly distinguished from dry AMD by the presence of choroidal neovascularization (CNV), which represents abnormal new blood vessel formation.

Loss of vision occurs through a variety of mechanisms, which in turn have important resulting clinical implications. At the level of the RPE and photoreceptors, alterations such as RPE hypopigmentation, depigmentation, atrophy, hypertrophy, and photoreceptor attenuation may manifest. In wet AMD, fluid or blood in the intraretinal, subretinal, or sub-RPE spaces may additionally result in decreased visual acuity, metamorphopsia, or other visual symptoms. Less commonly, massive subretinal hemorrhage or breakthrough vitreous hemorrhage may further reduce vision. Progression to geographic atrophy and disciform scarring occur in late stages of dry and wet AMD, respectively.

Examining the histopathology of AMD provides us with a better understanding of the pathogenic mechanisms that drive vision loss, which may ultimately aid in better determination of visual prognosis, patient counseling, and development of new treatments.

3.2 Histopathology and Anatomy of the Normal Retina, RPE, Bruch's Membrane, and Choroid

The retina is a thin, multi-layered sheet of tissue derived from the neuroectoderm. It represents the beginning of the visual pathway, responsible for transmitting visual stimuli from the external environment to the visual cortex, by way of the optic nerve [1, 2]. This approximately 0.2-mm-thick structure is comprised of the neuroretina, made of 9 separate layers, and the retinal pigment epithelium (RPE) [3]. As light enters the eye, it traverses the neuroretinal layers in the following order: (1) internal limiting membrane, (2) nerve fiber layer, (3) ganglion cell layer, (4) inner plexiform layer, (5) inner nuclear layer, (6) outer plexiform layer, (7) outer nuclear layer (the photoreceptor nuclei), (8) external limiting membrane, and (9) photoreceptor (the photoreceptor inner and outer segments) layer [1, 4]. Each layer includes neurons specialized in visual information processing, totaling more than 50 distinct types [2].

In the seventh and ninth layer**,** photoreceptors (i.e., rods and cones) convert photons of light into electrical impulses, thereby initiating signal transduction to the brain [2]. The photoreceptor layer comprises an outer and an inner segment: the outer segment includes the photoreceptor discs, which contain photopigment; the inner segment includes the structures responsible for intracellular metabolism and transport, like mitochondria (found in the outer ellipsoid), Golgi bodies, and ribosomes (found in the inner myoid) [5]. Photoreceptors extend from this layer to the outer nuclear layer, which houses the photoreceptor nuclei. In between lies the external limiting membrane, which is formed by tight junctions between photoreceptors and Müller cells and is not considered a true membrane. Axons extend from the photoreceptor cell bodies, synapsing with bipolar cells in the outer plexiform layer. The inner nuclear layer (INL) is comprised of a diverse set of neurons (their nuclei) primarily belonging to three classes—horizontal, bipolar, and amacrine cells—as well as Müller cells, a type of macroglial cell. Bipolar cell nuclei predominate in the INL, and are known to demonstrate different functional responses to light. Some bipolar cells respond at light onset, some at light offset, some transiently, and some in a sustained manner. Such ON bipolar cells terminate at the inner part, and OFF bipolar cells at the outer part, of the inner plexiform layer [1, 6]. Axons extend from the INL synapsing with ganglion cells in the inner plexiform layer.

The ganglion cell layer contains the nuclei of more than 10 types of ganglion cells [7]. Ganglion cell axons, which form the nerve fiber layer, are responsible for transmitting visual information to

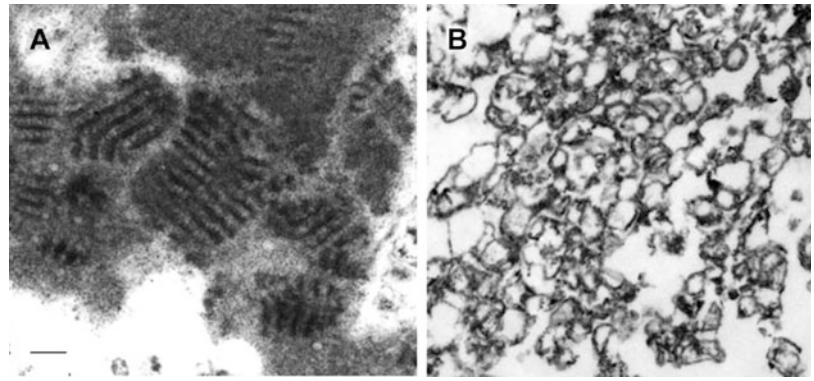

Fig. 3.1 Transmission electron micrography illustrating (**a**) basal laminar deposits (BlamD) and (**b**) basal linear deposits (BlinD) (bar = 0.5 μm)

higher visual centers by way of the optic nerve. Finally, the internal limiting membrane is a basement membrane formed by Müller cell footpads [1].

The RPE is a single layer of neuroectoderm-derived hexagonal epithelial cells primarily responsible for nourishing the overlying neurosensory retina. Its apical surface, which is covered in microvilli, faces the photoreceptors, helping in the continual turnover of their outer segments [4, 8]. The RPE basal lamina is the inner layer of Bruch's membrane, a 5-layered membrane overlying the choriocapillaris. Specifically, functions of the RPE include phagocytosis, recycling photoreceptor outer segments, converting all-trans retinal to 11-cis retinal as part of the visual cycle, secreting growth factors to maintain photoreceptor vasculature, transporting nutrients and waste between the photoreceptors and the choriocapillaris, and forming the outer blood-retinal barrier, which prevents molecules >300 kDa from passing into or out of the retina [4, 8].

Bruch's membrane, an acellular pentalaminar structure, separates the RPE and choroid. From the RPE to the choroid, the following layers are distinguished: (1) RPE basement membrane, (2) inner collagenous layer, (3) elastin layer, (4) outer collagenous layer, and (5) basement membrane of the choriocapillaris. Bruch's membrane serves as a semi-permeable filter to regulate the transport of molecules such as carbon dioxide, water, ions, waste products cleared by the RPE, photoreceptor outer segments and other substances between the retina and choroid [8].

The choroid is a vascular bed between Bruch's membrane and the sclera. Its innermost layer is the choriocapillaris, a single layer of fenestrated capillaries that supplies blood to the RPE and outer neurosensory retina. Sattler's Layer is comprised of intermediate arterioles and venules, connecting the choriocapillaris to the outer Haller's Layer. The choroidal vasculature is primarily supplied by the long and short ciliary arteries, which are branches of the ophthalmic artery. Of note, loss of endothelial cells of the choriocapillaris is an important contributor to the development of AMD [8].

3.3 Histological Changes During Normal Aging in the Retina

With non-pathological aging, the retina undergoes a number of changes such as drusen formation, Bruch's membrane thickening, photoreceptor loss, choroidal thinning, lipofuscin accumulation, and other RPE changes [9].

3.3.1 Drusen

Drusen, which are comprised of cellular debris and lipids, among others, accumulate within the Bruch's membrane with age. Drusen may be categorized by traits including their size, shape, and location, and are variably linked to disease states. Specifically, hard drusen accumulate as part of the normal retinal aging process. Typically, hard drusen are <63 micrometers with sharp borders, are

found at both the macula and periphery, and have not been causally linked to AMD development. By contrast, soft drusen are typically >125 micrometers, may have either sharp or indistinct borders, are only found at the macula, and significantly increase the risk of AMD [4, 9] (Fig. 3.2).

3.3.2 Thickening of Bruch's Membrane

Beyond drusen formation, Bruch's membrane thickening also occurs with age. In general, the five layers of Bruch's membrane become less sharply demarcated and the overall structure thickens [4, 10]. Previous work has demonstrated a linear relationship between Bruch's membrane thickness and age (from 2 mm in those <10 years old to 4.7 mm in those >80 years old) [8]. Further Bruch's membrane changes include lipid accumulation, reduced amino acid diffusion, diminished elasticity, and an increase in the expression of TIMP3, a regulatory protein that inhibits metalloproteases [9]. The implications of thickened Bruch's membranes in aging retina are a reduction in efficiency of exchange of nutrients and waste between the retina and choroid, potentially increasing susceptibility for development of retinal pathology.

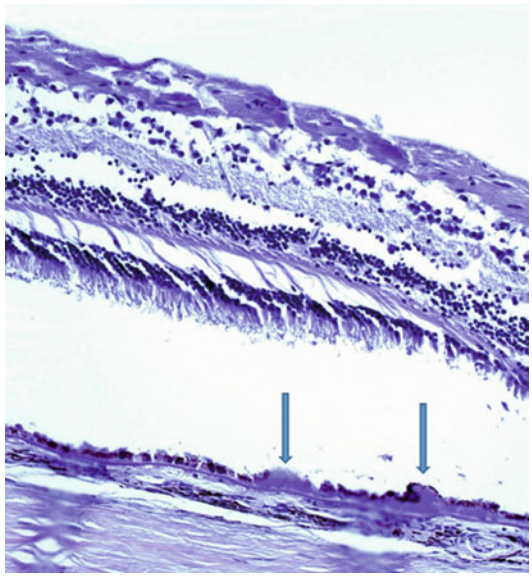

Fig. 3.2 Photomicrograph of an eye showing drusen (arrows) in the macular region (Periodic acid-Schiff (PAS), original magnification, ×100)

3.3.3 Loss of Photoreceptors

Another consequence of aging is photoreceptor loss, predominantly affecting rods more so than cones. It is estimated that 2 rods/mm^2 are lost per year in healthy retina; examination of donor retina tissue of patients over 90 has revealed minor cone loss in the fovea and 30% rod loss in the parafovea. Given their extremely high metabolic activity, photoreceptors are particularly susceptible to hypoxia. As a result, any degeneration in the choriocapillaris over time is expected to increase photoreceptors' vulnerability. Compared to older, healthy retina, more significant rod loss is observed in eyes with retinal disease, e.g., AMD (both geographic atrophy and CNV) [4, 9]. Given their role as mediators of vision in low-light conditions, loss of rods with age results in decreased scotopic sensitivity [11].

3.3.4 Choroidal Thinning

Choroidal thickness and choriocapillaris density decrease with age. Sub-foveal choroidal thickness has been observed to decrease by ~3 micrometers per year. Reduction in choriocapillaris blood flow has also been reported, which may increase risk for AMD and CNV [4, 9].

3.3.5 Lipofuscin Accumulation

Moreover, lipofuscin accumulation occurs as individuals' age. Lipofuscin is composed of granules that are byproducts of photoreceptor outer segment turnover. Accumulation in the RPE is observed in normal aging, reaching a detectable level in those over 40 years of age. Past work has not identified a significant difference in the degree of lipofuscin accumulation in patients with AMD vs. health- and age-matched controls—though this may be due to increased

cell death in AMD eyes and therefore loss of lipofuscin [8, 9].

3.3.6 RPE Changes

Finally, a number of RPE changes occur with age. Specifically, the following have been reported: reduced number of mitochondria, loss of cristae and matrix density, accumulation of lipofuscin (as previously noted), decrease in melanosomes, and increase in toxic visual cycle byproducts. Minor RPE cell loss has also been observed with age [4, 9].

3.4 Histopathology in Early Dry AMD

Clinically, a diagnosis of AMD requires the presence of extensive small drusen (Fig. 3.3), or the presence of any medium or large drusen (Fig. 3.4) in the posterior pole. Histologically, several other changes can be observed.

3.4.1 Changes in Bruch's Membrane

In AMD, thickening of the Bruch's membrane is greater than with aging alone, partly due to accumulation of inner collagenous material also known as BlamD [12]. In early AMD, further thickening and loss of normal architecture is seen within Bruch's membrane. Bruch's membrane is considered the structural analog of the vascular intima as it lies under the RPE and forms the inner margin of the choriocapillaris [13]. Analogous aging changes in the vascular intima of atherosclerosis were thought to relate to the pathogenesis of AMD in the Bruch's membrane [14].

3.4.2 Changes in the RPE

In the early stages of AMD, the RPE mosaic, a normally uniform hexagonal array, begins exhibiting pleomorphism [15]. There is also an exaggeration in RPE cell density decline, compared to the normal age-related decline [16]. As AMD progresses, RPE cells may die. In a study of the inflammatory roles of the RPE in AMD, Nussenblatt and Ferris discuss the importance of the downregulatory immune environment in the eye [17]. The hypothesis suggests that the natural environment of the eye is designed to downregulate inflammation while maintaining an equilibrium in the eye. The RPE cells downregulate the immune response in the eye, therefore in AMD it becomes a cycle of inflammatory response damaging the RPE, which subsequently decreases the downregulatory effect of the RPE leading to worsening RPE degeneration.

3.4.3 Various Deposits

Among the earliest pathological changes in early AMD are the appearance of BlamD and BlinD (Fig. 3.1). BlamD and BlinD, but not drusen, were found to have a positive association with CNV, disciform scarring, and visual loss [18].

3.4.3.1 Basal Laminar Deposits (BlamD)

BlamD are extracellular debris accumulating between the RPE and its basal lamina. They consist of membrano-granular material and foci of wide spaced collagen. Localized detachments of the BlamD result in the formation of soft drusen. BlamD stain light red with Mallory staining, and light blue with Masson's trichrome staining. Studies showed that BlamD are composed of type IV collagen, laminin, glycoproteins, glycosaminoglycans (chondroitin and heparin sulfate), N-acetylgalactosamine, esterified and unesterified cholesterol, and apolipoproteins B and E [19–21].

3.4.3.2 Basal Linear Deposits (BlinD)

BlinD are present in the superficial and deeper layers of Bruch's membrane, external to the RPE basement membrane. They are usually present in the inner collagenous layer, but can extend into the outer collagenous layer and even into the choriocapillaris pillars. They consist of lipid-rich vesicular material located in the inner collagenous

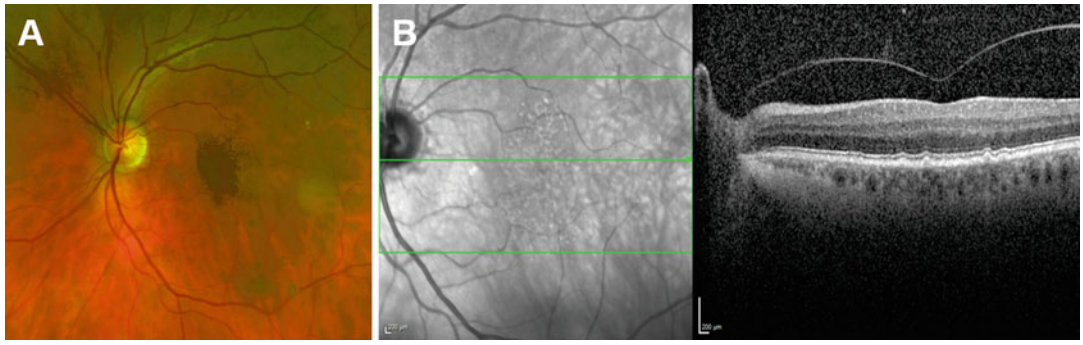

Fig. 3.3 Fundus photograph and optical coherence tomography (OCT) of a patient with early dry age-related macular degeneration. (**a**) Fundus photograph using Optos (Optos PLC, Dunfermline, Scotland) of the left eye reveals small drusen in the posterior pole. (**b**) OCT of the left eye using Heidelberg SPECTRALIS (Heidelberg Engineering, Heidelberg, Germany) reveals small drusen between retinal pigment epithelium and Bruch's membrane

zone of Bruch's membrane, which contributes to Bruch's membrane thickening. BlinD may represent a possible extension or progression of the abovementioned BlamD. They are found in association with soft drusen and RPE detachments. These deposits may not be evident on clinical examination in early AMD. They can sometimes be detected by very faint and late fluorescein staining, and inferred retinal function. They become clinically evident by secondary changes such as thinning of RPE, development of soft drusen or eventual choroidal neovascularization and disciform scarring. They are more specific for AMD than BlamD [12], and their amount is a more reliable indicator of the degree of RPE and photoreceptor degeneration [15, 22].

3.4.3.3 Drusen

Drusen are localized deposits between the RPE basement membrane and Bruch's membrane. In early AMD, they are frequently found as clusters within the macular region (Figs. 3.2 and 3.3). They vary in size and are split to small (<63 μm diameter) (Fig. 3.3), medium (63–125 μm diameter) (Fig. 3.4), and large (>125 μm diameter) drusen. The typical diameter of a retinal vein at the optic nerve head (125 μm) can be used as an estimate for classifying large drusen. Drusen also vary in shape, consistency, color, and distribution. Drusen usually increase in number with advancing age. Drusen are associated with thinning of the overlying RPE and become visible as yellow-white deposits on fundus examination.

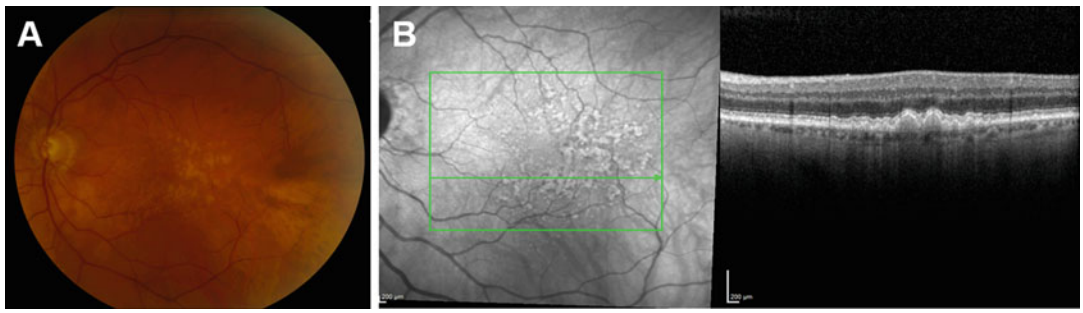

Fig. 3.4 Fundus photograph and optical coherence tomography (OCT) of a patient with intermediate dry age-related macular degeneration. (**a**) Fundus photograph using Optos (Optos PLC, Dunfermline, Scotland) of the left eye reveals large drusen in the posterior pole. (**b**) OCT of the left eye using Heidelberg SPECTRALIS (Heidelberg Engineering, Heidelberg, Germany) reveals large drusenoid pigment epithelium detachments

They are clinically classified into either hard drusen, soft drusen, or reticular pseudo-drusen. In early AMD, they are frequently found as clusters within the macular region (Fig. 3.3). Intermediate stage AMD is defined as the presence of extensive intermediate size (63–125 μm) drusen in the macula, or one large drusen (>125 μm) within 3000 μm of the foveal center (Fig. 3.4).

Types of Drusen

Hard drusen are small (less than 63 μm), yellowish punctuated deposits. They are globular in shape and stain with periodic acid-Schiff. They are not specific to AMD and are common in elderly patients even without AMD. The presence of a few small hard drusen is not an important risk factor for developing AMD.

Soft drusen are larger, paler, and more diffuse with blurry margins, and signify early AMD. They often represent localized accumulations of BlinD in the presence or absence of diffuse thickening of the inner aspect of Bruch's membrane [23]. Soft drusen are focal manifestations of a diffuse process; when there is diffuse BlamD, they may form focal accumulations that are represented as soft drusen. Through activated complement deposition in Bruch's membrane, soft drusen play a role in increased vascular endothelial growth factor (VEGF) production by RPE cells [24]. Large, soft, bilateral, and numerous drusen are significant risk factors for developing advanced AMD. The larger drusen with more RPE pigmentary changes seen in the macula confer a higher risk of progression to late AMD [25].

Reticular pseudo-drusen consist of an accumulation of extracellular debris between the apical processes of the RPE and the inner and outer segments of the photoreceptors [26]. They have been associated with a 4–8 greater risk of 5-year progression to late AMD compared to eyes with only drusen [27]. They were first described in 1990 as an outer macular yellow interlacing pattern with a 100 μm diameter that did not fluoresce on fluorescein angiogram, but instead had enhanced visibility in blue light [28]. The Wisconsin Age-Related Maculopathy grading scheme later described them as "ill-defined networks of broad interlacing ribbons" on color fundus photographs [29]. The term reticular pseudo-drusen was later introduced, described as a "yellow interlacing network 125–250 μm wide, appearing first in the superior outer macula and extending circumferentially and beyond" [30]. Curcio later named these deposits as subretinal drusenoid deposits, as spectral domain optical coherence tomography scans showed hyper-reflective deposits internal to the RPE [31]. Reticular pseudo-drusen share several common components with drusen, such as membranous debris, vitronectin, CFH, and apolipoprotein [32], but have a higher concentration of unesterified cholesterol compared to drusen. However, they do not contain opsins, glial fibrillary acid protein, or RPE marker proteins which are found in soft drusen [31]. Subretinal drusenoid deposits demonstrate the presence of complement and complement regulators [33]. Reticular pseudo-drusen are also present in other diseases such as pseudoxanthoma elasticum and Sorsby fundus dystrophy. Thickening of Bruch's membrane and pathologic changes to the Bruch's-RPE interface have been thought to play an important role in the pathogenesis of reticular pseudo-drusen [34, 35]. Reticular pseudo-drusen are dynamic structures that expand, enlarge, and/or regress over time. In early stages of AMD, they are usually located in the superior part of the macula between the superior temporal arcade and the fovea. The growth of these deposits becomes more rapid in advanced AMD, specifically in geographic atrophy [36].

Pathogenesis of Drusen

Several theories for the pathogenesis of drusen in early AMD have been proposed. Accumulation of BlinD may form a continuous layer of soft drusen which is sometimes referred to as diffuse drusen [23]. Autophagy, the process by which dysfunctional cellular components are degraded, may also play a role in early AMD pathogenesis. RPE cells exhibit reduced capacity for autophagy in AMD [37]. As the RPE is nondividing tissue, it may lead to accumulation of lipofuscin within RPE cells. Macrophage scavenging may also be impaired in AMD, resulting in decreased removal

of membranous debris. [38]. AMD donor eyes were found to contain markers for autophagy and exosomes, which suggests an increase in autophagy and intracellular protein release via exosomes by RPE as a contributor to drusen formation [39]. Apoptosis may also play a role in AMD-associated RPE and photoreceptor cell death [40]. The RPE-Bruch's membrane-choriocapillaris complex is predisposed to continuous oxidative stress, especially in the macular region [41]. Nuclear factor erythroid-2 related factor 2 (Nrf2), for example, is known to protect the RPE cells from oxidative injury [41], and smoking has been shown to suppress its upregulation [42]. Oxidative damage is thought to incite an inflammatory process, termed para-inflammation [43], which is in part mediated by the complement activation pathway at the level of RPE-Bruch's membrane-choriocapillaris [44].

Constituents of Drusen

Protein

AMD pathogenesis and drusen formation are likely multifactorial. Tissue metalloproteinase inhibitor 3, clusterin, vitronectin, and serum albumin are common drusen proteins, detectable in up to 80% of tissues in normal drusen and 60% of drusen in AMD eyes [45]. Clusterin and vitrenectin are complement pathway regulators. The identification of these and other complement system proteins (e.g., complement component 5 (C5), the membrane attack complex (MAC) containing C5b-9, and others) in drusen, coupled with genetic association studies, suggest a potential role of the complement pathway in AMD pathogenesis (please see Sect. 9.3: The Complement System) [24, 45–48]. Amyloid beta has also been identified in drusen and studies suggest that amyloid beta reduces complement factor I function, leading to chronic low-grade inflammation [49]. Moreover, protein modifications from lipoxidation and glyoxidation have been identified in drusen of AMD patients, suggesting a role for oxidative stress in AMD pathogenesis. [45]

Lipid

A variant of the hepatic lipase gene, Lipase C hepatic type (LIPC), has been found to have a significant genetic association with AMD [50]. Above, we briefly mentioned the Bruch's membrane changes in AMD that were thought to relate to changes in the vascular intima leading to the pathogenesis of atherosclerotic disease [14]. These associations were strengthened with similarities found in the protein molecular composition of drusen and atherioclerotic deposits [46]. Both conditions were found to have apolipoprotein B and cholesterol accumulation, with subsequent modification, oxidation, and aggregation. Given these associations, it was initially hypothesized that statins may affect AMD status and progression [51]. Statins are inhibitors of HMG-CoA reductase (the enzyme catalyzing the rate limiting step in cholesterol biosynthesis) and therefore suppress cholesterol synthesis. They also increase levels of liver LDL receptors, reduce apolipoprotein B synthesis, and suppress prenylation. Studies with AMD and statin use varied, and a 2015 Cochrane report concluded that there was insufficient evidence to conclude if statins have a role in the onset or progression of AMD [52]. A randomized, placebo-controlled study suggested that a daily 40 mg of simvastatin may slow the progression of early and intermediate AMD, especially with the CC genotype of the Y402H genotype of CFH. In an open-label prospective multicenter pilot study, 23 patients with large, soft drusenoid deposits were given 80 mg of atorvastatin daily for a year. Ten of those patients showed regression of drusen deposits on imaging with an associated vision gain of 3.3 letters [53].

Another study found an increased risk of neovascular AMD in patients with elevated plasma lipid levels with statin use for more than a year [54]. The authors however postulated that the risk was due to the patients' resistance to the statin treatment rather than the statins themselves causing the neovascular AMD.

RPE Alterations in AMD

Two of the common pigmentary changes seen in early AMD are RPE mottling and clumping. Pigment mottling consists of RPE attenuation, depigmentation, hypertrophy, hyperplasia, and eventual atrophy [18], while RPE clumping refers to the accumulation of pigmented cells in the subretinal space. In a study of three-dimensional OCT scans, AMD patients showed intraretinal RPE migration on OCT imaging. These areas of RPE migration corresponded with RPE pigment clumping on fundus photography. These areas of RPE clumping also showed a high incidence of underlying drusen, suggesting drusen might play a physical and catalytic role in facilitating RPE migration and the appearance of clumping [55].

3.5 Histopathology in Advanced Dry AMD with Geographic Atrophy

Advanced dry AMD is characterized by geographic atrophy, which are well-demarcated areas of confluent RPE atrophy through which underlying choroidal vessels are visible (Fig. 3.5). Geographic atrophy is characterized histologically by loss of overlying outer layers of neurosensory retina and underlying choriocapillaris (Fig. 3.6). Bruch's membrane may exhibit changes such as erosion of intercapillary pillars, but does not typically have breaks, which are the precursor to neovascular AMD [22, 56–58]. Macrophages have been reported to be the most prominent inflammatory cell type present in AMD, and have been found to be associated with drusen and areas of geographic atrophy [58]. Other cell types like melanocytes, fibroblasts, and multinucleated giant cells have likewise been reported to be associated with AMD [58].

3.5.1 Drusen and RPE Abnormalities

Drusen size and extent, along with the presence of RPE abnormalities, determine the risk of progression to geographic atrophy. However, although considered to be central to the initiation of RPE cell loss, drusen may disappear over time as AMD progresses, especially when geographic atrophy occurs [59–61].

RPE abnormalities that may precede more advanced geographic atrophy include hypopigmented areas of atrophy and areas of focal hyperpigmentation [59]. RPE abnormalities are often found on the anterior surface of drusen, but may also occur independently of drusen. As areas of discrete drusen and RPE abnormalities develop and increase in extent, adjacent areas may coalesce and form larger confluent patches. Retrospective analysis of fundus images prior to

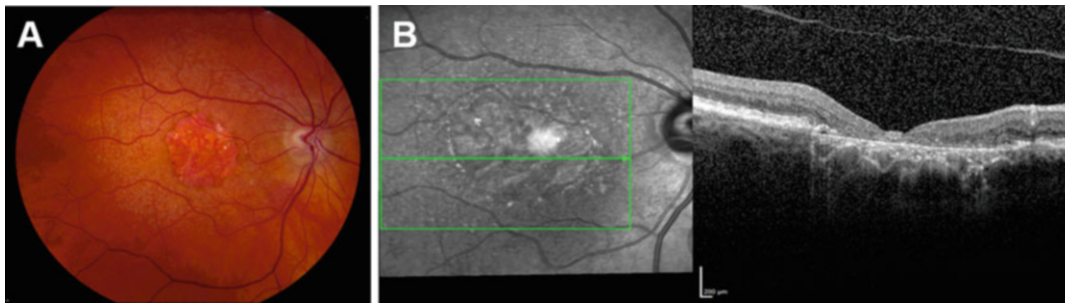

Fig. 3.5 Fundus photograph and optical coherence tomography (OCT) of a patient with advanced dry age-related macular degeneration with geographic atrophy. (**a**) Fundus photograph using Optos (Optos PLC, Dunfermline, Scotland) of the right eye reveals a geographic patch of hypopigmentation with visible underlying choroidal vessels. (**b**) OCT of the right eye using Heidelberg SPECTRALIS (Heidelberg Engineering, Heidelberg, Germany) reveals outer retinal and retinal pigment epithelium atrophy with adjacent drusen

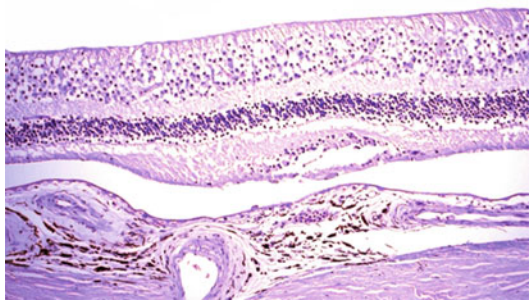

Fig. 3.6 Photomicrograph of a geographic AMD eye showing the macular lesion with total loss of RPE and mostly photoreceptor cells with a few remaining photoreceptor nuclei (asterisk) in the ONL (hematoxylin & eosin (H&E), original magnification, ×100)

the development of initial geographic atrophy revealed several retinal precursor lesions and a clinical sequence of events leading to the development of geographic atrophy [62]. This clinical sequence of events has been reported to begin with the development of large drusen (Fig. 3.4) and RPE hyperpigmentation, followed by regression of drusen, appearance of RPE hypopigmentation, loss of RPE, and ultimately by the development of geographic atrophy (Fig. 3.5). Geographic atrophy that develops from this evolution of RPE abnormalities often begins in the parafoveal region. Additionally, geographic atrophy may develop in association with drusenoid pigment epithelial detachments (PEDs), and independently from drusen in areas of RPE abnormalities. Progression and enlargement of geographic atrophy to involve the fovea typically results in precipitous decline of visual acuity.

3.5.2 Pathogenesis of Geographic Atrophy

The underlying mechanisms that lead to the eventual loss of the RPE and photoreceptors have not been fully elucidated. An exacerbated oxidative stress response is believed to occur, and treatment with antioxidants and omega-3 fatty acids has been touted as possible methods of maintaining RPE function. Healthy RPE function is known to maintain photoreceptor homeostasis, and when RPE function is compromised, photoreceptor damage and atrophy characteristic of AMD may occur (Fig. 3.6). Nevertheless, loss of photoreceptors, particularly rods, have been observed to occur prior to the development of RPE dysfunction [63]. The hypothesis that rod cell death occurs prior to RPE dysfunction is supported by the observation that greatest RPE and photoreceptor cell loss occurs in the parafoveal region, where rod density predominates [22]. It has been proposed that subclinical loss of rods without overt evidence of RPE disease may be the earliest manifestation of AMD, resulting in atrophy that encircles the fovea. Eventually, in susceptible individuals, RPE dysfunction exacerbates rod loss and cone loss begins.

3.5.3 Lipofuscin Accumulation

The role of lipofuscin accumulation in RPE cells further illustrates the close relationship between photoreceptor and RPE dysfunction. Lipofuscin is generated as a byproduct from the oxidation of unsaturated fatty acids, and enters the RPE through phagocytosis. Lipofuscin-containing phagosomes combine with lysosomes and are digested within the RPE. Abnormal molecular degradation of lipofuscin, as well as autophagy within non-dividing RPE cells, eventually leads to its accumulation over time, which may reduce RPE function and result in cell death [64]. A2E, a lipofuscin fluorophore internalized by RPE cells, has been shown to mediate membrane damage [65], and have the potential to induce activation of the complement system [66–68].

3.6 Histopathology in Choroidal Neovascularization

Choroidal neovascularization is the hallmark of neovascular AMD. It occurs as a growth of new choroidal blood vessels, and may extend through the RPE via breaks in Bruch's membrane (Fig. 3.7). These fragile new vessels are prone to

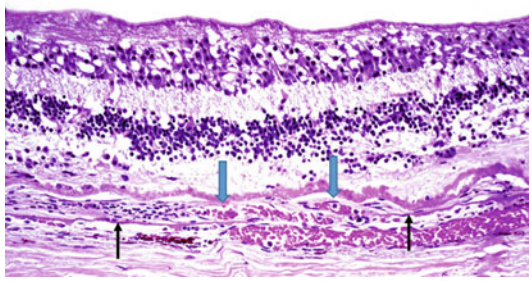

Fig. 3.7 Photomicrograph of a neovascular AMD eye showing the macular lesion with subretinal fibrovascular tissue containing many small vessels (thick blue arrows) through the broken Bruch's membrane (thin black arrows), most photoreceptor cells and RPE cells are missing (hematoxylin & eosin (H&E), original magnification, ×200)

leakage of intravascular contents due to the lack of barrier function present in mature vascular endothelial cells. Retinal edema from fluid, exudates from the deposition of proteins and lipids, and hemorrhage from erythrocytes may occur secondary to extravasation of intravascular contents. Less commonly, massive subretinal hemorrhage or breakthrough vitreous hemorrhage may occur, severely impairing visual function.

Decreased thickness and disruption of the elastic lamina of the Bruch's membrane may precede choroidal neovascularization of the sub-RPE space [69]. Calcification and breaks of the Bruch's membrane correlate with the presence of neovascular AMD [70]. Breaks in Bruch's membrane provide conduits that allow choroidal vessels to traverse the membrane into the sub-RPE space. Besides vascular endothelium, choroidal neovascular tissue consists of both cellular and extracellular components such as RPE, macrophages, lymphocytes, erythrocytes, fibrocytes, myofibroblasts, collagen, fibrin, and BlamD [71, 72].

3.6.1 Pro-Angiogenic Factors

A variety of components of choroidal neovascular tissue suggest a multifactorial etiology in its pathogenesis, with inflammatory and pro-angiogenic components contributing to its formation. Macrophages and lymphocytes have been reported to potentially have a role in the promotion of breaks in Bruch's membrane, induction of choroidal neovascularization, and increasing exudation of intravascular contents in new vessels [73]. Angiogenic factors such as vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) contribute to the neovascular process, and are pharmaceutical targets for the treatment of neovascular AMD.

3.6.2 Pigment Epithelial Detachments and Types 1/2/3 Neovascularization

Pigment epithelial detachments (PEDs) occur when the RPE separates from the underlying Bruch's membrane due to the presence of drusen, serous exudate, blood, or neovascular membrane [74]. On OCT imaging, drusenoid PEDs exhibit moderate hyper-reflectivity, serous PEDs appear hypo-reflective, and a mixed pattern of reflectivity may be seen in fibrovascular PEDs. Coalescence of soft drusen results in the formation of drusenoid PEDs (Fig. 3.4). Soft drusen may indirectly promote angiogenesis via macrophages [75], and are additionally implicated in increased VEGF production by RPE cells [24]. Serous PEDs can occur in the context of a non-neovascular process, but are often associated with an area of CNV underlying an intact Bruch's membrane in AMD. Active leakage of fluid has been postulated to increase hydrostatic pressure, leading to RPE separation from the inner collagenous layer of Bruch's membrane.

Fibrovascular PED, or type 1 neovascularization, occurs when neovascular tufts break through Bruch's membrane and extend laterally under the RPE (Fig. 3.7). Type 1 neovascularization is believed to be the predominant process corresponding to "occult" CNV as observed on fluorescein angiography. The horizontal growth of neovascular tissues is facilitated by a cleavage plane between BlamD and Bruch's membrane that contains accumulated lipids. Intravascular leakage from the type 1 choroidal neovascularization can result in serous or

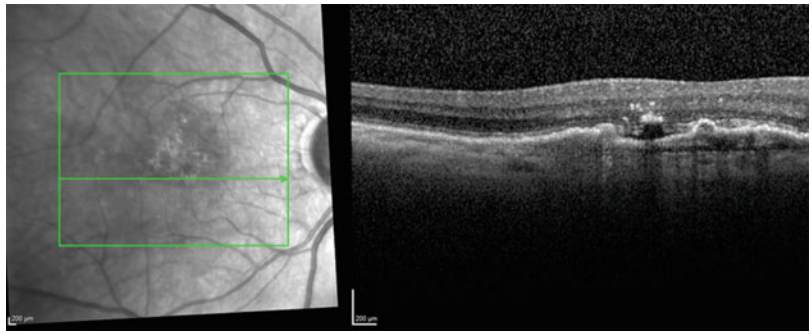

Fig. 3.8 Optical coherence tomography (OCT) of the right eye using Heidelberg SPECTRALIS (Heidelberg Engineering, Heidelberg, Germany) of a patient with mild wet age-related macular degeneration reveals a fibrovascular pigment epithelial detachment consistent with type 1 choroidal neovascular membrane with associated subretinal fluid

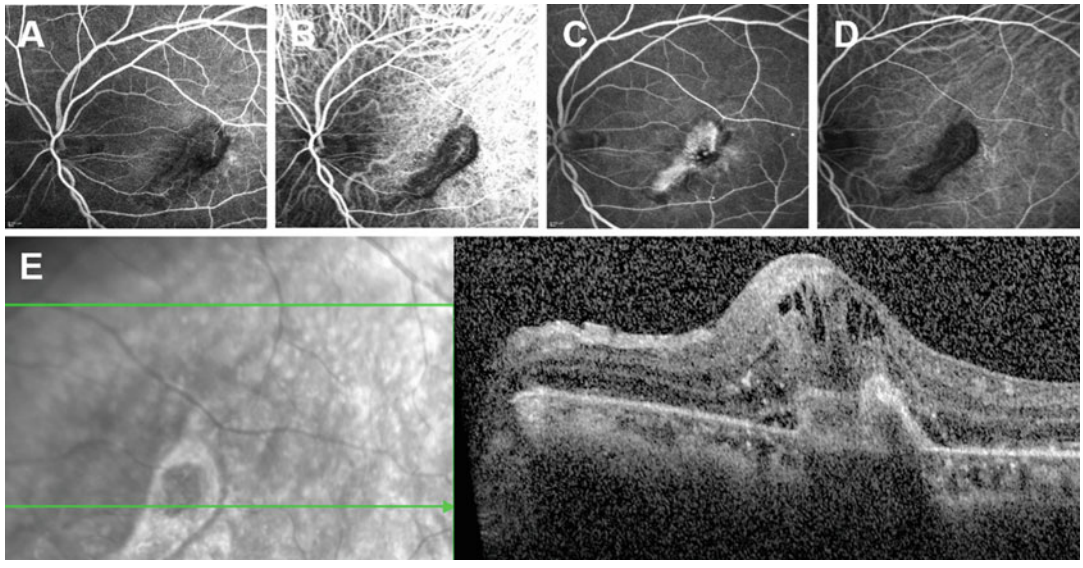

Fig. 3.9 Early and late phase fluorescein angiography (FA) and indocyanine green angiography (ICGA) along with optical coherence tomography (OCT) using Heidelberg SPECTRALIS (Heidelberg Engineering, Heidelberg, Germany) of a patient with advanced wet age-related macular degeneration. (**a**, **b**) Early phase and late (**c**, **d**) FA (**a**, **c**, respectively) ICGA (**b**, **d** respectively) of the left eye show a lacy net of hyperfluorescence with leakage consistent with choroidal neovascular membrane. (**e**) OCT reveals choroidal neovascular membrane with associated intraretinal and subretinal fluid

hemorrhagic PED components. Overlying retina is often intact, with comparably less visual symptoms, but secondary leakage of fluid, blood, or lipids into the retina can result in alteration of retinal structure (Fig. 3.8).

Type 2 neovascularization is located between the neurosensory retina and the RPE, and corresponds to the "classic" CNV appearance on angiography with leakage both under the RPE and into the outer retina (Fig. 3.9). Separation

from the RPE results in atrophy of photoreceptors and other outer layers of the neurosensory retina. Histologically, a reflected layer of inverted proliferating RPE onto the outer surface of type 2 choroidal neovascular membrane (CNVM) is present [71]. Inverted RPE cells may also be present on the inner surface of type 2 CNVM, and occur in association with a similar layer of RPE cells on the external surface of the CNVM [71].

Combined type 1 and type 2 choroidal neovascular growth patterns confer varying degrees of representation of each, resulting in "minimally classic" or "predominantly classic" appearances on angiography [76].

Type 3 neovascularization or retinal angiomatous proliferation (RAP) was described as a type of neovascular process that begins in the retina and not in the choroid, unlike type 1 and 2 neovascularization [77, 78]. Little information on the histopathology of type 3 neovascularization exists, but through clinical observation and optical coherence tomography findings, it reportedly occurs in three stages: (1) an intraretinal neovascular stage arising from the deep capillary plexus of the retina in the paramacular area, (2) a subretinal neovascular stage that may precipitate neurosensory retina and RPE detachments, and (3) choroidal neovascularization with the formation of retino-choroidal anastamoses [77].

3.6.3 Polypoidal Choroidal Vasculopathy

Polypoidal choroidal vasculopathy (PCV) is a distinct variant of AMD from which the primary abnormality lies within the choroidal vasculature [79]. PCV is characterized by an inner choroidal network of vessels that terminate in an aneurysmal bulge, resembling a polyp. Histopathologically, these vessels are located in the sub-RPE space and have been reported to exhibit extensive exudative changes and hyalinization of vessels [80, 81]. Inflammatory cells and positive expression of VEGF have been reported in cases of PCV [80]. Gross dilatation of the choroidal venules and capillaries in the sub-RPE neovascular membrane leads to the characteristic polyp structures, a unique clinical feature in PCV [82]. Recently, PCV pathogenesis is thought to occur through an initial stage mediated by proteolytic degradation of extracellular matrix protein by increased HTRA1 activity and a progression stage driven by inflammatory cascades [83].

Currently, common treatment modalities for PCV include intravitreal anti-VEGF monotherapy [84, 85], indocyanine green angiography-guided photodynamic therapy (PDT) with verteporfin [86, 87] and combined anti-VEGF and PDT [88]. Intravitreal anti-VEGF treatment can effectively reduce intraretinal and subretinal fluid in patients with PCV. In some patients however, choroidal vascular changes may persist, despite intravitreal anti-VEGF treatment [84]. PDT with verteporfin induces local choroidal vascular changes that eventually lead to the thrombosis and regression of PCV lesions. Although PDT has been shown to be effective, it may be associated with complications such as post-PDT subretinal hemorrhage and suprachoroidal hemorrhage [89, 90]. Combination therapy with anti-VEGF and PDT may lead to thrombosis of PCV lesions and regression of associated neovascularization via different mechanisms, and may be especially useful in refractory cases of PCV [88].

3.6.4 RPE Tears or Rips

Intravitreal anti-VEGF therapy has revolutionized the treatment of numerous retinal vascular diseases including AMD. Anti-VEGF pharmacotherapy is particularly effective in improving best corrected visual acuity by decreasing intraretinal and subretinal fluid. However, even though PEDs have been shown to improve with anti-VEGF pharmacotherapy, they are often resistant to anti-VEGF therapy. RPE tears or rips are also recognized as potential complications of anti-VEGF therapy [91, 92]. RPE tears are rare, but are potentially devastating to vision, and may also occur spontaneously or after photodynamic therapy [93, 94] or laser photocoagulation [93]. RPE tears lead to a zone of dehiscence of the RPE with

an adjacent scroll of retracted and irregular RPE monolayer. In AMD, RPE tears are most frequently associated with a vascularized PED, or type 1 neovascularization. It has been proposed that a type 1 neovascular membrane, tightly adherent to its surrounding structures, exerts a contractile effect on the undersurface of the RPE [95]. This contractile force is further increased after anti-VEGF therapy. Moreover, the increased hydrostatic force that contributed to the formation of these PEDs may lead to an acute rupture of the RPE, purportedly typically near the base of the PED. Nevertheless, even after an RPE tear occurs, continued monitoring for exudative changes warranting anti-VEGF therapy may stabilize VA, improve anatomical outcomes, reduce fibrosis, and decrease the risk of developing a large blinding end-stage exudative disciform scar [96].

3.7 Histopathology in Disciform Scarring of CNVM

End-stage neovascular AMD often progresses to a cicatricial stage with the formation of subretinal disciform scars, which are usually vascularized. Disciform scars are areas of fibrous tissue located within the Bruch's membrane, or between the RPE and retina (Fig. 3.10) [97, 98]. The location of the disciform scar with respect to the RPE, however, may become harder to determine due to destruction of the RPE over time. Fibroblasts, RPE hyperplasia, inflammatory cells, and small vessels are found within the fibrovascular tissue. Typically, there is loss of overlying retinal tissue, especially of the outer retinal cell layers, with corresponding visual impairment [99]. Particularly when surrounded by atrophy, disciform scars are generally considered clinically stable.

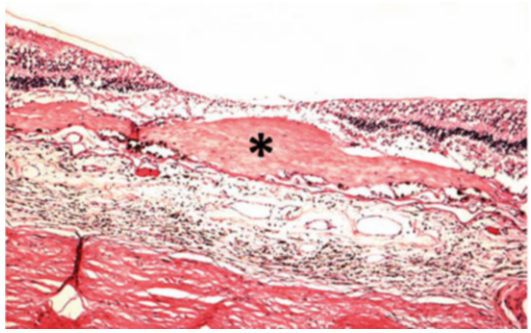

Fig. 3.10 Photomicrograph of an AMD eye with a large disciform scar of dense fibrous tissue in the central maculae (hematoxylin & eosin (H&E), original magnification, ×50)

3.8 Histopathology of Atrophy after Collapse of PED/CNVM as Compared to GA

Atrophy that occurs around disciform scars after the collapse of PEDs and CNVMs differs from atrophy associated with geographic atrophy [100]. Atrophy in geographic atrophy follows a distribution that corresponds to the presence of atrophic rod photoreceptors, drusen, and PEDs. Unlike disciform scars, geographic atrophy is usually confined to the macula, and does not commonly extend significantly further (Fig. 3.5). Atrophy in geographic atrophy is related to patient age, and generally has a later onset and slower progression than disciform scar-associated atrophy [100].

3.9 The Role of the Immune System in AMD

Histopathologically, immune cells have been found to be associated with numerous AMD lesions as detailed above, and play an important role in the pathogenesis of AMD.

3.9.1 Microglia

An extension of the brain, the retina contains many types of neurons and glial cells, including microglia. During development, microglia are distributed throughout the retina but localize to the inner layers as the retina reaches maturity [101]. Microglia form an important part of the retina's immune defense and response to tissue injury and more recently have been shown to also

be involved in the neural and vascular development of the retina [102–104]. Similarly to microglia in the brain, retinal microglia have been shown to express Toll-like receptors (TLRs) and Dectin-1 to recognize and promote clearance of infectious pathogens [105–107]. Activation of such receptors causes microglia to change shape, migrate from the inner to outer retinal layer, and secrete cytokines, chemokines, and neurotoxins [108, 109]. The ability of microglia to phagocytose dying neurons and remove cellular debris as a response to tissue injury has been well described in earlier studies [110, 111]. More recent studies have also shown that activation of TLR4 promotes phagocytosis of photoreceptor proteins and may contribute to retinal neurodegenerative diseases, including AMD [106].

Notably, microglia are absent from the outer layer and subretinal space in the healthy human eye [101]. With aging, however, microglia may migrate from the inner retinal layers to the subretinal space in older adults, resulting in an aberration of their usual distribution and numbers [112, 113]. In animal studies, aged microglia have been shown to have altered morphology, with reduced branching and shorter processes potentially compromising their ability to survey and interact with the surrounding environment and remove cellular debris. Functionally, they seem to have slower responses to tissue injury compared with younger microglia and synthesize excessive amounts of pro-inflammatory cytokines [113, 114]. It has additionally been shown that certain genes involved in neuroprotection are down-regulated in the aging retina [114], supporting a role for microglial senescence in retinal degenerative diseases such as AMD [115].

Consistent with this, activated microglia have been found in the outer retina and subretinal regions in patients with AMD, implying a potential pathogenic role for these cells [116]. In fact, in vivo studies have shown that activated microglia transplanted into the subretinal space caused displacement of additional microglia from the inner to outer layers, implying a potential positive feedback loop that promotes chronic neuroinflammation [117]. Indeed, results from in vitro studies have shown that activated microglia may have the ability to result in injury to healthy photoreceptor cells [118]. Recent studies in mice have supported these earlier findings and have shown that infiltrating retinal microglial cells secrete inflammatory mediators that contribute to the death of living photoreceptors [119, 120]. Genetic variants of the *CX3CR1* gene that produces the microglia chemokine receptor have been previously associated with an increased risk of AMD, and functional studies have suggested that inflammatory cells bearing this risk-conferring variant exhibit altered chemotaxis [121]. These data suggest that impaired microglial migration might contribute to AMD pathogenesis. Overall, dysregulation in migroglial distribution, morphology, and functionality seem to play a central role in the development of AMD.

3.9.2 Macrophages

Macrophages can be found in histological specimens of human AMD lesions, particularly in areas of choroidal neovascularization (CNV). They have similarly been noted in regions of RPE atrophy and breakdown of Bruch's membrane, suggesting a potential role in AMD pathogenesis [108]. Initial evidence implicating macrophages in the development of AMD comes from the studies of mice deficient in the macrophage chemoattractant protein CCL2/MCP-1, which exhibit decreased extravasation of monocytes from the circulatory system into the retina. In their study, Ambati et al. showed that mice with deficient CCL2-mediated macrophage recruitment exhibited AMD-like lesions, including geographic RPE atrophy, CNV, and drusen deposits, suggesting a protective role of macrophages in AMD [122]. Other earlier studies, however, have concluded the opposite, that decreasing CCL2-mediated macrophage recruitment to the retina lowers AMD risk [123], leaving it unclear whether macrophages accumulate near CNV lesions in a causative role or if they serve as an adaptive response in the pathogenesis of AMD.

One possibility is that macrophages play a complex role with both an anti-inflammatory and pro-inflammatory actions. The phenotypic plasticity of macrophages to be polarized into M1 and M2 subsets is well recognized and may contribute to an explanation for these findings. M1 macrophages are generally proinflammatory and secrete M1 chemokines, such as CXCL10. On the other hand, the M2 subset is less inflammatory, facilitating tissue repair and neovascularization, and secreting M2 chemokines, such as CCL2 [108]. In a pilot study of patients with and without AMD, Cao et al. found that increased levels of chemokines from both M1 and M2 subsets were present in AMD eyes as compared to eyes without AMD, suggesting an overall increase in both subsets of macrophage infiltration into the retina in AMD. When comparing eyes with the wet and dry forms of AMD, they found that eyes with the dry form had a greater expression of M1 cytokines, whereas those with the wet form had greater M2 cytokine expression. The authors speculate that the M1 macrophages might be implicated in AMD pathogenesis in the early stages with the M2 subset initially playing a protective role, with an eventual shift to M2 macrophage-induced fibrosis and angiogenesis in the later course of the disease [124]. In support of these findings, Yang et al. found that in mouse models of laser-induced CNV, M1-related markers were transiently upregulated in the early stages with a sustained M2 response in the later stages, leading them to conclude that the M2 subset likely plays a more important role in the development of CNV [125].

Recent studies have also suggested that age-related changes in macrophage function may contribute to numerous diseases of aging, including AMD. Lin et al. showed that miR-50, a microRNA found to be upregulated in macrophages from AMD patients, regulates macrophage-mediated inflammation and may mediate a switch from a healthy to a disease-promoting macrophage phenotype [126]. Indeed, the role of macrophages in the development and progression of AMD is likely multifaceted and may change throughout the disease process.

3.9.3 The Complement System

The complement system consists of over 40 proteins found in the systemic circulation and can be divided into three main pathways: the classical pathway triggered by antibody-antigen complexes, the lectin pathway triggered by polysaccharides (mannose and N-acetyl glucosamine) on microbial surfaces, and the alternative pathway triggered by binding to a host cell or pathogen surface. The function of this system is to create a proinflammatory response to provide defense against pathogens and remove apoptotic cells. It is well recognized that dysregulation of the complement system can lead to immune-mediated damage to host tissue. As such, it has been implicated in a wide spectrum of diseases. The complement system is continuously activated at low levels in the normal eye with tight regulation to maintain activity at a low level, providing microbial defense without host tissue damage [108].

Studies in human AMD eyes have suggested that the complement system may contribute to the pathogenesis of the disease. As noted above, complement components and regulators have been identified in drusen. One component of the system, complement factor H (CFH), seems to play a particularly important role in the development of AMD in both animal and human studies. CFH is known to be expressed in the human eye and acts as a negative regulator of the complement system, impairing activation of the alternative pathway. Impairments in CFH function might therefore result in overactivation of the alternative pathway and a proinflammatory state CFH was the first complement protein to be described in the pathogenesis of AMD following a functional variation in the *CFH* gene that was implicated in AMD in 2005 [47, 127–129]. CFH was also found to accumulate in drusen, RPE cells, sub-RPE cells, the inter-photoreceptor matrix, and the choroid [130]. A large population cohort (Beavers Dam Study) found that *CFH* gene polymorphisms were associated with macular pigmentary irregularities [131]. The Y402H missense variant in the *CFH* gene has been

associated with the presence of soft drusen in an Icelandic population [132], and has also been associated with the presence of bilateral intermediate-to-large drusen [133]. Homozygous individuals with the Y402H variant were also found to have more central and peripheral drusen, although there was no association with drusen size, location, or total area in this study [134]. In another study of 1107 subjects, the Y402H single nucleotide polymorphism was associated with peripheral drusen, while no known AMD-related polymorphisms were associated with the presence of 20 or more small hard drusen [135]. Studying the pathophysiology of the complement pathway and the genetics behind the *CFH* gene variations is important in understanding potential mechanisms for treatment of early AMD.

Animal studies similarly support the role of CFH in AMD pathogenesis. Using CFH knockout mouse models *($cfh^{-/-}$)* Coffey et al. demonstrated that $cfh^{-/-}$ mice had decreased visual acuity and impaired photoreceptor function compared with age-matched controls, suggesting a critical role of CFH for the health of the retina [136]. Similarly, there are variants in complement factor 3 (C3) that reduce its binding affinity to CFH and are associated with the development of AMD [137]. Rare variants in CFI, a factor that converts C3b and C4b to their inactive forms, have also been reported in association with the disease. These variants have been shown to result in diminished CFI production, interfering with the ability to regulate the alternative pathway leading to complement over activation [138]. Geerlings et al. showed that carriers of a C9 variant previously associated with an elevated risk for AMD have elevated concentrations of C9 in serum as compared to non-carriers. They hypothesize that increased C9 level results in elevated complement activation in patients with AMD, which may directly contribute to retinal destruction through lysis of target cells [139].

Given evidence supporting the role of the alternative pathway in AMD pathogenesis, more recent studies have investigated the effect of complement inhibition for the treatment of geographic atrophy (GA) associated with dry AMD. The monoclonal antibody lampalizumab, an inhibitor of factor D, which in turn is an activator of the alternative pathway upstream of CFH, was evaluated in the phase III CHROMA and SPECTRI studies. Both studies failed to meet their primary endpoints of reduction in the mean change in GA size [140]. The phase II FILLY trial investigated APL-2 for the treatment of GA associated with dry AMD. This compound inhibits C3, which plays a central role in all three complement pathways, potentially having a stronger anti-inflammatory effect in comparison to lampalizumab, which inhibits only the alternative pathway. Results from this trial showed a 29% reduction in growth of GA lesions at 12 months in the monthly treatment group (ClinicalTrials.gov Identifier: NCT02503332). As of the writing of this chapter, a phase III trial of APL-2 has begun in 2018. It is clear that the complement system, particularly the alternative pathway, plays a role in AMD pathogenesis. Future studies are needed to explore how selective targeting of this system may offer novel therapeutic strategies for AMD.

3.10 Conclusion

Aging is associated with a number of histological changes in the choroid, Bruch's membrane, RPE, and neuroretina. Outside of the normal physiologic aging spectrum of changes, abnormal deposits such as BlamD, BlinD, and soft drusen are known to be associated with AMD. Progression of AMD to advanced stages involving GA, CNVM, and/or disciform scar can result in debilitating vision loss.

Knowledge of the angiogenic pathway and its components that stimulate neovascularization has led to the development of a new paradigm of intravitreal anti-VEGF pharmacotherapy in the management of neovascular AMD. Currently however, there are no available treatments for the modification of disease progression in non-neovascular AMD, or for the treatment of

geographic atrophy. Further understanding of the histopathology of AMD and the molecular mechanisms that contribute to pathogenesis of the disease may reveal additional therapeutic targets.

References

1. Gupta MP, Herzlich AA, Sauer T, Chan CC (2016) Retinal anatomy and pathology. Dev Ophthalmol 55:7–17
2. Nag TC, Wadhwa S (2012) Ultrastructure of the human retina in aging and various pathological states. Micron 43:759–781
3. Masland RH (2012) The neuronal organization of the retina. Neuron 76:266–280
4. Chirco KR, Sohn EH, Stone EM et al (2017) Structural and molecular changes in the aging choroid: implications for age-related macular degeneration. Eye 31:10–25
5. Subhi Y, Forshaw T, Sørensen TL (2016) Macular thickness and volume in the elderly: a systematic review. Ageing Res Rev 29:42–49
6. Masland RH (2011) Cell populations of the retina: the proctor lecture. Invest Opthalmol Vis Sci 52:4581
7. Masland RH (2001) The fundamental plan of the retina. Nat Neurosci 4:877–886
8. Booij JC, Baas DC, Beisekeeva J et al (2010) The dynamic nature of Bruch's membrane. Prog Retin Eye Res 29:1–18
9. Ardeljan D, Chan C-C (2013) Aging is not a disease: distinguishing age-related macular degeneration from aging. Prog Retin Eye Res 37:68–89
10. Ramrattan RS, van der Schaft TL, Mooy CM et al (1994) Morphometric analysis of Bruch's membrane, the choriocapillaris, and the choroid in aging. Invest Ophthalmol Vis Sci 35:2857–2864
11. Grossniklaus HE, Nickerson JM, Edelhauser HF et al (2013) Anatomic alterations in aging and age-related diseases of the eye. Invest Ophthalmol Vis Sci 54: ORSF23–ORSF27
12. Curcio CA (1999) Basal linear deposit and large Drusen are specific for early age-related maculopathy. Arch Ophthalmol 117:329
13. Curcio CA, Millican CL, Bailey T, Kruth HS (2001) Accumulation of cholesterol with age in human Bruch's membrane. Invest Ophthalmol Vis Sci 42:265–274
14. Sivaprasad S, Bailey TA, Chong VNH (2005) Bruch's membrane and the vascular intima: is there a common basis for age-related changes and disease? Clin Exp Ophthalmol 33:518–523
15. van der Schaft TL, Mooy CM, de Bruijn WC et al (1992) Histologic features of the early stages of age-related macular degeneration. A statistical analysis. Ophthalmology 99:278–286
16. Panda-Jonas S, Jonas JB, Jakobczyk-Zmija M (1996) Retinal pigment epithelial cell count, distribution, and correlations in Normal human eyes. Am J Ophthalmol 121:181–189
17. Nussenblatt RB, Ferris F 3rd (2007) Age-related macular degeneration and the immune response: implications for therapy. Am J Ophthalmol 144:618–626
18. Green WR, Enger C (1993) Age-related macular degeneration histopathologic studies. The 1992 Lorenz E. Zimmerman lecture. Ophthalmology 100:1519–1535
19. van der Schaft TL, Mooy CM, de Bruijn WC et al (1994) Immunohistochemical light and electron microscopy of basal laminar deposit. Graefes Arch Clin Exp Ophthalmol 232:40–46
20. Kliffen M, van der Schaft TL, Mooy CM, de Jong PT (1997) Morphologic changes in age-related maculopathy. Microsc Res Tech 36:106–122
21. Malek G, Li C-M, Guidry C et al (2003) Apolipoprotein B in cholesterol-containing drusen and basal deposits of human eyes with age-related maculopathy. Am J Pathol 162:413–425
22. Sarks SH (1976) Ageing and degeneration in the macular region: a clinico-pathological study. Br J Ophthalmol 60:324–341
23. Bressler NM, Silva JC, Bressler SB et al (1994) Clinicopathologic correlation of drusen and retinal pigment epithelial abnormalities in age-related macular degeneration. Retina 14:130–142
24. Nozaki M, Raisler BJ, Sakurai E et al (2006) Drusen complement components C3a and C5a promote choroidal neovascularization. Proc Natl Acad Sci U S A 103:2328–2333
25. Klein R, Klein BEK, Tomany SC et al (2002) Ten-year incidence and progression of age-related maculopathy: the Beaver Dam Eye study1 1The authors have no proprietary interest in the products or devices mentioned herein. Ophthalmology 109:1767–1779
26. Zweifel SA, Spaide RF, Curcio CA et al (2010) Reticular pseudodrusen are subretinal drusenoid deposits. Ophthalmology 117:303–312.e1
27. Joachim N, Mitchell P, Rochtchina E et al (2014) Incidence and progression of reticular drusen in age-related macular degeneration: findings from an older Australian cohort. Ophthalmology 121:917–925
28. Mimoun G, Soubrane G, Coscas G (1990) Macular drusen. J Fr Ophtalmol 13:511–530
29. Klein R, Davis MD, Magli YL et al (1991) The Wisconsin age-related maculopathy grading system. Ophthalmology 98:1128–1134
30. Arnold JJ, Sarks SH, Killingsworth MC, Sarks JP (1995) Reticular pseudodrusen. A risk factor in age-related maculopathy. Retina 15:183–191
31. Curcio CA, Messinger JD, Sloan KR et al (2013) Subretinal drusenoid deposits in non-neovascular age-related macular degeneration: morphology,

prevalence, topography, and biogenesis model. Retina 33:265–276
32. Hageman GS, Luthert PJ, Victor Chong NH et al (2001) An integrated hypothesis that considers drusen as biomarkers of immune-mediated processes at the RPE-Bruch's membrane interface in aging and age-related macular degeneration. Prog Retin Eye Res 20:705–732
33. Rudolf M, Malek G, Messinger JD et al (2008) Sub-retinal drusenoid deposits in human retina: organization and composition. Exp Eye Res 87:402–408
34. Gliem M, Hendig D, Finger RP et al (2015) Reticular pseudodrusen associated with a diseased bruch membrane in pseudoxanthoma elasticum. JAMA Ophthalmol 133:581–588
35. Gliem M, Müller PL, Mangold E et al (2015) Reticular Pseudodrusen in Sorsby fundus dystrophy. Ophthalmology 122:1555–1562
36. Steinberg JS, Göbel AP, Fleckenstein M et al (2015) Reticular drusen in eyes with high-risk characteristics for progression to late-stage age-related macular degeneration. Br J Ophthalmol 99:1289–1294
37. Hyttinen JMT, Błasiak J, Niittykoski M et al (2017) DNA damage response and autophagy in the degeneration of retinal pigment epithelial cells-implications for age-related macular degeneration (AMD). Ageing Res Rev 36:64–77
38. Schachat AP, Wilkinson CP, Hinton DR et al (2017) Ryan's retina E-Book. Elsevier Health Sciences, New York
39. Wang AL, Lukas TJ, Yuan M et al (2009) Autophagy and exosomes in the aged retinal pigment epithelium: possible relevance to drusen formation and age-related macular degeneration. PLoS One 4:e4160
40. Dunaief JL (2002) The role of apoptosis in age-related macular degeneration. Arch Ophthalmol 120:1435
41. Zarbin MA (2004) Current concepts in the pathogenesis of age-related macular degeneration. Arch Ophthalmol 122:598
42. Cano M, Thimmalappula R, Fujihara M et al (2010) Cigarette smoking, oxidative stress, the anti-oxidant response through Nrf2 signaling, and age-related macular degeneration. Vis Res 50:652–664
43. Xu H, Chen M, Forrester JV (2009) Para-inflammation in the aging retina. Prog Retin Eye Res 28:348–368
44. Gehrs KM (2010) Complement, age-related macular degeneration and a vision of the future. Arch Ophthalmol 128:349
45. Crabb JW, Miyagi M, Gu X et al (2002) Drusen proteome analysis: an approach to the etiology of age-related macular degeneration. Proc Natl Acad Sci U S A 99:14682–14687
46. Mullins RF, Russell SR, Anderson DH, Hageman GS (2000) Drusen associated with aging and age-related macular degeneration contain proteins common to extracellular deposits associated with atherosclerosis, elastosis, amyloidosis, and dense deposit disease. FASEB J 14:835–846
47. Hageman GS, Anderson DH, Johnson LV et al (2005) A common haplotype in the complement regulatory gene factor H (HF1/CFH) predisposes individuals to age-related macular degeneration. Proc Natl Acad Sci U S A 102:7227–7232
48. Johnson LV, Leitner WP, Staples MK, Anderson DH (2001) Complement activation and inflammatory processes in Drusen formation and age related macular degeneration. Exp Eye Res 73:887–896
49. Wang J, Ohno-Matsui K, Yoshida T et al (2008) Altered function of factor I caused by amyloid : implication for pathogenesis of age-related macular degeneration from Drusen. J Immunol 181:712–720
50. Neale BM, Fagerness J, Reynolds R et al (2010) Genome-wide association study of advanced age-related macular degeneration identifies a role of the hepatic lipase gene (LIPC). Proc Natl Acad Sci U S A 107:7395–7400
51. Hall NF, Gale CR, Syddall H et al (2001) Risk of macular degeneration in users of statins: cross sectional study. BMJ 323:375–376
52. Gehlbach P, Li T, Hatef E (2015) Statins for age-related macular degeneration. Cochrane Database Syst Rev. https://doi.org/10.1002/14651858.CD006927
53. Vavvas DG, Daniels AB, Kapsala ZG et al (2016) Regression of some high-risk features of age-related macular degeneration (AMD) in patients receiving intensive statin treatment. EBioMedicine 5:198–203
54. VanderBeek BL, Zacks DN, Talwar N et al (2013) Role of statins in the development and progression of age-related macular degeneration. Retina 33:414–422
55. Ho J, Witkin AJ, Liu J et al (2011) Documentation of intraretinal retinal pigment epithelium migration via high-speed ultrahigh-resolution optical coherence tomography. Ophthalmology 118:687–693
56. Bressler NM, Bressler SB, Fine SL (1988) Age-related macular degeneration. Surv Ophthalmol 32:375–413
57. Green WR, Richard Green W, McDonnell PJ, Yeo JH (1985) Pathologic features of senile macular degeneration. Ophthalmology 92:615–627
58. Penfold PL, Killingsworth MC, Sarks SH (1985) Senile macular degeneration: the involvement of immunocompetent cells. Graefes Arch Clin Exp Ophthalmol 223:69–76
59. Sarks JP, Sarks SH, Killingsworth MC (1988) Evolution of geographic atrophy of the retinal pigment epithelium. Eye 2:552–577
60. Gass JD (1973) Drusen and disciform macular detachment and degeneration. Arch Ophthalmol 90:206–217
61. Sarks SH (1982) Drusen patterns predisposing to geographic atrophy of the retinal pigment epithelium. Aust N Z J Ophthalmol 10:91–97

62. Klein ML, Ferris FL 3rd, Armstrong J et al (2008) Retinal precursors and the development of geographic atrophy in age-related macular degeneration. Ophthalmology 115:1026–1031
63. Curcio CA, Medeiros NE, Millican CL (1996) Photoreceptor loss in age-related macular degeneration. Invest Ophthalmol Vis Sci 37:1236–1249
64. Dorey CK, Wu G, Ebenstein D et al (1989) Cell loss in the aging retina. Relationship to lipofuscin accumulation and macular degeneration. Invest Ophthalmol Vis Sci 30:1691–1699
65. Sparrow JR, Parish CA, Hashimoto M, Nakanishi K (1999) A2E, a lipofuscin fluorophore, in human retinal pigmented epithelial cells in culture. Invest Ophthalmol Vis Sci 40:2988–2995
66. Sparrow JR (2010) Bisretinoids of RPE lipofuscin: trigger for complement activation in age-related macular degeneration. Adv Exp Med Biol 703:63–74
67. Zhou J, Jang YP, Kim SR, Sparrow JR (2006) Complement activation by photooxidation products of A2E, a lipofuscin constituent of the retinal pigment epithelium. Proc Natl Acad Sci U S A 103:16182–16187
68. Zhou J, Kim SR, Westlund BS, Sparrow JR (2009) Complement activation by bisretinoid constituents of RPE lipofuscin. Invest Ophthalmol Vis Sci 50:1392–1399
69. Chong NHV, Keonin J, Luthert PJ et al (2005) Decreased thickness and integrity of the macular elastic layer of Bruch's membrane correspond to the distribution of lesions associated with age-related macular degeneration. Am J Pathol 166:241–251
70. Spraul CW (1997) Characteristics of Drusen and Bruch's membrane in postmortem eyes with age-related macular degeneration. Arch Ophthalmol 115:267
71. Grossniklaus HE, Gass JD (1998) Clinicopathologic correlations of surgically excised type 1 and type 2 submacular choroidal neovascular membranes. Am J Ophthalmol 126:59–69
72. Grossniklaus HE, Martinez JA, Brown VB et al (1992) Immunohistochemical and Histochemical properties of surgically excised subretinal neovascular membranes in age-related macular degeneration. Am J Ophthalmol 114:464–472
73. Penfold PL, Provis JM, Billson FA (1987) Age-related macular degeneration: ultrastructural studies of the relationship of leucocytes to angiogenesis. Graefes Arch Clin Exp Ophthalmol 225:70–76
74. Gass JD (1984) Pathogenesis of tears of the retinal pigment epithelium. Br J Ophthalmol 68:513–519
75. Killingsworth MC, Sarks JP, Sarks SH (1990) Macrophages related to Bruch's membrane in age-related macular degeneration. Eye 4 (Pt 4):613–621
76. Grossniklaus HE, Green WR (2004) Choroidal neovascularization. Am J Ophthalmol 137:496–503
77. Freund KB, Ho I-V, Barbazetto IA et al (2008) Type 3 neovascularization: the expanded spectrum of retinal angiomatous proliferation. Retina 28:201–211
78. Yannuzzi LA, Negrão S, Iida T et al (2001) Retinal angiomatous proliferation in age-related macular degeneration. Retina 21:416–434
79. Yannuzzi LA, Wong DW, Sforzolini BS et al (1999) Polypoidal choroidal vasculopathy and neovascularized age-related macular degeneration. Arch Ophthalmol 117:1503–1510
80. Terasaki H, Miyake Y, Suzuki T et al (2002) Polypoidal choroidal vasculopathy treated with macular translocation: clinical pathological correlation. Br J Ophthalmol 86:321–327
81. Nakashizuka H, Mitsumata M, Okisaka S et al (2008) Clinicopathologic findings in polypoidal choroidal vasculopathy. Invest Ophthalmol Vis Sci 49:4729–4737
82. Tso MOM, Suarez MJ, Eberhart CG (2018) Pathologic study of early manifestations of polypoidal choroidal vasculopathy and pathogenesis of choroidal neo-vascularization. Am J Ophthalmol Case Rep 11:176–180
83. Kumar S, Nakashizuka H, Jones A et al (2017) Proteolytic degradation and inflammation play critical roles in polypoidal choroidal vasculopathy. Am J Pathol 187:2841–2857
84. Gomi F, Sawa M, Sakaguchi H et al (2008) Efficacy of intravitreal bevacizumab for polypoidal choroidal vasculopathy. Br J Ophthalmol 92:70–73
85. Kokame GT, Yeung L, Lai JC (2010) Continuous anti-VEGF treatment with ranibizumab for polypoidal choroidal vasculopathy: 6-month results. Br J Ophthalmol 94:297–301
86. Chan W-M, Lam DSC, Lai TYY et al (2004) Photodynamic therapy with verteporfin for symptomatic polypoidal choroidal vasculopathy: one-year results of a prospective case series. Ophthalmology 111:1576–1584
87. Spaide RF, Donsoff I, Lam DL et al (2002) Treatment of polypoidal choroidal vasculopathy with photodynamic therapy. Retina 22:529–535
88. Koh A, Lee WK, Chen L-J et al (2012) EVEREST study: efficacy and safety of verteporfin photodynamic therapy in combination with ranibizumab or alone versus ranibizumab monotherapy in patients with symptomatic macular polypoidal choroidal vasculopathy. Retina 32:1453–1464
89. Hirami Y, Tsujikawa A, Otani A et al (2007) Hemorrhagic complications after photodynamic therapy for polypoidal choroidal vasculopathy. Retina 27:335–341
90. Ojima Y, Tsujikawa A, Otani A et al (2006) Recurrent bleeding after photodynamic therapy in polypoidal choroidal vasculopathy. Am J Ophthalmol 141:958–960
91. Gamulescu MA, Framme C, Sachs H (2007) RPE-rip after intravitreal bevacizumab (Avastin) treatment for

vascularised PED secondary to AMD. Graefes Arch Clin Exp Ophthalmol 245:1037–1040
92. Meyer CH, Mennel S, Schmidt JC, Kroll P (2006) Acute retinal pigment epithelial tear following intravitreal bevacizumab (Avastin) injection for occult choroidal neovascularisation secondary to age related macular degeneration. Br J Ophthalmol 90:1207–1208
93. Gass JD (1984) Retinal pigment epithelial rip during krypton red laser photocoagulation. Am J Ophthalmol 98:700–706
94. Gelisken F, Inhoffen W, Partsch M et al (2001) Retinal pigment epithelial tear after photodynamic therapy for choroidal neovascularization. Am J Ophthalmol 131:518–520
95. Nagiel A, Freund KB, Spaide RF et al (2013) Mechanism of retinal pigment epithelium tear formation following intravitreal anti-vascular endothelial growth factor therapy revealed by spectral-domain optical coherence tomography. Am J Ophthalmol 156:981–988.e2
96. Sarraf D, Joseph A, Rahimy E (2014) Retinal pigment epithelial tears in the era of intravitreal pharmacotherapy: risk factors, pathogenesis, prognosis and treatment (an American Ophthalmological Society Thesis). Trans Am Ophthalmol Soc 112:142–159
97. Grossniklaus HE, Green WR (1998) Histopathologic and ultrastructural findings of surgically excised choroidal neovascularization. Submacular surgery trials research group. Arch Ophthalmol 116:745–749
98. Grossniklaus HE, Miskala PH, Green WR et al (2005) Histopathologic and ultrastructural features of surgically excised subfoveal choroidal neovascular lesions: submacular surgery trials report no. 7. Arch Ophthalmol 123:914–921
99. Kim SY, Sadda S, Pearlman J et al (2002) Morphometric analysis of the macula in eyes with disciform age-related macular degeneration. Retina 22:471–477
100. Sarks J, Tang K, Killingsworth M et al (2006) Development of atrophy of the retinal pigment epithelium around disciform scars. Br J Ophthalmol 90:442–446
101. Santos AM, Calvente R, Tassi M et al (2008) Embryonic and postnatal development of microglial cells in the mouse retina. J Comp Neurol 506:224–239
102. Huang T, Cui J, Li L et al (2012) The role of microglia in the neurogenesis of zebrafish retina. Biochem Biophys Res Commun 421:214–220
103. Schafer DP, Lehrman EK, Kautzman AG et al (2012) Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner. Neuron 74:691–705
104. Checchin D, Sennlaub F, Levavasseur E et al (2006) Potential role of microglia in retinal blood vessel formation. Invest Ophthalmol Vis Sci 47:3595–3602
105. Kochan T, Singla A, Tosi J, Kumar A (2012) Toll-like receptor 2 ligand pretreatment attenuates retinal microglial inflammatory response but enhances phagocytic activity toward *Staphylococcus aureus*. Infect Immun 80:2076–2088
106. Kohno H, Chen Y, Kevany BM et al (2013) Photoreceptor proteins initiate microglial activation via toll-like receptor 4 in retinal degeneration mediated by all-trans-retinal. J Biol Chem 288:15326–15341
107. Maneu V, Yáñez A, Murciano C et al (2011) Dectin-1 mediates in vitro phagocytosis of *Candida albicans* yeast cells by retinal microglia. FEMS Immunol Med Microbiol 63:148–150
108. Patel M, Chan C-C (2008) Immunopathological aspects of age-related macular degeneration. Semin Immunopathol 30:97–110
109. Langmann T (2007) Microglia activation in retinal degeneration. J Leukoc Biol 81:1345–1351
110. Hume DA, Perry VH, Gordon S (1983) Immunohistochemical localization of a macrophage-specific antigen in developing mouse retina: phagocytosis of dying neurons and differentiation of microglial cells to form a regular array in the plexiform layers. J Cell Biol 97:253–257
111. Thanos S (1991) The relationship of microglial cells to dying neurons during natural neuronal cell death and axotomy-induced degeneration of the rat retina. Eur J Neurosci 3:1189–1207
112. Chinnery HR, McLenachan S, Humphries T et al (2012) Accumulation of murine subretinal macrophages: effects of age, pigmentation and CX3CR1. Neurobiol Aging 33:1769–1776
113. Damani MR, Zhao L, Fontainhas AM et al (2011) Age-related alterations in the dynamic behavior of microglia. Aging Cell 10:263–276
114. Ma W, Cojocaru R, Gotoh N et al (2013) Gene expression changes in aging retinal microglia: relationship to microglial support functions and regulation of activation. Neurobiol Aging 34:2310–2321
115. Ma W, Wong WT (2016) Aging changes in retinal microglia and their relevance to age-related retinal disease. Adv Exp Med Biol 854:73–78
116. Gupta N, Brown KE, Milam AH (2003) Activated microglia in human retinitis pigmentosa, late-onset retinal degeneration, and age-related macular degeneration. Exp Eye Res 76:463–471
117. Ma W, Zhao L, Wong WT (2012) Microglia in the outer retina and their relevance to pathogenesis of age-related macular degeneration. Adv Exp Med Biol 723:37–42
118. Roque RS, Rosales AA, Jingjing L et al (1999) Retina-derived microglial cells induce photoreceptor cell death in vitro. Brain Res 836:110–119
119. Zabel MK, Zhao L, Zhang Y et al (2016) Microglial phagocytosis and activation underlying photoreceptor degeneration is regulated by CX3CL1-CX3CR1 signaling in a mouse model of retinitis pigmentosa. Glia 64:1479–1491
120. Zhao L, Zabel MK, Wang X et al (2015) Microglial phagocytosis of living photoreceptors contributes to inherited retinal degeneration. EMBO Mol Med 7:1179–1197

121. McDermott DH, Fong AM, Yang Q et al (2003) Chemokine receptor mutant CX3CR1-M280 has impaired adhesive function and correlates with protection from cardiovascular disease in humans. J Clin Invest 111:1241–1250
122. Ambati J, Anand A, Fernandez S et al (2003) An animal model of age-related macular degeneration in senescent Ccl-2- or Ccr-2-deficient mice. Nat Med 9:1390–1397
123. Bojanowski CM, Shen D, Chew EY et al (2006) An apolipoprotein E variant may protect against age-related macular degeneration through cytokine regulation. Environ Mol Mutagen 47:594–602
124. Cao X, Shen D, Patel MM et al (2011) Macrophage polarization in the maculae of age-related macular degeneration: a pilot study. Pathol Int 61:528–535
125. Yang Y, Liu F, Tang M et al (2016) Macrophage polarization in experimental and clinical choroidal neovascularization. Sci Rep 6:30933
126. Lin JB, Moolani HV, Sene A et al (2018) Macrophage microRNA-150 promotes pathological angiogenesis as seen in age-related macular degeneration. JCI Insight 3. https://doi.org/10.1172/jci.insight.120157
127. Klein RJ, Zeiss C, Chew EY et al (2005) Complement factor H polymorphism in age-related macular degeneration. Science 308:385–389
128. Haines JL, Hauser MA, Schmidt S et al (2005) Complement factor H variant increases the risk of age-related macular degeneration. Science 308:419–421
129. Edwards AO, Ritter R 3rd, Abel KJ et al (2005) Complement factor H polymorphism and age-related macular degeneration. Science 308:421–424
130. Chen M, Forrester JV, Xu H (2007) Synthesis of complement factor H by retinal pigment epithelial cells is down-regulated by oxidized photoreceptor outer segments. Exp Eye Res 84:635–645
131. Thompson CL, Klein BEK, Klein R et al (2007) Complement factor H and hemicentin-1 in age-related macular degeneration and renal phenotypes. Hum Mol Genet 16:2135–2148
132. Magnusson KP, Duan S, Sigurdsson H et al (2006) CFH Y402H confers similar risk of soft drusen and both forms of advanced AMD. PLoS Med 3:e5
133. Tedeschi-Blok N, Buckley J, Varma R et al (2007) Population-based study of early age-related macular degeneration: role of the complement factor H Y402H polymorphism in bilateral but not unilateral disease. Ophthalmology 114:99–103
134. Droz I, Mantel I, Ambresin A et al (2008) Genotype-phenotype correlation of age-related macular degeneration: influence of complement factor H polymorphism. Br J Ophthalmol 92:513–517
135. Munch IC, Ek J, Kessel L et al (2010) Small, hard macular drusen and peripheral drusen: associations with AMD genotypes in the Inter99 Eye Study. Invest Ophthalmol Vis Sci 51:2317–2321
136. Coffey PJ, Gias C, McDermott CJ et al (2007) Complement factor H deficiency in aged mice causes retinal abnormalities and visual dysfunction. Proc Natl Acad Sci U S A 104:16651–16656
137. Seddon JM, Yu Y, Miller EC et al (2013) Rare variants in CFI, C3 and C9 are associated with high risk of advanced age-related macular degeneration. Nat Genet 45:1366–1370
138. Kavanagh D, Yu Y, Schramm EC et al (2015) Rare genetic variants in the CFI gene are associated with advanced age-related macular degeneration and commonly result in reduced serum factor I levels. Hum Mol Genet 24:3861–3870
139. Geerlings MJ, Kremlitzka M, Bakker B et al (2017) The functional effect of rare variants in complement genes on C3b degradation in patients with age-related macular degeneration. JAMA Ophthalmol 135:39–46
140. Holz FG, Sadda SR, Busbee B et al (2018) Efficacy and safety of lampalizumab for geographic atrophy due to age-related macular degeneration: CHROMA and SPECTRI phase 3 randomized clinical trials. JAMA Ophthalmol 136:666–677