Chapter 36
Is Age-Related Macular Degeneration a Microvascular Disease?

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Abstract Age-related macular degeneration (AMD) is a common, degenerative disease of the central retina affecting millions of elderly in the USA alone and many more worldwide. A better understanding of the pathophysiology of AMD will be essential for developing new treatments. In this review, we discuss the potential impact of complement complex deposition at the choriocapillaris of aging eyes and the relationship between choriocapillaris loss and drusen formation. We further propose a model that integrates genetic and anatomical findings in AMD and suggest the implications of these findings for future therapies.

Keywords Age-related macular degeneration · Choriocapillaris · Choroid · Complement · RPE

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36.1 Age-Related Macular Degeneration

Age-related macular degeneration (AMD) is a complex disease characterized by degenerative changes that are most severe in the macular region of the retina. It is characterized at its earliest stages by the formation of drusen beneath the retinal pigment epithelium (RPE) and may progress to severe atrophic degeneration, choroidal neovascularization, hemorrhage, and scarring. Due to its detrimental effect on central vision, AMD is a major societal problem in the developed world, with a substantial impact on quality of life in the elderly [1, 2].

Over the past several years, genetic insights into the risk factors that contribute to AMD have emerged. These include genes that encode members of the complement system (most notably complement factor H (*CFH*), a principal inhibitor of complement activation, but also including *CFHR* genes, as well as *CFB*, *C2*, *C3*, and in some reports *CFI* and *SERPING1*) (reviewed in [3]). Despite recent advances in genetics, the precise mechanism of AMD is not clear, and only very recently has the impact of high- and low-risk genotypes on ocular tissues been studied.

36.2 The Complement System

The complement system is an evolutionarily ancient defense mechanism against pathogens. It consists of a large array of proteins that are present in the circulation and can be localized to ocular tissues [4]. The complement system can be activated by multiple pathways [5]; however, regardless of the initial pathway, complement activation results in the formation of a membrane-spanning pore (the membrane attack complex, MAC), which can lyse target pathogens. Significantly, when unchecked, the same machinery that is designed to lyse microbes can similarly damage eukaryotic cells [6].

In view of the genetic finding of polymorphisms in complement genes in AMD, understanding where components of the complement system are localized (and where they assert their physiological effects) in the aging macula is an important step. While other byproducts of complement activation may have important biological activities (e.g., proinflammatory anaphylatoxins [7–9]), the MAC is intuitively the endpoint in complement activation that could have the most profound effect on ocular cells.

Solitary nodular drusen outside the macula show robust labeling with antibodies directed against the neoepitope of the MAC. Basal deposits in the macula, however, show variable immunoreactivity with anti-MAC antibodies. In contrast, we and others have shown that domains surrounding the endothelial cells (EC) of the choriocapillaris appear to be the main sites of MAC deposition in the macula [8, 10–12] (Fig. 36.1a). While this observation does not preclude RPE injury by the MAC as an important event in AMD pathogenesis, it does suggest that the cells under the greatest complement-mediated stress are choriocapillaris EC.

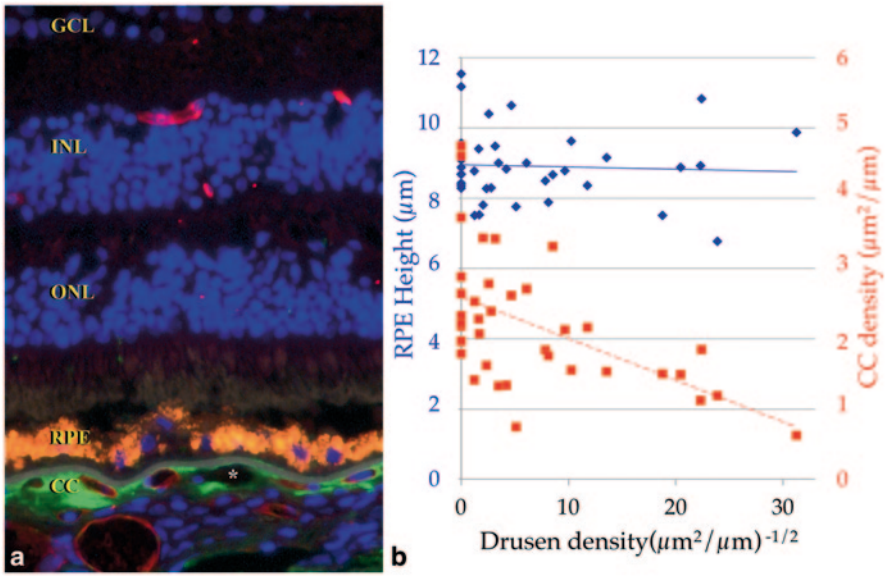

Fig. 36.1 **a** The choriocapillaris is the main site of MAC deposition in the macula (*green fluorescence*); *red fluorescence* is UEA-I lectin. RPE lipofuscin appears *orange*. The *asterisk* indicates a choriocapillaris ghost vessel. **b** Relationship between choriocapillaris endothelial cell density (*open squares, maroon dashed trend line*), RPE height (*closed diamonds*, *blue solid trend line*), and drusen density. Note that eyes with the lowest vascular density had the largest and most numerous drusen. (Modified from [20]; copyright held by ARVO)

36.3 High-Risk Genotypes Associated with Increased Complement Activation

The recent identification of genetic risk factors associated with AMD has created new opportunities to study disease pathophysiology. Notably, these findings may allow the discovery of molecular changes in the tissues of interest even before the onset of detectable disease. Studies on plasma and serum from AMD patients and controls have shown increased complement activation in individuals with high-risk genotypes [13]. Enzyme-linked immunosorbent assay (ELISA) analyses of the MAC in human genotyped RPE-choroid samples show ~70% increase in eyes homozygous for the high-risk CFH allele [14], with higher levels of MAC more closely associated with genotype than AMD status. These findings in aggregate may suggest that increased MAC deposition precedes an overt AMD phenotype, as discussed below.

36.4 Choriocapillaris EC Loss Occurs with Increasing Density of Drusen

In addition to being the principal site of MAC localization, anatomical and functional changes occur at the level of the choriocapillaris in AMD. Prolonged filling of the choroid in early AMD was noted by Pauleikhoff and coworkers using both indocyanine green and fluorescein [15]. In laser Doppler flow studies, choroidal blood volume and choroidal blood flow are decreased in association with increasing drusen abundance [16]. Proteomic analyses of age-matched control tissues compared to AMD tissues found loss of choriocapillaris proteins CA4 and HLA-A, with persistence of RPE proteins CRALBP and RPE65 [17]. In histological studies, McLeod and colleagues found loss of choriocapillaris and preservation of RPE outside of the neovascular lesion in eyes with wet AMD, whereas in atrophic AMD, preservation of choroid with loss of the RPE was observed [18]. We employed human donor eyes labeled with the EC-binding lectin from *Ulex europaeus* (UEA-I) to detect live EC and discriminate them from unoccupied, "ghost" vessels in the choriocapillaris, and related these features to the abundance of drusen. We also assessed the height of the RPE as a measure of RPE viability. Measurement of AMD and control eyes in a masked fashion revealed that as drusen density increases, there is loss of choriocapillaris area and increase in the number of ghost vessels [19]. Superimposing the height of the RPE onto these measurements shows no correlation between RPE height and advancing drusen abundance (Fig. 36.1b). However, it should be noted that the presence of an intact RPE monolayer of normal height does not mean that the RPE is completely normal. Morphological and biochemical changes in the RPE may be occurring in eyes with drusen that our measurements do not detect.

The relationship between vascular loss and accumulation of drusen suggests that vascular changes occur early in disease, especially since drusen tend not to form over vascular lumens. We recognize the alternative (and nonexclusive) possibility that the RPE, while still present, may fail to deliver necessary trophic factors to choriocapillaris when drusen develop, such that choriocapillaris loss occurs after drusen biogenesis. It is well known that injury to the RPE can lead to loss of the choriocapillaris as a secondary phenomenon. Nevertheless, our experiments do indicate that there is a correlation between death of choriocapillaris EC and increasing drusen number and size. Further studies of selective choriocapillaris ablation in animals (see below) will refine the relationship between the RPE, Bruch's membrane deposits, and death of the choriocapillaris.

36.5 Choriocapillaris Loss and Implications for Therapy

The photoreceptor cells, RPE, and choriocapillaris function as an interdependent unit (recently reviewed in [20]). In light of the many roles of the RPE in maintaining retinal health, replacement of the RPE may be beneficial in AMD, and a number of trials have begun with this goal. RPE cells derived from allogeneic, embryonic, or

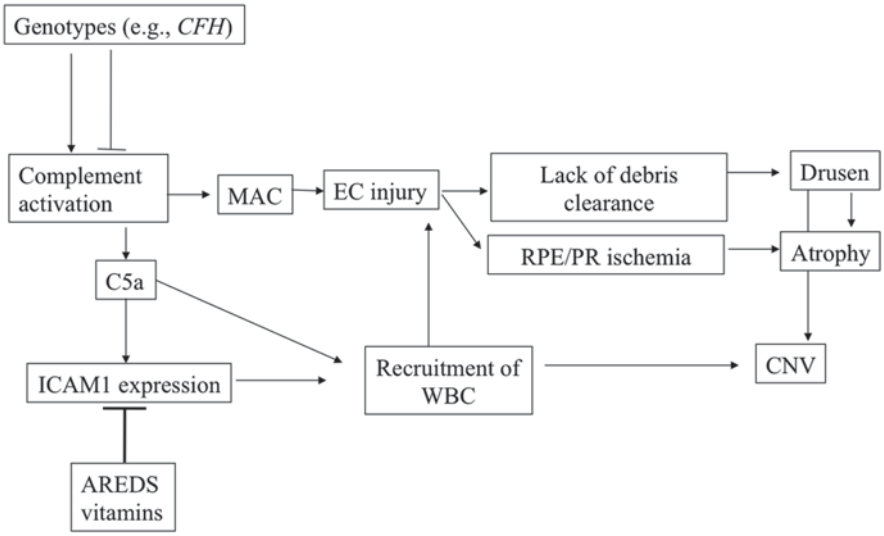

Fig. 36.2 Model for AMD pathogenesis (see text for details)

fetal sources, or more desirably from autogenic induced pluripotent stem cells, can now be placed into the subretinal space. However in the absence of patient-specific evidence of an intact choriocapillaris (becoming possible by improvements in optical coherence tomography (OCT) [21, 22]), we suggest caution with this approach. Replacement of photoreceptor cells and/or RPE cells on top of a depleted choriocapillaris may be fruitless.

36.6 A Model of Early AMD Based on Vascular Loss

A model that attempts to synthesize the role of genetics, complement, vascular loss, drusen, and AMD pathogenesis is depicted in Fig. 36.2. In this model, systemic and local complement activation occurs at the level of the choriocapillaris, and this activation is more pronounced in eyes with high-risk CFH genotypes, as we have observed [14]. Increased complement activation in the choriocapillaris yields C5a anaphylatoxin, which activates vascular endothelial growth factor (VEGF) expression in the RPE and intercellular adhesion molecule 1 (ICAM1) expression in the choriocapillaris, promoting angiogenesis and leukocyte recruitment, respectively [7, 8]. The generation of MAC over the course of decades leads to loss of choriocapillaris EC (which may have a more limited repertoire of defensive proteins than the RPE). Focal loss of the choriocapillaris results in failure to remove debris from the RPE/Bruch's membrane and development of drusen, as well as retinal and RPE hypoxia.

It is interesting in this context that a solution of AREDS vitamins has been shown to attenuate at least one step in this pathway (the upregulated ICAM1 expression by choriocapillary EC [23]).

36.7 Future Directions

One of the critical questions is the cause–effect relationship between drusen formation and EC loss in the choriocapillaris. A clear determination of the natural history of Bruch's membrane deposit formation and EC loss needs to be established. Genetically tractable models of vascular dropout will offer insight into this relationship. A better understanding of the mechanisms by which healthy EC are lost in early AMD and the molecular basis of why drusen form in areas depleted of capillary lumens are crucial. Moreover, as noted above, the loss of choroidal EC in AMD suggests that ideal treatments of early AMD may require replacement of lost choroidal EC, through, for example, induced pluripotent stem cells. Advances in the generation, phenotyping, and replacement of these EC will be important in providing cell-based therapies for this blinding disease.

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