Pathogenesis of Age-Related Macular Degeneration

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Abstract

Epidemiological, histopathological and biochemical evidence indicates that AMD is associated with oxidative damage, lipofuscin accumulation, chronic inflammation, and mutations in the complement system. Molecular targets have been identified that may serve as the basis for developing new, better treatments for AMD including prophylactic therapy and treatments for the late stage complications of geographic atrophy and choroidal neovascularization. Copyright © 2012 S. Karger AG, Basel

Age-related macular degeneration (AMD) is the most common cause of blindness in the industrialized world. Detailed reviews of its pathogenesis have been published [1–3]. In this chapter, the author will review epidemiological, biochemical, histological, and molecular biological data that shed light on the pathogenesis of this disease. The basic principles that emerge from this review are that AMD pathogenesis involves oxidative damage, lipofuscin accumulation, chronic inflammation, and mutations in the complement system with associated apoptosis.

AMD Is Associated with Oxidative Damage

Epidemiological data indicate that the main risk factors (i.e. those that increase risk by a factor of two or more consistently in different studies) for AMD include: age, white race, and smoking [4]. The Age-Related Eye Disease Study (AREDS) showed that patients with a minimum level AMD severity (i.e. extensive intermediate drusen, one large (>125 μm) soft druse, noncentral geographic atrophy in one or both eyes, choroidal new vessels in the fellow eye, or visual acuity <20/40 due to AMD) reduce the risk of moderate visual loss 19% during a 5-year period of follow-up by consuming a daily dose of vitamin C (500 mg), vitamin E (400 IU), zinc oxide (80 mg), cupric oxide (2 mg), and beta-carotene (15 mg), particularly among patients with the low-risk CFH TT genotype [5, 6]. Some of these components (e.g., zinc) are cofactors for antioxidant enzymes and some (e.g., vitamin C) are antioxidants. Based on this information, some researchers feel that the primary benefit of the supplement is via protection against oxidative damage. Biochemical and histological studies of AMD eyes also indicate that oxidative damage plays a role in AMD pathogenesis. For example, Shen et al. [7] demonstrated DNA strand breaks and lipoperoxidation in eyes with geographic atrophy. RPE antioxidant enzyme changes in AMD eyes indicate that the RPE are under oxidative stress [8]. Histological and clinical studies indicate that carotenoids (macular pigment, including lutein and zeaxanthin), which
scavenge free radicals and reduce phospholipid peroxidation, are decreased in AMD eyes [9]. Advanced glycation end products, carboxymethyl lysine (derived from lipoprotein peroxidation), and carboxyethylpyrrole protein adducts (derived from docosahexaenoic acid lipo-oxidation) all are present in drusen [10, 11]. Finally, chelatable iron accumulates in AMD Bruch’s membrane, and Fe2+ catalyzes the conversion of H2O2 to OH [12].

Mutations in the complement system linked to AMD (see below) probably are associated with increased risk of uncontrolled inflammation at the level of RPE-Bruch’s membrane-choroid. Inflammation can be associated with oxidative damage. Other AMD risk-enhancing mutations not involving the complement pathway may be linked to alterations in oxidative metabolism. Kanda et al. [13] identified a single nucleotide polymorphism (rs10490924) that was strongly associated with the risk of AMD and resulted in a nonsynonymous A695S alteration in the predicted protein LOC387715/ARMS2, which localizes to the mitochondrial outer membrane when expressed in mammalian cells. Jones et al. [14] assessed the association between mitochondrial haplogroups and AMD and found that haplogroup H was associated with a reduced prevalence of any AMD. Haplogroup J was associated with a higher prevalence of large, soft distinct drusen. Haplogroup U was associated with an increased prevalence of RPE abnormalities.

Lipofuscin Accumulation Is Associated with Increased Risk of AMD

Lipofuscin comprises a group of autofluorescent compounds present in neuronal and non-neuronal tissue. Lipofuscin accumulates within retinal pigment epithelium (RPE) cells during one’s lifetime and, in RPE, the major source of lipofuscin is the undegradable products of photoreceptor outer segment metabolism [15]. Lipofuscin accumulation is greatest in the RPE under the parafoveal retina, which may reflect the fact that the density of rod photoreceptors, which have a higher outer segment turnover rate than cones, is greatest in this area. N-retinylethene-N-retinylethanolamine (A2E) forms as a byproduct of the release of all-trans-retinal within outer segment discs, is a major chromophore in lipofuscin, and causes reactive oxygen species production when illuminated with high energy light (fig. 1) [16]. Excessive RPE lipofuscin (and A2E) accumulation may play an important role in AMD pathogenesis [16, 17]. Geographic atrophy
tends to develop in the parafoveal area and tends to spare the foveal center until the later stages of the disease [18]. Subfoveal RPE may be relatively spared from atrophy due to the presence of macular pigment, the high cone density in the foveola, and possibly other factors [15, 19, 20].

**AMD Is Associated with Chronic Inflammation**

Drusen contain many components of the activated complement cascade [21]. Histopathological studies demonstrate the presence of inflammatory cells in the RPE-Bruch's membrane-choriocapillaris of AMD eyes [22]. Bioactive fragments of C3 (C3a) and C5 (C5a) are present in drusen and induce VEGF expression in the RPE, which may explain why confluent soft drusen are a risk factor for choroidal new vessels (CNVs) in AMD eyes [23]. The presence of pro-inflammatory molecules in drusen creates a stimulus for chronic inflammation in the RPE-Bruch's membrane-choriocapillaris complex that may result in some features of late AMD.

Amyloid-β oligomers are toxic to cells (soluble monomers are not). Amyloid diseases typically exhibit abundant fibrils of various lengths. These fibrils are an end product of stepwise protein/peptide misfolding, and they accumulate as long-lived extracellular deposits. Drusen vesicles probably contain fibrillar amyloid composed in part of amyloid-β [24, 25]. Amyloid-β induces production of interleukin-1β and tumor necrosis factor-α by macrophages and microglia, which can cause increased expression of complement factor B in RPE [26] and may contribute to AMD progression.

**AMD Is Associated with Mutations in the Complement System**

Drusen, geographic atrophy, and CNVs are associated with mutations in components of the complement pathway, which is part of the innate immune system. There are four major pathways of complement activation: classical, alternative, lectin and intrinsic (fibrinolytic-activated). Activation of the complement system plays an important role in immunity, and inappropriate complement activation can damage tissue. Complement factor C3 is the critical point of convergence of all the activation pathways. Mutations in the following complement-related genes have been associated with AMD: complement factor H (CFH) [27–30], complement factor B (CFB) [31, 32], complement component 2 (C2) [31, 32], complement component 3 (C3) [33–36], complement factor I [37], FCN1 (a collagen-like ficolin gene involved in activation of the lectin pathway), F13B (F13b catalyzes formation of fibrin crosslinks and promotes stabilization of fibrin clots) [21, 38], and C9 [21]. In one study, 76% of the attributable risk of developing AMD was accounted for by single nucleotide polymorphism-type mutations in complement factor H (CFH Y402H), ARMS2 (A69S), and complement factor 3 (C3 R102G) [39]. If one has the low-risk genotype at these three loci, there is a 20-fold decreased risk of AMD versus the general population [40]. Although the details have not been established, it seems that many if not all of these mutations compromise the host’s ability to regulate activation of the complement system, which results complement attack and chronic inflammation at the level of the photoreceptor-RPE-Bruch's membrane-choroid. It may be of interest to note that zinc, one of the main therapeutic ingredients of the AREDS treatment, also affects the complement system. Zinc inhibits C3 convertase activity [41], and levels of C3a des Arg, which is a cleavage product of C3a and reflects complement activation, are higher in patients with AMD (including patients with early as well as late AMD) versus controls [42].

In humans, cells in the RPE-Bruch's membrane-choroid complex produce many (if not all) of the complement factors and regulatory molecules of the classical and alternative pathways (fig. 2) [21]. The choroidal cells seem to produce most
of these factors although the RPE, neural retina, and choroid all robustly produce membrane co-factor protein (MCP), which downregulates complement activation by fostering the cleavage and inactivation of surface-bound C3b and C4b via CFI [21]. The majority of components involved in the lectin pathway and the majority of the terminal pathway components involved in membrane attack complex formation seem to be derived from the circulatory system [21]. C4-binding protein (C4BP), working in conjunction with CFI, is a major fluid phase inhibitor of C3 convertase (C4b2a3b). Because the RPE and choroid do not produce C4BP, regulation of complement activation in the RPE-Bruch's membrane-choroid complex depends heavily on CFH (and possibly on

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**Fig. 2.** The complement cascade. Green and red circles identify molecules, mutations in which are associated with an increased risk of AMD. The critical control point for complement activation is C3.
C4BP derived from the plasma of the choroidal vasculature) [21]. Thus, the RPE-choroid may be relatively susceptible to damage in the setting of CFH mutations.

Some of these mutations seem to be linked to patients’ responses to therapeutic intervention. For example, progression to late-stage AMD with zinc treatment is reduced to a greater degree with the CFH TT genotype at position 402 than with the high-risk CC genotype [6]. Visual acuity outcomes seem to be worse among patients with CNVs and the CFH TT genotype (vs. TC or CC) who are treated with photodynamic therapy [43]. In one study, there was a 37% higher risk of needing additional ranibizumab injections among patients with the CFH CC genotype [44]. In another study, 54% of patients with the CFH TT and TC genotypes have improved vision with bevacizumab versus 11% with the CC genotype [45].

**Oxidative Damage Can Compromise RPE Regulation of the Complement System**

The alternative complement pathway is activated continuously in the fluid phase, and tissue surfaces require continuous complement inhibition to prevent spontaneous autologous cell injury [46]. The complement system is activated continuously in the eye [47]. Oxidative stress reduces the regulation of complement on the RPE surface in cell culture experiments (by reducing the surface expression of the complement inhibitors, decay accelerating factor (CD55) and CD59, and by impairing complement regulation at the cell surface by factor H [48]. Sublytic activation of the complement cascade also causes VEGF release from the cells, which compromises RPE barrier function. Oxidative stress also reduces the ability of interferon-γ (an inflammatory mediator) to increase CFH expression in RPE cells [49]. The products of A2E photo-oxidation in RPE cell cultures can serve as a trigger for the complement system [50]. Thus, the relative abundance of lipofuscin (and A2E) in the submacular RPE could predispose the macula to chronic inflammation and AMD. Hollyfield et al. [51] described an animal model that links oxidative damage and complement activation to AMD. Mice were immunized with mouse serum albumin adducted with carboxyethylpyrrole, a unique oxidation fragment of docosahexaenoic acid that is present in drusen of AMD eyes. Immunized mice developed antibodies to the hapten, fixed C3 in Bruch’s membrane, accumulated drusen, and developed lesions resembling geographic atrophy.

**Pathogenesis of AMD: Hypothesis**

The photoreceptor-RPE-Bruch’s membrane-choroid complex is a site of chronic oxidative damage that is most pronounced in the macula (fig. 3). This damage incites inflammation, mediated at least in part by complement activation, at the level of RPE-Bruch’s membrane-choroid. Patients with mutations in components of the complement system are less able to modulate the inflammatory response, resulting in excessive cellular damage and accumulation of extracellular debris (recognized, eventually, as drusen). These changes, which involve modification of the extracellular matrix, cause additional inflammation and cell damage. This chronic inflammatory response involves cellular components of the immune system as well as the classical and alternative pathways of the complement system. Accumulation of abnormal extracellular material (including membranous debris, oxidized molecules, extracellular matrix molecules, and components of the complement system) is thus a sign of chronic inflammatory damage, is manifest in part as drusen and pigmentary abnormalities, and fosters the development of the late sequelae of AMD in susceptible individuals, i.e., geographic atrophy and/or CNVs. Many treatments for AMD under investigation are based on concepts related to this hypothesis of pathogenesis.
The sequence above might account for the development of AMD, but it does not explain why some patients develop geographic atrophy, some develop CNVs, some develop neither, and some develop both. Data that may shed light on the pathobiology of geographic atrophy are as follows. Apoptosis is known to be involved in AMD-associated cell death [52]. Bone morphogenetic protein-4 (BMP-4) is an important regulator of cell differentiation, senescence, and apoptosis in many different cells and tissues. BMP-4 is involved, for example, in chemotherapy-induced senescence of lung and prostate cancer cells. BMP-4 acts as a mediator in oxidative stress-induced senescence. Via the Smad and p38 signaling pathway, BMP-4 increases and activates p53 and p21Cip1/WAF1 and decreases phospho-Rb. BMP-4 is highly expressed in the RPE and in adjacent extracellular matrix of patients with dry AMD [53]. In vitro studies show that sublethal oxidative stress increases BMP-4 expression in RPE, and both BMP-4 and persistent mild oxidative stress can induce RPE senescence through the p53- p21Cip1/WAF1- Rb pathway [53]. In contrast, in neovascular AMD lesions, BMP4 expression in RPE is low, possibly a result of local expression of pro-inflammatory mediators (see below). Transforming growth factor (TGF)-β is involved in mediating oxidative stress-induced premature senescence of fibroblasts. TGF-β mediates oxidative
stress-induced RPE cell senescence through the upregulation of p21^{WAF1/cip1} and down-regulation of phosphorylated Rb [54]. TGF-β and BMP-4 may have a synergistic effect in mediating oxidative stress-induced RPE senescence because neither TGF-β antibodies nor BMP-4 antagonists alone can completely block the expression of senescence marker genes to baseline in oxidative stress-treated RPE cells [53]. The microRNA processing enzyme DICER1 is reduced in the RPE of eyes with geographic atrophy [55]. Conditional ablation of DICER1 induces RPE degeneration in preclinical studies. The reduction in DICER1 activity is associated with accumulation of Alu RNA in the RPE of eyes with geographic atrophy [55] (DICER1 degrades Alu RNA). Preclinical experiments indicate that it is Alu RNA accumulation that induces RPE death [55]. Thus, Alu RNA-induced RPE cell apoptosis is triggered by DICER1 dysregulation in geographic atrophy. Of note, oxidative stress can induce DICER1 down-regulation [55].

RPE cells induced into senescence by chronic oxidative stress secrete 4-times higher interleukin-8 than nonsenescent RPE cells [56]. Interleukin-8 promotes angiogenesis by increasing the proliferation, survival, and migration of endothelial cells and promotes inflammation by increasing neutrophil chemotaxis and degranulation. Senescent heterogeneity combined with the effects of other cytokines (e.g., TNF-α inhibition of BMP-4 expression) may drive some cells to senescence with geographic atrophy and others to stimulate CNV formation [56].

Epidemiological, histopathological and biochemical evidence indicates that AMD is associated with oxidative damage, lipofuscin accumulation, chronic inflammation, and mutations in the complement system. Molecular targets have been identified that may serve as the basis for developing new, better treatments for AMD including prophylactic therapy and treatments for the late stage complications of geographic atrophy and choroidal neovascularization.

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