Progress and perspectives on the role of RPE cell inflammatory responses in the development of age-related macular degeneration

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Abstract: Age-related macular degeneration (AMD) is the leading cause of blindness in developed countries. The etiology of AMD remains poorly understood and no treatment is currently available for the atrophic form of AMD. Atrophic AMD has been proposed to involve abnormalities of the retinal pigment epithelium (RPE), which lies beneath the photoreceptor cells and normally provides critical metabolic support to these light-sensing cells. Cumulative oxidative stress and local inflammation are thought to represent pathological processes involved in the etiology of atrophic AMD. Studies of tissue culture and animal models reveal that oxidative stress-induced injury to the RPE results in a chronic inflammatory response, drusen formation, and RPE atrophy. RPE degeneration in turn causes a progressive degeneration of photoreceptors, leading to the irreversible loss of vision. This review describes some of the potential major molecular and cellular events contributing to RPE death and inflammatory responses. In addition, potential target areas for therapeutic intervention will be discussed and new experimental therapeutic strategies for atrophic AMD will be presented.

Keywords: age-related macular degeneration, danger signals, complement, inflammation, retinal pigment epithelial cells

Introduction

Age-related macular degeneration (AMD) is an idiopathic retinal degenerative disease and the cause of irreversible, profound vision loss in people over the age of 60 years (Evans and Wormald 1996). Due to a rapidly aging population, the number of people with AMD in the US is estimated to increase from 1.75 million in 2000 to 2.95 million in 2020 (Friedman et al 2004). Similar proportional increases in the worldwide prevalence of AMD can be expected placing an increasing burden on health services. The market for AMD therapies is projected to increase from $638 million in 2005 to $2722 million in 2015 (Augustin and Offermann 2006).

AMD occurs in two major forms: atrophic (dry) AMD and exudative (wet) AMD. Atrophic AMD, characterized by retinal pigment epithelial (RPE) cell atrophy and subjacent photoreceptor degeneration, accounts for approximately 25% of cases with severe central vision loss (Klein et al 1997). In contrast, exudative AMD is characterized by choroidal neovascularization (CNV) through the outer layers of Bruch's membrane under the basement membrane of the RPE or through the RPE into the subretinal space with subsequent retinal hemorrhage, detachment, and scarring, and accounts for approximately 75% of cases with severe central vision loss (Klein et al 1997). These two forms of AMD are both part of the same disease process and share similar risk factors for their development. In fact, patients may have CNV in one eye and geographic atrophy, the advanced form of atrophic AMD, in the second eye. In some cases, the dry form of AMD may progress to wet AMD

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(Sunness et al 1999). The specific biological processes that determine which form of advanced AMD occurs and why and how some of dry AMD later progress to wet AMD are still elusive. AMD is an aging-related syndrome caused by multiple factors including environmental, nutritional, and behavioral (Snodderly 1995; Seddon et al 1996) as well as different susceptibilities to these factors based upon an individual's genetic background (Heiba et al 1994; Seddon et al 1997). The early pathological changes of atrophic AMD begin with extracellular lipid-containing deposits in the macular area (Bressler et al 1990). Patients with drusen greater than 63 μm in diameter are diagnosed as having early AMD (Bird et al 1995; Curcio and Millican 1999). Large drusen can merge, become confluent and eventually lead to geographic atrophy of the central retina. Although the vision loss results from photoreceptor damage in the central retina and abnormalities of the photoreceptor cells or the choroid might contribute to the development of AMD, the initial pathogenesis of atrophic AMD has been proposed to involve the degeneration of RPE cells (Spraul et al 1996; Zarbin 1998). The specific genetic and biochemical mechanisms responsible for RPE degeneration in AMD have not been well determined. Recent progress in our understanding of atrophic AMD suggests that inflammation initiated by RPE cell death and RPE cell inflammatory responses plays an important role in drusen biogenesis and maybe central to the etiology of this disease (Hageman et al 2001; Rodrigues 2007).

The RPE is a monolayer of cuboidal cells located subjacent to the neural retina (Strauss 2005). The basal membrane of the RPE is in contact with Bruch's membrane, a multilayered matrix that separates the RPE from the underlying choroidal vasculature. The apical membrane of the RPE is intimately associated with the outer segments of retinal photoreceptor cells. The major function of RPE cells is to support the survival and normal functioning of photoreceptors by acting as part of the outer blood-retinal barrier to control exchange of nutrients, waste products, ions, and gases between the underlying choroidal blood vessels and overlying photoreceptors (McBee et al 2001). Additionally, the RPE is involved in the shuttling of retinoids required for visual pigment synthesis to photoreceptors (Thompson and Gal 2003), and in phagocytizing shed photoreceptor outer segment (POS) membrane discs for photoreceptor renewal (Nguyen-Legros and Hicks 2000). RPE cells also produce trophic factors necessary for photoreceptor survival, and immunological factors necessary for establishing immune privilege of the eye (Tanihara et al 1997; Streilein et al 2002). Injury to RPE cells causing failure of these functions may contribute to the retinal degeneration observed in AMD. Thus, there is considerable interest in elucidating the mechanisms responsible for RPE injury that would provide the basis for designing new strategies to treat or prevent AMD. In this review, the molecular and cellular events involved in RPE injury and inflammation, and potential therapeutic strategies are discussed.

Factors causing RPE cell injury

Oxidative stress

Histological staining with biomarkers of oxidative damage has demonstrated widespread oxidative damage in the retinas of patients with atrophic AMD and to a lesser extent also in the retinas of patients with wet AMD (Shen et al 2007). Moreover, intravitreal injection of oxidants results in lipid peroxidation and retinal degeneration in animal models (Cingolani et al 2006). RPE cells suffer from cumulative oxidative stress due to normal physiological activity as well as environmental factors (Figure 1). RPE cells generate reactive oxygen species (ROS) during phagocytosis (Miceli et al 1994). RPE phagocytosis takes up the tips of the shed POS that contain high concentrations of radicals, photo-damaged proteins and lipids. Some of these oxidative-damaged proteins and lipids are not completely digested by RPE lysosomes and accumulate within RPE cells as a major source of the photosensitizer, lipofuscin during RPE aging (Kennedy et al 1995). Once irradiated by light, lipofuscin produces ROS that damage surrounding tissues (Boulton et al 1993). Thus, the unique phagocytic function of RPE places an additional oxidative burden on the RPE. This is particularly true of RPE cells in the macula, where the predominant photoreceptors are cones that have a higher energy demand than rods (Perkins et al 2003). Additionally, the ratio of photoreceptors to RPE cells in the macula is significantly higher than in the para-macular region or the periphery (Dorey et al 1989).

In addition to the physiological oxidative stress, aging and pathological conditions can also contribute to oxidative damage to macromolecules via a reduction in defense mechanisms or an increase in ROS production or both (Ames et al 1993). Environmental factors such as cigarette smoke (Chan 1998), light exposure (Cruickshanks et al 1993), diet, and cataract surgery also enhance ROS generation and can increase the risk for AMD. Heavy cigarette smokers have significantly higher risks of AMD (Thornton et al 2005; Cong et al 2008). Furthermore, smoking can also cause a reduction in retinal and choroidal blood flow (Tamaki et al 1999;

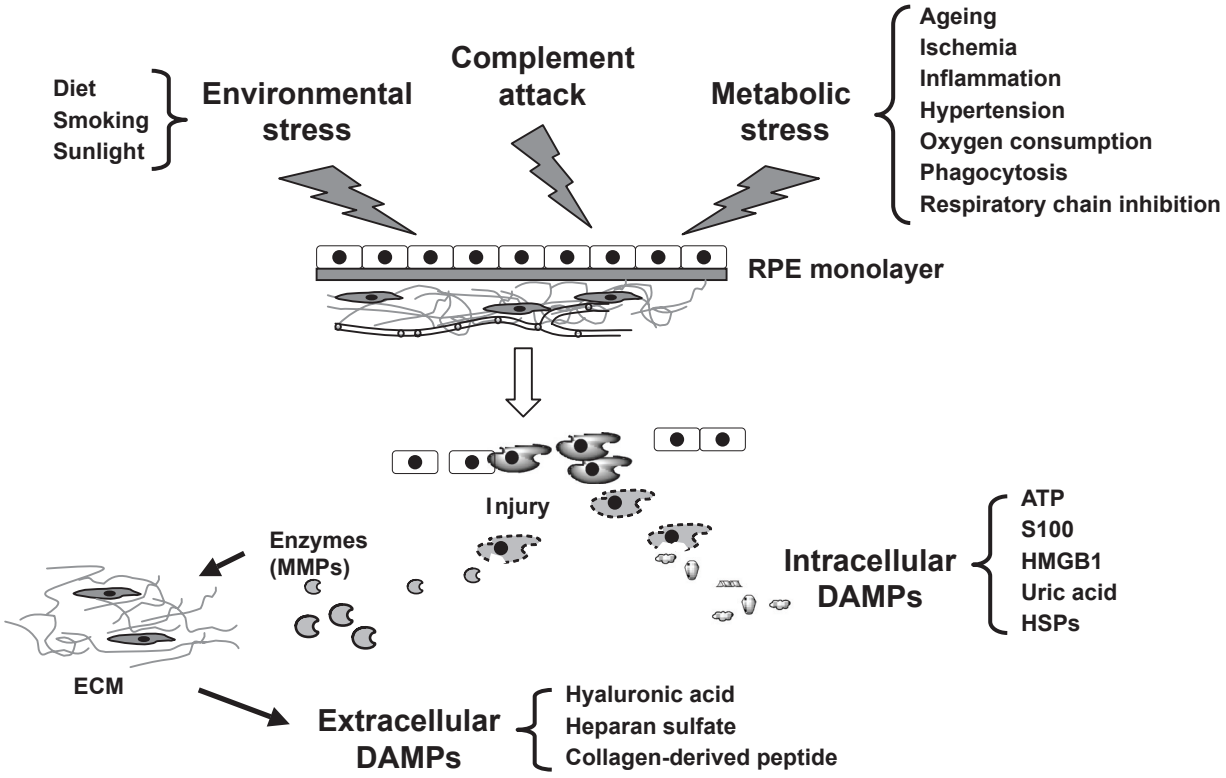

Figure 1 Conditions that trigger retinal pigment epithelial (RPE) cell injury and subsequent release of damage-associated molecule pattern (DAMP) molecules. RPE cell injury is triggered by environmental stress, metabolic stress, and complement attack. Injured or dying RPE cells then produce danger signals that are defined as DAMP molecules. Some of these danger signals are intracellular molecules and others are the cleaved products of extracellular components such as extracellular matrix (ECM).
Abbreviations: HMGB1, high mobility group box 1; HSPs, heat-shock proteins; MMPs, matrix metalloproteinases.

Wimpissinger et al 2004, 2005). The relative contribution of these two processes to AMD remains to be determined. Cataract surgery to remove the lens increases the amount of harmful ultraviolet and blue light reaching the retina. Epidemiological studies have revealed an increased risk of late AMD in patients with cataract surgery (Klein et al 2002; Ho et al 2008). Data analysis of participants of the Rotterdam study shows that a history of cataract surgery is linked to an increased incidence of atrophic AMD, but no correlation with wet AMD was observed (Ho et al 2008).

Recent evidence suggests that genetic factors may also contribute to the accumulation of oxidative stress. Polymorphisms in the age-related maculopathy susceptibility 2 gene (ARMS2, also known as LOC387715) were shown to be strongly associated with increased risk of AMD. The A69S polymorphism shows a strong association with risk of AMD (odds ratio [OR] 2.66 for GT heterozygotes and 7.05 for TT homozygotes) (Kanda et al 2007). A deletion-insertion polymorphism (del443ins54) in ARMS2 also causes an increased risk in individuals carrying single copy of this allele (OR, 2.9) or those that are homozygous (OR, 8.1) (Fritsche et al 2008). The ARMS2 gene codes for a hypothetical protein of approximately 12 ~ 13.5 kDa expressed in RPE and photoreceptor cells that is associated with the mitochondrial outer membrane, leading to the hypothesis that ARMS2 dysfunction could alter mitochondrial energy metabolism and homeostasis, thereby generating ROS (Kanda et al 2007; Fritsche et al 2008). Animal studies have also provided genetic evidence for a role of oxidative stress in AMD. Deletion of the superoxide dismutase 1 (SOD1) gene, the product of which is responsible for scavenging superoxide, results in mice that develop many of the hallmark features of AMD including drusen, RPE atrophy, and CNV (Imamura et al 2006; Hashizume et al 2008).

The factors described above suggest that oxidative stress is particularly significant in the RPE. Indeed, it has been demonstrated that a variety of these oxidative stressors can cause RPE cell injury and death in cultured RPE cell lines (Lu et al 2006; Qin 2007). Once the protection afforded by the antioxidant system has been overwhelmed, dysfunction and death of RPE cells can occur. Thus, boosting the antioxidant capacity of these cells has the potential to prevent or delay AMD progression (Snodderly 1995; Winkler et al 1999).

Complement-mediated bystander lysis

The activation of complement (McGeer and McGeer 2004) is a sophisticated attack system designed to destroy invaders, stimulate inflammation and assist in the phagocytosis of waste materials. A role for the complement pathway in AMD was first recognized through the biochemical characterization of the composition of drusen. By histochemical analysis and immunocytochemical studies drusen were found to contain numerous components of the complement pathway including complement activators, inhibitors, activation-specific complement fragments as well terminal pathway components (Hageman et al 2001; Anderson et al 2002). Complement cascades consist of the classical, alternative, and lectin pathways (Walport 2001). Antibodies are considered to be the chief activators of the classical complement pathway while molecules, such as lipopolysaccharides, activate the alternative complement pathway. Once the complement system is fully activated, it proceeds to assemble the terminal components (C5b, C6, C7, C8, C9) into the lytic macromolecule C5b-9, known as the membrane attack complex (MAC) that mediates the destruction of the foreign bacteria and viruses. However, if host cells are inadequately protected, they may also suffer a MAC attack in a process termed bystander lysis. Under normal physiological conditions, RPE cells protect themselves from complement-mediated bystander lysis, by expressing high levels of complement pathway regulators, such as complement factor H (CFH) (Chen et al 2007; Wu et al 2007) and CD46 (McLaughlin et al 2003; Vogt et al 2006) that inhibit activation of alternative pathway. However, acquisition of functional mutations or dys-regulation of these regulators during aging can lead to activation of alternate pathway. The Y402H polymorphism in CFH in particular has been proposed to be the major risk factor for acquiring AMD (Edwards et al 2005; Haines et al 2005; Klein et al 2005). In addition, CFH-related proteins (CFHR) including CFHR1, CFHR2, CFHR3, CFHR4, and CFHR5, which lack the complement regulatory activity of CFH can indirectly modulate activation of the complement pathway, likely via competing with CFH (Jozsi and Zipfel 2008). Deletion of CFHR1 and CFHR3 has been reported to be associated with decreased risk of AMD. Meta-analysis reveals that the ORs are 0.35 and 0.63 for a Caucasian population of 173 cases and 170 controls in the UK (Hughes et al 2006) and a Caucasian population of 780 cases and 265 controls in the US (Spencer et al 2008). Similarly, the R32G polymorphism in complement factor B (CFB), an activator of alternative pathway, is significantly associated with a reduced incidence AMD. In a Caucasian population of 698 cases and 282 controls evaluated at Medical Centers at Vanderbilt and Duke Universities, the R32Q variance was strongly associated with reduced risk of AMD (OR, 0.21) after controlling for age, the Y402H polymorphism, and smoking (Spencer et al 2007). A similar result is obtained in the combined Columbia and Iowa cohorts with 551 cases and 269 controls (OR 0.32) (Gold et al 2006). In contrast, genotyping demonstrated that a single nucleotide polymorphism in Complement factor 3 (C3) is significantly correlated with an increased risk of AMD (Maller et al 2007; Yates et al 2007; Spencer et al 2008). In combined English and Scottish groups with a population of 847 cases and 701 controls, the ORs for S/F heterozygotes and F/F homozygotes are 1.7 and 2.6, respectively (Yates et al 2007).

Together, these observations demonstrate a role for complement, particularly the alternative pathway, in the development of AMD. Consistent with this notion, it has been shown that *in vitro*, RPE cell synthesis of CFH is downregulated by direct hydrogen peroxide challenge (Wu et al 2007) or exposure to oxidized POS (Chen et al 2007). Furthermore, light exposure activates complement pathways in A2E-loaded RPE cells (Zhou et al 2006). Thus, it is reasonable to speculate that with aging, increased numbers of RPE cells could be subjected to bystander lysis due to oxidative stress-induced activation of the complement system. While the alternative pathway seems to play the major role in AMD, it was recently reported that mice immunized with mouse serum albumin (MSA) adducted with carboxylpyrrole, an oxidation fragment of docosahexaenoic acid (DHA) develop phenotypes mimicking atrophic AMD such as RPE cell lysis and drusen formation (Hollyfield et al 2008), suggesting that the classical pathway may also be involved in the development of atrophic AMD.

RPE cell inflammatory responses

Drusen biogenesis, in particular soft drusen, is a biological marker of atrophic AMD. Protein components of drusen include immunoglobulins, complement components, acute-phase inflammatory response molecules, and major histocompatibility complex class II antigens (Johnson et al 2000; Mullins et al 2000). Cellular components of drusen include RPE debris, lipofuscin, and dendritic cells (DCs) (Hageman et al 2001). The mechanisms by which drusen is formed are poorly understood. However, compelling evidence from a number of laboratories suggest that inflammatory responses of injured RPE cells serve as the initial events in drusen biogenesis (Hageman et al 2001; Anderson et al 2002). Potential triggers of RPE cell

inflammatory responses discussed here include molecules derived from necrotic RPE cells, complement attack, and oxidized adducts.

Pro-inflammatory danger molecules: DAMPs

Tissue damage arising from nutrient, oxidative, genomic, or mechanical stress is a principal stimulus of inflammation. Since this inflammation occurs without infection, it is defined as sterile inflammation to distinguish it from pathogen-induced inflammation. Although the mechanisms that stimulate the inflammatory response to injured cells remain poorly defined, progress has been made recently in our understanding of the process. In a fashion similar to defenses against invading microorganisms, the innate immune system alerts neighboring cells to the imminent danger by reacting to damage signals generated from dying cells called damage-associated molecular patterns (DAMPs). These signals lead to an inflammatory response by releasing mediators that dilate local vasculature and recruit leukocytes from the blood into the tissue. These DAMP molecules can be in general divided into two categories, intracellular and extracellular DAMPs, based on their origin and mechanism of action. Intracellular DAMPs are bioactive mediators of cellular origin that directly stimulate cells of the innate immune system. Extracellular DAMPs are the cleaved fragments of extracellular components generated by enzymes released from dead cells.

As shown in Figure 1, intracellular DAMPs include the high mobility group box 1 protein (HMGB1) (Klune et al 2008), heat-shock proteins (HSPs) (Chen et al 1999; Asea et al 2000), S100 proteins (Hofmann et al 1999; Foell et al 2007), nucleosomes (Muruve et al 2008), ATP (Idzko et al 2007), and uric acid (Martinon et al 2006). These molecules are expressed in all cell types. Most intracellular DAMPs exhibit a double life. As intracellular molecules, they play a role in cell homeostasis, for example as calcium binding proteins, chaperones, or chromatin-stabilizing molecules. Once released into the extracellular compartment as a result of cell injury, they become 'danger' signals which serve to warn other cells and tissues of imminent danger by activating innate and pro-inflammatory immune responses. For example, S100B, a member of the S100 family of calcium-binding proteins is a ligand for the receptor for advanced glycation endproducts (RAGE) and can stimulate expression of vascular endothelial growth factor (VEGF), transforming-growth factor-β1 and fibronectin in RPE cells (Kim et al 2007; Ma et al 2007). Induction of a variety of cytokines by intracellular DAMPs in RPE cells could contribute to disease progression and potentially the conversion of atrophic to wet AMD.

Extracellular DAMPs are generated through the cleavage of extracellular matrix molecules such as collagen, hyaluronic acid, and heparan sulfate into pro-inflammatory fragments (Wrenshall et al 1999; Johnson et al 2002; Taylor et al 2004; Scheibner et al 2006; Weathington et al 2006). Collagen, heparan sulfate, and hyaluronan are major components of RPE extracellular matrix that have been shown to be expressed in cultured RPE cells (Gross-Jendroska et al 1992; deS et al 2001). Degradation of extracellular matrix proteins is regulated by matrix metalloproteinases (MMPs), a family of zinc-binding, calcium-dependent enzymes (Alexander et al 1990). Release of MMPs from necrotic RPE cells would be expected to disrupt extracellular matrix, generating extracellular DAMPs. However, the extent to which these cellular triggers and the extracellular DAMPs that they generate contribute to the inflammatory response in AMD has not yet been addressed.

The detection of danger signals is made by antigen-presenting cells of the innate immune systems through receptors that recognize DAMP molecules. The known DAMP receptors include CD14, the NACHT-, LRR-, and Pyrin-domain-containing protein-3 (NALP3), P2 receptor, RAGE, and toll-like receptors (TLR) (Figure 2). CD14, NALP3, and P2 receptors detect HSPs (Chen et al 1999; Asea et al 2000), monosodium urate (Martinon et al 2006), and extracellular ATP (Idzko et al 2007), respectively. RAGE (discussed below) is a multi-ligand receptor for advanced glycation end (AGE) product, HMGB1, and S100 proteins (Barile and Schmidt 2007). TLRs recognize heparin suflate, HMGB1, HSP, and hyaluronate (Miyake 2007).

RPE cells make up the first line of defense against pathogens by expressing TLRs, which recognize microbial motifs. The ligation of TLRs leads to a cascade of events that culminates in the production of chemokines, and to the production of cytokines that can induce DC maturation. RPE cells express almost all TLR iso-forms except TLR-8 (Kumar et al 2004). While, activation of TLR-2, 4, and 9 have been demonstrated to result in cytokine production when cells are challenged with DAMP molecules in other systems (Scheibner et al 2006; Miyake 2007) it remains to be addressed whether TLRs have similar roles in mediating RPE cell danger responses.

Oxidized adducts

RPE cells are challenged with oxidative stress due to light irradiation, high oxygen consumption, and phagocytosis

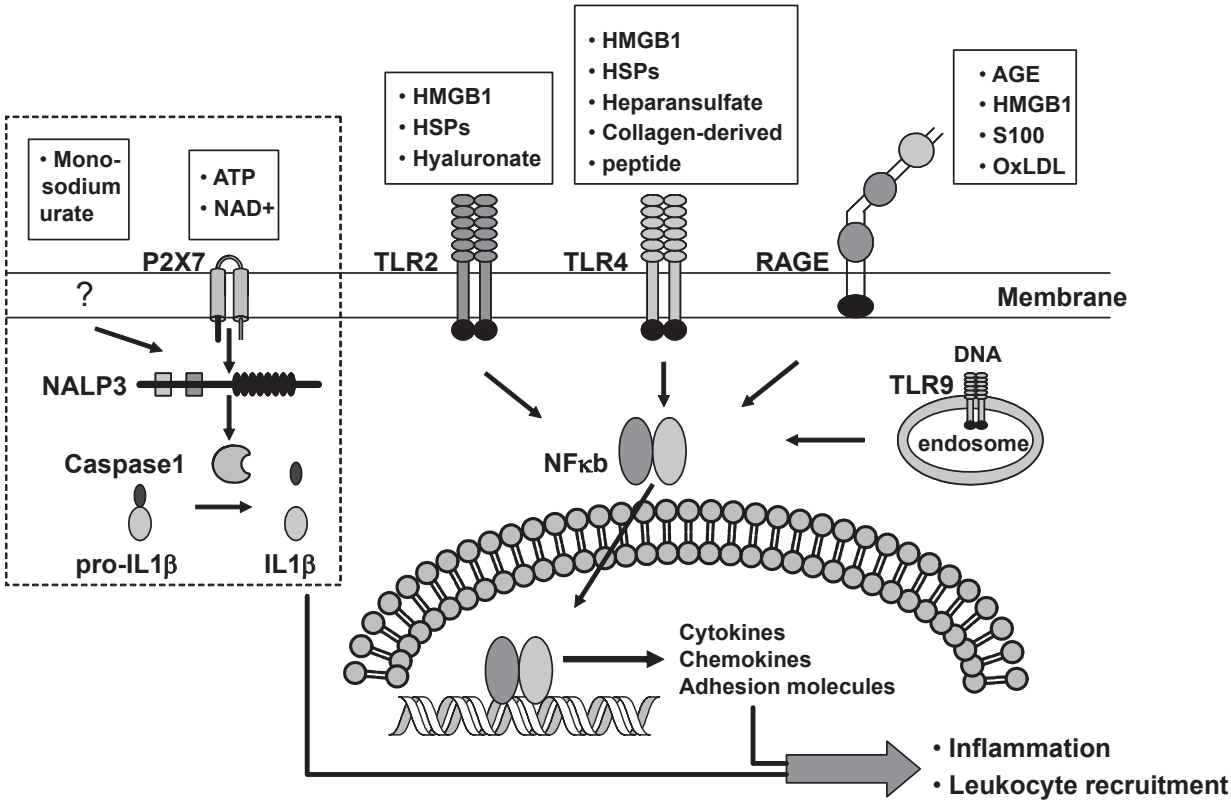

Figure 2 Receptors that sense danger signals released from neighboring injured retinal pigment epithelial (RPE) cells. Toll-like receptors (TLRs) and the receptor for AGE (RAGE) can detect danger signals released from or generated by dying RPE cells. Once stimulated, TLRs and RAGE activate the transcription factor nuclear factor kappa B (NFκB), thereby turning on the transcription of many pro-inflammatory genes and consequently inducing an inflammatory response. The DAMP molecules ATP and monosodium urate stimulate a pathway that works through Nod-like receptor NACHT, LRR, and PYD containing protein-3 (NALP3) and leads to the production of the proinflammatory cytokine interleukin-1β (IL-1β) and IL-18. Those pathways surrounded by dotted lines indicate a current lack of data supporting a functional pathway in RPE cells.
Abbreviations: AGE, advanced glycation endproducts; OxLDL, oxidized low density proteins.

of photo-bleached POS containing high levels of oxidized unsaturated fatty acids and free radicals. Many oxidized molecules, which are toxic to RPE cells, accumulate within and surround RPE cells with aging. Here we will focus our discussion on the relevance of AGE products, oxidized low-density proteins (oxLDL) and carboxyethyl pyrole (CEP) adducts to the etiology of AMD.

AGEs are an heterogeneous group of products derived from nonenzymatic glycation and oxidation of amino groups of proteins, lipids, and nucleic acids. AGEs accumulate during normal aging with their formation being accelerated in a setting of oxidative stress and inflammation (Schleicher et al 1997). There is little or no AGE products detected in normal retina, but expression of AGE products increases concomitantly with drusen formation and development of early atrophic AMD (Howes et al 2004). AGE products are also present in RPE lipofuscin, an enzymatically nondegradable heterogeneous mixture of numerous biomolecules (Schutt et al 2003). With the accumulation of AGE products during aging, RAGE, receptors for AGEs, are simultaneously induced (Yamada et al 2006). RAGE belongs to the immunoglobulin super-family of cell surface molecules that are constitutively expressed at very low levels in numerous cells, including Muller cells, photoreceptor cells, RPE cells, and vascular endothelial cells (Howes et al 2004; McFarlane et al 2005; Barile and Schmidt 2007). Cellular expression of RAGE increases upon ligand binding thus further amplifying cellular activation. *In vivo*, the levels of RAGE are correlated with drusen formation and early development of AMD (Howes et al 2004). Additionally, induction of AGE formation *in vivo* leads to the increased transcription of inflammatory genes in the RPE/choroid (Tian et al 2005). In cultured human RPE cells, activation of AGE-RAGE pathway stimulates expression of VEGF (Ma et al 2007), platelet derived growth factor-b (Handa et al 1998), and production of interlukin-8 (IL-8) and monocyte chemotactic protein-1 (MCP-1) (also known as CCL2) (Bian et al 2001). Moreover *in vitro*, ligand-dependent activation of RAGE can trigger RPE cell death (Howes et al 2004). Similarly, exposure of RPE cells to AGE-modified

matrix but not normal matrix triggers RPE cell death (Honda et al 2001). These observations suggest that activation of the RAGE axis is involved in disease progression and potentially in the conversion of atrophic to wet AMD. Consistent with this notion, transgenic expression of RAGE augmented blood-retinal barrier breakdown and leukostasis, accompanied by increased expression of VEGF and ICAM-1 in the retina in a murine diabetic model (Kaji et al 2007). Moreover, these changes were significantly inhibited by systemic administration of a soluble form of RAGE.

Oxidation of LDL generates malondialdehyde lysine protein adducts or 4-hydroxynoneal cysteine adducts that are the major modifications observed in RPE lipofuscin (Schutt et al 2003). OxLDLs have been shown to accumulate in Bruch's membrane in AMD patients (Yamada et al 2008). Significantly, treatment of RPE cells in culture with oxLDL alters phagosome maturation (Hoppe et al 2004) and inhibits phagocytosis of POS (Hoppe et al 2001), an important RPE cell function essential for outer segment renewal and survival of photoreceptors. Moreover, exposure of RPE cells to oxLDL induces transcriptional alterations in genes related to lipid metabolism, oxidative stress, inflammation and apoptosis (Yamada et al 2008). Consistent with this, ingestion of oxidized POS stimulates production of IL-8 and MCP-1 in cultured RPE cells (Higgins et al 2003). Additionally, as oxLDLs are ligands for scavenger receptors expressed on macrophages oxLDL accumulation leads to the recruitment of macrophages (Kamei et al 2007).

Proteomic analysis of drusen revealed the presence of a number of oxidized proteins (Crabb et al 2002). Most notable of these is CEP adducts. CEP adducts are uniquely generated by the oxidation of docosahexaenoate-containing lipids. Interestingly, docosahexaenoate is abundant in photoreceptors and is the most oxidizable fatty acid in humans (Fliesler and Anderson 1983). Western analysis and immunocytochemistry confirmed that CEP adducts are more abundant in photoreceptors in AMD than normal tissue (Crabb et al 2002; Gu et al 2003). Moreover, CEP immunoreactivity as well as autoantibodies to CEP was higher in plasma from AMD patients (Gu et al 2003). As previously noted, mice immunized with MSA adducted with CEP develop an atrophic AMD-like phenotype (Hollyfield et al 2008). Macrophages are observed in the interphotoreceptor matrix in these mice. Additionally, immunized mice fix C3 in Bruch's membrane (Hollyfield et al 2008). Interestingly, CEP modified human serum albumin was shown to stimulate angiogenesis in ex-vivo models and a subretinal injection of MSA-CEP exacerbated laser-induced CNV in mice (Ebrahem et al 2006). Together, these observations implicate oxidized molecules in the perturbation of RPE cell function and inflammatory responses observed in AMD.

Complement-driven inflammation

Activated complement will injure host cells and induce inflammatory responses under the conditions where loss of function mutations or downregulation of inhibitory regulators of the pathway occurs. In the retina, host cells are protected from complement attack by complement factors such as CFH, a negative regulator of the complement alternative pathway. The RPE is the major local source of CFH at the retina/choroid interface. Thus, mutations or downregulation of CFH may increase the chance of RPE cells being attacked by activated complement systems. During the process of complement cascade activation, the small fragments of cleaved complement components C3a, C4a, and C5a, known as anaphylotoxins, stimulate inflammation. C3a and in particular C5a, are potent inflammatory factors, recruiting leucocytes from blood and stimulating secretion of cytokines that lead to amplification of inflammatory responses (Ember et al 1994; Daffern et al 1995). In cultured RPE cells, treatment with C5a stimulates production of IL-8 (Fukuoka and Medof 2001; Fukuoka et al 2003) and MCP-1 (Ambati et al 2003). Furthermore, C3a and C5a have been shown to be present in drusen (Johnson et al 2001; Ambati et al 2003), supporting the idea that RPE cells are constantly stimulated by the presence of C3a and C5a, which induce the release of inflammatory mediators that subsequently cause further damage to retina *in vivo*. In addition, C3a and C5a are generated early in the course of laser-induced CNV and activation of C3aR or C5aR has been shown to be required for CNV formation (Nozaki et al 2006). Moreover, the alternative pathway has been suggested to play a central role in the CNV development in laser-induced murine model (Bora et al 2006). Taken together, these results suggest that complement-driven inflammation is involved in the etiology of both dry and wet AMD as well as progression of dry to wet AMD.

Chronic infection

RPE cells can be infected *in vitro* with viruses and bacteria such as cytomegalovirus (Bodaghi et al 1999) and *Bacillus cereus* (Moyer et al 2008). The presence of viruses in RPE cells has also been demonstrated in patients (Henderly et al 1988) and in animal models (Vann and Atherton 1991). Additionally, pathogen replication and toxin production can cause RPE death (Vann and Atherton 1991; Moyer et al 2008). Evidence is accumulating that pathogen-induced

chronic infection may be a novel risk factor in the etiology of AMD. An increase in the prevalence of higher-titer antibody to *Chlamydia pneumoniae* was found more often in wet AMD than in dry AMD (Miller et al 2004). Subjects with high antibody titers had a threefold greater risk of progression than those with low antibody titers (Robman et al 2005). Pathogen infection may stimulate macrophage activation and entrapment between the choroid and RPE layer. Local release of cytokines from these macrophages in concert with cytokine release from overlying RPE cells might be involved in the manifestation of certain AMD phenotypes. This is supported by the observation that infection of macrophages and RPE cells with *C. pneumoniae in vitro* induces production of cytokines such as VEGF, IL-8, and MCP-1 (Kalayoglu et al 2005). Moreover, the risk of AMD progression was increased by about 12-fold when, in addition to having the CFH C-risk allele, subjects also presented with high antibodies titers to the bacterial pathogen *C. pneumoniae* (Baird et al 2008). These observations suggest that infection-induced complement activation may participate in AMD progression, although a causal and functional tie between infection and AMD remains to be established.

Recruitment of inflammatory cells

Injury to RPE cells caused by oxidative stress induced by a number of processes including ischemia, photooxidative damage, phagocytosis, and lipofuscin toxicity, serves as the critical seeding event in the initiation of drusen formation by secreting soluble cytokines that initiate recruitment of macrophages and DCs (Hageman et al 2001; Holtkamp et al 2001). Activated macrophages and DCs have been found to accumulate in the subretinal space of patients with AMD (Gupta et al 2003). However, it is unclear whether endogenous macrophages serve protective or destructive functions in these patients. Recruited macrophages have been shown to be required for clearance of drusen and other debris (Duvall and Tso 1985). While activated macrophages and DCs phagocytize RPE debris they also produce mediators that amplify local inflammation (van der Schaft et al 1993; Hageman et al 2001; Penfold et al 2001). In support of a protective role for these cells, impairment in recruitment of macrophages leads to manifestations of atrophic AMD in CCL2 (MCP-1) knockout mice (Ambati et al 2003). Consistent with this, an age-dependent increase in the expression of CCL2 (MCP-1) in the RPE and in macrophage infiltration is observed in the choroids of wild-type mice (Ambati et al 2003). Additionally, IL-$10^{-/-}$ mice have significantly reduced laser-induced CNV formation with increased macrophage infiltrates compared to wild type mice. Moreover, direct injection of macrophages into the eye of these IL-$10^{-/-}$ mice inhibits CNV (Apte et al 2006). However, depletion of macrophages is also reported to inhibit experimental CNV (Espinosa-Heidmann et al 2003; Sakurai et al 2003). These findings suggest that macrophages can serve in both pro- and anti-inflammatory capacities, and that dysregulation of clearance functions may fuel disease progression.

DCs are powerful antigen-presenting cells that participate in the induction of immunity. Histochemical studies have revealed that DCs are present in drusen and are thus proposed to play a role in drusen biogenesis (Hageman et al 2001). According to this hypothesis, injured RPE cells recruit and activate DCs which sustain and amplify the local inflammation by producing inflammatory mediators, activating complement system, and degrading extracellular matrix. The CX3C chemokine receptor 1 (CX3CR1) is expressed in retinal microglia cells (macrophages and DCs) and mediates migration and adhesion of these cells in response to its ligand. Interestingly, mice deficient in CX3CR1 develop some cardinal features of AMD with accumulation of microglia cells in the subretinal space at the sites of retinal degeneration and CNV (Combadiere et al 2007). Manifestations of AMD-like retinal lesions are exacerbated by further knockdown of the chemokine, CCL2 (MCP-1). AMD lesions are found in 6-week-old CX3CR1/CCL2 double-deficient mice while none of the single knockout mice develop retinal lesions at this age (Tuo et al 2007). This may be because of the retention of CX3CR1-deficient microglia cells at the sites of cell and/or tissue injury leading to sustained inflammation. In the laser-induced CNV model, DCs infiltrate transiently into the lesion sites (Eter et al 2008; Nakai et al 2008) and intravenous DC transplantation augments CNV (Nakai et al 2008), supporting a contribution of DCs to the development of AMD.

Recent evidence suggests that lymphoid cells may also be involved in the development of experimental atrophic AMD. When RAG-deficient mice lacking mature lymphocytes were challenged with MSA adducted with CEP, unlike wild-type mice they failed to produce antigen-specific antibodies and fix C3 in Bruch's membrane. Nor did these animals develop an AMD-like phenotype (Hollyfield et al 2008).

Potential therapeutic approaches for atrophic AMD

Damage caused by oxidative stress and inflammation lead to progressive loss of cell function and thus contributes to the development of atrophic AMD. Protecting RPE cells by

neutralizing ROS, promoting cell survival, and inhibiting inflammatory responses could potentially delay the onset or progression of this disease. Discussed below are two treatment strategies: cytoprotection and anti-inflammation therapy.

Cytoprotection

RPE cell death is believed to constitute the primary events in atrophic AMD and subsequent dysfunction and degeneration of photoreceptor cells lead to the loss of cone-mediated high resolution central and color vision. Thus, preservation of RPE and photoreceptor cells could help prevent progression of AMD.

ROS scavengers

Antioxidants have the capacity to alleviate or reduce the detrimental effects of ROS in response to a variety of stress stimuli. Extensive laboratory investigations have demonstrated the role of oxidative stress and the inhibitory effects of antioxidants on RPE cellular function (Choudhary et al 2005; Fernandez-Robredo et al 2005; Volobouéva et al 2005). Clinically, antioxidant supplements may delay the onset of AMD and progression to advanced AMD. The Age-related Eye Disease Study (AREDS) was comprised of 3640 participants who were randomized into cohorts receiving daily high-dose antioxidants (vitamin C 500 mg, vitamin E 400 IU, β-carotene 15 mg), or zinc (80 mg zinc oxide, 2 mg copric oxide), or antioxidants plus zinc or placebo (AREDS. 2001). With an average 6.5-year follow-up, comparison with placebo showed a statistically significant reduction in the development of advanced AMD in those patients receiving antioxidants plus zinc (AREDS. 2001). At 5 years in the absence of supplementation of antioxidants plus zinc, the estimated probability of progression of participants with advanced AMD or vision loss due to nonadvanced AMD to advanced AMD in the second eye was 43%. The 5-year estimated probability of progression to advanced AMD in either eye with extensive intermediate drusen, large drusen, or noncentral geographic atrophy is 18% (AREDS 2001). Thus, people at risk of developing advanced AMD lowered this risk by about 25% when treated according to the AREDS recommendations. It has been estimated that if the 8 million people in the USA at risk of advanced AMD receive AREDS-recommended supplements, 329, 000 of them would avoid developing advanced AMD during the next 5 years (Bressler et al 2003). In addition, dietary lutein/zeaxanthin intake has been shown to be inversely associated with the occurrence of geographic atrophy and large or extensive intermediate drusen (SanGiovanni et al 2007). Taken together these data demonstrate that dietary supplementation with antioxidants reduce the risk of progression of AMD. OT-551, a catalytic antioxidant with anti-inflammatory activity has been shown to functionally and morphologically protect photoreceptor cells against acute light-induced damage in an animal model (Tanito et al 2007) and is currently being assessed in clinical trials in AMD patients with geographic atrophy (Table 1).

Induction of phase 2 antioxidant enzymes including SOD, catalase, glutamate cysteine ligase, and heme oxygenase-1 by phase 2 enzyme inducers provides an alternative way to protect cells against ocular oxidative stress. High amounts of catalase (Tate et al 1995), SOD (Oliver and Newsome 1992), and glutathione (Newsome et al 1994; Beatty et al 2000) can efficiently detoxify and remove ROS once they are generated in RPE cells. GSH synthesis can be induced in cultured RPE cells by a number of naturally occurring compounds that induce phase 2 detoxification enzymes, including the rate-limiting enzyme for GSH synthesis, glutamate cysteine ligase (Nelson et al 1999; Volobouéva et al 2005; Ha et al 2006; Qin et al 2006). RPE cells can be protected from peroxide-induced damage by pre-treatment with a number of agents

Table 1 Developing therapies for atrophic age-related macular degeneration

Compound	Indications	Company	State of development	Mechanism of activation
OT-551	Geographic atrophy	Othera	Phase 2	Antioxidants and anti-inflammation
CNTF (NT-501)	Geographic atrophy and retinitis pigmentosa	Neurotech	Phase 2	Anti-apoptosis
JPE-1375	Geographic atrophy	Jerini	Pre-clinical testing	C5aR antagonist
PMX-53	AMD and arthritis	Arana	Pre-clinical testing	C5aR antagonist
POT-4	Geographic atrophy and inflammatory ocular diseases	Potentia	Phase 1	C3 inhibitor
Fenretinide	Geographic atrophy	Sirion	Phase 2	Vitamin A receptor antagonist

that induce GSH synthesis including dimethylfumarate (Nelson et al 1999), 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (Qin et al 2006), (R)-α-lipoic acid (Volobouéva et al 2005), or zinc (Ha et al 2006). Sulforaphane, a potent inducer of phase 2 antioxidant enzymes, has been demonstrated to protect RPE cells from photooxidative damage *in vitro* (Gao and Talalay 2004; Tanito et al 2005; Zhou et al 2006) and *in vivo* (Tanito et al 2005). A single dose of oltipraze, another potent phase 2 enzyme inducer, significantly stimulated GSH level in lymphocytes isolated from human blood (Gupta et al 1995), and induced a four- to fivefold increase in mRNA transcripts for glutamate cysteine ligase and quinone reductase in colon mucosa (O'Dwyer et al 1996). These findings provide a strong rationale to test whether phase 2 enzyme inducers, such as sulforaphane and oltipraz, can elevate the antioxidant enzymes in the retina and whether they can prevent oxidative damage in humans at risk for AMD.

Antiapoptotic approaches

In addition to the maintenance of antioxidant capacity, RPE cells and photoreceptors can be persevered by stimulating cell survival signaling and/or inhibiting death pathways.

RPE cell survival

Cell viability is precisely regulated by the balanced expression of pro- and antiapoptotic factors such as the Bcl-2 family of proteins. As expected, ectopic expression of antiapoptotic proteins Bcl-2 renders RPE cells resistant to A2E-mediated toxicity (Sparrow and Cai 2001) whereas knockdown of Bcl-xL expression leads to RPE cell death (Zhang et al 2007). Neuroprotectin D1 (NPD1), a metabolite of DHA that is rich in the retina, has been shown to protect against RPE cell apoptosis induced by A2E, a byproduct of phototransduction (Mukherjee et al 2007), as well as hydrogen peroxide and tumor necrosis factor-α (Mukherjee et al 2004). NPD1 and DHA protect RPE cells via upregulation of antiapoptotic proteins and downregulation of pro-apoptotic proteins of Bcl-2 family (Mukherjee et al 2004, 2007). Pigment epithelium-derived factor (PEDF), secreted by RPE cells, is a trophic factor required for RPE cell function and survival under stress conditions. PEDF can protect RPE cells from oxidant-mediated barrier dysfunction (Tsao et al 2006) and death (Ho et al 2006). ERK activation and NPD1 production stimulated by PEDF appear to be involved in mediating PEDF's cyto-protective effects (Tsao et al 2006; Mukherjee et al 2007). Interestingly, PEDF levels in RPE cells are significantly reduced in AMD patients (Bhutto et al 2006). Thus, increases in PEDF levels could potentially delay the onset of RPE abnormalities and progression to RPE cell atrophy.

Photoreceptor cell survival

RPE cell death initiates the development of atrophic AMD. However, loss of vision is caused by the death of rod and cone photoreceptors. Thus, the preservation of macular photoreceptors is a valid anti-AMD strategy. Many trophic factors can support photoreceptor survival (LaVail et al 1992; Steinberg 1994). Among them, PEDF and ciliary neurotrophic factor (CNTF) have demonstrated consistent photoreceptor protection. PEDF protects cultured retinal neurons from hydrogen peroxide injury (Cao et al 1999) and inhibits macrophage-driven inflammation (Zamiri et al 2006). *In vivo*, intravitreal injection or intraocular gene transfer of PEDF rescues photoreceptors from light-induced damage (Cao et al 2001; Imai et al 2005). Moreover, a PEDF (82–121) peptide is as effective as full-length PEDF in protecting retina from ischemia and longer protection can be achieved if delivered in poly(lactide-co-glycolide) (PLGA) nanospheres (Li et al 2006). Taken together with the RPE protective effects, these observations suggest that PEDF is a promising therapeutic treatment for AMD.

CNTF is another trophic factor that has reached clinical development for retinal degeneration (Sieving et al 2006). Injection or gene transfer of CNTF protects photoreceptor degeneration in animal models (LaVail et al 1998; Huang et al 2004). Delivery of CNTF by encapsulated cell-based technology reduces photoreceptor degeneration in animal model of retinitis pigmentosa (Tao et al 2002). This delivery system, which can deliver CNTF to the vitreous for at least 1 year (Thanos et al 2004), is currently being evaluated clinically.

Anti-inflammation therapy

Steroids

Clinical studies have indicated that corticosteroid, triamcinolone acetonide (TA), has the capacity to reduce the permeability of the outer blood–retinal barrier (Antoszyk et al 1993), promote resorption of subretinal exudation (Penfold et al 1995) and downregulate inflammatory stimuli (Tanner et al 1998). After 18 months of treatment with TA in patients with exudative AMD, the rate of development of severe visual loss in treated patients was 30%, compared with 55% for historical controls (Challa et al 1998). Anecortave acetate is another angiostatic steroid that might be clinically efficacious for maintaining vision, preventing severe vision loss, and inhibiting subfoveal CNV lesion growth (Russell et al 2007). Anecortave acetate was approved for use in wet AMD in Australia in 2005 with approval pending in the US. A third steroid, Posurdex (dexamethasone implant) is in

phase III clinical trial for retinal vein occlusion and diabetic macular edema. Although no clinical data are available yet, the role of chronic inflammation in atrophic AMD pathogenesis (Hageman et al 2001; Anderson et al 2002) has led to the consideration that atrophic AMD patients might be expected to benefit from anti-inflammatory therapy that attenuates the local inflammation, thereby preventing progression of the disease.

Nonsteroidal anti-inflammatory drug

Epidemiology and animal studies suggest that nonsteroidal anti-inflammatory drugs (NSAIDs) can delay the onset and progression of Alzheimer's disease (AD) (Townsend and Pratico 2005; McGeer and McGeer 2007). Amyloid-β (Aβ), a major trigger of AD pathogenesis, is present in drusen from retinas with AMD (Dentchev et al 2003). Owing to the compositional similarities between drusen and AD-associated plaques, atrophic AMD patients might be expected to benefit from NSAID therapy that may attenuate local inflammation (Mullins et al 2000; Luibl et al 2006). Consistent with this, accumulation of Aβ causes RPE atrophy and basal deposit formation, characteristic features of human AMD (Yoshida et al 2005). Moreover, the addition of Aβ to cultured RPE cells upregulates expression of VEGF (Yoshida et al 2005; Ma et al 2007) and downregulates PEDF expression (Yoshida et al 2005). The anti-inflammatory compounds eicosapentaenoic acid and astaxanthin have been shown to inhibit cytokine production, macrophage activation, and CNV formation in mice (Koto et al 2007; Izumi-Nagai et al 2008). Consistent with these observations, the prevalence of AMD in rheumatoid arthritis patients with long term NSAID use is about 10-fold lower than in general populations of similar racial origin (McGeer and Sibley 2005). Thus, additional studies of the potential utility of NSAIDs for the treatment of AMD patients are warranted.

Complement pathway regulators

As discussed in the section III, certain CFH haplotypes are associated with increased frequency of AMD (Hageman et al 2005; Hughes et al 2006). In contrast, the L9H variant of CFB, which participates in the amplification of the complement alternative pathway, confers a significantly reduced risk for AMD (Gold et al 2006). Conceivably, delivery of a protective variant of the CFH or CFB through gene therapy or as a biologic could block activation of the alternative pathway and serve as an anti-AMD strategy. Alternatively, as CFB and CFD are both serine proteases small-molecule, inhibitors of these proteins could be used to inhibit activation of the alternate pathway.

Reduction of the CFH-associated AMD may also be achieved by inhibiting targets downstream the point of conversion for all three complement pathways. JPE-1375 and PMX53 are two small molecule peptidomimetic antagonists targeting the C5a receptor (Schnatbaum et al 2006) and are in preclinical evaluation for AMD. POT-4 is a cyclic peptide capable of binding to human C3 and prevents its activation, resulting in broad and potent complement activation inhibition (Nilsson et al 1998). POT-4 is the first complement inhibitor that has entered into a Phase I clinical trial for AMD (Table 1).

Other novel therapeutic approaches

Besides antioxidant and anti-inflammation therapies, other novel therapeutic approaches are currently under development for the treatment of atrophic AMD. Lipofuscin in RPE cells is an autofluorescent lipid-retinoid aggregate derived from visual cycle retinoids in photoreceptor cells. The major component of lipofuscin is A2E, a product of condensation of two molecules of all-trans-retinal with one molecule of phosphatidylethanolamine (Sparrow et al 2003). Lipofuscin accumulation in RPE cells is the key feature of the aging retina (Sparrow and Boulton 2005). *In vitro*, A2E can inhibit lysosomal function, perturb cholesterol metabolism, cause light-induced oxidative stress to RPE cells as well as activate the complement pathway (Bergmann et al 2004; Zhou et al 2006; Lakkaraju et al 2007). Therefore, reducing A2E accumulation in RPE cells by inhibiting the visual cycle could lead to delay in visual loss in the patients with atrophic AMD. Fenretinide, a synthetic nontoxic retinoid, competes with all-trans-retinol binding to retinol binding protein, thereby reducing ocular all-trans-retinol uptake and inhibiting the visual cycle (Radu et al 2005). Thus, administration of fenretinide effectively blocks A2E production and lipofuscin accumulation (Radu et al 2005). Fenretinide is currently in clinical trials to evaluate its efficacy on AMD patients with geographic atrophy.

Issues and challenges

Etiology of atrophic AMD

Despite an increase in our understanding of the immunological factors involved in atrophic AMD, this knowledge has yet to be translated into new treatments. One possible reason for this is our lack of understanding of disease chronicity, and the causative rather than risk factors that contribute to disease. Do drusen materials in AMD represent an abnormal or normal accumulation of metabolic debris? Why do soft drusen appear in macular area? What specific factors lead

to atrophy of the RPE and photoreceptors? The answers to these questions could one day be exploited to provide ideas for novel therapies. Another issue is the variability in disease characteristics at different stages of progression. As our understanding of the natural history of atrophic AMD grows, eligibility criteria for clinical trials might be tailored depending on the mechanism of action of a particular drug candidate.

Animal models

A variety of animal models have been developed to investigate the pathogenesis of atrophic AMD and test therapeutic compounds to ameliorate the pathology. Inflammatory models of AMD have been generated to elucidate the role of inflammation in the etiology of AMD. One such model can be induced by immunization of mice with MSA adducted with CEP, an oxidation fragment of DHA (Hollyfield et al 2008). Mice challenged with the CEP produce antigen-specific antibodies, develop drusen under the RPE and lesions mimicking atrophic AMD. Moreover, macrophages are observed in the interphotoreceptor matrix in these mice (Hollyfield et al 2008). As complement deposition is also observed below the RPE in this model the phenotype appears to be driven by activation of this pathway. Other inflammatory models include mice with knockouts of the chemokine receptor CX3CR1 with CCL2 (MCP-1) or CCR2 in combination with its ligand, CCL2 as well (Ambati et al 2003; Combadiere et al 2007; Tuo et al 2007). These knockout mice display many of the features of AMD including the accumulation of lipofuscin, A2E, drusen as well as photoreceptor atrophy and CNV. Additionally, in the case of the CX3CR1 knockouts, subretinal accumulation of microglial cells was observed (Combadiere et al 2007; Tuo et al 2007).

There are also a number of noninflammatory models that have been developed. The sodium iodate model involves the selective induction of RPE necrosis followed by photoreceptor degeneration in a pattern resembling gyrate atrophy of the choroid and retina (Kiuchi et al 2002). The mcd/mcd mouse was generated using a dominant negative form of Cathepsin D which is required for RPE phagocytosis of shed POS membrane discs (Rakoczy et al 2002). This transgenic mouse model manifests features of AMD including lipofuscin accumulation and both photoreceptor and RPE atrophy. The SOD1-deficient mouse represents a novel model of ROS-mediated retinal degeneration, which similarly displays features of AMD such as drusen, RPE atrophy, and CNV (Imamura et al 2006; Hashizume et al 2008).

The extrapolation of experimental data obtained from animal studies to explain the *in vivo* pathological conditions must be done with caution. First, the mouse retina does not contain a specialized macula, thus, a murine model is not an exact replica of the human condition. Moreover, the components of drusen described in many animal models have not been molecularly characterized to confirm a similarity with human drusen. In this regard, some progress has been made to identify a nonhuman primate model of AMD. A cynomolgus monkey pedigree has been identified that develops early onset macular degeneration (Umeda et al 2005). Interestingly, many components in the drusen of these animals are comparable to those found in human drusen (Umeda et al 2005). Furthermore, the loss of ultrastructure and function as well as progression of the disease is similar to that observed in humans (Dawson et al 2008).

AMD is a multi-factor disease; however, the available animal models only evaluate the contributions of one or at most two risk factors. Thus this limits the utility of these animal models of AMD for drug testing. Frequently, in the case of genetically modified animals the penetrance of the observed phenotype is not complete. Moreover, the appearance of many of the AMD-like features occurs over a protracted period. Thus, a large number of animals and a prolonged treatment regimen would be required to test potential drug candidates. Efforts to accelerate the manifestation of the measurable features of these models are ongoing. Crossing various transgenic and knockout mice may accelerate the course of the disease. Alternatively, modification of environmental factors such as diet or the addition of other risk factors could similarly hasten disease progression. For example, the addition of paraquat to SOD1-defficient mice has been shown to result in changes in electroretinography measurements within two weeks (Dong et al 2006). These efforts have the potential to enhance the utility of these models for drug development significantly.

Conclusions and future studies

Currently, most treatments for AMD such as laser photocoagulation, photodynamic therapy, surgery, and anti-VEGF antibodies benefit patients in advanced stages of the disease. The progressive nature of atrophic AMD and the extensive cell damage in affected individuals at the time of diagnosis indicates that the future of atrophic AMD therapeutics is in the prevention of progression of the disease. Any drug for disease prevention needs to fulfill two crucial requirements. First, the drug should target a key mechanism responsible for the initiation or progression of the disease process. Second, the drug should have minimal side

effects and be tolerated for extended periods. To date no such therapy is available for atrophic AMD. Thus, educating people about the risk factors associated with this disease such as smoking and diet is an important consideration at present. The development of pharmacological approaches to prevent onset or progression of AMD faces significant hurdles including a lack of understanding of the molecular mechanisms underlying the disease and the paucity of experimental models for testing potential drugs. Although a causal relationship based upon mechanistic studies of oxidative injury and inflammation has not been completely established, the cumulative evidence presented in this review suggests a strong correlation between oxidative stress, inflammation and AMD. This represents a significant step towards the goal of identifying more effective treatments for this disease.

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