Special Issue

Dry Age-Related Macular Degeneration: Mechanisms, Therapeutic Targets, and Imaging

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Age-related macular degeneration is the leading cause of irreversible visual dysfunction in individuals over 65 in Western Society. Patients with AMD are classified as having early stage disease (early AMD), in which visual function is affected, or late AMD (generally characterized as either "wet" neovascular AMD, "dry" atrophic AMD or both), in which central vision is severely compromised or lost. Until recently, there have been no therapies available to treat the disorder(s). Now, the most common wet form of late-stage AMD, choroidal neovascularization, generally responds to treatment with anti–vascular endothelial growth factor therapies. Nevertheless, there are no current therapies to restore lost vision in eyes with advanced atrophic AMD. Oral supplementation with the Age-Related Eye Disease Study (AREDS) or AREDS2 formulation (antioxidant vitamins C and E, lutein, zeaxanthin, and zinc) has been shown to reduce the risk of progression to advanced AMD, although the impact was in neovascular rather than atrophic AMD. Recent findings, however, have demonstrated several features of early AMD that are likely to be druggable targets for treatment. Studies have established that much of the genetic risk for AMD is associated with complement genes. Consequently, several complement-based therapeutic treatment approaches are being pursued. Potential treatment strategies against AMD deposit formation and protein and/or lipid deposition will be discussed, including anti-amyloid therapies. In addition, the role of autophagy in AMD and prevention of oxidative stress through modulation of the antioxidant system will be explored. Finally, the success of these new therapies in clinical trials and beyond relies on early detection, disease typing, and predicting disease progression, areas that are currently being rapidly transformed by improving imaging modalities and functional assays.

Keywords: drusen, complement, autophagy, functional imaging, therapeutic targets

Age-related macular degeneration (AMD) is the leading cause of blindness in people 65 years of age or older in developed countries.[1,2] Currently, patients with AMD are classified as having early AMD, intermediate, and late AMD based on the appearance of the macula.[3] In early and intermediate AMD, drusen and pigmentary changes are visible and visual function is often affected. In late or advanced stage disease, neovascularization and/or atrophy are visible in the macula and central vision is more often severely compromised. Typical symptoms in late stage disease include decreased night vision and progressive loss of central vision. Loss of vision is attributed to macular drop out of RPE and photoreceptors (Fig. 1), termed geographic atrophy (GA); or from the invasion of RPE and/or retina by abnormal blood vessels, termed neovascular, exudative, or "wet" AMD as this involves choroidal neovascularization (CNV).[4] In this review, we will focus on emerging therapeutic interventions in early AMD (Fig. 2A) and also GA (Fig. 2B), which accounts for 20% of legal blindness related to AMD and produces reduced visual function (e.g., reduced reading speed) due to ring-shaped vision loss sparing the center.[5–7]

Progress in understanding CNV pathogenesis has led to development of several Food and Drug Administration (FDA)-approved therapies for wet AMD, including anti-VEGF agents, and emerging therapies targeting vessel maturation and remodeling versus angiogenesis.[8–10] In contrast, the pathogenic mechanisms in GA are still unclear and there is no existing FDA-approved therapy. Presently, there is no treatment available to repair damaged RPE cells or photoreceptor cells. Thus, treatment approaches will likely be focused on early intervention that precedes RPE cell loss and in later stages, replacement with stem cell–derived RPE or photoreceptor cells.

Diagnosis of AMD is primarily achieved through various ocular-imaging techniques that have evolved considerably over the past few years and are facilitating development of evidence-based classification system for early forms of AMD as well as refining classification of late AMD.

Dry AMD Deposits

The pathogenesis of early AMD is characterized by thickening of Bruch membrane (BrM) due to lipid and protein accumulation that lead to formation of sub-RPE deposits that occur as discrete accumulations, called drusen, which can be hard or soft, or as continuous accumulations. The lipid build up is thought to primarily interfere with the fluid efflux from the RPE across BrM, thereby inflicting stress on the RPE.[11] These and other stressors (e.g., oxidative stress from smoking and aging)

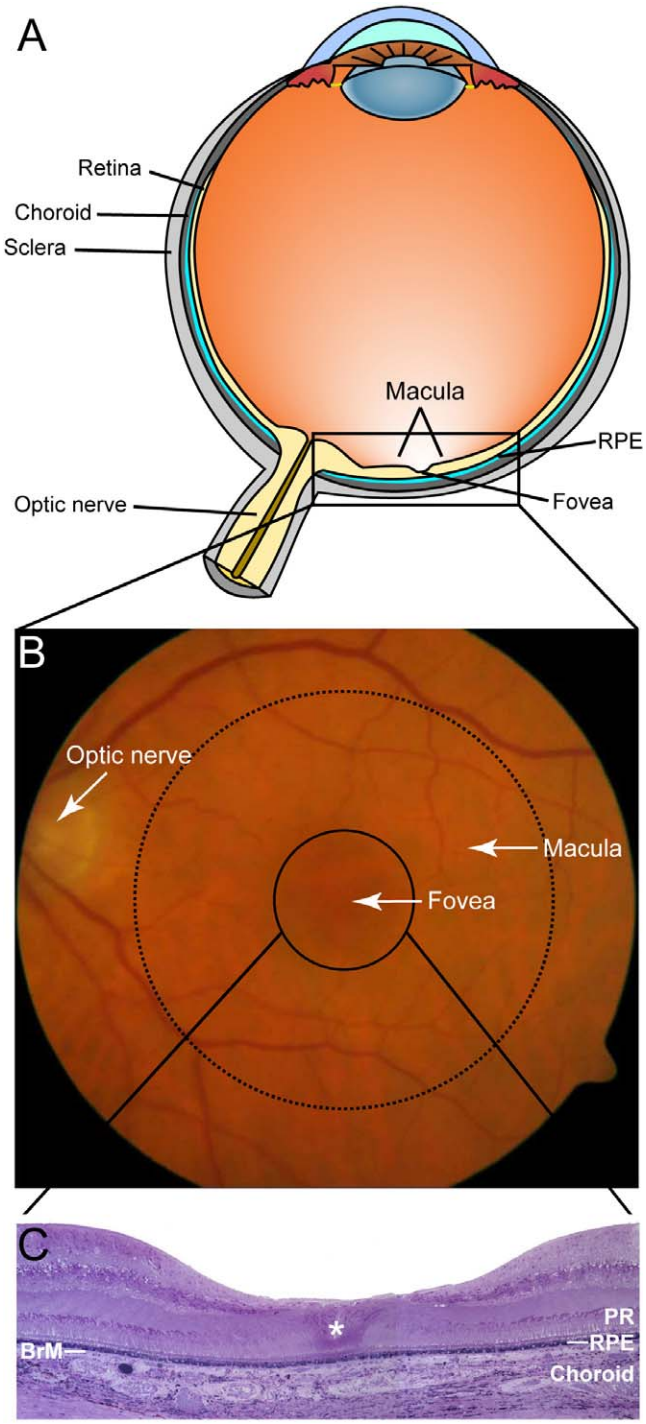

Figure 1. Ocular anatomy of a healthy eye relevant to AMD. (**A**) Cross-section schematic of a human eye showing major structures. (**B**) Color fundus photograph from an elderly patient covering the area indicated in (**A**) showing a healthy macula (*dashed line*, ø = ~6 mm), healthy fovea (*solid line*, ø = ~1.5–2 mm), and healthy optic nerve head (ø = ~1.5–2 mm), respectively (photograph courtesy of Eleonora Lad). (**C**) Immunohistochemical cross-section of the foveal region indicated in (**B**), foveola (ø = 0.2–0.3 mm, indicated by *), which contains only cone photoreceptors and no rods, is located at the center of the foveal pit. Photoreceptor layer (PR), RPE, BrM, and choroid, are indicated (fovea image courtesy of Christine Curcio).

result in an increased accumulation of lipofuscin in RPE cells, which in turn affect lysosome function and cholesterol metabolism.[12] Cells under stress are known to increase the release of membranous vesicles such as exosomes and it is possible that this process is in part responsible for the deposits in the sub-RPE region.[13–16] In addition, a number of proteins found in drusen are serum proteins, suggesting that impairments in fluid transport across BrM also might play a more direct role in drusen formation and deposition.[17,18]

Sub–retinal pigment epithelial deposits are classified as basal laminar deposits (BlamD) or basal linear deposits (BlinD). Basal laminar deposits consist of membranous material and wide- or long-spaced collagen between the plasma membrane and basal lamina of the RPE.[11] Basal linear deposits consist of vesicular material located in the inner collagenous layer of BrM. Basal linear deposits (0.4–2 μm) and soft drusen (30–300 μm) are considered differently sized assemblies (layer and protrusion) of the same aggregate.[11] Hard drusen (<63 μm) have higher apolipoprotein content than soft drusen and are much less fragile upon dissection.[11,19–21]

The composition of drusen has been investigated and described by a number of investigators,[18,22–26] and are discussed in more detail below for their potential as targets for AMD therapies. The role of drusen in the pathogenesis of AMD has not been clarified, although it has long been known that they constitute hallmark lesions of AMD. Studies focused on delineating their components and origin have provided insights into pathways associated with early AMD,[23] including the complement pathway and amyloid deposition discussed later.

As a result of the decreased flow of nutrients across BrM and the physical displacement caused by drusen, areas of hypopigmentation of the RPE monolayer on histologic tissue sections can be observed in the macula. Hyperpigmented areas are often located adjacent to hypopigmented regions, and have been proposed to be due to RPE cell proliferation as a response to RPE cell loss.[27] On RPE flat mounts of AMD eyes the macular region contain many large and multinucleate cells (≥2 nuclei) as opposed to a healthy cell monolayer composed of mostly equally-sized mononucleate and a small proportion binucleate (~3%) RPE cells.[28–31] These areas of RPE cell heterogeneity may be due to RPE cell death and dropout. Ultimately, areas of confluent RPE cell loss can occur, which can be visualized by fundus autofluorescence (FAF) and SD-OCT, and are classified as GA (Fig. 2D, 2F).

Current Treatment Option

Age-Related Eye Disease Study Formulation

A number of studies in the 1980s and 1990s identified a link between antioxidant status, zinc levels, and risk of AMD.[32–36] To further investigate these associations, the Age-Related Eye Disease Study (AREDS) was implemented. Results reported from the first AREDS (AREDS1) documented slowed progression to the late wet form of AMD when taking a formulation of beta-carotene, vitamin C, vitamin E, and zinc.[37] However, the benefit of zinc in this formulation has been debated.[38–41] A recent meta-analysis on the effect of zinc supplementation in prevention of AMD also concluded that available data are inconclusive.[42] Recently, it has been shown that daily zinc supplements for 3 months in AMD patients lowered complement activation as measured by C3d/C3 ratio in serum, but this effect was only statistically significant in patients that already had high complement activation at the start of treatment.[43] While intriguing, it is unknown whether this systemic zinc-mediated effect on complement activation also occurs in the eye and if so, how AMD progression is altered.

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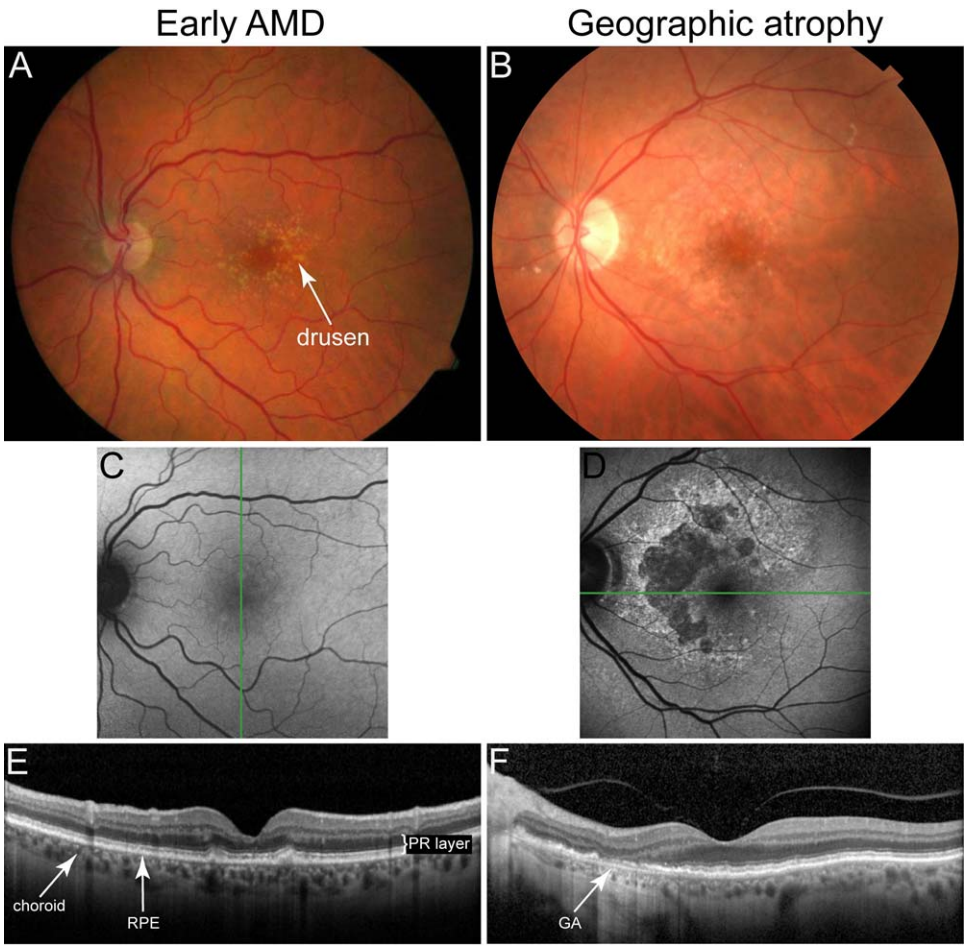

Figure 2. Fundus photography, FAF, and SD-OCT images of early (**A**, **C**, **E**) and late cases (**B**, **D**, **F**) of dry AMD. Fundus photographs of early AMD (**A**) and GA (**B**) are not easily differentiated. However, in FAF images (**C**, **D**) the difference is striking. In SD-OCT B-scan images the RPE and PR layers can be delineated in early stage dry AMD (**E**) while these layers are missing in the area of GA (*arrow* in [**F**]). Spectral-domain OCT scans were acquired from the location indicated by the green lines on the FAF images (images courtesy of Eleonora Lad).

Based on data from earlier studies suggesting protective effects of lutein plus zeaxanthin and omega-3 fatty acids in AMD,[35,44,45] these ingredients were added to the AREDS formulation in AREDS2 and the results of AREDS2 supplementation are discussed in a companion chapter in this supplement by Emily Chew. To date, the only treatment for dry AMD consists of daily intake of an AREDS formulation.

Drusen-Associated Proteins

As discussed above, drusen, BlamD, and BlinD constitute some of the clinical hallmarks of AMD.[11] These deposits consist of proteins and lipids that are also found in plaques and deposits in other age-related degenerative disorders such as atherosclerosis,[26] Alzheimer's disease,[46,47] and a subset of Prion diseases,[48,49] among others.[50] The deposited proteins include vitronectin, clusterin (ApoJ), apolipoprotein E (ApoE), (serum) amyloid P (SAP), complement components, and amyloid-β (Aβ).[18,51] The appearance of a small number of hard drusen (< 63-μm diameter) is a normal age-related change in the eye.[20,52] However, soft "diffuse" drusen correlate with progression of AMD.[53–55]

It is not known if drusen are the primary cause for the degeneration of RPE in AMD, but they do ultimately affect RPE health due to impaired transport across BrM.[11] Thus, elimination of these soft drusen present as an obvious therapeutic target to slow or inhibit AMD progression. The direct approach to target individual components of drusen has demonstrated some promise.[12,56] Recently, we demonstrated that systemic treatment with an antibody against Aβ protects against RPE damage and vision loss in an AMD mouse model.[29,57] Clearance of Aβ from sub-RPE deposits coincided with protection of visual function and structural preservation of the RPE, identifying Aβ as a druggable target. Another approach to remove Aβ from these deposits could be to induce higher expression of ApoE. Apolipoprotein E promotes proteolytic degradation of Aβ,[58] and this effect is thought to be the mechanism for the enhanced Aβ clearance in the brain of some models of Aβ deposition,[59] although this study is being revisited due to recently published conflicting data.[60–64]

Other constituents of drusen being targeted in preclinical and clinical trials include components of the complement system (e.g., C3 and C5).[65] In addition, there are a number of drusen components that are potential drug targets that have not yet been pursued in treatment of AMD. Some obvious targets include vitronectin and clusterin, molecules involved in the acute-phase response to inflammation such as amyloid P component, and elements of lipid metabolism in addition to ApoE such as Apolipoprotein B, and peroxidized lipids.[19,25,26,66–68]

Complement Pathway in AMD

The identification of polymorphisms in genes coding for complement factor H (CFH),[69–72] factor B,[73] and C3,[74] which confer greater "risk" for developing AMD, supported earlier pathobiologic investigations that led to the identification of numerous complement proteins in drusen.[25,75,76] These studies implicated the complement system as an important biological pathway in development of AMD. The complement system is a component of our antigen-nonspecific defense mechanism or innate immunity and it consists of three pathways, classical, lectin, and alternative that all converge on C3.[77]

It is now apparent that dysregulation of the complement cascade, and of the alternative pathway in particular,[78,79] is a critical predisposing step in AMD development. Although the precise triggering event(s) that provokes RPE–choroidal pathology are unknown, it is clear that a major consequence is the deposition and sequestration of cellular and acellular debris in the sub-RPE space that leads to drusen formation.

Complement activation products, produced as part of the inflammatory response, can have beneficial effects by facilitating phagocytosis and removal of cellular debris, or they can be detrimental by causing bystander damage to surrounding tissues. Currently, the bystander damage through complement dysregulation is suspected in AMD, where those patients lacking sufficient alternative pathway-modulating activity have subsequently sustained complement attack, bystander injury to neighboring cells, continued formation of drusen and other sub-RPE deposits, and eventually vision loss.[65,79] This is supported by the ocular phenotype in *CFH* knock-out mice, generated to model factor H deficiency in humans,[80] which revealed that old *CFH* knock-out animals exhibit an age-related decrease in visual acuity (VA) with evidence of C3 deposition.[81]

CFH, which is implicated as the strongest genetic risk factor for AMD,[69–72] is an inhibitor of the alternative pathway of complement. Despite knowing for many years that CFH variants confer AMD risk, the underlying molecular mechanisms attributing to the risk factor activity of CFH remains unclear. CFH circulates at high concentrations in plasma but is also synthesized in the eye by the RPE,[79] and the relative contribution of these sources of CFH to local complement regulation within sub-RPE deposits is not known. Recently, a study has shown that CFH may neutralize the pro-inflammatory effects of malondialdehyde (MDA)-induced inflammation in an AMD-risk associated manner.[82] This study suggests that CFH also functions to attenuate oxidative stress insults leading to AMD, which may explain why clinical trials using complement inhibition-based treatment of AMD (targeting C3 or C5, e.g.) have produced disappointing outcome data that show minimal improvement in VA or reduction in disease progression.[65]

Oxidative Stress

The retina is particularly susceptible to oxidative stress because of its high consumption of oxygen, large amounts of polyunsaturated fatty acids concentrated in the photoreceptors, and continual exposure to visible light.[83] Oxidative stress has long been hypothesized to play a substantive role in the development of AMD due to the high oxidative stress environment of the fundus. Oxidative stress in the retina is aggravated by lipofuscin, which accumulates in the RPE with age,[84] especially in AMD eyes.[85] Cigarette smoking, considered a strong oxidative stressor, remains the most consistent preventable risk factor for AMD across all studies[86]; considering these stressors, it is remarkable that most individuals maintain homeostasis throughout life. This is likely due to the presence of a range of efficient antioxidants and repair systems. For example, the macular pigment, formed by lutein and zeaxanthin, is a natural barrier protecting the central retina against oxidative damage believed to limit retinal oxidative damage by absorbing incoming blue light and/or quenching reactive oxygen species.

Antioxidants beyond vitamins and minerals (i.e., AREDS supplements, which have only been shown to be efficacious in the eye) are also being investigated as AMD therapies and may be effective by acting as neuroprotective agents, preventing toxicity in the retina as well as interfering with cell death pathways. Agents that augment the intrinsic antioxidant functions of the retina are promising candidates for the prevention and treatment of early AMD. These compounds exert their protective effects by modulating nuclear factor erythroid 2-related factor 2 (Nrf2), commonly involved in regulating the expression of genes encoding various antioxidant and antistress proteins.[87,88]

Autophagy

Autophagy is an essential lysosomal pathway that degrades cytoplasmic proteins and damaged organelles.[89] This is especially important in highly metabolically active and highly phagocytic nondividing cells such as the RPE, which phagocytose photoreceptor outer-segment discs daily. As discussed above, RPE cells run a high risk of oxidative damage due to their exposure to high levels of lipid peroxidation products from photoreceptors, constant exposure to light stimuli, and their high oxygen utilization.[90] There are three autophagic pathways (chaperone-mediated, micro-, and macroautophagy), of which macroautophagy is the primary route for transport of organelles and protein aggregates to the lysosome.[91] The focus, for this review, will be on the macroautophagy pathway, which will be referred to as autophagy. Autophagy is initiated by the formation of the autophagosome, a double-membrane vesicle containing lipids, damaged organelles and/or cytoplasmic proteins. After their formation, autophagosomes undergo a strictly controlled fusion process with lysosomes where their contents are degraded by lysosomal enzymes.[92]

Autophagy pathways also have intracellular quality control functions, especially in the turnover of proteins that are prone to aggregation.[93] It has been suggested that aggregate formation is prevented by autophagic degradation into oligomers and monomers.[94] Thus, disturbances in autophagy in RPE cells may be a contributing factor in generation of protein aggregates seen in sub-RPE deposits and drusen in AMD.

During RPE cell aging, autophagic, and lysosomal degradation pathways lead to the accumulation of lysosomal lipofuscin, which is considered to contribute toward the pathogenesis of AMD.[95,96] There is considerable evidence that lipofuscin and one of its major fluorophores, A2E, can increase lysosomal pH and reduce lysosomal degradation which, in turn, further reduce the functional capacity of the RPE and increase cellular stress[97–100]; thus, lipofuscin accumulation is a hallmark of RPE cell aging, which adversely affects the lysosomes' capacity to degrade proteins.[97–100] Numerous studies have shown a strong association between lipofuscin accumulation and retinal degenerations such as AMD.[95] Furthermore, as a form of protein quality control, protein aggregates are delivered to the lysosome by autophagy pathways.[101,102] This might be one of the few ways to remove large, preformed aggregates from the cell. Thus, disturbances in lysosomal function by accumulated lipofuscin[97,98] may increase the misfolding of intracellular proteins by reducing the efficiency of this clearance mechanism.[92] Though the precise mechanisms by which the RPE becomes compromised by aging and in AMD has not been

elucidated, a decrease in the removal and replacement of aggregated proteins and damaged intracellular organelles is likely to play a significant role.

It has recently been demonstrated that lysosomal activity decreases, and markers of autophagy accumulate in human AMD donor samples.[16] In addition, as discussed in an earlier section, it is known that stressed RPE releases exosomes that are coated with complement and can bind CFH,[15,16] suggesting that this may be a mechanism for sub-RPE deposit formation. Preservation of autophagic activity might be associated with a decrease of intracellular accumulation of damaged proteins, which may delay the RPE aging process.[103,104] Conversely, autophagy may trigger cell death by excessive self-digestion.[105] Thus, dysregulation of autophagy can result in cellular dysfunction. AMD has degenerative characteristics including protein deposits, and in certain cases, proliferative characteristics as occurs in wet AMD; thus, there is no consensus as to whether autophagy inhibitors or activators would be beneficial in AMD therapy, and how they should be used for different phenotypes of AMD.

A recently identified target for modulation of autophagy in RPE cells is the serine/threonine kinase mammalian target of rapamycin (mTOR) that regulates the damaging hypertrophy and dedifferentiation of RPE cells exposed to oxidative stress. Rapamycin-induced inhibition of mTOR can prevent these effects and preserve photoreceptor functions.[106] However, rapamycin has a number of off-target effects, which have limited its practicality in age-related neurological disorders.[107]

Intraocular delivery of drugs could potentially circumvent a number of systemic side effects that would otherwise be an issue with many autophagy-modulating therapies. Gene therapy for treatment of AMD targeting autophagic pathways is also an interesting future option.[108]

Several environmental and genetic risk factors for AMD progression may be associated with lysosomal dysfunction, including buildup of sub-RPE deposits, drusen, lipofuscin, and chronic inflammation and likely lead to decreased autophagy flux in RPE cells. Thus, autophagy may represent an important therapeutic target in AMD.

Immune Cells in AMD Microglia

Microglia are resident immune cells in the central nervous system (CNS) and retina and constitute the main immune defense in CNS.[109,110] They enter the retina during development and are activated by retinal injury and degeneration, transforming from quiescent stellate-shaped cells into large amoeboid-shaped cells. Activated microglia proliferate, migrate to areas of damage, degrade and clear debris, and secrete pro-inflammatory cytokines and chemokines.[110] Long-term activation of microglia results in chronic neuroinflammation. Studies of AMD retinas suggest that widespread activation and migration of microglia does not occur during early stages of AMD. However, at intermediate to late stage dry AMD and GA, RPE and photoreceptor damage leads to accumulation of activated microglia at the site of macular injury suggesting that these may be relevant targets in late disease.[111]

Macrophages

Though AMD is not a classical inflammatory disease, increased numbers of macrophages have been detected in areas of BrM damage and RPE atrophy.[112–116] Importantly, macrophages are also found in the choroid of healthy human eyes, but do not express the activation marker inducible nitric oxide synthase (iNOS). However, choroidal macrophages (as well as endothelial cells and pericytes) do express iNOS when they have been recruited to BrM in early AMD eyes with soft drusen or thick continuous BlamD. Macrophages recruited to active disciform scars also express iNOS.[117]

There are two subtypes of macrophages: the pro-inflammatory M1 macrophages, and the mostly anti-inflammatory M2 macrophages, whose main function is scavenging and tissue remodeling. M2 macrophages are thought to perform the beneficial, long-term housekeeping role of scavenging deposits such as degrading and removing drusen in early stages of the disease. M1 macrophages on the other hand, might incite and exacerbate the inflammatory responses to retinal injury.[118] Interestingly, a recent study by Sene et al.[119] found that altered cholesterol homeostasis due to decreased ABCA1 expression in aging macrophages promoted a switch from the M1 to the M2 phenotype. In the paradigm of CNV, the pro-angiogenic properties of M2 macrophages has been shown to promote progression of wet AMD.[119] However, in the case of dry AMD, it is entirely unknown which of the two macrophage subtypes would be the most beneficial for degradation of drusen and prevention of RPE cell loss. Thus, the mechanisms of polarization of macrophages into M1 or M2 subtypes represent an interesting target for cellular intervention in different stages (early versus late) of dry AMD pathogenesis and warrants further investigation.

Modulation of the recruitment of microglia and macrophages to the site of injury is also a potential target for AMD treatment. Polymorphisms in the CX3CR1 chemokine receptor found on microglia and macrophages have been associated with increased risk of AMD.[120,121] The CX3CR1 polymorphisms result in decreased affinity for its ligand (CX3CL1), which in turn negatively affects microglial and macrophage migration.[122] In vitro studies suggest that accumulation of microglia due to impaired migration may cause direct damage to the photoreceptors.[123] In the case of macrophages, impairments in migration may interfere with recruitment from the circulation into the choroid and BrM in order to clear deposits at the site of injury.[121,122] Previous reports on mice lacking expression of both the chemokine, CCL2, and the receptor, CX3CR1, described a phenotype similar to that seen in human AMD.[124] However, the intraretinal rather than subretinal lesions seen in this mouse model suggest that the phenotype is due to the rd8 mutation, which is now known to be present in the background mouse strain used in these studies.[125–127] The rd8 mutation is a single nucleotide deletion in the *Crb1* gene that can cause a distinct clinical ocular phenotype due to formation of retinal folds, pseudorosettes, as well as focal retinal dysplasia and degeneration.[128]

There are still many details about the roles of microglia and macrophages in the pathophysiology of dry AMD that are unknown. Consequently, further studies will be needed to clarify whether microglia and macrophages do indeed represent feasible druggable targets in AMD. Mast cells have also emerged as immune cells of the choroid that may play a role in AMD. Lutty and colleagues recently described an accumulation of degranulated mast cells around GA lesions in the choroid of human donor eyes, suggesting that mast cell degranulation may contribute to the damage of this tissue in GA (Lutty GA, et al. *IOVS* 2013;54:ARVO E-Abstract 3051). This is particularly intriguing since activated complement stimulates degranulation of mast cells. Other immune cells and targets, including the inflammasome, will not be discussed in this review as they were addressed at length in a recent review by Ambati et al.[65]

Cell-Based Therapies

A large body of evidence supports the concept that RPE cell loss precedes photoreceptor loss in AMD.[12] Thus, it follows that if the RPE loss can be mitigated, visual function can most likely be maintained. A number of fetal and stem cell–based

approaches to replenish the RPE in vivo have been attempted in the last 20 years, resulting in varying levels of reconstitution and/or visual function rescue in animal models.[129,130] RPE cells are a highly polarized cell type and some studies suggest that for transplanted RPE cells to survive, they need to be polarized prior to transplantation.[131] Therefore, subretinal implantation of polarized RPE cells on biocompatible supports was pursued, with some success.[131–134] However, the major drawbacks using this approach are the traumatic implantation procedures and the possibility of rejection of the support/scaffold if it is not sufficiently immunologically inert.[131]

Recently, studies using an approach based on adult hematopoietic stem cells (HSC) showed promising results in restoring damaged RPE layers in vivo.[135,136] The authors reprogrammed adult HSCs by lentiviral expression of *RPE65*, which drove these cells to develop into RPE-like cells. Furthermore, systemic delivery via intravenous injection resulted in homing of these cells to the subretinal space in a mouse model of sodium iodate–induced RPE damage.[135] This is significant since, in a human clinical setting, intravenous injection provides higher safety and ease of use than subretinal delivery. In addition, the prospect that the patient's own HSCs could be used is particularly appealing as it avoids any issues related to transplant rejection. A logical next step will be to determine whether use of systemically delivered RPE-like cells will also home to the retina in a mouse model of early AMD in which there is more subtle RPE damage, and, hence, are likely to release less chemokines.[137] Such a mouse model mimics the human disease more closely and a successful outcome may be a more relevant proof of concept for human trials in AMD. Unfortunately, there are no good animal models of GA but the current advances in RPE cell-based therapies suggest these cells could be ideal for saving, and potentially restoring vision in patients with GA.

The challenge of replacing lost photoreceptors is more daunting than replacing RPE as the transplanted cells not only have to develop normally, but they must also integrate into the damaged retina and, potentially most difficult, establish the nerve connections required to transmit the visual information to the brain. Recently, successful photoreceptor transplantation has been achieved in mice by harvesting rod precursor cells from healthy mouse retina to restore functional vision in a mouse model of stationary night blindness (alpha-transducin knock-out, *GNAT1*$^{-/-}$).[138] The authors showed, for the first time, that the transplanted rods acquired the proper morphology including a classical triad synapse, had properly integrated into retinal circuits, and that signals from these cells were transmitted to the visual cortex.[138] In order for photoreceptor cell transplantation to be a viable therapy in humans, a source of cells other than photoreceptor precursors will be required. Progress to this end was made by a team led by Robin Ali that established that embryonic stem cells can provide a source of photoreceptors for retinal cell transplantation.[139] Although the percentage of integrated rod photoreceptors was low this is an important milestone toward developing cell therapy for regenerating lost photoreceptors.

Techniques for Diagnosis and Prediction of Dry AMD Progression

Currently, clinicians monitor morphological changes in the retina/RPE/choroid by fundus exam, color fundus photography, FAF, OCT, and infrared (IR) reflectance. In the past two decades, OCT[140] imaging technologies have had a profound impact on early detection, monitoring of progression, and treatment-efficacy evaluation of dry AMD. The current generation of commercialized OCT systems, SD-OCT, provides volumetric and cross-sectional views of the retina facilitating the visualization, measurement, monitoring, and phenotyping of the retinal layers and RPE,[141–143] hyperreflective foci,[144] GA,[145,146] and drusen[147] in eyes with dry AMD (Figs. 2E, 2F). While SD-OCT imaging is currently widely used in clinics as the standard of care for dry AMD diagnosis and prognosis, some emerging developments in OCT technology may lead to paradigm shifts in the way that dry AMD imaging is performed for research and clinical applications. One such technology is the polarization-sensitive OCT (PS-OCT) with tissue-selective imaging capabilities.[148] In PS-OCT imaging, the RPE layer appears distinct from other tissue layers and thus PS-OCT may provide complimentary information about RPE health, drusen subtyping, and GA progression.[149] In the next decade, advances in OCT technology are expected to continue to impact our understanding of dry AMD pathologies. Development of swept-source OCT (SS-OCT) systems with speeds exceeding 1,000,000 A-scans/s (as compared with the current generation of ~30,000 A-scans/s commercial SD-OCT systems) will facilitate imaging and image analysis by reducing image acquisition time, noise, and motion artifacts.[150] Moreover, SS-OCT systems are well suited to operate at approximately 1060-nm wavelength, which allows for enhanced visualization and monitoring of the choroidal structure, thickness, and vasculature pattern,[151] as well as the inner retina's distinct retinal capillary beds.[152]

Indeed, other modern and classic, noninvasive, imaging systems such as en face color fundus photography[153] and FAF, which may predict the rate of GA progression,[154–157] provide important and complementary information to OCT about dry AMD.[158] It has been proposed that the in vivo hyperfluorescence detected by FAF around GA lesions (Fig. 2D), represents dying RPE cells full of lipofuscin, which is the main source of FAF in these cells.[154] Recently, however, Rudolf et al.[159] published evidence against this hypothesis by measuring the histologic autofluorescence in human donor eyes with GA as a means to establish the cellular basis of the hyperfluorescence in these GA border zones. They found that the areas with highest histologic autofluorescence at GA borders were often associated with vertically superimposed RPE cells and, therefore, that the increased FAF was not necessarily a harbinger of cell death. Their data also suggests that lipofuscin itself may not be a relevant target for AMD treatment.[159]

Integration of adaptive optics (AO) into modern scanning laser ophthalmoscope (SLO) systems has made the in vivo visualization of individual photoreceptors (initially cones[160–163] and very recently rods[164]) and RPE cells[165] in dry AMD eyes possible (Fig. 3). On another front, while SS- and SD-OCT create images based mainly on the scattering properties of the tissue, a novel three dimensional (3-D) imaging technology called photoacoustic ophthalmoscopy (PAOM) provides complementary information by creating images based on the absorption properties of the tissue. Photoacoustic ophthalmoscopy, which may operate in multiple wavelengths (e.g., 532 nm and 1064 nm), is especially suitable for melanin-related imaging (e.g., RPE), choroid capillary network imaging, and for measuring oxygen saturation in retinal microvasculature.[166]

The large quantity of data created by these novel imaging technologies are often too large to be fully analyzed and interpreted manually. Thus, considerable work has been done in recent years to automate the segmentation of the imaging biomarkers of dry AMD. Due to the wide clinical applications of SD-OCT, most recent efforts have been focused on development of automatic segmentation algorithms to quantify individual retinal layer thicknesses, drusen, and GA in presence of dry AMD pathology (Fig. 4A).[167–171] We have identified efficient quantitative imaging biomarkers to automatically

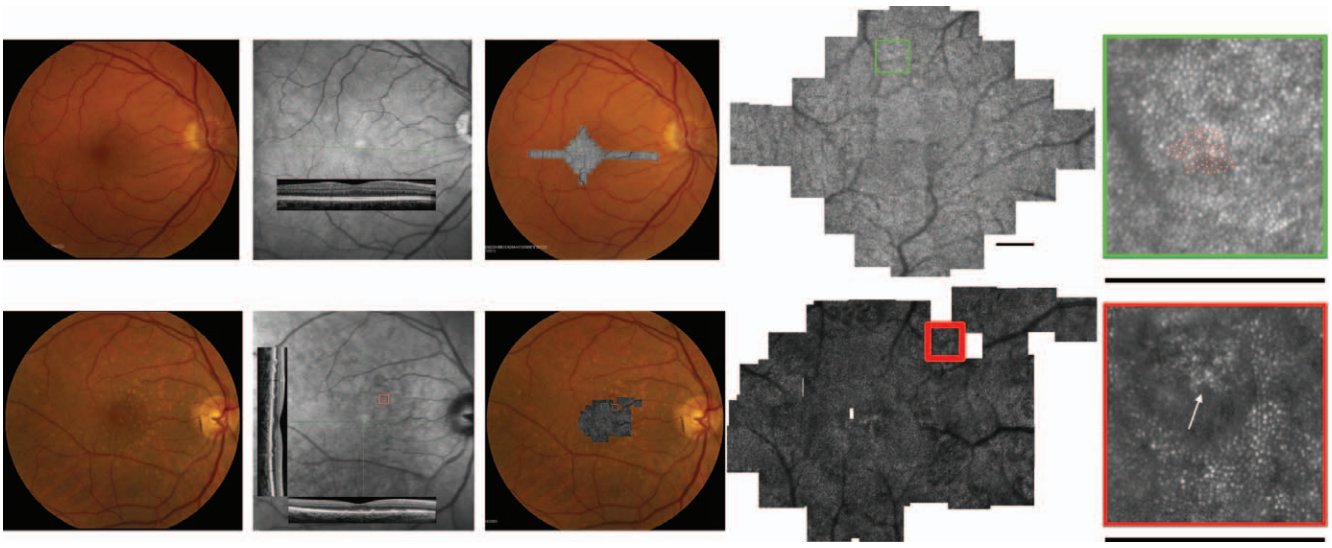

Figure 3. Cone structures in a 61-year-old healthy subject (*top panels*) and a 64-year-old subject with dry AMD (*bottom panels*). The panels show (*left* to *right*) color fundus photos, infrared fundus photos with SD-OCT superimposed, color fundus photo with montage of AO-SLO images superimposed, AO-SLO image with a box highlighting a magnified AO-SLO image (*far right panel*). In the healthy subject, the far *right panel* shows *red crosses* where cones were quantified and cone spacing fell within normal limits for the eccentricity shown. In the AMD patient the *red box* includes a region over a druse, which can be seen on the SD-OCT. The highly magnified AO-SLO image shows coarse cones over the surface of the druse (*arrow*). *Scale bars*: 1° (figure courtesy of Jacque L. Duncan and her colleagues Katrina A. Woo, Shiri Zayit-Soudry, and Austin J. Roorda [Woo KA, et al. *IOVS* 2011;54:ARVO E-Abstract 1672]).

distinguish intermediate AMD from healthy eyes by analyzing the topographic distribution of healthy and abnormal retinal layer thicknesses (Figs. 4B, 4C).[147] On the laboratory science front, automated segmentation of RPE cells in confocal microscopy images of flat-mounted AMD mouse models yielding cell count and mean cell area measurements has drastically sped up the experiment and analysis time (Fig. 4D).[172] Despite recent advances in automated segmentation of cones[173–176] and rods[173] in healthy eyes (Fig. 4E), lack of automated software is still one of the main obstacles in large-scale clinical utilization of adaptive optics scanning ophthalmoscope (AO-SLO) systems for diagnosis and prognosis of dry AMD. Moreover, regardless of the imaging modality or segmentation algorithm used, for many dry AMD patients with severe pathology (e.g., patients with cataracts which reduces the quality of captured images), automated image analysis methods fail to provide reliable measurements. Novel image enhancement methods have been demonstrated to significantly improve the quality of SD-OCT images of dry AMD patients,[177,178] and may improve the performance of automated segmentation methods.

Functional Assessments of Vision in AMD

Functional changes in vision of AMD patients reflect early dysfunction of the neurosensory retina and the supporting RPE. These changes are assessed by a variety of methods including measuring VA at normal and low luminance and/or reading speed, and by using microperimetry, assaying dark adaptation and contrast sensitivity. As with fundus imaging, these tests of retinal function may also be confounded by media opacity such as progressive cataract.

One of the earliest complaints of patients with AMD is difficulty with vision under dark-adapted conditions and is in most cases due to a decrease of rod photoreceptor function.[179,180] Thus, methods that could precisely distinguish the early changes in function are an obvious outcome measure for testing the efficacy of therapies targeting early AMD. To date, widespread use of a single method for functional testing has been hampered by the time to test a patient, the reproducibility of the test between instruments, testing methods, and patient visits. Measurements of dark adaptometry are underway in a longitudinal clinical trial of early and intermediate AMD (National Eye Institute, NCT01352975).[181–184] Low luminance VA deficit, poorer foveal dark-adapted sensitivity, and reduced reading rate have all been shown to predict subsequent VA loss in eyes with atrophic AMD that started with good acuity.[185] Contrast sensitivity changes in standard photopic lighting have been used for many years in assessment of AMD progression, especially to neovascularization; more recently mesopic testing has been explored.[180] Recently, advances in microperimetry suggest greater utility in early AMD when nonphotopic testing is performed. Several methods to obtain non-photopic microperimetry have been proposed and larger scale studies are needed to evaluate these methods. To support future therapeutic studies, simplified tests that are reproducible and can be commonly used across centers for functional assessment will be important. Equally important will be assessing the relationship of these tests to the other systemic and ocular measures of disease.

Conclusions

In general terms, therapeutic approaches to GA should be aimed at (1) reducing or stopping the stimuli of continuing damage, which depends on continued progress in identifying and characterizing relevant targets; (2) protecting remaining cells from further damage; and (3) repairing, replacing, or regenerating damaged cells. Currently, the third approach is gaining traction with the advances in RPE and photoreceptor cell–based therapies described earlier. That being said, the first two areas are experiencing steady progress. Together, the current and continued advances in understanding the molecular pathogenesis of early AMD and GA, which are identifying relevant therapeutic targets, coupled with the advances in detecting and measuring disease progression, should expedite breakthroughs in developing therapies that block and/or

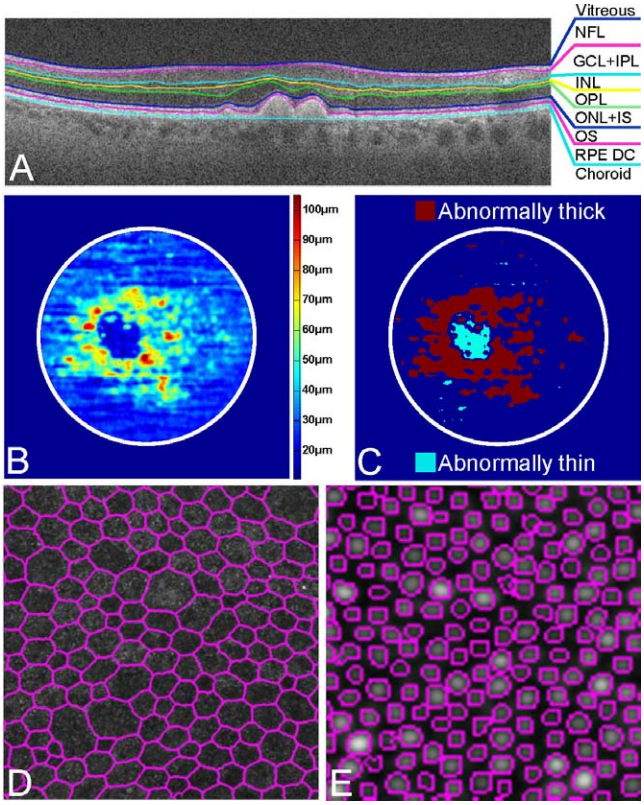

Figure 4. Application of novel automated segmentation algorithms for analysis of the anatomical and pathologic biomarkers of dry AMD. (**A**) Automated segmentation of the eight retinal boundaries on an SD-OCT image of a dry AMD patient with drusen using DOCTRAP software[186] delineating the vitreous (at the *top* of the image) from the nerve fiber layer (NFL, *blue line*), NFL from ganglion cell layer and inner plexiform layer (GCL+IPL) complex (*pink line*), GCL+IPL from inner nuclear layer (INL, *aqua line*), INL from outer plexiform layer (OPL, *yellow line*), OPL from outer nuclear layer and inner segment (ONL+ IS) of the photoreceptor layer (*green line*), ONL+ IS from outer segments (OS) of the photoreceptor layer (*blue line*), OS from the RPE and drusen complex (RPE DC, *pink line*), and the RPE DC from the choroid (*aqua line*).[169] The *top* and *bottom* boundaries correspond to the inner limiting membrane (ILM) and the Bruch membrane, respectively. (**B**) Example of a 5 mm in diameter RPE DC thickness map centered at the fovea from a dry AMD patient. Thickening around the fovea (*red* and *yellow regions*) is indicative of drusen, while thinning (*blue regions*) is representative of GA.[147] (**C**) DOCTRAP software automatically extracts areas of abnormally *thin* (*cyan region*) and *thick* (*red region*) RPE DC from the thickness map in (**B**), which we use to automatically distinguish AMD from healthy eyes.[147] (**D**) Automatically segmented confocal fluorescence image of the RPE cells in a flat-mounted *APOE4* mouse retina.[172] (**E**) Automatically segmented AO-SLO image of the cone photoreceptors in a healthy human subject.[173]

reverse early AMD and GA. The new imaging, automated segmentation, and advances in visual function testing are directly impacting the great unmet need for devising therapeutic strategies for early AMD, which has been plagued by a lack of understanding of how to sub-classify types of early stage disease based on initial presentation and subtle changes over time. These new and evolving technologies are, for example, simplifying imaging and quantifying drusen (Figs. 4C, 4D) and should allow us to test whether changes in appearance of drusen (e.g., appearance and disappearance) can be correlated with vision effects and whether these are predictors of rates of progression. These advances will also have a large impact on the success of clinical trials as they will facilitate sub-stratification of early, intermediate and late AMD patients, by prediction of progression rates and appearance as well as refine quantifiable outcome measures of visual function changes and recovery. While there are many challenges and unmet needs in understanding and treating early and atrophic AMD this is an exciting time to be working in this area due to the convergence of advances in understanding retinal physiology, genetics, and technology.

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Investigative Ophthalmology & Visual Science

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