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Introduction to the issue regarding research regarding age related macular degeneration

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Blindness is the second greatest fear among the elderly. Age-related macular degeneration (AMD) is the leading cause of vision loss among the elderly in most industrialized nations. AMD first compromises central high acuity vision. Subsequently, all vision may be lost. AMD is a progressive retinal degeneration that develops after age 50 and affects 30% of people age 70 or older. At least sixty million people worldwide are affected. Over 10 million people are affected in the US, and it is predicted that this number will double by 2050. Globally, the number will triple in 13 years and persons over 60 represent the fastest growing segment of the population, and people around the world are living longer. Given the cost in terms of quality of life and with financial costs in excess of \$340 US billion, AMD will remain a significant and growing public health problem for the foreseeable future. Clearly, it is critical to find means to diminish risk for, and to delay the onset or progress of AMD.

In this issue leading AMD researchers summarize our current knowledge regarding the etiology of the disease. They also suggest directions that should lead to new means to diminish the burden of this devastating condition. These articles are complementary. When one article discusses material that is found in another article in this issue, the complementary article is mentioned. This is done a limited number of times per article, in order to save space.

There are two major forms of AMD. Atrophic (also called “dry”) AMD is defined by atrophy and loss of retinal pigmented epithelial cells (RPE), as well as starvation and loss of the overlying photoreceptors and other parts of the neurosensory retina. Atrophic AMD afflicts approximately 85–90% of AMD victims. There is no treatment for the millions of people who suffer from this form of AMD. The other devastating form of AMD is called neovascular, “wet”, or “exudative” AMD. This is caused when choroidal blood vessels in the back of the retina break through Bruch’s membrane and invade the space that is normally occupied by RPE. This is associated with vascular leakage, hemorrhage and scarring. Whereas atrophic AMD is much more common than neovascular, neovascular AMD accounts for the majority of the vision loss. Generally, there is progression from atrophic to neovascular disease. Progression from the early (see below, and article by

Bhutto) to the late stage of AMD can occur in as little as 5 years. Although AMD is encountered in the elderly, it is assumed that the initiating insults are experienced earlier in life. While treatment regimens are available for some neovascular AMD patients, they are deployed only after the patients have already lost some vision. Clearly, it would be of enormous advantage to avoid the onset or limit progress to stages of disease that compromise vision.

Most research is focused around the RPE because the RPE is a locus of early AMD-related pathology. Additionally, in the normal retina, the RPE performs many critical functions including the nutritional and metabolic support of the photoreceptor cells that receive and transduce light energy to chemical and then electrical signals that are sent to the brain to create vision. The RPE is also part of a polarized barrier between the choroid and photoreceptors. Each night, the outer 10% of segments of photoreceptors are shed and engulfed by the RPE, which lies posterior to the photoreceptors. Due to the requirement of one RPE cell to service more than thirty photoreceptors, the RPE has among the highest degradative burden in the body. Importantly, a common insult appears to be oxidative damage. This results in the accumulation of altered proteins, lipids, and/or genetic material that may be cytotoxic and may accumulate in ways or at sites that result in displaced dysfunctional RPE.

Nutrients from the choriocapilaris (CC) blood supply must cross Bruch's membrane (BrM), a pentalaminal structure composed of several layers of elastic fibers and collagen, to enter the RPE and photoreceptors. The flow of nutrients into the retina, and debris out of the retina through the RPE is crucial, since photoreceptors do not have their own blood supply.

This series of articles starts with a clear summary of the various cell types that are involved in retinal function and AMD-related dysfunction. Bhutto notes that within the retina there is a mutualistic symbiotic relationship between the components of the photoreceptor/RPE/BrM/CC complex that is lost in AMD. Which component in the photoreceptor/RPE/BrM/CC complex is affected first appears to depend on the type of AMD. In atrophic AMD, it appears that large confluent drusen formation (see below) and hyperpigmentation (presumably dysfunction in RPE) are an initial insult and the resorption of these drusen and loss of RPE (hypopigmentation) can be predictive for progression to geographic atrophy (GA). The death and dysfunction of photoreceptors and CC appear to be secondary events to the loss of RPE.

In neovascular AMD, the loss of choroidal vasculature may be the initial insult to the photoreceptor/RPE/BrM/CC complex. Loss of CC may be due to reduction in blood supply because of large vessel stenosis. Furthermore, the environment of the CC, basement membrane and intercapillary septa, is a proinflammatory milieu with accumulation of complement components as well as proinflammatory molecules like CRP during AMD. In this toxic milieu, capillaries of the choroid die or become dysfunctional making adjacent RPE hypoxic. These hypoxic cells then produce angiogenic substances like VEGF that stimulate growth of new vessels from the CC, resulting in choroidal neovascularization. The loss of CC might also be a stimulus for drusen formation since the disposal system for retinal debris and exocytosed material from RPE would be limited. Ultimately, the

photoreceptors die of lack of nutrients, leakage of serum components from the neovascularization, and scar formation. Indicators of aging and early AMD include basal laminar deposits, basal linear deposits, and drusen. The combination of inadequate nutrition, stress and the inability to properly degrade and dispose of cellular debris may contribute to the formation of deposits in the RPE-BrM region. Basal laminar deposits accumulate in the RPE cell, between the RPE basement membrane (BrM) and the RPE plasma membrane. Basal linear deposits accumulate between the RPE basal lamina and the inner collagenous layer of BrM. Deposition of basal linear deposits is thought to precede the formation of drusen, the latter being clinical indicators for early AMD. Drusen are larger deposits that are often found between the RPE and the choroid. Mass spectrometric analysis indicates that drusen contain a variety of lipids, proteins, including ubiquitin and advanced glycation end products, as well as inflammatory mediators (also see review by Shang in this issue). Damaged photoreceptors and RPE cells may also accumulate and accelerate the formation of drusen, further exacerbating disease.

Among the many factors that make AMD research so challenging is that the disease is clearly multifactorial, having genetic, environmental and as yet other unknown influences. Many hypotheses have been proposed to explain the etiology and mechanisms of AMD. Most of the articles in this issue present data to support at least one hypothesis. Most of these hypotheses include oxidative stress as one of the insults that is etiologic for AMD.

In order to directly address the hypothesis that oxidative stress is causally related to elevated risk for AMD and in order to provide therapy for the vast majority of AMD victims for whom there is no treatment by the least invasive modalities, a myriad of studies sought to determine if risk for AMD is diminished upon (a) supplementation with antioxidant vitamins, (b) consuming diets that are rich in antioxidants (c) diets that have higher levels of omega 3 fatty acids or (d) lower glycemic index diets that form lower levels of advanced glycation end products.

The series continues with an evaluation of the epidemiologic record regarding the use of various micronutrients or nutrient status to diminish risk for AMD. Vitamins C and E, carotenoids and Zn are involved in antioxidant systems. Data from the AREDS intervention trial indicate that use of a high potency supplement with vitamins C and E, Zn^{2+} , Cu and beta carotene delays the progress of intermediate to advanced AMD. However a critical need remains to delay onset or abate AMD at earlier stages before vision is altered. Observational studies indicate that maintaining adequate levels of omega-3 fatty acids (e.g. with 2 servings/wk of fish) or a low glycemic index diet may be particularly beneficial for early AMD and that higher levels of carotenoids may be protective, most probably, against neovascular AMD. Many additional studies suggest a benefit of micro and macronutrients with respect to AMD, as well as other age-related debilities, with few, if any, adverse effects. These findings are summarized in this issue in Weikel's comprehensive, unbiased review of the epidemiologic record.

The strongest modifiable risk factor for AMD is smoking, which increases risk for AMD up to 7-fold. Obesity is also a significant, largely modifiable, risk factor for AMD: a 3%

reduction in waist-hip ratio decreases risk for AMD by 29%. A tenuous correlation between hypertension and risk for wet AMD has also been reported.

Newer morphologic concepts regarding the etiology of atrophic AMD are presented in Mettu's review. They postulate that the pathogenesis and progression of atrophic macular degeneration is characterized by three distinct stages with morphologic involvement and ramifications. First, endogenous or exogenous oxidative stress causes extrusion of RPE cell membrane "blebs". This is coincident with decreased activity of matrix metalloproteinases. This results in bleb accumulation as basal laminar deposits under the RPE. The RPE cells are subsequently stimulated to increase synthesis of MMPs and other molecules responsible for extracellular matrix turnover (i.e. producing decreased collagen), affecting both RPE basement membrane and BrM. This process leads to progression of basal laminar deposits into basal linear deposits and drusen by admixture of blebs into BrM. Subsequently a new basement membrane forms under the RPE to trap these deposits within BrM. Interestingly, various hormones and other plasma-derived molecules related to systemic health cofactors are implicated in this stage. Finally, macrophages are recruited to sites of RPE injury and drusen formation. Macrophage recruitment may be beneficial or harmful depending upon their activation status at the time of recruitment. Nonactivated or scavenging macrophages may remove deposits without further injury. Conversely, activated or reparative macrophages may promote complications and progression to the late forms of the disease through the release of inflammatory mediators, growth factors, or other substances.

Major contributions regarding our understanding of roles for oxidative stress in the etiology of AMD have come from the Boulton laboratory. Jarrett informs that the combination of a very high flux of oxygenated blood and exposure to photosensitizers and light in the retina creates an ideal environment for the generation of reactive oxygen species (ROS) and resultant oxidative damage. Furthermore, the high concentrations of critically located and physiologically functional polyunsaturated fatty acids are extremely prone to lipid peroxidation. Such oxidative stress, combined with diminished antioxidant capacities upon aging, results in increased cytotoxic burden.

Decrements in proteolytic capacities due to age and stress exacerbate the accumulation of dysfunctional and obsolete materials and the associated cytotoxicity within the RPE (see Shang's review). Together, these decreases and the deficiency of reparative systems result in retinal dysfunction associated with AMD. Jarrett further elaborates the nature of oxidative damage in AMD pathobiology by describing roles for mitochondria and mitochondrial oxidation in AMD pathobiology. Particularly vulnerable to oxidative damage is mitochondrial DNA (mtDNA). This is exacerbated by the high transcription rate for mtDNA, and oxidative stress-limited efficacy of repair, resulting in increased probability of mutations and/or deletions. Since mtDNA does not have introns, genomic injury will impact coding regions for critical components of mitochondrial translational machinery or the electron transport chain. Further, the mtDNA genome is in close proximity to the electron transport chain that leaks ROS together with metal ions within the inner mitochondrial membrane. This provides the potential for Fenton chemistry and generation of $\text{OH}^\bullet$. Thus it is not surprising that ROS-induced mtDNA damage is positively correlated with progression of AMD, while repair capacity is negatively correlated. Furthermore, a genetic

component of mitochondrial stress appears to be in play. Mitochondrial oxidative stress is further enhanced by phagocytosis of photoreceptor outer segments, presumably through the burst of ROS generated during ingestion, and by exposure to blue light.

Sparrow observes that an important source of stress on RPE takes origin from the bisretinoids that accumulate as the lipofuscin of these cells. One of this group of compounds is A2E. It is noted that while these di-retinal molecules undergo photooxidation, photooxidized species do not accumulate with time. Rather, continued photooxidation leads to photofragmentation. Harmful effects mediated by photooxidation-induced lysis of bisretinoid result from the release of aldehyde-bearing cleavage products. The latter includes methylglyoxal and glyoxal, small, reactive oxo-aldehydes well known to form advanced glycation end (AGE) products. Interestingly, methylglyoxal and accumulation of AGEs are also associated with RPE and retinal damage in carbohydrate – stressed cells and in tissues from animals fed high glycemic index diets (see reviews by Weikel and Shang in this issue).

Handa's review provides another important contribution to our understanding of how oxidative stress is related to risk for AMD. Whereas his earlier efforts focused on effects of smoking, in this review, as well as in several others, the ramifications of excessive innate immune system activation and inflammation with regard to AMD pathobiology are considered. Significantly, Handa covers newer data indicating that the RPE has developed a robust antioxidant system driven by the transcription factor Nrf2. Impaired Nrf2 signaling can lead to oxidative damage or activation of the innate immune response, both of which can lead to RPE apoptosis, a defining change in AMD. Some of the features of AMD were simulated in mouse models using environmental stressors or by targeting specific antioxidant enzymes such as superoxide dismutase or Nrf2. Further, Handa observes that while reactive oxygen species are short-lived, oxidatively damaged molecules termed oxidation specific epitopes (OSEs), can be long-lived and a source of chronic stress that activates the innate immune system through pattern recognition receptors (PRRs). The macula accumulates a number of OSEs including carboxyethylpyrrole, malondialdehyde, 4-hydroxynonenal, and advanced glycation endproducts, as well as their respective neutralizing PRRs. Excessive accumulation of OSEs results in pathologic immune activation. For example, mice immunized with carboxyethylpyrrole develop cardinal features of AMD and mice that consumed high glycemic index diets show systemic accumulation of AGEs, including within the retina and RPE. Regulating reactive oxygen species in the RPE by modulating antioxidant systems or neutralizing OSEs through an appropriate innate immune response are potential modalities to treat or prevent early AMD.

The accumulation of damaged or post synthetically-modified proteins has long been considered etiologically related to RPE stress, formation of drusen and its sequela. With an enormous proteolytic burden imposed by the need to degrade photoreceptors nightly, the RPE has among the highest proteolytic burdens in the body. The ubiquitin proteasome pathway (UPP) plays crucial roles in protein quality control, cell cycle control, signal transduction, responses to oxidative stress, and eye development. Shang's review sets the ubiquitin proteasome system against a perspective of cellular proteopoeis: the sum of protein synthesis, modification and degradation. Selective degradation of aberrant proteins by the UPP is essential for timely removal of potentially cytotoxic damaged or otherwise abnormal

proteins. Age- or stress induced- impairment the UPP or insufficient UPP capacity appears to contribute to the cytotoxic accumulation of abnormal proteins and the associated pathogenesis of AMD. Insufficient UPP capacity in retina and RPE could also result in dysregulation of signal transduction. Aberrant UPP function also impacts the autophagic lysosomal pathways. Further, improper cell signal transductions in the retina/RPE may trigger abnormal inflammatory responses and neovascularization. Thus, there seems to be a vicious cycle of oxidative stress, damage to protein and the proteolytic machinery, and exacerbated accumulation of altered proteins resulting in accelerating cytotoxicity. Drugs that modulate the proteolytic capacity, both via the UPP and lysosomal pathways, are making their way into new generation of pharmacotherapies for delaying age-related diseases and may be useful to prevent AMD.

A significant finding of the last decade is that genetics, specifically involving unanticipated genes, may predict a large proportion of the risk for AMD. The genetics of AMD began with the appreciation of familial aggregation and increased risk and expanded with the initial association of APOE variants with the disease. Starting with identification of risk for AMD associated with the CFH variants Y402H, then ARMS2/HTRA1 locus, the field has exploded in the past 5 years. This work is summarized well with Gorin's rich insights into- and cautions regarding- AMD diversity and causality. Several other reviews summarize these genetic data and pursue the associated biochemistry (see Sparrow's review). These findings spurred efforts to understand the function and pathobiology of complement and inflammation in the retina. Together, these findings hold the promise of enabling clinicians to provide better risk assessments for individuals as well as to develop and selectively deploy new therapeutics to either prevent or slow the development of disease and lessen the threat of vision loss.

As noted above, AMD is a complex disease that involves the interaction of both genetic and environmental factors with the unique anatomy of the human macula. A major challenge has been to develop suitable models of human AMD. Pennesi observes that models in mice, rats, rabbits, pigs and non-human primates have recreated many of the histological features of AMD and provided much insight into the underlying pathological mechanisms of this disease. However, no single model yet recapitulates all of the features of human AMD. Furthermore, and most laboratory animals do not have a macula. Additionally, most of the mouse genetic variants bear little resemblance to known etiologic genes for human AMD. Nevertheless, these models have helped reveal the roles of chronic oxidative damage, inflammation and immune dysregulation, and lipid metabolism in the development of AMD. Models for induced choroidal neovascularization have served as the backbone for testing new therapies. This article reviews the diversity of animal models that exist for AMD as well as their strengths and limitations.

In summary, preservation of vision would enhance quality of life for millions of elderly people, and alleviate the personal and public health financial burden of AMD. The articles in this issue of *Molecular Aspects in Medicine* describe the "state of the art" of AMD research. Thus, this issue serves as an invaluable resource for stakeholders, investors and investigators with interests in AMD research and those seeking new pharmacologic and nutritional cures for this devastating disease.

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