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A2E accumulation influences retinal microglial activation and complement regulation

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Abstract

Age-related macular degeneration is an outer retinal disease that involves aging and immune dysfunction. In the aging retina, microglia aggregate in the outer retina and acquire intracellular autofluorescent lipofuscin deposits. In this study, we investigated whether accumulation of A2E, a key bisretinoid constituent of ocular lipofuscin, alters the physiology of retinal microglia in pathologically relevant ways. Our findings show that sublethal accumulations of intracellular A2E in cultured retinal microglia increased microglial activation and decreased microglial neuroprotection of photoreceptors. Increased A2E accumulation also lowered microglial expression of chemokine receptors and suppressed microglial chemotaxis, suggesting that lipofuscin accumulation may potentiate subretinal microglial accumulation. Significantly, A2E accumulation altered microglial complement regulation by increasing complement factor B and decreasing complement factor H expression, favoring increased complement activation and deposition in the outer retina. Taken together, our findings highlight the role of microglia in the local control of complement activation in the retina and present the age-related accumulation of ocular lipofuscin in subretinal microglia as a cellular mechanism capable of driving outer retinal immune dysregulation in age-related macular degeneration pathogenesis.
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1. Introduction

Age-related macular degeneration (AMD), a neurodegenerative retinal disease of the elderly (Jager et al., 2008), is the leading cause of legal blindness in persons 60 years and older in the developed world (Congdon et al., 2004) and represents a major challenge to healthy vision worldwide. Central vision is lost in AMD as a result of neurodegenerative atrophy affecting photoreceptors and retinal pigment epithelial (RPE) cells associated with characteristic extracellular lesions between these cells and their blood supply. Although therapies targeting vascular endothelial growth factor have demonstrated efficacy in limiting pathological neovascular growth in a subset of cases, comprehensive prevention and treatment measures for AMD remain elusive. In part, a lack of knowledge of AMD pathogenesis have impeded the development of new therapies (Donoso et al., 2010; Zarbin and Rosenfeld, 2010).

In AMD, 2 particular factors, chronic neuroinflammation and aging, appear central in disease risk and progression (Augustin and Kirchhof, 2009; Donoso et al., 2006; Hyman and Neborsky, 2002; Kanda et al., 2008). For the first, the involvement of chronic neuroinflammation has been underscored by genetic analyses implicating individual molecules of the immune system, particularly those in the complement

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system, as conferring risk for AMD (Donoso et al., 2010; Gehrs et al., 2010; Swaroop et al., 2009). However, how these immune molecules participate in pathogenic cellular mechanisms in AMD is unclear. For the second, advanced age constitutes the largest and most significant risk factor for AMD (Friedman et al., 2004), reflecting the importance of senescent effects on relevant cellular mechanisms. Taken together, these findings indicate that aging effects within immune cells of the outer retina, the tissue locus where AMD develops, are likely to be significant in disease progression.

Microglia, the primary resident immune cell of the retina and a critical regulator in its immune environment (Boehm et al., 2011), have been implicated in the etiology of AMD (Karlstetter et al., 2010; Xu et al., 2009). In the mouse retina, while retinal microglia in young healthy animals are confined to the inner retinal layers and are spatially removed from photoreceptors and RPE cells (Chen et al., 2002), they undergo a remarkable translocation into the outer retina in aged animals to accumulate in the subretinal space (Damani et al., 2011; Xu et al., 2008). Indeed, similar accumulations of subretinal microglia have also been characterized in human AMD (Gupta et al., 2003; Penfold et al., 2001), as well as in AMD-relevant retinopathies in mouse models (Combadière et al., 2007; Luhmann et al., 2009; Tuo et al., 2007). These findings imply that these accumulations of senescent microglia may dysregulate immune interactions and drive AMD progression (Ma et al., 2009), linking the cellular mechanisms of aging and chronic neuroinflammation in the outer retina.

The aging phenotype of central nervous system (CNS) microglia, the mechanisms underlying microglia aging, and microglial contributions to age-related CNS neurodegenerative diseases are topics of current investigation (von Bernhardi et al., 2010). However, these cellular changes may differ between different CNS compartments and the related pathologies induced in each region may vary (Olah et al., 2011). In the mouse retina, the senescent microglial phenotype is characterized not only by their outer retinal translocation but also by the development of intracellular lipofuscin granules whose spectral characteristics are similar to those found in aged RPE cells (Xu et al., 2008). In RPE cells, the age-related accumulation of ocular lipofuscin, and a constituent bisretinoid in particular, A2E (Lamb and Simon, 2004), has been related to deleterious effects observed in in vitro studies. These include membrane disruption (Sparrow et al., 1999), lysosomal dysfunction (Holz et al., 1999), loss of antioxidant activity (Shamsi and Boulton, 2001), phototoxicity (Schütt et al., 2000; Sparrow and Cai, 2001; Sparrow et al., 2000), and immune dysregulation through complement activation (Zhou et al., 2006, 2009). Whether lipofuscin accumulation in aged subretinal microglia induces parallel alterations that may contribute to age-related retinal pathology has not previously been investigated.

In this study, we address the hypothesis that A2E accumulation in retinal microglia induces significant alterations in microglial physiology that can contribute to immune dysregulation relevant to AMD progression. We have employed an in vitro model of A2E-loaded cultured retinal microglia cells and assessed their activation status, chemotaxis, neuroprotective properties, and immune regulation as a function of their A2E accumulation. Additionally, by using an in vivo cell transplantation model, we assessed the effect of A2E-laden microglia in the subretinal space on complement regulation and photoreceptor apoptosis. These studies enabled us to explore the significance of increasing ocular lipofuscin in retinal microglia and to investigate the mechanisms underlying how aged microglia, altered in their retinal location and physiology in senescence, may contribute to cellular mechanisms driving AMD.

2. Methods

2.1. Experimental animals

Wild type C57BL/6J and heterozygous CX3CR1$^{+/GFP}$ transgenic mice (created by breeding CX3CR1$^{GFP/GFP}$ mice [Jung et al., 2000] to C57BL/6J mice) were used. Animals were purchased from the Jackson Laboratory (Bar Harbor, ME, USA) and from the National Institute of Aging-Aged Rodent Colony (Bethesda, MD, USA). Mice were bred and housed in a National Institutes of Health animal facility in a temperature and light controlled environment with a 12-hour day-light cycle. Animal procedures were conducted according to protocols approved by the local Institutional Animal Care and Use Committee and in concordance with the ARVO Statement for the Use of Animals in ophthalmic and Vision Research.

2.2. Human eye tissue

Eyes from 2 donors with atrophic form of advanced AMD (geographic atrophy) (aged 82 and 85 years) and 4 donors without history and signs of AMD (aged 45–77 years) were obtained from eye banks through a tissue resource center (National Disease Research Initiative, Philadelphia, PA, USA). The AMD diagnoses in these eyes were derived from donor records and from gross examinations of the fundus prior to tissue sectioning. The eyes were fixed in 4% paraformaldehyde, and the maculas dissected. A portion of the macular tissue was processed by cryosectioning (8–10-μm thick sections). In other macular areas, the retina was dissected free of the choroid-RPE complex and flat mounted with the photoreceptor layer uppermost. Eye tissue was collected under applicable regulations and guidelines with proper consent, protection of human subjects, and donor confidentiality.

2.3. Synthesis of A2E

A2E was synthesized essentially as described previously (Parish et al., 1998). Briefly, a mixture of all-transretinal