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Microglia in the Outer Retina and their Relevance to Pathogenesis of Age-Related Macular Degeneration (AMD)

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Abstract

Age-related macular degeneration (AMD), the largest cause of legal blindness in the elderly in the Western world, is a disease whose pathogenesis is incompletely understood and for which therapeutic challenges remain. The etiology of AMD is thought to involve chronic neuroinflammation of the retina but the details of relevant cellular mechanisms are still not fully understood. Retinal microglia are the primary resident immune cell in the retina and are normally absent from the outer retina, the locus of AMD. Their migration and infiltration into the outer retina under conditions of advanced age and disease implicate their involvement in the neuroinflammatory etiology of AMD. We propose that interactions between microglia and RPE cells in the subretinal space result in significant alterations in the structure and physiology of RPE cells that in turn transforms the environment of the retino-choroidal interface into one conducive for the progression and advancement of AMD. In particular, microglia induce RPE alterations that result in a more chemoattractive, pro-inflammatory, and pro-angiogenic environment that increases the recruitment and activation of immune cells and fosters the growth of neovascular vessels into the retina. Microglia-to-RPE influences may represent a cell-cell interaction that may be targeted for therapeutic strategies to treat and/or prevent AMD.

XX.1 Introduction

Age-related macular degeneration (AMD), the leading cause of legal blindness in the elderly in the industrialized world (Klein *et al*. 1997; Friedman *et al*. 2004), is a disease whose pathogenesis remains unclear (Zarbin 2004). Although multiple mechanisms are likely to contribute to the onset and progression of AMD, the role of inflammation has been given much consideration in the etiology of AMD (Donoso et al. 2006; Augustin and Kirchhof 2009). However, the nature of the immune cell interactions that drive cellular and tissue changes in AMD remain incompletely understood.

Retinal microglia are the primary immune cell type in the retina and comprise of a population of resident cells normally present in the retina (Provis et al. 1996). Distributed in a regular array throughout the inner retina, retinal microglia, through their dynamic process movements, carry out constant and dynamic immune surveillance of the extracellular environment (Lee et al. 2008), and can respond rapidly to tissue injury by altering their activation state, acquiring capabilities of migration and proliferation, and secreting

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inflammatory mediators and neurotrophic agents (Hanisch and Kettenmann 2007). Found throughout the CNS, microglia are capable of carrying out diverse sets of housekeeping functions under normal conditions and also executing adaptive functions under conditions of tissue injury (Ransohoff and Perry 2009). However, maladaptive inflammatory responses of microglia have been implicated in the progression of various chronic neurodegenerative diseases including Alzheimer's disease and Parkinson's disease (Brown 2009; Lucin and Wyss-Coray 2009). In the retina, how microglia may contribute to the pathogenesis of AMD is a subject of interest and an area of investigation in our laboratory.

XX.2 AMD Pathology in the Retino-Choroidal Interface

It has been well established that the locus of AMD is situated in the outer retina, in particular the retino-choroidal interface. The hallmarks of early and intermediate AMD include drusen, which are located between Bruch's membrane and the retinal pigment epithelial (RPE) layer (Green and Key 1977), and pigmentary changes, which are comprised of RPE hyperplasia and atrophy (Green 1999). The lesions of advanced AMD are also in the same locus; the atrophic form of advanced AMD (or geographic atrophy) is defined by photoreceptor and RPE atrophy (Sarks et al. 1988), while the exudative (or "wet") form involves choroidal neovascularization which extends through Bruch's membrane into the sub-RPE and subretinal space (Green 1999). Recent studies have also highlighted the presence of drusenoid deposits located within the subretinal space adjacent to the apical surface of RPE cells (Rudolf et al. 2008). These deposits, which form part of early/intermediate AMD, are clinically visible as reticular drusen (Zweifel et al. 2010a) and may be associated with advancement of AMD (Cohen et al. 2007; Zweifel et al. 2010b). The location of these AMD-associated lesions indicates that cellular interactions that drive their formation and progression are also likely to be located in the region of the RPE, subretinal space, and sub-RPE space.

In the young healthy retina, microglia are found distributed in regular horizontal arrays in the inner retina, with their ramified processes concentrated in the inner plexiform layer (IPL) and the outer plexiform layer (OPL) (Provis et al. 1996; Santos et al. 2008). Interestingly, retinal microglia are notably excluded from the outer nuclear layer and subretinal space, indicating that the outer retina may be distinguished as a specialized zone of particular immune privilege (Streilein et al. 2002). Under normal conditions, the outer retina, for reasons that are unclear, appears to be exempt from direct immune surveillance by resident microglia, which occupy the inner retina and much of the entire CNS. How this peculiar microglia-free zone is maintained in the outer retina, and what functional implication this entails are unknown but are likely to be important in the processes of neuroimmune regulation required by the specialized environment of the retinochoroidal interface.

The normal distribution of retinal microglia is however perturbed under conditions of advanced age and pathology. In the aged retina, microglia are found to be displaced in increasing numbers into the subretinal space, acquiring morphological and immunohistochemical features of activation (Xu et al. 2008; Xu et al. 2009). In histopathological specimens of AMD, retinal microglia have also been found in contact with drusenoid deposits (unpublished data) and in association with advanced AMD lesions

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(Gupta et al. 2003). Also, in genetic mouse models of AMD, in which chemokine ligands/receptors, CX3CR1 and/or CCL2 have been genetically ablated, activated microglia have also been found to accumulate in the subretinal space and become associated with drusen-like accumulations, RPE degeneration, photoreceptor atrophy, and choroidal neovascularization (CNV) (Combadiere et al. 2007; Tuo et al. 2007). The age-related accumulation of microglia in the outer retina and the association between AMD-associated lesions and displaced microglia in both mouse models and human disease give rise to the hypothesis that altered microglial distribution in the outer retina perturbs tissue homeostasis and promotes chronic neuroinflammation, leading eventually to alterations that constitute AMD advancement.

XX.3 Microglia-RPE Interactions in the Outer Retina

We hypothesize that the presence of displaced and activated microglia in the outer retina results in altered cellular interactions that help drive AMD pathogenesis and progression. In addressing this hypothesis, we have investigated in particular the nature of microglia-RPE cell interactions in this context. RPE cells and microglia are normally found in anatomically disparate locations but become uniquely juxtaposed in senescent and pathological situations. Although epithelial in nature, RPE cells play significant immune regulatory roles (Holtkamp et al. 2001) and alterations in RPE structure constitute hallmark lesions in both early and advanced AMD. Thus, contact between microglia and RPE cells in pathological situations may result in cell-cell interactions relevant to the inflammatory etiology of AMD.

We explored the nature of microglia-induced effects on RPE cells by using: (1) an *in vitro* co-culture model in which cultured murine retinal microglia, before and after activation with lipopolysaccharide (LPS), were co-cultured with primary murine RPE cells, and (2) an *in vivo* model of microglia transplantation in which cultured activated murine retinal microglia were transplanted into the subretinal space, adjacent to the RPE layer. We found that activated retinal microglia induced multiple structural and functional alterations in primary RPE cells. RPE cells upon exposure to activated microglia decreased their expression levels of the visual cycle protein, RPE65, and also tight-junctional proteins (ZO-1 and claudin-1) (Ma et al. 2009). In both *in vitro* and *in vivo* models, RPE cells, under the influence of activated microglia, also exhibited a loss of tight-junction contacts, becoming more disorganized and haphazard in distribution. These RPE changes may be analogous to alterations seen in early/intermediate AMD, where RPE hypertrophy and disorganization in the form of pigment clumping are described, and constitute a separate risk factor for AMD advancement (Ferris et al. 2005).

We observed that on exposure to activated microglia, RPE cells *in vitro* altered their gene expression in significant ways (Ma et al. 2009). RPE cell expression levels of chemotactic cytokines (CCL2, CCL5, SDF-1), which are capable to attracting immune cells, and adhesion molecules (VCAM-1 and ICAM-1), which are capable of retaining immune cells, were both increased. Functionally, supernatants from exposed RPE cultures were capable of inducing increased microglia migration in in *vitro* assays compared to controls. *In vivo*, activated microglia transplanted into the subretinal space were also associated with the displacement of endogenous microglia from the inner retina to the outer retina. These data

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suggested the possibility of a positive feedback mechanism in which subretinal microglia induces changes in RPE cell gene expression which in turn produces a more chemoattractive environment in the outer retina for the attraction and retention additional microglia that can further influence RPE cells. This progressive accumulation of subretinal microglia may incrementally abrogate the immune privileged environment of the outer retina, promoting processes of chronic neuroinflammation relevant for AMD pathogenesis.

Lastly, RPE cells under the influence of activated microglia also expressed higher levels of proinflammatory cytokines (IL-1β, TNF-α, IL-6) and pro-angiogenic molecules such as VEGF and metalloproteinases (MMP-1, -2, -9). Supernatants from exposed RPE cultures were found to promote higher levels of angiogenesis in several *in vitro* assays (endothelial proliferation assay, endothelial migration assays, and aortic ring assay) compared to controls. Significantly, transplantation of activated microglia into the subretinal space also markedly promoted the growth of choroidal vessels into the area of subretinal microglial transplantation in the form of choroidal neovascularization. These results indicated that RPE cells, under the inductive influence of subretinal microglia, may be altered significantly in both function and structure so as to promote a more pro-inflammatory, pro-angiogenic environment in the outer retina that encourages that formation of choroidal neovascularization, a hallmark lesion of advanced “wet” AMD.

XX.4 Therapeutic perspectives

Advancements in the treatment of AMD have occurred in recent years but many therapeutic challenges remain. Comprehensive prevention measures for early and advanced AMD are lacking, and there is still no proven treatment for advanced atrophic AMD. Current anti-VEGF therapies for advanced “wet” AMD have markedly improved visual outcomes but are not without their limitations (Mousa and Mousa, 2010). While multiple molecules and mechanisms have been implicated in AMD pathogenesis (Zarbin and Rosenfeld 2010), the cellular mechanisms at play in the locus of AMD disease are still incompletely understood. We posit that microglia-induced influences in the retinochoroidal interface may be of pathogenic significance and the inhibition of these influences represents a potential therapeutic strategy. A more comprehensive understanding of the factors that influence (1) the initial displacement of microglia into the outer retina, (2) the activation of microglia in the outer retina, (3) the increasing recruitment of microglia with age and disease, and (4) the cellular interactions that microglia have with photoreceptors and RPE cells, will be helpful in elucidation of the retinal cell biology underlying AMD and in the design of future treatments.

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