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Adv Exp Med Biol. Author manuscript; available in PMC 2017 January 01.

Published in final edited form as:

Adv Exp Med Biol. 2016 ; 854: 73–78. doi:10.1007/978-3-319-17121-0_11.

Aging Changes in Retinal Microglia and their Relevance to Age-related Retinal Disease

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Abstract

Age-related retinal diseases, such as age-related macular degeneration (AMD) and glaucoma, contain features of chronic retinal inflammation that may promote disease progression. However, the relationship between aging and neuroinflammation is unclear. Microglia are long-lived, resident immune cells of the retina, and mediate local neuroinflammatory reactions. We hypothesize that aging changes in microglia may be causally linked to neuroinflammatory changes underlying age-dependent retinal diseases. Here, we review the evidence for (1) how the retinal microglial phenotype changes with aging, (2) the factors that drive microglial aging in the retina, and (3) aging-related changes in microglial gene expression. We examine how these aspects of microglial aging changes may relate to pathogenic mechanisms of immune dysregulation driving the progression of age-related retinal disease. These relationships can highlight microglial aging as a novel target for the prevention and treatment of retinal disease.

XX.1 Introduction

Common retinal diseases, such as AMD and glaucoma, contribute significantly to vision loss in the US and worldwide (Congdon et al. 2003; Congdon et al. 2004). They however have an intriguing age-dependence in their prevalence which increases markedly with aging (Friedman et al. 2004a; Friedman et al. 2004b). The causes for their association with aging are not well-understood, but because these diseases are characterized by an early emergence of retinal neuroinflammation (Wax and Tezel 2009; Buschini et al. 2011), it has been hypothesized that an age-related dysregulation of immune response in the retina can contribute to disease pathogenesis (Xu et al. 2009; Wong 2013). As microglia are the primary resident immune cell in the retina, and are long-lived cells that persist across long periods of chronological time (Albini et al. 2005; Ajami et al. 2007), senescent changes occurring within aging microglia may be one cause of immune response “failure”, conferring upon the retina an age-dependent vulnerability to disease. Here we review the evidence that retinal microglia in fact demonstrate aging-dependent physiological and molecular changes, and speculate on the drivers and consequences of microglia aging in the retina.

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XX.2 Aging Phenotypes of Retinal Microglia

Microglia in the young healthy retina demonstrate an orderly laminar distribution in which individual cells are evenly spread out in a regularly tiled distribution in the inner and outer plexiform layers but are intriguingly excluded from the outer retina (Santos et al. 2008). Each microglial cell possesses ramified, branching processes that exhibit rapid, constitutive motility that enables the cell to effectively survey the extracellular milieu in its vicinity (Lee et al. 2008). While microglial somata are evenly spaced and relatively stationary in the uninjured state, microglia following focal injury promptly polarize their processes and migrate in the direction of injury to cluster around the injury site. In our studies, we found that these phenotypic features of retinal microglia are not static but change progressively with aging. Compared to the young (3–4 month old) mouse retina, the aged (18–24 month old) retina contains a slightly but significantly greater density of microglia; each of these aged microglia have a significantly smaller ramified dendritic arbor on average, with fewer branches and shorter total process lengths (Damani et al. 2011). In addition, the constitutive movements of aged microglial processes were significantly slower than those in their younger counterparts. Similar observations were also found for microglia in the cortex (Hefendehl et al. 2014) and hippocampus (Mouton et al. 2002) of the brain, indicating that CNS microglia may decline in their ability to perform everyday functions of immune surveillance and synapse maintenance with aging, which may translate to an increasing vulnerability to neurodegenerative disease (Streit and Xue 2009).

In addition to microglial phenotypes in the steady state, we found that the nature and extent of microglial responses to injury become altered with aging. While young retinal microglia responded dynamically to exogenous applications of ATP, an injury-related signal, by increasing motility and the degree of branching in their processes, aged microglia demonstrated a converse response by decreasing both process motility and ramification (Damani et al. 2011). In a laser model of focal retinal injury, we found that aged microglia failed to upregulate their process motility in the immediate aftermath of focal injury (minutes to hours) in a manner observed in young microglia. Aged retinal microglia also migrated to the injury site more slowly compared with young microglia. In the longer term, while young microglia demonstrated dispersal from the injury site 16 days after laser injury, aged microglia remained clustered at the laser burn with a reduced rate of dispersal. These data indicated while microglial injury responses in the young retina have a prompt and rapid initiation upon the onset of injury, followed by an expeditious downregulation upon injury resolution, those in the aged retina are slower to initiate but are also slower to reverse and return to the resting state. These dysregulated responses may thus contribute defects in efficient homeostasis and help contribute to a more chronically active neuroinflammatory state in the retina.

The exclusion of microglia from the young healthy outer retina is a unique feature that indicates the outer retina as a special zone of immune regulation where the spatial segregation of microglia from outer retinal cell types is required. However, with aging, this zone of exclusion is increasingly transgressed by microglia that translocate into the outer retina to accumulate in the subretinal space (Xu et al. 2008; Chinnery et al. 2012). In the young retina, physical contact and interaction between microglia and RPE cells are highly

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infrequent, but in the aged retina, these RPE-microglia contacts increase monotonically as a function of aging. Microglia accumulating in the subretinal space demonstrate morphological and molecular markers of increased activation (Xu et al. 2008; Ma et al. 2013b), indicating their ability to contribute to an increased pro-inflammatory local environment. These changes were similarly observed in aged and AMD human retinas (Ma et al. 2013a). While the factors that drive this translocation are unclear, these increasing age-dependent RPE-microglia interactions result in changes in RPE cells that induce further immune dysregulation at this outer retinal interface and promote pathological changes similar to those observed in AMD (Ma et al. 2009; Ma et al. 2012). From observations *in vitro* and *in vivo* systems, we found that activated retinal microglia induced in RPE cells 1) changes in RPE structure and distribution, 2) increased expression and secretion of pro-inflammatory, chemotactic, and pro-angiogenic molecules, and 3) an increased ability to promote *in vivo* choroidal neovascularization. As such, we speculate that the migration of retinal microglia into the subretinal space induces significant changes in RPE cells that perpetuate further microglial accumulation, increase inflammation in the outer retina, and fosters an environment conducive for the formation of neovascular changes in wet AMD.

XX.3 Potential factors driving the aging microglial phenotype in the retina

How do aging-related phenotypes arise in retinal microglia? Elucidation of the drivers of microglial aging can not only enable an understanding of microglial physiology but also present therapeutic opportunities for modulating of these phenotypes to inhibit or reverse vulnerabilities to aging-related retinal disease. Factors influencing microglial phenotypes may arise from the environment of the aging retina or otherwise from intrinsic age-related changes within microglial cells themselves. Genetic expression profiling of the entire retina have shown that retinal aging involves gene sets involved in the regulation of local inflammatory responses, particularly those involved with the innate immune system (Chen et al. 2010), suggesting that modulatory influences onto microglia, possibly from neighboring retinal cells such as Müller cells (Wang et al. 2011; Wang et al. 2014; Wang and Wong 2014), may change with aging. On the other hand, microglia themselves demonstrate intrinsic age-dependent changes such as the accumulation of lipofuscin, which are likely built up as a function of continuing phagocytosis of byproducts of the visual cycle. We discovered that the accumulation of A2E, a primary bisretinoid of lipofuscin, has the effect of increasing microglial activation, suppressing microglial chemotactic responses, and altering complement gene expression to favor complement activation. As such, lipofuscin buildup in aging microglia may constitute one potential driver of pathogenic aging microglial phenotypes.

We found by microarray analysis of microglia isolated *ex vivo* from the mouse retina that patterns of gene expression in microglia demonstrate progressive change with aging (Ma et al. 2013b). In particular, molecular pathways involving immune function and regulation, angiogenesis, and neurotrophin signaling demonstrated age-related changes. Interestingly, expression levels of complement genes C3 and CFB, which have been associated with AMD, also increased with aging, indicating that microglia, which can contribute to local complement regulation (Rutar et al. 2011), may falter in their ability to limit complement activation with aging. Indeed, we also found immunohistochemical and mRNA evidence of

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increased C3 and CFB expression, as well as complement activation in the aging, relative to young, retina (Ma et al. 2013b). Therefore, intrinsic changes in complement gene expression, combined with outer retinal accumulation, may constitute a mechanism by which aging microglia alter the immune environment in ways pathologically relevant to AMD.

XX.4 Conclusions and Perspectives

Microglia in the young healthy animal have a highly ordered, regular and laminar distribution in the retina, in which they conduct constitutive activities of synapse maintenance and immune surveillance via highly dynamic processes. They also express multiple inflammatory proteins, including complement and complement regulatory proteins, indicative of their role in the immune regulation of the retinal environment. With retinal aging, these phenotypes demonstrate age-related changes that result in a disordered microglial distribution in the retina, deficient constitutive microglial function, and abnormal microglial injury responses. These alterations, combined with molecular and gene expression aging changes within microglia, indicate that aged microglia may be less capable of maintaining homeostasis in the immune environment, particularly of the outer retina. Further study into the factors in the aging retinal environment influential on microglial phenotype and into the key molecular regulators of microglial function will be helpful in understanding how microglial aging can be modulated or reversed. The ability to successfully modulate microglial aging phenotype has the promise of “rejuvenating” the immune environment of the retina in ways that may be protective against the progression of age-related retinal diseases.

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