ANNUAL REVIEWS

Annual Review of Vision Science

Microglia in the Retina: Roles in Development, Maturity, and Disease

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Annu. Rev. Vis. Sci. 2018. 4:45–77

First published as a Review in Advance on May 31, 2018

The *Annual Review of Vision Science* is online at vision.annualreviews.org

https://doi.org/10.1146/annurev-vision-091517-034425

Keywords

microglia, retina, development, homeostasis, disease, therapy

Abstract

Microglia, the primary resident immune cell type, constitute a key population of glia in the retina. Recent evidence indicates that microglia play significant functional roles in the retina at different life stages. During development, retinal microglia regulate neuronal survival by exerting trophic influences and influencing programmed cell death. During adulthood, ramified microglia in the plexiform layers interact closely with synapses to maintain synaptic structure and function that underlie the retina's electrophysiological response to light. Under pathological conditions, retinal microglia participate in potentiating neurodegeneration in diseases such as glaucoma, retinitis pigmentosa, and age-related neurodegeneration by producing proinflammatory neurotoxic cytokines and removing living neurons via phagocytosis. Modulation of pathogenic microglial activation states and effector mechanisms has been linked to neuroprotection in animal models of retinal diseases. These findings have led to the design of early proof-of-concept clinical trials with microglial modulation as a therapeutic strategy.

1. INTRODUCTION

Microglia, the primary resident population of innate immune cells in the neural parenchyma, is a key constituent of the glial populations in the central nervous system (CNS). Formerly mysterious and largely ignored, microglia have over the last decade been discovered to play crucial roles in the CNS that extend beyond more traditional concepts of immune defense to functions in CNS development and homeostasis (Kierdorf & Prinz 2017). Significantly, studies of CNS pathology have found microglia to be centrally involved in pathogenic mechanisms in multiple neurodegenerative diseases (Salter & Stevens 2017), prompting investigation into new therapeutic strategies for which microglia constitute the central cellular target (Peña-Altamira et al. 2017).

In the retina, microglia similarly constitute a prominent part of the resident glial population. Microglia in the adult retina resemble those throughout the CNS in their ramified morphology and dynamic process motility (Lee et al. 2008). However, they possess some distinguishing features in the laminated structure of the retina, such as their distribution as horizontal arrays of cells that are concentrated in the synaptic plexiform layers of the inner retina and their interactions with specialized glia and neurons, such as Müller cells and photoreceptors (**Figure 1**). While there are fewer studies specifically examining microglia in the retina than elsewhere in the CNS, the retina represents an advantageous and unique system for studying microglia. The close juxtaposition of retinal microglia with orderly arrays of retinal synapses in the plexiform layers can facilitate the study of microglia-synapse interactions. Microglia in the retina are uniquely optically accessible without intervention and can be directly and noninvasively examined using in vivo fundus imaging. Lastly, the ability to evoke neuronal responses in the retina by visual stimuli that can be measured with electroretinography enables neuron-microglia interactions in neural circuits to be studied under natural conditions. As such, while microglia in the retina may possess specialized differences, their study can provide general insight into microglia in the rest of the CNS.

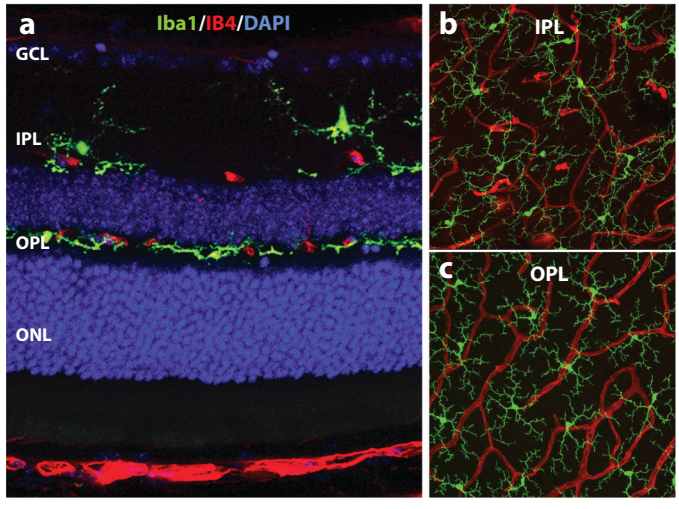

Figure 1

Distribution and morphology of microglia within the laminated retina. (*a*) Cross-section of the adult mouse retina, showing microglia (labeled with Iba1) distributed in a laminar pattern in the inner plexiform and outer plexiform layers (IPL and OPL, respectively), alongside vascular plexi (labeled with IB4). Microglial distribution is largely excluded from the outer retinal layers, such as the outer nuclear layer (ONL). (*b*,*c*) En-face views of microglia in flat-mounted retinal tissue as horizontally ramified cells that tile the IPL and OPL in a nonoverlapping manner. Additional abbreviation: GCL, ganglion cell layer.

Our aim in this review is to provide a current survey of the state of knowledge regarding microglia in the retina in physiological and pathological situations. In the physiological context, we have focused on microglia with respect to their presence, organization, and function during development, when they serve to help organize the formation of the nervous system, and during adulthood, when they help to maintain homeostasis. In the pathological context, we review the main themes underlying microglia involvement in degenerative conditions of the retina, focusing on glaucoma, retinitis pigmentosa (RP), and age-related macular degeneration (AMD) as illustrative examples. We review current strategies targeting microglia in animal models of disease and in early proof-of-concept clinical trials. Our comments derive primarily from studies performed in the retina but also draw from studies performed elsewhere in the CNS when helpful.

2. ROLES OF MICROGLIA IN RETINAL DEVELOPMENT: "SCULPTORS" OF CELL POPULATIONS AND CIRCUITS

During development, microglia enter and colonize the CNS parenchyma, exerting effects on the development of surrounding neurons, synapses, and blood vessels. These events are discussed in the following sections.

2.1. Microglial Entry and Colonization of the Developing Retina and Brain

The origin of microglia in the CNS, a former subject of debate (Ginhoux & Prinz 2015), has been confirmed to derive from primitive hematopoietic progenitors originating from the extraembryonic yolk sac. These early cells, generated around embryonic day (E) 8.0 in the mouse, differentiate under the control of transcription factors Pu.1 and Irf8, colonizing the developing brain to give rise to embryonic microglia at E9.0–9.5 (Ginhoux et al. 2010, Kierdorf et al. 2013a, Schulz et al. 2012). Developmentally distinct from CNS neurons and macroglia, microglia have been likened to "immigrants from another world" (Prinz & Mildner 2011). Following colonization, they exist as a closed, long-lived population under normal conditions, separated by the blood-brain barrier from circulating monocytes or other bone marrow–derived progenitors (Ajami et al. 2007).

While microglia show regional diversity across the CNS (De Biase et al. 2017), it is likely that retinal microglia are ontogenically similar to other CNS microglia. Upon entry into the developing retina, the colonization and spread of microglia across multiple species, including the mouse (Hume et al. 1983, Santos et al. 2008), quail (Marin-Teva et al. 1998, 1999a,b; Navascues et al. 1995; Sanchez-Lopez et al. 2004), rabbit (Ashwell 1989), rat (Ashwell et al. 1989), and human (Diaz-Araya et al. 1995, Provis et al. 1996), follow a similar general pattern. Amoeboid cells immunopositive for microglia/macrophage markers first emerge in the vitreous and on the vitreal surface of the embryonic retina at around E11.5 in the mouse, near the optic nerve and in the peripheral retina (Provis et al. 1996, Santos et al. 2008). These cells migrate in the horizontal/tangential plane in the central-to-peripheral and circumferential directions (Marin-Teva et al. 1998, 1999b) to occupy all retinal areas and then migrate radially from the inner to outer retinal layers. They develop polarized morphologies and ramified processes that fasciculate closely with the radial Müller cell processes that may provide a migratory substratum (Sanchez-Lopez et al. 2004). These migrating microglia undergo concurrent proliferation, expanding overall numbers (Marin-Teva et al. 1999a) to achieve final mature densities. Notably, the outer retina, from the outer nuclear layer (ONL) to the retinal pigment epithelium (RPE), are consistently devoid of microglia throughout development, designating it as a specialized microglial exclusion zone.

Transcriptional profiling has revealed that developing brain microglia in addition to changes in their position with migration proceed through progressive stages [early (until E14),

premicroglia (E14–P9), and adult] characterized by distinct transcriptional and epigenomic signatures (Matcovitch-Natan et al. 2016). In the developing brain, local signals appear important in guiding microglial migration and maturation. Disruption of CX3CL1-CX3CR1, a neuron-to-microglia signaling chemokine, slowed microglia colonization in the hippocampus (Paolicelli et al. 2011) and somatosensory cortex (Hoshiko et al. 2012), and retarded the acquisition of mature physiological and morphological features (Pagani et al. 2015). Ablation of IL-34, a neuronally secreted ligand to the CSF1R microglial receptor, decreased developmental microglial proliferation (Wang et al. 2012). In the developing subventricular zone, neural progenitors and radial glia use CXCL12 and MIF, respectively, to signal to developing microglia, guiding their migration and proliferation (Arno et al. 2014). Overall, microglial colonization appears to require guidance from developmentally staged signals in the developing neural parenchyma. In the developing retina, while these guidance signals are not precisely known, it is likely that environmental cues also serve to guide aspects of tangential and radial migration, as well as exclusion from the outer retina.

2.2. Microglial Influence on the Survival and Death of Developing Retinal Neurons

As microglia distribute themselves in the developing retina, they are brought into close proximity to concurrent developmental events, including programmed cell death (PCD), neurogenesis, synaptic refinement, and vascular development. Early microglia transiently express activation markers typically absent in the healthy adult retina, including F4/80, isolectin, CD45, CD68, and iNOS (Hume et al. 1983, Santos et al. 2008, Sierra et al. 2014), suggesting they may play distinct developmental roles. These markers are lost as microglia mature and tile the horizontal aspect of the retina in a mosaic pattern (Santos et al. 2008). The sections below examine the evidence that microglia participate actively in "sculpting" the neuronal and vascular organization of the retina, as well as fine-tuning the synaptic circuitry in the retinal plexiform layers, helping shape the full complexity of the mature retina.

The organization of superfluous numbers of early neurons into the final complement of cells requires the retention of selected neurons and the elimination of unwanted cells by PCD (Oppenheim 1991). Examples of microglia-mediated trophic influences on neurons in vitro include increased proliferation (Morgan et al. 2004), survival (Nagata et al. 1993), and neurite outgrowth (Chamak et al. 1994) of embryonic neurons cultured with microglia-conditioned media. The loss of microglia also negatively impacted neuronal survival in the developing brain. In vivo microglial depletion in the developing cortex decreased the survival of cortical neurons in layer 5 (Ueno et al. 2013), and depletion of CD11c+ microglia from the neonatal brain decreased myelination and neurogenesis via IGF-1-dependent mechanisms (Wlodarczyk et al. 2017). In the retina, this trophic role of microglia is also evident. Targeted knockdown of microglial *Csf1r* decreased colonizing microglia numbers in the zebrafish retina, which also severely reduced neuronal proliferation and differentiation, causing microphthalmia (Huang et al. 2012). Recovery of CSF1R levels enabled microglial colonization to resume, which was followed by a partial rescue of retinal neurogenesis, strengthening the correlation between microglial presence and neuronal survival. In organotypic cultures of postnatal (P10) mouse retinas, partial elimination of microglia using clodronate liposomes also decreased neuronal viability (Ferrer-Martin et al. 2015). These findings indicate that early microglia can provide trophic support to developing neurons.

The mechanisms underlying the trophic influence in developing retinal microglia are incompletely understood but are likely related to their specialized activation states. When microglial activation was modulated with minocycline treatment in the developing retina (Ferrer-Martin et al. 2015) or subventricular zone (Shigemoto-Mogami et al. 2014), the trophic influence of

microglia was diminished, contrasting with minocycline's ability to decrease microglial neurotoxicity in adult retinal pathologies (Bosco et al. 2008, Krady et al. 2005, Zhang et al. 2004, Zhao et al. 2011). The prosurvival effects of retinal microglia have been attributed to (*a*) neurotrophic factor production, either secreted directly by microglia or indirectly induced by microglia in Müller cells (Harada et al. 2002, Wang et al. 2011) and (*b*) the maintenance of a retinal environment supportive of cell survival by the clearance of dying neurons via microglial phagocytosis, averting the release of noxious factors from dying cells (Napoli & Neumann 2009). Progranulin-a is an example of a microglia-derived growth factor with roles in the trophic support of neuronal differentiation and as an autocrine signal guiding microglial colonization in the developing zebrafish retina (Walsh & Hitchcock 2017).

Counter to their prosurvival role, microglia are found in intimate association with aspects of developmental cell death in the retina. Microglial colonization coincides spatiotemporally with PCD in the brain (Ashwell 1991, Swinnen et al. 2013), spinal cord (Caldero et al. 2009), and retina (Cuadros & Rios 1988, Hume et al. 1983). Disruption of PCD, either by the overexpression of *Bcl-2*, a prosurvival gene (Xu et al. 2016), or inhibition of Caspase-3, an apoptotic-associated protein via genetic (Casano et al. 2016) and pharmacological means (Martin-Estebane et al. 2017), was found to decrease microglial colonization in the developing brain and retina. This suggests that degenerating neurons produce so-called find-me signals (Medina & Ravichandran 2016) that help guide microglial entry and distribution in the CNS. One example is lysophosphatidylcholine, which is released from apoptotic cells following CASP3 activation (Lauber et al. 2003) and signals to developing microglia via G2A G-protein-coupled receptors (Xu et al. 2016). Also, extracellular ATP and UTP nucleotides released from dying cells, either through membrane breakdown or via nucleotide-permeable Pannexin-1 channels (Chekeni et al. 2010), can signal via microglial-expressed P2X and P2Y purinergic receptors (Koizumi et al. 2013) to induce microglial motility and tropism in the brain (Casano et al. 2016) and the retina (Martin-Estebane et al. 2017).

As developing microglia are brought into the locus of PCD, they appear to perform at least two functional roles. One involves clearing out dead cells by phagocytosis, enabling a clean removal of cellular so-called corpses without leakage of cellular contents that promote inflammation and tissue necrosis (Ravichandran 2003). The numerous molecular mechanisms connecting the so-called eat-me signals on dying cells to phagocyte receptors have been reviewed in detail (Park & Kim 2017). In the developing retina, phagocytic microglia can be found in proximity to dying neurons (Pearson et al. 1993), internalizing fragmented DNA (Egensperger et al. 1996) and cell membranes (Bodeutsch & Thanos 2000). The other function involves the potentiation and specification of PCD in developing neurons. This is evidenced in some contexts by decreased developmental apoptosis when microglia are eliminated with clodronate liposomes (Marin-Teva et al. 2004) or specifically altered by the genetic deletion of *CD11b* or *DAP12* (Wakselman et al. 2008). These prodeath influences are driven by mechanisms that include the production of superoxide ions (Marin-Teva et al. 2004, Wakselman et al. 2008), secretion of proinflammatory cytokines such as TNFα (Sedel et al. 2004), removal of nonapoptotic differentiated neurons by phagocytosis (termed phagoptosis) (Brown & Neher 2014), and the phagocytic clearance of neural precursor cells (Cunningham et al. 2013). In the developing chick retina, microglia secrete nerve growth factor, which induces PCD of retinal neurons via the p75 neurotrophin receptor (Frade & Barde 1998), an interaction that is modulated by parallel TGFβ signaling (Dunker et al. 2001). Taken together, developing microglia play both prosurvival and prodeath roles in shaping neuronal development, sculpting the initial population of early neurons to the organized subsets found in maturity.

2.3. Microglial Roles in Shaping the Development of Neuronal Circuits

Following the specification of the correct type and size of neuronal populations, the development of neural systems requires the generation of the precise patterns of synaptic connectivity between the final set of neurons. This involves (*a*) correct spatial positioning of neuronal partners for synaptic contact, (*b*) proper extension and juxtaposition of axons and dendrites between neuronal partners, (*c*) formation of early synapses, followed by the selective elimination of supernumerary synapses, and (*d*) functional maturation of the final subset of synapses. Studies have underscored microglial participation in individual examples of each of these steps. In the developing mouse forebrain, microglial perturbation, either by pharmacological depletion, lipopolysaccharide (LPS)-mediated activation, or inactivation of microglial genes *Cx3cr1* and *Tyrobp*, prevented the correct positioning of Lhx6-expressing interneurons and disrupted the axonal outgrowth of midbrain dopaminergic neurons (Squarzoni et al. 2014). These effects appeared specific in context, as laminar positioning of Reelin-expressing neurons and the outgrowth of thalamic axons remained unchanged. In the developing retina, the effects of microglia on neuroblast migration or neurite elaboration have not yet been specifically examined. When developing microglia colonize the retina, they extend their ramified processes within the nascent inner plexiform and outer plexiform layers (IPL and OPL, respectively), indicating a spatiotemporal coincidence between neurite outgrowth and microglial ramification (Santos et al. 2008). The interaction between developing microglia and neuronal dendritic remodeling in the inner retina merits future investigation.

After developing neurons and their neurites move into the correct positions, synaptogenesis between the juxtaposed partners follows. Excess early synapses are subjected to activity-dependent elimination in which stronger synapses are retained and weaker synapses removed (Goda & Davis 2003). Evidence suggests microglia can play supporting roles in both the formation and elimination of these early synapses. In the cortex, dynamic contact between microglia processes and the dendrites of developing pyramidal neurons can induce filopodia and synapse formation, which was reduced when microglia were genetically ablated (Miyamoto et al. 2013). Conversely, microglia also participate in the developmental elimination of nascent synapses, primarily by phagocytic engulfment and clearance (Hong & Stevens 2016, Paolicelli et al. 2011). Dynamic contact between microglial processes and synapse-bearing dendritic processes (Tremblay et al. 2010, Wake et al. 2009), guided by neuronal activity and molecular cues, can culminate in microglial engulfment and elimination of unwanted synapses (Schafer et al. 2012), as documented in the retinogeniculate system (Schafer et al. 2012, Stevens et al. 2007) and visual cortex (Tremblay et al. 2010). One central mechanism involves the action of complement molecules C1q and C3 in differentially "tagging" synapses according to their activity status, promoting their recognition by microglia via complement receptor CR3 and subsequent elimination by phagocytosis (Schafer et al. 2012, Stevens et al. 2007). This role of microglia in synaptic refinement can be modulated by neuron-glia signaling occurring via TGFβ (Bialas & Stevens 2013), serotonin (Kolodziejczak et al. 2015), and CX3CL1 (Paolicelli et al. 2011).

Finally, microglia can further participate in the functional maturation of these synapses by regulating the expression of synaptic receptors. Dysfunctional microglia appear to fail in this task in the brains of CX3CR1-deficient mice, which demonstrate altered physiology and delayed colonization of target brain regions, resulting in synapses with abnormal receptor expression and functional properties (Hoshiko et al. 2012, Pagani et al. 2015, Paolicelli et al. 2011). These mechanisms may vary across brain regions. Ablation of microglial CX3CR1 was recently found not to influence developmental synaptic plasticity in the visual cortex (Lowery et al. 2017, Schecter et al. 2017). With respect to the retina, neuronal activity has been demonstrated to influence synapse formation between retinal neurons (Kerschensteiner et al. 2009) as well as the functional

maturation of these synapses (Dunn et al. 2013). However, while there is evidence to indicate that microglia in the retina are responsive to neuronal activity (Fontainhas et al. 2011), whether and how retinal microglia serve to shape and mature synapses in the developing plexiform layers remain to be elucidated.

2.4. Microglial Roles in Shaping the Development of Retinal Vasculature

While this review centers on microglia-neuron interactions, it is worth mentioning that myeloid cells in the eye are additionally involved in shaping vascular development. As in neuronal development, these cells can exert both prodeath and prosurvival influences. In the developing vitreous, regression of the transient hyaloid capillary network has been associated with vitreal myeloid cells called hyalocytes (Balazs et al. 1980). Genetic or pharmacological ablation of vitreal macrophages preserved the normally transient hyaloid vasculature, which can be driven to regress when subsequent bone marrow–derived macrophages were reintroduced (Diez-Roux & Lang 1997). The underlying mechanism is thought to involve macrophage-mediated induction of apoptosis in vascular endothelial cells via WNT7b-mediated WNT signaling (Lobov et al. 2005). Retinal microglia, which are intimately associated with developing retinal vessels (Provis et al. 1997), can play supportive and guidance roles. Converse to the hyaloid vasculature, elimination of developing microglia reduced retinal vascular growth, while their restoration with intravitreally delivered exogenous microglia resumed growth (Checchin et al. 2006). In supporting studies, genetic deficiency or pharmacological inhibition of CSF-1 reduced retinal microglial numbers and decreased developmental branching of retinal vasculature (Kubota et al. 2009). Retinal microglia-endothelial cell signaling via secreted soluble factors can also help shape vascular growth and branching (Rymo et al. 2011). Examples include CD95L, which potentiates vascular growth and complexity (Chen et al. 2017), and Flt1, which conversely limits vascular branching (Stefater et al. 2011).

Taken together, microglia colonization and development, which are spatiotemporally coordinated with key events in neurovascular development, are prominently influential in developmental processes throughout the CNS, including the retina. Developmental microglial morphology and distribution, which are dissimilar to those in adulthood, appear specialized to carry out functions in directing, supporting, and eliminating developing cells and cellular structures, acting to sculpt the neural anlage to its mature form. These mechanisms may potentially reveal the pathogenesis of neurodevelopmental diseases (Arcuri et al. 2017) and provide insights into regenerative strategies.

3. ROLES OF MICROGLIA IN THE HEALTHY MATURE RETINA: CONSTANT "ELECTRICIANS" IN CONSTITUTIVE MAINTENANCE

In the healthy adult brain and retina, microglia make up a stable and highly ordered network of ramified cells that are thought to carry out constitutive maintenance functions. The evidence for their long-lived status, homeostasis, and constitutive functions are summarized in the sections below.

3.1. Stability and Turnover of Microglia in the Adult Retina and Central Nervous System

As retinal development approaches maturity, microglia acquire their final adult topographical distribution of uniformly spaced cells tiling the IPL and OPL that provide comprehensive coverage of the retinal milieu through their dynamically motile ramified processes (Santos et al. 2008).

Previous studies employing bone-marrow transplantation techniques have posited that mature retinal microglia are continuously turned over and replaced by bone marrow–derived monocytic precursors (Xu et al. 2007). However, subsequent studies demonstrated that monocytic entry was induced secondarily from irradiation measures and absent under healthy conditions (Ajami et al. 2007, Kierdorf et al. 2013b). Recent cell-fate mapping studies confirmed that adult retinal microglia exist as a self-sustaining, closed population in the absence of disease or injury (Ma et al. 2017, O'Koren et al. 2016). While there is current consensus on this view, the precise rate and extent of steady-state self-renewal of microglia are less well defined. A decades-old study employing ^{3}H-thymidine incorporation and autoradiography to track cell replication had reported that brain microglia turnover occurs at a very low basal rate (Lawson et al. 1992), with individual microglia demonstrating residence times of nearly a lifetime. Studies using cell-fate mapping (Tay et al. 2017) and BrdU incorporation (Askew et al. 2017) have detected that microglial self-renewal occurs stochastically in the mouse brain, with different CNS compartments demonstrating different turnover rates. Estimates of the time taken for microglia to completely turn over have varied between studies and brain regions, ranging from 3 (Askew et al. 2017) to 41 months (Tay et al. 2017) in the mouse brain. Human brain data obtained from labeling of microglia with nucleotide analogs used in cancer treatment estimate turnover periods to be on the timescale of years to decades (Reu et al. 2017), corroborating the concept of microglia as long-lived cells whose permanence extends across a substantial fraction of an animal's life span. Estimates for the longevity of retinal microglia are yet unavailable.

3.2. Homeostasis of Microglia Presence and Organization in the Adult Retina and Central Nervous System

In the adult CNS, the number, distribution, and physiology of microglia in each region are highly ordered and robust to perturbations (De Biase et al. 2017), invoking mechanisms that safeguard the homeostasis of microglial presence and organization. Cell-fate mapping experiments have shown that individual cells show considerable stability in the overall network (Tay et al. 2017). If individual cells were to undergo apoptosis stochastically, local proliferation of nearby microglia is induced to provide replacements (Askew et al. 2017). This regenerative property appears general to all microglial cells, and a designated proliferative microglial niche has not been evident. Short-term live-cell imaging experiments in both the brain (Davalos et al. 2005, Nimmerjahn et al. 2005) and the retina (Lee et al. 2008) support this scenario of stability in microglial organization. Individual microglia demonstrate dynamic constitutive motility in their processes while the positions and overall regularity of their somata remain relatively stable over time.

Various lines of evidence indicate microglial homeostasis is not cell-autonomously determined but occurs under tight regulation by constitutive signals arising from surrounding neurons. Microglia survival in the adult brain requires constitutive signaling between neuronally derived cytokine IL-34 and the microglial receptor CSF1R (Greter et al. 2012). This applies to retinal microglia also as pharmacological inhibition of CSF1R by PLX-5622 results in widespread microglia depletion (Hilla et al. 2017). The maintenance of a baseline activation state for CNS microglia also arises from a balance of multiple constitutively active, neuronally derived factors (Biber et al. 2007, Bohlen et al. 2017). TGFβ signaling is critical in conferring the characteristic molecular signature of adult microglia (Butovsky et al. 2014). In the retina, constitutive signaling from neuronally expressed CX3CL1 regulates the physiological state of microglia. In the absence of signaling, as in the genetic deletion of CX3CR1, aberrant activation results in microglia displacement to the subretinal space (Combadiere et al. 2007) and exacerbated microglial responses in retinal injury models (Kezic et al. 2013a, Roche et al. 2017, Zabel et al. 2016). Likewise, CD200

expression on neurons and vascular endothelium in the retina signal through microglia-expressed CD200R to regulate microglial number and activation state under normal conditions and suppress excessive activation in response to injury (Broderick et al. 2002).

The significance and effectiveness of mechanisms maintaining microglial homeostasis have been underscored by the recent discovery that CNS microglia can rapidly regenerate the full complement of microglial cells following near-complete depletion (Bruttger et al. 2015, Elmore et al. 2014). In the retina, work performed in our laboratory has also demonstrated a prominent repopulation response following depletion using genetic and pharmacological methods (Zhang et al. 2018). We found that microglial repopulation following depletion occurred in a center-to-peripheral, IPL-to-OPL, direction that was driven by a dynamic in situ proliferation and migration of residual microglia. This response fully recapitulated original microglial organization in terms of overall density, laminar distribution, and morphological structure. Repopulated microglia recapitulated endogenous microglia in terms of the quantitative motility of their processes, the ability to respond to light injury by activation, migration, inflammatory cytokine production, and the function of maintaining synaptic function and integrity. The ability of microglial organization in the CNS to precisely recover following perturbation underscores (*a*) the presence of prominent signaling mechanisms in the CNS parenchyma that specify and maintain microglial organization and (*b*) the functional importance of the overall presence and organization of microglia in the adult CNS.

3.3. Constitutive Role of Microglia in Regulating Neuronal Activity and Synaptic Integrity in the Retina

What is the nature of the microglial requirement in the brain and retina that necessitates high-fidelity maintenance of microglial presence and organization? While the answers to this question are incompletely understood, there are indications that microglia play functional roles in mature systems. Owing to the location of microglial processes in the plexiform layers, microglia frequently and dynamically contact dendritic, axonal, and synaptic compartments of neurons and are well positioned to influence their structure and function. Conversely, there is evidence that retinal neurons can exert influences on microglia, regulating morphology and dynamic behavior using neuronally derived signals such as CX3CL1 (Liang et al. 2009) and ATP (Fontainhas et al. 2011). Indeed, microglial process behavior is coordinated with the level of neuronal activity as regulated via glutamatergic and GABAergic signaling. These forms of neurotransmission influence the release of extracellular ATP, which then signals directly to microglia via P2 receptors (Eyo et al. 2014, Fontainhas et al. 2011). Taken together, the existence of this constitutive, bidirectional communication between neurons and microglia in the retina posits a consequential ongoing interaction between these cell populations.

We investigated the constitutive function of microglia in the adult mouse retina by examining the consequences of prolonged microglial depletion over the course of one month (Wang et al. 2016c). We found the histological structure of the retina, in terms of its thickness, lamination, and organization of its vasculature, were quantitatively unchanged, with no detectable increase in cellular apoptosis. The number and morphology of neuronal subsets and macroglia (astrocytes and Müller cells) were also stable, indicating that retinal microglia did not exert a trophic effect on cell survival nor were influential in the organization of adult neurons and vasculature as during development. However, increasing durations of microglial depletion were associated with progressive decrements in the electroretinographic (ERG) response to light stimuli, characterized particularly by decreased amplitude of the b-wave component. This physiological change was correlated with an increase in synaptic degeneration on electron microscopy, indicating microglia

in the adult retina are necessary for maintaining synaptic integrity and function, and thus the retina's normal physiological responses to light. This role of synaptic maintenance has also been indicated in the adult brain; ablation of microglia or microglia-derived brain-derived neurotrophic factor (Parkhurst et al. 2013) in early adulthood using genetic methods resulted in reductions in synapse elimination during motor learning and decreased performance on learning tasks. However, the depletion of brain microglia in another study using pharmacological methods failed to demonstrate similar cognitive or behavioral changes (Elmore et al. 2014), indicating a need for closer examination of microglia-synapse interactions in mature systems, perhaps using more precise electrophysiological assessments.

Another contribution that microglia make to CNS function is to regulate adult generation of neurons and glia. Microglia in the hippocampus (Sierra et al. 2010) and the subventricular zone (Fourgeaud et al. 2016) can conduct phagocytic clearance of apoptotic neuroblasts, maintaining the homeostasis of the adult neurogenic niche. Microglial depletion in the adult brain was also found to decrease the number of $NG2^+$ oligodendrocyte precursor cells in the brain, suggesting that mature microglia can provide neurotrophic effects on progenitor populations (Hagemeyer et al. 2017). The mechanisms underlying these trophic effects and how they may differ from those found in development await further elucidation.

The studies described in this section together describe an intriguing place for microglia in the everyday function of the CNS. Beyond simply acting as immune sentinels, microglia play modulatory and maintenance functions in adult systems without which neuronal connections, which subserve sensory perception and cognitive processing, may fail. These constitutive microglial functions provide clues as to how microglia may fail, such as with senescence, as previously reviewed (Wong 2013), and contribute toward increased vulnerability to age-related neurodegeneration.

4. MICROGLIAL INVOLVEMENT IN DEGENERATIVE CONDITIONS IN THE RETINA

In the following sections, we review the evidence that retinal microglia are involved in, and make pathologic contributions to, neurodegeneration in three major retinal diseases: glaucoma, retinitis pigmentosa (RP), and AMD. These serve as examples for microglial involvement in retinal pathologies.

4.1. Microglia in Glaucoma

Glaucoma, a set of heterogenous diseases featuring progressive degeneration of retinal ganglion cells (RGCs), is a leading cause of visual field loss and blindness worldwide (Quigley & Broman 2006). While factors that drive loss of RGCs are incompletely known, neuroinflammatory changes in the retina and optic nerve have been implicated in influencing the rate of degeneration (Williams et al. 2017). Findings of microglial involvement in glaucoma have been documented in human disease and in multiple animal models (Agarwal & Agarwal 2017). Interest in understanding and targeting neuroinflammation in glaucoma (Williams et al. 2017) is premised on the causative role of immune cells, particularly microglia, in augmenting RGC degeneration, rather than demonstrating adaptive or bystander effects. The most prevalent association has been one relating to microglial activation in the retina during disease progression. In human glaucomatous eyes, degenerative changes at the optic nerve head have been spatially associated with morphologically amoeboid microglia immunopositive for markers of activation (HLA-DR, CD68), inflammatory mediators and cytokines (TNFα, NOS-2, COX-1), and metalloprotease enzymes (Neufeld 1999, Yuan & Neufeld 2001). Proteomic and immunohistochemical analysis of glaucomatous human

retinas also highlighted increased microglial expression of TLR2, -3, and -4, indicating increased proinflammatory Toll-like receptor (TLR) signaling (Luo et al. 2010). In the genetic DBA/2J model of pigmentary glaucoma (Bosco et al. 2011), and in models involving elevated intraocular pressure (IOP) (Bordone et al. 2017, Ebneter et al. 2010, Kezic et al. 2013b), the premise that microglial responses contribute causally to glaucoma progression is supported by observations that microglial activation responses temporally precede RGC degeneration (Bosco et al. 2011). Also, the extent of microglial activation was found to be correlated with the severity of subsequent RGC degeneration (Bosco et al. 2015). Further support is provided by studies showing that genetic alterations in mouse models that are specific to, or occur predominantly in, microglia significantly affect disease phenotypes. Deletion of *Cx3cr1*, which increases microglial activation, resulted in more extensive RGC loss in an IOP elevation model (Wang et al. 2014) and greater glaucoma-related RGC axon transport dysfunction in the DBA/2J model (Breen et al. 2016). Genetic deletion of either CD11b, a marker for microglia, or TNFα, a proinflammatory cytokine expressed by microglia, decreased RGC loss in an IOP-elevation model (Nakazawa et al. 2006). These findings indicate that the products of microglial activation can potentiate RGC degeneration via proinflammatory and oxidative stress pathways. Pharmacological blockade of these pathways has been found to protect RGCs in animal models, as had been previously reviewed (Wang et al. 2016b, Williams et al. 2017).

Another feature of the microglial response to glaucoma is the elevated expression of complement molecules. Profiling of messenger RNA (mRNA) expression in retinas from DBA/2J (Howell et al. 2011, Steele et al. 2006) and IOP elevation rodent models (Ahmed et al. 2004), as well as proteomic analysis in human glaucomatous retinas (Mirzaei et al. 2017, Tezel et al. 2010), have found upregulated expression of complement cascade components. Microglia are a prominent source of complement components, particularly in the aging and diseased retina (Ma et al. 2013a, Natoli et al. 2017a). Aberrant expression of C1q emerges in the synaptic IPL layer of DBA/2J retinas prior to RGC loss and in human glaucomatous retina (Stevens et al. 2007). In the developing visual system, complement molecules C1q and C3 have been found to opsonize supernumerary synapses, enabling their recognition and phagocytic elimination by CR3-expressing microglia (Schafer et al. 2012, Stevens et al. 2007). As synapse and dendritic loss occur prior to RGC somatic degeneration in glaucoma models (Williams et al. 2016), microglia may inappropriately recapitulate this developmental function in early glaucoma, secreting complement molecules into the IPL and conducting pathological pruning of RGC synapses and dendrites (Rosen & Stevens 2010). Genetic deletion of complement components in the DBA/2J model were also consequential to disease progression, implicating complement-mediated microglia-RGC interactions as a disease-relevant mechanism. Interestingly, studies found that while C1q deletion decreased degeneration (Williams et al. 2016), C3 ablation conversely increased degeneration (Harder et al. 2017), suggesting that mechanisms involving complement in glaucoma may be complex and involve both deleterious and protective roles, implicating the need for complement-targeted therapies to be both specific and nuanced (Xu & Chen 2016).

Finally, microglia interact with RGCs in the context of glaucoma in the later stages through phagocytic clearance of degenerating cells. In models of optic nerve injury involving axotomy, activated retinal microglia were noted to overtly phagocytose dying RGCs, as evidenced by specific transcellular staining of microglia following the carbocyanine retrolabeling of axotomized RGCs (Thanos et al. 1992). Indeed, each microglia cell is thought to be able to phagocytose multiple RGCs over a sustained period during degeneration (Schuetz & Thanos 2004). This may be an adaptive mechanism for efficiently removing apoptotic cells, to allow for homeostasis of the retina environment (Sierra et al. 2013) by preventing DNA release from dying cells (Egensperger et al. 1996). Conversely, microglial phagocytosis may accelerate RGC degeneration by the

inappropriate clearance of living RGCs by phagoptosis (Brown & Neher 2014). As such, the net contribution of microglial phagocytosis to overall disease progression deserves additional study.

There is evidence that the neuroinflammatory environment in the glaucomatous retina can also be influenced in consequential ways by the systemic immune system, which includes lymphocytes of the adaptive immunity system and circulating monocytes, and by the retinal macroglial populations of astrocytes and Müller cells (Howell et al. 2012, Tezel 2013). While these topics lie outside the scope of this review, the interactions between microglia and these cell populations in glaucoma constitute an interesting area for future study.

4.2. Microglia in Inherited Photoreceptor Degeneration (Retinitis Pigmentosa)

RP represents a group of typically monogenic, hereditary retinal diseases in which photoreceptors undergo degeneration, leading to visual field loss and eventually to complete blindness (Hartong et al. 2006). The precipitating cause for degeneration arises from mutations in genes expressed predominantly in photoreceptors or RPE cells. The causative mutations are diverse, affecting a large (>200) set of genes (Daiger et al. 2013). While the causative genes in RP are not typically expressed in microglial cells, photoreceptor degeneration has been spatiotemporally associated with prominent microglial responses. In human histopathological analyses of RP, microglial responses consist of a prominent displacement of microglia from the inner retina to the ONL, a transition from a ramified to amoeboid morphology, and the development of cytoplasmic inclusions with rhodopsin-immunopositive contents (Gupta et al. 2003, Zhao et al. 2015). In rodent models of RP that contain related pathogenic mutations, a similar microglial translocation and activation response is present (Roque et al. 1996, Thanos 1992). These activated microglia undergo proliferation (Zeiss & Johnson 2004), secrete proinflammatory (e.g., TNFα and IL-1β) (Appelbaum et al. 2017, Yoshida et al. 2013b) and chemotactic (CCL2 and CCL3) cytokines (Zeng et al. 2005), and produce biologically active compounds like nitric oxide (de Kozak et al. 1997, Yang et al. 2007) and reactive oxygen species (Zeng et al. 2014). The ability of microglial responses in human RP to alter the ocular inflammatory environment is indicated by elevated cytokine levels in the aqueous and clinical signs of mild chronic inflammation (Yoshida et al. 2013a). Although microglial responses are secondary to events induced by photoreceptor gene mutations, there is evidence that activated microglia can potentiate degeneration in a non–cell autonomous manner. Genetic manipulation of microglia-specific genes that control activation (e.g., *Cx3cr1*) (Zabel et al. 2016) and pharmacological suppression of general activation such as with minocycline (Peng et al. 2014, Yang et al. 2007), tamoxifen (Wang et al. 2017), or progesterone (Roche et al. 2016) have been shown to alter the rate of degeneration. Also, measures that deplete microglia (Zhao et al. 2015) or decrease their recruitment into the photoreceptor layer via the suppression of chemoattractive signaling (e.g., CCL3 and CCL2-CCR2) (Guo et al. 2012, Kohno et al. 2014) can also ameliorate degeneration. Taken together, the release of microglial activation products in the context of RP has the effect of facilitating and perpetuating microglial presence in the outer retina, enabling neurotoxic and apoptosis-inducing proinflammatory influences to be exerted on photoreceptors that speed their demise.

In addition to the release and secretion of factors, microglia in the RP retina gain physical access to photoreceptors, enabling increased contact-mediated interactions. Live-cell time-lapse imaging of outer retinal microglia in retinal explants from the rd10 mouse model of RP demonstrated that these amoeboid microglia migrate across the ONL and make repeated contact with photoreceptors via motile processes that often terminate in phagocytic cups (Zhao et al. 2015). Following multiple probing contacts, ONL microglia rapidly engulf photoreceptor somata, translocating them to phagosomes for destruction. While this phagocytosis activity facilitated the clearance of

photoreceptors that have undergone apoptosis, many of the photoreceptors phagocytosed were negative for multiple markers of apoptosis, indicating the role of microglial phagoptosis in removing viable photoreceptors. Inhibition of microglial phagocytosis was able to slow the structural and functional photoreceptor degeneration in rd10 retinas (Zabel et al. 2016, Zhao et al. 2015), underscoring this as a mechanism underlying microglial contributions to degeneration in RP.

The signals that initiate early microglial responses in the RP retina and sustain microglial activation across degeneration are incompletely understood. The initial migration of microglia into the ONL in various RP models occurs early in the course of photoreceptor degeneration, when apoptosis is newly initiated and before extensive photoreceptor loss is present (Karlstetter et al. 2014, Zhao et al. 2015). Interestingly, the upregulation of mRNA transcripts of inflammatory genes (Appelbaum et al. 2017) could be detected prior to photoreceptor apoptosis, indicating that the initiating signal to microglia may be generated from the altered physiology of mutation-bearing photoreceptors, rather than from their overt death. One candidate signal is extracellular ATP, which can be released by stressed retinal cells (Notomi et al. 2011), leading to microglial chemotaxis and activation via purinergic receptors such as P2Y12 and P2X7 (Reichenbach & Bringmann 2016). As activated and phagocytic microglia gain access to the ONL, they may be able to sustain the ongoing program of microglial recruitment and activation. Microglial phagocytosis of photoreceptor proteins has been described to increase the inflammatory activation via a TLR4-mediated mechanism (Kohno et al. 2013). Activated microglia in the ONL can further recruit and attract other microglia from the inner retina via the secretion of CCL2 and CCL3 (Kohno et al. 2014). In this light, microglia-photoreceptor interactions may trigger a perpetuating cycle of microglial activation that sustains its pathologic contribution to photoreceptor degeneration.

In contrast to microglia-mediated mechanisms that confer neurotoxicity to photoreceptors, there is evidence that part of the microglial response to photoreceptor degeneration may be adaptive in nature. In vitro activation of retinal microglia has been demonstrated to induce the expression of neurotrophic factors in cocultured Müller cells. Conditioned media from these Müller cells can exert neuroprotection in stressed photoreceptors in vitro (Wang et al. 2011). The sustained presence of microglia in neonatal retinal explants was found to promote photoreceptor survival, indicating a trophic influence (Ferrer-Martin et al. 2015). In the specific context of a mouse model for RP, retinal microglia have also been linked to neuroprotective effects involving IGF-1 signaling (Arroba et al. 2011). As such, microglial involvement in RP pathobiology is likely to involve a balance of neuroprotective and neurotoxic influences, which deserve consideration in the design of microglial modulatory therapeutic strategies.

The involvement of systemically derived monocytes, relative to that of retina-derived microglia, in the context of human RP and RP models is unclear and awaits further study. In the rd10 mouse model, CCR2-expressing monocytes infiltrate the subretinal space during degeneration (Zhao et al. 2015), indicating possible monocyte-photoreceptor interactions. Cell-fate mapping techniques in transgenic mice that enable microglia and infiltrating monocytes to be distinguished have also documented the entry of monocytes into the retina in surrogate models involving light-induced photoreceptor injury (O'Koren et al. 2016) and sodium iodate-induced RPE injury (Ma et al. 2017). Whether and how these two cell populations differ in their involvement in the RP retina are not fully understood. Recruitment of bone marrow–derived myeloid cells in the rd10 retina had been associated with photoreceptor neuroprotection, but the results may be confounded by the use of irradiation and bone-marrow transplantation (Sasahara et al. 2008). Conversely, genetic deletion of CCR2 in the rd10 model had been associated with decreased monocyte recruitment to the retina and decreased photoreceptor degeneration, indicating a neurotoxic role (Guo et al. 2012). Live-imaging of CCR2+ and CCR2− myeloid cells in the rd10 retina showed active phagocytosis of photoreceptors in CCR2− cells but not in CCR2+ cells,

indicating that monocytes, prior to their downregulation of CCR2 expression, may demonstrate a distinct dynamic behavior from infiltrating microglia (Zhao et al. 2015). Further clarification of the roles of these two cell populations can help guide local versus systemic considerations for potential immunomodulatory therapy of RP.

4.3. Microglia in Age-Related Macular Degeneration

AMD, a leading cause of central vision loss in older patients around the world (Wong et al. 2014), is a disease of progressive stages. Early and intermediate stages of AMD are characterized by the formation of sub-RPE deposits called drusen. These serve as precursor stages for the evolution of late AMD, which consists of two distinct phenotypes: (*a*) geographic atrophy, characterized by progressive degeneration of the RPE, photoreceptors, and choriocapillaris, and (*b*) neovascular AMD, characterized by the formation of a choroidal neovascularization (CNV), which causes structural disruption of the retina (Jager et al. 2008). The role of inflammation as a pathogenic factor in AMD is supported by studies demonstrating that polymorphisms in multiple inflammatory genes confer genetic risk for AMD (Fritsche et al. 2014), and inflammatory cells, including microglia, are found in close association with AMD lesions (Combadiere et al. 2007, Gupta et al. 2003, Lad et al. 2015, Sennlaub et al. 2013). As the involvement of microglia and other mononuclear phagocytes (MPs) in AMD has been recently reviewed in comprehensive detail (Guillonneau et al. 2017), we restrict the commentary below to general themes.

What findings implicate microglial involvement in early and intermediate AMD? While the presence of immune cells is limited to microglia in the inner retina during homeostasis, histopathological analyses of AMD retinas have localized MPs to large drusen, both on the apical surfaces of RPE cells overlying drusen and within drusen themselves (Eandi et al. 2016, Gupta et al. 2003, Hageman et al. 2001, Lad et al. 2015, Sennlaub et al. 2013). While RPE cells alone appear capable of synthesizing many drusen components (Johnson et al. 2011), it has been hypothesized that associated MPs can also directly contribute to drusen components and/or induce physiological changes in RPE cells that promote drusenogenesis (Hageman et al. 2001, Johnson et al. 2011). The cellular sources of AMD-related MPs are unclear and consist of (*a*) retinal microglia, (*b*) systemically recruited monocytes, and/or (*c*) choroidal macrophages/dendritic cells. Some of these cells in the AMD retina are CCR2+ (Sennlaub et al. 2013) and likely involve recruited monocytes, at least in part. However, to the extent that age-related accumulation of myeloid cells in the subretinal space in aging mice may serve to model AMD-related processes (Raoul et al. 2010), cell-fate mapping techniques have indicated that resident microglia (Ma et al. 2017) may be attracted from the inner retina to the subretinal space by age-related compositional changes in the outer retina (Indaram et al. 2015). The combination of cell-autonomous changes occurring within aging microglia (Ma et al. 2013a, Wong 2013), the displacement of microglia into the outer retina with aging (Xu et al. 2007), and the transformation of microglia to a more proinflammatory and procomplement activated phenotype upon contact with factors present in the aged outer retina (Indaram et al. 2015, Ma et al. 2013b) may induce RPE cells to transition to a more drusenogenic mode in early and intermediate AMD.

Can microglia contribute to progression to advanced AMD? The clinical progression from intermediate AMD to geographic atrophy (GA) is heralded by drusen regression and RPE clumping in the GA lesion site. Once arisen, GA progresses as a growing area of overt RPE and photoreceptor demise (Klein et al. 2008). Local changes in RPE physiology, as indicated by findings using optical coherence tomography (Sleiman et al. 2017) and fundus autofluorescence imaging (Toy et al. 2013), are apparent following drusen regression and prior to GA formation. It may be hypothesized that the inflammatory events surrounding the clearance of drusen may relate to

ensuing RPE changes. Studies involving microglia-RPE coculture in vitro and mouse models of subretinal microglia accumulation have demonstrated that contact with activated microglia can induce physiological changes in RPE cells, including alterations in structure, inflammatory gene and chemokine expression, and autophagy (Ma et al. 2009, Natoli et al. 2017b, Nebel et al. 2017, Wang et al. 2009). One of the relevant signals in this interaction may be via TNFα secretion from activated mononuclear cells. TNFα can suppress RPE expression of OTX2, which regulates genes involved in the visual cycle, potentially decreasing the ability of RPE cells to facilitate dark adaptation (Flamendorf et al. 2015, Mathis et al. 2017). These inflammatory influences can also drive the overt demise of RPE and photoreceptor cells (Combadiere et al. 2007, Devarajan et al. 2016, Ma et al. 2013b) and likely involve neurotoxic effects mediated by IL-1β secretion from myeloid cells triggered by ATP release and inflammasome activation (Eandi et al. 2016, Hu et al. 2015).

Inflammatory myeloid cells in the outer retina have also been associated with the transition to neovascular AMD. Multiple histopathological studies have correlated increased inflammatory cell presence in regions of CNV (McLeod et al. 2016, Penfold et al. 2001). Their positive contribution to CNV formation have been supported by studies in which an increase in the number of subretinal myeloid cells potentiates CNV formation (Combadiere et al. 2007) while their depletion decreased it (Espinosa-Heidmann et al. 2003). The mechanisms that enable myeloid cells to promote neovascularization have been related to aging (Kelly et al. 2007) and cholesterol-mediated effects (Sene et al. 2013). The specific relevant sources of potentiating myeloid cells in CNV formation are not completely understood. It appears that infiltrating CCR2+ monocytes are a source (Liu et al. 2013, Tsutsumi et al. 2003), but the size of the contribution that can be attributed specifically to retinal microglia requires further confirmation.

5. MICROGLIA MODULATION AS A THERAPEUTIC STRATEGY

With the implication of microglia as cellular contributors to the progression of CNS neurodegenerative disease, it is apt that there is considerable interest in the therapeutic targeting of microglia, particularly with respect to the modulation of their activation status (Peña-Altamira et al. 2017). In retinal diseases, this strategy is already being explored (Karlstetter et al. 2015). Therapies aimed at the modulation of microglia-mediated effects may be categorized into those that modulate the general activation state of microglia in the disease context and those that modulate specific molecular pathways through which microglia exert pathologic effects. We review here some of the interventions as employed in animal models for these two respects, as well as clinical trials for which this strategy had been pursued.

5.1. Glucocorticoids

Glucocorticoids (GCs) have been prevalently used as a way to generally lower microglial activation in CNS disorders. Synthetic GCs bind to endogenous GC receptors to powerfully inhibit activation of the innate immune system through the negative regulation of proinflammatory molecular pathways (Glezer & Rivest 2004). Local retinal delivery of GCs such as triamcinolone acetonide or fluocinolone acetonide has demonstrated significant suppressive effects on microglial activation and numbers that are correlated with decreased neuronal degeneration. Examples include the relative preservation of RGCs in the optic nerve crush (Wang et al. 2016a) and excitotoxic injury (Singhal et al. 2010) models, and decreased photoreceptor degeneration in ischemia-reperfusion and RP models (Glybina et al. 2009). In clinical care, GCs delivered via intravitreal injections or a sustained-release implant have been a mainstay in the treatment of diabetic macular edema

(Pearson et al. 2011) and retinal vein occlusions (Ip et al. 2009). However, the direct contributions of microglial modulation to the overall effects of the therapy are unclear. The broad expression of GC receptors by different ocular tissues and cell types (Sulaiman et al. 2017) also complicates efforts at specific modulation of retinal microglia. Owing to the complications of cataract formation and increased IOP associated with long-term GC use in the eye (Holekamp et al. 2005), the utility of GCs as a sustained microglial modulator to provide neuroprotective effects in diseases such as glaucoma and RP may be limited. However, more selective and facilitated targeted delivery of GCs to activated retinal microglia via dendrimer nanoparticles may improve the efficacy of microglia modulation and enable a more favorable side-effect profile (Kambhampati et al. 2015).

5.2. Minocycline

Minocycline is a tetracycline derivative that acts as a broad-spectrum antibiotic for the treatment of infections but has also been used clinically as an anti-inflammatory agent for the treatment of rosacea (van der Linden et al. 2017) and periodontitis (Williams et al. 2001). Owing to its high bioavailability in the CNS following oral administration, and its ability to suppress microglial activation via mechanisms involving the inhibition of MAPK (Du et al. 2001) and $PKC_{\alpha/\beta II}$ (Nikodemova et al. 2007) pathways, it has been employed in multiple CNS studies despite uncertainties regarding its mechanism of action and the selectivity of its inhibitory effects (Moller et al. 2016). Minocycline effects on microglia include significant reductions in cytokine and nitric oxide release, decreased proliferation, and downregulation of activation markers (Zemke & Majid 2004). Minocycline has been employed as a therapeutic microglial inhibitor in multiple models of retinal disease, resulting in the neuroprotection of RGCs in models of glaucoma, optic nerve transection, and excitotoxic injury (Bosco et al. 2008, Levkovitch-Verbin et al. 2006, Levkovitch-Verbin et al. 2014, Shimazawa et al. 2005) and of photoreceptors in models of light injury, RP, retinal detachment, and subretinal hemorrhage (Peng et al. 2014; Yang et al. 2007, 2009; Zhang et al. 2004; Zhao et al. 2011). Similarly, minocycline administration resulted in decreased retinal capillary degeneration and neuronal apoptosis in animal models of diabetic retinopathy (Krady et al. 2005, Vincent & Mohr 2007). Studies involving selective nanoparticle-mediated targeting of activated microglia using dendrimer-conjugated minocycline demonstrated enhanced cellular availability and suppression of TNFα and nitric oxide production, indicating opportunities for microglial targeting using this approach (Sharma et al. 2017).

Minocycline as a microglial modulator in human retinal disease was first evaluated in a phase I/II clinical trial of diabetic macular edema (Cukras et al. 2012) in which oral minocycline as monotherapy was associated with a modest but progressive improvement in mean central macular edema and an improvement in vascular leakage on fluorescein angiography. In another phase I/II trial in which minocycline was similarly employed to treat patients with cystoid macular edema that was associated with RP, modest reductions of mean central macular edema were also observed (NCT02140164). Ongoing trials of oral minocycline for the treatment of central and branch retinal vein occlusion (NCT01468844, NCT01468831) and GA associated with AMD (NCT02564978) are currently being conducted.

Doxycycline, another associated tetracycline derivative described to suppress microglial activation via similar pathways (Santa-Cecilia et al. 2016), has been evaluated in a randomized clinical trial of diabetic retinopathy. Patients with more severe disease, but not those with mild or moderate disease, demonstrated subclinical functional improvement (Scott et al. 2014a,b). A separate randomized trial testing doxycycline in patients with GA is currently underway (NCT01782989).

5.3. Modulation of Microglial Phagocytosis

Microglial phagocytosis serves multiple homeostatic functions in removing debris and apoptotic cells but can also contribute to pathologic neuronal loss via phagoptosis of living cells, speeding degeneration (Brown & Neher 2014). As such, the modulation of microglial phagocytosis to reduce phagoptosis may be considered as a therapeutic strategy, in which components of the underlying molecular mechanism may be targeted. One target may involve milk fat globule factor-E8 (MFG-E8), which acts as an extracellular adaptor protein, adhering to neurons and also binding to the phagocytic vitronectin ($\alpha_v\beta_5$ integrin) receptor to facilitate phagoptosis of stressed neurons (Neniskyte & Brown 2013). In the rd10 retina, MFG-E8 was secreted by infiltrating microglia and found on the extracellular surfaces of vulnerable photoreceptors, indicating its role in photoreceptor phagoptosis. Another target is the phagocytic vitronectin receptor; inhibitory binding by the cyclic Arg-Gly-Asp (cRGD) peptide decreased microglial phagocytosis of photoreceptors in the rd10 retina and slowed structural and functional degeneration (Zhao et al. 2015). However, as these molecules are also involved in photoreceptor outer segment phagocytosis by RPE cells (Nandrot et al. 2007), effective long-term interventions will have to account for potential effects on other retinal cells.

As microglial phagocytic activity is increased with microglial activation, decreasing microglial activation may also reduce pathological phagoptosis. CX3CR1-deficient microglia in the rd10 retina demonstrated dysregulated activation as well as increased phagocytosis of photoreceptors, as demonstrated in vitro and in vivo. Increased signaling through CX3CR1, as induced by the exogenous intravitreal delivery of recombinant CX3CL1 to rd10 retinas, was found to decrease microglial phagocytosis of photoreceptors, which correlated with improved structural and functional outcomes (Zabel et al. 2016).

5.4. Microglial Ablation

The role of microglia in neurodegenerative disease has been studied in animal models using experimental paradigms that enable their ablation from the CNS. These paradigms have involved cellular toxins that are selectively taken up by or targeted to myeloid cells (e.g., clodronate liposomes, Mac1-conjugated saporin), synthetic CSF1R inhibitors (e.g., PLX3397, PLX5622), and the use of transgenic mice that can be induced to express toxic proteins (e.g., diphtheria toxin A) specifically in myeloid cells (Waisman et al. 2015). Microglial depletion may be sustained by the maintenance of the inducing stimulus; however, if the depleting stimulus is only transiently applied, spontaneous microglia repopulation can occur rapidly, reconstituting the neural parenchyma with a new array of microglial cells (Bruttger et al. 2015, Elmore et al. 2014). These experimental paradigms, when used in retinal studies, have demonstrated successful ablation and repopulation of retinal microglia (Wang et al. 2016c).

If microglia can be causal in potentiating neurodegeneration, can these approaches be employed as therapeutic strategies in modulating microglia? The underlying therapeutic rationale will be to remove from the brain or retina pathological microglia, which may be physiologically altered during aging or disease, and to allow subsequent repopulation by so-called new microglia to resume the needed maintenance functions of microglia. Although certain microglia-depleting agents have been evaluated in clinical trials (Butowski et al. 2016), challenges for this approach remain. First, the efficacy of microglia depletion in disease likely depends on the balance of positive versus negative contributions that microglia make in the disease context. This role can vary; in the retina, transient depletion in the rd10 model of RP was found to exert positive effects (Zhao et al. 2015), while a similar intervention in a model of optic nerve crush was not (Hilla et al. 2017). Also,

current methods do not deplete microglia specifically and involve the depletion of other myeloid cell populations. Therefore, the relative contributions of systemic monocytes versus microglia in each disease context needs to be considered. There is concern regarding the physiological state and functions of repopulating microglia following depletion, which relates to whether these new cells can continue to carry out the constitutive functions of the original cells. Further experimentation in animal models of retinal disease will be instrumental in determining the promises and caveats of this approach to better characterize its translational potential.

Strategies that generally target microglial activation or presence, while efficacious in certain models, may exert broad physiological changes that impinge on both pathological, as well as homeostatic, effects of microglia. An alternative strategy is to more precisely target molecular pathways underlying specific microglial effector functions, as discussed in the sections below.

5.5. Targeting Complement-Mediated Effects

The role of complement in neurodegenerative conditions in the retina has received significant attention as multiple lines of evidence have implicated their involvement in disease progression. In AMD, polymorphisms related to genes for complement and complement regulatory proteins were found to confer genetic risk for the disease (Fritsche et al. 2014). Increased deposition of complement-related proteins has also been found in drusen and the choriocapillaris, the loci of AMD involvement (Fett et al. 2012, Johnson et al. 2001). In glaucoma, upregulated expression of complement proteins was found on proteomic analyses of human eyes (Tezel et al. 2010), and increased complement expression and deposition were found on immunohistochemical studies in glaucoma models (Howell et al. 2011, Jha et al. 2011). In models of retinal disease where complement expression or deposition was experimentally decreased or ablated, measures of neurodegeneration were ameliorated (Howell et al. 2013, Silverman et al. 2016), indicating that complement activation can play a pathologic role in driving disease progression.

While the mechanisms connecting pathologic neurodegeneration and complement are not fully understood, it is likely that retinal microglia may be involved in complement-mediated mechanisms. Retinal microglia express significant levels of complement molecules in both in vitro (Luo et al. 2011) and in vivo (Natoli et al. 2017a) in models of retinal disease. In the context of aging and exposure to aging-related A2E, retinal microglia also changed their expression levels of complement and complement regulatory proteins favorable for complement activation (Ma et al. 2013a,b). In addition, microglia can express complement receptors, such as C3aR, C5aR, and CR3 (Schafer et al. 2012, Song et al. 2017), and likely respond to complement activation products. In this light, retinal microglia can act as local producers and regulators of complement activation, as well as carry out complement effector functions such as immune cell chemotaxis and complement-opsonized phagocytosis. CFH, as expressed by MPs in the retina, has been recently found to demonstrate a novel role in regulating the clearance of myeloid cells from the subretinal space, and therefore in immune homeostasis in the outer retina (Calippe et al. 2017). As such, strategies that specifically target complement-mediated microglial activities may be influential in controlling neurodegenerative processes, but a full understanding of these await further experimentation in defined model systems.

Despite the lack of clear knowledge regarding cellular mechanisms, numerous clinical trials targeting complement have been conducted in retinal diseases, particularly for GA associated with AMD, as recently reviewed (Xu & Chen 2016). These have included the targeted inhibition of central complement components, such as C3 (APL-2, NCT02503332), of more so-called downstream components of the alternative pathway, such as C5 (eculizumab) (Yehoshua et al. 2014), and of complement regulatory proteins, such as CFD (lampalizumab) (Yaspan et al. 2017). Different

rationales have been articulated for targeting each point in the complement cascade. However, a clear choice of the optimal approach has been difficult to make as complement-mediated pathologic mechanisms, as well as the effect of treatment on retinal and microglial physiology, is not well understood at this point.

5.6. Targeting Proinflammatory Cytokine Signaling

The pathogenicity of activated microglia in the context of retinal disease has been linked to microglial production of proinflammatory neurotoxic cytokines. A prominent example is IL-1β, which has been thought to perpetuate pathological neuroinflammation and contribute to neurodegeneration (Mendiola & Cardona 2017). Increased expression of IL-1β in models of retinal disease has been linked to induction of increased photoreceptor apoptosis (Appelbaum et al. 2017, Hu et al. 2015, Kohno et al. 2013, Zhao et al. 2015). Microglia have been identified as the primary retinal source, although RPE cells likely contribute (Tseng et al. 2013). Pharmacological inhibition of IL-1 signaling and the genetic deletion of *Il1r1* have demonstrated therapeutic effects in animal models of retinal disease (Kowluru & Odenbach 2004, Kowluru et al. 2011, Lavalette et al. 2011). In particular, anakinra, an FDA-approved IL-1 receptor antagonist (IL-1RA) capable of blocking the biologic activity of endogenous IL-1, demonstrated a promising safety profile in retinal tissue (Ranjbar et al. 2017) and improved vascular and neurodegenerative pathology in vivo (Hu et al. 2015, Olson et al. 2009, Rivera et al. 2013, Zhao et al. 2015).

TNFα is another potent proinflammatory molecule widely implicated in retinal pathology for which activated microglia is a significant source (Dong et al. 2014). Intravitreal delivery of TNFα was found to induce neurotoxicity to RGCs, implicating its role in neurodegeneration (Kitaoka et al. 2006). Inhibition of microglia-mediated retinal neurodegeneration via TNFα neutralization may be an approachable strategy, as pharmacological inhibitors approved for nonocular inflammatory conditions exist and are available for drug repurposing. These include etanercept (a dimeric fusion protein), infliximab (a chimeric monoclonal antibody), and adalimumab (a fully humanized antibody) (Mirshahi et al. 2012). Experimental animal models and off-label treatment in small cohorts of uveitis patients have demonstrated intravitreal administration of etanercept and adalimumab are nontoxic and well tolerated (Kivilcim et al. 2007, Paula et al. 2015). Additionally, they provided rescue to PRs and RGCs in animal models of retinal disease and suppressed caspase activity, while modulating the inflammatory response of activated microglia (Bae et al. 2016, Joussen et al. 2009, Martinez-Fernández de la Camara et al. 2015, Roh et al. 2012). Off-label use of these three agents in patients with exudative AMD or diabetic macular edema have found that only infliximab was associated with a risk for intraocular inflammation while the others were well-tolerated; however, overall efficacy measures were minimal or mixed (Pascual-Camps et al. 2014).

5.7. Other Therapeutic Approaches to Microglia Modulation

In addition to the areas outlined above, new directions in microglia-targeted therapies are under current development. One involves the design of new methods for selective drug delivery to microglia for the treatment of neuroinflammation. Dendrimer nanoparticles, which can distribute themselves throughout the CNS parenchyma, can be internalized by endocytic or phagocytic activity to preferentially deliver conjugated therapeutics to microglia (Nance et al. 2016). This technology of precise cell targeting can increase drug availability and minimize side effects (Zhang et al. 2016). Another direction involves the genetic targeting of microglia, potentially with viral vectors (Cucchiarini et al. 2003), that can enable a modulation of the microglial activation state by

the expression of suppressive factors (Chen et al. 2005) or restore lysosomal function by genetic repair of a mutated enzyme (Aronovich & Hackett 2015). Finally, the successful derivation of microglia-like cells from induced pluripotent stem cells opens up the possibility for cell-based therapy in which microglia can be exogenously introduced to replace or supplement endogenous populations in pathological situations (Takata et al. 2017). These approaches, if successful, can significantly expand the means by which microglial pathogenicity can be therapeutically modulated.

SUMMARY POINTS

1. Early microglia-neuron interactions help to shape neuronal-glia organization in the developing retina. Retinal microglia can exert trophic influences to support the survival of developing neurons but can be also involved in inducing programmed cell death and the clearance of apoptotic cells.
2. Microglia in the healthy adult retina form a closed, self-sustaining population of cells that are kept separate from circulating monocytes behind the blood-retinal barrier. Neuron-microglia interactions help maintain homeostasis in the number and distribution of microglia in the retina; when depleted of microglia, the retina can spontaneously regenerate its microglial population from residual cells, recapitulating structural and functional features of original microglia.
3. Microglia in the adult retina are required in the maintenance of normal synaptic structure and function that underlie the retina's normal electrophysiological response to light.
4. Microglia can play a causal role in driving neurodegeneration in retinal diseases such as glaucoma, RP, and AMD. The mechanisms of microglial pathogenicity include the production of neurotoxic proinflammatory cytokines that increase neuronal apoptosis and the phagocytic clearance of stressed but still living cells (i.e., phagoptosis).
5. Modulation of microglial physiology in the context of retinal disease holds therapeutic promise in ameliorating neurodegeneration. General therapeutic approaches involve the overall suppression of pathogenic microglial activation states and the specific inhibition of neurotoxic microglial effector mechanisms.

FUTURE ISSUES

1. The spatiotemporal coincidence of microglial development and synaptogenesis in the retina strongly implicate microglial involvement in the formation of retinal synapses and circuits. The orderly spatial organization of retinal synapses in the plexiform layers and the well-characterized system of retinal circuits present an advantageous system for the future elucidation of mechanisms underlying microglial involvement in synaptogenesis.
2. Although microglial dysregulation has been linked to neurodegeneration, the positive functions of microglia in maintaining the health and homeostasis of the retina under normal and pathological conditions are less well understood and deserve greater investigation.

3. A more detailed understanding of the pathological mechanisms underlying microglia involvement in processes of synaptic loss, neurodegeneration, vascular compromise, and neovascularization will be key in the development of targeted therapies that modulate microglia to minimize their deleterious effects, while maximizing their neuroprotective and adaptive contributions.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

This study is supported by funds from the National Eye Institute Intramural Research Program.

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