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Microglia: Friends or Foes in Glaucoma? A Developmental Perspective

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Abstract

Glaucoma is the most prevalent form of optic neuropathy where a progressive degeneration of retinal ganglion cells (RGCs) leads to irreversible loss of vision. The mechanism underlying glaucomatous degeneration remains poorly understood. However, evidence suggests that microglia, which regulate RGC numbers and synaptic integrity during development and provide homeostatic support in adults, may contribute to the disease process. Hence, microglia represent a valid cellular target for therapeutic approaches in glaucoma. Here, we provide an overview of the role of microglia in RGC development and degeneration in the backdrop of neurogenesis and neurodegeneration in the central nervous system and discuss how pathological recapitulation of microglia-mediated developmental mechanisms may help initiate or exacerbate glaucomatous degeneration.

Key words: microglia; retinal ganglion cell; neurogenesis; degeneration; glaucoma.

Graphical Abstract
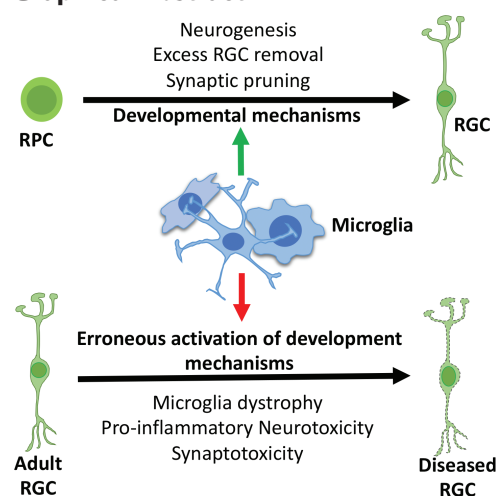
Significance Statement

Glaucoma, where retinal ganglion cells degenerate, is the leading cause of irreversible blindness. Evidence suggests that microglia, which play a homoeostatic role in the regulation of RGC development and maintenance, may determine the initiation and progression of RGC degeneration. Therefore, microglia represent a valid therapeutic target for glaucomatous degeneration.

Introduction

Glaucoma is a complex group of disorders with a common pathology of retinal ganglion cell (RGCs) degeneration.[1] The progressive loss of these projection neurons that connect the retina to the brain for visual perception is the leading cause of irreversible blindness, estimated to affect 118 million people by 2040 worldwide.[2] Advanced age, positive family history, and elevated intraocular pressure (IOP) are recognized risk factors for primary open angle glaucoma (POAG), the most common glaucoma subtypes.[1] While these risk factors

have helped in the diagnosis, monitoring, and management of the disease, their contributions to RGC degeneration are not well understood. These cells are generated and reside in close proximity of microglia, the resident immune cells in the CNS. In the adult retina, as elsewhere in the central nervous system (CNS), microglia surveil the environment to maintain the structural and functional homeostasis. They get activated in response to external pathogens/toxins or internal danger signal generated by neurons under duress and mount a swift inflammatory response to regain the structural and physiological balance.[3] Their actions often consist of the redeployment of mechanisms, which help regulate the number of excess neurons generated and fine tune the wiring during development. However, the beneficial aspect of microglia becomes counterproductive to repair and remodeling in the degenerative diseases if their activation persisted for too long. Thus, the role of microglia is akin to that of a beat patrol, surveilling the neighborhood, talking to the community, and exchanging information to maintain normalcy. When there is a disturbance in the neighborhood the patrol gets into action, mediating and removing the cause of the chaos. The beneficial effects of the patrol are dependent upon whether or not the action is calibrated and its duration. When it is excessive and relentless it has an adverse effect on the community, which may become depressed and may not recover and regain the normalcy. The relationship outcome between microglia and glaucomatous RGCs may be equally contextual, which may play out all along the visual pathway, beginning with the proximal connections of RGCs within the retina and distally with the neurons in the superior colliculus (SC) and lateral geniculate nucleus (LGN), and axons in between. Since this relationship is traced back to RGC generation, in this review we will examine the role of microglia in RGC development and degeneration in the backdrop of neurogenesis and neurodegeneration in the CNS in general. The activation of the microglia-mediated developmental mechanisms during glaucoma may influence the initiation and or progression of the disease.

Development and Migration of Microglia

Lineage tracing studies have demonstrated that microglia are the immigrant cells from the embryonic yolk sac (YS) and begin to colonize the developing mouse brain, starting around embryonic day 9 (E9) till the formation of the blood brain barrier approximately at E14.[4] These studies, along with the recent transcriptional profiling, have shed valuable light on different stages of development of the YS progenitors into mature microglia.[5-7] The broadly defined stages, based on the stage-specific regulators and markers, are early microglia (recently immigrant microglia till ~E14; they share properties and markers with the YS macrophages, demonstrating their nascent stage and are enriched in genes associated with cell proliferation), pre-microglia (microglia, spanning ~E14 to early postanal weeks, displaying predominantly amoeboid morphology), and adult microglia (few weeks after birth into adulthood; these are predominantly ramified) (Fig. 1). These 3 stages correspond to the developmental function of microglia in supporting neurogenesis, refining the wiring in the developing brain (early- and pre-microglia), and maintaining the surveillance homeostasis in the adult brain (adult microglia). The differentiation of YS progenitors into migratory YS pre-macrophage is regulated by the transcription factors Runx1,

PU.1, and interferon regulatory factors 8 (IRF8).[8,9] These transitory cells begin to express the fractalkine (CX3CL1) receptor, CX3CR1 to facilitate their migration into the brain. Their differentiation into microglia once inside the embryonic brain is facilitated by transforming growth factor beta (TGFβ), colony stimulating factor 1 (CSF-1), and interleukin-34 (IL-34) mediated signaling.[10,11] The retinal microglia are likely to go through similar developmental stages, identified by the corresponding markers, as the retina follows similar developmental trajectories like the rest of the CNS. Microglia precursors migrate into developing retina around the same time as in the developing brain because they are already present in the E11.5 mouse retina, the earliest stage studied.[12] As they expand pre- and postnatally and the neuroblastic layer progressively differentiates into a laminar structure, microglia preferentially locate to the inner and outer plexiform layers (IPL, OPL), where synapses are formed and the ganglion-cell layer (GCL) and the inner nuclear layer (INL), where excess neurons undergoing apoptosis are removed in the first 2 postnatal weeks[12,13] (Fig. 1). Given the temporally and spatially changing niche in the developing retina and microglia being highly responsive to their immediate environment, they may exist in different subtypes specific to the development and function of the retina. This premise has been temporally analyzed in the context of the mouse brain, where scRNA seq revealed 9 transcriptionally distinct clusters found across 5 different ages, at E14.5, PN4/5, PN30, PN100, and PN450.[14] The diversity of the clusters was greatest at embryonic and early postnatal age versus adult. The transcriptional signature of these clusters reflected the functional state of microglia at that age. For example, the early postnatal microglia (PN4/5) were uniquely characterized by a cluster (cluster 4) that expressed high levels of osteopontin gene (*Spp1*) along with transcripts corresponding to insulin like growth factor 1 (*Igf1*), glycoprotein NMB (*Gpnmb*), immunomodulators of galectin family, galectin-1 (*Lgals1*) and galectin-3 (*Lgals3*), and a lysosomal glycoprotein, CD68 (*Cd68*), enriched in phagocytotic microglia and macrophages. They were associated with the pre-myelinated axon tracts and are thought to clear the debris before axons are myelinated[14] and/or engulf oligodendrocytes.[15] This subpopulation of amoeboid and phagocytotic brain microglia shares transcriptional signature with activated retinal microglia in the animal models of glaucoma[16] and a subset of those exposed to apoptotic retinal neurons during development,[17] suggesting the recruitment of developmentally regulated genes by microglia in response to degenerative changes in the retina.

Influence on Neurogenesis

The timing of the microglia precursors' entry into the brain coincides with the early stage of neurogenesis. The experimental elimination of microglia from the developing brain, including retina, demonstrated their influence on the outcome of neurogenesis. The influence appeared contextual, depending upon when during retinal development microglia were eliminated. For example, when microglia precursors were prevented from migrating into the retina from the YS in Zebrafish by morpholino-based silencing of CSF-1R the manipulation caused microphthalmia, accompanied by the increased proliferation and reduced differentiation of the retinal progenitors.[18] Similar observations were made for the cortical progenitors in the small brain phenotype when
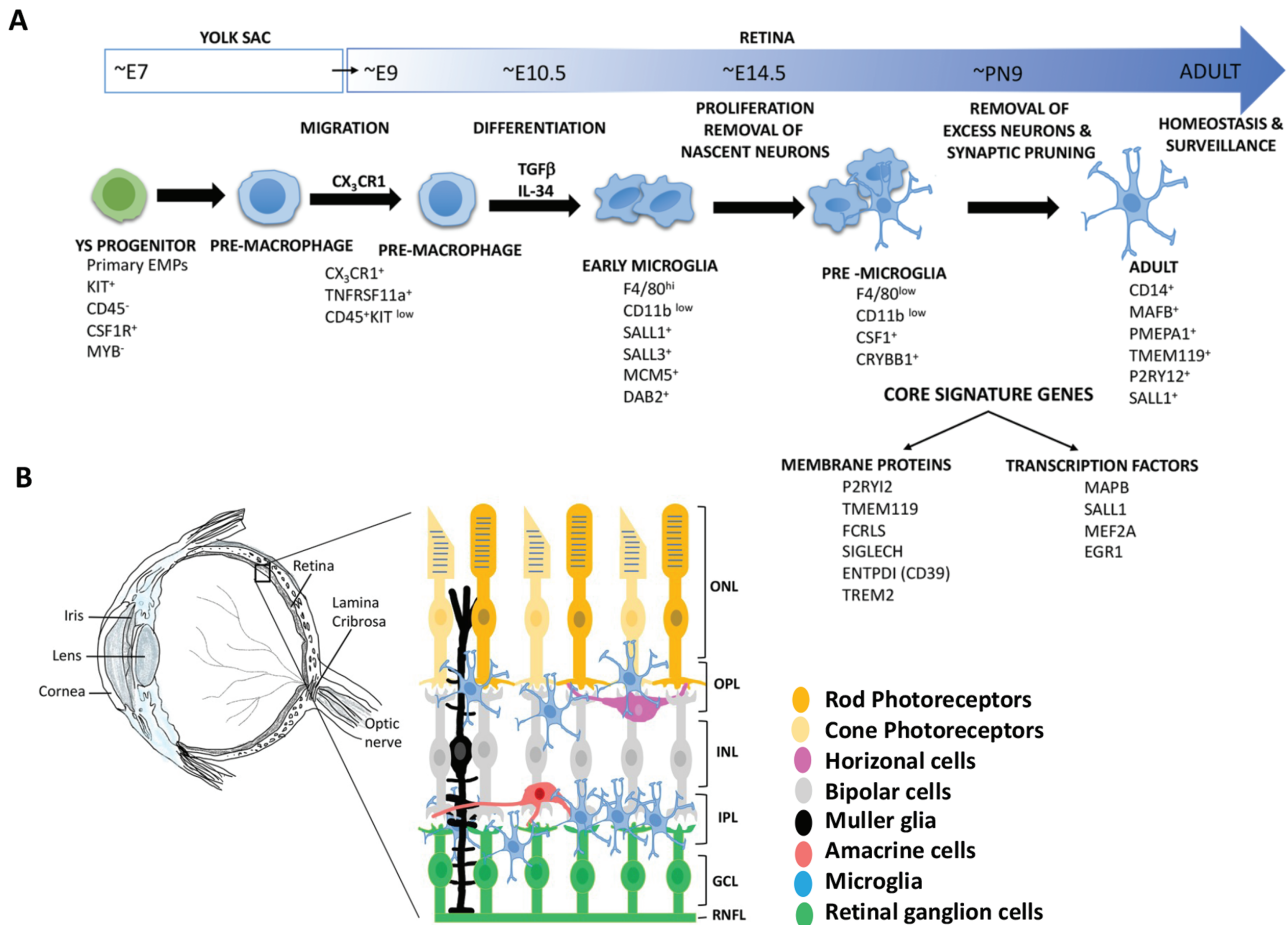
Figure 1. (**A**) A schematic representation of microglia development with associated markers. The scheme is adapted from Li and Barres[6] and include information from Butovsky et al.[10] (**B**) A schematic representation of the adult eye and the laminar localization of microglia in the adult retina.

microglia were depleted in the developing brain by eliminating the *Csf-1R* gene,[19,20] demonstrating that microglia may positively influence the neuronal differentiation. However, when microglia were eliminated after they had colonized the retina, the effect on the retina was rather subtle; the number of the newly born RGCs was increased, suggesting that microglia remove the nascent RGCs during early retinal neurogenesis,[21] presumably regulating the neurogenic output by eliminating a subset of post-mitotic cells. This subset were tagged with C1q, the initiation component of the classic complement signaling pathway and removed by microglia by complement receptor3 (CR3)-mediated phagocytosis,[21] the mechanism used in the postnatal pruning of RGC synapses.[22] The eliminated cells were non-apoptotic like those removed by microglia from the developing cortex,[23] and the hippocampal sub granular zone (SGZ) during adult neurogenesis.[24] What makes the subset of cells destined for elimination during neurogenesis is currently not well understood. Evidence has emerged that microglia may target another subset of cells during development and eliminate them by causing apoptosis. For example, nascent neurons are removed in the developing cerebellum and hippocampus by microglia-mediated apoptosis, initiated by reactive oxygen species (ROS).[25,26] Microglia-mediated apoptosis has also been observed in the developing retina.[22] However, the apoptotic signal is different; it consists of microglia-derived NGF, which promotes programmed cell death through p75 neurotrophic receptor.[27] Together, these observations point to

an important role of microglia in homeostatic regulation of neurogenesis through active elimination of subsets of cells in different stages of development.

Regulation of RGC Number

About 50 percent of cells generated in the developing CNS are eliminated, the majority of them through apoptosis.[28-30] The retina is no exception, and the chronology of generation and elimination of cells is well-documented. For example, cell loss in the postnatal retina is observed to be 5%, 50%, and 51% in ONL, INL, and GCL, respectively.[31] From the viewpoint of glaucoma, the cells in the GCL are important. At birth almost all cells in the GCL are RGCs.[32] These are the first-born neurons in the retina the majority of which are generated between ~E9 and ~E19 in rats and ~E7 and ~E16 in mice.[33,34] RGC axons are detected in the optic nerve within 24-48 hours after their birth and reach SC and LGN by approximately 3 and 5 days, respectively, after their generation.[35] However, axons of late born RGCs have to travel longer distance and thus take ~8 days to reach SC.[36] Almost half the population of the RGCs are eliminated in the first postnatal week (the peak of elimination falling between PN2 and PN4) after they have reached their targets in the mouse retina.[12,37,38] The space is taken over by the displaced amacrine cells (AC) such that in the adult, RGCs and ACs comprise 45% and 51% of cells in the GCL, respectively.[32] Early evidence of microglia-mediated

elimination of the apoptotic RGCs was provided by Hume et al, who demonstrated microglia, identified by F40/80 immunoreactivities, migrating to the GCL and in phagocytotic association with the pyknotic neurons in the first postnatal week.[39] The mechanism by which microglia gravitate toward the dying neurons is not well understood, but evidence points toward chemoattraction through factors released by the dying cells, called the "find me" signals.[40] These signals are exemplified by lisophosphatidylcholine (LPC) and ATP, recognized by G-protein-coupled receptor 132 (Gpr132) and purinergic receptor, P2Y12, respectively, on microglia.[41,42] Once in the vicinity, microglia recognize the dying neurons for phagocytosis by the display on their surface phospholipid phosphatidylserine, the major "eat-me" signal.[43]

Modulation of Synaptic Structures and Activities

Microglia play an essential role in fine tuning the wiring in the developing brain by pruning inactive and/or immature synapses as a mean to remove inappropriate and excessive connections.[6,44] They achieve this through the complement system, used systematically for the removal of cellular debris.[6] This is best exemplified in the pruning of synapses between RGC axons and cells in the LGN in the first postnatal week in rodents.[22,45] The strength of the retinogeniculate synapses is activity driven and relatively weak synapses, tagged with the complement components C1q and C3, are recognized by microglia and promptly phagocytosed through the interactions with the microglial complement receptor, CR3.[22,45] Microglia also assist in the elimination of immature synapses in the developing hippocampus,[46] supporting the notion that the sculpting of synapses is their essential function in the developing nervous system, including retina,[47] and along the visual pathway.[48] Therefore, a pathological activation of complement-mediated synapse pruning by microglia may lead to an abnormal or a loss of function. For example, there is early synapse loss in the hippocampus in the animal model of Alzheimer's disease (AD) due to an inappropriate activation of microglia by the amyloid beta (Aβ) protein, suggesting that the loss of cognition in AD is due to a complement-mediated pathological pruning.[49,50] Similarly, it is thought that the complement-mediated excess removal of synapses underlies the pathology of fewer synapses than normal in schizophrenic patients.[51] Recent studies suggest that microglia can discriminate between the excitatory versus inhibitory synapses for the remodeling of connections, and their inappropriate removal has behavioral consequences.[52] Evidence is emerging that abnormal complement-mediated pruning in the retina and along the visual pathway may also compromise normal function. For example, microglia have been observed to engulf postsynaptic elements in the OPL in a complement-dependent manner following rod photoreceptor degenerationin in the animal model of retinitis pigmentosa (RP).[47] Conversely, under-pruning of synapses in the developing visual cortex due to the elimination of purinergic receptor *P2Y12*, which allows microglia to scan the parenchyma for pruning, results into immature synapses, abrogating the ocular dominance plasticity.[48]

Microglia and Glaucomatous Degeneration

Given the role of microglia in maintaining homeostasis in the CNS and its macrophage lineage it is expected that these cells would react to degenerative changes in neurons and hence, the activation of microglia and proinflammatory responses are associative features in almost all neurodegenerative diseases[53] including glaucoma.[54] That glaucoma is associated with microglia activation was demonstrated by Neufeld,[55] who observed clusters of amoeboid microglia in the compressed lamina cribrosa (Fig. 1), encircling the blood vessels in the paraffin sections of the glaucomatous eyes with optic nerve damage. Later, these ameboid cells were shown to express the activation marker (HLA-DR), phagocytotic markers (TSP, CD68), inflammatory cytokine (TNFα), inflammatory mediators (NOS-2, COX-1), proliferation marker (PCNA), and several metalloproteases confirming their proliferative and activated status in glaucoma.[56] Examination of microglia in the glaucomatous retina also revealed enhanced expression of Toll-like receptors, suggesting active signaling through these receptors, which may lead to pro-inflammatory changes.[57] Although the association of microglia activation with glaucoma is well established, it is not clearly understood whether it precedes or follows the disease and therefore what is its effect on the onset and progression of the disease. The animal models of glaucoma have begun to shed light on these issues, with a caveat that some of the emerging information may not be clinically relevant given the species difference in ocular anatomy and genes/pathways involved. A detailed comparison of the relative merit of different rodent models of glaucoma is given in excellent reviews on the topic by Johnson and Tomarev and Pang and Clark.[58,59] Microglia activation has been observed in both the ocular hypertension (OHT) models and a chronic model of pigmentary glaucoma, exemplified by the inbred DBA/2J (D2) mouse.[58] For example, in an episcleral vein occlusion mediated OHT model, activation of microglia was observed coincidental with RGC degeneration.[60] In the trans-limbal laser photocoagulation OHT model, activated microglia were observed in the retina, optic nerve, and in the optic tract, where the degree of activation of microglia was correlated with the axonal damage.[61] In the D2 mice, where mutation in *Gpnmb* and *Tyrp1* genes are associated with iris atrophy and pigment dispersion,[58] RGC loss was detected maximally between 11 and 18 months of age, preceded by the elevation in the IOP that occurs approximately at 6-7 months of age.[62,63] However, the degenerative changes in RGC dendrites in terms of synaptic loss and axonal dysfunction can be detected much earlier around 5 months of age.[45,64] That microglia may have a causal role in the RGC degeneration was demonstrated by Bosco et al,[65] when they observed microgliosis in D2 mice at an early age of 3 months, much before the onset of degenerative changes in RGCs. Activated microglia were specifically detected in the retina and unmyelinated regions of the optic nerve. The mechanism underlying the transition of homeostatic to proinflammatory or neurodegenerative microglial phenotype in glaucoma remains poorly understood. However, a recent study, carried out in both the OHT and D2 mouse models, demonstrated an important role of apoE-galectin3 signaling in conferring the neurodegenerative phenotype on microglia and that this phenotype may precede RGC degeneration.[16] Based on the emerging role of activated microglia in animal models of neurodegeneration such as AD and microgliosis observed in the OHT models and D2 mice 3 broad pathways[53] can be envisioned for microglia influencing the onset and progression of glaucomatous degeneration (Fig. 2).
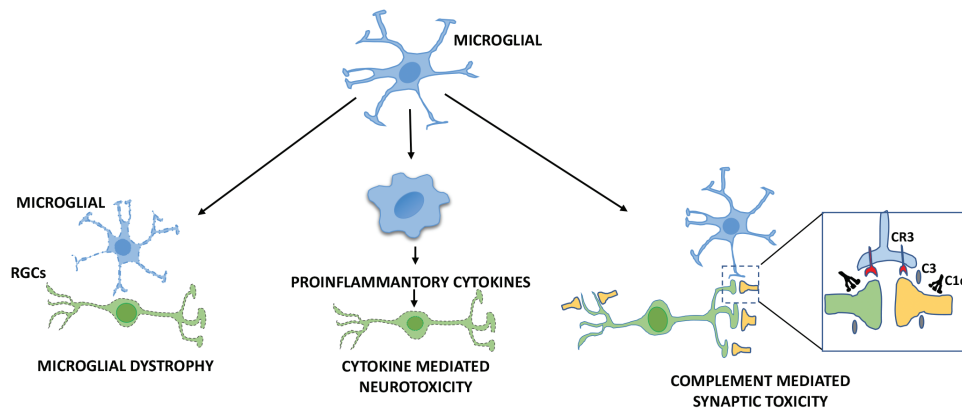
Figure 2. A schematic representation of 3 broad pathways for microglia influence on the onset and progression of glaucomatous degeneration. The dystrophic microglia represent those that are inherently abnormal due to mutations in genes expressed in microglia, exemplified by the TREM2 phenotype.[66,67] C3 and C1q are complement proteins mediating complement-dependent synaptic toxicity via complement receptor, CR3.

Microglial Dystrophy

It is possible that the activation of microglia and associated inflammatory changes may not be secondary to neuronal degeneration, but due to the abnormalities inherent in microglia themselves, which may be manifested upon aging, contributing to neuronal degeneration. For example, Nasu-Hakola disease (NHD), which leads to a lethal presenile dementia is secondary to the dystrophic changes in microglia due to a mutation in *TREM2*, a gene that is exclusively expressed in microglia and involved in phagocytosis.[66] Postmortem of NHD patients reveal demyelination of subcortical white matter. Examination of the TREM2 phenotype in *Trem2*^{-/-} mice revealed glia with abnormal morphology which were impaired in responding to age-related myelin damage for the removal of the debris, thus presumably compromising the remyelination by oligodendrocytes.[67] The non-cell autonomous influence of microglia on the progression of neuronal degeneration is also exemplified by the role of dystrophic microglia in amyotrophic lateral sclerosis (ALS), where there is a progressive loss of the motor neurons due to mutations in the ubiquitous super oxide dismutase 1 (*SOD1*) gene.[68] As mentioned, the first evidence that microgliosis may be causal to glaucomatous degeneration emerged when the activation of microglia was observed prior to any overt neurodegenerative changes in the D2 mice,[65] even before the onset of the IOP and aging, 2 of the prominent risk factors for glaucomatous degeneration. This early activation, preceding the disease, may reflect microglial dystrophy similar to that seen in NHD, which may be due to the mutation in genes that are exclusively or predominantly expressed in microglia. One such mutated gene in D2 mice is *Gpnmb*, which has an immune suppression role[69] and thus may contribute to microglia dysfunction. Therefore, the emergence of the cell-autonomously activated microglia with hyperactive immune response in the retina and optic nerve may be the tipping point for the neurodegenerative changes. Another candidate gene underlying the microglial dystrophy preceding glaucoma could be *Trem2*, whose expression is decreased in the optic nerve head microglia in D2 mice.[70] Given the essential role of this gene in the microglia-mediated homeostasis in the brain, a decrease in its expression will add to the dysfunction of the cells already burdened with the mutation in the immune suppressor gene (*Gpnmb*), making microglia proinflammatory to the detriment of the optic nerve and RGCs.

Pro-inflammatory Cytokines-mediated Neurotoxicity

Pro-inflammatory cytokines released by microglia could either be beneficial or harmful depending upon the context of time and space.[71] In disease conditions, these cytokines create a neurotoxic environment, adding to the progression of the neurodegenerative changes. One of the molecular axes of the microglia-mediated homeostasis, whose dysregulation leads to the neurotoxic microglia activation, is the CX3CR1-fractalkine signaling pathway.[72-74] Fractalkine, primarily secreted by neurons including RGCs,[66] has been shown to be neuroprotective by suppressing the inflammatory response by binding to CX3CR1 on microglia.[72,74] Thus, when the fractalkine-CX3CR1 axis was dysregulated by the homozygous deletion of *Cx3cr1* gene neuronal loss was observed exacerbated in the animal models of PD and ALS.[73] Disruption of the CX3CR1-fractalkine signaling has been observed to have an adverse effect on RGC survival and function as well. The extent of the effects of the disruption depends upon whether the signal is disrupted in acute or chronic model of glaucoma. When IOP was transiently elevated following ischemia reperfusion in the *Cx3cr1*^{-/-} mouse, the microglia became amoeboid and expressed CD68, a lysosomal glycoprotein enriched in phagocytotic miroglia and macropghages, accompanied by significant degenerative changes in soma and dendrites of RGCs.[75] The activated microglia were first observed in the central retina consistent with the observations of activated microglia recruitment in the optic nerve head in glaucomatous human eyes and animal models of glaucoma.[60,61,65] These changes were alleviated in mice treated with minocycline-a tetracycline derivative that suppresses chronic microglial activation and neuroinflammation[76] -linking RGC degeneration with *Cx3cr1*^{-/-} microglia activation.[75] However, in D2 mice the absence of *Cx3cr1* degenerative changes in RGCs were confined to axon transport dysfunction,[77] and similarly, minocycline-mediated suppression of microglia activation was neuroprotective of RGC axons.[78] The association of the activated microglia with the dysfunction of the axons is informative in understanding the disease progression because the axonal transport is compromised before the overt cell loss in D2 mice.[64,79] For example, the failure of the anterograde transport from RGCs to the superior colliculus (SC) is detected as early as 3-5 months of age with complete failure of the transport between 11 and 12 months and to all other central targets (ie, LGN) after 12 months.[64] The axonal transport
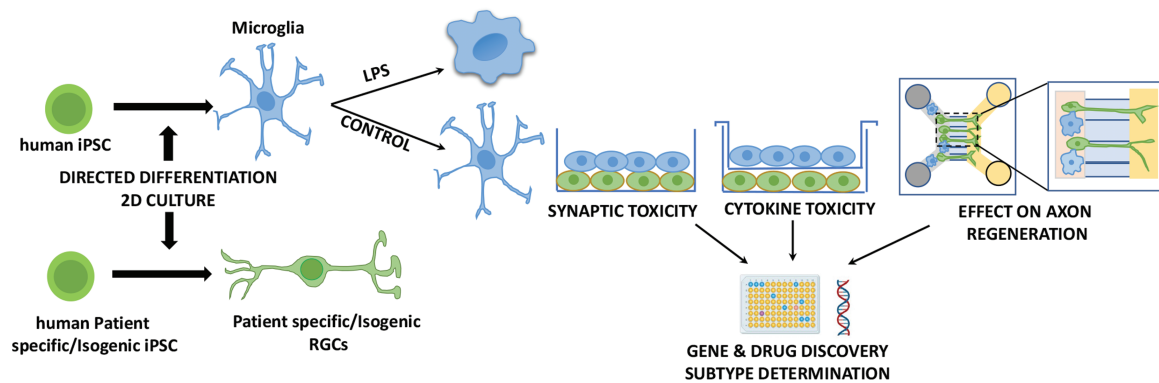
Figure 3. A schematic representation of stem cell modeling of microglia-RGC interactions. Both microglia and RGCs are generated from iPSCs by directed differentiation in 2D culture. Glaucoma patient-specific and isogenic control RGCs are co-cultured with LPS-activated/control microglia together across a membrane to examine synaptic and cytokine toxicity of microglia, respectively. The co-culture paradigm is adapted in a microfluidic model of optic nerve regeneration (Teotia et al.[89]) to examine the effects of microglia on axonal transport and regeneration. Each experimental paradigm is analyzed by population and single-cell RNA seq analysis for gene/drug discovery and subtype-based causes and effects, respectively.

deficit may reflect an early state in the disease progression, as observed in the animal model of ALS, where it is inflammation induced.[80] This suggests that a neuroinflammatory lesion is causing the early axonal dysfunction, which may also underlie the early transport deficits seen in glaucoma. However, it is important to keep in mind from a therapeutic viewpoint the observation that CX3CR1 deficiency is not always deleterious, but could be neuro protective, depending on the stage of degenerative changes. For example, during the early stage of AD when Aβ accumulates, blocking of the CX3CR1-fractalkine signaling reduces the Aβpathology, presumably through the phagocytosis of the extracellular Aβaggregates, whereas in the late stage the dysregulation of the signaling may add to the progression of the disease by the microglia-mediated aggregation of microtubule associated protein tau (MAPT).[81]

Complement-mediated Synaptotoxicity

As mentioned, the complement-mediated synapse pruning by microglia refines the wiring in the developing brain.[44] A pathological re-activation of this mechanism of phagocytosis by microglia in response to the degenerative changes may initiate or add to the progression of the degenerative changes.[44] This was demonstrated in the animal model of AD, where microglia, activated by Aβoligomers, phagocytosed synapses tagged with the fluorescent Homer protein.[49] The phagocytosis was complement-mediated as the Aβ-induced synaptic loss was not observed when *CR3* gene was deleted from the microglia.[49] Of note, Aβ has been observed elevated in the animal models of glaucoma and glaucoma patients,[82] raising the possibility of Aβ-mediated synaptotoxicity in the disease process. Further evidence of synaptotoxicity due to the aberrant activation of the complement in microglia was obtained in the animal model of autosomal dominant frontotemporal dementia (FTD) with the mutation in *progranulin* (*Prgn*) gene.[83] The loss of the *Prgn* gene was associated with the age-dependent up regulation of lysosomal (*Cd68*) and complement (*C1*, *C3*) genes; this increased the complement production and synaptic phagocytosis by microglia, which preferentially eliminated the inhibitory synapses in the ventral thalamus, causing hyperexcitability in the thalamocortical circuits.[83] A clear evidence of microglia in the aberrant synaptic pruning as observed in the animal models of AD and FTD has yet to emerge in glaucoma.

However, the upregulation of the complement components has been observed in both acute and chronic models of glaucoma and associated with the early degenerative changes in RGCs. For example, the induction of the complement cascade genes is one of the earliest identifiable molecular changes in the glaucomatous D2 mouse retina, where the synaptic and dendritic atrophy precedes any detectable degenerative changes seen in the axon or soma of RGCs.[44,84,85] That the complement cascade is involved in these degenerative changes was demonstrated when genetic ablation (D2 mice) or pharmacological inhibition (acute OHT mice) of C1q preserved the synaptic and dendritic integrity.[85] In the postnatal LGN, where microglia refine the synaptic input by the complement-mediated pruning,[22,45] optic nerve crush caused the activation of the complement cascade to remove the debris due to the Wallerian degeneration of the RGC axons.[86] In both these cases however, the direct involvement of the activated microglia in the synaptic toxicity was not demonstrated, which may be attributed to the observations that the dysregulated complement-mediated synaptic pruning may be contextual, depending on the mode of activation, location, and time.[83]

Stem Cell Modeling of Human Microglia-RGC Interactions

Animal models of glaucoma have shed valuable light on the association of activated microglia with the glaucomatous RGC dysfunction and degeneration.[75,77,78] However, the premise that activated microglia make RGC vulnerable to the disease remains unexplored in humans because of the inaccessibility to the human microglia and RGCs. With the advent of the induced pluripotent stem cells (iPSCs) technology, fibroblast/blood cells obtained from patients and healthy donors can be reprogrammed into iPSCs. Neurons/glia can be generated from the patient-specific or isogenic control iPSCs in 2D or 3D culture to establish disease models in which the mechanism of RGC degeneration can be examined in controlled conditions[87] (Fig. 3). Our lab developed a disease in a dish model of glaucomatous degeneration using iPSCs reprogrammed from the POAG patients[88] and microfluidic model of optic nerve regeneration to identify genes and pathways as potential targets for axon regeneration.[89] Both these models can be leveraged to examine the role of the human microglia in RGC dysfunction

and degeneration in a co-culture paradigm. Several methods have been developed recently to generate microglia from the ES/iPSC cells and used to examine the role of microglia in neurodegeneration.[90] From the disease modeling perspective, the ex vivo generated microglia display disease-specific phenotypes. For example, reduced phagocytosis is observed in the iPSC-derived microglia from PD patients with synuclein alpha triplication[91] and those containing the *TREM2* mutation.[92] The human iPSC-derived microglia display the function specific-morphology (ameboid vs ramified), can be activated by the diseased neurons, mount inflammatory response, and induce synaptic loss.[90,91,93] Hence, disease modeling using human iPSC-derived microglia and RGCs has the potential for determining the role microglia play in RGC dysfunction and degeneration from all 3 mechanistic possibilities, ie, microglia dystrophy, proinflammatory insults, and synaptotoxicity in controlled conditions. These mechanisms can be evaluated at the dendrite, soma, and axon levels and may reveal targets for diagnostics and therapeutic purposes.

Conclusion

The temporal and spatial relationship of microglia with RGCs suggests that they may not just be a bystander when the degeneration overtakes these output neurons in the retina. Whether the degeneration is due to the developmental susceptibility[87,88] or an age-related degenerative process secondary to certain risks factors such as the high IOP, microglia are well positioned to affect the disease process. If the disease had a developmental origin, microglia could influence the process as they help calibrate the neurogenic output, determine the RGC number, and sculpt their post- and pre-synaptic connections within the retina and brain. In the adult retina, they maintain close surveillance of RGCs because of their proximity and expression of a battery of receptors whose ligands are expressed by RGCs.[94] Therefore, microglia can read the disease state of RGCs effectively and, in initial state activate their developmental mechanism(s) of providing trophic support and removing toxic cellular debris to rescue cells under stress. Here, microglia execute the role of a friendly beat patrol, restoring normalcy through communicatiocations and calibrated actions. If microglia were inherently dysregulated because of a gene mutation (as observed in NHD) or they activated developmental mechanism (s) that were abberant and dysregulated (as observed in animal models of neurodegeneration), it would accelerate the degenerative changes, adding to the disease process. Here, microglia emerge as an overbearing beat patrol, adding to the disturbance by uncalibrated and relentless actions. Therefore, the key to microglia-dependent therapeutics in glaucoma may require a calibrated re-activation of their developmental mechanism(s) and/or suppression of their dysregulated functions toward restoring homeostasis.

Conflict of Interest

The authors indicated no financial relationships.

Author Contributions

I.A.: conceptualization and design, financial support, administrative support, provision of study material or patients, collection and/or assembly of data, data analysis and interpretation, manuscript writing, final approval of manuscript. M.S.: collection and/or assembly of data, data analysis and interpretation, manuscript writing.

Data Availability

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

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