

Unraveling the role of microglial activation and immune regulation in glaucomatous neurodegeneration: insights into its dynamic interactions with retinal cells

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Abstract

Glaucoma is a common ocular disease characterized by elevated intraocular pressure (IOP). Despite the efforts to manage IOP, it still cannot prevent the development of glaucomatous neurodegeneration in the retina. Microglia, the principal resident immune components in the eyes, serve as the concertmaster in the progression of glaucoma. They are activated and polarized in response to the stimuli, including increased IOP attack and advancing neurodegeneration. In previous studies, microglia not only target retinal ganglion cells, which are the victims of glaucomatous neurodegeneration, but also interplay with the other cells in the retina, including Müller cells and astrocytes. These interactions can either accelerate or decelerate the glaucomatous progression. In these complex interactions, directly targeting or inhibiting part of them may become a focal point for future therapies. Therefore, microglial activation and their mutual regulation with the other retinal cells have become a breakthrough point. This review explores the role of microglia in glaucoma pathogenesis and concentrates on the microglial activation and immune regulatory functions. The current study focuses on the immune network orchestrated by microglia and their interactions, to shed light on potential avenues for developing novel neuroprotective therapies that target microglia.

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Introduction

Glaucoma is a blinding neurodegenerative disease characterized by the progressive death of retinal ganglion cells (RGCs), and it is predicted that primary open-angle glaucoma and primary angle-closure glaucoma will affect over 100 million individuals globally by 2040^[1]. Among various risk factors associated with RGC degeneration in glaucoma, elevated intraocular pressure (IOP) stands out as the most consequential factor^[2]. Approaches including surgical intervention and topical medications have been adopted for IOP reduction^[2]. However, despite efficient IOP management, the possibility of persistent degeneration for RGCs remains. It implies that numerous factors, besides IOP, affect the survival of RGCs in glaucoma. The neurodegenerative changes in glaucoma, with the feature of increased IOP, likely involve a complex interplay among multiple triggers, cell types, and molecular pathways.

The immune system is assumed to play a pivotal role in the neurodegenerative cascade, and it is also applicable in the case of glaucoma. Microglia, the resident immune forces in the retina, is indispensable for neuroinflammation and immunoregulation^[3]. When the retina is exposed to the elevation of IOP, microglia are rapidly activated, followed by remarkable alterations that initiate the onset and progression of glaucomatous lesions^[4]. Notably, microglia exhibit remarkable alterations including enlarged cell soma, retracted cellular processes, and changing expression of phagocytosis-related proteins and myeloid cell markers, all of which serve as indicators of subsequent behaviors and functions of microglia^[5]. Furthermore, if the over-aggressive microglial activities follow, it can trigger a self-perpetuating toxic cycle that damages the tissue^[6]. As the primary sources of inflammatory signals, microglia release excessive neurotoxic and pro-inflammatory factors

that amplify the overall inflammatory response^[6,7].

Whether in a homeostatic or overactive state, microglia are not solitary players in glaucoma. Instead, they are highly dynamic and engage in social interactions with other cells. For example, recent investigations have elucidated that the interplay between microglia and Müller cells intensifies the inflammatory response within the glaucomatous retina^[8]. Hence, understanding this dynamic and complex cellular interaction is crucial to deciphering microglial immunoregulatory roles within the retina attacked by glaucoma or ocular hypertension (OHT). Establishing a comprehensive map of microglial interaction with retinal cells could help to elucidate the signaling cascades and provide key insights into preventing or treating glaucomatous neurodegeneration.

Microglia dynamic activation and functional heterogeneity in glaucoma

Microglia are the primary resident immune components of the eyes and maintain ocular immune privilege. Under the physiological condition, the static microglia continuously scan and monitor their local environment^[9]. When facing OHT, the retinal homeostasis is disrupted, leading to the activation of microglia and increasing relative biomarkers, which is an early event in the development of glaucoma^[10,11]. With the retraction of cell processes, enlarged cell volume, and increased cell density, static microglia immediately respond to the injury signals, activate into different subpopulation, and dynamically perform their function^[8,9,11].

Historically, these activated microglia have been traditionally described as the M1/M2 polarization pattern. With the pro-inflammatory properties, the classical M1 microglia are induced by many

aspects, such as the production of NLRP3 inflammasome, ROS and iNOS^[9,12,13]. Besides, microglia could be triggered by STAT1 signaling pathway and Toll-like-receptor also involves in microglial activation which then NF- κ B signal pathway reveals a wave-like activation pattern after acute OHT attack^[14,15]. These activated M1 microglia enhance the inflammatory response, generate the cytotoxic molecules and pro-inflammatory factors, including nitric oxide (NO), interleukin (IL)-12, IL-6, IL-1 α , IL-1 β , Tumor necrosis factor (TNF)- α , leading to neuronal damage^[15–17]. Opposite to the effect of M1 microglia, M2 microglia, with its anti-inflammatory properties, could be activated by cytokines, like IL-4, initiated STAT6 signaling pathway promoted migration toward injury sites, and secreting neurotrophic and anti-inflammatory factors, including Insulin-like growth factor 1 (IGF 1), transforming growth factor β (TGF β), and IL-10^[15,18].

The M1/M2 classification, primarily derived from macrophages, oversimplifies the complexity of activated microglia, which are dynamic immune cells characterized by unique functions, distinct gene expression, and remarkable plasticity. A more diverse range of approaches is required to effectively classify microglia. Recently, a study concluded the possible microglial communities analyzed in the brain and hippocampus of unstimulated adult mice based on single-cell RNA sequencing studying, including 'Satellite' microglia, KSPG microglia, Hoxb8 microglia, CD11c microglia, 'Dark' microglia, and so on^[9]. Therefore, emerging advanced throughput techniques could also provide unprecedented insights into microglial context-dependent functional heterogeneity of microglial activation states in glaucoma.

Recently, single-cell sequencing studies identified a new reactive subpopulation with neuritic A β -plaques named disease-associated microglia (DAM), which was first mentioned in the Alzheimer's disease (AD) model^[19,20]. A similar DAM phenotype was also found in glaucoma. In the silicone oil model with OHT, CD74⁺ microglia, identified as DAM in the brain, was also observed in the optic nerve (ON) and correlated phosphoinositide 3-kinase (PI3K) signaling in response to OHT^[21]. With the unique treatment targeting CD74⁺ microglia, lipoxin B₄ (LXB₄) treatment reduces the expression of CD74 shifting the reactive CD74⁺ microglia toward the homeostatic subtype^[21]. Similarly, in microbead-injected glaucomatous retina, another microglial neurodegenerative phenotype (MGnD) elevated expression of Lgals3 and upregulated complement-related genes, mediating neurotoxicity by TREM2-Apolipoprotein E (APOE) signaling^[22]. Interestingly, in the early stages of the microbead-injected glaucoma model, the APOE-positive microglia predominantly express APOE4, and inversely correlates with RGC loss which is converse to AD^[22]. Upregulation of APOE4 suppressed the microglia conversion to MGnD, thereby reducing Lgals3 expression and conferring neuroprotective effects on RGCs^[22]. Notably, APOE also plays a key role in DAM immunometabolism, suggesting that MGnD may not be completely distinct from DAM. DAM is a transition from homeostasis to activation via a specific signaling pathways while MGnD is specifically associated with neurodegenerative diseases, and they both downregulate the homeostatic genes. The elevated APOE in early DAM may prime its progression to MGnD while the APOE knockout prevents MGnD transformation, indicating that MGnD likely shares a common DAM origin with ON-specific CD74⁺ microglia and the reactive microglia subtypes differentiate along a continuum in glaucoma.

High-resolution transcriptomic analysis technologies, such as single-cell sequencing, dissect the dynamic phenotypes of microglia under various pathological conditions and reveal their functional heterogeneity. This approach has overcome the limitations of the

traditional M1/M2 polarization model and identified disease-specific molecular markers and precise therapeutic targets, thereby providing strategies for targeted intervention of microglial phenotypes and functions (Fig. 1).

Microglial proliferation, migration, and phagocytosis in glaucoma

Microglial recruitment has been identified as one of the initial responses following exposure to OHT^[23]. This recruitment involves the proliferation and migration of microglia and the recruited microglia will remove degenerative cells or debris through phagocytosis.

It has been reported that microglial proliferation precedes RGC injury in OHT. In the model of chronic ocular hypertension (COH), the proliferative response is found to be mediated through purinergic receptors expressed on microglia. For instance, ATP could modulate microglial proliferation via the P2X7 receptor (P2X7R) in the COH model established by microbeads-injection^[24,25]. Specifically, this process is further regulated through the ATP/P2X7R/mitogen-activated ERK-regulating kinase (MEK)/extracellular signal-regulated protein kinase (ERK) pathway^[24–26]. Both the knockout of the P2X7R gene and the pharmacological blockade of P2X7R have demonstrated significant inhibition of microglial proliferation within the COH retina^[24]. These findings highlight the pivotal role of P2X7R and the related downstream pathway in microglial proliferation, providing potential targets to promote appropriate proliferation while preventing over-proliferative behaviors that exacerbate RGC injury. Although the proliferative mechanism in the COH model is described in the research of Xu et al., the specific mechanisms in other glaucoma models are complex and largely unknown^[24].

With the elevated IOP, there is a notable accumulation of microglia within the ganglion cell layer (GCL) and the nerve fiber layer (NFL), which is primarily attributed to microglial proliferation and migration^[24]. Upon activation, microglia rapidly extend the processes toward the injured sites or migrate to the damaged locus such as GCL and ON head, to modulate the spread of the lesion^[11,27]. Similar to proliferation, microglial migration is also related to purinergic receptors. *In vitro*, adenosine participates in microglial migration when mimicking OHT, hinting at the involvement of purinergic receptors^[28]. *In vivo*, extracellular ATP and nucleotides released from injured or dead neurons can activate the purine receptors on microglia, initiating migration^[11]. Notably, Müller cells contribute to the microglial migration through ATP-activated purinergic receptors, mainly P2X4 receptor (P2X4R) in the context of the COH model^[24]. Furthermore, cytokines, chemokines, and growth factors may act as navigational cues to direct microglia toward the site of injury during microglial migration^[29].

Phagocytosis is the primary mechanism by which microglia execute immune function. Reactive microglia can beneficially phagocytose cellular debris or apoptotic cells to maintain microenvironment homeostasis, however, similar to the previously dysregulated proliferation discussed, the overactive phagocytosis towards damaged RGCs may result in the adverse and paradoxical effects on visual function. For instance, in the acute ocular hypertension (AOH) model that maintains IOP at 110 mmHg for 60 min, phospholipid scramblase 1 (PLSCR1) is upregulated to promote cellular apoptosis and microglial phagocytosis^[30]. This process further increases the proportion of pro-inflammatory microglia, which in turn enhances the subsequent apoptosis of damaged RGCs and their phagocytosis by microglia^[30]. As the core of retinal function, RGCs may still retain some visual capabilities even when damaged. However, when these damaged RGCs are cleared away by microglia, both retinal visual function and visual acuity are directly

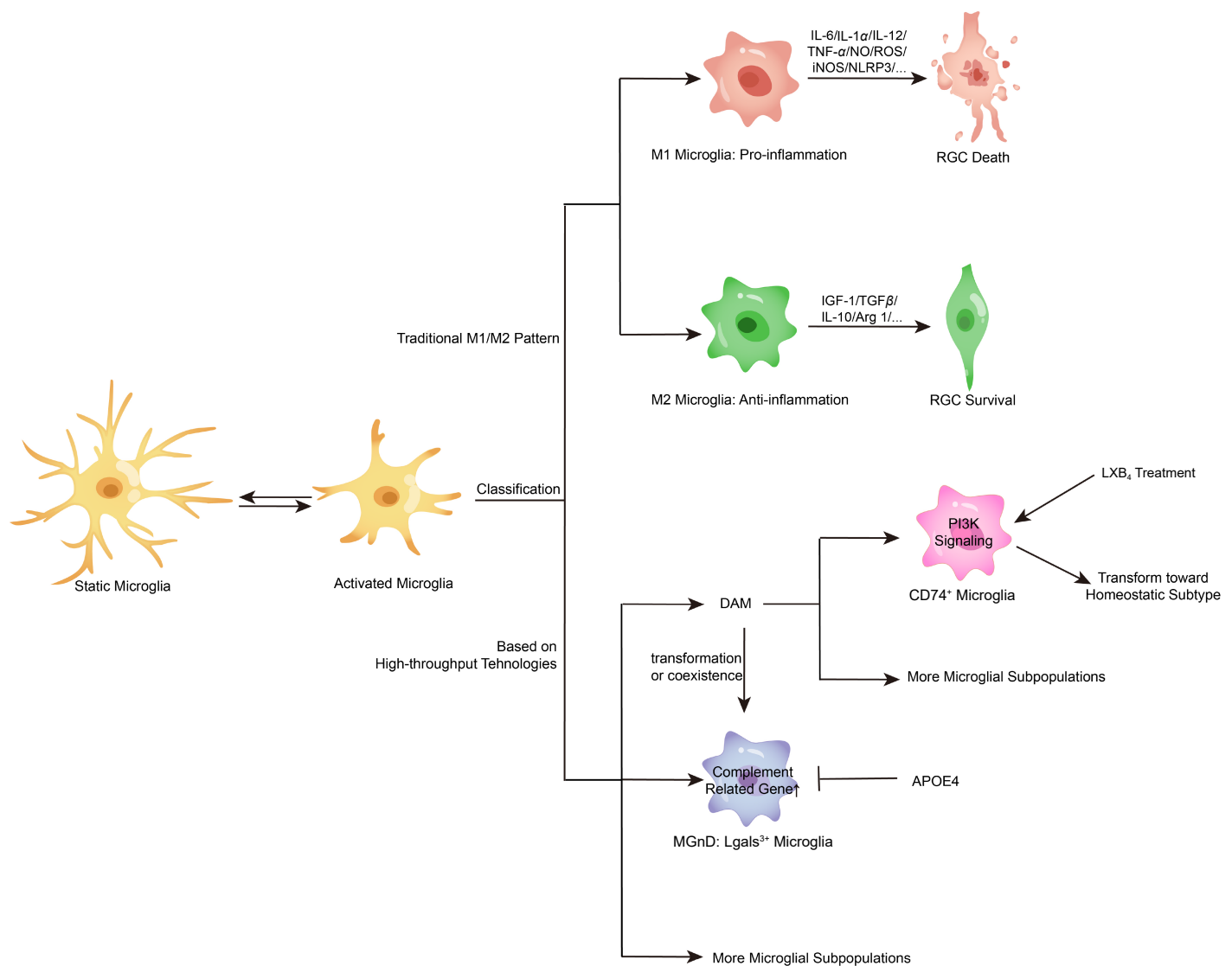


Fig. 1 Dynamic microglial activation in traditional or novel classification. The static microglia serve as guardians that monitor the microenvironment and are activated into different subtypes in glaucoma. Historically, the M1/M2 paradigm established for macrophages has been applied to microglial classification. The M1 phenotype mediates pro-inflammatory responses via cytotoxic factors and inflammasomes, leading to RGC loss, while M2 phenotype plays a neuroprotective role in promoting RGC survival. Advances in high-throughput technologies enabled identification of more precise microglial subpopulations: CD74⁺ microglia from DAM drive neuroinflammation whereas LXB₄ treatment promote their transformation to homeostatic microglia; MGNd (Lgal3⁺ microglia) can be suppressed by APOE4 upregulation, thereby contributing to RGC protection. Notably, MGNd may be derived from DAM or coexistence with DAM.

disrupted. Therefore, to rescue the remaining RGCs and save visual function, it is possible to regulate the overzealous phagocytic behavior of microglia towards damaged RGCs by targeting the efficiency of phagocytosis. Recently, one interesting research paper has shown that exosomes, an emerging hot spot for treatment, from microglia are also involved in phagocytosis and its efficiency^[31,32]. *In vitro*, microglia can actively secrete the extracellular vesicles, among which a subset identified as exosomes affect microglial phagocytosis^[32]. Upon exposure to elevated IOP, microglia-derived exosomes trigger phagocytic activity and contribute to the enhancement of phagocytic efficiency, subsequently, increasing the inflammatory cytokines and reactive oxygen species (ROS), which are detrimental to retinal health^[32]. Moreover, when these exosomes are artificially injected into the eyes, the damage inflicted by microglia is exacerbated in RGCs and retina^[32]. Despite the lack of research addressing the effects of timely reduction or inhibition of phagocytosis-related exosomes, recent studies have explored the

potential of intravitreal exosomes for glaucomatous management^[31,33]. Hence, it's possible for exosomes to provide a potential and emerging therapeutic approach for regulating microglia in appropriate phagocytosis to protect retinal health.

Interplay between microglia and other retina cells in glaucoma

Regardless of whether it is activated, proliferating, or migrating, microglia are not isolated during the development of glaucoma. Hence, we focus on the interaction of microglia with other retinal cells. Nowadays, the interplay between activated microglia and glaucomatous RGCs degeneration and dysfunction has been well-documented in glaucomatous animal models (including pathways, complement system, cytokines, receptors, etc.), and the details of how activated microglia contribute to the fragility of RGCs in glaucoma still need further exploration^[27,34,35]. Meanwhile, microglia also

show a synergistic effect with other cells (mainly astrocytes and Müller cells) so that they indirectly affect the function of RGCs and constitute a complex and sophisticated network in glaucoma^[8,36–39]. These interactions form a positive feedback loop between microglia, RGCs, and macroglia in glaucoma^[8,24,40,41]. Obviously, in these interactions, researchers have noted various mechanisms in different glaucomatous models, highlighting the possible therapeutic targets for different types of glaucoma in the future.

RGCs

RGCs are the output neurons that reside in the retina and are responsible for transmitting visual information from the retina to the brain. They also serve as the core cells involved in the pathological damage of glaucoma, which has the closest interaction with microglia^[42]. Given that various subtypes of glaucoma share the common feature of RGCs damage and loss, it is necessary to explore the interplay between microglia and RGCs within the background of glaucoma, which contributes to neuroprotective mechanisms or neurodegenerative processes.

As mentioned above, there is a direct interaction between microglia and RGCs in glaucoma. Activated microglia can be attracted by damaged RGCs, migrate to GCL and directly engulf RGCs^[30]. Furthermore, once localized to the GCL, microglia also produce excessive pro-inflammatory factors, such as TNF- α , NO, and IL-1 β , leading to intensified neuroinflammation and RGC toxicity^[24,43]. Notably, microglial pyroptosis plays a remarkable role in IL-1 β -mediated RGC death in the context of the AOH model characterized by elevated IOP^[13]. During this process, the N-terminal fragment of gasdermin D (GSDMD), which is cleaved in a caspase-1-dependent manner, forms pores in the cellular membrane^[44]. Consequently, it facilitates enhanced release of IL-1 β and leakage of lactate dehydrogenase from microglia, further contributing to the RGCs loss^[13]. Intriguingly, IL-1 β provides the positive feedback to promote the microglial pyroptosis response axis, caspase 8-HIF-1 α -NLR family^[13]. The leakage of inflammatory factors due to microglial pyroptosis, coupled with the accelerated positive feedback from IL-1 β in microenvironment, leads to neurotoxic and accelerate the axon neurodegeneration and RGC death in glaucoma.

It should be noted that these pro-inflammatory factors participate in the expansion of the local inflammatory microenvironment, thus, limiting the microglia-mediated inflammatory response will be conducive to rescuing RGCs. In the context of OHT, microglia create local inflammation through the activation of the NF- κ B and ERK signaling pathways^[45–47]. Meanwhile, the aryl hydrocarbon receptor (AhR) co-localized with microglia has a neuroprotective role by downregulating NF- κ B and ERK signal and then restricting microglial inflammatory response in downstream, thereby protecting RGCs^[45,47,48]. Furthermore, the endogenous tryptophan metabolite can activate the AhR, alleviate the retinal inflammation, delay the retinal thinning or RGC loss, and ultimately block the propagation of neuroinflammation^[45,49,50]. Hence, the positive effect of tryptophan metabolites in restraining microglia-mediated local inflammatory amplification provides a new strategy for saving RGCs in glaucoma.

Facing the OHT environment in glaucoma, the nucleotides released from damaged neurons can directly upregulate the potency of purinergic receptors on microglia, enhancing their inflammatory response, phagocytosis, and migration^[51,52]. Therefore, it is admitted that the purinergic receptors on microglia present key points for initiating the microglial inflammatory response. In the COH model, P2X4R and P2X7R are expressed on microglia as purinergic receptors and could be activated by Müller-derived ATP, but only P2X7R contributes to RGCs death via activating the P2X7R-NLRP3 pathway^[25,53]. Consistent with this,

pharmacological inhibition of P2X7R using antagonists, like JNJ47965567, has demonstrated neuroprotective effects on RGCs in DBA/2J model, suggesting a potential therapeutic strategy to mitigate retinal degeneration in optic neuropathy^[54].

In summary, the interplay between microglia and RGCs is more intricate than currently understood. In addition to the pathways directly impacting the microglia-RGCs interplay, the crosstalk between microglia and other cells can also indirectly affect the survival of RGCs in the context of glaucoma. Cytokines such as IL-1 β are released through intercellular communication between microglia and other cells, such as Müller cells and astrocytes, aggravating the inflammatory response and RGC injury^[8,46].

Müller cells

Müller cells are important macroglia in the retina which take part in regulating and maintaining the function of RGCs^[55]. With the feature of upregulating the glial fibrillary acidic protein (GFAP), glial cytoskeletal proteins, and vimentin, reactive Müller cells have been detected in glaucoma and involved in neurodegeneration via inflammatory cytokines releasing like IL-1 β and TNF- α ^[37,55–58]. This function parallels that of microglia, suggesting potential intercellular communication between Müller cells and microglia. Furthermore, recent studies have reported that Müller cells collaborate with microglia to create an inflammatory microenvironment in neurodegenerative diseases including glaucoma.

Müller cells and microglia are actively associated through receptor-ligand interaction in activation, migration, and proliferation, indicating their co-immunoregulation in glaucoma^[24]. As a superior source of extracellular ATP, Müller cells affect microglia primarily through ATP mediation^[8,59]. In COH-affected glaucomatous retina, extracellular glutamate can activate the group I metabotropic glutamate receptor (mGluR I)/Gq/PI-PLC/PKC signaling pathway in Müller cells to increase ATP release^[60–62]. These ATP, released through the connexin43 (Cx43) hemichannel, trigger P2X7R to induce microglia activation^[8]. When P2X7R is inhibited, the release of ATP is suppressed, or the activation of Müller cells is restrained, the biomarker of activation like translocator protein (TSPO) is sharply decreased in microglia, suggesting that most of the microglial activations are induced by activated Müller cells^[8].

In the COH model induced by microbead injection, the ATP released from Müller cells also triggers microglial activation and then regulates the release of inflammatory mediators via the ATP/P2X7R/Ca²⁺/NFAT (nuclear factors of activated T cells)/NF- κ B pathway, leading to cytokine release, like IL-6, iNOS, and TNF- α ^[8,25,63,64]. In turn, these inflammatory factors upregulate the pro-inflammatory gene expression in Müller cells at the mRNA level, synergistically amplifying the inflammatory response with microglia^[8]. Interestingly, the ATP released from Müller cells also elevates the mRNA level of anti-inflammatory factors in microglia, which may link to the initial functions of microglia^[8]. However, the protein concentration of these anti-inflammatory factors remains too low to sustain a comprehensive neuroprotective impact^[8].

Activated Müller cells also participate in the aggregation of microglia via the release of ATP which activates both P2X7R and P2X4R on microglia to promote their migration and proliferation in the microbead-induced COH model^[24,65]. For instance, with the stimulation of ATP, P2X7R contributes to microglial proliferation through the ATP/P2X7R/Ca²⁺/MEK/ERK pathway in COH^[8,24,66]. Moreover, the adhesive interaction between microglia and Müller cells suggests that Müller cells may directly play a role in microglial migration as a scaffold^[67–69]. Müller cells are radially-oriented cellular structures through the entire retina while microglia are predominantly horizontal^[68]. The activated microglia modify surface

molecules on Müller cells, inducing positive secretion of adhesive chemokines and molecules, which in turn enhances adhesion between Müller cells and microglia^[67,68]. Therefore, it is inferred that activated microglia can adopt a vertical orientation and migrate radially across the retinal layers via Müller cells (Fig. 2).

Microglia may affect the function of Müller cells via cytokine crosstalk. Co-culture with lipopolysaccharide-activated microglia induce Müller cells, which adopt more multipolar and elongated spindle shapes, to deregulate the vimentin and glutamate aspartate transporter (GLAST), while upregulating the neuroprotective factors such as leukocyte inhibitory factor^[70,71]. Notably, recent research has demonstrated that exocellular TNF- α partly mediated NF- κ B pathway in Müller cells and modulated the expression of CCL2, IL-6, iNOS, and vascular cell adhesion molecules while microglia serve as the key source of TNF- α ^[70]. It is suggested that TNF- α may participate in the interaction between microglia and Müller cells. Additionally, the expression of glial cell line-derived neurotrophic factor (GDNF) and basic fibroblast growth factor in Müller cells is regulated by brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), and ciliary body-derived neurotrophic factor (CNTF) from activated microglia, and microglia-derived IL-1 β also can modulate the expression of CXCL1, CCL2, and CXCL10 from Müller cells^[72,73]. Moreover, microglia-derived molecules also play a role in the behavior of Müller cells via receptor-ligand interaction. In experimental COH glaucoma, microglia-derived osteopontin (OPN) acts on the Itg α v β 3/CD44 receptor complex to inhibit the autophagy of Müller cells through the p38-MAKP signaling pathway^[74]. With this dysfunction of Müller cells, Müller cell-mediated neuroprotection and homeostatic support is disturbed, including the decrease of neurotrophic factors, resulting in the parallel loss of RGCs and further retinal neurodegeneration^[74]. Meanwhile, the dysfunction of Müller cells also disturbs microglial activation, leading to sustained pro-inflammatory states. Collectively, these studies highlight the

close interaction between microglia and Müller cells in glaucomatous neurodegeneration, while the signaling pathways and mechanisms involved warrant further research.

Astrocytes

Astrocytes are another type of macroglia usually associated with neurodegenerative diseases. In healthy retina, astrocyte activities can be detected in the ON, retinal nerve fibers, and GCL^[75]. Exposed to stimuli such as elevated IOP, astrocytes can be activated into different reactive states, A1 pro-inflammatory and A2 neuroprotective types, similar to microglial traditional M1/M2 polarization^[38]. A1 reactive astrocytes obtain neurotoxic and pro-inflammatory functions when losing their ability to promote synapse formation and phagocytosis at the same time^[76,77]. Conversely, A2 astrocytes upregulate neurotrophic factors to promote a neuroprotective environment^[78]. In fact, A1 astrocytes are the major effectors of neurodegeneration diseases.

Astrocytes and microglia are closely interdependent in the pathogenesis of glaucoma. It has been demonstrated that microglia play a role in astrocyte activation primarily through different cytokine interactions. Microglia-derived TNF- α , IL-1 α , and C1q serve as critical stimulants for A1 astrocytes, and downregulate their production can effectively reduce A1 astrocytes polarization^[17,79–81]. Meanwhile, M1 microglia increase mitochondrial fission and release damaged or dysfunctional mitochondria, activating A1 astrocytes and exacerbating neuroinflammation, inducing the innate immune response in glaucoma^[82]. Conversely, M2 microglia secrete IL-4 and IL-10 to mediate astrocyte polarization towards A2 phenotypes, which secrete TGF β and other neurotrophic factors to support neuroprotection^[17,38]. It is understood that the microglial activation states may directly affect the polarization and degeneration of astrocytes^[38].

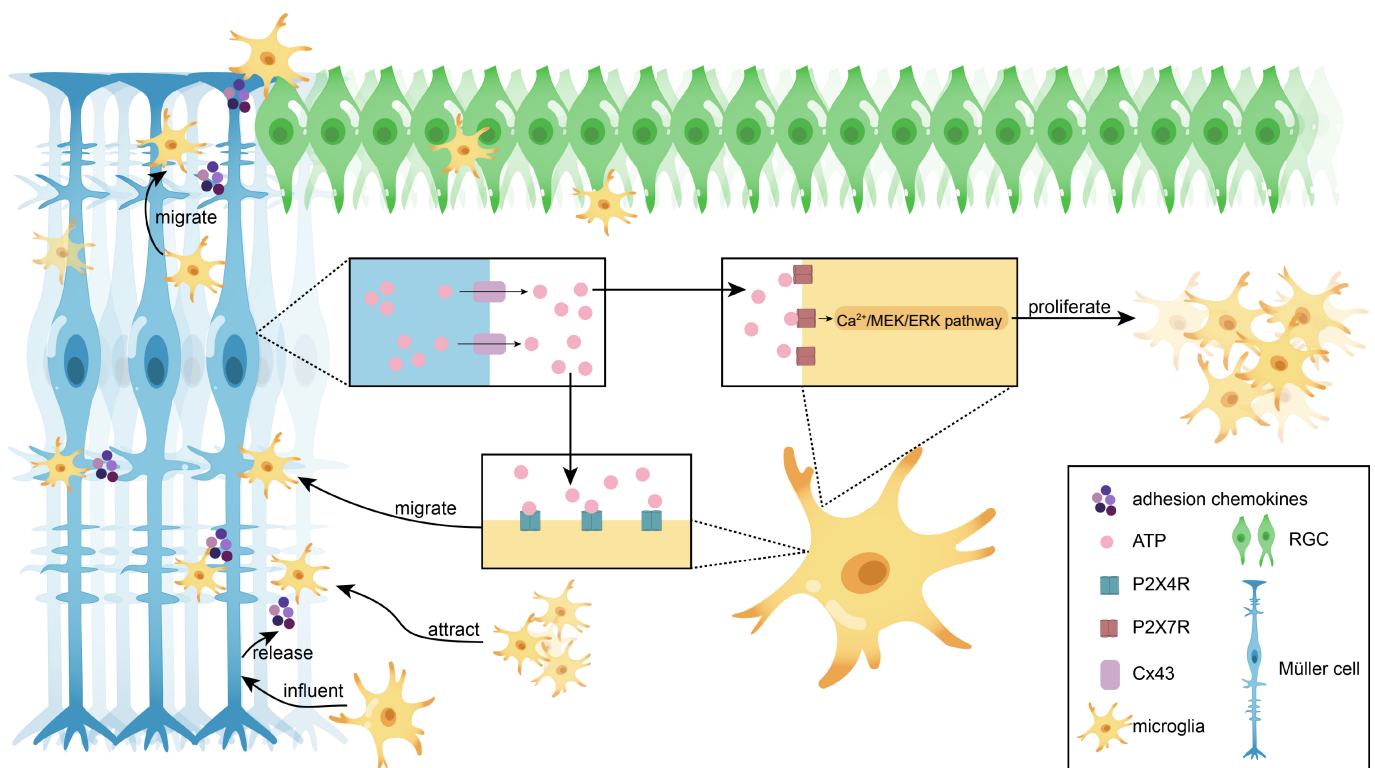


Fig. 2 Müller cells play a role in microglial proliferation and the migration of Müller cells are mainly involved in the proliferation and migration of microglia through ATP releasing and primarily mediate P2X7R and P2X4R on microglia. In addition, after being stimulated by microglia, Müller cells release sufficient adhesion factors to attract microglia, promoting their vertical migration across the retina to GCL.

Furthermore, activated microglia also modulate astrocytes function by releasing IL-1, resulting in the upregulation of IL-6, TNF- α and colony stimulating factors in astrocytes, which comprise a positive feedback loop to affect microglia^[83]. Moreover, it is also inferred that microglia-released TNF- α regulates the expression of astrocytic tenascin-C (Tnc), resulting in harmful astrogliosis in glaucoma^[84]. In turn, astrocyte-derived Tnc enhances the glial reactivity, including support for pro-inflammatory microglia, whereas the deficiency of Tnc in an experimental autoimmune glaucoma (EAG) rat model may encourage the microglial polarization towards a neuroprotective state, leading to decreased axonal fiber damage and RGC death^[84–86].

Similar to the interaction between microglia and Müller cells, astrocytes also induce microglial activation by the release of ATP via the Cx43 hemichannel and interplay with the correlated receptor, which is a phenomenon that reactive astrocytes positively feedback to recruit and activate microglia^[87–89]. Notably, pro-inflammatory microglia specifically trigger the C3a receptor 1 by C3, a characteristic marker of A1 astrocytes, then initiating the IL-10 related signaling pathway in microglia in DBA/2J mice^[77,90,91]. Furthermore, astrocytes also influence the functionality of microglia via receptor-ligand interaction. Astrocyte-derived S100B attaches to the receptor for advanced glycation end products (RAGE) on microglia, activating the NF- κ B signaling pathway and modulating IL-1 β release, ultimately enhancing ON damage via an IL-1 β -related positive feedback loop in the EAG model^[40,41,92]. Based on previous discussions, these synergistic effects and the close relationships between microglia and astrocytes in glaucoma have been emphasized. Nonetheless, further investigation is warranted to ascertain which glial cell type responds most rapidly, as this understanding could inform targeted approaches to modulate immune responses in glaucoma treatment.

Oligodendrocytes

Oligodendrocytes are one type of macroglia that myelinate RGC axons in ON. It is reported that microglia affect oligodendrocytes in the context of ischemic stroke, which may be detrimental or beneficial, depending on the microglial subtype^[93–95]. It may have microglia-oligodendrocyte interactions in glaucoma with RIR similar to other macroglia. However, oligodendrocytes are mainly distributed in the myelination transition zone, which is spatially separated from the RIR area directly induced by OHT^[96]. Therefore, this spatial disparity precludes direct comparison with microglia-oligodendrocyte interactions observed in ischemic stroke. Besides, activated microglia could influence oligodendrocytes by releasing TNF- α and IL-1 β which both result in oxidative stress and inflammation in the microenvironment^[97]. Generally, the studies in microglia-oligodendrocyte interactions in the glaucoma model has been limited to TNF- α and IL-1 β , leaving room for a fuller understanding of the interaction.

Retinal pigment epithelium (RPE) cells

RPE cells are located between choroid and photoreceptor cells and offer nutrition to the retina. The interplay or physical contact between microglia and RPE cells are rarely observed in young healthy retina, but they become more frequent when aging. Previous studies have presented that the age-dependent microglia-RPE cell interplay causes changes in RPE cells and then mediates enhanced immune dysregulation at the outer retinal layer, resulting in analogical pathological changes such as age-related macular degeneration (AMD)^[98,99]. Glaucoma is also an age-related disease. Therefore, it is necessary to consider whether age-related increases in microglia-RPE cell interactions affect the core interactions between microglia and other cells in glaucoma. For example,

synergistic effects may share biological pathways, or common targets within the interactions between microglia and RPE cells, which predispose microglia in elderly glaucoma patients become more aggressive or more friendly towards other retinal cells. Exploring the shared genetic expression between RPE cells and other retinal cells may reveal similar interactions with microglia when the microglia may target the same or similar protein expressed. Taking RGCs as an example, RGCs are the primary target cells of microglia. Microglia both have close interaction with RPE cells and RGCs. Nowadays, optineurin, an adaptor protein that interacts with myosin VI, has been identified in RGCs, nerve fibers, and RPE cells, which can be regarded as a basis for further research on whether the similar interplay between microglia and both RGCs and RPE cells can affect glaucoma progression^[42,100–102].

Photoreceptor, amacrine, and bipolar cells

The interactions between microglia and other retinal cells such as photoreceptor cells, bipolar cells, and amacrine cells have been described in other neurodegenerative diseases^[43,103,104]. Their performance in glaucoma remains unexplored or unreported.

The crosstalk between microglia and photoreceptor cells mainly occurs in retinitis pigmentosa (RD), while there is limited research about their interaction in glaucoma^[43,105]. In RD, activated microglia quickly respond to the condition and phagocytose dying photoreceptors^[106]. When microglia are depleted, photoreceptor death increases, suggesting that microglia play a role in producing nutritional factors and maintaining reactive photoreceptors^[68,106,107]. For example, NADPH oxidase, which is closely linked to microglia, can generate ROS, contributing to microglia-mediated neurotoxicity in photoreceptor cells^[108,109]. In the OHT model and the contralateral eyes, activated microglia in amoeba-like shapes have been observed in the photoreceptor outer segment (OS) layer^[110,111], but there are few reports investigating the interaction between microglia and photoreceptor cells in glaucoma.

Amacrine and bipolar cells are significant neurons that transfer visual signals to RGCs in the inner retinal layer. In glaucomatous animal models, their numbers have been observed to decrease, bipolar cell synapsis has a partial loss, and amacrine cells are particularly susceptible to damage resembling that of glaucoma after RGCs death^[104,112–115]. It is not clear however, whether the involvement of microglia is directly associated with such damage of amacrine and bipolar cells or whether they are affected by changes in the ocular microenvironment in glaucoma.

Conclusions and prospects

In glaucoma, the microglia can be directly activated by OHT, and the various types of cells in eyes also play a role in microglial activation. Traditionally, it has been considered that the activated microglia polarize into two major categories M1 microglial (neurotoxic), and M2 microglia (neuroprotective). However, microglia have been found to exhibit a range of distinct activation states by high-throughput technology with different markers, highlighting their complex functions and dynamic interactions with other cells. In glaucomatous pathology, microglia primarily target RGCs, exerting both damaging and protective effects simultaneously. Additionally, microglia interplay extensively with Müller cells and astrocytes, ultimately targeting RGCs and influencing the progression of glaucoma. Interestingly, oligodendrocytes, another type of glial cells, have minimal direct interaction with microglia due to their spatial distribution, and are mainly affected by the inflammatory microenvironment induced by microglia. The interactions between microglia and other retinal cells may primarily manifest in glaucomatous patients comorbid with other diseases, while the interactions within the

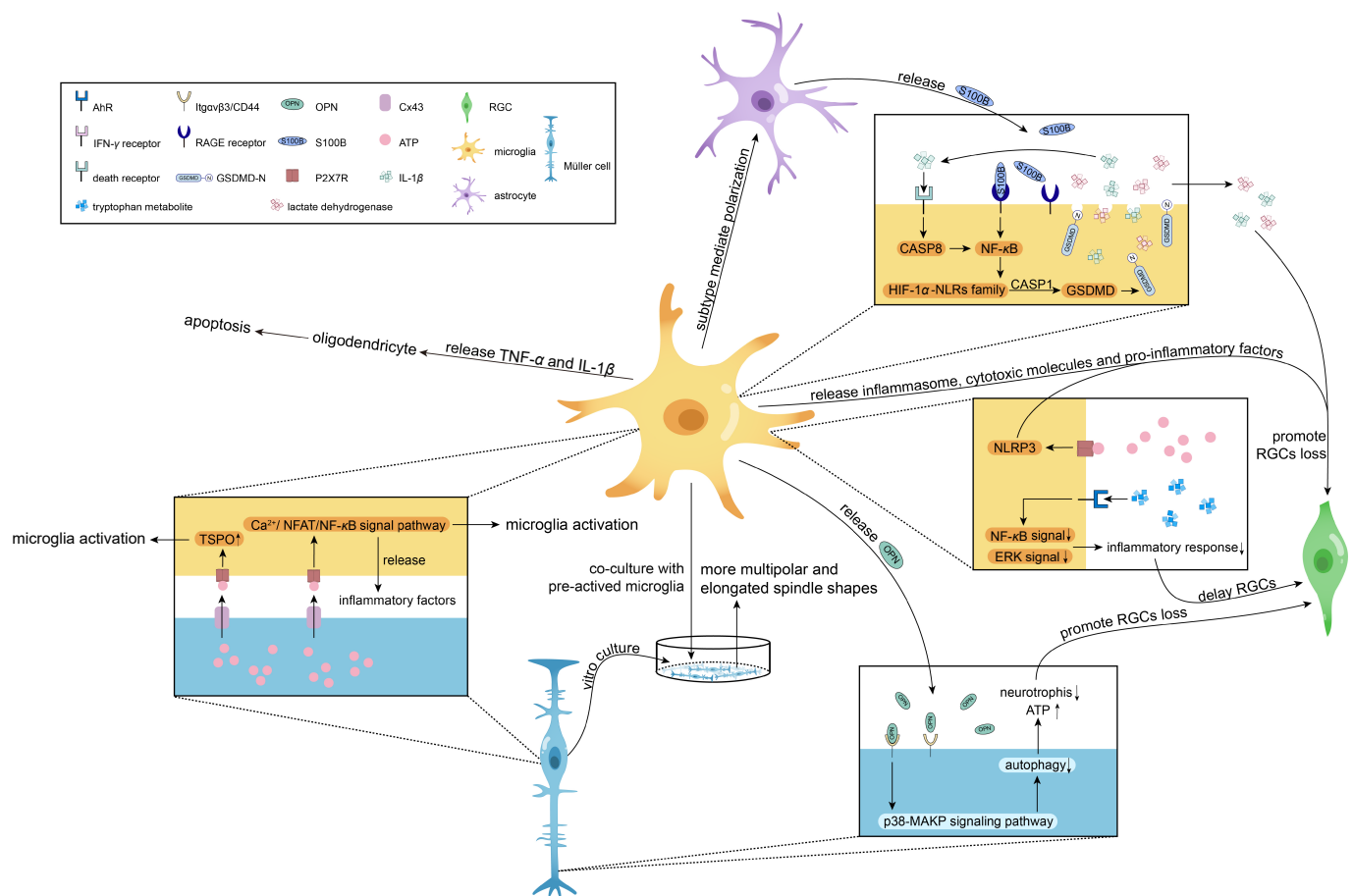


Fig. 3 A network of the interplay between microglia and intraretinal cell in glaucoma. Microglia interplay with a variety of retinal cells. RGCs, Müller cells, and astrocytes are the leading interactive cells when oligodendrocytes are also affected to a small extent. These interactions affect the microglial function, lead to neuroprotective or neurodegenerative lesions, and ultimately affect pathological outcomes within glaucoma. The interplay of other retina cells with microglia is mainly reflected in other disease models and awaits further explanations in glaucoma.

context of pure glaucoma warrant further investigation and consideration of microglial-related interactions.

For the development of potential clinical therapies aimed at suppressing the process of glaucomatous neurodegeneration, it is crucial to focus on modulating microglial activities and protecting RGCs from detrimental microglial interactions. Identifying the possible pharmacological targets within these pathways or disrupting the cytokine crosstalk is of paramount importance. For instance, ATP serves as a critical mediator of Müller cells-microglia interactions and initially activates microglia through P2X7R in the COH model. The antagonist A740003 effectively prevents the harmful activities from microglia at the beginning stage of inflammation and saves RGC loss^[116]. Furthermore, potential solutions may also include the components from traditional Chinese medicine. Chinese herb extracts may alleviate indirect RGC damage by microglia by down-regulating the expression of inflammatory factors^[117–119]. Taking saffron as an example, it has been reported to prevent the release of pro-inflammatory factors while restoring the release of anti-inflammatory factors, possibly by reducing microglial activity and proliferation in an OHT model, providing a novel neuroprotective strategy for glaucoma treatment^[117,119].

Systemic administration risks unintended effects on the central nervous system (CNS) microglia and other immune cells, given their shared involvement in neuroinflammation. Meanwhile, the existence of blood-retina barrier poses a significant challenge for drug presentation when translating the potential treatments into

clinically viable oral or intravenous formulations. Therefore, the topical administration of glaucomatous treatment, such as intravitreal injection, may offer a more feasible therapeutic precision. The specific markers for microglial subpopulation have been identified through high-throughput technologies and serve as potential targets for the targeted drug delivery via intravitreal injection^[21,120]. Astrocytes-derived LXB₄ play a pivotal role in deregulating microglial sensing and reactivity, mainly typically targeting CD74⁺ microglia subpopulations^[21]. LXB₄-related therapeutic strategies including exogenous supplementation of LXB₄ via intravitreal may promote a microglial phenotypic shift toward a homeostatic subtype^[21]. By selectively targeting glial cell interactions, the specific P2X7R antagonist JNJ47965567 or the supplement of tryptophan metabolites may demonstrate superior therapeutic precision in topical treatment, such as intravitreal injection, compared to systemic administration, hence mitigating the potential off-target effects on CNS microglia^[45,49,50,54].

Despite there being many advancements in microglia research, translating preclinical findings into clinical applications remains challenging. In glaucomatous experiments, animal models are useful for studying microglial activities and interactions within the retinal environment, but differences in microglial genetics and immune responses between species hinder clinical translation, resulting in inconsistent immunotherapy outcomes in clinical trials^[121]. Meanwhile *in vitro* cultures of human-derived microglia, from fresh tissue or induced pluripotent stem cells (iPSCs), lack

in vivo-like maturity and traits due to the simplified environment. To overcome the above, researchers are developing co-culture systems and human retinal organoids (ROs). To better integrate resident microglia into retinal layers *in vitro*, Usui-Ouchi et al. developed a new microglia-containing tissue model by co-culturing ROs and iPSCs-derived macrophage precursor cells, then the RNA-seq confirmed the enrichment of microglia markers, and microglia are similar to the development of the *in vivo* environment in morphology and behavior^[122]. This model bridges species and micro-environment gaps, enabling deeper studies of microglial functions and interactions in intraocular conditions more similar to those of glaucoma patients^[122].

Collectively, this review offers a comprehensive analysis of microglial activation and function, the current state of knowledge on the microglial interplay, highlights the gaps in our understanding, articulates a regulatory framework centered around microglia, and provides probable novel perspectives for future therapeutic targets (Fig. 3).

Ethical statements

Not applicable.

Author contributions

The authors confirm contribution to the paper as follows: study conception and design: Lin Y, Xu J; writing - original draft: Lin Y; visualization: Lin Y, Heng X, Yang Z; writing - review and editing: Lin Y, Xu J, Huang Q; supervision: Xu J; project administration: Zhang H; funding acquisition: Zhang H. All authors reviewed the results and approved the final version of the manuscript.

Data availability

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

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Conflict of interest

The authors declare that they have no conflict of interest.

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