

The role of microglia in the progression of glaucomatous neurodegeneration- a review

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Abstract

• **Glaucoma is a serious leading cause of irreversible blindness worldwide. Reducing intraocular pressure (IOP) does not always stop glaucomatous neurodegeneration and the optic nerve may continue to be damaged in the normal IOP. Microglial activity has been recognized to play essential roles in pathogenesis of the central nervous system (CNS) as well as retinal ganglion cell (RGC) survival. The relationship between the neurodegeneration and the microglia cells in glaucoma is very complicated and still remains unclear. In the present review, we summarize the recent studies of mechanisms of microglia in glaucoma neurodegeneration, which might provide new ways to treat glaucoma.**

• **KEYWORDS:** glaucoma; microglia; neuroinflammation

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INTRODUCTION

Glaucoma is the leading cause of irreversible blindness worldwide, and the vision loss often occurs gradually over a long period of time^[1]. The major pathological features of glaucoma are loss of retinal ganglion cells (RGCs) and degeneration of their axons.

The World Health Organization reported that glaucoma affects over 60 million people worldwide^[2]. And it is expected that approximately 80 million people would suffer from glaucoma by the year of 2020, leading to 11.2 million cases of bilateral blindness^[3-4].

Although many studies and investigations have shown some effect factors which related to glaucoma, the specific etiology

and pathogenesis still remains unclear. Among these factors, elevated intraocular pressure (IOP) is one of the primary and important one, while other factors have also been identified that associated with the disease, including low diastolic ocular perfusion pressure, other ocular hemodynamic parameters, and systemic diseases such as sustained high blood pressure or diabetes, ethnic group, age, myopia, migraine, as well as the nutritional state of RGCs^[5].

Besides IOP, other factors which related to immunological mechanisms also play key roles in the pathogenesis of glaucoma^[6]. Microglia are immune cells that normally residing in the central nervous system (CNS). Recent studies showed that microglia play essential roles in the interactions between the CNS and the immune system^[7]. By scavenging, phagocytosis, extracellular signaling and other functions, microglia might be an important mediator of immune response and maintain homeostasis within the CNS.

Acute glial hyperplasia promotes the survival of neurons by reconstructing the protection and recruitment of nerve tissue in the extracellular medium. However, the uncontrolled response occurs in most neurodegenerative diseases like glaucoma, would have a negative impact on the tissue^[8].

Therefore, the role of microglia in the pathogenesis of glaucoma as well as its possible mechanism(s) are discussed in this review.

INTERACTION OF MICROGLIA AND GLAUCOMA

In the eyes of patients with glaucoma, microglial cells with a variety of morphology, gathered at the lamina cribrosa and its surrounding blood vessels, suggesting that microglial cells on the blood retina barrier (BRB) protective effect^[9]. Furthermore, microglia also have an important function to continuously serve the microenvironment and respond rapidly to neuronal injury by the phagocytosis of potentially harmful neuronal debris to limit damage, the secretion of local inflammatory mediators and the signal transmission with other potential immune effector cells^[10]. However, microglial cells may have beneficial effects in the presence of acute inflammation, whereas in chronic inflammation the activation of microglia is often detrimental, leading to the pathogenesis of neurodegenerative disorders^[11].

In animal models of ocular hypertension^[12] and chronic glaucoma^[13], microglia become reactive and redistribute in the retina, optic nerve, and optic tract as early alterations,

which may contribute to the disease onset or progression. Nevertheless, it was observed that microglia proliferated near the RGCs^[14], and recruitment as well as activation of microglia occurred before RGC death^[13]. This provides direct evidence for the involvement of microglia in RGC death in glaucoma. In addition, in glaucomatous animal models, the CD200, which is closely associated with microglial activation, was early detected and increased in the retina, indicating this process accompanies ongoing axonal degeneration^[15]. Microglia reactivity in glaucoma is not limited in the retina. In glaucomatous monkeys, activated microglia in the lateral geniculate nucleus (LGN), the primary processing center for visual information received from the retina, can be observed through positron emission tomography^[16]. Other studies of neurodegeneration showed that glaucoma occurred in the LGN, perhaps with microglial-related activations^[17-18].

In addition, a tetracycline derivative, minocycline, known to inhibit the activation of microglia, also inhibited RGCs neurodegeneration in ischemia and glaucoma models^[19-21] and improved the integrity of the optic nerve, supporting further evidence of the role of microglia in neuropathy in glaucoma. In the early stage of glaucomatous model, minocycline can also inhibit the axonal transport deficit contribute to microglial reactivity^[22]. Furthermore, high doses of irradiation can reduce microglia activation and proliferation in the central retina, the optic nerve head (ONH) region in glaucoma animal models, leading to a decrease of degeneration in RGC and an improvement of the structural and functional integrity of RGC axons^[23].

In an experimental autoimmune glaucoma animal model, IgG autoantibody is accompanied with the loss of RGCs and could be a useful glaucoma biomarker, found in co-localization with activated microglia cells^[24].

Microglia and Neuroinflammation in Glaucoma As with many neurodegenerative diseases, there are similar inflammatory responses in glaucoma. Microglia activation not only occurs in the eyes with high IOP, but also occurs in the contralateral healthy eye^[25], indicating complex mechanisms are involved in the pathogenesis of glaucoma.

Microglia perceive the signal from microenvironment and protect neurons from disturbances. Accompanied with changes in signaling and gene expression, activated microglial cells with morphous altered would proliferate and migrate to the site of injury^[26]. However, in order to limit the damage, the persistent impairment may activate the neurotoxic phenotype of microglia^[27]. The prolonged and excessive activation of retinal microglia is related to the degeneration of neurons, especially the loss of RGC, which is a characteristic of glaucoma^[21,28].

The activations of microglial cells is closely regulated by several inhibitory pathways^[29-30]. Fractalkine, also known as chemokine (C-X3-C motif) ligand 1 (Cx3cl1), is a membrane-

bound chemokine primarily expressed by neurons, while microglia is the mainly expression of its anti-inflammatory receptor Cx3cr1. These molecules are important to maintain microglial function in physiological and pathological conditions. In mouse Parkinson and amyotrophic lateral sclerosis (ALS) models, the lack of Cx3cr1 leads to microglia neurotoxicity and neuronal vulnerability^[31]. Following the loss of Cx3cr1 in microglia, a selective worsening of axon transport dysfunction in RGCs can be caused in glaucoma mouse model^[32]. Similarly, Cx3cr1 deficiency evokes subretinal microglia accumulation and leads to age-related macular degeneration (AMD)^[33]. On the other hand, Cx3cr1 deficiency showed a prevention of neuron loss in Alzheimer's disease (AD), a neurodegenerative condition^[34]. Therefore, under different conditions of neuroinflammatory, inhibition of the receptor have different effects on proinflammatory role of microglia. Thus, suppression of neuroinflammatory responses could be a potential treatment for glaucoma.

Microglia and Cytokines in Glaucoma Microglia are thought to play important roles in the inflammatory response of glaucoma. Over activation of microglia would result in the production of proinflammatory cytokines and increase the oxidation and nitrification reactions, thereby endangering the retinal neurons^[35]. Indeed, stimulating by these cytokines, fibroblasts, endothelial cells and macrophages could produce chemokines, recruit neutrophils and macrophages to the retina, which leads to more severe tissue damage and chronic inflammatory response.

Tumor necrosis factor-alpha (TNF- α), which is produced by macrophage and microglia in the optic nerve and ONH^[36], is related with innate immune responsiveness^[37-39]. TNF- α was considered to be an important mediator of RGC death in glaucoma, and the up-regulation of TNF- α and its receptor were involved in the process of glaucomatous neurodegeneration^[40]. Indeed, further *in vitro* studies have shown that anti-TNF- α attenuates ischemia or ocular hypertension-induced RGC apoptosis^[41]. Moreover, while TNF- α antagonized by etanercept, inflammation and RGC loss in a glaucoma animal model was attenuated^[42].

Other investigations supported that TNF- α is beneficial and protective to neurons. TNF- α appears to protect RGCs in the early stage of optic nerve crushed in mice, perhaps with some indirect mechanisms^[43-46].

Interleukin-1 β (IL-1 β) has been considered to be an essential pro-inflammatory cytokine which produced by activated microglia in glaucoma patients, and are thought to promote the progression of glaucoma^[47-48]. IL-1 β has also been reported to increase the generation of ROS^[49] and nitric oxide synthesis (NOS)^[50] and is involved in RGC damage which leads to neurodegeneration^[51-52]. Indeed, tetrandrine can effectively suppress the activity of microglia^[53] and inhibit the production

of IL-1 β and TNF- α , suggesting that it may be effectively suppressing over activated microglia and protecting RGCs in glaucoma^[49,54].

Interleukin-6 (IL-6) is a key component of pressure-induced retinal microglia response^[55-57]. In animal models of glaucoma and aging retina, stressor-dependent of IL-6 and IL-6 receptors have been detected^[58]. Similarly, in the iris specimens from patients of neovascular glaucoma, mRNA level of IL-6 was significantly increased^[59]. Nevertheless, it has been demonstrated that IL-6 increased the survival of RGCs challenged with pressure, and the stimulus for IL-6 synthesis arose from axonal injury rather than ocular hypertension^[57].

Microglia and Adenosine Receptors Adenosine is a neuromodulator, which also exerts important functions in the immune-inflammatory system^[60]. Microglial cells express all subtypes of adenosine receptors, A1, A2A, A2B, and A3 receptors and particular attention has been paid to adenosine A2A receptors (A2AR).

A2AR is associated with neurodegeneration as the blockade of A2AR providing protection against a variety of deleterious conditions^[61-65]. It is speculated that the neuroprotective effect of A2AR antibodies is thought to control microglia-mediated neurodegeneration^[66-67].

A2AR blockade was provided to prevented retinal microglia reactivity and neuroinflammation^[67-68]. This might be concerned with the ability of A2AR controlling the formation and release of cytokines such as IL-1 β and/or TNF, as previously observed in different brain preparations^[69-73].

Microglia and Oxidative Stress Nitric oxide (NO) is known to be secreted by microglia^[74] and inflammation upregulated inducible nitric oxide synthase (iNOS) can raise the production of NO^[75]. Upregulated iNOS and increased NO levels were found in the ONH of glaucomatous patients^[76] and in the retina and ONH of glaucoma animal models^[77-79]. Inhibition of iNOS with aminoguanidine confers neuroprotection to RGCs in an animal model of glaucoma^[80], supporting the existence of a role of NO in the pathophysiology of glaucoma.

Nicotinamide-adenine dinucleotide phosphate (NADPH) oxidase, which is closely related to microglial cells, is capable of generating reactive oxygen species ROS that also associated with microglia-mediated neurotoxicity in photoreceptor cells^[81].

Microglia and Complement The complement system is part of innate immune defense, consists of a number of small proteins that can eliminate alien cells and debris^[82]. Complement proteins are expressed in the normal physiological processes of the retina^[83], and complement activation would increase in pathological conditions like inflammation^[84] and ageing^[85].

The classical pathway is triggered by activation of the C1-complex and the upregulation of complement protein C1q was detected in some neurodegenerative diseases including glaucoma^[86-87]. Most of the secreted C1q is released by microglia,

which express C1q mRNA strongly. The complement cascade is thought to present a target for subsequent elimination by microglial engulfment^[87]. Besides, dendritic and synaptic architecture can be protected in genetic knockout of C1qa [D2.C1qa (-/-) mouse] or pharmacological inhibition of C1^[88]. In complement depleted rats model with increased IOP, the apoptosis of RGCs in retina was decreased and the activation of both caspase-8 and caspase-9 was inhibited^[89]. These findings suggested that complement mediated apoptosis plays a pivotal role in glaucomatous neurodegeneration.

Microglia and Fas Ligand Fas ligand (FasL) is associated with activation of microglia-induced RGCs. FasL could be divided into two types, the truncated soluble product (sFasL) and membrane-bound FasL (mFasL). Considerable data proved that in animal models the mFasL is proinflammatory and proapoptotic, while sFasL is anti-inflammatory and non-apoptotic^[90-91]. In the animal model of chronic glaucoma, ectogenic sFasL provided complete and sustained neuroprotection, reduced production of TNF- α , and decreased apoptosis of RGCs and loss of axons^[92]. The opposing activities of mFasL and sFasL further suggest that FasL cleavage, mediated primarily by matrix metalloproteinases (MMPs) and their inhibitors (TIMPs), is a major mechanism for limiting the neurotoxic activity of FasL in the eye^[93]. In addition, MMPs and TIMPs were expressed by retinal microglia, RGCs, and their axons^[94], indicated that microglia participates in the division of FasL.

M1/M2 Polarization of Microglia Similar to macrophages, microglia can be categorized into at least two phenotypes: M1 and M2. The activation of different phenotypes depending on the disease stages and severity can produce either cytotoxic or neuroprotective effects^[95]. Microglia conduct similar functions in CNS compared with peripheral macrophages. Therefore, different phenotypes of microglia are considered to participate in the degeneration of the CNS. In AD models, the conversion of microglia from M1 to M2 lead to decreased toxicity, while the ability to swallow β -amyloid increased^[96]. Furthermore, M1 activation may have association with dopaminergic cell death in Parkinson's disease^[97]. Aiming at the transformation of different subtypes of microglia could also provide new therapeutic targets. Further investigations should be made in the roles played by M1/M2 microglia in glaucoma.

However, recently some opinions claimed that the evidence of microglial M1/M2 polarization is inadequate^[98]. There are other transcriptional profiles failed to fit with these two phenotypes^[99]. New markers to distinguish microglia from macrophages will be a significant task in future^[100].

Microglia and MicroRNAs MicroRNAs (miRNAs), as important epigenetic regulators, are small noncoding single-stranded RNA molecules regulating gene expression post transcriptionally^[101]. In an animal model of acute ocular

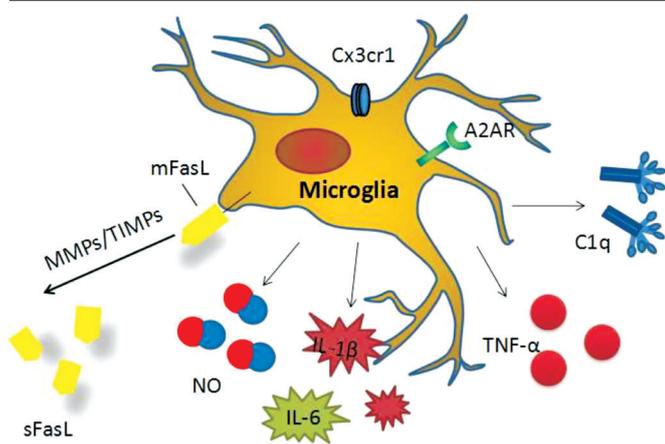


Figure 1 Factors and cytokines related to microglia in glaucoma.

hypertension (AOH), the loss of RGCs was associated with an activation of retinal microglial cells and thirty-one miRNAs significantly changed. For instance, miR-350/MAPK14, miR-539/MAP3K8 and miR-93/MAPK9 altered in AOH eyes could regulate the mitogen-activated protein kinases (MAPKs) signaling pathways, which could lead to inflammation and RGCs death^[102].

CONCLUSION

Microglia, involved in inflammatory factors, cytokine activation, complement cascade as well as FasL cleavage, are closely related to glaucoma neurodegeneration (Figure 1). In the early stages of the disease, microglia-mediated inflammatory response may have protective effects on glaucoma in patients with RCG injury. While in the process of chronic disease, inhibition of microglia activity and its metabolites, can reduce glaucoma progress. Although its subtype classification is still controversial, microglial cells could be potential targets in treating glaucoma, especially normal-tension glaucoma.

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