



# Immunomodulation as a Neuroprotective Strategy for Glaucoma Treatment

Mine Barış<sup>1</sup> · Gülgün Tezel<sup>1</sup>

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## Abstract

**Purpose of Review** This review aims to highlight the current knowledge about inflammatory mechanisms of neurodegeneration in glaucoma with emphasis on potential immunomodulation strategies.

**Recent Findings** Glaucomatous retina and optic nerve present multiple evidences of inflammatory responses of astroglia, microglia, and blood-borne immune cells. Although adaptive/protective responses of resident or systemic immune cells can support neurons and promote tissue repair mechanisms after injurious insults, prolonged inflammatory processes can also produce neurotoxic mediators. Treatments targeting these neurodestructive outcomes may restore immune homeostasis and protect neurons from inflammatory injury. Due to widespread and chronic nature of neuroinflammation in glaucoma, immunomodulation offers a treatment strategy to protect different neuronal compartments of RGCs during the chronic and asynchronous course of neurodegeneration. Uncovering of distinct molecular responses and interactions of different immune cells that determine the neuroinflammatory phenotype and participate in neurodegenerative outcomes will be critical to develop effective strategies for immunomodulation in glaucoma.

**Summary** Neuroinflammation has increasingly been recognized to play an important role in glaucomatous neurodegeneration, and its modulation appears to be a promising treatment strategy for neuroprotection.

**Keywords** Glaucoma · Immunoregulation · Immunomodulation · Neuroinflammation · Neurodegeneration · Neuroprotection

## Introduction

Glaucoma, a leading cause of blindness in the world, is a chronic neurodegenerative disease characterized by degeneration of optic nerve axons and loss of retinal ganglion cell (RGC) somas and synapses. Although increased intraocular pressure and advanced age are the main risk factors for initiation and progression of neurodegeneration in glaucoma, genetic and epigenetic predispositions also take part. Advancement of neuron loss despite efficient control of

increased intraocular pressure in many patients suggests that mechanisms other than intraocular pressure-related biomechanical and/or ischemic injury may enhance the neurodegenerative process. Among a number of mechanisms that have been linked to the pathophysiology of neurodegeneration in glaucoma, emerging evidence supports an important role of inflammatory processes [1–3].

As reviewed herein, multiple studies focusing on the immunogenic aspects of glaucoma have revealed a prominent activation of resident and systemic immune responses at early stages of the disease, and inhibition of some of these responses has provided protection against neuron injury. As summarized later below, findings of these studies have also provided a list of immune mediators/regulators that may serve as molecular targets for new treatments to restore immune homeostasis. Evidently, neurodestructive consequences of inflammatory responses may lead to injury of optic nerve axons, including proximal and distal axon degeneration, and loss of RGC somas, dendritic tree, and synapses. Immunomodulatory treatments may therefore provide widespread protection against inflammatory injury at different neuronal compartments.

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✉ Gülgün Tezel  
gt2320@cumc.columbia.edu

Mine Barış  
mb4537@cumc.columbia.edu

<sup>1</sup> Vagelos College of Physicians and Surgeons, Department of Ophthalmology, Columbia University, New York, NY, USA

This review will provide an overview of the present evidence for immune system involvement in glaucomatous neurodegeneration and the potential of immunomodulation as a therapeutic approach for neuroprotection. Recent trends and advances in the field will be briefly highlighted, rather than including a comprehensive literature review.

## Cell Types Involved in Neuroinflammation in Glaucoma

Similar to many other neurodegenerative diseases, chronic glial activation is recognized as a sufficient hallmark of neuroinflammation in glaucoma. This is mainly due to the immune privileged status of brain tissues, in which inflammatory responses are dampened to an intermediate state called parainflammation [4]. This intermediate state characterized by sustained low-grade inflammation relying on reactive glia may represent an adaptive response to primary stress stimuli and/or neuron injury in glaucoma as the body's natural attempt to minimize injury and help tissue healing. However, prolonged inflammatory activation of the glia, along with a failure in the regulation of immune response pathways, may shift the initial beneficial state towards a neurodegenerative process and promote secondary injury to neurons. It seems quite possible that the danger signals arising from stressed and injured tissues over a chronic period (along with the aging-related, genetic, epigenetic, and/or other systemic vulnerabilities to develop autoimmunity) may compromise the immune homeostasis in glaucoma [1–3]. Thus, chronic tissue stress, neuron injury, glial and systemic immune responses, and sustained release of neurotoxic mediators may create a vicious cycle that may promote progressing neuron injury.

Both astroglia (retina and optic nerve astrocytes, and retinal Müller cells) and microglia resident in the retina and optic nerve (head) profoundly respond in glaucoma. Owing to the intimate relationship of these neurosupport cells with RGCs at different neuronal compartments, glial responses are evident through the entire projection of RGCs from the retina to upper brain centers. However, despite their widespread nature, inflammatory responses in glaucoma may vary between different compartments that present differences in populations of glial subtypes that exhibit morphological, molecular, and functional characteristics [5, 6].

Astroglia normally preserve tissue homeostasis, maintain synapses, recycle neurotransmitters, deliver neurotrophic factors and metabolites, control blood flow and vascular barriers, and participate neurogenesis. These neurosupport cells quickly recognize and respond to stress and injury signals as a neuronal defense and recovery mechanism. Astroglial cells become highly reactive both in the retina [7] and optic nerve head [8] in glaucomatous human donor eyes and animal

models with glaucoma [9, 10]. Even a short period of time with ocular hypertension may be sufficient to initiate astrocyte reactivity in experimental glaucoma [10]. The early activation response of astroglia to glaucoma-related insults is portrayed by morphological alterations [8, 9, 11–13], and molecular responses [5, 14–16] that may be detectable even before the damage to RGCs and axons.

Reactive astrogliosis may insulate the injured tissue to protect uninjured neurons from damaging effects. However, as evident in the glaucomatous optic nerve head, astroglial responses leading to tissue remodeling and glial scarring [10] may also create biomechanical stress on optic nerve axons. In addition, reactive astrocytes execute a self-survival program that may result in inadequate mechanic, trophic, and bioenergetic support to RGCs [9]. Mac-2-expressing phagocytic astrocytes in the myelination transition zone which have a tissue-cleaning function also show reduced capacity in glaucoma [17]. Moreover, astrocytes after gaining the inflammatory phenotype that is characterized by increased production of pro-inflammatory and neurotoxic molecules may generate neurodestructive signals and induce the death of neurons and oligodendrocytes [18, 19••]. Besides driving innate inflammatory activity that can promote neuron injury, reactive glia, including astrocytes, may also function as antigen-presenting cells to prime T cells for activation [20, 21]. Even more, glaucoma-related alterations may compromise astrocyte functions in controlling the blood-brain barrier [22] and may thereby play an additional role in increased access to systemic immune cells into the retina and optic nerve.

Similar to astroglia, microglial cells demonstrate increased reactivity in the retina and optic nerve head in experimental models [5, 16, 23–25] and human donor eyes with glaucoma [7, 26, 27]. Microglia normally provide neurotrophic support, and their scavenger and phagocytosing functions promote tissue cleaning and healing; however, inflammatory responses of microglial cells may also contribute to neuron injury [28–30].

As much as the individual contribution of astroglial and microglial cells in neuroinflammation, their interplay is also critical for the inflammatory outcomes. There is an intricate inter-relationship between astroglia and microglia in the induction of the inflammatory and neurotoxic phenotype. For example, activated microglia induce neurotoxic A1 astrocytes by secreting pro-inflammatory cytokines [19••], while astroglial cytokines and chemokines chemoattract and activate microglia.

Based on studies of experimental models, widespread responses of glia may even include the contralateral eyes with normal intraocular pressure [31, 32], and bilateral spread of inflammatory responses and activation of astrocytes and microglia in both ocular hypertensive and normotensive fellow eyes may be detectable through the RGC projection sites, including the superior colliculus [33•].

Another group of resident immune regulatory cells includes dendritic cells [34–36]. These cells are known to be early responders of neuron injury; however, their roles in glaucomatous neurodegeneration are not well-studied.

Myelin-producing oligodendrocytes constitute another important cell type that joins astrocytes and microglia behind the optic nerve head. Immune regulatory functions of oligodendrocytes are not fully understood; however, they can provide a physical barrier for immune cell infiltration into the optic nerve, and their loss may result in the breakdown of this barrier [37, 38].

In addition to resident glia, blood-borne immune cells, such as monocytes, that can invade the optic nerve tissue, may also be related to neuroinflammation in glaucoma. For example, in hereditary DBA-2J glaucoma with a strong inflammatory component, transendothelial monocyte migration has been linked to the inflammatory component of neurodegeneration [29].

Thus, the complex process of neuroinflammation involves several cell types, and its outcomes may affect neuron survival at different compartments of RGCs, including dendritic tree, soma, axon, and synapses. Ongoing studies are expected to improve the molecular and mechanistic understanding of inflammatory responses and interactions of different cell types at different sites and different stages of neurodegeneration to enable immunomodulation as a neuroprotection strategy in glaucoma. The next section summarizes the current knowledge on molecular regulation of neuroinflammation in glaucoma, which founds the basis for further work and provides new directions for progress.

## Molecular Pathways of Neuroinflammation

Multiple studies using transcriptional [14–16, 39–41] or proteomics profiling [42–45] have identified early upregulation of numerous molecules linked to inflammation pathways in the retina and optic nerve head of human donor eyes or animal models with glaucoma. Identified molecules have included a number of sensors/inducers, transducers, and effectors of neuroinflammatory processes leading to the production of neurotoxic mediators.

Glial cells can recognize stress or damage-associated molecular patterns (DAMPs) released from stressed or dying cells [46]. Among various DAMPS, heat shock proteins (HSPs) are a major group of stress proteins upregulated in the glaucomatous retina and optic nerve head [47], and these highly antigenic molecules have been associated with the immune responses in glaucoma [48, 49]. An important class of pattern recognition receptors (PRRs) that can sense DAMPs is toll-like receptors (TLRs) [50]. Different TLRs display increased expression on astroglia and microglia in human glaucoma [43], experimental animals with induced ocular hypertension [45], and DBA-2J mice with hereditary glaucoma [16]. Based on *in vitro*

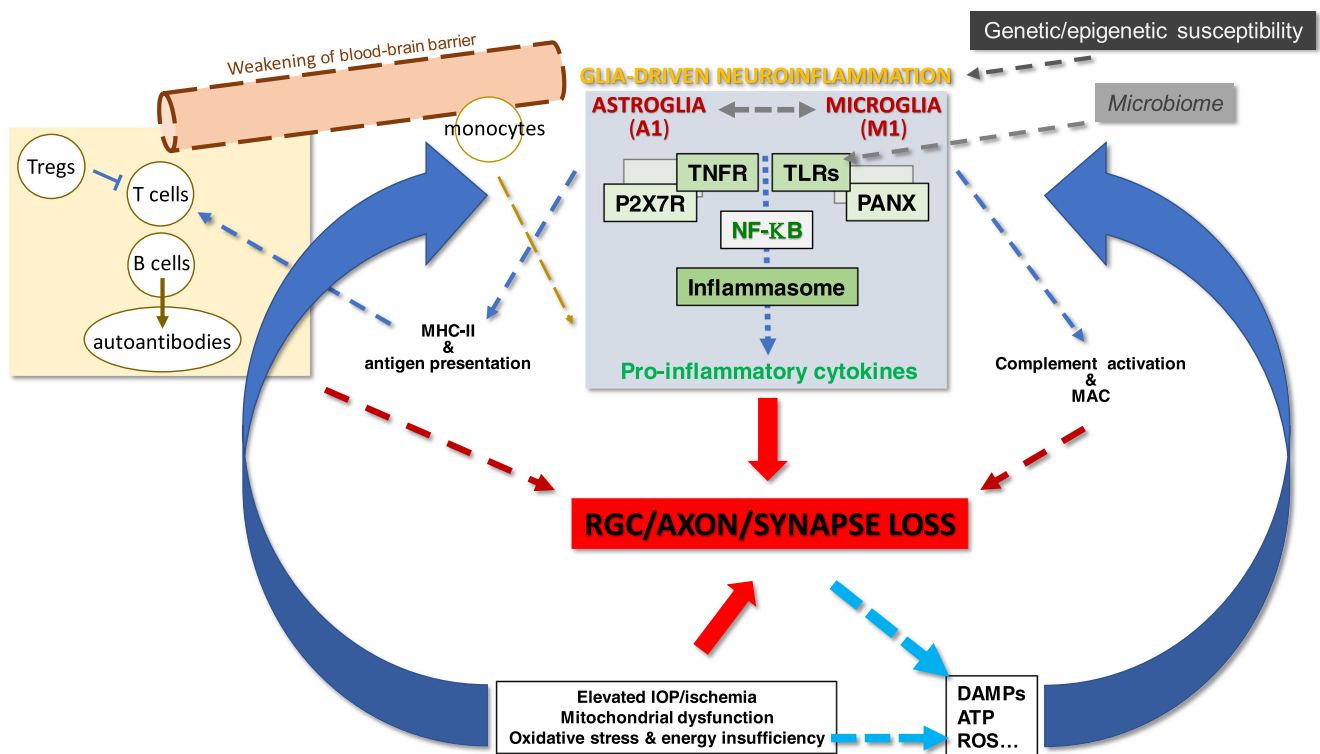
experiments, components of the glaucomatous tissue stress, including upregulated HSPs and oxidative stress end-products, may function as intrinsic ligands for glial TLRs. Recognition of these endogenous stress-associated molecular patterns by TLRs initiates MyD88-dependent signaling that leads to nuclear factor-kappa B (NF- $\kappa$ B) activation, cytokine production, and stimulation of co-cultured T cells [43]. Tenascin C, another intrinsic ligand for TLRs, is also upregulated in the glaucomatous optic nerve head [51]. In addition, TLRs can recognize mitochondrial DAMPS, including the mitochondrial DNA released from cell debris [52]. The TLR signaling initiated by endogenous danger signals has been linked to innate and adaptive immune responses in various diseases with the autoimmune origin [53].

As a matter of fact, recent observations suggest that not only intrinsic stimuli but also conventional ligands for TLRs, namely pathogen-associated molecular patterns, may signal for TLR activation in glaucoma. This is because, increased loads in the microbiome have been linked to immune responses and neurodegeneration in human glaucoma and animal models [54, 55••]. As microbial and human proteins share significant homologies, the cross-reactivity between microbial and human antigens may also influence immune responses through TLR signaling in glaucoma [56, 57], similar to many autoimmune diseases.

Nucleotide-binding oligomerization domain-like (NOD-like) receptors, another class of PRRs, are components of the multiprotein complex, named inflammasome. Inflammasome formation, an early innate response to cell stress, activates an inflammatory cascade that promotes proteolytic activation and secretion of cytokines. Glial NOD-like receptors and inflammasome components are also among the molecules upregulated in the retina of human donors [44] and experimental animals with glaucoma [45].

While glial cells are equipped to sense stressed or injured neurons to initiate inflammatory signaling through DAMPs/PRRs, these cells may also directly respond to increased intraocular pressure and mechanical strain by sensing through mechanosensitive ion channels, such as purinergic receptors (P2X7R), transient receptor potential vanilloid (TRPV), and pannexin channels (PANX) [58, 59, 60••]. However, inflammation-promoting mechanosensitive receptor signaling may also be activated by the ATP released from damaged RGCs. A principal role of nerve injury in the stimulation of glial responses is supported by the attenuation of retinal glial responses after optic nerve crush when RGC death was blocked by deletion of the *Bax* gene [61••].

Thus, as schematized in Fig. 1, glial inflammatory responses may be triggered by neuron stress/injury (by DAMPs through PRRs, by ATP through mechanosensitive ion channels), as well as directly by glaucoma-related insults, such as increased intraocular pressure (through mechanosensitive ion channels).



**Fig. 1** Glia-driven neuroinflammation may be initiated by both neuron injury (by DAMPs and ROS through PRRs, by ATP through mechanosensitive ion channels) and glaucoma-related insults, such as elevated intraocular pressure (through mechanosensitive ion channels).

Thus, chronic tissue stress, neuron injury, glial and systemic immune responses, and sustained release of neurotoxic mediators create a vicious cycle that promotes further injury to retinal ganglion cells at different neuronal compartments

Additionally, it is important to note that the neuron damage in glaucoma is asynchronous over a prolonged period, and the chronic disease process may also generate recurring waves of inflammatory stimulation.

After sensing the tissue stress and/or neuron injury, cooperative actions of different pathways initiate cascades of events that involve NF-κB, the key transcriptional activator of inflammatory mediators. This master regulator of the transcriptional program for inflammation [62] is activated in human glaucoma and animal models [44, 45]. Various inflammation pathways that are activated in the glaucomatous glia, including TNFR and TLR signaling and inflammasome formation [43–45], are commonly linked to NF-κB.

In addition to stress/damage sensors (such as PRRs, TLRs), and inflammatory transducers (such as NF-κB, MyD88), up-regulated molecules in glaucoma also include effectors and amplifiers of inflammation with neurotoxic outcomes, such as pro-inflammatory cytokines and chemokines. In glaucoma, reactive glia usually gain the pro-inflammatory phenotype characterized by increased production of pro-inflammatory cytokines, such as TNF-α, IL-1, IL-12 [16, 44], as opposed to anti-inflammatory cytokines. The glial pro-inflammatory cytokine imbalance in glaucomatous tissues is most prominent by increased production of TNF-α [63, 64], which is also linked to RGC death [18, 21, 63–65]. Evidently, TNFα/TNFR1

signaling can induce RGC death through proteolytic caspase cascade, mitochondrial dysfunction, oxidative stress [18, 44, 66], and calcium-permeable AMPA receptor activation [67]. Besides direct neurotoxicity of TNF-α produced by reactive glia [18, 68], downstream NF-κB activation leads to transcriptional activation of more cytokines and other immune mediators, further activate glial responses, and thereby amplify the vicious cycle of inflammation. Additionally, TNF-α may weaken the blood-brain barrier and directly facilitate glia-T cell interactions to stimulate differentiation of T cells into effector cells of systemic immune responses [21].

Parallel to increased expression of pro-inflammatory cytokines in the glaucomatous retina and optic nerve, analysis of glaucomatous aqueous humor and blood samples has also detected increased levels of these cytokines [69–72]. Furthermore, proteins encoded by the current collection of glaucoma-related genes include those linked to cytokine signaling [73]. It is evident that not only genetic predispositions but epigenetic susceptibility factors may also affect the glia-driven neuroinflammation in glaucoma. For example, individual variations detected in the retinal expression pattern of TNF-α-induced protein-3 (TNFAIP3, a regulator of the inflammatory outcomes of TNFα/TNFR1 signaling) among glaucomatous human donors have been found correlated with increased methylation of the TNFAIP3 promoter [44].

Another important immunostimulatory stimulus in glaucoma includes oxidative stress. As well as the age dependence of glaucoma and key roles of oxidative stress in aging-related deterioration, the glaucoma-related amplification of oxidative stress (through mitochondrial dysfunction or other pathogenic processes) has been implicated in neurodegeneration [74]. Apparently, many consequences of oxidative stress may also be immunostimulatory in glaucoma [1, 75]. For example, oxidative stress stimulates glial cytokine production [21], activates glial TLR signaling [43], and induces complement dysregulation [42]. In addition to the co-stimulatory role of reactive oxygen species in antigen presentation of glial cells to T lymphocytes [21], protein oxidation [76] may modify the antigenic features of retina and optic nerve proteins [77]. Moreover, advanced glycation end-products (AGEs) that accumulate through oxidative stress-dependent processes in glaucomatous tissues [78] may act as a persistent antigenic stimulus and activate immunostimulatory signaling through specific receptors (such as RAGE, another sensor receptor of tissue stress). It is also noteworthy that the NF- $\kappa$ B-regulated transcriptional program for inflammation is redox-sensitive. In support of these immunostimulatory roles of oxidative stress in glaucoma, antioxidant treatment has been found immunomodulatory. Parallel to neuroprotective outcomes, antioxidant treatment has decreased the production of pro-inflammatory cytokines and activation of NF- $\kappa$ B in experimental glaucoma [79•].

Complement activation has also been associated with glaucomatous neurodegeneration. Various components of the complement activation cascade, including the cytolytic membrane attack complex, have been detected in glaucomatous eyes [16, 42, 80–82]. This critical mechanism for immune surveillance and clearance has been linked to synapse elimination and dendrite remodeling in glaucoma [82]. In addition, transgenic or pharmacologic treatments inhibiting complement components have provided protection against RGC loss in animal models [83, 84, 85••]. Although many evidences support neurodestructive consequences of uncontrolled complement activation in mouse glaucoma, inflammatory outcomes through complement anaphylatoxins, C3a, C5a, remain poorly defined.

## Systemic Immune Responses

Besides innate immune responses, glial cells (together with other resident immune cells) also have the ability to stimulate systemic immune responses. The neuroinflammatory environment enriched with pro-inflammatory cytokines appears to favor the interaction of glia with T lymphocytes to elicit expanded immune responses. Additionally, increased expression of highly antigenic and immunostimulatory stress proteins [47, 48], increased antigenicity due to protein modifications [76, 77], and increased exposure of antigens due to neuron

injury may help create a proper setting for autoimmunity. Growing data from gene or protein expression studies of glaucoma also support a marked upregulation of various chemotaxis and adhesion molecules needed for the glia-T cell interaction [14–16, 39–41, 44, 45]. Furthermore, major histocompatibility complex II molecules are increased on reactive glia in human glaucoma [20] and animal models [23, 86], and stress-associated co-stimulatory molecules in glaucomatous tissues, including reactive oxygen species [21], may enhance the ability of glial cells for antigen presentation to T lymphocytes. As supported by animal models, activated T cells may be recruited to the eye, infiltrate the site of injury [55••, 87, 88], and produce inflammatory outcomes that are neurotoxic to RGCs [49, 55••, 88, 89].

Indeed, studies of experimental models have provided data supportive of stimulated T cell responses with neurodegenerative potential [49, 55••, 88, 89]. Likewise, studies of blood samples from patient groups with glaucoma have detected a shift in T cell subset distribution towards the pro-inflammatory Th1 cytokine phenotype [90, 91•]. Recent studies have also pointed to an imbalance of the regulatory subset of T cells (Treg), indicating the lack of efficient T cell suppression in some patients with glaucoma (presented at the Annual Meeting of the Association for Research in Vision and Ophthalmology, 2018, Abstract # 3733). These observations are consistent with increased titers of pro-inflammatory cytokines in the glaucomatous human blood [71].

Numerous studies have also documented a complex repertoire of circulating autoantibodies that react to a variety of retina and optic nerve proteins, and the accompanying deposition of immunoglobulins in glaucomatous retinas [89, 92]. Whether these antibody responses are an outcome or a pathogenic mechanism of neurodegeneration is the subject of ongoing debate. Based on experimental studies, HSP antibodies, when exogenously applied at concentrations similar to that detected in the glaucomatous blood, may be internalized by retinal neurons and facilitate their death in the human retina, *ex vivo* [93]. Although antibody-mediated processes may not produce inflammatory outcomes, which is the focus of this review, autoantibody production and ocular entrance may be the consequence of glia-driven inflammatory responses that prepare the conditions for antigen presentation (leads to autoantibody production) and blood-brain barrier permeability (facilitates the access of autoantibodies).

While reactive T lymphocytes may be able to invade through the intact blood-brain barrier [55••, 94], glaucoma-related alterations in astrocyte functions may also weaken the perivascular barriers [22]. It seems quite possible that a breach in the **blood-retina barrier** as evident in small optic disc hemorrhages [95] or parapapillary chorioretinal atrophy areas [96], both of which are commonly detectable in glaucomatous eyes in association with disease progression, may also allow increased access of circulating **immune**

cells, or non-cellular components (like autoantibodies) into the retina and optic nerve. Interestingly, activated microglia have been found strategically positioned within the regions of parapapillary chorioretinal atrophy in glaucomatous eyes [26, 27]. A longitudinal study of serum autoantibody reactivity has also shown an increase over time in glaucoma patients with optic disc hemorrhages, but no change has been detected in patients without optic disc hemorrhages [97•]. Consistent with these observations, transfer of the mononuclear cells obtained from glaucoma patients with optic disc hemorrhages into immune-deficient mice has resulted in a marked decrease in RGC density (presented at the Annual Meeting of the Association for Research in Vision and Ophthalmology, 2018, Abstract # 3731).

## Potential Treatments for Immunomodulation

With respect to the neurodegenerative potential of neuroinflammation, its efficient control can protect neurons from inflammatory neurotoxicity and enhance endogenous recovery processes. Due to the widespread nature of neuroinflammation through different neuronal compartments, immunomodulation may offer a treatment strategy to protect RGC somas, axons, and synapses during the course of neurodegeneration in glaucoma. As being major players of neuroinflammation, glial cells appear to be excellent candidates for targeting to restore immune homeostasis and improve the outcome of glaucoma. Chronic nature of the astroglial response, as opposed to temporary responses of microglia, during glaucomatous neurodegeneration makes them particularly important as a treatment target. As also noted above, ongoing tissue stress and asynchronicity of neuron injury over the chronic disease period may re-amplify glial inflammatory responses in glaucomatous eyes. Therefore, lowering of elevated intraocular pressure, or other neuroprotective treatments, may also have anti-inflammatory outcomes.

Recent experimental studies of therapeutic approaches have targeted different molecules to modulate neuroinflammation in glaucoma. Genetic or pharmaceutical inhibition of TNF- $\alpha$ , a major pro-inflammatory and pro-apoptotic cytokine, has provided protection against RGC and axon degeneration in experimental models of glaucoma [68, 98]. However, it should be clarified that TNF- $\alpha$  signaling may lead to protective or destructive outcomes depending on the type of receptor preferentially used, and the cross-talk between multiple signaling pathways. In addition, soluble TNF- $\alpha$  more than its membrane-bound form is required to generate neuroinflammation, which is the principal ligand for TNFR1, the primary receptor for the majority of inflammatory responses [65]. Different effects of TNF- $\alpha$  on neuron survival or glia-driven inflammatory outcomes may also be time-dependent [99, 100], as well as depending on interactions between different molecular pathways [44].

As a treatment approach towards sensing and inducing of inflammation, selective inhibition of TLR4 signaling with TAK-242 (resatorvid) has also reduced astrocyte activation and RGC death after optic nerve crush injury in mice [101•].

Regarding the transduction of inflammation signaling, inactivation of astroglial NF- $\kappa$ B, the key transcriptional activator of inflammatory mediators downstream of TNF- $\alpha$ /TNFR and TLR signaling pathways, has reduced pro-inflammatory genes and promoted RGC survival after retinal ischemia [102]. Recent experimental findings from the cre/lox-based deletion of I $\kappa$ K $\beta$  (the main activating kinase for NF- $\kappa$ B in the canonical pathway with inflammatory outcomes) in experimental mouse glaucoma have also supported immunomodulatory and neuroprotective outcomes of astroglial NF- $\kappa$ B inhibition in the glaucomatous retina and optic nerve head (presented at the Annual Meeting of the Association for Research in Vision and Ophthalmology, 2018, Abstract # 6140). Although NF- $\kappa$ B appears to be a promising target for immunomodulation, it should be emphasized that besides inflammatory mediators, NF- $\kappa$ B also activates critical anti-apoptotic genes and regulates a broad range of processes essential for neuron survival [62, 103]. These opposing aspects of NF- $\kappa$ B point to the need for glia-targeting transgenic strategies because the lack of cell specificity of NF- $\kappa$ B-inhibiting pharmacological treatments would potentially cause adverse effects on neuron survival. This is also supported by NF- $\kappa$ B1 knockouts that develop optic neuropathy and progressive RGC loss [104].

Consistent with various roles of oxidative stress in induction of glia-driven inflammatory responses in glaucoma [74], antioxidant treatment has lowered pro-inflammatory cytokine production and NF- $\kappa$ B activation in the ocular hypertensive rat retina and optic nerve [79•].

Various studies have also targeted different cellular components of neuroinflammation in experimental models. Decreasing the astroglial and microglial reactivity with intraocular administration of a cAMP phosphodiesterase inhibitor (ibudilast) has resulted in decreased production of pro-inflammatory mediators and increased survival of neurons in ocular hypertensive rat eyes [105•]. Other studies testing different treatments to deactivate retina or optic nerve head microglia at early stages of experimental glaucoma have also reported to reduce neurodegeneration [28, 106, 107]. Additionally, inhibition of monocyte entry into the optic nerve head by irradiation has provided protection against neuron injury in DBA/2J mice [29]. Treatments targeting endothelin-2, a damaging mediator produced by monocytes (bosentan) combined with anti-complement treatment (C1qa mutation) have also been found protective [84]. Furthermore, findings of a more recent study have indicated that the extensive neuroinflammation in DBA/2J glaucoma, but not in induced microbead occlusion model of glaucoma that lacks pre-existing inflammation, may contribute to Ranvier node pathology and neurophysiological deficits, and immunosuppressive treatment with fingolimod can provide some protection [108••].

Treatments towards systemic immune responses may also be considered to restore immune homeostasis in glaucoma. The neurodegenerative potential of reactive T cells, along with the altered T cell profile with inefficient Treg suppression in patients' blood, may promise for T cell-based immunotherapies. Antigenic vaccination has been suggested to boost protective T cell responses for neuroprotection in glaucoma models but not been pursued further [109]. Similar to many autoimmune neurodegenerative diseases, boosting of the endogenous Treg response, or adoptive cell therapy by transferring purified induced Treg [110•], may be further tested as an immunomodulation strategy for glaucoma.

Moreover, stem cell-based strategies may be useful to modulate inflammatory responses. For example, mesenchymal stem cell-derived exosomes have been reported to deliver trophic and immunomodulatory factors, suppress the migration of inflammatory cells, attenuate pro-inflammatory cytokine secretion, and promote RGC survival [111, 112•].

Despite favorable results of recent experimental studies, development of immunomodulatory treatments is highly challenging when respecting the dynamic nature of inflammatory responses and the complexity of cellular and molecular components of neuroinflammation. Since beneficial and detrimental outcomes of inflammatory processes involve shared molecules (as noted above for TNF- $\alpha$  signaling or NF- $\kappa$ B activation), protective versus destructive pathways of glial responses should be carefully outweighed. Various potential treatments may also require the development of glia-targeting strategies. Further elucidation of the molecular components and time course of specific responses of glial subtypes and interactions of different immune cells in governing the final outcome will be crucial to develop effective treatment modalities.

## Conclusion

Accumulating evidence from studies of human donor eyes and animal models supports prolonged roles of resident and systemic immune responses in glaucomatous neurodegeneration and points to immunomodulation as a neuroprotective treatment strategy for glaucoma. Since neurodestructive outcomes of glia-driven neuroinflammation may affect RGC survival at different neuronal compartments, glia-targeting strategies are likely to provide widespread protection against inflammatory injury to RGC somas, dendrites, axons, and synapses during the chronic and asynchronous course of glaucomatous neurodegeneration. Ongoing efforts are expected to further enhance the molecular understanding of specific responses and interactions of different immune cells at different sites and different stages of neurodegeneration so that improved strategies can be developed for immunomodulation in glaucoma.

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## Compliance with Ethical Standards

**Conflict of Interest** Gülğün Tezel reports grants from National Eye Institute, during the conduct of her studies. Mine Barış declares no conflict of interest.

**Human and Animal Rights and Informed Consent** This article does not contain any studies with human or animal subjects performed by any of the authors.

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