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Manipulating Glia to Protect Retinal Ganglion Cells in Glaucoma

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1. Introduction

Increasing evidence supports both direct and indirect roles for retinal glia in the pathogenesis of glaucoma. To complicate these roles is the realization that glial activity can be both beneficial and detrimental to the survival of retinal ganglion cells (RGCs) and their axons. The contribution of glia to glaucoma pathogenesis also varies by compartment; glia in retina react differently to disease-induced stressors than glia in the optic nerve head or in the optic nerve. We will describe the evidence to date for the various roles of glia in each of these compartments. From this foundation, we have explored two hypotheses: whether manipulating gliosis can protect RGCs or their axons; and whether manipulating the anti-oxidant supportive role of retinal glia could prevent RGC degeneration and preserve vision. Encouragingly, we have observed that retinal gliosis can be altered to positive effect for RGCs. Improving glial support of RGCs has also increased RGC and optic nerve axon survival.

Glia greatly outnumber neurons in the CNS, but due to their reputation as secondary support cells, their study has lagged that of neurons. Within the retina, there are three types of glia: Astrocytes and Müller glia (the macroglia), and microglia. The Müller glia form the structural scaffolding of the retina, with endfeet that comprise both the inner and outer limiting membranes. The astrocytes reside among the retinal ganglion cells and their axons, while the microglia exist in non-overlapping tiled arrangements throughout the neuronal and synaptic layers of the inner retina (Bosco et al., 2011). Astrocytes and Müller glia provide homeostatic support to retinal neurons, including neurotransmitter and ion buffering, and anti-oxidant, nutrient and growth factor provision. Microglia, the resident immune cells, survey the retinal environment and respond to changes or threats. Müller glia, a macroglia subtype specific to the retina, serve all of the functions of parenchymal astrocytes, but with additional unique qualities such as transdifferentiation after specific kinds of injury (Bringmann & Reichenbach, 2001).

Glia have garnered attention in the visual system through their emergence as fascinating arbiters of health and disease. Glia respond quickly to even the slightest homeostatic alterations, including pressure, electrical activity, infection, degeneration, and pH changes. Astrocytes and Müller glia undergo gliosis in response to many of these stimuli, a cellular hypertrophy that includes, but is not limited to, upregulating the intermediate filament proteins glial fibrillary acidic protein (GFAP) and vimentin. GFAP expression is always apparent in astrocytes, but Müller glia only express this intermediate filament in times of

stress (Kim et al., 1998). In some contexts, gliosis is accompanied by proliferation, but not in glaucoma. Of the glia subject to review here, only microglia proliferate in the DBA/2J murine model of glaucoma (Inman & Horner, 2007). Gliosis and the accompanying hypertrophy rearranges glial processes which can change glial connectivity and position. Microglia responding to environmental change often increase their secretion of matrix metalloproteinases (MMPs) and become motile, retracting processes and upregulating their expression of Iba1, a Ca²⁺-binding protein (Ito et al., 1998; Bosco et al., 2008). Gliosis and microglial response are the earliest signs of pathology in glaucoma models that include intraocular pressure (IOP) increase (Inman & Horner, 2007; Bosco et al., 2011). Increased IOP, like age, is a major risk-factor in developing glaucoma (Flanagan, 1998). Glia possess mechanoreceptors that could transduce the pressure signal for the retina (Gottlieb et al., 2004). Some mechanoreceptors flux ions and likely initiate signal transduction that can lead to changes in glial production of intermediate filaments (GFAP, vimentin) and proteoglycans of extracellular matrix.

Both astrocytes and Müller glia manage glucose metabolism, maintain the blood-retinal-barrier, control ion and water homeostasis (Bringmann et al., 2006), and contribute to signal processing by recycling neurotransmitters and modulating neuron excitability (Stevens et al., 2003). Fundamental to the role of retinal glia is their exchange of substrates (pyruvate, glutamine) and their uptake of byproducts (glutamate, CO₂) from neurons. Retinal glia often rely on anaerobic glycolysis which generates lactate; conversion of lactate to pyruvate then release from the glia via a monocarboxylate transporter, MCT2 (Lin et al., 1998) supplies neurons that take up the pyruvate and use it as a substrate in their own Krebs cycle. Like astrocytes, Müller glia also have glycogen deposits (Kuwabara & Cogan, 1961) that could provide a ready substrate during ischemia or glucose shortage.

Of the several glutamate transporters identified in the CNS, retinal astrocytes and Müller glia primarily express GLAST (glutamate-aspartate transporter). The importance of glutamate transport in retinal glia extends beyond managing neurotransmitter levels in the extracellular milieu. Glutamate is an important stimulator of glycolysis in glia, via its co-transport of Na⁺. In addition, glutamate, through the glial enzyme glutamine synthetase, gets converted to glutamine in glia, which then provides the glutamine to neurons for their production of glutamate and GABA (Pow & Crook, 1996). More important, however is the use of glutamate in the production of glutathione (GSH). This ubiquitous and quickly metabolized anti-oxidant is present in high concentrations in the astrocyte (up to 20mM) and released to the extracellular space. Once there, a glia membrane-bound ectoenzyme, γ -GT (γ -glutamyltranspeptidase), breaks GSH into the cysteine-glycine dipeptide that can be taken up by neurons. This reaction is key because neurons maintain GSH at low levels in the cytoplasm (<1mM) and they cannot import it directly. Neurons require GSH to reduce side-products of oxidative phosphorylation and detoxification pathways. The cysteine-glycine precursors for GSH are provided to neurons solely by astrocytes or Müller glia. Mechanisms of neurodegeneration related to glutamate handling and oxidative stress have been implicated in glaucoma, discussed in greater detail below.

In this chapter, we review the role of glia by compartment of the visual system— retina, optic nerve head (ONH) and optic nerve (ON)— which encompass the potential sites of glaucoma initiation and progression. Astrocytes and microglia appear in each compartment while Müller glia are confined to the retina. The overlapping function of astrocytes and Müller glia reinforces common mechanisms for disease pathogenesis across visual system compartments; however, the unique environment in each compartment allows for distinct

causes and consequences of disease-related pathology. The various environments and stresses experienced by glia demand that we understand the time line of glial activation and find ways to manipulate that activation for RGC neuroprotection. Once we have discussed the mechanisms of glaucoma involving glia by compartment, we review the methods we have used glia to limit glaucoma pathology and protect RGCs and visual function.

2. Retina

Glaucomatous changes that result in RGC death occur in the retina. This general hypothesis for the pathogenesis of glaucoma draws strength from the fact that the retina contains the RGC somas and the glia that support them. The intraocular pressure (IOP) increases that pose a risk factor for glaucoma translate directly into key concerns for the RGCs in the retinal compartment.

2.1 Astrocytes

Astrocytes in the retina migrate in from the optic nerve as the retina develops (Huxlin et al., 1992). Likely a reflection of their role in the blood-retinal-barrier, astrocytes are only present in the retinas of mammals with vascularized retinas (Stone & Dreher, 1987). They form a syncytium across the nerve fiber layer with processes that extend into the ganglion cell layer. Astrocytes ensheath RGC axons and have processes that converge on the initial segments of RGCs (Hollander et al., 1991). In places where vessels and axon bundles prevent Müller glia endfeet from forming the glia limitans of the inner limiting membrane, astrocytes extend the glial coverage over both vessels and axon bundles. Adherent junctions form between astrocytes and Müller glia, but gap junctions form only between astrocytes, never between astrocytes and Müller glia. The glial sheaths around RGCs are primarily comprised of Müller glia processes, though the occasional astrocyte process has been observed. Astrocytes (but not Müller glia) have processes that contact node-like specializations of RGC axons (Hollander et al., 1991). In human retina, there is limited astrocyte support in the macula, the region of tightly-packed multi-layered RGCs responsible for central high resolution vision. Vasculature also avoids this region; these facts have implications for glaucoma pathogenesis since the arcuate pattern of RGC loss in human glaucoma follows the arc of vessels and astrocytes beyond the macula (Araie, 1995). Astrocytes respond to even subtle changes in retinal homeostasis in ways that can be protective or detrimental. The most overt response of astrocytes to glaucoma in the retina is gliosis, which includes hypertrophy of the astrocytic cytoskeleton along with various context dependent changes in gene expression and astrocyte function. In some regions of the CNS, gliosis includes astrocyte proliferation and migration, but gliosis in glaucomatous retina is simply reactive (Inman & Horner, 2007). As RGCs die in glaucoma, hypertrophied astrocyte and Müller glia processes fill in the empty spaces. Increased proteoglycan production concomitant with GFAP and vimentin over-expression occurs in the DBA/2J mouse retina with early glaucoma (Figure 1). These proteoglycans, important to retinal development for neural patterning (Brittis et al., 1992), significantly inhibit axon growth or cellular migration when re-expressed (Inatani et al., 2000; Inman & Horner, 2006). Proteoglycan deposition restricts the ability of the retina to engage in any endogenous repair. Two signal transduction pathways, the JAK/STAT and NF κ B, have been implicated in the reactive gliosis observed in retinal astrocytes and Müller glia. Astrocytes in the DBA/2J mouse retina express STAT3 and phosphorylated STAT3 protein at higher levels as glaucoma progresses (Lupien & Horner,

2007). Retinal astrocytes and Müller glia *in vitro* upregulate GFAP expression through the STAT3 pathway, as determined by increased GFAP expression with the addition of the STAT3 activators ciliary neurotrophic factor (CNTF) or leukemia inhibitory factor (LIF) (Lupien et al., 2008). GFAP expression in retinal glia decreased when inhibitors of NF κ B were introduced four weeks after glaucoma induction (Lupien et al., 2009). We have manipulated these pathways to understand the role of gliosis in glaucoma (see below).

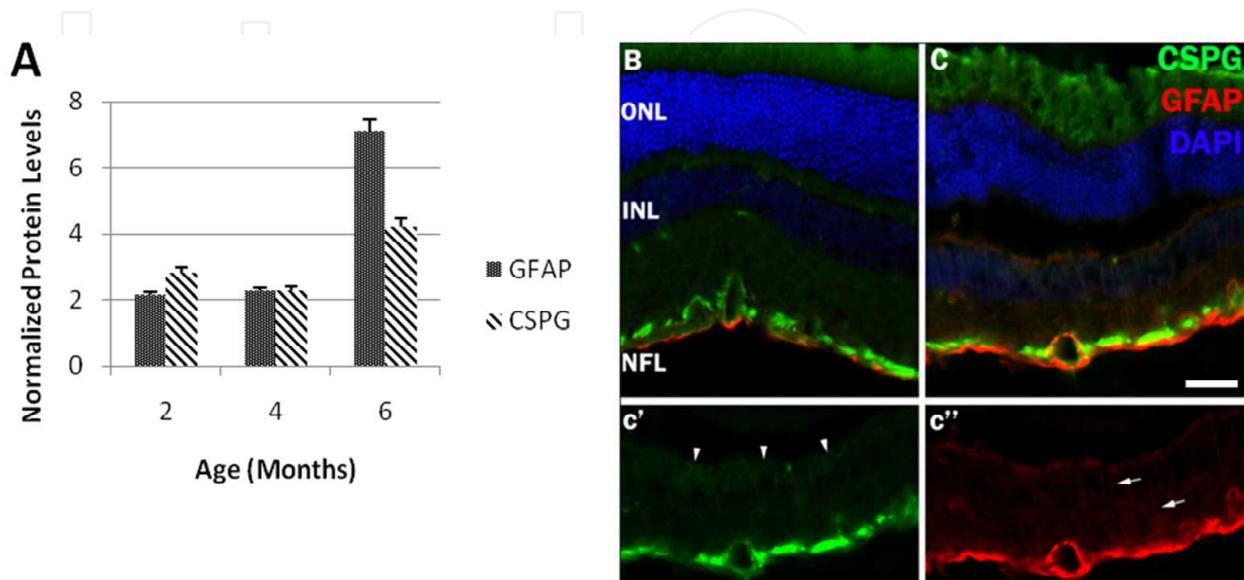


Fig. 1. **A**. Western blot of whole retina from DBA/2J mice from 2 to 6 months of age shows a significant increase in GFAP and CSPG expression concomitant with early increases in IOP (5-6 months of age, $p < 0.05$). **B & C**. Immunolabeling of retina with antibodies against CSPG (green) and GFAP (red) show significant increases in GFAP expression in astrocytes of the nerve fiber layer (NFL) and significant upregulation of CSPG in Müller glia endfeet when comparing 4 month (**B**) and 8 month (**C**) DBA/2J retinal sections. Arrowheads point to Müller glia somas expressing CSPG in the inner nuclear layer (INL) in **c'** and arrows point to GFAP expression in Müller glia processes (**c''**) Scale bar=50 microns. repair.

Retinal astrocytes release cytokines and chemokines in response to various stimuli, and changes in many of these signaling molecules have been observed in glaucoma. Astrocytes decrease their release of IL-6 when cultured under hydrostatic pressure, a condition designed to recapitulate the increased IOP observed in glaucoma (Sappington & Calkins, 2006). Lower IL-6 expression may be detrimental to cell survival in retina because astrocyte-derived IL-6 has been shown to regulate expression of metallothionein I and II, potent antioxidants in the CNS (Penkowa et al., 2003). RGCs import astrocyte-derived metallothioneins through their megalin receptors; their import has been associated with subsequent axon regeneration (Chung et al., 2008). Regardless, IL-6 activates astrocyte gliosis through the activity of the JAK/STAT pathway, a potentially autocrine mechanism that may be helpful or detrimental depending on the context and timing of activation.

Analysis of human glaucoma retina shows mRNA and intense immunolabel for TNF α in glia—likely both astrocytes and Müller cells, while the TNF-R1 was observed primarily on RGCs (Tezel et al., 2001). TNF α is a pro-inflammatory cytokine that can be released by microglia, astrocytes or Müller glia, and its effect depends upon the intracellular signals induced after binding to the TNF α -R1 or 2. For example, the TNF α -R1 receptor has a cell

death domain that promotes apoptosis, but only if NF κ B anti-apoptotic mechanisms are also inhibited (Kraft et al., 2009). TNF α binding that leads to NF κ B nuclear translocation enables transcription of inhibitors of apoptosis, thereby promoting cell survival (Beg & Baltimore, 1996). Robust and sustained activation of JNK, which occurs when NF κ B is suppressed, would indicate the TNF α -R1 directed apoptosis as opposed to survival. One model of experimental glaucoma found no evidence of JNK activation in RGCs or other cells of the retina (Levkovitch-Verbin et al., 2005), while JNK expression was observed in RGCs from glaucoma donor eyes, though the timing or persistence of expression is unknown (Tezel et al., 2003). TNF α -R1 can also activate cell protective heat shock proteins such as hsp70 in response to cellular stress (Heimbach et al., 2001), and this activity involves cross-talk with NF κ B. TNF α represents just one signaling system between glia and neurons with finely-tuned, context-dependent effects on RGC survival. TNF α undoubtedly can cause RGC apoptosis, but for which cells and when, and in which glaucoma-related contexts, remains a topic of active investigation.

Astrocytes that surround synapses actively modulate synaptic activity in a Ca²⁺-dependent, glutamate mediated way (Jourdain et al., 2007). Early studies showed how the amplitude of Ca²⁺ waves moving through retinal astrocytes and Müller glia modulated the spike activity of RGCs (Newman & Zahs, 1998). Since glutamate antagonists reduce the inhibition of neuronal activity associated with glial Ca²⁺ waves, inhibition is likely mediated by inhibitory interneurons stimulated by glutamate release from glial cells. In the hippocampus, TNF α has been shown to regulate the glial release of glutamate that can escape glial reuptake and bind to neuronal NMDA receptors (Santello et al., 2011). TNF α improvement of glutamate exocytosis from astrocytes could be quite detrimental if the transport mechanisms to prevent glutamate RGC overstimulation are compromised. On the other hand, we have already outlined how loss of TNF α could limit the ability of astrocytes to support activity-dependent RGC survival. Well-timed glutamate release from glia activates glutamate transporters, potentially extending the interval of extracellular glutamate signaling. TNF α also works through the TNF α -R1 to increase AMPA-R insertion in the post-synaptic membrane. This multi-pronged modulation of glutamate signaling through TNF α significantly complicates our understanding of the role of TNF α in glaucoma. Müller glia, through their more intimate association with retinal synapses in which TNF α modulation of glutamate release would occur, will be the object of intense study of these phenomena.

TNF α contributing to glutamate dysregulation implicates glutamate-induced excitotoxicity as a glial-related mechanism of glaucoma. Evidence for neurotoxic levels of glutamate has been observed in the vitreous of canines (Brooks et al., 1997) but not in monkeys (Carter-Dawson et al., 2002) with induced glaucoma. However, focal depletion of glutamate in glaucomatous canine retina occurred concomitant with significantly lower levels of glutamine and glutamine synthetase (GS) (Madl et al., 2005; Chen et al., 2008). Reduced glutamate disrupted important anti-oxidant systems, making oxidative stress secondary to glutamate dysregulation a potential mechanism of RGC loss in glaucoma. In support of this concept, anti-oxidant treatment α -luminol restored glutamate, glutamine and GS levels and protected RGCs in the DBA/2J mouse model of glaucoma (Gionfriddo et al., 2009). Further detail regarding the role of oxidative stress appears in the Müller glia section below.

In a microarray study that compared 3 and 8-month retinas from DBA/2J mice prior to outright glaucoma, a few of the mRNAs that underwent the greatest decrease were members of the crystallin family (Steele et al., 2005), a group of proteins with diverse

function but primary expression in the lens. The β A3/A1crystallin subtype, which was down 2-fold in 8-month DBA/2J, is also expressed in retinal astrocytes. Elimination of β A3/A1crystallins results in structural and functional defects in astrocytes that lead to abnormalities in vascular development and maturation (Sinha et al., 2008). Though these observations were made in young knockout mice, the implications of eliminating an astrocyte-derived vascular modulator in the DBA/2J glaucoma mice could have a significant effect on retinal function and RGC survival. Autoantibodies against α -crystallin have been observed in normal-tension glaucoma patients, and these antibodies could cause apoptosis of RGCs *in vitro* (Tezel et al., 1998). These data as well as data described in the microglia section raises the possibility of an auto-immune component to glaucoma. Evidence for pathogenic glial interactions in glaucoma continues to mount.

2.2 Müller glia

Müller glia have overlapping roles with astrocytes, including redox homeostasis, maintenance of extracellular milieu through ion buffering and growth factor provision. However, by virtue of their role in retina structure, their contact of all retinal neurons, and their anatomical link between the vitreous of the inner retina and the pigment epithelium of the outer retina, Müller glia form a functional unit with a much more critical role to play in retina than astrocytes. Müller glia contribute more to the glia limitans and to wrapping cell somas in the inner retina than do astrocytes (Hollander et al., 1991). Through their expression of myriad receptors, ion channels, and transporters, Müller glia engage in extensive interplay (energetic and signaling) with their neuronal neighbors (Bringmann et al., 2006).

The response of Müller glia to glaucomatous changes resembles that of astrocytes in that many activities are neuroprotective, but these macroglia can also severely damage the retina through the release of cytokines, nitric oxide, and proteoglycans or by failing to release growth factors. Beyond the non-specific responses to retinal injury which include upregulation of GFAP, vimentin (Bringmann & Reichenbach, 2001) and activation of the extracellular signal-regulated kinases (ERKs) (Tezel et al., 2003), Müller glia have specific responses like upregulation of glutamine synthetase (GS) by mRNA and protein, which we have observed in the DBA/2J mouse retina at ages of accelerating RGC loss (10 months; see Figure 2). A similar upregulation of GS expression occurs in hepatic retinopathy where the GS detoxifies the retina of accumulated ammonia (Reichenbach et al., 1995). Upregulation of GS in Müller glia is neuroprotective against glutamate neurotoxicity (Gorovits et al., 1997), suggesting that the increased expression in DBA/2J retina may be a protective mechanism. As noted in the retinal astrocyte section, changes in GS have implications for managing oxidative stress and maintaining appropriate glutamate levels in retina.

A strong neuroprotective role for Müller glia emerges in their management of the neurotransmitter glutamate. Müller glia use GLAST to efficiently move glutamate from the extracellular space in the course of normal retinal function but also in pathological conditions such as ischemia, a proposed contributor to the mechanism of glaucomatous damage. Patients with primary open angle glaucoma had glial activation that correlated well with vascular dysregulation (Grieshaber et al., 2007), a potential source of retina ischemia. Blocking GLAST or GLT-1 in the retina of normal rats led to glutamate increases and RGC death (Vorwerk et al., 2000), establishing that RGCs are sensitive to excitotoxicity. While Müller glia endeavor to maintain signaling homeostasis, RGCs do not necessarily sit

passively by: RGCs expressed one isoform of the GLT-1 transporter (GLT-1c) upon induction of glaucoma in the rat eye (Sullivan et al., 2006). This unusual expression might be a protective response to increases in extracellular glutamate. GLAST can reverse glutamate and aspartate transport when extracellular K^+ concentration is high, as would be the case with high neuronal activity or Müller glia depolarization (Marcaggi et al., 2005). However, an electrophysiological analysis in the DBA/2J mouse model of glaucoma determined that neither membrane currents nor membrane potentials were altered in the Müller glia in mice with clear glaucoma pathology (Bolz et al., 2008). Evidently, concrete evidence for excitotoxicity in the glaucomatous retina is elusive; the evidence for oxidative stress as a driver in glaucoma, which can be secondary to glutamate dysregulation, is much stronger.

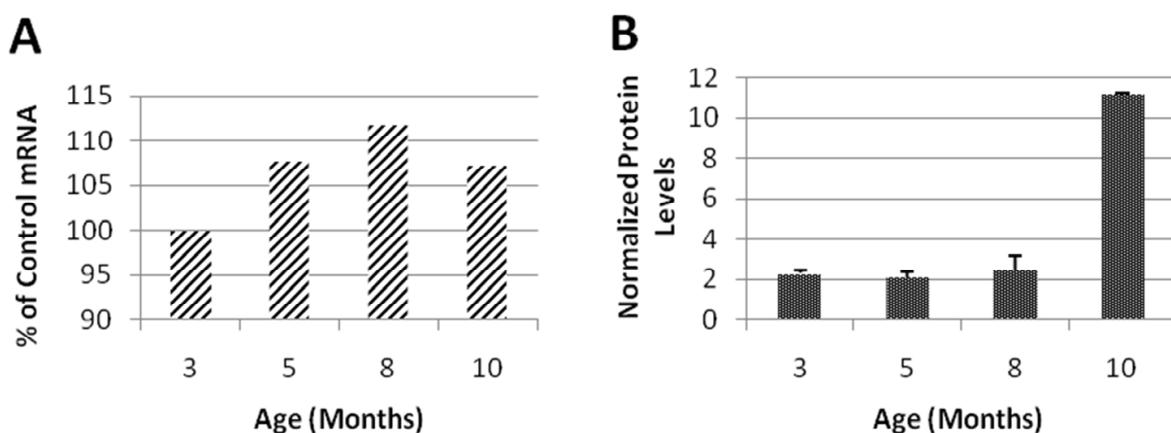


Fig. 2. **A.** Glutamine synthetase (GS) mRNA in whole retina from DBA/2J mice from 3 to 10 months of age, as percent of control (3 month levels). GS mRNA levels peak at 8 months, coincident with significant gliosis but prior to significant RGC loss. **B.** GS protein levels in whole retina from DBA/2J mice from 3 to 10 months of age. The protein levels were normalized to β -actin. GS peaks at 10 months ($p < 0.05$) when RGC loss is accelerating.

Retinal glia have a fundamental role in maintaining redox homeostasis so are equipped with the tools to resolve redox imbalance. Oxidative stress occurs when reactive oxygen species (ROS) creation outpaces its clearance by anti-oxidant enzymes and compounds in a cell. Oxidative stress can result from exposure to stimuli that range from environmental toxins, infection and energy dysregulation to ischemia; it has been implicated in several neurodegenerative diseases, including glaucoma (Gmitterova et al., 2009). Resolution of oxidative stress can halt neurodegeneration (De Luca et al., 2008). As will be discussed at length in the section on optic nerve head, increased IOP can affect blood flow to the retina. Lack of nutrient delivery and waste removal via the circulation leads to ischemia. Müller glia upregulate iNOS in times of ischemia and the subsequent NO can dilate blood vessels (Goldstein et al., 1996), but NO can also be quickly converted to peroxynitrite that can attack proteins. Protein thus nitrated can disrupt normal cellular function and lead to increased energy demand. ATP energy in the CNS comes from glycolysis or oxidative phosphorylation. Non-reduced diatomic oxygen (O_2^-), known as superoxide anion, is the primary mitochondrial reactive oxygen species (ROS) generated from oxidative phosphorylation (Skulachev, 2006). Other ROS can be derived from superoxide production. ROS attack glutamate transporters on redox-sensing cysteine residues (Aschner, 2000), leading to higher extracellular glutamate, lower glutamine (and therefore, glutathione)

production as well as compromised energy availability, a dangerous feedback loop. The loss of the anti-oxidant glutathione hinders the cell's ability to limit ROS damage. Cellular components accumulate ROS-related changes (oxidation, nitration, lipidation) and cease to function. As a sign of extreme distress, Müller glia exposed to ROS express major histocompatibility class II molecules, allowing these glia to present antigen and T cells to create antibodies against the presented protein (Tezel et al., 2007). Autoantibodies appear in the serum of glaucoma patients (see microglia section below). Oxidative stress can be initiated in several ways; it is certainly a secondary degenerative process in glaucoma, but it has the ability to amplify and extend damage at every level of cellular regulation, making it a formidable foe.

Müller glia from human glaucoma eyes showed increased levels of advanced glycation end products (AGE), an inflammatory stimulant, as well as the receptor for advanced glycation end products (RAGE) (Tezel et al., 2007). Müller glia express proinflammatory cytokines IL-6 and TNF α (Tezel & Wax, 2000) in response to AGE exposure. TNF α release has been shown to impact the blood-retinal-barrier through increased vesicular transport of serum proteins by vascular endothelial cells. Serum proteins accumulate in Müller glia, perivascular microglia and pericytes after TNF α exposure, suggesting that these glia protect the retina by acting as secondary barriers (Claudio et al., 1994). However, Müller glia are just as likely as retinal microglia and astrocytes to be a source of the TNF α . Similarly to astrocyte-derived TNF α , Müller glial TNF α can dictate cell death or survival based on the complement of receptors and second messenger systems available to it.

2.3 Microglia

Microglia function in environment surveillance, synapse elimination, cytokine/chemokine release, innate immunity and debris clean-up. The release of specific cytokines and synapse elimination are two mechanisms of glaucoma in which microglia would play a significant role, possibly driving pathogenesis. In order to implicate microglia in the development of glaucoma, one research group used minocycline to reduce microglial activation and document changes in glaucoma disease progression. A significant decrease in microglial activation occurred with minocycline treatment, as shown through morphology and gene expression, and there was significant improvement of retrograde transport in RGC axons (Bosco et al., 2008). Minocycline inhibits the expression of NOS (Amin et al., 1996) and exerts a stimulus-dependent decrease or increase the production of IL-6 through TNF- α (Kloppenburger et al., 1996). Microglia in DBA/2J mouse retina have been shown to release the cytokine IL-6. These observations suggest protection of RGCs by decreasing microglial activation in glaucomatous retina, but it does not rule out a contribution of astrocytes, Müller glia or even neurons due to the systemic delivery of the minocycline treatment.

Microglia increase their expression and release of IL-6 when cultured under hydrostatic pressure, conditions meant to mimic the retina at increased IOP (Sappington & Calkins, 2006). Microglia-derived IL-6 rescued RGCs cultured under hydrostatic pressure from death (Sappington et al., 2006). Interestingly, retinal microglia express TRPV channels, cation-selective transient receptor potential vanilloid-1 receptors sensitive to mechanical stimuli like pressure (Sappington & Calkins, 2008). IL-6 release from microglia under pressure required Ca²⁺ increase, mediated by both intracellular ryanodine receptors and TRPV channels (Sappington & Calkins, 2008). The same group also demonstrated that IL-6 increases were preceded by sustained NF κ B nuclear translocation, suggesting that NF κ B

drives IL-6 changes in retinal microglia (Sappington & Calkins, 2006). Antagonism of Ca^{2+} release reduced IL-6 release and NF κ B activation in retinal microglia. These findings reflect the complexity of glial response during disease circumstances; not only is IOP translated by glia in myriad ways, but those ways can be in opposition, depending on the glial subtype.

Additional studies have underscored the complexity of glial-associated pathogenesis by showing the give and take of astrocytes and microglia. Astrocyte-derived signals (possibly ROS) initiated Nrf2 activity and expression of heme-oxygenase in microglia (Min et al., 2006). Conditioned media from those astrocytes suppressed interferon- γ (IFN- γ)-induced ROS production, leading to reduced iNOS expression and NO release. These data illustrate the feedback loop of ROS production that exists between these glia. Feedback loops regulated by astrocytes in microglia work in reverse too: Microglia can activate the Nrf2-ARE (anti-oxidant response element) in astrocytes and change the redox level there.

An autoimmune component to glaucomatous optic neuropathy has emerged from observations of serum autoantibodies against proteoglycans in open-angle glaucoma patients (Tezel et al., 1999), and against heat shock proteins (hsp) hsp27 and hsp60 (Wax et al., 2008) in patients with normal tension glaucoma (NTG). Microglia have a complex, context-dependent role in autoimmunity. They can express MHC class II proteins which allow them to present antigen. Their expression of Fas ligand (FasL), a cytokine in the TNF superfamily, leads to apoptosis in cells expressing the FasL receptor. Immunization of rats with hsp27 or hsp60 activated microglia and upregulated FasL receptor expression in RGCs and cells in the INL (Wax et al., 2008). Heat shock proteins are upregulated in times of stress and function as protein chaperones or assembly of protein complexes (Young & Elliott, 1989). In NTG patients, antibody against hsp27 may reduce the ability of native hsp27 to stabilize the cytoskeleton, thereby initiating apoptosis in hsp27-expressing cells (Tezel & Wax, 2000). These data suggest that activated microglia could initiate apoptosis in cells targeted by autoantibodies. Conversely, the expression of FasL proteins by retinal microglia may be a mechanism of maintaining ocular immune privilege and avoiding damaging inflammatory reactions in the eye (Griffith et al., 1995) by inducing apoptosis of invading T-cells. Ocular immune privilege certainly limits T-cell travel in the retina unless coincident with blood-retinal-barrier breach, a circumstance that has been demonstrated only in canine glaucoma that is secondary to uveitis (Reilly et al., 2005; Mangan et al., 2007). Glaucoma experimental models that document persistent microglial activation do not also find T-cells (Ebner et al., 2011). Glaucoma T-cell entry to the retina is receptor-mediated even in cases of clear autoimmune retinal pathological mechanisms such as in glaucomas secondary to sarcoidosis (Hamanaka et al., 2002) or B-cell lymphoma (Cockerham et al., 2000), in which T-cell or B-cells were observed in Schlemm's canal and trabecular meshwork. Therefore in retina, these studies suggest microglia would be the primary agents of autoimmune-related FasL-induced apoptosis capable of dispatching significant numbers of RGCs. Further investigation into which cells present antigen, what comprises that antigen and which cells express the FasL receptor would improve our understanding of potential autoimmune processes in glaucoma.

Based on evidence from innate immunity research, microglia and astrocytes coordinate management of synapses in the inner retina. The initiating member of the classical complement cascade, complement component 1q (C1q), coats dead cells, debris or pathogens and initiates a protease cascade that results in complement C3 deposition. C3 can activate receptors on microglia that signal the cells to phagocytose any C3-coated elements. Synapse elimination during CNS development proceeds by this mechanism (Stevens et al.,

2007), with immature astrocytes signaling to RGCs to express C1q. Not normally observed in the adult retina, C1q is nevertheless upregulated in glaucomatous eyes (Steele et al., 2005; Stasi et al., 2006), suggesting that activated astrocytes have overcome their developmental limitations and released a factor to which the RGCs respond by making C1q. Immunolabeling of the inner plexiform layer where RGCs synapse with amacrine and bipolar cells shows C1q upregulation coincident with synapse loss (Stevens et al., 2007). An unknown stimulus triggers the astrocytic release of the factor that leads to C1q upregulation in the glaucomatous retina. Microglia respond by eliminating the targeted synapses, reducing RGC connectivity. Evidence for dendritic arbor shrinkage and loss (Weber et al., 1998; Pavlidis et al., 2003; Shou et al., 2003) in glaucoma supports this mechanism of degeneration.

A study in which a bone marrow transplant after irradiation rescued RGCs from cell death in the DBA/2J has intrigued many, and may be evidence for a vital role of microglia in glaucoma. Since microglia comprise the largest group of proliferating cells in the retina (Inman & Horner, 2007), the irradiation would have destroyed them and any endothelial cells. The bone marrow transplant repopulated the immune system and irradiation created a leaky blood-retinal-barrier, allowing peripheral immune cells to infiltrate. No one understands the mechanism of protection against optic neuropathy in this study, but the results highly suggest eliminating endogenous retinal microglia enabled RGC rescue (Anderson et al., 2005).

3. Optic nerve head

3.1 Locus of degeneration

A long-held hypothesis of the mechanism of RGC damage in glaucoma states that compression and rearrangement in the lamina cribosa cribriform plates, the connective tissue collagen plates through which the ganglion cell axons pass to form the optic nerve, impinge upon the axons, disrupting axon transport to a degree that leads to axon degeneration and RGC death (Quigley & Addicks, 1981). Supportive evidence for this hypothesis includes failure of retrograde delivery of target-derived growth factors in monkey and rat glaucoma models (Pease et al., 2000; Quigley et al., 2000) as well as the considerable extracellular remodeling that alters the lamina and deposits significant amounts of collagen and proteoglycans in humans (Tengroth & Ammitzboll, 1984; Tengroth & Ammitzboll, 1984) and in experimental glaucoma models (Johnson et al., 1996). The optic nerve head (ONH) undergoes considerable deformation in response to the diurnal fluctuation in IOP, and these conformational changes impact both glia and RGC axons. There are several mechanosensitive receptors on ONH astrocytes that could respond directly to changes in IOP (Oh, 1997).

Despite lacking a lamina cribosa, the DBA/2J mouse provides additional evidence for the optic nerve head as the site of initial damage in glaucoma. Fasciculated RGC axons travel through glial columns (glia lamina) in the mouse until myelination within the optic nerve. The glial columns are astrocytes arranged parallel to the axons and run caudal from the scleral boundary for about 200 microns (Howell et al., 2007), beyond which the ganglion cell axons become myelinated by optic nerve oligodendrocytes (May & Lutjen-Drecoll, 2002). Dystrophic neurites, with swollen and accumulated organelles and neurofilament breakdown, were observed in the glia lamina while the corresponding axons in the pre-lamina and in the retinal nerve fiber layer were intact (Howell et al., 2007). It appears that

axons underwent degeneration as entire fascicles, leaving sectors of ganglion cells axotomized and destined for degeneration within the retina (Jakobs et al., 2005; Howell et al., 2007). These data raise two interesting possibilities: for one, doubt is cast on the idea that the lamina cribosa pinches the axons to initiate glaucomatous degeneration; but secondly, the data implicates astrocytes in the lamina as prime drivers of pathology in this disease. The pattern of degeneration, that of ganglion cell loss in a pattern dictated by the position of axons lost, mirrors that observed in the human retina and proximal optic nerve. Astrocytes and microglia are the sole cell types in the lamina, so how might these cells initiate glaucoma? Recent evidence suggests mechanisms of action that include increased endothelin release, axon impingement from changes in extracellular matrix and astrocyte migration, oxidative stress from intermittent ischemia, and anterograde axon transport deficit of unknown etiology.

3.2 ONH astrocytes

As RGC axons undergo degeneration, the optic cup deepens, enabled by astrocyte remodeling and migration. The prelaminar portion of the optic nerve loses glial columns and astrocytes migrate between axon bundles (Hernandez & Pena, 1997). The ONH has both Type1A and Type1B astrocytes, distinguished primarily by the expression of NCAM in Type1B; both types express GFAP. Type1B astrocytes appear to be responsible for extracellular matrix production in ONH (Ye & Hernandez, 1995). Astrocytes deposit new extracellular matrix (ECM) in the course of glaucoma development. Astrocytes isolated from the human optic nerve head upregulated their expression of neural cell adhesion molecule (NCAM) two-fold by 6h of exposure to 60mmHg hydrostatic pressure (Ricard et al., 2000). NCAM participates in cell anchoring, so documented changes in its expression in glaucoma (Ricard et al., 1999) suggest a potential point of intervention if astrocyte migration contributes to the incipient optic neuropathy. Integrins join GFAP and vimentin at focal adhesion complexes on astrocytes that connect the cells to ECM, giving them a role in cell migration and transmitting mechanical signals to the astrocyte (such as increased IOP). Integrins also undergo considerable changes in expression in glaucoma (Morrison, 2006). Altered ECM makeup means potential loss of compliance of this dynamic tissue.

As demonstrated in the retina (see Figure 1), glia overexpress proteoglycans during gliosis in glaucoma. Serum autoantibodies obtained from patients with normal tension glaucoma and those with increased IOP bind to chondroitin sulfate and heparan sulfate glycosaminoglycans, proteins upregulated in the optic nerve head of glaucoma patient tissue (Tezel et al., 1999). However, chondroitin sulfate proteoglycan 4 (CSPG4) and CSPG1 (aggrecan) mRNA stood out on one ONH astrocyte microarray for being downregulated 35-fold and 29-fold, respectively in glaucoma patients (Hernandez et al., 2002). If there are feedback mechanisms that regulate proteoglycans, the mRNA downregulation might be a response to the persistence of the protein within the tissue. Optic nerve head astrocytes also degrade extracellular matrix as they migrate *in vitro* after exposure to hydrostatic pressure (Tezel et al., 2001).

Astrocytes in the optic nerve head (and retina) express G-protein-coupled endothelin receptors ET_A and ET_B , as do retinal vessels. Endothelin, a vasoactive and neuroactive peptide, has been shown to produce optic neuropathy when injected intravitreally. Patients with primary open-angle glaucoma have significantly higher levels of aqueous humor endothelin than age-matched normal patients (Tezel et al., 1997; Iwabe et al., 2010).

Endothelin leads to intracellular Ca^{2+} increases in astrocytes and can increase astrocyte hypertrophy and proliferation (Prasanna et al., 2003). This effect was mediated by the ET_A receptor since it could be blocked by an ET_A -specific antagonist. Ischemia and mechanical pressure can induce endothelin secretion from astrocytes. ET_A has greatest affinity for ET-1 and ET-2 while ET_B has equal affinity for all three isoforms of ET (1, 2 and 3). Endothelin can disrupt anterograde axon transport, making its upregulation in optic nerve head astrocytes (even retinal astrocytes) an important potential mechanism of glaucoma. Key to understanding the role of endothelin is recognizing that it had effects on specific transport types and times; early after intravitreal injection of 2nM endothelin, it increased fast anterograde axon transport of tubelovesicles, but by 28h after injection, it decreased transport of mitochondria and decreased slow anterograde transport of cytoskeletal proteins at 4 days (Stokely et al., 2002). A straightforward mechanism for optic neuropathy initiated by endothelin would combine its vasoconstriction (through the ET_A) that leads to ischemia with the energy dysregulation in RGC axons, a result of the ET_B -mediated deficit in mitochondrial anterograde transport. Not surprisingly, $\text{TNF}\alpha$ stimulates ET-1 release in ONH astrocytes exposed for 24h to hypoxia (Desai et al., 2004).

Some of the genes downregulated in ONH astrocytes cultured from human glaucoma patients have negative ramifications for cell survival. The loss of glucose transporter mRNA (GLUT5) and monocarboxylate transporter (MCT3) (Hernandez et al., 2002) would challenge astrocytes to maintain energy levels and exchange pyruvate or lactate with neurons. Fuel energy dysregulation would negatively impact membrane potentials and therefore action potential propagation in the neighboring RGC axons. Axonal Na^+ overload occurs when energy substrates to decrease and Na^+ initiates Ca^{2+} accumulation in the axon through reverse operation of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger (Stys et al., 1992). Subsequent to Na^+ overload, K^+Cl^- co-transporters, with subtypes present on both astrocytes and axons, release K^+ and Cl^- from the axons, disrupting the Cl^- gradients that contribute to resting optic nerve membrane potential (Malek et al., 2003). Axons unable to conduct action potentials will fail to transmit the visual signal from RGCs to the target regions in brain. Energy depletion would be complete as ATP-dependent processes such as axon transport would cease. This proposed mechanism supports many of the observations of generalized axon transport failure (Buckingham et al., 2008) or transport failure at the ONH (Minckler et al., 1977; Quigley et al., 2000). However, important observations of axon transport deficit in the DBA/2J mouse model of glaucoma showed that axon transport in glaucoma fails first in the target regions of the RGC axons, the superior colliculus (Crish et al., 2010). There are many activity-dependent regulatory mechanisms that maintain synapses and transmit sustaining growth factors back to the RGC soma. One might anticipate loss of synaptic connections with axon conduction loss, though the authors were able to demonstrate in the DBA/2J that collicular synapses were intact despite lack of transport to the target (Crish et al., 2010). Certainly distal transport failure highlights the logistical difficulties that RGCs face in getting cargo to remote synapses and highlights a potentially novel mechanism of glaucoma pathology.

Understanding the mechanism of optic neuropathy in glaucoma is complicated by the frequent overlap of expression and activation of potentially damaging intermediates by both astrocytes and microglia. Cytokines and chemokines expressed by glia have been implicated in neurodegeneration, and the feedback loops or escalation of inflammatory processes are difficult to tease apart. Astrocytes in human glaucomatous ONH express $\text{TNF}\alpha$ and its receptor, TNF-R1 (Yuan & Neufeld, 2000). Upregulation of $\text{TNF}\alpha$ and TNF-R1 occurred concomitant with increasing optic nerve degeneration; in severe glaucoma, $\text{TNF}\alpha$ and TNF-R1

R1 were also expressed on activated microglia (Yuan & Neufeld, 2000). One consequence of TNF-R1 activation is NOS-2 induction, in both astrocytes and microglia (Neufeld, 1999). NOS-2 induction also occurs in reactive astrocytes from human ONH as a result of epidermal growth factor (EGF) receptor agonism (Liu & Neufeld, 2003). ONH astrocytes exposed to hydrostatic pressure showed increased EGF-R expression and phosphorylation, as did reactive, but not quiescent, astrocytes in human glaucoma ONH (Liu & Neufeld, 2003). A microarray analysis of EGF-R activation in ONH astrocytes showed upregulation of many genes associated with astrocyte activation, including various proteoglycans, ET_A, leukemia inhibitory factor (LIF), insulin-like growth factor (IGF), fibroblast growth factor 2 (FGF-2), nerve growth factor (NGF), transforming growth factor- β (TGF β) and tissue inhibitor of matrix metalloproteinase (TIMP) (Liu et al., 2006). Others have shown ONH astrocytes express TGF β (Pena et al., 1999) which can upregulate ECM molecule expression, both possibly as a result of EGF-R activation. These data suggest that EGF is a potent astrocyte activator, but ironically, many of the upregulated genes are growth factors which generally support cell survival. Seven months delivery of an EGF-R tyrosine kinase inhibitor or NOS-2 inhibitor protected RGCs from cell death in a chronic rat model of glaucoma (Liu et al., 2006). The treatments sought to eliminate EGF-R activation and its target NOS-2 in astrocytes. Due to systemic delivery of the inhibitors, any cells with EGF-R would be antagonised, though the implication is that tempering glial reactivity protected RGCs from glaucoma-related cell death. However, RGC number and not function was the only outcome measure.

3.3 ONH microglia

Microglia are activated in glaucomatous human ONH (Neufeld, 1999) and very early at the ONH in chronic glaucoma models, indicating either an unusual responsiveness to stressors there or an initiation of degenerative processes (Bosco et al., 2011). Microglia often appear activated and in high numbers near the peripapillary chorioretinal region, a possible site of tenuous blood-retinal-barrier, and in close proximity to retinal vessels (Neufeld, 1999). These cells' position and activation state suggest interaction with the periphery; perhaps the observed autoantibodies against proteins found in the ONH are generated with the assistance of ONH microglia. Microglia in ONH of humans with glaucoma (but not controls) express TNF α , TGF β and shows signs of proliferation by being immunopositive for PCNA, the proliferating cell nuclear antigen (Yuan & Neufeld, 2001). Cytokine expression targets astrocytes, the primary cell type in the ONH besides microglia, and modulates many of the pathological processes already described. TNF α induces NOS-2 which leads to nitric oxide (NO) production in microglia. Microglia can affect axon transport of synaptic vesicle precursors by releasing nitric oxide (Stagi et al., 2005). This ability would be especially destructive in the ONH where the RGC axons do not have a protective myelin sheath and activated microglia have been observed very early in the DBA/2J mouse model of glaucoma (Bosco et al., 2011). Microglia constitutively express several matrix metalloproteinases (MMPs) that get upregulated with glaucoma progression. These enzymes can contribute to much of the documented tissue remodeling that occurs in the ONH.

4. Optic nerve

All glaucomas, regardless of etiology, share optic nerve (ON) degeneration. Despite this fact, the ON has not been a focus of research into the pathogenesis of glaucoma. This may change with recent evidence of phagocytic astrocytes and energy dysregulation in ON.

4.1 ON astrocytes

Unlike in the retina, astrocytes proliferate in the glaucomatous optic nerve, as well as increase their expression of vimentin (Son et al., 2010). There are age-related changes in optic nerve astrocytes that likely alter their ability to function. One recent glaucoma hypothesis posits that phagocytic astrocytes in a particular region of the optic nerve, an area just rostral to the beginning of myelination (the myelination transition zone, or MTZ), become dysregulated. These phagocytic MTZ astrocytes express Mac-2 and upregulate it with IOP increase; the Mac-2 levels correlated with the number of RGCs with damaged axons in the retina (Nguyen et al., 2011). Spheroids in the MTZ contained γ -synuclein; their numbers increased in DBA/2J mice with glaucoma. The MTZ astrocytes were observed with large, γ -synuclein-positive axon inclusions within their cytoplasm (Nguyen et al., 2011). These data suggest that glaucoma might be a synucleinopathy, with the γ -synuclein axon spheroids in the MTZ mimicking the α -synuclein accumulations in Lewy bodies that contribute to cell death in Parkinson's disease. Immunolabeling for γ -synuclein was observed in human optic nerve (on axons and GFAP+ astrocytes) from glaucoma patients (Surgucheva et al., 2002). In models of glaucoma, phagocytic astrocytes in the myelination transition zone (MTZ) may become hyperactive in a bid to control γ -synuclein accumulation, or they may be responding to pressure signals that encourage hyperphagocytosis and lead to premature axon destruction.

Also of note, one study of glaucomatous ON showed an inverse relationship between IOP and available ATP. Increased IOP correlated with significantly decreased ATP in ON (Baltan et al., 2010), suggesting a metabolic vulnerability in glaucoma. Given that astrocytes comprise 28 percent of the ON (Perge et al., 2009), it may be the case that ATP decrease occurs in ON astrocytes. This possibility, combined with the observation of Glut5 and MCT downregulation in ONH astrocytes (Hernandez et al., 2002) supports an astrocyte energy dysregulation mechanism of optic neuropathy. Efforts to resolve whether ATP decrease in extrinsic (astrocytes) or intrinsic (axons) to the ON in glaucoma continue.

4.2 ON microglia

Microglia also occupy non-overlapping areas in the optic nerve (ON). Microglia may have a role in the glaucomatous ON that resembles their function in the ONH. Microglia-derived TNF α recruited macrophages to the sciatic nerve during Wallerian degeneration (Liefner et al., 2000), in part by inducing MMP activity that allows migration of these cells to sites of debris. As discussed in the ONH section, TNF α figures prominently in the response of microglia to the increased IOP and intermittent ischemia that occurs at the ONH. In the glaucomatous optic nerve, microglia have been observed full of lipid droplets, the result of ingesting myelin debris (Figure 3), but only in ON undergoing significant degeneration. It has yet to be determined if ON microglia have a role to play in glaucoma pathogenesis.

5. Manipulating glia

5.1 Intracellular pathways

Manipulating gliosis is one strategy for diminishing any negatives, and improving upon any positive effects, of glial response on RGC survival and function in glaucoma. We have changed the course of gliosis in mice with glaucoma by manipulating two intracellular signaling pathways implicated in gliosis, the JAK/STAT and the NF κ B pathways. In one

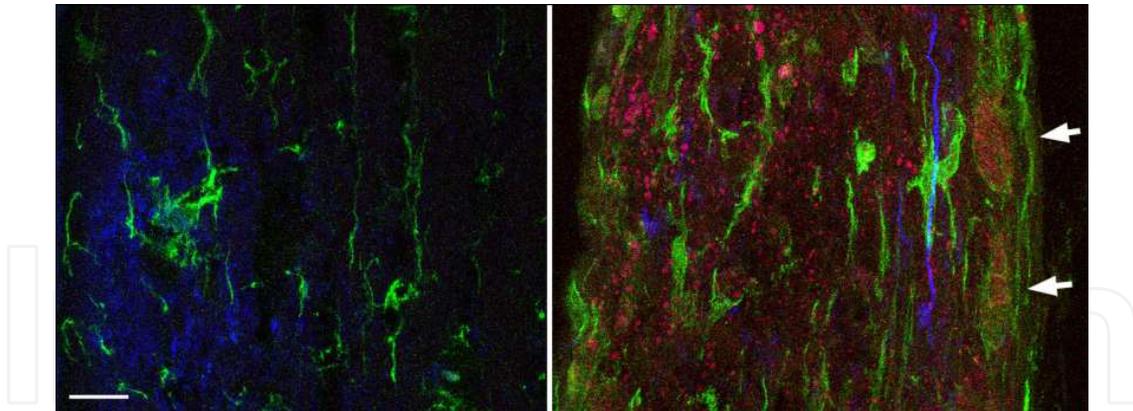


Fig. 3. Cross-sections of DBA/2J optic nerve at 3 months (left) and 12 months (right) shows green Iba1-positive microglia (arrows) filled with phagocytosed myelin debris (red) as labeled by a dye that stains lipid droplets (Oil Red O). Axons are labeled blue with an antibody against neurofilament-200 (NF-H) Scale bar=100 microns.

transgenic mouse, STAT3 is knocked out in GFAP+ cells (Herrmann et al., 2008); in the other, NF κ B in GFAP+ cells is prevented from translocating to the nucleus (Brambilla et al., 2005).

STAT3, a critical regulator of astrocyte intermediate filament upregulation and hypertrophy (Herrmann et al., 2008), transduces the intracellular signal for IL-6, CNTF and LIF, cytokines released after CNS injury. STAT3 has been observed in the nuclei of Müller cells, RGCs and astrocytes (Thanos & Naskar, 2004; Wang et al., 2010). In general, STAT3 activation is present in neurons in the acute phase of neural tissue injury and in astrocytes during the chronic phase, though severe injury may disturb this distinction (Yi et al., 2007). Furthermore, contradictions about the protective nature of STAT3 activation abound. For example, Luo et al., found that *inhibition* of JAK/STAT promoted RGC survival and axon regeneration (Luo et al., 2007), while a separate study showed that *activation* of STAT3 protected RGCs (Ji et al., 2004). In the former, RGC survival accompanied inhibition of macrophage recruitment, suggesting that JAK/STAT inhibition protected RGCs by preventing immune cell infiltration. To further clarify the role of STAT3, we subjected the GFAP+ -STAT3-KO mouse (Herrmann et al., 2008) to the microbead occlusion glaucoma model (Sappington et al., 2009). The STAT3-KO had decreased GFAP and proteoglycan expression in retinal glial cells and optic nerve when compared to control. Moreover, RGCs were significantly spared from degeneration in the STAT3-KO mice (Lupien et al., 2010).

NF κ B is a transcription factor expressed in neuronal and glial cells (Kitaoka et al., 2006) that regulates the expression of genes involved in inflammation, cell survival and apoptosis (Hayden & Ghosh, 2004). NF κ B is multi-talented, able to upregulate anti-apoptotic factors but also activate pro-apoptotic pathways. This dual role is reflected in retinal research; for example, deletion of the p50 subunit of NF κ B led to accelerated age-related RGC death (Takahashi et al., 2007). Also, ONH astrocytes from glaucoma patients express higher levels of NF κ B mRNA and show greater nuclear translocation than controls (Agapova et al., 2006). Conversely, GFAP-I κ B α -dn mice given retinal ischemia achieved significant survival of RGCs and reduction of pro-inflammatory gene expression (Dvorianchikova et al., 2009). Our research would echo the latter findings in that we observe decreased retinal gliosis *in vitro* and *in vivo* by using NF κ B inhibitors in a glaucoma mouse model (Lupien et al., 2009). By subjecting the GFAP-I κ B α -dn mice to acute glaucoma, we also observed a significant

decrease of GFAP expression in retinal glial cells that coincided with RGC survival and improved visual function (unpublished data).

5.2 Oxidative stress pathways

Manipulating essential functions of glia can augment glial support of retinal neurons. One major glial support function is the management of retinal redox homeostasis. Through delivery of exogenous anti-oxidants or the manipulation of anti-oxidant pathways within the retina, we have altered the neural-glial interaction in a way that improves RGC survival and function. In many circumstances, retinal glia provide protection from oxidative stress through the ROS-mediated liberation of a transcription factor, Nrf2, that binds to the anti-oxidant response element (ARE) (Itoh et al., 1999) and promotes the expression of anti-oxidants such as heme-oxygenase, ceruloplasmin (Miyahara et al., 2003; Lambert et al., 2006), peroxiredoxin 1, catalase, glutathione peroxidase, superoxide dismutase and thioredoxin (Lee et al., 2003; Kobayashi & Yamamoto, 2005). Nrf2 controls expression of genes encoding the catalytic (GCLC) and modifier (GCLM) subunits of glutamate-cysteine ligase, the rate-limiting enzyme in glutathione (GSH) biosynthesis (Wild et al., 1999). Glutathione is such an important component of redox homeostasis that it comprises two percent of the total protein in the Müller glia, a level that decreases with age (Paasche et al., 1998). As oxidative stress increases in glaucoma (Tezel et al., 2005; Wang et al., 2005), endogenous antioxidants are likely insufficient to maintain redox homeostasis.

Our first efforts at decreasing oxidative stress in glaucoma delivered exogenous lipoic acid, an organosulfur compound also made by mitochondria, over several months to DBA/2J mice with glaucoma. Lipoic acid protected RGCs from cell death and improved retrograde transport; indices of anti-oxidant activity were also increased (Lambert et al., 2008). In researching the mechanism of lipoic acid neuroprotection, we determined that the compound was working through the transcription factor Nrf2. In other contexts, lipoic acid was able to restore GSH levels in the aged liver by increasing Nrf2 nuclear translocation and its binding to the anti-oxidant response element (Suh et al., 2004). Nrf2 overexpressing astrocytes, when cocultured with motor neurons expressing mutant hSOD1, protected the neurons from cell death through GSH-enabled reduction of ROS (Vargas et al., 2008). These data demonstrate that increasing Nrf2-ARE activity decreases ROS generation through upregulation of anti-oxidant enzymes. We hypothesized that Nrf2 activation in retinal glia could provide additional anti-oxidant capacity to the retina, thereby protecting RGCs from pressure and ischemia-induced cell death. First, we subjected GCLM knockout mice, which have just 10 to 15 percent of their normal production of glutathione (Yang et al., 2002), to acute glaucoma. GCLM^{-/-} mice with glaucoma experienced a significant increase in RGC death and decreased retrograde axon transport in the remaining RGCs compared to wildtype littermates. This implicated glutathione as important to RGC survival in glaucoma. Our next experiments will target astrocytes and Müller glia to overexpress Nrf2. Glaucoma will provide the initial stimulus for Nrf2 activation, but the additional transcripts will enhance Nrf2 promotion of anti-oxidant genes.

6. Conclusions

Glaucoma is a complex disease. Diverse etiology has challenged researchers to determine disease mechanism, and several hypotheses have provided exciting ideas about how to

achieve neuroprotection. The latest research supporting the symbiotic relationship of neurons and glia suggest the pursuit of glial manipulation as a worthwhile means to protect RGCs in glaucoma.

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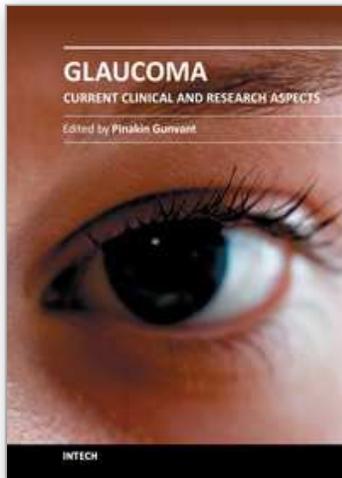
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This book summarizes current literature about research and clinical science in glaucoma and it is a synopsis and translation of the research conducted by individuals who are known in each of their respective areas. The book is divided into two broad sections: basic science and clinical science. The basic science section examines bench- and animal-modeling research in an attempt to understand the pathogenesis of glaucoma. The clinical science section addresses various diagnostic issues and the medical, laser and surgical techniques used in glaucoma management.

How to reference

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