The Complex Role of Neuroinflammation in Glaucoma

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Glaucoma is a multifactorial neurodegenerative disorder affecting 80 million people worldwide. Loss of retinal ganglion cells and degeneration of their axons in the optic nerve are the major pathological hallmarks. Neuroinflammatory processes, inflammatory processes in the central nervous system, have been identified in human glaucoma and in experimental models of the disease. Furthermore, neuroinflammatory responses occur at early stages of experimental glaucoma, and inhibition of certain proinflammatory pathways appears neuroprotective. Here, we summarize the current understanding of neuroinflammation in the central nervous system, with emphasis on events at the optic nerve head during early stages of glaucoma.

Glaucoma is a complex neurodegenerative disease that results in the degeneration of retinal ganglion cells (RGCs) and their axons in the optic nerve. Age and elevation of intraocular pressure (IOP) over baseline are major risk factors for glaucoma (Quigley 1993, 2011). Strong evidence suggests that an early insult occurs to RGC axons at the optic nerve head (ONH) (Jakobs et al. 2005; Schlamp et al. 2006; Howell et al. 2007; Soto et al. 2008; Burgoyne 2011; Quigley 2011; Nickells et al. 2012). The exact mechanisms by which RGC axons are insulted and ultimately degenerate are not clear, although early neuroinflammatory responses by astrocytes, microglia, and other blood-derived immune cells are observed in the ONH, suggesting a primary role of inflammation in glaucoma (Howell et al.

2012; Nickells et al. 2012; Tezel 2013). Furthermore, some evidence suggests that other RGC compartments, such as dendrites, synapses, and soma, are impacted early in the disease and that these events may also involve, even be mediated by, inflammatory responses in the retina (Howell et al. 2011a; Nickells et al. 2012; Tezel 2013). Therefore, the role of inflammation in glaucomatous neurodegeneration is currently of great interest.

In this review we focus on the role of inflammation in glaucomatous RGC loss (referred to as neuroinflammation). In particular, we discuss the proposed roles of astrocytes, resident microglia and other monocyte-derived cells in early stages of glaucoma in the optic nerve head. Evidence is suggesting these cell types are criti-

Editors: Eric A. Pierce, Richard H. Masland, and Joan W. Miller
Additional Perspectives on Retinal Disorders: Genetic Approaches to Diagnosis and Treatment available at www.perspectivesinmedicine.org

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Cite this article as *Cold Spring Harb Perspect Med* 2014;4:a017269

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cal players in the early neuroinflammatory responses in glaucoma.

NEUROINFLAMMATORY RESPONSES IN THE CNS RELEVANT TO GLAUCOMA

Although there is still much to be explored, neuroinflammatory responses identified to date in glaucoma show some hallmarks of classic inflammatory responses to disease, injury or infection in the central nervous system (CNS). Therefore, we can learn from what has been studied in other diseases, particularly neurodegenerative diseases. The CNS, which includes the brain, retina, and optic nerve, is an immune privileged tissue (Glass et al. 2010; Lampron et al. 2013); in contrast to other tissues, communication between the CNS and the systemic immune system is relatively limited (Glass et al. 2010; Ransohoff and Brown 2012; Lampron et al. 2013). Therefore, immune responses in the CNS are generally mediated by a limited number of cell types.

Major Players in CNS Immune Responses

The CNS includes resident astrocytes and microglial cells (collectively termed glial cells) that perform immune surveillance and mediate primary inflammatory responses to infection, disease, or injury. Astrocytes are glial cells that are in contact with neurons, blood vessels and other glial cells to provide an array of functions including metabolic support, modulation of synaptic activity, regulation of extracellular ion concentrations, and maintenance of the blood–brain barrier (Ullian et al. 2001; Simard and Nedergaard 2004; Iadecola and Nedergaard 2007; Pellerin et al. 2007; Rouach et al. 2008). Microglial cells, myeloid-derived cells that reside in the CNS, are generated from the yolk sac before vascularization and possess phagocytic and antigen-presenting capabilities, although these are more limited than those of peripheral antigen-presenting cells (Ginhoux et al. 2010; Glass et al. 2010; Ransohoff and Brown 2012; Lampron et al. 2013). Blood-derived monocytes, dendritic cells and other immune cells are generally excluded from the CNS parenchyma un-

der healthy conditions (Ransohoff and Brown 2012; Ransohoff and Engelhardt 2012). However, following proinflammatory stimulation, glial cells are activated and produce cytokines and chemokines to recruit infiltrating blood-derived immune cells to amplify the inflammatory response in the CNS (Ransohoff et al. 2003; Callahan and Ransohoff 2004).

Triggering Inflammatory Responses: PAMPs, DAMPs, and Their Sensors

The main role of astrocytes and microglia is to recognize and respond to perturbations in the environment. Perturbations can arise from two major sources: invading microbial pathogens and age- or disease-related stress or injury. Astrocytes and microglial cells possess signaling mechanisms for host defense that are activated by the recognition of structural characteristics found in pathogens, known as pathogen-associated molecular patterns (PAMPs) (Glass et al. 2010; Lampron et al. 2013). Astrocytes and microglial cells also recognize signals released from damaged or stressed cells, known as damage-associated molecular patterns (DAMPs) (Rifkin et al. 2005; Yu et al. 2010; Zhang et al. 2010; Zhu et al. 2011). It has been hypothesized that DAMPs are preexisting intracellular adjuvants that are released when necrotic cell death occurs or when apoptotic cells are not rapidly cleared (Kono and Rock 2008). These DAMPs can also be delivered to the cell surface of damaged cells after injury (Zhu et al. 2011). Molecules identified as bona fide DAMPs include heat shock proteins (HSPs), uric acid, high-mobility group box-1 protein and double stranded DNA (Kono and Rock 2008). The existence of additional DAMPs cannot be ruled out, and efforts are ongoing to identify them. Both PAMPs and DAMPs are recognized by pattern recognition receptors (PRRs) on astrocytes and/or microglia that trigger and mediate the inflammatory response (Fig. 1).

An important class of pattern recognition receptors is the toll-like receptors (TLRs) (Gorina et al. 2009; Downes and Crack 2010; Lehnardt 2010). TLRs can recognize a diverse group of pathogenic molecules that are not present

Figure 1. Inflammatory responses in the CNS are mediated by resident astrocytes and microglia. The neurovascular unit comprises neurons (green), astrocytes (blue, with processes) and components of the blood–brain barrier such as endothelial cells (red, vessel). In addition, microglia (red, with processes) sense environmental changes. Glial cells (astrocytes and microglia) express pattern recognition receptors (PRRs) such as toll-like receptors (TLRs), purinergic receptors (PRs) and scavenger receptors (SRs) to respond to DAMPs released by cells during injury or disease. The activation of these receptors promotes proinflammatory signaling that leads to the production of cytokines and chemokines. These cytokines and chemokines induce changes in the endothelial cells and blood–brain barrier integrity, resulting in recruitment of blood-derived immune cells (blue) and amplification of the innate immune response.

in the host (e.g., lipopolysaccharide and viral RNA), but are also able to recognize endogenously derived molecules that are released from injured or dying cells such as HSP60, HSP70 and α B-crystallin (Takeuchi and Akira 2010; Zhang et al. 2010; Ransohoff and Engelhardt 2012). Whereas all thirteen TLRs are expressed on microglial cells, only TLR2, TLR3, TLR4, TLR5, and TLR9 are expressed on astrocytes (Farina et al. 2007). On activation, TLRs recruit the downstream signal adaptor proteins

MyD88 and TRIF, which lead to the activation of kinases I κ B and MAPK and their downstream group of transcription factors that include members of the NF- κ B, AP-1 and interferon regulator factor families resulting in the transcription of several amplifiers and effectors (Glass et al. 2010; Takeuchi and Akira 2010). Among these amplifiers and effectors are cytokines, such as tumor necrosis factor (TNF)- α , interleukin (IL)-1 β , and IL-6, as well as an array of chemokines (e.g., CCL2, CXCL1, CXCL10).

A second class of pattern recognition receptor is the NOD-like receptors, specifically the NALP family, which are components of the multiprotein complexes termed the “inflammasome” that can modulate the inflammatory response (Takeuchi and Akira 2010; Ransohoff and Brown 2012). Cooperative interactions between TLRs and the inflammasome lead to the maturation and secretion of cytokines such as IL-1 β and IL-18. Other types of pattern recognition receptors include the mannose receptor, scavenger receptor, and the ionic purinergic receptor. The mannose receptor is a C-type lectin that recognizes surfaces that are glycosylated with a mannose moiety (Gazi and Martinez-Pomares 2009) and can trigger immune responses including the activation of the complement cascade (Kerrigan and Brown 2009). Scavenger receptors are involved in the recognition and uptake of oxidized proteins and lipids released by damaged cells (Husemann et al. 2002). The ionic purinergic receptors (such as P2X7R) are activated by ATP released from damaged cells and facilitate formation of the inflammasome (Takeuchi and Akira 2010; Ransohoff and Brown 2012).

Under conditions of injury or disease, astrocytes and microglia become reactive and their PRRs are activated, leading to the generation of innate inflammatory mediators that include members of the complement pathway, cytokines, and several chemokines (such as those listed above). Certainly these proinflammatory molecules affect neuronal function and amplify the inflammatory response of microglial cells during pathological conditions (Glass et al. 2010). Activation of proinflammatory mediators by microglia can result in a cascade of

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events, including proliferation, migration, expression of adhesion molecules on endothelial cells, and the promotion of leukocyte infiltration into the CNS parenchyma through the blood-brain barrier (Ransohoff and Brown 2012).

Transendothelial Migration in the CNS

Migration (or infiltration) of leukocytes (immune cells) into the CNS is a highly regulated process involving interactions between circulating leukocytes and endothelial cells (Fig. 2) (Ransohoff et al. 2003). Transendothelial migration of leukocytes is a multistep process and involves: (1) tethering and rolling of leukocytes on the endothelial cells; (2) activation of key molecules on both endothelial cells and leukocytes; and finally (3) paracellular or transcellular migration of leukocytes into the CNS. Leukocyte infiltration is a common event in CNS disease and injury, and is thought to occur after chronic stress (Ransohoff et al. 2003; Callahan and Ransohoff 2004; Ransohoff and Brown 2012). Leukocyte infiltration is commonly initiated by the release of cytokines and chemokines from glial cells (Carson et al. 2006; Ifergan et al. 2008). Studies using intravital microscopy found that activation of leukocytes was not sufficient to promote interaction of endothelial cells and leu-

kocytes. However, leukocyte tethering was observed when endothelial cells were activated with lipopolysaccharide or TNF- α (Piccio et al. 2002). It is thought that after cytokine stimulation, tethering and rolling occurs as a result of the up-regulation of selectins (e.g., P-selectin) and integrin ligands (e.g., VCAM-1 and ICAM-1) on the lumen of endothelial cells that are sensed by selectin ligands (e.g., PSGL1) and integrins (e.g., LFA1) present on the surface of circulating leukocytes (Lampron et al. 2013). These signaling processes between endothelial cells and leukocytes promote the loosening of the tight junctions between the endothelial cells, facilitating migration of leukocytes into the CNS (Ransohoff et al. 2003; Lampron et al. 2013).

The cellular and molecular mechanisms by which transendothelial migration occurs in the CNS during injury and disease are complex and still not completely understood. More research is needed to elucidate the identity and role of these infiltrating cells during neurodegenerative diseases.

The Complement Cascade Modulates Inflammatory Responses

The complement cascade was originally named as it “complements” immune responses. How-

Figure 2. Transendothelial migration in the CNS. Schematic representation of the different steps involved in leukocyte extravasation. Selectin ligands and molecular adhesion molecules that are up-regulated in endothelial cells at the ONH interact with selectins and integrins present at the cell surface of leukocytes, leading to leukocyte infiltration.

ever, recent and emerging evidence suggests it plays a much more complex role in the CNS in health and disease. Components of the complement cascade play a significant role in immune surveillance and inflammatory processes, both peripherally and in the CNS (Janeway et al. 2001). In addition to its involvement in pathogen targeting and elimination, the complement system is also involved in synapse elimination and clearance of potential mediators of damage or injury (Ricklin et al. 2010; Rosen and Stevens 2010).

The complement system is a cascade of three separate pathways known as the classical, alternative, and lectin pathways (Ricklin et al. 2010). A strong complement response includes opsonization of the foreign pathogen or apoptotic cell/cellular compartment by complement fragments, induction of proinflammatory signaling by anaphylatoxins that recruit macrophages, and enable phagocytosis and the formation of the membrane attack complex (MAC), which leads to the lysis of the targeted cell (Ricklin et al. 2010). The classical pathway is initiated by the C1 complex that recognizes pathogens or DAMPs, leading to the formation of the C3 convertase that cleaves the C3 protein to generate the active fragments C3a and C3b. C3a induces proinflammatory signaling and C3b deposition induces cleavage of the C5 protein to C5a and C5b by C5 convertases. C5a is a potent anaphylatoxin, whereas C5b is a fundamental member of the MAC, a cell lysis-inducing channel. Other complement factors in the MAC are C6, C7, C8, and C9. The C3 convertase is also activated by the alternative and lectin pathways, and all three pathways can result in the activation of the MAC (Ricklin et al. 2010). Activation of the complement system has been observed in the CNS after injury and in neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and glaucoma (Bonifati and Kishore 2007).

Beneficial and Damaging Effects of the Immune Response in the CNS

As described above, the immune response is a complex series of events involving resident glial

cells (e.g., astrocytes and microglia), components of the blood–brain barrier (e.g., endothelial cells), and infiltrating blood-derived cells (e.g., monocytes). The early immune responses are likely the body's natural attempts to minimize damage after an initial injury or insult in chronic and age-related CNS diseases. Later immune responses, as the disease enters a chronic phase, are likely more detrimental. In some cases, these beneficial and detrimental events involve molecules in the same pathway, such as the complement cascade. Therefore, when considering therapies, it is important to fully understand the beneficial and detrimental natures of inflammatory processes in CNS injury and disease.

NEUROINFLAMMATORY RESPONSES IN GLAUCOMA

So far, we have described immune responses as they relate generally to the CNS in response to infection, injury, or disease. We now describe what is known about the role of the immune system in glaucoma, and highlight areas for further investigation.

Activation of Astrocytes and Microglia in the Optic Nerve Head in Early Stages of Glaucoma

Retinal cells and glial cells in the ONH respond rapidly and early to glaucomatous insults, including elevation of IOP. Changes in astrocyte morphology, such as enlargement of the cell body and processes, as well as up-regulation of cytoskeletal and extracellular matrix proteins, have been observed in the retina and ONH in human glaucoma (Wang et al. 2002; Inman and Horner 2007; Hernandez et al. 2008; Kompass et al. 2008; Nikolskaya et al. 2009). Several studies have demonstrated that this reactivity and remodeling of astrocytes, and the increased deposition of extracellular matrix occur in early stages of experimental glaucoma (Johnson and Morrison 2009; Howell et al. 2011a; Johnson et al. 2011; Lye-Barthel et al. 2013; Qu and Jakobs 2013). In fact, just a short term elevation of IOP induces rapid and reversible morphological

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changes in astrocytes of the ONH that include hypertrophy, process retraction, and simplification of the shape without changing gene expression (Sun et al. 2013). Furthermore, changes in gene expression in astrocytes are observed at early stages of experimental glaucoma before RGC axon damage (Johnson et al. 2000, 2011; Howell et al. 2011a; Qu and Jakobs 2013). This early up-regulation of extracellular matrix in astrocytes of the ONH may function to repair or prevent damage to the blood–brain barrier in response to high IOP. This has been shown in other settings (Sofroniew and Vinters 2010). For instance, inhibition of reactive astrogliosis by the conditional deletion of the STAT3 protein in models of spinal cord injury promotes massive infiltration of blood-derived leukocytes and increased neurodegeneration, suggesting that astrocyte reactivity is a protective response in CNS injury (Okada et al. 2006; Herrmann et al. 2008). Consistent with this idea is the observation that components of the basement membrane of the blood–brain barrier, such as collagen, are up-regulated in the ONH in human glaucoma and in experimental models of the disease (Hernandez et al. 1990, 1994; Morrison et al. 1990; Johnson et al. 2011). On the other hand, the overexpression of cell adhesion proteins, including several integrins, by astrocytes in glaucoma could also promote the adhesion and migration of immune cells into damaged areas of the ONH (Howell et al. 2011a; Johnson et al. 2011; Tanigami et al. 2012). Furthermore, ONH astrocytes in human glaucoma and experimental models up-regulate tenascin-C (Pena et al. 1999; Howell et al. 2011a; Johnson et al. 2011), an extracellular matrix glycoprotein that supports proinflammatory responses through TLR4 signaling (Midwood et al. 2009), suggesting a proinflammatory role of astrocytes in glaucoma.

Activation of microglial cells in the ONH and retina has also been observed in experimental models of glaucoma (Neufeld 1999; Yuan and Neufeld 2001; Johnson and Morrison 2009; Ebner et al. 2010; Bosco et al. 2011; Taylor et al. 2011). This microglial reactivity is characterized by increased proliferation, increased phagocytic activity (revealed by an

increase in the phagocytosis-related protein CD68), changes in morphology, and expression of proinflammatory molecules, such as members of the complement cascade, major histocompatibility complex class I, and major histocompatibility complex class II (Ebner et al. 2010; Howell et al. 2011a; Bosco et al. 2012). Although the role of microglia in glaucoma progression is not clear, it is notable that the extent of microglia activation is closely related to axonal degeneration in the ONH. In fact, reduction in microglial number is evident when axonal degeneration (but not high IOP) is prevented by radiation treatment of the eye or head in a mouse model of glaucoma (Howell et al. 2012; Bosco et al. 2012).

What Triggers Inflammatory Responses in Glaucoma?

Early neuroinflammatory responses by astrocytes and microglia occur in glaucoma, but it is not known which stresses or damage-associated molecules trigger the responses. One possibility is that DAMPs are released or presented by stressed or damaged RGC axons in the ONH. In glaucoma, heat shock proteins, a type of DAMP, are up-regulated in response to elevation in IOP (Tezel et al. 2004; Tezel 2011), and levels of a variety of heat shock proteins were also increased in human glaucomatous retinas (Luo et al. 2010). A second possibility is that astrocytes and/or microglia produce DAMPs independent of or before changes in RGCs. One candidate for this is tenascin-C, which is up-regulated in astrocytes of the glaucomatous ONH (Pena et al. 1999; Howell et al. 2011a; Johnson et al. 2011). Tenascin-C is an endogenous activator of the TLR4 in inflammation associated with arthritis (Midwood et al. 2009). Identification of initiator(s) of inflammatory events is a key area of study in glaucoma research.

Inflammatory Signaling Pathways in Glaucoma

Although the specific triggers for inflammatory responses in glaucoma remain poorly defined,

inflammatory processes, mediated in part by astrocytes and resident microglia, clearly play a crucial role in glaucoma. Several studies using transcriptional profiling have identified early up-regulation of genes associated with inflammatory pathways in the retina and ONH in experimental models of glaucoma (Ahmed et al. 2004; Johnson et al. 2007, 2011; Yang et al. 2007; Kompass et al. 2008; Nikolskaya et al. 2009; Panagis et al. 2010; Howell et al. 2011a, 2012). Collectively, these studies showed an up-regulation of inflammatory inducers/sensors (PRRs, e.g., TLRs), transducers (e.g., *Mapk*, *Trif*, *MyD88*) and amplifiers (e.g., *Il1*, *Il6*, *Tnf*, *Ccl2*) in human glaucoma (Luo et al. 2010; Yang et al. 2011; Tezel et al. 2012) and in early stages of experimental glaucoma (Howell et al. 2011a; Johnson et al. 2011). In our study using DBA/2J mice, an inherited model of glaucoma, we were able to define early molecular changes that occur before detectable loss of RGCs and axons (Howell et al. 2011a). Gene ontology analysis revealed that genes associated with “immune response,” “leukocyte activation,” and “chemotaxis” were among the genes up-regulated earliest in mice with high IOP but no detectable loss of axons.

Evidence from both human glaucoma patients and animal models of glaucoma suggest that immune responses are mediated, at least in part, by TLRs. For instance, proteomic analysis of glaucomatous human retinas revealed up-regulation of TLR signaling (Luo et al. 2010). Expression of TLR2, TLR3, and TLR4 was observed in microglia and astrocytes from glaucomatous retinas. In early stages of DBA/2J glaucoma, 11 of the 13 TLRs were up-regulated in the ONH at early stages of DBA/2J glaucoma (Howell et al. 2011a,b). These included *Tlr3*, *Tlr7*, and *Tlr9*, whose proteins detect nucleic acids released from damaged cells, and *Tlr2* and *Tlr4*, whose gene products sense host cell HSPs (Rifkin et al. 2005; Yu et al. 2010; Zhang et al. 2010). The TLR adaptor protein MyD88 and members of the MAPK pathway were also up-regulated in retinas from glaucoma patients and in the ONH of DBA/2J mice (Luo et al. 2010; Howell et al. 2011a,b).

As mentioned previously, engagement of TLRs by DAMPs can lead to the activation of

NF- κ B. Not surprisingly, proteomic analysis of glaucomatous retinas from humans and rats found increased expression of kinases such as RIPK, NIK, and I κ K that are involved in the activation of the NF- κ B pathway (Yang et al. 2011; Tezel et al. 2012). Activation of NF- κ B results in the transcription of genes from the IL-1 cytokine family. Secretion of these cytokines can promote production of a secondary cascade of inflammatory cytokines in microglia (e.g., TNF- α) and astrocytes (e.g., IL-6) that amplifies the immune response by recruiting other cells to the site of damage. In support of this sequence of events, members of the IL-1 family were found to be up-regulated in the ONH at early stages of DBA/2J glaucoma (Howell et al. 2011a,b).

Studies have also suggested an important role for tumor necrosis factor (TNF) family members in the pathogenesis of glaucoma. Increased levels of TNF- α have been found in the aqueous humor, retina, and optic nerve microglia and optic nerve astrocytes from glaucoma patients (Yan et al. 2000; Yuan and Neufeld 2000, 2001; Tezel et al. 2001; Sawada et al. 2010). Activation of the signaling pathway downstream from TNF- α in glaucomatous human retinas has also been reported (Yang et al. 2011). Several ligands and receptors of the TNF family were overexpressed at early stages of DBA/2J glaucoma, although not as early as was observed for the *Il1*-related cytokines (Howell et al. 2011a,b). Genetic and pharmaceutical inhibition of TNF- α activity in experimental models of glaucoma can prevent microglia activation, axonal degeneration and RGC loss (Fig. 3) (Nakazawa et al. 2006; Roh et al. 2012). Another member of the TNF family implicated in glaucoma pathogenesis is the proapoptotic protein Fas ligand, which was found to be damaging to RGCs in DBA/2J glaucoma (Gregory et al. 2011). Inhibition of Fas ligand activity prevented death of RGCs after intraocular injection of TNF- α , indicating that Fas ligand mediates TNF- α cytotoxicity in RGCs (Gregory et al. 2011).

In summary, it has been clearly demonstrated that proinflammatory pathways (likely activated by DAMPs) are important contributors to the early progression of glaucoma. Further

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Figure 3. Inhibition of TNF- α activity reduces microglial activation and axonal degeneration in the optic nerve after ocular hypertension. (A) Ocular hypertension (OHT) increased the number of Iba-1-positive microglial cells in the ONH and induced TNF- α expression in these cells. Treatment with the TNF- α inhibitor Etanercept (Etan.) significantly reduced the number of microglia and the expression of TNF- α . (B) Axonal degeneration in the optic nerve is reduced by Etanercept treatment in ocular hypertension (Roh et al. 2012). Mann-Whitney U test, ** $P < 0.01$ comparing control vs. Etanercept treated ocular hypertension; †† $P < 0.01$ comparing vehicle vs. Etanercept treated OHT. (Adapted from data in Roh et al. 2012.)

studies are needed to identify the precise roles of the different cell types at different stages of disease, keeping in mind that inflammatory molecules can mediate both damaging and beneficial responses.

Transendothelial Migration of Leukocytes in Glaucoma

One of the earliest processes to be significantly up-regulated in our transcriptional profiling of DBA/2J mice was transendothelial migration of leukocytes (Howell et al. 2012). In DBA/2J glaucoma, up-regulation of selectins (e.g., P-selectin), adhesion molecules (e.g., VCAM-1) and chemokines (e.g., CCL2) was observed at early stages of disease (Howell et al. 2012). Flow cytometry experiments confirmed the entry of monocyte-derived cells into the ONH (Fig. 4A)

(Howell et al. 2012). No other type of immune cell (including B cells or T cells) was found in DBA/2J glaucomatous tissue, although one study has shown B cells in human glaucomatous retinas (Gramlich et al. 2013). Monocyte infiltration was apparently blocked by DBA/2J mice treated with radiation (Fig. 4B) indicating that monocyte infiltration could be an important mediator of RGC and axonal damage in glaucoma (Howell et al. 2012). In support of this, a second study using a different experimental mouse model of glaucoma found that deletion of CD11b prevented the activation of monocyte-derived cells and loss of RGCs after induction of high IOP (Nakazawa et al. 2006). However, this study was not able to distinguish between resident microglia and infiltrating monocytes. The mechanism(s) by which monocytes affect RGCs in glaucoma has not been

Figure 4. Monocyte infiltration occurs early in DBA/2J glaucoma. (A) Flow cytometry revealed that the major blood-derived immune cell detected in the ONH of DBA/2J glaucomatous mice was the CD11b⁺Cd11c⁺ monocyte. These cells were completely absent in radiation-treated DBA/2J eyes (Rad-D2) or control (D2-Gp) eyes. (B) Cell infiltration was also assessed using the injection of a fluorescent tracer into the spleen (CFDA, green). Spleen-derived CFDA⁺ cells entered the optic nerves of untreated DBA/2J eyes but not Rad-D2 eyes or control eyes (Howell et al. 2012). (From Howell et al. 2013; adapted, with permission, from the authors.)

elucidated and further studies are needed to define the contribution of these infiltrating monocytes in glaucoma progression.

The Complement Cascade in Glaucoma

Components of the complement cascade are induced in human and animal models of glaucoma, suggesting a key role for this system in progression of the disease (Ahmed et al. 2004; Kuehn et al. 2006, 2008; Stasi et al. 2006; Steele et al. 2006; Stevens et al. 2007; Ren and Danias 2010; Tezel et al. 2010; Howell et al. 2011a). In experimental models of glaucoma, induction of complement components in the retina and

ONH was one of the earliest signaling responses to high IOP (Howell et al. 2011a; Johnson et al. 2011). Up-regulation of *C1qa*, a member of the C1 complex that triggers activation of the classical pathway, was observed in microglial cells in the ONH before detection of axonal damage in DBA/2J glaucomatous mice (Howell et al. 2011a). Deposition of C1QA protein was also observed in RGC dendrites in glaucomatous DBA/2J retinas as well as in primate and human glaucomatous retinas (Fig. 5A) (Kuehn et al. 2006; Stasi et al. 2006; Stevens et al. 2007; Tezel et al. 2010), suggesting an involvement of the complement cascade in pathological synapse elimination and/or dendrite remodeling in

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Figure 5. The complement system is activated in human and animal models of glaucoma. (A) C1Q protein is increased in RGCs and in the inner plexiform layer of human (Tezel et al. 2010), primate (Stasi et al. 2006), and mouse (Howell et al. 2011a) retinas in response to high IOP. (B) MAC deposition is found in RGCs from C5-sufficient glaucomatous DBA/2J.C5^{B6} mice (Howell et al. 2013). TUBB3, tubulin β -3; NOE, normal or early.

glaucoma. Importantly, DBA/2J mice deficient in C1QA were protected from glaucomatous RGC loss, demonstrating an important and damaging contribution for *C1qa* in glaucoma (Howell et al. 2011a).

A second component of the complement cascade that likely plays a damaging role in glaucoma is complement component C5, a necessary component in generation of the MAC. Significant deposition of MAC was found in glaucomatous RGCs in human eyes and in experimental

models of glaucoma (Kuehn et al. 2006; Jha et al. 2011; Howell et al. 2013). Drug inhibition of complement activation reduced MAC deposition and apoptosis of RGCs in a rat model of glaucoma (Jha et al. 2011). Furthermore, C5-deficient DBA/2J mice showed reduced neurodegeneration compared with C5-sufficient DBA/2J mice (Howell et al. 2013). Although it is not clear how C5 contributes to glaucoma, significant deposition of MACs was observed in RGCs and in dystrophic neurites in the optic

Figure 6. A model of early neuroinflammatory responses in glaucoma. We hypothesize that initiation of these immune responses occurs after the release of DAMPs from RGCs, glial cells or both. The TLRs expressed in glial cells activate the production and secretion of cytokines such as those of the IL-1 family. A secondary expression of cytokines, such as TNF- α in microglia and IL-6 in astrocytes, is induced, leading to an amplified inflammatory response. These neuroinflammatory responses are likely modulated by complement proteins, such as C1qa, in the ONH. In addition, intrinsic up-regulation of complement molecules in RGCs (such as C1qa and C3) occurs early and mediates synaptic dysfunction. The cell types shown here are described in the legend for Figure 1.

nerve (Fig. 5B), suggesting a detrimental contribution of C5b to glaucoma progression.

Much work is being done (in our laboratory, the Danias laboratory, and others) regarding the role of the complement cascade in glaucoma. Areas of active research include assessment of other key components of the cascade such as C3 and C4. Complement proteins are expressed in multiple cell types and it will be important to understand the cell-specific roles of complement components in glaucoma.

CONCLUDING REMARKS

In recent years a critical role for neuroinflammatory processes mediated by astrocytes, microglia, endothelial cells, infiltrating monocytes and other cell types in the pathogenesis of glaucoma has been demonstrated (Fig. 6). However, many challenges remain. The potential for

beneficial and detrimental inflammatory processes occurring at different stages of glaucoma make developing therapies that target these processes a complex but, in our view, solvable problem. Furthermore, these cells do not function in isolation, and understanding the changes to the neurovascular unit as a whole will also be critical. Development of a detailed spatial and temporal understanding of these neuroinflammatory events in humans and in multiple animal models is critical as we move toward new neuroprotective treatments for human glaucoma.

ACKNOWLEDGMENTS

The authors thank Pete Williams and Jeffrey Harder for critical comments. This work was supported by The Glaucoma Foundation (GRH) and EY021525 (GRH).

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Cold Spring Harb Perspect Med 2014; doi: 10.1101/cshperspect.a017269 originally published online July 3, 2014

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