Understanding Glaucomatous Optic Neuropathy: The Synergy Between Clinical Observation and Investigation

Harry A. Quigley

Glaucoma Center of Excellence, Wilmer Institute, Johns Hopkins University School of Medicine, Baltimore, Maryland 21287; email: hquigley@jhmi.edu

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Keywords

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Abstract

Glaucoma is a complex disorder of aging defined by the death of retinal ganglion cells and remodeling of connective tissues at the optic nerve head. Intraocular pressure-induced axonal injury at the optic nerve head leads to apoptosis. Loss of retinal ganglion cells follows a slowly progressive sequence. Clinical features of the disease have suggested and corroborated pathological events. The death of retinal ganglion cells causes secondary loss of neurons in the brain, but only as a by-product of injury to the retinal ganglion cells. Although therapy to lower intraocular pressure is moderately effective, new treatments are being developed to alter the remodeling of ocular connective tissue, to interrupt the injury signal from axon to soma, and to upregulate a variety of survival mechanisms.

1. INTRODUCTION

IOP: intraocular pressure

OAG: open-angle glaucoma

RGC: retinal ganglion cell

ONH: optic nerve head

NFL: nerve fiber layer

Our understanding of glaucoma has improved substantially during the past two decades. Previously, its features were known only through pathology specimens of blind, painful eyes (Friedenwald et al. 1952, pp. 306–7). Recent improvements in diagnostic testing (Bussel et al. 2014, Harwerth et al. 2010, Heijl et al. 2013, Medeiros et al. 2012) and data from population-based surveys (Hollows & Graham 1966, Tielsch et al. 1991) have clarified clinical phenotypes and risk factors (Leske 2007). The level of intraocular pressure (IOP) is causally related to open-angle glaucoma (OAG), but many people with OAG have normal IOP (Drance 1972). IOP lowering slows glaucoma damage, even with normal baseline IOP (Collaborative Normal-Tension Glaucoma Study Group 1998). OAG is caused by neurodegeneration of retinal ganglion cells (RGCs) and most patients are not seriously impaired (Chauhan et al. 2014), but it is sufficiently prevalent that it represents the second leading cause of worldwide blindness (Tham et al. 2014).

Glaucoma is present when there is an excavated optic nerve head (ONH) and concomitant defects are identified during visual field testing (**Figure 1**) (Foster et al. 2002). Normal ONH structure (Anderson 1969) is progressively altered in glaucomatous eyes (Emery et al. 1974), with outward collapse of the supporting connective tissue. In primary human OAG, RGCs are the only retinal neurons to die (Kendell et al. 1995), although photoreceptor alterations have been suggested in animal models (Lei et al. 2011, Nork et al. 2000) but not confirmed (Kielczewski et al. 2005, Wygnanski et al. 1995, Jakobs et al. 2005). This review summarizes knowledge of the pathogenesis of RGC loss in glaucoma and suggests that better understanding of this loss can lead to new therapies.

2. RGCs AND OPTIC NERVE HEAD DEVELOPMENT

RGCs are the first neurons to differentiate in the embryonic retina under the genetic influence of sonic hedgehog, Pax6, Ath5, Pou4f2, and Wt1. Some genes determine the initial RGC phenotype and others act to develop RGC dendrites and axon growth (Isenmann et al. 2003). During the first trimester, RGC axons traverse the superficial retina, exit the ONH, correctly choose the proper path at the chiasm, and synapse predominately with midbrain (rodent) or thalamic (primate) targets under the influence of genetic expression (Kunzevitzky et al. 2011), patterns of electrical activity (Penn et al. 1998, Xu et al. 2011, Goldberg et al. 2002), and associated glia (Ray & Kay 2015). There are specific topographical maps of the retina preserved in the thalamus and cortex. Thus, glaucoma injury to RGC axons at the ONH causes loss of neighboring RGC bodies in the retina and their adjacent groups of RGC axons in the nerve fiber layer (NFL) (Radius & Anderson 1979, Minckler 1980). Clinicians can, therefore, determine with some precision the location of a lesion using the known topography of RGC axons within the anterior visual pathway (Hoyt & Luis 1962).

The majority of embryonic RGCs die by apoptosis due to failure to reach their target neuron (Perry et al. 1983, Crespo et al. 1985, Galli-Resta & Ensini 1996). RGCs that find an appropriate partner are provided with sustaining neurotrophins, which inhibit programmed cell death (Raff et al. 1993). Genetic knockout of genes participating in apoptosis, such as *Bax*, results in a doubling of the normal number of RGCs (Mosinger Ogilvie et al. 1998). Thus, the baseline number of RGCs depends on the number originally generated minus the proportion that fails to survive initially.

The area (diameter) of the normal human ONH varies over a 5-fold range (**Figure 1*a*,*b***) (Quigley et al. 1990). In addition, the portion of the ONH occupied by RGC axons (the so-called rim area) increases proportionately with larger disc diameter (Caprioli & Miller 1987). Hence, the normal number of RGCs and their axons varies over an order of magnitude. RGC axon outgrowth occurs during the first trimester, but the peripapillary sclera and Bruch's membrane

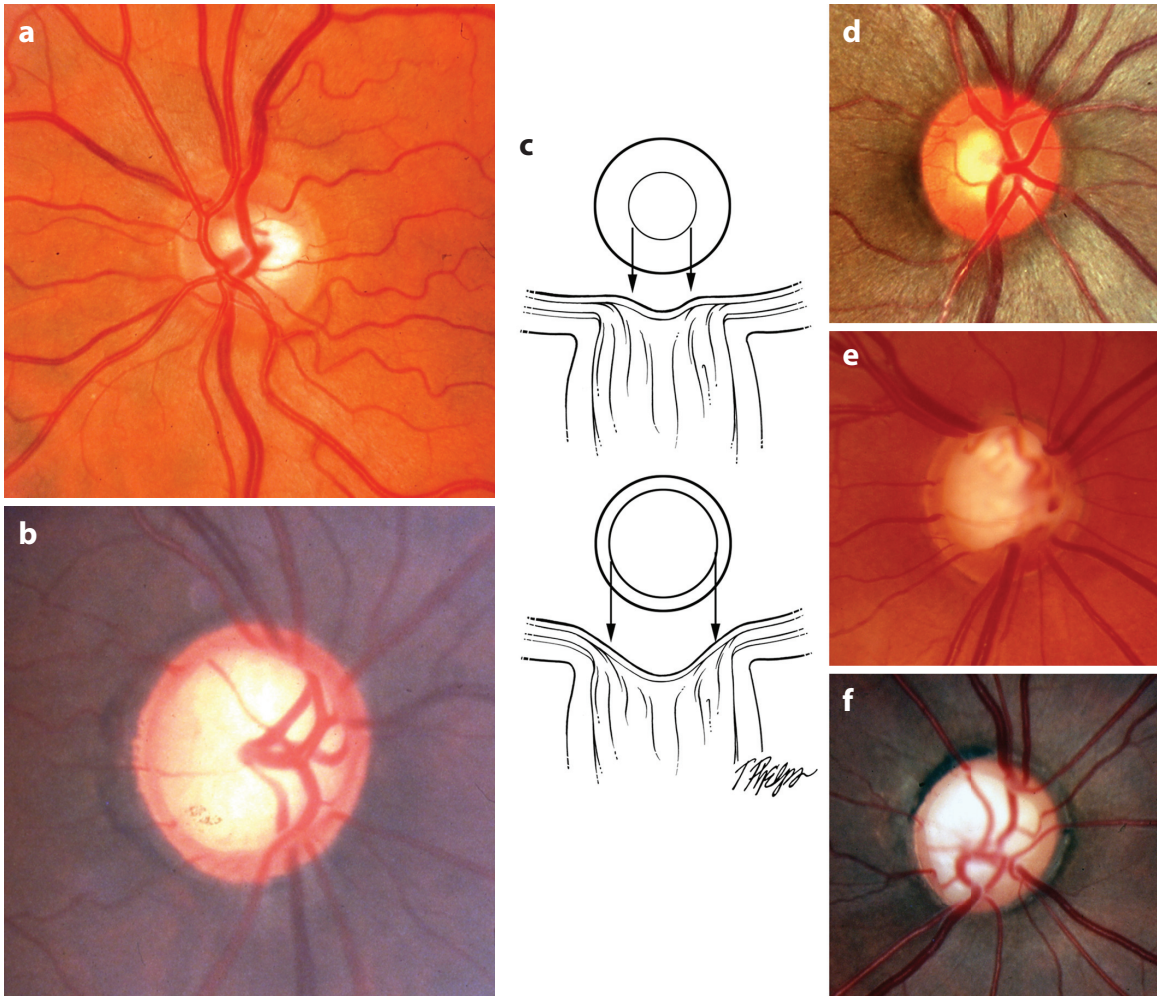

Figure 1

The clinical view of the optic nerve head (optic disc) is of a round to oval structure from which retinal blood vessels emanate. There is a central depression (the cup) representing space not occupied by the axons of retinal ganglion cells (RGCs). (*a*) A normal disc, small in diameter. (*b*) A normal disc that is large in diameter, showing how large the cup area can be in such large-diameter discs. (*c*) In glaucoma, the RGC axons atrophy, enlarging the cup (narrowing the rim of axonal tissue), as seen in the lower schema. (*d–f*) A series of discs from normal at the top to (*e*) moderate glaucoma and (*f*) severe glaucoma damage.

develop only in late gestation (Quigley 1977). It is logical that larger diameter ONHs have more axons because axons have exited to the brain embryologically before the collagenous sclera forms to surround them. Having more RGCs at baseline is clearly a buffer against losing them to glaucoma. Interestingly, larger ONHs, despite having more RGCs, are at greater risk from OAG (Healey & Mitchell 2000). This is consistent with biomechanical considerations because a larger ONH opening would be subject to more strain at a given level of IOP. African-derived persons have both a larger ONH area and less rim area at the same ONH area compared with European-derived persons (Varma et al. 1994). Both attributes may contribute to the relatively greater OAG prevalence among African-derived persons (Sommer et al. 1991).

RGC numbers decline with age in rodents (Danias et al. 2003) and humans, whose rate of decline accelerates after age 50 (Kerrigan-Baumrind et al. 2000). In population-based data, the higher the IOP, the lower the rim area (Varma et al. 1995), suggesting that there may be IOP-related RGC loss in many persons that never reaches the threshold to produce the clinical OAG phenotype. The loss of RGCs is found in many other disorders, such as ischemic optic neuropathy, hereditary optic atrophies, and systemic disorders such as Alzheimer's disease. However, each differs from glaucoma in the pattern and time course of RGC loss and, critically, all of the nonglaucoma optic neuropathies lack excavation of ONH connective tissue (Quigley et al. 1982, Danesh-Meyer et al. 2010).

LGN: lateral geniculate nucleus

CNS: central nervous system

3. SIX ZONES OF THE RGC

RGCs interact with more cell types than any other retinal neuron. With cell body diameters of 10–30 μm, their axon, only 1 μm wide, stretches 50 mm from retina to brain. The axon has 10 times the volume of the cell body and 125 times larger surface area, so distant maintenance and local mechanisms of repair are vital to its survival. Similarly, weakness in the ability to recover from injury to the axon may underlie glaucoma susceptibility in some persons. Although RGCs are outnumbered by photoreceptors 130 to 1, each RGC synapses at its dendrites with bipolar and amacrine cells. In addition, RGCs are electrically coupled to one another (Vaney 1991). At the axon terminal, most RGCs synapse with lateral geniculate nucleus (LGN) neurons and interneurons, but a minority terminates in other brain centers. In addition, RGCs interact with astrocytes, microglia, and Müller cells in the retina; with astrocytes and oligodendrocytes in the optic nerve and tract; and with central nervous system (CNS) glia in the LGN. There are six distinct zones for RGCs, each with unique structure and function that are relevant to glaucoma (Yu et al. 2013).

3.1. RGC Dendrites

RGC dendrites synapse with bipolar and amacrine cells in the inner plexiform layer, collecting information in their receptive fields from rods and cones. More than one dozen types of RGC are recognized by size and dendritic spread in the primate retina, the majority (80%) focused on color and detailed information. RGCs and their dendritic fields are small near the fovea and larger toward the periphery. Their dendrites occupy either the inner or the outer half, or both, of the inner plexiform layer, depending upon whether they increase (on cells) or decrease (off cells) firing with center field stimulation (Nelson et al. 1978). Large and small RGCs are arranged in regular, superimposed bilevel mosaics across the mammalian retina (Wassle et al. 1981). There is minimal overlap between adjacent RGCs of the same type, although different types freely occupy the same physical and functional spaces. Larger RGCs are estimated to have convergent input from 15,000 cones and make 10,000 dendritic synapses. Foveal cells, by contrast, receive input from eight cones and four bipolars (Kolb & Nelson 1993). Thus, clinical visual field studies of human glaucoma with fixed test spot sizes stimulate different numbers and types of RGCs at different eccentricities. RGCs share synchronous synaptic signals from amacrine and bipolar cells that are distributed onto multiple RGCs. So although RGCs have their own individual anatomic turf, their physiological responses result from highly correlated firing patterns among neighboring cells. This is an important factor to consider when attempting to correlate structural and functional measures of glaucoma damage.

3.2. RGC Bodies

RGC bodies are found mostly in the ganglion cell layer, although a minority (Nadal-Nicolas et al. 2014) resides in the inner nuclear layer (the so-called displaced RGCs). These displaced RGCs are

not included in quantifications by optical coherence tomography (OCT) that measure ganglion cell and NFL thicknesses. In humans, RGC somas are stacked five high near the border of the macular capillary-free zone, comprising 100 μm in thickness in OCT images. Their density falls off peripherally. Most RGCs are physically centered on their receptive fields, except foveal RGCs, which are displaced outside the fovea, a factor that must be accounted for in direct structure–function correlations in glaucoma tests (Raza et al. 2014). The axon hillock, where spike-like action potentials (APs) are generated, has the highest concentration of voltage-sensitive sodium channels and mitochondria to serve its high energy consumption (Wollner & Catterall 1986). The inner two-thirds of the retina, including RGC dendrites, somas, and axons in the NFL, is nourished by the retinal vascular circulation, which has a blood–brain barrier that keeps most proteins from its extravascular space. In addition, stable retinal blood flow is maintained by vigorous autoregulation when there are alterations in IOP and blood pressure (Arciero et al. 2013). Abnormalities in vascular nutrition at the ONH may contribute to glaucoma damage, although defects in retinal blood flow alone do not reproduce the clinical picture of glaucoma (Cherecheanu et al. 2013).

OCT: optical coherence tomography

AP: action potential

3.3. Unmyelinated RGC Axons in the Retina

From the RGC soma, unmyelinated, unbranched axons course to the ONH (**Figure 2**). Those from RGCs in the central–temporal 15° pass over or under the fovea. The RGC body carries out much of the nucleic acid and protein synthesis for RGCs, although extranuclear RNA translation and mitochondrial DNA expression occur along the axon. If the RGC soma were basketball-sized, the corresponding axon would be half a mile long. To communicate along this vast distance, proteins and other molecules are carried by anterograde axonal transport to the brain and by retrograde transport back to the soma. Vesicles and mitochondria move at faster and slower rates through this energy-requiring transport process. Axonal transport is clearly abnormal in glaucoma at the ONH (see below) (Anderson & Hendrickson 1974).

RGCs fire continuous trains of APs along the axon, and changes in AP frequency are the RGC signaling language. APs move slower along the unmyelinated retinal axon than in the retrobulbar nerve, where myelination allows saltatory conduction. The first three RGC compartments are contained within the eye and thus are not exposed to pressure differentials between the IOP and pressure outside the eye (IOP-induced mechanical stress). There is no evidence that changes in eye pressure have a direct, hydrostatic influence on these three intraocular zones. Type 1 astrocytes are found in the NFL, but at the ONH there is greater astrocytic variety (Hernandez 2000). With RGC death in glaucoma, the remaining astrocytes comprise a residual NFL thickness of 50 μm, as seen in OCT images.

In some diseases, the RGC axons of the retina and superficial ONH thicken due to the backup of fast and slow phases of axonal transport. One such cause is vascular insufficiency (anterior ischemic optic neuropathy); another cause is elevated cerebrospinal fluid pressure, which alters transport due to the reduction or reversal of the trans-lamina cribrosa pressure differential (Minckler et al. 1976b). Even though the microscopic and biochemical abnormalities caused by OAG are within the ONH, no clinical signs of this initial injury are detected by present diagnostic techniques until there is frank RGC axon loss seen in the retinal NFL and loss of the ONH rim tissue. The single exception is an occasional flame-shaped hemorrhage in the NFL at the ONH rim, known to be an epiphenomenon strongly associated with ongoing NFL damage (Drance 1989).

This RGC zone comprises the tissue marked as the cup to disc ratio. The vast majority of the ONH rim consists of unmyelinated RGC axons, and only when axons die in considerable numbers is their absence clinically detectable. Due to substantial variation in the size and configuration of the human ONH, the precision with which glaucoma damage can be detected or followed is

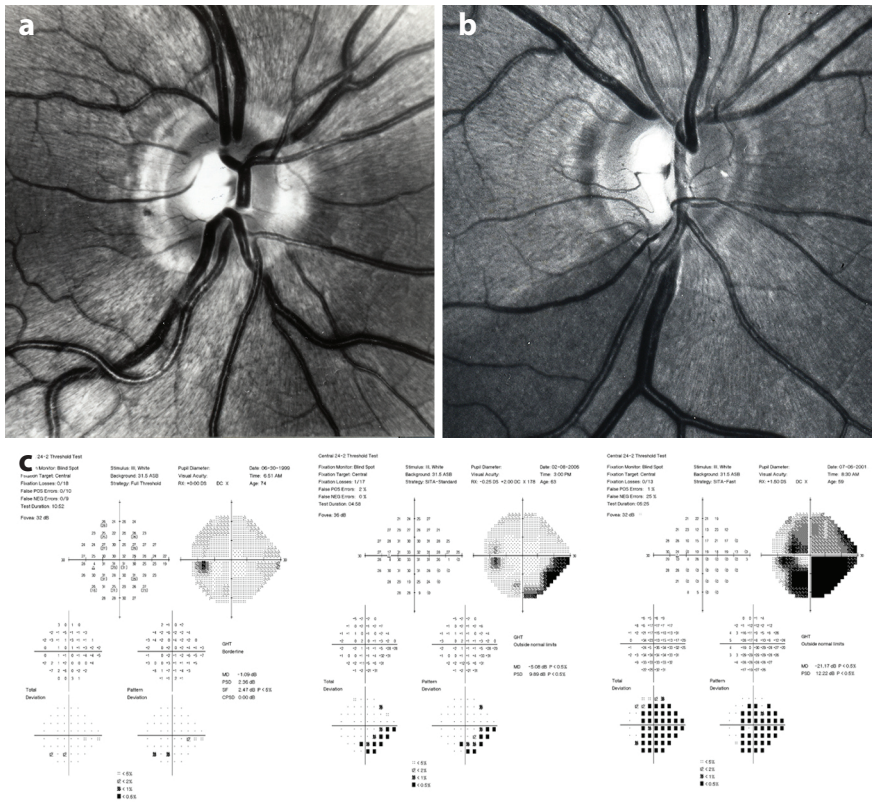

Figure 2

(*a*) The retinal ganglion cell axons are seen as white, linear reflexes arching into the normal disc. (*b*) In this glaucomatous eye, there is focal loss of axons in a dark wedge at the six to eight o'clock position. (*c*) Results of visual field testing from patients with normal (*left*) to mild inferior loss (*middle*) to severe upper and lower field loss (*right*).

less than ideally precise. OCT imaging has significantly improved the quantitative assessment of this area (Chauhan et al. 2013). The physical changes in the ONH rim in glaucoma are a combination of both the loss of RGC axons and connective tissue modifications. Because the connective tissue remodeling does not occur in nonglaucomatous optic neuropathy, this represents the most important diagnostic differentiation of glaucoma from neurological optic atrophy. When the IOP level changes, the ONH supportive tissue retains sufficient elasticity to exhibit reversible movements (Lee et al. 2012). Because these first three zones of RGC structure are those that can be imaged with greatest resolution, it would be extremely useful to identify reversible features of axonal abnormality in glaucoma that occur prior to axon loss. As yet, no method of imaging the NFL appears to satisfy this criterion (Fortune et al. 2013, Xu et al. 2013).

3.4. Unmyelinated Axons in the Lamina Cribrosa

At the ONH, in monkey and human eyes, RGC axons remain unmyelinated until the posterior limit of the lamina cribrosa, but in rodents, myelin begins about 1 mm behind the eye. Axons must undergo a 45–135° turn into the optic nerve, depending upon the particular ONH configuration.

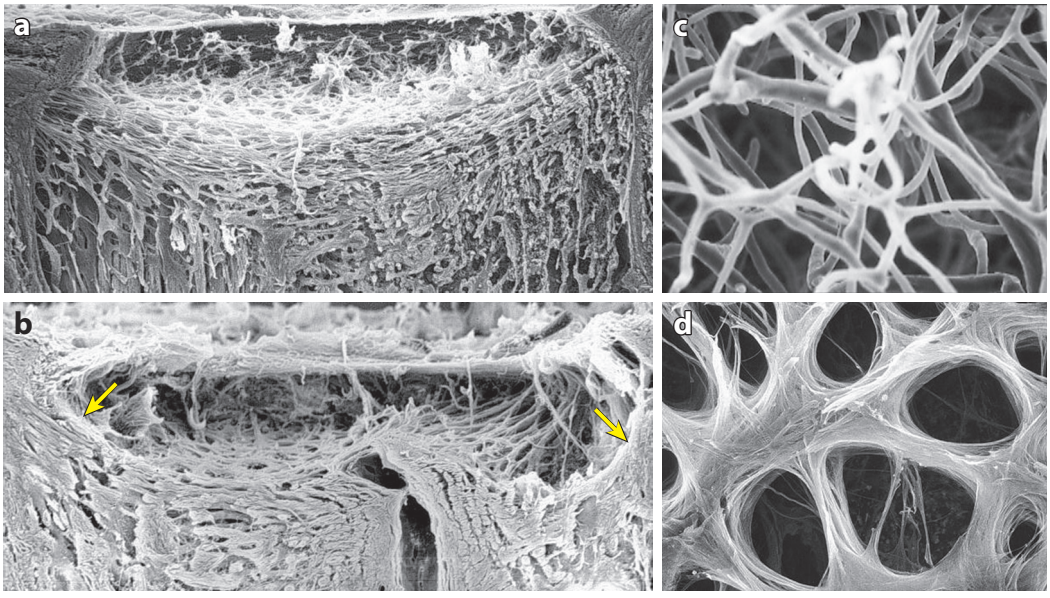

Figure 3

Scanning electron micrographs of the optic disc sectioned along its superior–inferior axis, with nerve tissues digested to show lamina cribrosa connective tissue beams. (*a*) Normal nerve head. (*b*) Glaucomatous nerve head with remodeling of connective tissues into bowed outward zones in upper (*left*) and lower (*right*) portions of the nerve head (*arrows*). (*c*) Capillaries from an epoxy cast of the nerve head are found within the connective tissue beams; (*d*) seen at high power in the same orientation.

This area continues to undergo dramatic alteration in overall connective tissue structure even into the teenage years (Kim et al. 2012). At the lamina, RGC axons are separated into bundles by astroglial columns at the level of the choroid and by connective tissue beams at the scleral level (**Figure 3**). Detailed study of the ONH in experimental monkey and human glaucoma has shown that it is at the level of scleral laminar beams that axonal abnormality is microscopically visible (Quigley & Anderson 1976).

The lamina beams are lined by astrocytes and their basement membranes, within which lie connective tissue, with collagen, elastin, and fibroblasts, and the capillaries supplying local nutrition (**Figure 3**). ONH capillaries are continuous with retinal and optic nerve capillaries and have tight junctions similar to CNS capillaries. However, laminar axons are exposed to many proteins leaking from choriocapillaries at the ONH periphery. These beams are attached to the sclera at the ONH canal wall, and they are subject to mechanical stress produced by two forces: the circumferentially directed hoop stress of the peripapillary sclera generated by IOP and the stress produced by the translaminar gradient between normally higher IOP and lower optic nerve tissue pressure (Morgan et al. 1998). Thus, the laminar capillaries and the axons must withstand mechanical stresses not seen in either the retina or retrobulbar optic nerve. Furthermore, at the posterior lamina border is the first node of Ranvier. The high concentration of mitochondria at this site has been interpreted as showing a high metabolic requirement (Minckler et al. 1976a). Astrocytes have many processes among laminar axons, and their production of cytokines in glaucoma may affect the RGC axons both beneficially and detrimentally.

There is a physiological flow of extracellular fluid from the vitreous humor to the extracellular optic nerve (Quigley et al. 1979). In diseases causing rapid optic atrophy, the spaces vacated by axons in the optic nerve accumulate glycoproteins from the vitreous through this flow, historically

referred to as Schnabel's cavernous atrophy. This was formerly thought to be characteristic of OAG, but it is seen histopathologically only in unusual, secondary glaucomas with very high IOP and in acute, severe optic neuropathies, and thus it is not a typical feature of OAG (Quigley & Green 1979).

3.5. Myelinated Optic Nerve

At the posterior limit of the lamina cribrosa, oligodendrocytes myelinate RGC axons. This region also has astrocytes and microglia, and its connective tissue beams widen and take on a wavier course in histological section, suggesting freedom from the mechanical stress at the lamina itself. In experimental monkey glaucoma, connective tissue septa from the myelinated nerve may be recruited (Roberts et al. 2009), straightening as IOP transmits stress to them via indirect connections through the meninges. Although the anterior lamina cribrosa limit is easily visible in spectral domain OCT images, its posterior limit cannot be identified precisely with clinical imaging. RGC axons generally are arranged in an organized fashion in the NFL, with the more peripherally located RGC axons in the outer NFL and the more posterior ones in the inner NFL. At the ONH, the posterior and central RGC axons occupy the temporal and central pores. Behind the globe, they rearrange along the nerve and tract, as axons from the nasal RGCs cross at the chiasm to the opposite side of the brain. Myelination confers a lower energy requirement and 10 times faster conduction for APs than in the retina (Ogden & Miller 1966). This is the longest, and volumetrically largest, zone of RGCs, extending approximately 5 cm from the eye to the thalamus.

3.6. Synaptic Zone in the Lateral Geniculate Nucleus

Most RGC axons in monkeys and humans terminate at the LGN, some go to the superior colliculus and other centers, including melanopsin-containing RGCs that control pupil movement. Some axons may have branches to more than one location. At the six layers of the LGN, axons from each eye end at one of three layers specific to their cell type (two parvocellular and one magnocellular), and they are also arranged topographically by retinal location. Because the layers from the two eyes are adjacent but separate, it is not until the second synapse at the occipital cortex that binocular representation converges onto single neurons. The intimate relationship between LGN neurons and RGCs is vital to the survival of RGCs in embryological life because RGCs that are not properly connected by LGN layer and region do not receive sufficient neurotrophic support and die by programmed cell death. The loss of neurotrophic support due to axonal transport interruption is hypothesized to be one mechanism of glaucomatous RGC death (Martin et al. 2003). The death of RGCs is associated with the secondary loss of LGN (Chaturvedi et al. 1993, Weber et al. 2000, Yücel & Gupta 2008) and occipital cortex neurons through anterograde degeneration, which is detectable by magnetic resonance imaging (Gupta et al. 2009). There is no evidence that this indicates a primary CNS pathology in glaucoma, and statements indicating that glaucoma is a brain disease are without foundation. Although anterograde atrophy in OAG is routine, there is no retrograde loss of neurons in the inner and outer nuclear layers. Yet in primary photoreceptor disease, consecutive neuron loss and disorganization ensue in the mid- and inner retina (Fariss et al. 2000).

RGCs with larger soma and axon diameters, which project to the magnocellular LGN layers, are quantitatively lost first in monkey and human OAG (Quigley et al. 1987). This selectivity has been exploited in the development of a functional test (Maddess & Severt 1999) using the frequency doubling phenomenon that is embodied in an existing commercial instrument. However, larger RGCs represent only 10% of all RGCs in the retina, so by the time even one-third of RGCs are

gone, more small RGCs have died than larger ones. This may explain some failed attempts to devise psychophysical tests that better identify OAG based on the selective loss of RGCs. There is no question that the clinical features of OAG are different from those of other optic neuropathies, even those such as Leber's optic neuropathy (Yu-Wai-Man et al. 2011) and nutritional neuropathy, that affect RGCs near the ONH; the latter conditions specifically affect foveal (midget) RGCs that subserve central visual acuity and color vision, which are spared until late in OAG.

4. RGC INJURY IS (AT LEAST) AT THE DISC

Many human and animal studies of glaucoma have detected primary RGC abnormality in axons at or near the scleral level of the ONH, where both anterograde and retrograde axonal transport are interrupted in mice, rat, monkey, and human glaucoma (**Figure 4**) (Minckler et al. 1978,

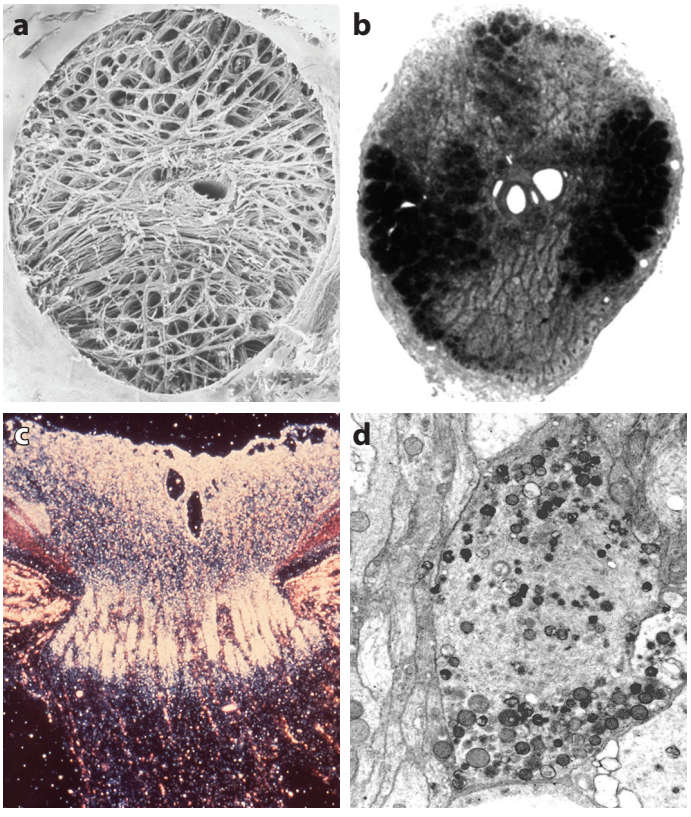

Figure 4

(*a*) Scanning electron micrograph of optic disc digest showing connective tissues as seen from the inside of the eye, similar to the clinical view. Note that the pores are larger and the connective tissue is less dense both superiorly and inferiorly. (*b*) Cross section of myelinated optic nerve posterior to the eye in glaucoma, with typical loss of axons (decrease in dark myelin stain) superiorly and inferiorly, matching the lack of connective tissue support in the lamina cribrosa (*a*). (*c*) Blockade of axonal transport (*white*) of radioactive protein in monkey glaucoma model. The site of the block is within the lamina cribrosa of the nerve head. (*d*) Axonal transport block seen by transmission electron microscopy in retinal ganglion cell axons of experimental mouse glaucoma eye at the area just behind the scleral level of the nerve head, indicated by the local accumulation of organelles in a dilated axon.

Quigley & Addicks 1980, Quigley et al. 1981, Salinas-Navarro et al. 2010, Crish et al. 2010). This is so fundamental that animal models that are claimed to mimic human OAG should demonstrate more than simple RGC death. The injury typical of glaucoma that animal models must duplicate is axonal injury at the ONH leading to the selective loss of RGCs with sparing of remaining retinal neurons, including amacrine cells (Kielczewski et al. 2005) and photoreceptors (Kendell et al. 1995). An additional characteristic feature is the widening of the optic nerve canal and alteration of astrocytic or collagenous lamina cribrosa structure. There are many similarities among optic nerve crush and transection models and glaucoma models induced by experimental IOP elevation, although differences are apparent as well (Yang et al. 2007). The production of RGC death by intravitreal injection of toxins (e.g., glutamate or *N*-methyl D-aspartate) produces RGC damage, but is far from a simulation of glaucoma.

The physical reconfiguration at the ONH lamina cribrosa in glaucoma both defines its clinical picture (optic disc excavation) and provides an explanation for the selective loss of RGCs, whose axons pass through the upper and lower ONH (Emery et al. 1974, Radius et al. 1978, Quigley & Addicks 1981). It is in these two areas where the connective tissue support is least and where axons die first. Biomechanical modeling studies have pointed to the ONH and peripapillary sclera as the zones of the greatest IOP-induced strain (Norman et al. 2011, Grytz et al. 2012, Coudrillier et al. 2012). Any disease that injures RGC axons at the ONH would exhibit sectoral patterns of functional loss, as found in monkey, rat, and mouse eyes when IOP is experimentally elevated, despite species differences in ONH anatomy. Anterior ischemic optic neuropathy injures RGC axons at the ONH, but that condition lacks the connective tissue excavation of OAG and thus the ONH has a different clinical appearance (Danesh-Meyer et al. 2010).

Direct mechanical compression of neuronal processes can block axonal transport (Hahnenberger 1980). Yet multiple causes probably underlie glaucoma damage, and the contribution of various factors surely differs among glaucoma patients. There is a high RGC energy requirement at the ONH, indicated by its high concentration of mitochondria (Minckler et al. 1976a, Andrews et al. 1999, Yu et al. 2013). In experimental monkey glaucoma, mitochondrial transport is impeded at the ONH (Gaasterland et al. 1978), and altered mitochondrial structure has been observed in experimental glaucoma (Ju et al. 2008). There is strong epidemiological evidence for an association between poor vascular perfusion and OAG (Leske 2009). It is intriguing that the capillaries supplying RGC axons at the ONH lie only within its connective tissue beams. Thus, mechanical strain on beams would be logically linked not only to direct axonal compression but also to capillary impairment and, potentially, additional insult. The astrocytes of the ONH line the laminar beams and are known to be directly responsive to IOP-induced stress, releasing molecules that may be either protective or toxic (Tezel et al. 2001, Nakazawa et al. 2006, Hernandez et al. 2008). However, experimental studies have detected that within hours of IOP elevation, alterations are already apparent in both RGC soma and CNS synaptic zones, which is clear evidence of prompt injury signaling through axonal transport. Thus, in human OAG, at any given time it may be impossible to define the earliest events of disease because the ongoing noxious stimulation rapidly produces active responses throughout the zones of the RGC. Hence, the survival of RGCs may depend not only on the type and degree of the initial injury to the axon but also on important processes in the retina and retrobulbar optic nerve.

There is increasing evidence that separate processes govern survival and death in the RGC soma and its axon (Levkovitch-Verbin et al. 2007, Calkins 2012). Fish and amphibians regrow a new axon after injury (Perry et al. 1987), showing that they can derepress the genetic program for axon growth, as is seen in the peripheral nervous system. They must also have a permissive environment for regrowth in the optic nerve, tract, and CNS target cells. Mammals repress these

abilities and undergo both somal and axonal loss after injury and in OAG. With significant axon injury, the axon dies first, then the soma (Quigley et al. 1977).

In glaucoma models, it has been experimentally possible to avoid the death of either the RGC soma or its axon by separate manipulations. Knocking out both alleles of the *bax* gene allows long-term survival of RGC somas despite the loss of their axons distal to the ONH (Libby et al. 2005). The parallel experiment, preservation of the axon with death of the cell body, has been demonstrated in the Wld^S mutant rodent (Beirowski et al. 2008). The myelinated RGC axon injured at the ONH dies by Wallerian degeneration (Wang et al. 2012), possibly due to a loss of axonal trophic factors (Lubińska 1982), including Nmnat2 (Gilley & Coleman 2010). The prosurvival gene products IAP1 and XIAP are activated in the RGC soma, but not in axons of glaucomatous rats (Levkovitch-Verbin et al. 2013). The overexpression of IAP is neuroprotective in rat glaucoma (McKinnon et al. 2002). From the clinical perspective, it is meaningless to save an axonless cell body or a disembodied axon. Neuroprotective strategies for both soma and axon will likely be found and may be necessary to maximize RGC rescue.

BDNF: brain-derived neurotrophic factor

CNTF: ciliary-derived neurotrophic factor

5. APOPTOSIS IS A MECHANISM OF RGC BODY DEATH

RGCs have been shown to die by apoptosis in experimental glaucoma in rats and monkeys (**Figure 5**) (Garcia-Valenzuela et al. 1995, Quigley et al. 1995) and in human glaucomatous eyes (Kerrigan et al. 1997). Although there may be several pathways to this final common mode of RGC death, one mechanism has been proposed that suggests the withdrawal of neurotrophic support is a significant contributor to the initiation of programmed cell death. Both anterograde and retrograde axonal transport are decreased at the ONH in glaucoma. It is a general property of neurons that they require neurotrophic support, particularly in embryological life. Neurons survive when provided with neurotrophins from target neurons in the brain, and those that fail to target properly activate the apoptotic program and follow a sequence including mitochondrial membrane potential loss, caspase activation, and phagocytosis by local glia (Almasieh et al. 2012). There is abundant evidence that in glaucoma brain-derived neurotrophic factor (BDNF) and ciliary-derived neurotrophic factor (CNTF) are blocked at the ONH from reaching RGC soma (Pease et al. 2000). Viral vector–derived overexpression of BDNF or CNTF is neuroprotective in rat glaucoma (Pease et al. 2009). This explanation of RGC death in glaucoma can be expressed as pathology recapitulates ontogeny. Clinical trials in human glaucoma are ongoing using chronic intraocular delivery of CNTF. CNTF may be more effective than BDNF at stimulating RGC axon growth after injury (Leaver et al. 2006). Glial cell line–derived neurotrophic factor is also protective against RGC death in DBA/2J glaucoma mice (Ward et al. 2006). The loss of target-derived neurotrophins may be, at least temporarily, replaced by retina-derived sources (Murphy & Clarke 2006). It would be useful to study the effect of the conditional deletion of neurotrophin genes in normal and glaucomatous adult animals.

The process of apoptosis, once initiated, is completed rapidly, typically in less than 24 hours (Cellerino et al. 2000). There may be a premonitory stage of cell injury, but this phase is unlikely to be associated with general failure of RGC function. There is no significant reversible functional improvement from any present glaucoma treatment. It would be extremely helpful to find such a reversible structural or functional feature of RGCs prior to their death, as this would give a more rapid method for identifying neuroprotective agents. At present, proof of neuroprotection depends on demonstrating a slower rate of visual field loss, which takes 2–3 years of testing, even under ideal conditions (Quigley 2012). A method for identifying dying RGCs by fluorescent labeling with annexin V has been proposed (Cordeiro et al. 2004), but has not yet been proven to be practical or helpful. Calculating the number of RGCs dying at any given time does not inspire

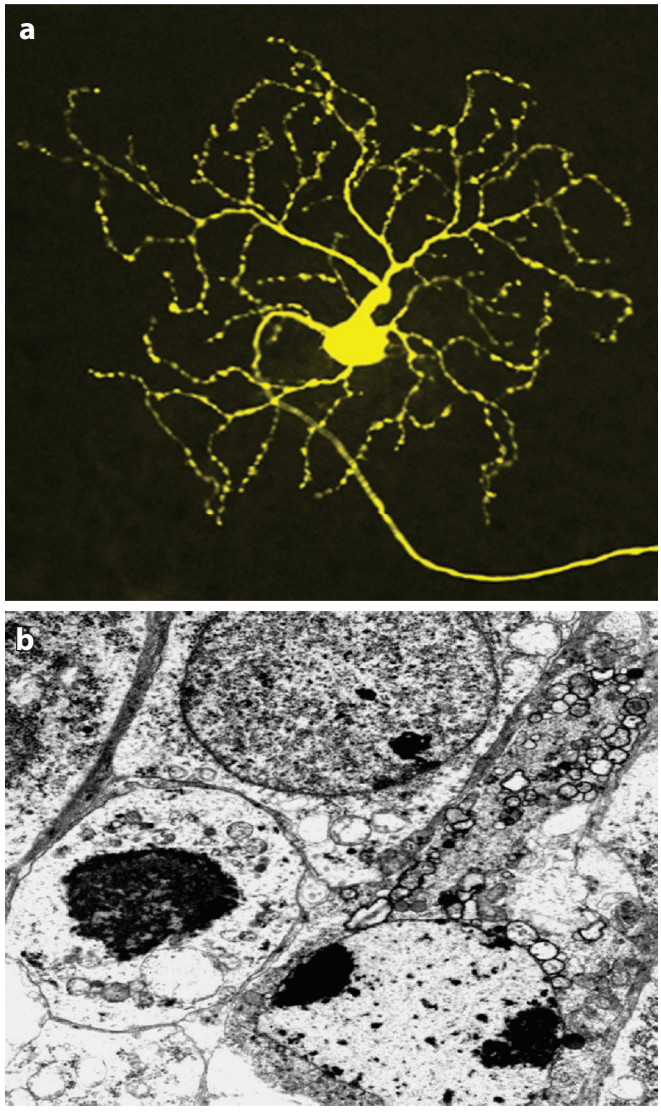

Figure 5

(*a*) Dendritic tree and axon exiting to the lower right from retinal ganglion cell expressing yellow fluorescent protein in mouse retina. (*b*) Transmission electron micrograph showing typical apoptosis of lower two retinal ganglion cell nuclei in experimental monkey glaucoma retina.

confidence that the study of already dying RGCs will be useful, as fewer than one dozen RGCs are in the apoptotic process at any given time.

There are likely to be other mechanisms leading to apoptosis in glaucoma, and the possibility that necrosis or autophagy may have a role has been proposed. However, any substantial amount of necrotic cell death would lead to significant inflammation in the retina. No clinical evidence for such inflammatory events is seen in OAG. Thus, the apoptotic pathway serves as a major avenue for the identification of new therapeutic targets. One principle that seems logical is to look

for candidate therapies that act at early stages of RGC injury and death, often called upstream therapies.

6. ADDITIONAL TARGET AREAS FOR RGC NEUROPROTECTION

Because the effect of IOP at the ONH is a well-known initiating event, altering IOP-induced stress by altering the peripapillary sclera surrounding the ONH has been a subject of interest and recent success in mouse models. In mice, stiffening the sclera by using local treatment with glyceraldehyde made RGC loss worse (Kimball et al. 2014), but altering scleral remodeling events by using the angiotensin II receptor type 1 inhibitor, losartan, was significantly protective (Quigley et al. 2015). The transforming growth factor-β pathway seems to play a key part in how the eye responds to IOP, and its modification has already been shown to have value. Consistent with this conclusion, losartan had no benefit in optic nerve crush, and produced a specific decrease in axonal transport block at the ONH. The attractiveness of this approach stems both from its action on a major early event in glaucoma and from the possibility that treatment could be delivered locally to the sclera by subconjunctival injection, avoiding systemic side effects. Because glaucoma patients are largely unaware of their disease until late, treatments must have very few side effects.

A second approach is to intercept the injury signal received at the axon before terminal events are initiated in the RGC body. The mitogen-activated kinases are part of general pathological events in injured neurons (Almasieh et al. 2012), and our laboratory and several other groups have shown upregulation in members of this pathway, including c-Jun upregulation in rodent (Levkovitch-Verbin et al. 2005) and human glaucoma (Tezel et al. 2003). Small interfering RNA–mediated knockdown of c-Jun resulted in RGC survival after optic nerve lesion (Lingor et al. 2005), and an inhibitor of c-Jun N-terminal kinase (JNK) conferred moderate RGC protection in acute IOP elevation (Sun et al. 2011). Mice lacking both JNK2 and 3 had improved RGC survival after injury (Fernandes et al. 2012). Welsbie et al. (2013) showed substantial RGC neuroprotection in rat glaucoma by inhibiting an upstream activator of JNK, dual leucine zipper kinase, using a sustained delivery, intravitreal injection of tozasertib. The profound benefit of this approach was also seen in that it prolonged RGC life both in dissociated culture and after optic nerve crush. Interrupting the death signal may be an important avenue for human glaucoma clinical trials.

With respect to several other areas of RGC survival, beneficial effects have been seen, although in many cases the link between glaucoma and the proposed mechanism is not clear. Grosskreutz and coworkers (Huang et al. 2005) found that cleavage of the autoinhibitory domain of calcineurin in rodent glaucoma could dephosphorylate Bad, making apoptosis more likely. A calcineurin inhibitor, tacrolimus, delivered systemically, reduced RGC death in glaucoma, but had no effect after optic nerve crush, suggesting its effect (like that of losartan's scleral neuroprotection) was specific to the RGC injury of glaucoma, not simply protection against any RGC injury. Tacrolimus has major systemic side effects, so its use would need to be modified from systemic application.

Additional pathways whose inhibition has shown benefit in animal models of glaucoma have involved tumor necrosis factor-α (Nakazawa et al. 2006), β-amyloid (Salt et al. 2014), free radicals (Neufeld et al. 1999), glutamate (Hare et al. 2004), and endothelin (Howell et al. 2014). The site of activation of the pathological process for these molecules is often thought to be the astrocytes of the ONH, retinal Müller glia, or macrophages and microglia. Presumably, the detrimental effect from their action would be secondary to the injury to RGCs. Only one of these approaches, inhibition of glutamate toxicity with oral memantine, has been tested in a human clinical trial, but the data were never published.

Dendrites of injured neurons are pruned by glia (Stephan et al. 2012), and this process has been proposed as part of the pathology in glaucomatous monkeys (Weber et al. 1998). General dendritic

arborization shrinkage has not been consistently found before RGC death in glaucomatous mice (Li et al. 2011, Kalesnykas et al. 2012), but subgroups of RGCs may undergo a loss of dendritic spread and develop functional defects prior to somal death (Della Santina et al. 2013, El-Danaf & Huberman 2015). In mammalian retina, RGCs do not successfully regenerate an axon after significant injury, but there is evidence for an abortive regenerative attempt (Soto et al. 2011). Protecting either dendrites or injured axons would potentially provide adjunctive avenues for glaucoma treatment.

Although it is common to read introductions to publications that state how little is known about the pathogenesis of glaucoma, recent decades have dramatically increased our knowledge, linking the clinical events of glaucoma to the molecular pathways leading to RGC loss. We are on the threshold of new treatments, independent of IOP lowering, to maintain vision and to avoid functional impairment in glaucoma.

FUTURE ISSUES

1. What factors cause RGC axons to die from injury at the ONH while axons in most persons survive insults?
2. Why do smaller, foveal RGCs survive longer than larger RGCs, despite having a greater energy requirement?
3. What cellular events link IOP-induced stress on RGC axons and interruption of their axonal transport?
4. Will it be possible and beneficial (or detrimental) to activate the axonal regrowth program of RGCs?
5. Can we identify a reversible structural or functional defect in RGCs prior to their irreversible loss in glaucoma?
6. Can we develop a treatment that protects RGCs from glaucoma damage by altering the scleral and ONH responses to IOP-induced strain?
7. Can we produce neuroprotection of RGCs in addition to lowering IOP as glaucoma therapies?

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