Differential Gene Expression in Glaucoma

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In glaucoma, regardless of its etiology, retinal ganglion cells degenerate and eventually die. Although age and elevated intraocular pressure (IOP) are the main risk factors, there are still many mysteries in the pathogenesis of glaucoma. The advent of genome-wide microarray expression screening together with the availability of animal models of the disease has allowed analysis of differential gene expression in all parts of the eye in glaucoma. This review will outline the findings of recent genome-wide expression studies and discuss their commonalities and differences. A common finding was the differential regulation of genes involved in inflammation and immunity, including the complement system and the cytokines transforming growth factor β (TGF β) and tumor necrosis factor α (TNF α). Other genes of interest have roles in the extracellular matrix, cell–matrix interactions and adhesion, the cell cycle, and the endothelin system.

The term glaucoma refers to a group of diseases that lead to the degeneration and eventual death of ganglion cells, the retina's projection neurons. There is wide agreement that elevated IOP and age are the most important risk factors (Quigley 2011), and that the ganglion cells die by apoptosis (Quigley et al. 1995; Qu et al. 2010). However, the exact sequence of events that leads to ganglion cell degeneration is not fully understood.

Although it is the loss of ganglion cells that leads to visual impairment, pathological changes in glaucoma have been demonstrated in most structures of the eye. In the anterior chamber, the major structures regulating production and outflow of aqueous humor, including the ciliary body, the trabecular meshwork, and Schlemm's canal, can be affected (Johnson 2006; Coca-Pra-

dos and Escibano 2007; Tamm 2009). The retina and, in particular, the ganglion cells themselves (Danias et al. 2003; Jakobs et al. 2005; Schlamp et al. 2006; Morgan 2012) are affected. Finally, the optic nerve head (ONH), including the lamina cribrosa (Hernandez 2000; Hernandez et al. 2008; Roberts et al. 2009; Burgoyne 2011), the optic nerve proper (Nakazawa et al. 2006), and the central projection targets of the ganglion cells in the lateral geniculate body and the superior colliculus (Gupta et al. 2007; Liu et al. 2011) all can display pathological changes in glaucoma.

The advent of animal models for glaucoma and the wide availability of genome-wide expression analysis techniques have led to a considerable number of recent studies that address gene expression changes associated with glauco-

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T.C. Jakobs

ma (Yang and Zack 2011). Transcriptome-wide expression studies are generally better at generating hypotheses than at elucidating mechanisms, and the differences in animal models and expression profiling methods have introduced a lot of experimental variation. However, several common themes have emerged. This review will summarize recent advances in gene expression profiling studies of glaucoma, considering each anatomical location in turn.

DIFFERENTIAL GENE EXPRESSION IN STRUCTURES OF THE ANTERIOR EYE

The production and outflow of aqueous humor has a direct relation to IOP, and pharmacological interventions to lower IOP are still the first-line treatment for glaucoma. Aqueous humor is produced by the ciliary epithelium. Although there is at present no published study which specifically compares ciliary epithelia from glaucomatous and nonglaucomatous eyes, gene expression profiling of the normal epithelium or the whole ciliary body has been carried out (Coca-Prados et al. 1999; Janssen et al. 2012; Wagner et al. 2013). It has been demonstrated that the ciliary epithelium is a source of proteins in the aqueous humor (Escribano et al. 1995; Escribano and Coca-Prados 2002), and that several glaucoma-related genes are expressed in this tissue, most notably myocilin and CYP11B1 (Coca-Prados and Escribano 2007; Janssen et al. 2012). In addition, the ciliary epithelium produces a variety of neuroendocrine factors, such as natriuretic peptides, neurotensin, and somatostatin (Coca-Prados and Escribano 2007). Their potential role in regulating the production of aqueous humor and their influence on outflow resistance is an intriguing topic for further research.

The trabecular meshwork and the wall of Schlemm's canal are major points of resistance for the outflow of aqueous humor (Tamm 2009), and morphological changes of the trabecular meshwork in glaucoma are apparent using both light and electron microscopy (Lutjen-Drecoll et al. 1981; Rohen et al. 1981; Tektas and Lutjen-Drecoll 2009; Tektas et al. 2010). Microarray studies of gene expression have been conducted using isolated trabecular meshwork

cells, organ cultures, and fresh tissue from a variety of animal models and humans (Gonzalez et al. 2000; Ishibashi et al. 2002; Lo et al. 2003; Vittitow and Borrás 2004; Zhao et al. 2004; Diskin et al. 2006; Liton et al. 2006; Rozsa et al. 2006; Fan et al. 2008; Wang et al. 2008a; Danias et al. 2011; Liu et al. 2013). The conditions assayed in these studies were primary open angle glaucoma, elevated pressure, and treatment of trabecular meshwork cells with steroids or TGF β . Because of the differences in experimental models and microarray platforms used in these studies, the results have varied considerably. For instance, myocilin was found to be induced by dexamethasone treatment of cultured human trabecular meshwork cells (Ishibashi et al. 2002; Lo et al. 2003; Fan et al. 2008), confirming other studies (Polansky et al. 1997; Nguyen et al. 1998), but was not observed in the bovine trabecular meshwork after steroid treatment (Danias et al. 2011). Several studies identified mediators of inflammation or immunity in the trabecular meshwork, such as up-regulation of interleukin (IL) 6 by elevated IOP (Gonzalez et al. 2000), up-regulation of the IL6 receptor in glaucoma (Diskin et al. 2006), and up-regulation of IL8 in response to steroid treatment (Lo et al. 2003). One study, which investigated differential regulation at the mRNA and protein level after treatment of human trabecular meshwork cells with TGF β 1 and TGF β 2, found that the classes of genes most up-regulated by TGF β encoded extracellular matrix proteins and other extracellular proteins, including versican (Zhao et al. 2004). In glaucoma, TGF β 2 levels in the aqueous humor are elevated compared to controls (Inatani et al. 2001; Picht et al. 2001), and TGF β 2 is also involved in the regulation of some matricellular proteins like secreted protein, acidic and rich in cysteine (SPARC) (Kang et al. 2013). The up-regulation of extracellular proteins in response to TGF β is an interesting mechanical link between high IOP and increased outflow resistance.

DIFFERENTIAL GENE EXPRESSION IN THE GLAUCOMATOUS RETINA

As retinal ganglion cells are the main cell type vulnerable to glaucomatous degeneration, many

studies have been undertaken to find the underlying molecular causes of their vulnerability and the processes that are responsible for their eventual death. In these studies, several different models and species have been employed. In one model, usually used in rats but more recently also in mice, elevated IOP results from obliteration of the episcleral veins caused by an injection of hypertonic saline solution (Morrison et al. 1997; Walsh et al. 2009). Translimbal laser photocoagulation of the trabecular meshwork has also been used to increase the IOP in one eye of rats and monkeys (Miyahara et al. 2003; Yang et al. 2007). Retinal ganglion cells degenerate reproducibly after axotomy (Berkelaar et al. 1994). Although not models of glaucoma per se, optic nerve crush and optic nerve transection have been used to induce ganglion cell death (Yang et al. 2007; Agudo et al. 2008). Finally, the mouse strain DBA/2J develops glaucoma secondary to iris stroma disease caused by two mutations in the genes *Gpnmb* and *Tyrp1* (Anderson et al. 2002). These mice develop an increase in IOP at ~6 mo of age, followed by a sectorial degeneration of retinal ganglion cells (Danias et al. 2003; Jakobs et al. 2005; Libby et al. 2005; Schlamp et al. 2006). Among the studies that used an ocular hypertension model, some have assayed differential gene expression in the whole retina (Miyahara et al. 2003; Ahmed et al. 2004; Walsh et al. 2009), and others have analyzed cells isolated from the ganglion cell layer by laser capture microdissection (Guo et al. 2010, 2011; Wang et al. 2010). For microarray analysis in the inherited glaucoma models in mice (DBA/2J) and rats (RCS-rdy⁻), whole retinas were used (Naskar and Thanos 2006; Steele et al. 2006; Panagis et al. 2010, 2011; Howell et al. 2011). This is an important consideration because the retina is a complex tissue, and ganglion cells make up only 1% of the neurons in the retina (Jeon et al. 1998). In addition to the neuronal cells, there are also macroglia (Mueller cells and astrocytes), microglia, and vascular cell types.

Despite these differences in models and sample preparation, the studies share many common findings. One of them is the prominent up-regulation of genes involved in the in-

flammatory and immune response. For instance, several studies reported up-regulation of lipocalin 2, both after an increase of IOP and in DBA/2J mice (Ahmed et al. 2004; Steele et al. 2006; Yang et al. 2007; Guo et al. 2010, 2011; Howell et al. 2011). Lipocalin 2 is a general marker of tissue response to injury and is up-regulated among others by IL1 β , IL17, and TNF α (Li and Chan 2011). It has also emerged as a marker of astrocyte reactivity in the brain (Zamanian et al. 2012). Other immune response genes found to be up-regulated in glaucoma models include α -2-macroglobulin, MHC class II RT1.u-D- α chain, Interferon-induced transmembrane protein 1 (Ifitm3/Fgls), and Chemokine (C-C motif) ligand 12 (Ahmed et al. 2004; Steele et al. 2006; Guo et al. 2010). Of particular interest among the immune response genes are members of the complement cascade. Most studies identified at least one member of the complement cascade as significantly up-regulated (Miyahara et al. 2003; Ahmed et al. 2004; Piri et al. 2006; Steele et al. 2006; Yang et al. 2007; Panagis et al. 2010; Wang et al. 2010; Howell et al. 2011). Importantly, complement up-regulation seems to precede morphological signs of ganglion cell degeneration in the DBA/2J model (Howell et al. 2011). The complement system was recently found to mediate synapse elimination in the CNS (Stevens et al. 2007). Synapses are decorated with complement and stripped off the dendrites by microglia (Schafer et al. 2012). This mechanism, physiological during CNS maturation, may be pathologically reactivated in the glaucomatous retina and lead to synapse loss followed by dendritic remodeling (Stephan et al. 2012). Members of the complement cascade are expressed at the mRNA and protein levels in several models of glaucoma, as well as in other types of retinal insult, such as a direct tear injury (Vazquez-Chona et al. 2004; Kuehn et al. 2006, 2008; Stasi et al. 2006). Finally, genetic disruption of the complement cascade is highly protective of ganglion cells in the DBA/2J model of glaucoma (Howell et al. 2011).

Ganglion cells die by apoptosis in glaucoma and after axotomy (Garcia-Valenzuela et al. 1994, 1995; Quigley et al. 1995; Qu et al. 2010). In retinal ganglion cells isolated after induc-

T.C. Jakobs

tion of elevated IOP, genes involved in apoptosis and response to oxidative stress (such as *Angptl4*, *Ednrb*, and *Pnkp*) were among the most up-regulated, whereas genes involved in neuronal development and function (including *Gja1*, *Snap25*, and *Gabrg2*) were down-regulated (Wang et al. 2010). The same study found that several prosurvival genes were significantly down-regulated, but the prosurvival gene *STAT3* was up-regulated at the mRNA, and protein levels and glaucomatous retinas showed increased labeling for the phosphorylated, active form of *STAT3* (Wang et al. 2010). The up-regulation of *STAT3* seems to be relatively specific for glaucomatous damage, as it was not observed after optic nerve transection or ischemia/reperfusion injury (Yoshimura et al. 2003; Yang et al. 2007). A simultaneous up-regulation of proapoptotic and antiapoptotic genes (like the caspase inhibitor *IAP1*) was also observed in a rat model of increased IOP and optic nerve transection (Levkovitch-Verbin et al. 2006b). This suggests that there is a balance between protective and detrimental processes when the ganglion cells are threatened by injury. Conceivably, this balance could be tilted in favor of the protective side by timely therapeutic intervention. As an example of such a strategy, minocycline, an anti-inflammatory and neuroprotective second-generation tetracycline, was found to delay retinal ganglion cell death in a rat model of glaucoma (Levkovitch-Verbin et al. 2006a). Both ocular hypertension and optic nerve transection lead to up-regulation of immediate early response genes. *C-Jun*, *Junb*, and *Atf3* (activating transcription factor 3) were up-regulated in both models (Yang et al. 2007; Guo et al. 2010). These factors are downstream from JNK, and the phosphorylated, active form of JNK is present at increased levels in glaucoma (Kwong and Caprioli 2006; Levkovitch-Verbin et al. 2007). Therefore, blocking the activation of the JNK pathway may also be of therapeutic benefit in glaucoma. In an attempt to identify other pathways that are involved in ganglion cell degeneration and death and that may be amenable to drug treatment, Welsbie and coworkers recently identified dual leucine zipper kinase (DLK) via RNAi-based screening (Welsbie et al. 2013). DLK inhibition

promotes ganglion cell survival in in vitro cultures and in vivo after optic nerve injury. Importantly, a small molecule inhibitor of DLK, tozasertib, administered intraocularly with drug-eluting microspheres, protected ganglion cells after optic nerve transection (Welsbie et al. 2013).

Another pathway that was differentially regulated in several studies is the endothelin/endothelin receptor system (Ahmed et al. 2004; Yang et al. 2007; Panagis et al. 2010; Howell et al. 2011). Endothelin 2 (*Edn2*) up-regulation occurs in the retina and the ONH before morphological signs of glaucomatous damage are observed in DBA/2J mice (Howell et al. 2011). Endothelins exist in three isoforms and exert their effects through two types of receptors (Davenport 2002). They are potent vasoconstrictors and have been found to be elevated in the aqueous humor of glaucoma patients (Noske et al. 1997; Tezel et al. 1997). Intraocular administration of endothelin 1 leads to acute blockage of axonal transport (Taniguchi et al. 2006; Wang et al. 2008b), optic neuropathy, and ganglion cell loss (Chauhan et al. 2004; Sasaoka et al. 2006). It was recently shown that endothelin 2, which binds to the same receptors as endothelin 1, also causes ganglion cell loss and optic nerve damage (Howell et al. 2011). In DBA/2J mice, endothelin 2 is expressed by retinal microglia, and administration of bosentan, an inhibitor of both types of endothelin receptors, protects ganglion cells in this model (Howell et al. 2011).

Microglial activation in the retina and the ONH are among the earliest changes in the DBA/2J mouse model of glaucoma (Bosco et al. 2008, 2011). Consistent with this, an up-regulation of *Aif1* was observed in retinas after elevation of IOP (Ahmed et al. 2004). In a mouse model of ocular hypertension that assayed gene expression before the onset of retinal cell apoptosis, serum amyloid A (SAA), too, was found to be up-regulated in retinal microglia (Walsh et al. 2009). It is interesting in this context that SAA is also up-regulated in the glaucomatous trabecular meshwork (Wang et al. 2008a). Microglial activation may be detrimental to ganglion cell survival. A possible mechanism involves $\text{TNF}\alpha$, whose levels are elevated in the retina and

ONH of glaucoma patients (Yan et al. 2000; Yuan and Neufeld 2000, 2001; Sawada et al. 2010). In a mouse model of glaucoma, TNF α was found to mediate the cytotoxic effects of elevated IOP on ganglion cells, and injection of TNF α led to ganglion cell death and optic neuropathy (Nakazawa et al. 2006). The source of the TNF α in the retina seems to be a population of microglia that resides around the ONH and becomes activated in ocular hypertension (Roh et al. 2012). Importantly, the TNF α -decoy receptor etanercept prevents ganglion cell loss and optic nerve pathology in this model (Roh et al. 2012).

GENE EXPRESSION IN THE ONH IN GLAUCOMA

Experimental evidence points to the ONH as the site of initial insult to retinal ganglion cell axons in glaucoma. Most importantly, the topology of ganglion cell loss in glaucoma and the resulting visual field defects (radial in humans, sectorial in rodents), is difficult to explain without reference to the ONH because there the ganglion cell axons run in bundles with topological relationship to the retina (Danas et al. 2003; Jakobs et al. 2005; Schlamp et al. 2006; Howell et al. 2007; Soto et al. 2011; Nickells et al. 2012). In humans this region contains the lamina cribrosa, a stack of collagenous plates with pores through which the ganglion cells' axons pass (Quigley and Addicks 1981). An increase in IOP leads to increased pressure across the lamina cribrosa and mechanical strain within it (Burgoyne 2011). This may cause direct mechanical injury to the axons, as they are compressed against this rigid structure. Indeed, blockage of axonal transport has been identified in the region of the lamina cribrosa (Quigley and Addicks 1980; Quigley et al. 1981; Johansson 1983, 1988; Balaratnasingam et al. 2007). This may lead to an interruption of retrograde transport of neurotrophic factors, such as brain-derived neurotrophic factor (BDNF), to the ganglion cell body, followed by degeneration of the cell (Pease et al. 2000; Quigley et al. 2000). However, a rigid lamina cribrosa is not necessary to develop glaucoma. Rodents are susceptible to the disease, yet mice do not

show staining for any collagen other than collagen 4 associated with blood vessels, and the ONH of rats contains only sparse collagenous septa (Morrison et al. 1995; May and Lutjen-Drecoll 2002; Sun et al. 2009). A commonality between ONH of humans and all other species, including rodents, is that this region contains an abundance of astrocytes that line the pores of the lamina cribrosa and ensheath the axons, or, in rodents, form glial tubes around axon bundles that visually resemble a lamina cribrosa so much that they are referred to as the "glial lamina" (Morcos and Chan-Ling 2000; Howell et al. 2007; Sun et al. 2009). In addition to astrocytes, the human laminar region contains lamina cribrosa cells (Hernandez et al. 1988). Although negative for the astrocyte marker glial fibrillary acidic protein (GFAP), lamina cribrosa cells have many similarities to ONH astrocytes, and may be a subtype of astrocyte (Lambert et al. 2001; Zode et al. 2007; Nickells et al. 2012). The ONH also contains microglia/macrophages and endothelial cells. Given the importance of the ONH in glaucoma, several studies have addressed differential gene expression in this tissue. Cultured astrocytes and lamina cribrosa cells and whole tissues from a variety of models, including ocular hypertension following episcleral vein injection, the DBA/2J mouse, and human glaucoma, have been used.

In reaction to injury to the CNS, astrocytes become reactive. Originally, astrocyte reactivity was described as an up-regulation of GFAP and cellular hypertrophy, but it has become clear that astrocyte reactivity is a complex process that involves differential gene expression and morphological changes (Sofroniew 2005; Sofroniew and Vinters 2010; Sun and Jakobs 2012). In glaucoma, too, ONH astrocytes show signs of reactivity (Hernandez and Pena 1997; Hernandez 2000; Lye-Barthel et al. 2013). To characterize astrocyte reactivity in glaucoma, Hernandez and coworkers (2002) cultured astrocytes from normal human eyes and from donors with primary open angle glaucoma and subjected them to microarray screening. They found an up-regulation of genes involved in cell motility and migration, including gelsolin, periplakin, and LIM binding protein 1, and a down-regulation

T.C. Jakobs

of genes associated with attachment, such as several integrins and chondroitin sulfate proteoglycan 4. Astrocytes from glaucomatous donors did indeed migrate faster than did normal astrocytes in an in vitro assay of migration. In glaucomatous astrocytes there is also evidence for increased signaling by insulin-like growth factor II and the TGF β receptor (Hernandez et al. 2002). More recently, microarray screening and pathway analysis was applied to immunopurified ONH astrocytes from normal and glaucomatous donors (Nikolskaya et al. 2009). Processes that were up-regulated in glaucoma were the complement system, signal transduction (e.g., NF- κ B, SP1, VDR, AP-1, STAT5), the inflammatory and immune response, nervous system development, and cell adhesion. Fewer pathways were down-regulated; among them were platelet-derived growth factor signaling, membrane trafficking, and signal transduction via G- α 1. Some of these changes could be interpreted as being neuroprotective, such as the up-regulation of VDR and AP-1, whereas others, namely the activation of NF- κ B and complement, may contribute to a neurotoxic activity of ONH astrocytes toward the axons of ganglion cell (Nikolskaya et al. 2009). A different approach, also making use of astrocytes cultured from human donors, was taken in a recent study that compared the transcriptome of normal astrocytes from African American versus Caucasian American donors (Miao et al. 2008). As African Americans are affected by primary open angle glaucoma more often than are Caucasians (Leske 2007), this study aimed at finding underlying differences in the astrocytes of these populations. Astrocytes from African American donors showed higher expression of genes that are associated with cAMP signaling (adenylate cyclase 3 and 9), intracellular vesicular transport, G protein signaling (such as regulator of G protein signaling 5, RGS5), and protein phosphatases. Astrocytes from African Americans also showed decreased cell adhesion and increased cell migration, and expressed significantly more elastin. Finally, glutathione-metabolizing enzymes were up-regulated in astrocytes from African American donors, and glutathione levels were lower, which may hint at a compro-

mised antioxidant response in these cells (Miao et al. 2008). Although these results do not fully explain the higher vulnerability of African Americans to primary open angle glaucoma, it is possible that in this population the ONH astrocytes exhibit an increased tendency to become reactive, which may be associated with higher cell mobility in the ONH, and are less able to withstand oxidative stress and protect ganglion cell axons from threatened injury.

One of the characteristics of primary open angle glaucoma is a remodeling of the ONH accompanied by deposition of fibrotic material (Hernandez and Ye 1993), including collagens I, IV, and VI, and the disruption of normal elastin fibers (Morrison et al. 1990; Quigley et al. 1991a,b; Fukuchi et al. 1992; Hernandez 1992; Hernandez et al. 1994; Hernandez and Pena 1997). Lamina cribrosa cells synthesize extracellular matrix in the human ONH. In a recent study, Kirwan and coworkers (2009) performed microarray screening on lamina cribrosa cells cultured from normal and glaucomatous human optic nerves. Among the functional categories most significantly up-regulated were extracellular matrix, collagen, and extracellular space. The most highly regulated gene was periostin, a secreted protein involved in adhesion and differentiation that is up-regulated under conditions of tissue stress such as myocardial pressure overload (Litvin et al. 2005). Other genes up-regulated in glaucomatous lamina cribrosa cells included dystrophin, cartilage linking protein, SPARC, thrombospondin 1, TGF β 1, and collagens I, V, and XI; in contrast, fibulin 1, decorin, and collagen VIII were down-regulated (Kirwan et al. 2009). These results are indicative of a profibrotic state of the glaucomatous lamina cribrosa cells. Recently, the same data set was used in a graph-clustering approach that identified three clusters of differentially regulated genes, including genes involved in axon guidance (such as BDNF), the basal cell carcinoma pathway (such as frizzled family receptor 2 and 7), and cell adhesion (such as versican and integrin α 4) (Luo et al. 2013).

Cell cultures from ONH astrocytes have the advantage that they contain only one defined cell population. On the other hand, cells in cul-

ture tend to undergo changes in their gene expression profile. Several studies have therefore used whole ONH tissue for analysis. Although the majority of the cells in the ONH are astrocytes, microglia, and endothelial cells, axons are also present; and each of them contribute to the total RNA being analyzed. Johnson and coworkers (2007, 2011) used unilateral injection of hypertonic saline into the episcleral veins in rats to induce ocular hypertension and compared the transcriptome of the experimental eye with the control eye. Biological processes that were significantly regulated in the ocular hypertension group included cell proliferation (cyclin D1 was up-regulated 3.5-fold), immune response (including all members of the complement 1 complex, F_C receptor, and β 2 microglobulin), ribosomal and lysosomal genes, and extracellular matrix (including periostin and tenascin C). The isoforms of TGF β were differentially regulated: expression of TGF β 1 increased while that of TGF β 2 decreased slightly with elevated IOP. The up-regulation of cell proliferation genes corresponded to an increase in cellularity of the ONH (Johnson et al. 2007). In an attempt to characterize early changes in response to ocular hypertension, Johnson and coworkers used microarray analysis of ONHs with only minimal axon damage (Johnson et al. 2011). Cell cycle genes displayed the greatest up-regulation, followed by cytoskeletal genes and immune process genes. Several cytokines were among the most highly up-regulated genes, for example, leukemia inhibitory factor (LIF), Ccl1, and IL6. The up-regulation of these IL6-like cytokines could have a variety of effects on the ONH: all three can induce astrocytic differentiation from precursor cells (Klein et al. 1997; Uemura et al. 2002) and they may be neuroprotective (Leibinger et al. 2009; Suzuki et al. 2009). LIF has recently been shown to mediate the neuroprotective effect of preconditioning with moderate oxidative stress in the retina (Chollangi et al. 2009). The expression of IL6-like cytokines in the early phase of ocular hypertension may therefore be indicative of a protective role of the ONH glia. Whole ONHs were also used for microarray screening in a recent study that made use of the ocular laser-

induced hypertension model in cynomolgus macaques (Kompass et al. 2008). The most important finding was that early in the course of the disease, before the appearance of visual field defects, retinal ganglion cells make an attempt at regeneration as evidenced by the appearance of GAP43⁺ and pNEFH⁺ growth cones. Astrocytes apparently facilitated this attempt by expressing neuroprotective factors such as apolipoprotein E, cellular retinol binding protein 1, and pigment epithelium—derived factor (Kompass et al. 2008).

Using the DBA/2J inherited mouse model of glaucoma, Howell and coworkers (2011, 2012) characterized the early stages of molecular changes in the ONH. Before the onset of morphological signs of axon degeneration, over 400 genes were differentially expressed compared to a control strain that does not develop glaucoma (D2-Gpnmb⁺). Pathways that were up-regulated early in the disease process involved the immune response, chemotaxis, and leukocyte activation. Cell–matrix interaction genes were up-regulated at a slightly later stage in the disease; these included tenascin C, which has also been identified in the glaucomatous optic nerve by immunohistochemistry (Pena et al. 1999), as well as various integrins, fibronectin 1, Timp 1 and 2, and several collagens. Up-regulation of endothelin 2 and several members of the complement cascade was observed at early stages in the optic nerve as well as in the retina (Howell et al. 2011). A pathway that is activated very early in the optic nerve of DBA/2J mice compared to nonglaucomatous controls is the leukocyte transendothelial migration pathway, characterized, e.g., by the genes for P- and E-selectins (Howell et al. 2012). It was shown recently that whole-body irradiation protects ganglion cells from glaucomatous degeneration (Anderson et al. 2005). In an extension of this study, Howell and coworkers (2012) found that irradiation of the eye was equally protective, and that in irradiated eyes the up-regulation of P-selectin and monocyte entry into the eye did not occur. Irradiation also had a mitigating effect on microglial activation in the ONH while having minimal effect on the astrocytes (Bosco et al. 2012). These important results underscore the impli-

T.C. Jakobs

cation of neuroinflammatory events in the pathogenesis of glaucoma.

The crush model of damage to the optic nerve has the advantage that the onset of the injury is clearly defined and it lends itself to time-course studies. Astrocyte reactivity after optic nerve crush follows a biphasic temporal pattern: in the first phase (lasting days) after the injury, astrocytes reduce their special coverage, retract processes and become “amoeboid” in shape; in a second phase (> 14 d after injury), they re-extend long processes and resume a more normal appearance (Sun et al. 2010). Analysis of differentially expressed genes after optic nerve crush revealed that the changes occur in three partially overlapping waves: a first wave of genes involved in inflammation and immunity (1–7 d), a second wave of genes involved in cell cycle and proliferation (3–7 d), followed by a slower wave of genes involved in extracellular matrix restructuring, cell–matrix interaction, and debris removal. By 3 mo after the optic nerve crush, the tissue reached a stationary phase that reflects structural changes in the axons (Qu and Jakobs 2013). Cell labeling with BrdU revealed that it was primarily the microglial population that proliferated rather than the astrocytes in this model (Qu and Jakobs 2013).

GENE EXPRESSION IN THE OPTIC NERVE PROPER

In contrast to the glial lamina, in which astrocytes ensheath the unmyelinated axons, the optic nerve proper contains myelinating oligodendrocytes in addition to astrocytes, microglia, and NG2-cells. Oligodendrocyte death may be an early event in optic nerve damage caused by increased IOP. In a mouse model of laser-induced ocular hypertension, TNF α levels were increased in the retina followed by oligodendrocyte loss in the optic nerve that preceded ganglion cell death (Nakazawa et al. 2006). Although no study so far has compared gene expression in the optic nerve proper with that of the glial lamina in glaucoma, in some studies parts of the myelinated optic nerve were analyzed together with the glial lamina (Johnson

et al. 2007; Qu and Jakobs 2013). Not surprisingly, optic nerve damage is accompanied by down-regulation of oligodendrocyte-specific genes. For example, among the genes that were permanently down-regulated after optic nerve crush was oligodendrocyte transcription factor 1, myelin-associated oligodendrocytic basic protein, and myelin basic protein (Qu and Jakobs 2013). Recently, Nguyen and coworkers identified a novel function of astrocytes in the myelination transition zone behind the glial lamina. These astrocytes express the phagocytic marker Mac-2. The astrocytes up-regulate this protein in glaucoma, and they engulf and internalize evulsed material from the ganglion cell axons (Nguyen et al. 2011). Astrocytes have been shown to be phagocytically active in vitro and in vivo (Kalmar et al. 2001; Ito et al. 2007), and constitutively express several genes involved in phagocytosis (Cahoy et al. 2008). Thus, dysregulated phagocytosis of axonal material may play a role in glaucomatous neuropathy.

GENE EXPRESSION IN THE CENTRAL PROJECTING TARGETS OF THE GANGLION CELLS

At present, no transcriptome-wide expression study has targeted the superior colliculus or the lateral geniculate nucleus in glaucoma. Although dendritic changes in the lateral geniculate nucleus have been described in glaucoma, their molecular mechanisms remain unclear (Liu et al. 2011). The lateral geniculate nucleus of monkeys with experimental glaucoma also shows signs of reactive gliosis, as GFAP and S100 β expression was increased (Dai et al. 2012).

CONCLUDING REMARKS

Despite the experimental differences, a number of common themes have emerged. A consistent finding is the involvement of immune and inflammatory pathways in all tissues of the eye in glaucoma. An example of this is the up-regulation of members of the complement cascade in retina and ONH. Up-regulation of complement is of course not specific for glaucoma. Complement factors have been identified as constitu-

ents of drusen in age-related macular degeneration, and several genetic studies have associated variants of complement factor genes with this disease (Anderson et al. 2010). Complement up-regulation is also a factor in Alzheimer's disease and many other neurodegenerative diseases (Stephan et al. 2012). As complement has been shown to play a role in synapse elimination, its up-regulation in the glaucomatous retina may mediate synapse elimination followed by remodeling of ganglion cell dendrites (Stevens et al. 2007). Complement factors are also up-regulated in the glaucomatous ONH, although its role there is presently unclear (Howell et al. 2011). Another common finding is the up-regulation of proinflammatory cytokines, most notably TNF α and TGF β , and of genes regulated by them. This is of importance for new treatment approaches to glaucoma. A TNF α antagonist has already been used in animal studies to ameliorate ganglion cell loss caused by ocular hypertension (Roh et al. 2012). As TNF α antagonists are already approved for therapy of rheumatoid arthritis, these drugs could have a place in glaucoma therapy as well. Another pathway that has been implicated in the pathogenesis of glaucoma by several studies is the endothelin/endothelin receptor system. This pathway, too, is a potential target for therapeutic intervention.

There is much evidence that glial reactivity, both of astrocytes and microglia, is a feature of the glaucomatous optic nerve, but it is less clear whether glial reactivity is a helpful or a deleterious response. Astrocytes produce TNF α as a response to elevated IOP (Tezel and Wax 2000), which may in turn stimulate the production of nitric oxide (Neufeld 1999; Shareef et al. 1999; Yuan and Neufeld 2000). This has led to the concept of "misbehaving glia" in glaucoma (Neufeld and Liu 2003). The mechanism of ganglion cell damage through up-regulation of nitric oxide synthetase is still controversial, however, as other studies have found no evidence of enhanced nitric oxide production (Pang et al. 2005; Libby et al. 2007). On the other hand, reactive astrocytes have been shown to exert beneficial effects on neurons (Bush et al. 1999; Faulkner et al. 2004; Myer et al. 2006; Okada et al. 2006), and astrocyte reactivity per se is

not immediately harmful to ganglion cell axons (Sun et al. 2013). It is possible that the initial reaction of astrocytes to elevated IOP is indeed neuroprotective and only if this response is overwhelmed, as is the case in severe or prolonged insult, do the astrocytes react by forming a permanent glial scar.

Genome-wide microarray expression screening has unearthed a wealth of information about the reactions of different tissues of the eye to increased IOP and glaucoma. Several new leads to novel therapeutic approaches to the disease have been identified and the coming years may see some of them come into clinical practice.

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Differential Gene Expression in Glaucoma

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T.C. Jakobs

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