Retinal Ganglion Cells Resistant to Advanced Glaucoma: A Postmortem Study of Human Retinas with the Carbocyanine Dye DiI

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PURPOSE. The present study was conducted to examine whether the morphology of the retinal ganglion cells is altered in advanced glaucoma. Perikaryal, axonal, and dendritic alterations were monitored in glaucoma-resistant retinal ganglion cells by postvitam application of the fluorescent dye DiI.

METHODS. The retinas of four amaurotic glaucomatous eyes and four normal eyes enucleated after death were used in this study. The retinas were freed from surrounding tissue, prepared as flatmounts on a nitrocellulose filter, and fixed overnight in 4% paraformaldehyde. The retinal ganglion cells were labeled by introducing crystals of the fluorescent carbocyanine dye DiI, into the optic fiber layer. This dye diffuses along membranes of ganglion cell axons, completely labeling them and their cell bodies and dendrites. Further characterization of the retinas and optic nerves included hematoxylin-eosin and van Gieson histochemical staining as well as immunohistochemistry against glial fibrillary acidic protein.

RESULTS. Because of the advanced stage of the disease, the retinas were almost completely depleted of ganglion cells, which had degenerated and therefore could not be stained. The few remaining ganglion cells were considered to be resistant to glaucoma. They showed drastic morphologic alterations, such as abnormal axonal heading, the cell bodies were normal in size but had irregular silhouettes or swellings, and there were fewer dendritic bifurcations. The size of the dendritic trees was smaller, implicating pruning of smaller dendritic branches. Glial cells were also detected immunocytochemically indicating their involvement in the pathologic course of glaucoma.

CONCLUSIONS. The data suggest that the few ganglion cells that survive the elevated intraocular pressure associated with loss of visual function display morphologic changes that are manifested both on the cell body and on their intraretinal processes, including axons and dendrites. (Invest Ophthalmol Vis Sci. 2003;44:5196–5205) DOI:10.1167/iovs.03-0614

Glaucoma is one of the most common causes of blindness, with prevalences of 0.15% in the total population and of approximately 2% among individuals older than 40 years. The glaucomas constitute a diverse group of disorders characterized by the slow, progressive degeneration of retinal ganglion cells that causes visual field loss, eventually leading to blindness. Although several hereditary and acquired forms of the disease exist, the major risk factor common to almost all forms of glaucoma is abnormal elevation of intraocular pressure (IOP) that damages the optic nerve directly or indirectly, resulting in characteristic optic nerve head cupping and visual field loss. If the disease is insufficiently treated or left untreated, advancement of cellular changes is unavoidable. Elevated IOP affects the retina evenly, but is most deleterious at the optic disc, perhaps affecting both the ganglion cell axons and the microvasculature. Consequently, when blood vessels are compressed, the retina becomes ischemic, resulting in secondary intraretinal changes.1–3 Larger retinal ganglion cells (RGCs) are more susceptible to glaucomatous damage.4 However, at advanced stages of the disease, horizontal cells and photoreceptors undergo degenerative changes as shown with 1,1-dioctadecyl-3,3,3,3-tetramethylindocarbocyanine perchlorate (DiI) labeling6,7 and accompanying histologic examination.8 It has yet to be established whether the degenerative effects in RGCs are a consequence of optic nerve damage4–9 or are caused by the direct pressure on ganglion cells and their axons10,11 or indeed by a combination of both mechanisms. Intravital analysis of these mechanisms is extremely difficult, however, due to the inaccessible nature of RGCs. Animal models of glaucoma that have been developed in various species,12 such as the monkey13,15 or rats14,16 and transgenic mice,15 are essential for characterizing the cellular effects of glaucoma. In addition, eyes obtained at autopsy or from eye banks17–20 may help in understanding the advanced stages of human glaucoma at the cellular level.

Two major classes of RGCs with subtypes can be distinguished in the human retina: midget and parasol cells with large or wide-field parasol cells in the retinal periphery and displaced ganglion cells located in the inner nuclear layer (INL).16–19 In the monkey, midget cells constitute approximately 80% of all RGCs and have the smallest dendritic fields at any retinal location.20–24 Each midget cell close to the fovea receives its dominant input from a midget bipolar cell, which in turn synapses with a single cone photoreceptor. Midget cells constitute two subgroups (bushy branching and simple branching), which stratify in the inner and outer regions of the inner plexiform layer (IPL), and their axons project to the parvocellular layers of the lateral geniculate nucleus (LGN) and to the pulvinar.25,26 Parasol cells form two subgroups (medium and large), have large dendritic fields, which are stratified near the center of the INL.26–28 They project to the magnocellular layers of the LGN, the pulvinar, and possibly the superior colliculus in the midbrain. Although the morphology and size of these major types of RGCs are well known, there have been few investigations showing cellular alterations in such pathologic situations as glaucoma.1 A comparison of the distribution of the parasol cell dendritic fields of normal and glaucomatous monkey retinas reported a significant decrease in dendritic field size of both midget and parasol cells in glaucoma.25,26
This study focused on the morphology of glaucoma-resistant midget and parasol cells in the human glaucomatous eye. To visualize the ganglion cell dendrites, paraformaldehyde-fixed retinas were labeled with the carbocyanine dye DiI.6–8 To assess possible morphometric changes, the number of dendritic bifurcations and the extent of dendritic branching of both midget and parasol cells were examined throughout the retinal eccentricity.

**METHODS**

This study was based on two enucleated eyes obtained from patients aged 53 and 60 years who had been diagnosed with congenital glaucoma and lacked the ability to perceive light (e.g., amaurosis at last examination) and two eyes of patients aged 32 and 52 years who had therapy-resistant absolute secondary glaucoma with amaurosis and painful chronic IOP higher than 40 mm Hg. Four nonglaucomatous eyes obtained postmortem from the Eye Bank of the University of Münster served as the control. Within 20 minutes after enucleation or release from the eye bank the eyes were transferred into 0.1 M phosphate-buffered saline (PBS, pH 7.4) and stored overnight at 4°C.
For retrograde staining of the RGCs, retinal flatmounts were rinsed in PBS several times before applying crystals of the fluorescent carboxy-cyanine dye Dil obtained from Molecular Probes (Eugene, OR) at 10 central and 10 peripheral positions on the retina, as follows (Figs. 1A, 1B). Tiny incisions were made on the retinal surface, into which small crystals of the dye (~50 μm in diameter) were inserted with a scalpel blade. The retinas were stored at 4°C for a period of 4 to 6 months in PBS containing 0.5% to 1% parafformaldehyde; and the success of staining was evaluated twice a week by low-magnification microscopy.

The rationale for using intraretinal labeling was based on data obtained from several pilot experiments conducted to label RGCs from the optic nerve stump. All of them failed to delineate RGCs, both in animal (rat, monkey) and human specimens. The lamina cribrosa represents the transition zone, where the myelinated axons of the optic nerve become unmyelinated in the retina, thus making it impossible for the dye to be retrogradely transported under postmortem conditions. The only feasible method to outline RGCs with their dendrites was to deposit the dye onto the nonmyelinated portion of the axons (i.e., directly on the nerve fiber layer around the optic nerve head).

For fluorescence microscopy analysis, the retinal flatmounts were placed on glass slides and coverslipped using medium for fluorescence microscopy (Mowiol; Merck, Darmstadt, Germany) and observed with the aid of a fluorescence microscope (AxioCam; Carl Zeiss Meditec, Oberkochen, Germany) at an excitation wavelength of 560 to 590 nm (rhodamine filter). Dil completely labels the ganglion cell soma as well as the dendrites, which were photographed digitally with a camera attached to the microscope (AxioCam; Carl Zeiss Meditec). Morphometric analysis was performed on the digital negatives, with a computer-assisted image-analysis system (Axiovision KS 300; Carl Zeiss Meditec). For this purpose, the bifurcations of the dendrites were marked and counted. Because RGC size varies with retinal eccentricity, a central and a peripheral position with reference to the fovea was determined. The central ganglion cells were located within a radius of 5 mm from the fovea, whereas all ganglion cells that were located beyond this border were considered to be peripheral ganglion cells. Only those cells that met the criteria of being either midget or parasol cells, based on previous studies using silver staining or Dil labeling in the human retina, were analyzed in this study.

The dendritic field diameter was measured using computer-aided graphical analysis (Axiovision KS 300; Carl Zeiss Meditec). Each photographed cell (total magnification 200× or 400×) was analyzed. A convex polygon was traced around the perimeter of the dendritic tree by connecting the tips of all peripheral arbors, and the area was calculated automatically by the computer software. The dendritic field diameter was expressed as the diameter of a circle with the same area as that of the polygon. Soma size was similarly expressed in terms of equivalent diameter.

The individual stages of ganglion cell axon and optic nerve atrophy were determined by examining transverse sections of retinal flatmounts and optic nerves stained with the hematoxylin-eosin and van Gieson stain, respectively. For this, the retinal flatmounts were fixed overnight in 4% paraformaldehyde and processed for paraffin embedding. Transverse sections through the retina and transverse and longitudinal sections through the optic nerves (5 μm) were cut and collected on gelatinized slides. The slides were then deparaffinized in xylene for 5 minutes, hydrated by passing them through a series of alcohol concentrations, dipped in hematoxylin for 5 minutes, and washed in running tap water. The sections were blued in lithium carbonate, rinsed in tap water, placed in eosin for 5 minutes, rinsed in tap water, dehydrated in alcohol, cleared in xylene and mounted in aqueous medium (Aquatex; Merck). For the van Gieson stain, the slides were deparaffinized in xylene for 5 minutes and hydrated with alcohol. The sections were dipped in celestine blue for 3 minutes, washed in water, dipped in alum hematoxylin for 3 minutes, and rinsed in water to blue the sections. This was followed by differentiation in 1% acid alcohol and washing in water. Then the sections were stained in van Gieson’s solution for 5 minutes, dehydrated in alcohol, cleared in xylene and mounted and cover-slipped (Aquatex; Merck). The sections were then examined for glaucoma-associated changes and documented.

Immunohistochemistry was performed using the gial fibrillary acidic protein (GFAP) antibody to identify glial cells. Frozen sections (12 μm) were cut, collected on gelatinized slides and stored at −20°C. The sections were fixed in cold acetone for 10 minutes, washed three times for five minutes each in phosphate-buffered saline (PBS) and blocked with 10% fetal calf serum (FCS) for 30 minutes. The polyclonal rabbit anti-rat GFAP antibody was diluted in fetal calf serum (FCS; dilution 1:100) and the sections incubated overnight at 4°C. After the slides were rinsed three times for five minutes each in PBS, the sections were incubated with an anti-rabbit Cy2 antibody (dilution 1:200 in FCS; Dianova, Hamburg, Germany) for 30 minutes at room temperature (RT) and washed three times for five minutes each in PBS. The sections were rinsed again in PBS (three times for five minutes each). Finally, the slides were cover-slipped using Mowiol (Merck) and viewed with the appropriate filter (band pass 560–570 nm) under a microscope equipped with epifluorescence (Axiohot; Carl Zeiss Meditec). Control experiments were conducted without the primary antibodies.

Results are expressed as mean ± SD throughout the text. Statistical analysis was performed using ANOVA. Significance was assumed at the 95% level of confidence of 95% (P < 0.05) using Student’s t-test.

**RESULTS**

**Labeling of RGCs and Other Retinal Cells with Dil**

All retinas analyzed were preserved well with the fixative, and RGCs were labeled to their full extent including axons, cell bodies, and dendrites. The highly lipophilic nature of Dil results in its immediate intercalation into the cell and axon membranes (Figs. 1C–F) subsequently diffusing intramembranously into the soma and dendrites, resulting in complete, “silver-stain-like” delineation of ganglion cells. RGCs in the perifoveal retina (Fig. 1G) and peripheral to the site of dye deposition (Fig. 1H) were intensely labeled within a distance of 2 to 5 mm from the point of dye application. Anterograde and retrograde diffusion of the dye completely stained the axonal segments (Figs. 1C–F). Retrograde labeling of the axons and the cell bodies both in glaucomatous and normal RGCs was achieved within 4 weeks. Intense labeling of the RGC dendrites took 4 to 5 weeks in control retinas, whereas glaucomatous ganglion cells needed almost 8 weeks for uniform dendritic labeling. Beyond 6 months, no further improvement in either the quantity or quality of labeling was observed. Therefore, the cells examined in this study were analyzed 6 months after application of the dye. In addition to the RGCs, other cells such as horizontal cells (Figs. 2A–D), amacrine cells (Fig. 2A) and sometimes glia (data not shown) were also observed in close vicinity of dye crystal (Fig. 2). However, the RGCs (Figs. 2E, 2F) were unequivocally distinguishable from horizontal (Figs. 2B–D) and amacrine cells (Fig. 2A), in both normal and glaucomatous retinas (Figs. 2E, 2F).

Deposition of the dye around the optic nerve head of normal retinas (Figs. 1A, 1B) resulted in labeling of 3000 ± 230 cells/mm² (n = 4) around the fovea, as determined from images shown in Figure 1G. In contrast, Dil deposited into the mid periphery of the retina (Figs. 1A, 1B) labeled 16 ± 7 cells/mm² in the outermost area of normal retinas. These densities comprised less than 15% of the total number of RGCs reported to be found in the retina (approximately 20,000 cells/mm² in the central and 1000 cells/mm² in the peripheral retina). The limitation of the methodology in quantitative studies has been reported. This methodological restriction therefore did not allow the quantification of the total number of RGCs either in the normal nor in the glaucomatous retinas. However, when deposited onto glaucomatous retinas, the dye labeled 25 ± 8 cells/mm² within the central retina and 7 ± 4
cells/mm² (n = 4) within the peripheral. When these densities were compared with the densities of RGCs obtained from normal DiI-labeled retinas, they led to the estimation that less than 1% of DiI-stainable RGCs remained in the diseased retinas. The morphometric data reported herein are based on 123 ganglion cells that were completely labeled with DiI. Of these, 61 (28 midget, 33 parasol) were from control retinas, and 62 (39 midget, 23 parasol) from glaucomatous retinas. The low number of glaucomatous RGCs was as expected, because of the advanced stage of degeneration and to the scarcity of human glaucoma donor eyes. Complete quantification of all glaucoma-resistant RGCs was not possible, because the dye stains only those cells with whose axons it comes in direct contact (Figs. 1A, 1B). Anterogradely stained axons that were visible over few millimeters in control retinas had axon bundles of different calibers with regularly distributed varicosities resembling transportation vesicles (Figs. 1C, 1D). In contrast, in all glaucomatous retinas, drastic axonal depletion was observed within the nerve fiber layer (Figs. 1E, 1F). Labeled axons had irregular shapes and exhibited variations in caliber, having large varicosities that were unevenly distributed along the axons (Figs. 1E, 1F).

**Midget Cells**

Examples of normal midget cells that were unequivocally identifiable are shown in the images in Figure 3. These neurons were characterized by their small cell bodies, smaller and more compact dendritic arbors, and relatively thin proximal axon segments. In addition, the dendritic trees of virtually all midget cells originated from a single primary dendrite and do not extend radially around the cell body. In some cases, it was necessary to focus into the IPL to visualize the entire dendritic tree, because some branching points were out of the plane of focus as seen in the single photographs (Fig. 3). The fine dendritic processes varied in both size and length. In addition to their small size, the bifurcation of the dendritic branches decreased with increasing eccentricity (Fig. 3). Whereas the number of branches was 19.1 ± 2.4 per cell (mean ± SD; n = 28) in central normal midget cells, it dropped significantly to 10.8 ± 1.9 per cell (n = 30 from four retinas, P < 0.05) in glaucomatous retinas. Likewise, the branching frequency in peripheral midget cells decreased from 30.7 ± 2.4 per cell (n = 28 from four retinas) to 24.7 ± 2.5 per cell (n = 28 from four retinas, P < 0.05) in the glaucomatous retinas.

Despite the limitations of the methods for quantitative staining of all RGCs, the intensity of DiI labeling in individual RGCs was sufficient to allow cell classification and morphometric analysis. Both types of RGCs could be identified in the glaucomatous retinas, although both were extremely rare and comprised less than 1% of the stainable RGC population when compared with normal retinas. The size of the diseased midget ganglion cells (Fig. 3) was comparable to that of normal midget cells. A scatterplot of the cell body sizes revealed a comparable distribution between glaucomatous and normal retinas (Fig. 4A). The extent of the dendritic field of glaucomatous RGCs was slightly reduced (Fig. 4B), perhaps due to the pruning of
peripheral arbors. However, the large degree of overlapping in the scatter diagram (Fig. 4B) and in the histogram (Fig. 4C) indicates that no changes were evident in cell body sizes between the normal and diseased state.

**Parasol Cells**

The size distribution of parasol cells showed that their diameter increased with increasing retinal eccentricity (Figs. 4C, 4D) although most parasol cells in the glaucomatous retinas had smaller diameters than the control parasol cells (Fig. 4D). In accordance with this, on an average, the size of the glaucomatous parasol cell bodies was comparable to that of normal parasols (Fig. 4E), whereas the average diameter of the dendrites was significantly smaller (Fig. 4F).

Normal parasol cells had variably large cell bodies of sizes directly proportional to increasing eccentricity (Fig. 4). They had radially oriented and almost symmetrically placed dendritic trees that originated from three to four primary dendrites. In contrast to the midget cells, parasol cells typically exhibited a symmetrical extension of dendrites and clearly increasing dendritic field diameter with increasing distance from the fovea (Fig. 5).

As expected from the functional blindness of the eyes included in this study, only a few parasol cells with completely outlined dendritic arbors were found in the glaucomatous retinas. However, the cells included in this study showed marked differences in the dendritic pattern (Figs. 5B, 5C, 5E, 5F, 5H, 5I–K) compared with normal parasol cells (Figs. 5A, 5D, 5G, 5J). In particular, cells in the periphery appeared to extend far fewer branches than normal cells of the same type. Morphologically, the arbors appeared beaded. The average branching frequency dropped from $36 \pm 3.4$ per cell in the normal central retinas ($n = 28$ in four retinas) to $23.7 \pm 5.5$ per cell in the glaucomatous central retinas ($n = 28$ in four retinas).
A more pronounced reduction of branching frequency was observed in the peripheral parasol cells. The average frequency of branching decreased from 58.7 ± 5.1 per cell (n = 28 from four retinas) in normal retinas to 26.3 ± 5.2 per cell (n = 28 from four retinas) in glaucomatous retinas (P < 0.01).

**Histologic Aspects**

In cross-sections the glaucomatous retinas appeared thinner, largely due to a marked decrease in the number of photoreceptors and RGCs (Fig. 6). In addition, profiles of shrunken, degenerating photoreceptor inner and outer segments were observed. There seemed to be a decrease in the thickness of the inner and outer plexiform layers. No morphologic sign of active degeneration was observed within the INL (Fig. 6).

van Gieson–stained glaucomatous optic nerves showed a loss of parenchyma (Fig. 7A), myelin substance, and axonal cylinders (Fig. 7B) compared with normal eyes. The normally spongy texture of the optic nerve was altered. Longitudinal hematoxylin-cosin–stained sections of the glaucomatous optic nerve (Figs. 7C-F) showed the typical structural deformation of oligodendroglial cell nuclei seen typically after atrophy. Anti-GFAP labeling of macroglial cells, revealed a marked proliferation of astrocytes, which is a sign of advanced gliosis (Fig. 7G) that was not observed in the control optic nerve (Fig. 7H).

**DISCUSSION**

We have examined the few RGCs that are resistant to the advanced stages of glaucoma by staining them in functionally blind eyes obtained after medically indicated enucleation due to the disease. The study was based on retrograde labeling with the carbocyanine dye DiI, which can label neurons in formalin-fixed tissue. (1) Despite the inability of this method to label all RGCs, a comparison between normal and glaucomatous retinas revealed that few RGCs, comprising far less than 1% of the stainable population, are still present in the amaurotic retinas; (2) the glaucoma-resistant RGCs can be categorized to the two major groups of midget and parasol cells; (3) whereas the cell bodies did not differ from normal RGCs, the dendritic branches were pruned, thus indicating degenerative changes; and (4) at the histologic level, glaucomatous changes were observed in the plexiform layer, the photoreceptor layer, and within the optic nerve.

The loss of RGCs in glaucoma is well-documented.1–5,10,11,25, 26,28–30 However, the mechanisms involved in the degeneration of the RGCs remain to be analyzed. One aspect of this complex process may lie in the ability of some RGCs to resist hypertension. Although morphometric data on ganglion cells were collected from only four eyes, and were limited in number due to the scarce material, they represented cells from four independent individuals. To our knowledge, this study documents for the first time that even amaurotic retinas possess axons that may connect the retina with the brain. The clear identification and categorization of RGCs...
in all the functionally blind eyes indicates that both major morphologic types of RGCs are represented, that some RGCs can survive abnormally high and chronic IOP, and that those surviving the high IOP are not sufficient to enable light perception.

The method used to label RGCs in formalin-fixed retinas is relatively simple and is based on the ability of DiI to diffuse intramembranously and label the cell bodies and processes of ganglion cells, which can be visualized with conventional fluorescence microscopy. The method’s applicability in the diseased retina indicates that the integrity of dendrites remains intact. However, pruned dendritic branches cannot be seen using this method, because they have been disconnected from Figure 5. Normal parasol cells within control (A, D, G, J) and glaucomatous (B, C, E, F, H, I, K) retinas (eccentricity increases from top to bottom). Note the marked decrease of the arborization in the glaucomatous cells. (L) Quantitative comparison of branching frequency between control and glaucomatous parasol cell in the center and periphery. Scale bar: 50 μm.

Figure 6. Histologic assessment of the degenerative changes associated with the late stages of glaucoma. (A) Normal retina obtained 24 hours after death. (B) Glaucomatous retina of the buphthalmic eye. Note the gliosis-like, irregular shapes of the NFL and GCL, the reduction in the number of photoreceptor cell nuclei, and the disappearance of photoreceptor outer segments. PRL, photoreceptor layer. Scale bar: 50 μm.
The sensitivity of axonal transport to various retinal diseases was also demonstrated in the Royal College of Surgeons rat.\textsuperscript{32} The irregular, swollen beads along the axons may represent aggregates of transportation vesicles, although blockage of axonal transport has been assumed to be involved in initial stages of RGC loss. By injecting lucifer yellow intracellularly, Weber et al.\textsuperscript{25} reported on the absence of axonal beads, which had been described by Kolb et al.\textsuperscript{33} based on Golgi impregnation, and thereby confirmed earlier\textsuperscript{23} observations of such beading. This difference may be of a methodological nature, because lucifer yellow diffuses into the axonal lumen, whereas the Golgi method stains argyrophilic compartments of the axon, including the beads. Therefore, the present data, which were obtained with a dye that is known to stain all parts of the axons, clearly shows a beading of normal axons and increased beading of glaucomatous axons, thus suggesting that this is an effect of advanced glaucoma's effects on axonal transport.

According to several studies, because glaucoma causes degeneration of RGCs, it comonly induces some glial proliferation.\textsuperscript{34} This was confirmed in the present study by showing the proliferation of astrocytes as a sign of advanced gliosis in the periaxonal space of the optic nerve. Furthermore, evidence of retinal damage and glial proliferation was shown in retinal cross sections. In addition to glial cells, the deeper layers of the retina were affected, and even a marked loss of photoreceptor cell nuclei and outer segments was observed, thus supporting previous findings.\textsuperscript{8,32,35} Glial proliferation is known to accompany various retinal diseases that are associated with loss of neurons. Such proliferation is probably non-specific and is considered a secondary effect.

Both major classes of RGCs\textsuperscript{18,20–22} exhibited reductions in the number of dendritic bifurcations. Changes at the level of the dendritic arbors are not surprising because they represent the early signs of glaucomatous neuropathy, as shown in the monkey model of glaucoma.\textsuperscript{25} Consequences of this pruning are the reduction of arbors and general decrease of the complexity of the dendritic tree. Compared with the monkey retina that shows several degrees of optic nerve damage and included earlier stages of the disease,\textsuperscript{34} the present study only dealt with extremely advanced stages of the disease and could not be used to discern dynamic changes. It is important to note, however, that loss of dendritic branches, which probably occurred at much earlier stages of the disease, did not result in apoptosis of the RGCs. The high degree of dendritic disintegration does not permit evaluation of whether midget or parasol cells are more vulnerable to IOP elevation. Former studies from different animal models revealed, however, that larger RGCs—presumably large parasol cells—are more susceptible to glaucoma-induced damage.\textsuperscript{5}

This was confirmed by other investigators who have also reported a selective loss of larger retinal ganglion cells when soma sizes were considered.\textsuperscript{5,10,11} Psychophysical studies in humans have revealed a diminution in the pattern electroretinogram and pattern visual evoked potentials, in response to stimuli of low spatial and high temporal frequencies, thus confirming vulnerability of parasol cells,\textsuperscript{36,52} a reduction in motion detection,\textsuperscript{26} and high-frequency flicker sensitivity\textsuperscript{55} in the glaucoma eyes. However, it is uncertain whether a selective damage of the parasol first and then the midget cells alone represent the earliest degenerative changes in glaucoma. Irrespective of these studies, some parasol cells can survive chronic IOP, just as midget cells do. This is in line with the detailed study of Weber et al.\textsuperscript{25} who found that the magnitudes of dendritic, axonal, and perikaryal changes were similar when comparing different stages of the disease with respect to differential changes between parasols and midget cells. The present study does not exclude differential changes, nor does the main dendritic tree or because they have degenerated and the debris was cleared away. Regardless of the final fate of these branches, their disappearance is probably pathognomonic in glaucoma, and most likely indicates that the RGCs not only atrophy retrogradely because of compression at the optic nerve head, but may also be directly influenced by the increased IOP. Morphologic changes in RGCs have been reported in induced glaucoma in rodents and primates.\textsuperscript{25} It has been recognized that acute IOP elevation obstructs orthograd and retrograde axonal transport in experimental monkey models.\textsuperscript{29–31} This blockage may involve the trophic supply to the RGCs, as shown by the inhibition of brain-derived neurotrophic factor transport after an acute increase in IOP.\textsuperscript{12} The morphologic correlate of diseased axons in the glaucomatous retinas in the present study may be altered axoplasmic transport, although dynamic studies have yet to demonstrate this.
it support such changes, because it dealt only with the aspect of resistance to advanced glaucoma within blind eyes.

The changes observed within all intraretinal segments of the RGCs are related to each other, and dendritic changes have been assumed to have some significance for the survival of the RGCs. Weber et al. suggested that as a first response to injury, RGCs spare their most distal dendrites to conserve energy and maintain homeostasis at the level of the soma. They also suggested that concomitant deprivation of retrogradely transportable neurotrophic factors ultimately results in apoptosis.

Pruning of dendritic spines and arbors alone does not suffice to induce apoptosis, perhaps because dendritic plasticity is a common feature of central nervous system neurons. The changes described in the present study are more robust than in normal neurons and should be considered pathologic. Dendritic spines, in contrast, are known to be extremely motile elements, providing a structural mechanism for synaptic plasticity.

The high metabolic and kinetic activity of the dendrites in the central nervous system is accompanied by a high vulnerability of the dendritic spines. Many forms of neuronal degeneration in the central nervous system occur with dendritic morphologic abnormalities, as in Huntington’s disease, in epileptic hippocampal neurons, in the cerebral cortex of patients with Alzheimer’s disease, and in the LGN of the monkey after eye removal. Deafferentation seems to be a reason for attenuation of dendritic transport, thus supporting the view that if both the axonal flow is interrupted and the dendrites are pruned, the RGCs may activate signals for apoptosis. It should be noted that all cells included in this study had an intact intraretinal axon segment, and no cells were included without the axons because the dye was applied distantly from the cell body. Although unlikely, we cannot exclude that some axonless RGCs were labeled in the retinas.

Retinal ganglion cells that survive an axotomy have been reported in several species such as mice, rats, and rabbits. In cats, approximately 2% to 5% of the RGC population appeared viable 2 months after optic nerve axotomy. Dendritic arbors of surviving RGCs, as revealed by horseradish peroxidase labeling, showed that some dendrites lacked branches and were smaller caliber. Other dendrites had many spiny processes and bulbous swellings. Why are these RGCs able to survive for such a long time after injury? von Bussmann et al. postulated that the population of ganglion cells that are injury resistant in the rat retina express high cytochrome oxidase (CO) activity. The CO-positive cells include both large type-I-like cells and smaller ganglion cells. After an intraorbital lesion, the RGCs are related to each other, and dendritic changes have been assumed to have some significance for the survival of the RGCs. Weber et al. suggested that as a first response to injury, RGCs spare their most distal dendrites to conserve energy and maintain homeostasis at the level of the soma. They also suggested that concomitant deprivation of retrogradely transportable neurotrophic factors ultimately results in apoptosis.

In summary, to our knowledge, the present study shows for the first time, that some ganglion cells of identifiable morphology are resistant to chronic and abnormally high IOP. However, the remaining population is insufficient to mediate light perception. In future studies, we will seek to determine the minimum essential population of RGCs required for residual light perception, and to determine the molecular mechanisms underlying the resistance to abnormally high IOP as a potential target of therapeutic measures.

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References