ELSEVIER

Immunolocalization of Heat Shock Proteins in the Retina of Normal Monkey Eyes and Monkey Eyes with Laser-induced Glaucoma

Miyako Sakai* Hiroshi Sakai*,
Yoshimi Nakamura*, Takeo Fukuchi† and Shoichi Sawaguchi*

*Department of Ophthalmology, School of Medicine, University of the Ryukyus, Okinawa, Japan; †Department of Ophthalmology, School of Medicine, Niigata University, Niigata, Japan

Purpose: To examine the expression and localization of heat shock proteins (HSPs) in the retinas of normal and experimentally induced primate glaucoma eyes. These proteins are known to be produced in response to a variety of stresses.

Methods: Experimental glaucoma was induced in the right eyes of three adult monkeys by repeated applications of argon laser to the chamber angle. Immunostaining with a panel of antibodies against HSP 90, 70, 60, 47, and 27 was performed on retinal sections prepared from the normal and glaucomatous monkey eyes.

Results: The intensity of immunostaining for HSP 90, 60, and 27 was greatly enhanced in the retinas of glaucomatous eyes. Prominent reactivity was observed in the inner retinal layers, especially in the ganglion cell and nerve fiber layers. The staining intensity for HSP 70 was also moderately increased, while immunoreactivity against HSP 47 remained almost unchanged in glaucomatous retinas. Immunostaining against glial fibrillary acidic protein was increased and the immunolabeling pattern appeared to be identical with that of HSP 90 in glaucoma retinas.

Conclusions: The level of HSP 90, 70, 60, and 27 in primate retinas was increased in experimentally induced ocular hypertension. The differences in expression pattern suggest that each HSP may have its unique role in responding to damage or injury related to intraocular pressure elevation. **Jpn J Ophthalmol 2003;47:42–52** © 2003 Japanese Ophthalmological Society

Key Words: Experimental glaucoma, heat shock protein, immunohistochemistry, retina.

Introduction

Glaucoma is generally characterized by elevated intraocular pressure (IOP), a typical excavated appearance of optic disc, and loss of retinal ganglion cells. Current research indicates that retinal ganglion cell death is at least in part mediated through apoptotic pathways.[1–4] Recently, in an experimental rat model, detailed molecular mechanisms of ganglion cell apoptosis triggered by transient acute high IOP have been disclosed.[5,6] Chronic IOP elevation also induces apoptotic retinal ganglion cell death in humans, primates, and rabbits.[1,3] In the glaucoma field, considerable interest and efforts are being focused on anti-apoptotic regimens for the prevention of retinal ganglion cell death both in vivo and in vitro.[7–9]

Heat shock proteins (HSPs), also called stress proteins, are highly conserved proteins constitutively expressed in most cells, including neural cells under physiological conditions.[10,11] They are classified into family members according to their molecular weights, for example, the 90-kDa HSP (HSP 90), 70-kDa HSP (HSP 70), 60-kDa HSP (HSP 60), 47-kDa HSP (HSP 47), and the small 27-kDa HSP (HSP 27). In response to environmental stresses such as heat,

Received: December 5, 2001
Correspondence and reprint requests to: Miyako SAKAI, MD, Department of Ophthalmology, School of Medicine, University of the Ryukyus, 207 Azauehara Nishihara-cho, Okinawa 903-0215, Japan

Published by Elsevier Science Inc.
0021-5155/03/$–see front matter
PII S0021-5155(02)00627-5

anoxia, and a variety of cytokines, neuronal and non-neuronal cells express large amounts of HSPs.[12,13] Because of their cellular protective capability, the increased levels of HSPs may help the cells to survive under stressful environment and also to promote recovery from stress.[14–17] For instance, HSP 27 was upregulated in a time-dependent manner after damage or transection of the peripheral nerve.[18] HSPs are also expressed in the retina and regulated through ocular organogenesis.[19,20] HSP 70 is rapidly induced by hyperthermia, light exposure, and ischemic injury in the retina.[21–23] Investigation of retinal HSP expression in ocular diseases, especially glaucoma, is limited. Herein we describe an immunohistochemical study to examine the expression and localization of a panel of HSPs in the retinas of experimentally induced glaucomatous monkey eyes. Our results agree with a very recent report[24] that HSP 60 and 27 were increased in the retina and optic nerve head of primary open-angle glaucoma (POAG) and normal pressure glaucoma (NPG) eyes. In addition, we observed increased HSP 90 staining. In this study, the expression pattern of HSP 90 in the monkey paralleled that of the glial fibrillary acidic protein (GFAP) seen in Müller cells.

Materials and Methods

Experimental Glaucoma Models

Three adult Macacus fuscatus monkeys, MK44, 66, and 73, each weighing 8–10 kg, were used in this study. All animals were treated in accordance with the Association for Research in Vision and Ophthalmology Resolution on the Use of Animals in Research. A baseline examination showed all monkey eyes had normal anterior segments and optic nerve heads. The IOP measured by pneumatic tonography (Alcon, Fort Worth, TX, USA) was also within normal range (20–25 mm Hg). Argon laser photocoagulations were applied repeatedly to chamber angles of the right eyes of these monkeys to induce glaucoma. The contralateral eyes were used as controls. The detailed laser treatment was as described previously.[25,26] Briefly, the monkeys were anesthetized by intramuscular injection of 9 mg/kg of ketamine hydrochloride and intravenous injection of 11 mg/kg of sodium pentobarbital. They were placed in front of the slit-lamp of an argon laser system (Biophysic, Paris, France), and the eyes were anesthetized with 0.4% oxybuprocaine hydrochloride. Approximately 200–400 circumferential laser burns were made with a small gonioscopic lens, aimed at the middle of the trabecular meshwork, with a 100-μm beam diameter, 0.2-second duration, and 600–800 mW power. The laser treatment was repeated weekly for 3–5 weeks. Ophthalmologic examinations were performed every 1–2 weeks. After treatment, the IOPs of laser-treated eyes increased up to 50 mm Hg. The mean IOP of the experimental eyes of MK44, 66, and 73 monkeys was respectively, 32.1, 31.5, and 31.2 mm Hg, and that of control eyes was 22.9, 25.2, and 19.4 mm Hg (Table 1).

Tissue Preparation

Monkeys were sacrificed by exsanguination after an overdose administration of ketamine hydrochloride and sodium pentobarbital. All experimental eyes had advanced to severe glaucomatous cupping (Table 1). At the time of sacrifice, the IOP of laser-treated eyes of monkeys MK44, 66, and 73 was, respectively, 47, 29, and 42 mm Hg and that of control eyes was 28, 24, and 20 mm Hg. All eyes were gently enucleated and parts of the retinas were removed and fixed in 10% neutralized buffered formalin, fixed overnight, dehydrated in graded ethanol, and

Table 1. Intraocular Pressure (IOP) and Optic Nerve Damage

Monkey Eyes	Diagnosis	IOP Range (mm Hg)	Mean IOP (mm Hg)	Duration of Glaucoma*	C/D Ratio†
MK 44					
R	Glaucoma	21–48	32.1	4.5 mo	0.9
L	Normal	21–28	22.9		
MK 66					
R	Glaucoma	22–50	31.5	3.5 mo	0.8
L	Normal	24–26	25.2		
MK 73					
R	Glaucoma	20–44	31.2	5.0 mo	>0.9
L	Normal	16–21	19.4		

*mo: month.
†C/D: cup-to-disc ratio.

Jpn J Ophthalmol
Vol 47: 42–52, 2003

processed for paraffin embedding. Sections (6-μm thick) were cut onto poly L-lysine-precoated glass slides.

Immunohistochemical Study

For immunostaining, polyclonal goat antibodies against human HSP 90, 70, 60, 47, and 27 (Santa Cruz Biotechnology, Santa Cruz, CA, USA) were used at 1:800 dilutions. Normal goat serum (Sigma-Aldrich, St. Louis, MO, USA) at the same dilution was used in place of the primary antibodies to create negative controls. For immunoperoxidase staining, sections from normal and glaucomatous eyes were deparaffinized, rehydrated, and pretreated with 0.3% hydrogen peroxide in phosphate-buffered saline (PBS) to quench endogenous peroxidase activity. After blocking with 10% bovine serum (Sigma-Aldrich), the slides were incubated at 4°C overnight with primary antibodies in a humidified chamber, followed by 20-minute incubation with biotinylated rabbit anti-goat antibody (Zymed Lab, San Francisco, CA, USA) at 1:1000 dilution. Sections were then allowed to react with streptavidin-horseradish peroxidase conjugate (DAKO, Kyoto) for 30 minutes. Immunoreactivity was visualized by incubation with peroxidase substrate 3,3′-diaminobenzidine tetrahydrochloride (DAKO) for about 5 minutes. For GFAP staining, polyclonal rabbit anti-cow GFAP (DAKO) was used at 1:500 dilution.

Densitometric Analysis

For quantitative evaluation, the intensity of immunohistostaining was measured with the DAB analysis system (Carl Zeiss, Oberkochen, Germany) as

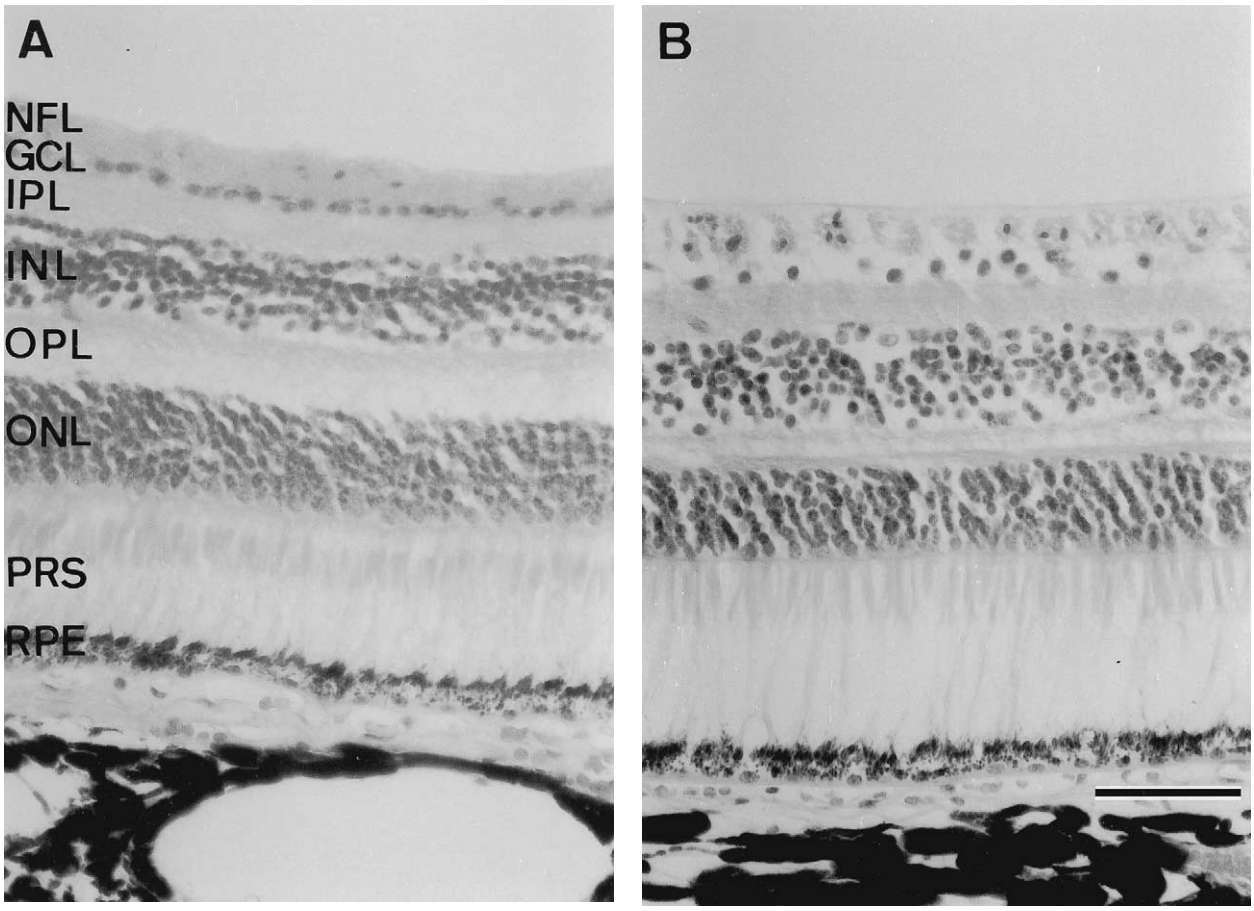

Figure 1. Histopathology of the retina in eyes of monkey MK73 with (**B**) or without glaucoma (**A**). Note the loss of retinal nerve fiber layer and ganglion cell layer in glaucomatous retina (**B**) compared with control retina (**A**). Original magnification was ×50. Bar = 100 μm; hematoxylin and eosin stain. NFL: nerve fiber layer, GCL: ganglion cell layer, IPL: inner plexiform layer, INL: inner nuclear layer, OPL: outer plexiform layer, ONL: outer nuclear layer, PRS: photoreceptor segment, RPE: retinal pigment epithelium.

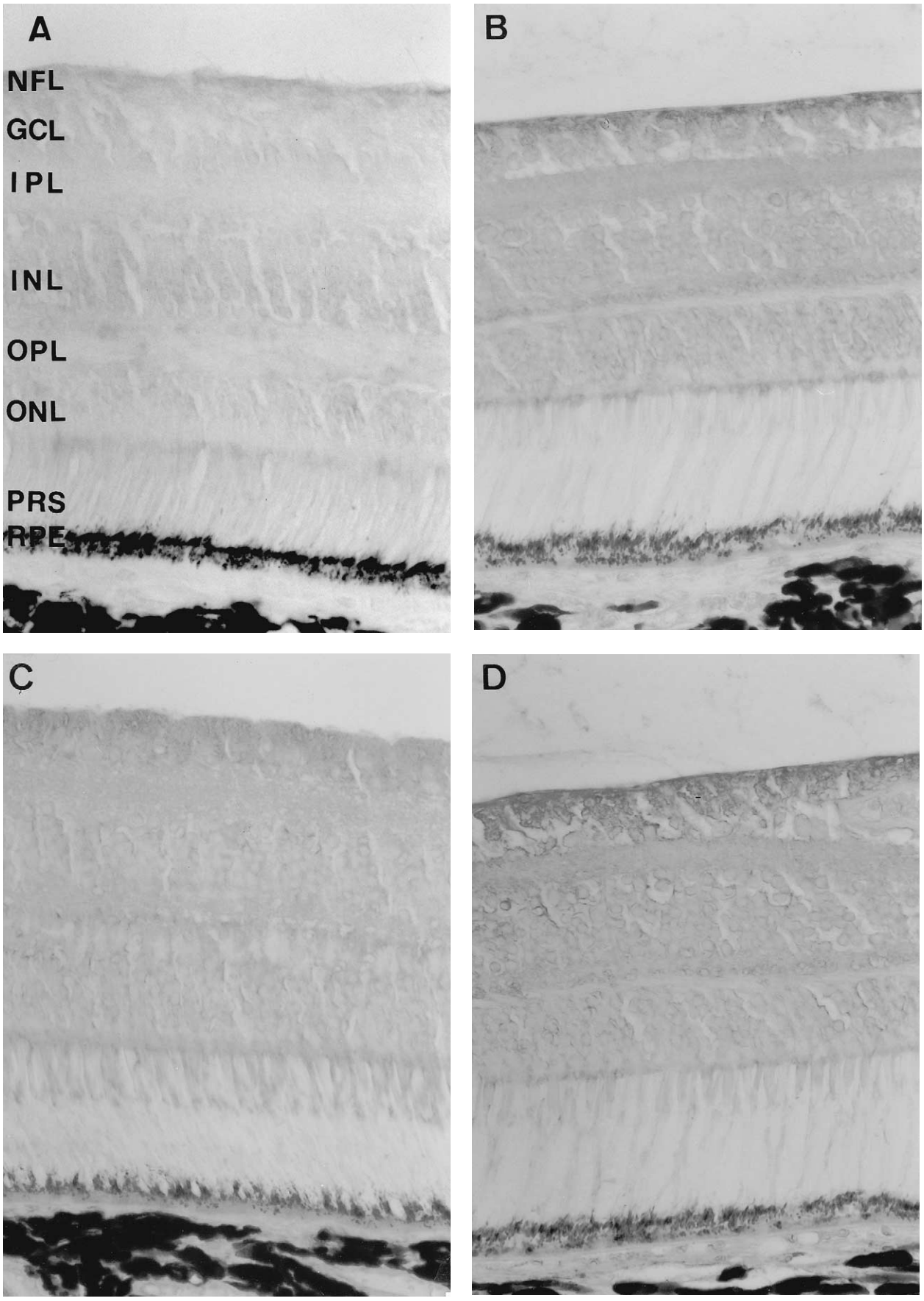

Figure 2. Continued.

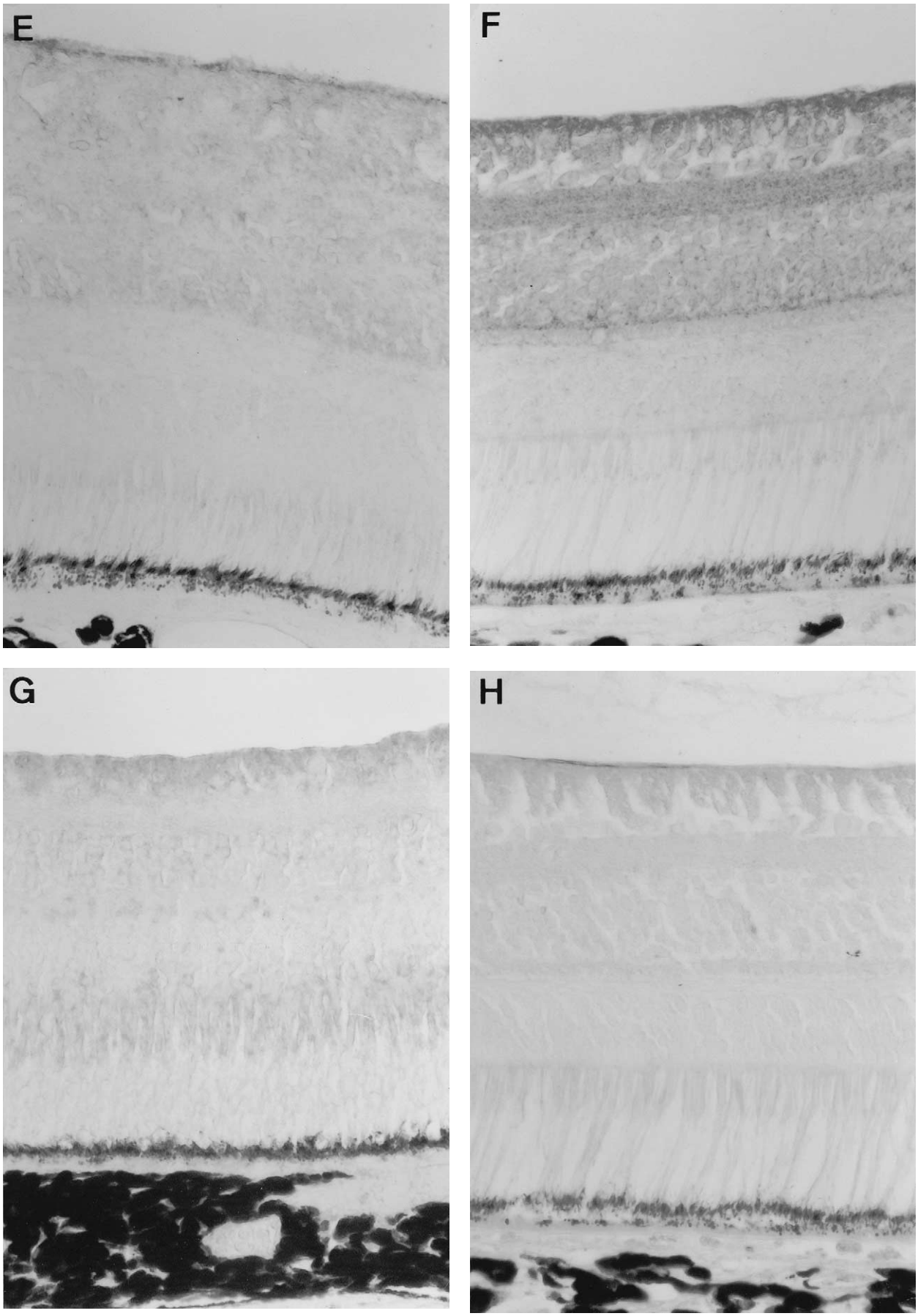

Figure 2. Continued.

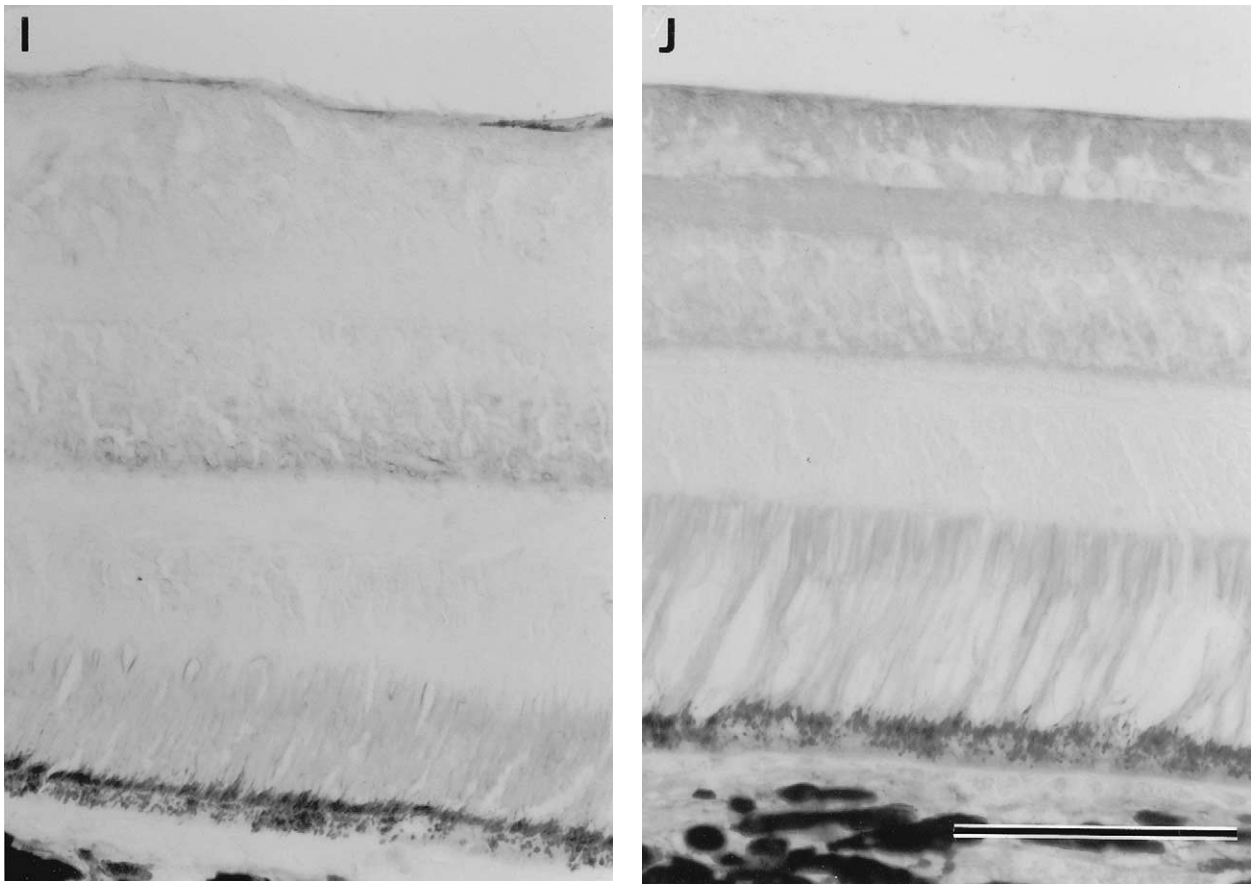

Figure 2. Immunohistochemistry of heat shock proteins (HSPs) in the retina of control (**A**, **C**, **E**, **G**, and **I**) and glaucomatous (**B**, **D**, **F**, **H**, and **J**) eyes of monkey MK44. Immunostaining was performed with antibodies to HSP 90 (**A** and **B**), HSP 70 (**C** and **D**), HSP 60 (**E** and **F**), HSP 47 (**G** and **H**) and HSP 27 (**I** and **J**). Note that the immunoreactivity for HSP 90 (**B**), HSP 70 (**D**), HSP 60 (**F**), and HSP 27 (**J**) was increased in glaucomatous retina compared to normal retina. A nearly identical immunolabeling pattern between HSP 90 and HSP 70 in glaucomatous retinas (**B** and **D**) was demonstrated. Also, retinal ganglion cell layer (GCL) and nerve fiber layer (NFL) showed the most prominent immunoreactivity. Little change with glaucoma was seen with antibody against HSP 47. Original magnification was ×50. Bar = 200 μm. IPL: inner plexiform layer, INL: inner nuclear layer, OPL: outer plexiform layer, ONL: outer nuclear layer, PRS: photoreceptor segment, RPE: retinal pigment epithelium.

described previously.[27] The images of stained sections were scanned by light microscope and the processing DAB analysis system. The measurements were made as the optical density (OD) of the light source of the light microscope set on fixed luminous and exposure time. The reference line of OD was made the average value of the empty background field. For each retinal layer, at least three measurements were made on focused areas of different fields. Data were presented as the ratio of the average value of OD in the measured area to the value of OD in the reference area. Grouped Student *t*-tests were used for statistical analysis.

Results

The clinical data that include experimental periods, average IOP values, and the degree of glaucomatous optic nerve damage are shown in Table 1. All three experimental eyes displayed typical advanced to end-stage glaucomatous discs compared to those of the control eyes. Retinal sections stained with hematoxylin and eosin showed marked reduction of retinal ganglion cells and thinning of the inner retinal layer, especially of the nerve fiber layer and ganglion cell layers (Figure 1). Immunostaining experiments performed indicated that in normal monkey retina, the immunoreactivity for HSPs var-

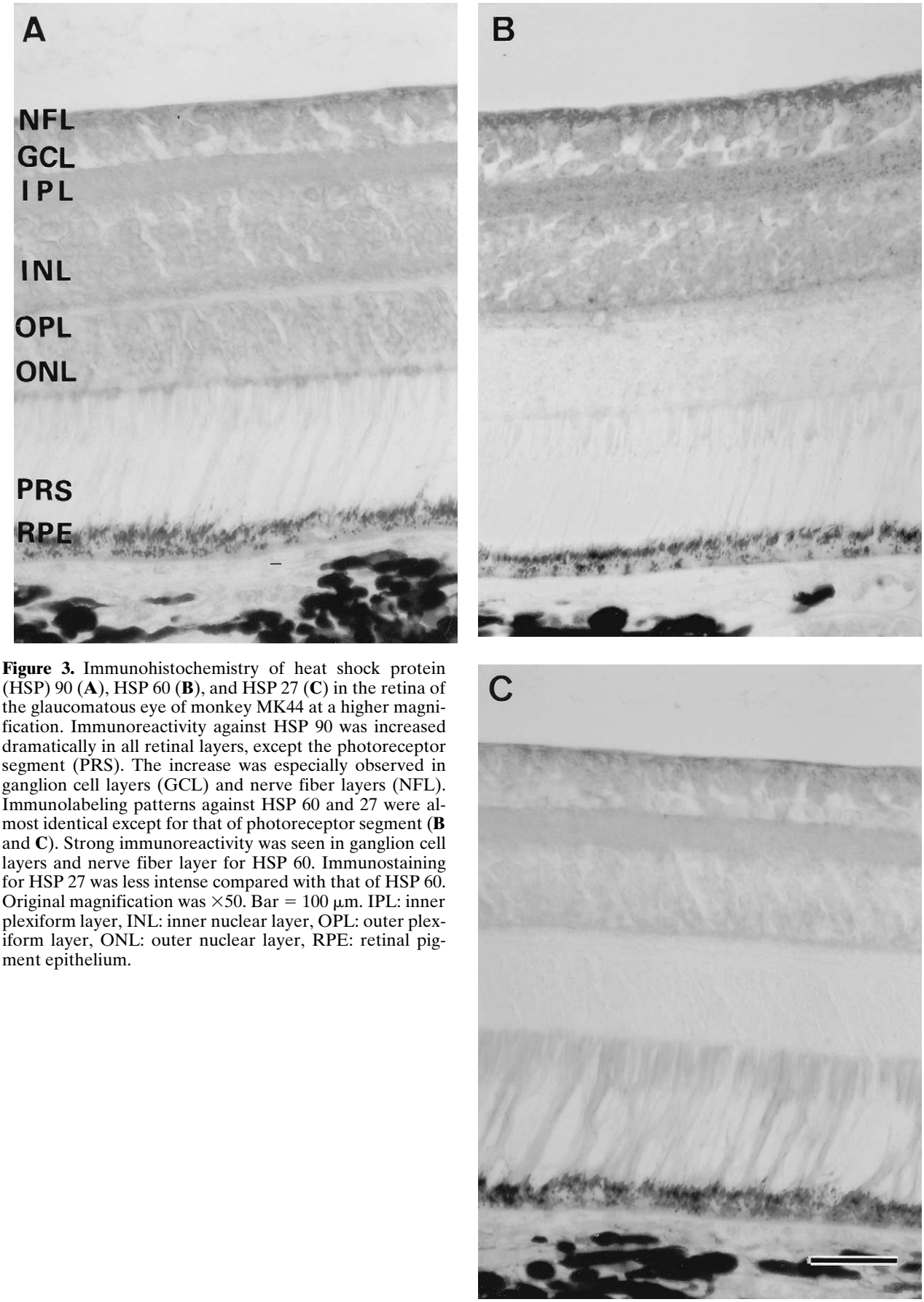

Figure 3. Immunohistochemistry of heat shock protein (HSP) 90 (**A**), HSP 60 (**B**), and HSP 27 (**C**) in the retina of the glaucomatous eye of monkey MK44 at a higher magnification. Immunoreactivity against HSP 90 was increased dramatically in all retinal layers, except the photoreceptor segment (PRS). The increase was especially observed in ganglion cell layers (GCL) and nerve fiber layers (NFL). Immunolabeling patterns against HSP 60 and 27 were almost identical except for that of photoreceptor segment (**B** and **C**). Strong immunoreactivity was seen in ganglion cell layers and nerve fiber layer for HSP 60. Immunostaining for HSP 27 was less intense compared with that of HSP 60. Original magnification was ×50. Bar = 100 μm. IPL: inner plexiform layer, INL: inner nuclear layer, OPL: outer plexiform layer, ONL: outer nuclear layer, RPE: retinal pigment epithelium.

Table 2. Intensities of Immunostainings of Each Heat Shock Protein (HSP) in the Retina of Normal and Glaucomatous Monkey Eyes*

	HSP90		HSP70		HSP60		HSP47		HSP27	
Retinal Area†	Normal Eyes	Glaucoma Eyes	Normal Eyes	Glaucoma Eyes	Normal Eyes	Glaucoma Eyes	Normal Eyes	Glaucoma Eyes	Normal Eyes	Glaucoma Eyes
NFL	1.24 ± 0.12	1.68 ± 0.26‡	1.57 ± 0.14†	2.27 ± 0.34‡	1.45 ± 0.37	1.99 ± 0.21§	1.26 ± 0.30	1.51 ± 0.16	1.56 ± 0.08	2.27 ± 0.36§
GCL	1.14 ± 0.10	1.48 ± 0.17‡	1.36 ± 0.04	1.78 ± 0.13‡	1.40 ± 0.35	1.78 ± 0.28‡	1.17 ± 0.23	1.37 ± 0.24	1.31 ± 0.15	1.75 ± 0.23‡
IPL	1.05 ± 0.09	1.33 ± 0.07‡	1.28 ± 0.07	1.55 ± 0.19§	1.24 ± 0.16	1.60 ± 0.23‡	1.09 ± 0.13	1.20 ± 0.10	1.18 ± 0.12	1.49 ± 0.17§
INL	1.07 ± 0.09	1.28 ± 0.06§	1.23 ± 0.06	1.48 ± 0.17§	1.16 ± 0.09	1.52 ± 0.19‡	1.06 ± 0.13	1.15 ± 0.07	1.10 ± 0.06	1.28 ± 0.09‡
OPL	1.03 ± 0.11	1.45 ± 0.19‡	1.23 ± 0.02	1.56 ± 0.14‡	1.16 ± 0.13	1.23 ± 0.12	1.04 ± 0.10	1.17 ± 0.12	1.14 ± 0.14	1.18 ± 0.11
ONL	1.11 ± 0.11	1.30 ± 0.10§	1.20 ± 0.04	1.50 ± 0.14‡	1.13 ± 0.14	1.16 ± 0.09	1.05 ± 0.09	1.11 ± 0.05	1.09 ± 0.07	1.11 ± 0.05
PRS	1.07 ± 0.10	1.21 ± 0.07¶	1.21 ± 0.06	1.36 ± 0.13¶	1.06 ± 0.02	1.22 ± 0.19¶	1.06 ± 0.09	1.25 ± 0.07§	1.27 ± 0.12	1.42 ± 0.16§

*Measured by the DAB analysis system (Carl Zeiss), the intensities of immunohistostaining are calculated from the ratio of the mean intensities in the measured area to the densities in the measured areas to the densities in the reference area in each slide. Grouped Student *t*-tests were used for statistical analysis (*P* values are not shown).

†NFL: nerve fiber layer, GCL: ganglion cell layer, IPL: inner plexiform layer, INL: inner nuclear layer, OPL: outer plexiform layer, ONL: outer nuclear layer, PRS: photoreceptor segment.

‡$P < .001$ compared with normal retinas.

§$P < .01$ compared with normal retinas.

¶$P < .05$ compared with normal retinas.

ied from weak (HSP 90, Figure 2A), and mild (HSPs 60, 47, and 27, Figures 2E, 2G, and 2I) to moderate (HSP 70, Figure 2C). The staining pattern for each HSP in the retinal layers was also somewhat different. For example, the staining for HSP 70 (Figure 2C) was moderate in the ganglion cell layer and nerve fiber layer, mild in the inner nuclear, outer nuclear, and outer plexiform layers, but very weak in the outer photoreceptor segment. HSP 60 immunoreactivity was mostly in the inner retinal layers (Figure 2E). The normal retina was mildly positive for HSP 47 at the ganglion cell and nerve fiber layers, and at the outer nuclear layer (Figure 2G); while staining for HSP 27 (Figure 2I) was seen at the inner nuclear layer, near the outer plexiform layer and at the photoreceptor segment. Negative control specimens exhibited little to no immunoreactivity.

In glaucoma eyes, enhanced immunoreactivity was observed for all HSPs examined except HSP 47. Increased staining intensity was seen for HSP 90 (Figures 2B, 3A), HSP 70 (Figure 2D), HSP 60 (Figures 2F, 3B) and HSP 27 (Figures 2J, 3C) at especially the ganglion cell and nerve fiber layers. Both anti-HSP 90 and anti-HSP 70 demonstrated positive reactivity at the outer nuclear layer of glaucomatous eyes (Figures 2B and 2D). Staining at this layer, however, was not observed for HSP 60 and HSP 27 (Figures 2F, 2J, 3B, 3C). Also, anti-HSP 27 but not anti-HSP 60 and HSP 90, yielded positive staining in the photoreceptor segment of glaucoma retinas (Figures 2F, 2J, 3B, 3C). In the case of HSP 47, the positive immunostaining at ganglion cell and nerve fiber layers of normal eyes appeared to be unchanged by glaucomatous damage, except for increased mild immunostaining at photoreceptor segments (Figures 2G, 2H). Analysis of OD measurements showed almost similar results (Table 2). Staining for GFAP was also more prominently observed in all layers except in the photoreceptor segment of the retina in experimental glaucoma eyes (Figure 4B) compared to that of the control eyes (Figure 4A). The GFAP staining pattern was similar to that of HSP 90 (Figures 2A, 2B, 4A, 4B). Results from the three experimental animals were nearly identical.

Discussion

HSPs are known to be expressed and to be produced in response to various environmental and physiological stresses in all cells, from bacteria to mammalian cells, in order to enhance cell survival. Induced expression of HSPs has been shown to heighten neuronal tolerance and promote survival of neuronal tissue after retinal ischemic insult, light-induced retinal damage[22,28] and neuro-excitotoxicity.[29,30] HSPs are classified into family members according to their molecular weights and different functions. Briefly, HSP 90 is necessary for cell proliferation or cell survival and plays a role in the regulation of tyrosine kinase activity.[31] It is induced in retinal Müller cells also by heat shock stress.[32] HSP 70 is the most common HSP member and is known as mo-

Jpn J Ophthalmol
Vol 47: 42–52, 2003

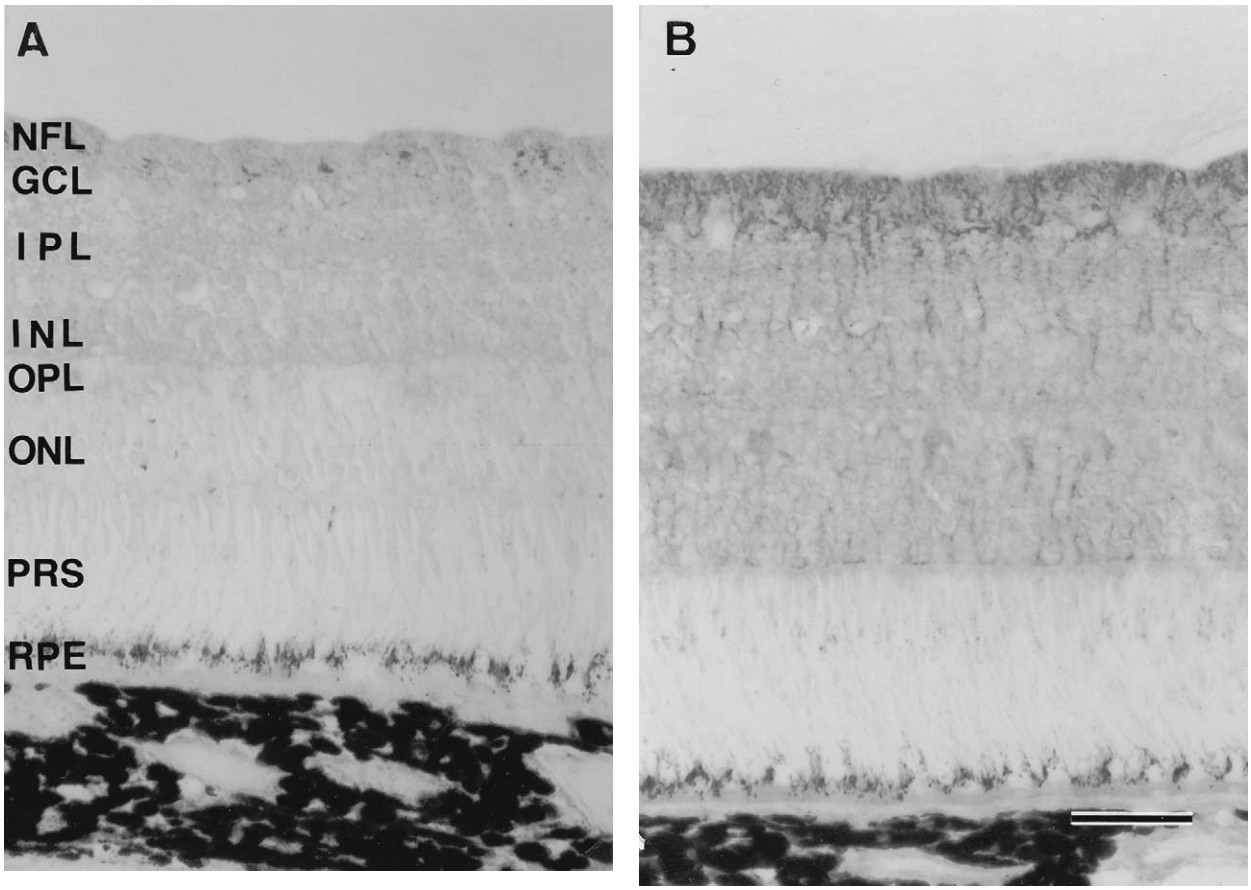

Figure 4. Immunohistochemistry of glial fibrillary acidic protein in the retina of normal (**A**) and glaucomatous retina (**B**) from monkey (MK66) eyes. Markedly enhanced immunoreactivity was seen in all layers except photoreceptor segment (PRS) layer of glaucomatous retina (**B**). Original magnification was ×50. Bar = 100 μm. NFL: nerve fiber layer, GCL: ganglion cell layer, IPL: inner plexiform layer, INL: inner nuclear layer, OPL: outer plexiform layer, ONL: outer nuclear layer, RPE: retinal pigment epithelium.

lecular chaperone and possesses cellular protective anti-apoptotic ability.[33,34] HSP 60 plays a critical role in the routing of proteins to the mitochondrial intermembrane space.[35,36] HSP 47 is basically involved in the tissue remodeling processes and functions as a collagen-specific molecular chaperone.[37,38] HSP 27 protects cells from apoptotic death by inactivating caspase-8 and -3, which disintegrate actin cytoskeleton.[33,39,40] This HSP member also prevents Fas/Apo1-induced DNA fragmentation and morphological changes, and acts as a cellular inhibitor of Fas/Apo1-induced apoptosis.[41]

By immunohistochemical analysis, the present study demonstrated the expression and localization of the various HSPs in the retinas of control and experimentally induced glaucomatous eyes. Immunostaining for HSPs 90, 60, and 27 was found to be much stronger and that for HSP 70 was moderately elevated in the glaucomatous retinas compared to the control retinas, consistent with the principle that the apoptotic pathway is one of the key mechanisms involved in glaucomatous neuropathy, and that HSPs are upregulated perhaps to inhibit apoptosis and rescue neuronal cells. Among the HSPs examined, HSP 47 was the only exception to the rule that little change in staining intensity or pattern was detected in any layer of the glaucomatous retinas, with a mild increase at photoreceptor segments. As discussed above, HSP 47 has a specialized function in procollagen synthesis and assembly[37,38] but may not be directly involved in cell protection during stress. Although the staining pattern was somewhat varied for each HSP, the increased immunoreactivity for HSPs 90, 70, 60, and 27 in glaucomatous retinas was

in all cases prominently seen at the ganglion cell and nerve fiber layers. The GFAP immunolabeling was similar to that of HSP 90 in both normal and glaucomatous eyes.

Recently, studies that explored the role of HSPs in glaucomatous neuropathy have been reported. Serum autoantibodies against HSP 60 and 27 were shown to be significantly elevated in POAG and NPG patients.[42] Immunostaining of HSP 60 and 27 in the retina and optic nerve heads of POAG and NPG patients was also more intense than in the age-matched normal counterparts.[24] Furthermore, induction of HSP 72, a member of the HSP 70 family, in a rat glaucoma model by heat stress or zinc administration increased the survival of retinal ganglion cells.[43]

The different expression patterns of HSPs observed suggest that each HSP may respond differently to IOP-related damage or injury in the retina and each may have a unique role. Interestingly, the retinal localization and expression of HSP 90 paralleled that of GFAP, a glial cell marker, in glaucoma eyes. It is known that retinal glial cells, especially Müller cells, are activated in glaucomatous neuropathy.[44] Müller cells have also been reported to produce HSP 90 under heat shock stress[32] and to generate neuroprotective cytokines such as ciliary neurotrophic factor after retinal injury.[9] These cells may thus hold an inherent neuroprotective capability in the retina. The identical staining pattern of HSP 90 and GFAP suggests that activated Müller cells may be an important source of the HSPs. HSP 70, moderately enhanced in ganglion cell and nerve fiber layers, may also exert neuroprotective effects in experimental glaucoma, because members of the HSP family display anti-apoptotic activities.[33,34,43] An HSP 70 family member appeared in the rat retina immediately after IOP elevation and its expression declined 7 days later.[43] In experimental monkey glaucoma, the IOP fluctuated and at the time of sacrifice (3–5 months after laser treatment), the IOPs were usually only mildly to moderately elevated. One may speculate that, analogous to the rat glaucoma model, the expression of human HSP 70 may be very high shortly after acute IOP elevation. It is also possible that in the current primate study, the increased immunoreactivity for HSPs 90, 70, 60, and 27 was IOP-dependent. However, increased expression of HSP 60 and 27 in the human eye has also been observed in NPG eyes, suggesting an IOP-independent pathway. While their mechanisms remain to be elucidated, such HSP members as HSP 90 and HSP 70, because of their anti-apoptotic activities, may be used therapeutically in conjunction with IOP-reducing modalities for the management of glaucomatous neuropathy.

Part of this study was presented at the ARVO annual meeting in Fort Lauderdale, FL, USA, in May 2000. This study was supported in part by a Grant-In-Aid for Scientific Research (no. 13671845) from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

References

1. Quigley HA, Nickells RW, Kerrigan LA, Pease ME, Thibault DJ, Zack DJ. Retinal ganglion cell death in experimental glaucoma and after axotomy occurs by apoptosis. Invest Ophthalmol Vis Sci 1995;36:774–786.
2. Garcia-Valenzuela E, Shareef S, Walsh J, Sharma SC. Programmed cell death of retinal ganglion cells during experimental glaucoma. Exp Eye Res 1995;61:33–44.
3. Okisaka S, Murakami A, Mizukawa A, Ito J. Apoptosis in retinal ganglion cell decrease in human glaucoma eyes. Jpn J Ophthalmol 1997;41:84–88.
4. Nickells RW. Apoptosis of retinal ganglion cells in glaucoma: an update of the molecular pathways involved in cell death. Surv Ophthalmol 1999;43(Suppl):151–161.
5. Szabo ME, Droy LeFair MT, Doly M, Carre C, Braquet P. Ischemia and reperfusion-induced histologic changes in the rat retina. Invest Ophthalmol Vis Sci 1991;32:1471–1478.
6. Selles-Navarro I, Villegas-Perez MP, Salvador-Silvia M, Ruiz-Gomez JM, Vidal-Sanz M. Retinal ganglion cell death after different transient periods of pressure-induced ischemia and survival intervals: a quantitative in vivo study. Invest Ophthalmol Vis Sci 1996;37:2002–2014.
7. Katai N, Yoshimura N. Apoptotic retinal neuronal death by ischemia reperfusion is executed by two distinct caspase family proteases. Invest Ophthalmol Vis Sci 1999;40:2697–2705.
8. Ikeda K, Tanihara H, Honda Y, Tatsuno T, Noguchi H, Nakayama C. BDNF attenuates retinal ganglion cell death caused by chemically induced hypoxia in rats. Invest Ophthalmol Vis Sci 1999;40:2130–2140.
9. Honjo M, Tanihara H, Kido N, Inatani M, Okazaki K, Honda Y. Expression of ciliary neurotrophic factor activated by retinal Müller cells in eyes with NMDA- and Kainic acid-induced neuronal death. Invest Ophthalmol Vis Sci 2000;41:552–560.
10. Subjeck JR, Shyy TT. Stress protein systems of mammalian cells. Am J Physiol 1986;250:C1–C17.
11. Welch WJ. Mammalian stress response: cell physiology structure/function of stress proteins and implications for medicine and disease. Physiol Rev 1992;72:1063–1081.
12. Iwaki T, Iwaki A, Tateishi J, Sakaki Y, Goldman JE. αβ-Crystallin and 27 kilodalton heat shock protein are regulated by stress conditions in the central nervous system and accumulate in Rosenthal fibers. Am J Pathol 1993;143:487–495.
13. Kato H, Liu Y, Kogure K, Kato K. Induction of 27-kda heat shock protein following cerebral ischemia in a rat model of ischemic tolerance. Brain Res 1994;634:235–244.
14. Lindquist S. The heat-shock response. Annu Rev Biochem 1986;205:1151–1191.
15. Lindquist S, Craig EA. The heat shock proteins. Annu Rev Genet 1988;22:631–677.
16. Ellis RJ, Van Der Vies SM. Molecular chaperones. Annu Rev Biochem 1991;60:321–347.

17. Parsell DA, Lindquist S. The functions of heat shock proteins in stress tolerance: degradation and reactivation of damaged proteins. Annu Rev Biochem 1993;27:437–496.
18. Hoskins DA, Plumier JC, Currie RW. Induction of the 27-kDa heat shock protein (Hsp 27) in the rat medulla oblongata after vagus nerve injury. Exp Neurol 1988;153:173–183.
19. Tanaka Y, Kobayashi K, Kita M, Kinoshita S, Imanishi J. Messenger RNA expression of heat shock proteins (HSPs) during ocular development. Curr Eye Res 1995;14:1125–1133.
20. Kojima M, Hoshimaru M, Aoki T, et al. Expression of heat shock proteins in the developing rat retina. Neurosci Lett 1996;205:129–1235.
21. Tytell M, Barbe MF, Brown IR. Induction of heat shock (stress) protein 70 and its mRNA in the normal and light-damaged rat retina after whole body hyperthermia. J Neurosci Res 1994;38:19–31.
22. Caprioli J, Kitano S, Morgan JE. Hyperthermia and hypoxia increase tolerance of retinal ganglion cells to anoxia and excitotoxicity. Invest Ophthalmol Vis Sci 1996;37:2376–2381.
23. Lewdon O, Garcher C, Assem M, Morales C, Rochette L, Bron AM. Changes of the inducible heat shock protein 70 mRNA level in rat retina after ischemia and reperfusion. Ophthalmic Res 1998;30:291–294.
24. Tezel G, Hernandez MR, Wax MB. Immunostaining of heat shock proteins in the retina and optic nerve head of normal and glaucomatous eyes. Arch Ophthalmol 2000;118:511–518.
25. Fukuchi T, Sawaguchi S, Yue BYJT, Iwata K, Hara H, Kaiya T. Sulfated proteoglycans in the lamina cribrosa of normal monkey and monkey eyes with laser-induced glaucoma. Exp Eye Res 1994;58:231–244.
26. Sawaguchi S, Yue BYJT, Fukuchi T, et al. Collagen fibrillar network in the optic nerve head of normal monkey eyes with laser-induced glaucoma: a scanning electron microscopic study. Curr Eye Res 1999;18:143–149.
27. Sawaguchi S, Yue BYJT, Sugar, J, Gilboy JE. Lysosomal enzyme abnormalities in keratokonus. Arch Ophthalmol 1989;107:1507–1510.
28. Barbe MF, Tytell M, Gower DJ, Welch W. Hyperthermia protects against light damage in the rat retina. Science 1988;241:1817–1819.
29. Rodorf G, Koroshetz WJ, Bonventre JV. Heat shock protects cultured neurons from glutamate toxicity. Neuron 1991;7:1043–1051.
30. Lowenstein DH, Chan PH, Miles MF. The stress protein response in cultured neurons: characterization and evidence for a protective role in excitotoxicity. Neuron 1991;7:1053–1060.
31. Simon MA, Bowtell DDL, Dodson GS, Laverty TR, Rubin GM. Ras 1 and putative guanine nucleotide exchange factor perform crucial steps in signaling by the sevenless protein tyrosine kinase. Cell 1991;67:701–716.
32. Wakakura M, Foulds WS. Response of cultured Müller cells to heat shock—an immunocytochemical study of heat shock and intermediate filament proteins in response to temperature elevation. Exp Eye Res 1989;48:337–350.
33. Samali A, Cotter T. Heat shock proteins increase resistance to apoptosis. Exp Cell Res 1996;223:163–170.
34. Lacthman DS. Heat shock proteins: protective effect and potential therapeutic use (review). Int J Mol Med 1998;2:375–381.
35. Koll H, Guiard B, Rassow J, et al. Antifolding activity of hsp 60 couples protein import into the mitochondrial matrix with export to the intermembrane space. Cell 1992:68:1163–1175.
36. Martin J, Horwich AL, Hartl FU. Prevention of protein denaturation under heat stress by the chaperoning Hsp 60. Science 1992;258:995–998.
37. Hart DA, Reno C, Le Graverand MPH, Hoffman L, Kulyk W. Expression of heat shock protein 47 (Hsp 47) mRNA levels in rabbit connective tissues during the response to injury and in pregnancy. Biochem Cell Biol 2000;78:511–518.
38. Gu X, Ko MK, Kay EP. Intracellular interaction of Hsp 47 and type I collagen in corneal endothelial cells. Invest Ophthalmol Vis Sci 1999;40:289–295.
39. Tezel G, Wax MB. The mechanisms of hsp 27 antibody-mediated apoptosis in retinal neuronal cells. J Neurosci 2000;10:3552–3562.
40. Tezel G, Wax MB. Inhibition of caspase activity in retinal cell apoptosis induced by various stimuli in vitro. Invest Ophthalmol Vis Sci 1999;40:2660–2667.
41. Mehlen P, Schlze-Osthoff K, Arrigo A-P. Small heat shock proteins as novel regulators of apoptosis: heat shock protein 27 blocks FAS/APO1 and staurosporine induced cell death. J Biol Chem 1996;271:16510–16514.
42. Tezel G, Seigel GM, Wax MB. Autoantibodies to small heat shock proteins in glaucoma. Invest Ophthalmol Vis Sci 1998;39:2277–2287.
43. Park KH, Cozier F, Ong OC, Caprioli J. Induction of heat shock protein 72 protects retinal ganglion cells in a rat glaucoma model. Invest Ophthalmol Vis Sci 2001;42:1522–1530.
44. Tanihara H, Hangai M, Sawaguchi S, et al. Up-regulation of glial fibrillary acidic protein in the retina of primate eyes with experimental glaucoma. Arch Ophthalmol 1997;115:752–756.