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Two Cases of Primary Open Angle Glaucoma with Serum Autoantibody Against Retinal Ganglion Cells

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Purpose: To present two cases of primary open angle glaucoma (POAG) with serum autoantibody against retinal 25 kDa or 50 kDa antigen.

Methods: Ocular examinations were performed on a 60-year-old man (case 1) and a 74-year-old woman (case 2). We also performed serological and immunological examinations including Western blot analysis and immunocytochemical analysis.

Results: In Western blot analysis, they were found to have serum autoantibody against retinal 25 kDa or 50 kDa antigen, and immunocytochemical analysis revealed that retinal ganglion cell layers were specifically labeled by each patient's IgG.

Conclusion: We report two interesting cases of POAG with serum autoantibody against retinal 25 kDa or 50 kDa antigen. **Jpn J Ophthalmol 2000;44:648–652** © 2000 Japanese Ophthalmological Society

Key Words: Apoptosis, autoantibody, normal tension glaucoma, primary open angle glaucoma, retinal ganglion cells.

Introduction

Glaucoma is a disease of the optic nerve characterized by loss of retinal ganglion cells and their axons, excavated appearance of the optic nerve head, and progressive loss of visual field sensitivity. Clinically, two major forms have been identified: Primary open angle glaucoma (POAG) and normal tension glaucoma (NTG), which are associated with elevated and normal intraocular pressure (IOP), respectively.[1] It has been suggested that apoptosis of retinal ganglion cells has been implicated in the pathology of glaucomatous optic neuropathy, on the basis of experimental glaucoma models[2,3] and pathological studies using human glaucomatous eyes[4,5] With regard to the molecular mechanisms triggering the apoptosis, several factors such as deprivation of neurotrophic factors,[2] ischemia,[6] chronic elevation of glutamate,[7] and disorganized nitric oxide synthase[8] have been considered. In addition, it was recently suggested that autoantibodies against some retinal components, such as rhodopsin,[9] 60 kDa heat shock protein (hsp 60),[10] 27 kDa heat shock protein (hsp 27), and α-crystallin[11] may also be involved in the apoptosis mechanism in glaucomatous optic neuropathy.

Here, we report two cases of POAG with serum autoantibody against retinal ganglion cells. Clinically, the first case showed a significant difference between the eyes in optic disc cupping and visual field loss although there was only a slight difference in IOP. The second case demonstrated progressive deterioration of her visual field unless her intraocular pressure was controlled at relatively lower levels by medication.

Materials and Methods

The studies were performed in accordance with the Declaration of Helsinki on Biomedical Research Involving Human Subjects, and protocols were approved by our Medical School's Committee for the Protection of Human Subjects. Bovine retinas were obtained from a local slaughterhouse and rat eyes for immunocytochemistry were enucleated, after sacrifice by CO_2 inhalation, according to the guidelines for the treatment of animals of the Animal Care Committee of Sapporo Medical University.

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Isolation of IgG from Peripheral Blood of Patients

Peripheral venous blood samples were withdrawn from these glaucoma patients with informed consent, and immediately subjected to serum separation. Then, IgG was isolated from the serum by using a protein G Sepharose column chromatograph, employing the protocol described by the manufacturer, and stored at −80°C before use.

Immunocytochemical Analysis

Unfixed freshly dissected rat retinas were infiltrated with 30% sucrose in phosphate-buffered saline (PBS) at 4°C, cryosectioned at 4-μm thickness, mounted on subbed slides, air-dried, and stored at −80°C before use. The sections were treated with ice-cold acetone for 10 minutes, air-dried, and plastic rings were mounted around the sections to form incubation walls. The sections were incubated with diluted patient IgG at 1:100 in PBS for 1 hour and rinsed with PBS three times for 5 minutes each. Then the sections were incubated with goat anti-human IgG labeled with Cy3 at 1:400 in PBS with 0.3% Tween 20 at room temperature for 1 hour. The sections were then rinsed three times with PBS for 5 minutes, and cover-slipped in 90% glycerol in PBS containing 2% 1,4-diazabicyclo (2,2,2) octane. The sections were photographed using a Cy3 filter set.

Western Blot Analysis

Western blot analysis was carried out as described previously.[12] For isolation of bovine retinal soluble protein fractions, 10 frozen bovine retinas were homogenized in 10 mM hepes buffer (pH 7.5) containing 100 mM NaCl, 1 mM benzamidine and 0.1 mM leupeptine, and centrifuged at 50,000 *g* for 1 hour. The supernatant containing approximately 20 μg protein was analyzed by sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) using a 12.5% polyacrylamide gel. Separated proteins in the gel were electrotransferred to polyvinylidene difluoride membranes in 10 mM bis-tris-propane buffer (pH 8.4), and 10% methanol solution. After blocking nonspecific binding by 2% skim milk in PBS, the membrane was probed successively with diluted patient serum and horseradish peroxidase-labeled anti-human IgG (Funakoshi, Tokyo). Specific antigen/antibody binding was visualized by the ECL system (Amersham Pharmacia Biotech, Buckinghamshire, England).

Case Reports

Case 1. A 60-year-old man visited our clinic for further examination of right optic nerve atrophy in January 1996. He had a history of decreasing visual acuity in his right eye for almost 1 year and had been diagnosed by a local ophthalmologist as having right optic nerve atrophy. At the initial examinations in our clinic, the best visual acuity of his right and left eyes was 0.2 and 1.0, respectively. The IOP was 24 mm Hg in the right and 20 mm Hg in the left eye and gonioscopic examination revealed normal open angles. Funduscopic examination showed severe glaucomatous optic changes, such as vertical cup:disc ratio of 1.0 in the right eye and 0.7 in the left eye (Figure 1). Visual fields tested by Goldmann perimeter revealed a double arcuate scotoma close to the fixation (Aulhorn-Greve's Stage 2) in the right eye and a paracentral scotoma (Aulhorn-Greve's Stage 0–1) in the left eye. Magnetic resonance imaging disclosed no abnormalities consistent with optic neuropathy. Laboratory findings were unremarkable except for hyperglycemia. He had a family history of glaucoma. As a result of the above clinical findings he was diagnosed as having POAG. Administration of 0.12% isopropyl unoprostone (Rescula®, Fujisawa, Osaka) twice a day was started, but his IOP remained in the high 10s. In addition to the isopropyl unoprostone medication, 0.5% betaxolol hydrochloride (Betoptic®, Alcon, Tokyo) and then 0.1% dipivefrine hydrochloride (Pivalephrine®, Santen, Osaka) were started. Although his mean IOP had been 18.9 ± 3.6 mm Hg in the right eye and 17.6 ± 3.0 mm Hg in the left during the previous 3 years of follow-up, the latest visual fields tested by a Humphrey Field Analyzer, program 30-2 (Humphrey Instruments, San Leandro, CA, USA) disclosed deterioration of visual sensitivity in both eyes.

Case 2. A 74-year-old woman visited a local ophthalmologist in 1993 complaining of a floating spot in the left eye. She was first diagnosed as having POAG and administration of 0.5% timolol maleate (Timoptol®, Banyu, Tokyo) was started twice a day. In addition to timolol medication, 0.12% isopropyl unoprostone was started in 1995, and then 0.1% dipyvalyl dipivefrin (Santen) in 1997. She was referred to our clinic in November 1998 because of the substantial progression of visual field loss. At the initial examinations, the best visual acuity of her right and left eyes was 1.0 and 1.2, respectively. The IOP was 19 mm Hg in the right and 20 mm Hg in the left eye and gonioscopic examination revealed normal open angles. Funduscopic examination showed severe glaucomatous optic changes, such as vertical

Jpn J Ophthalmol
Vol 44: 648–652, 2000

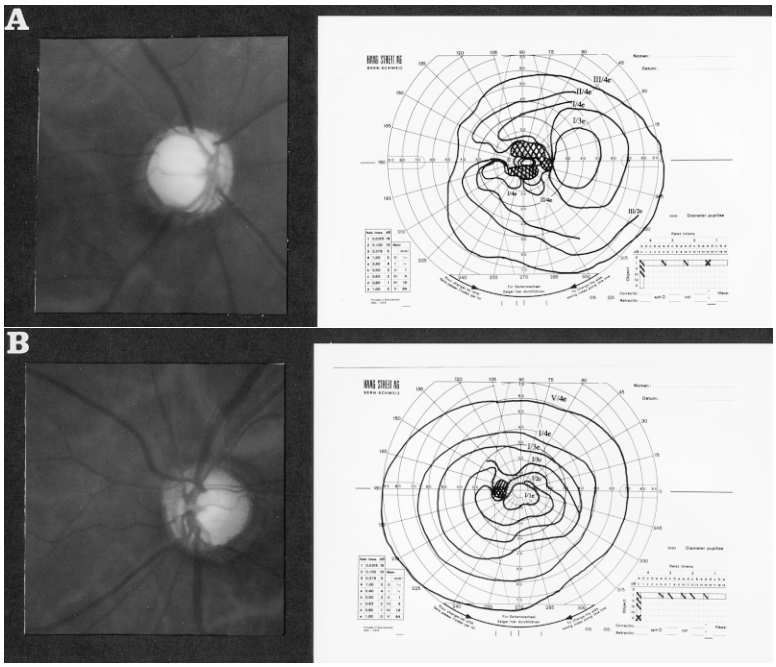

Figure 1. Optic discs and visual fields of case 1 (60-year-old man). (**A**) Right eye, (**B**) left eye. Glaucomatous optic disc atrophy is recognized, accompanied by visual field defects in both eyes, especially in right eye.

cup:disc ratio of 0.9 in the right eye and 0.8 in the left eye. Visual fields tested by a Humphrey Field Analyzer, program 30-2 showed that the mean deviation was −2.11 DB in the right and −6.29 DB in the left eye. Laboratory findings were unremarkable except for hyperglycemia. She had hypertension and hyperglycemia and a family history of glaucoma. Based upon the clinical findings, she was diagnosed as having POAG and was followed up every month after undergoing argon laser trabeculoplasty once and receiving one more administration of 1% pilocarpine hydrochloride (Sanpilo®, Santen, Osaka) four times a day in both eyes. Although her mean IOP was 15 to 19 mm Hg in both eyes at the initial examination, her visual field defect as shown by the Humphrey Field Analyzer program 30-2 worsened by June 1999 (Figure 2).

Results

For the identification of the autoantibodies in our patients, bovine or rat retinas were used instead of human retinas, following the same method as used in previous studies.[9–12] In Western blot analysis, 25 kDa and 50 kDa antigens were specifically recognized by serum from case 1 and case 2, respectively (Figure 3). To determine the localization of these antigens within the retinal layers, we performed immunocytochemical analysis and found that retinal ganglion cell layers were specifically labelled in each patient by one of the autoantibodies (Figure 4).

Discussion

In the present report, we describe two interesting cases of POAG with serum autoantibodies against the retinal ganglion cell layer, which is a specific target in glaucoma. In terms of the relationship between autoimmune response and glaucoma, Yamamoto et al[13] reported that female patients with collagen diseases are highly susceptible to NTG and POAG. In addition, Wax and his associates reported that autoantibodies against α-crystallin or heat shock proteins were recognized in approximately 20% of

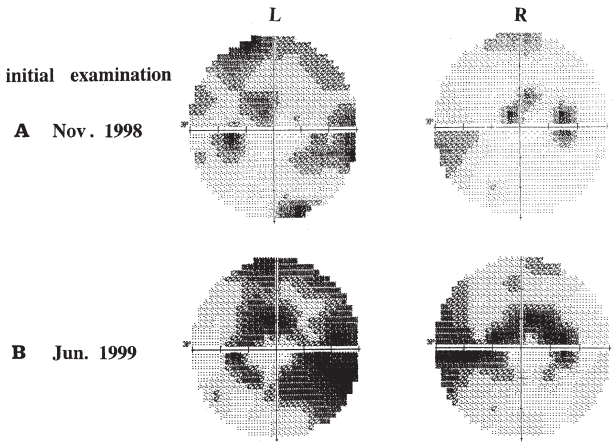

Figure 2. Visual fields of case 2 (74-year-old woman). (**A**) At initial examination in November 1998. (**B**) At latest test in June 1999.

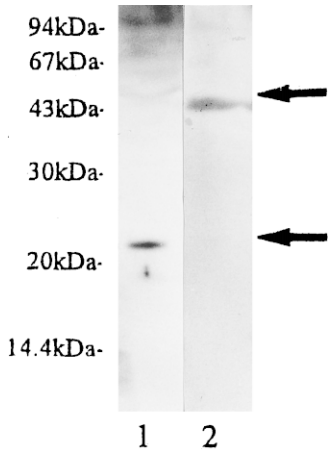

Figure 3. Western blot analysis of patients' serum using retinal soluble fractions. At serum dilution of 1:400, prominent bands of 25 kDa and 50 kDa (indicated with arrows) are recognized by case 1 and case 2 patient serum, respectively.

NTG patients.[11] Here, case 1 showed asymmetry of optic nerve features, namely a difference of more than 0.2 in cup:disc ratio between the 2 eyes even though there was little difference between the eyes in terms of IOP. Case 2 demonstrated progressive visual field defects even though her IOPs were controlled at relatively low levels. Therefore, their clinical courses caused us to speculate that some other unknown factors such as autoimmunity, in addition to IOP, must be involved in the pathogenesis of their glaucomatous optic neuropathy. If this is possible, it is feasible that the autoimmune response toward the 25 kDa or 50 kDa protein may have enhanced the IOP-induced retinal damage resulting in the asymmetry of optic nerve features seen in case 1 and the progressive visual field defect in case 2. To date, we have examined sera from 79 glaucoma patients (POAG: n = 56; NTG: n = 23) and 60 age-matched healthy controls by Western blot and immunocytochemical analysis. We found the presence of the 50 kDa antibody reacting with retinal ganglion cells in approximately 20% of POAG and NTG patients, but this antibody was observed in only 2 healthy control subjects. The 25 kDa autoantibody was recognized in only 4 of the above 79 glaucoma patients.[14]

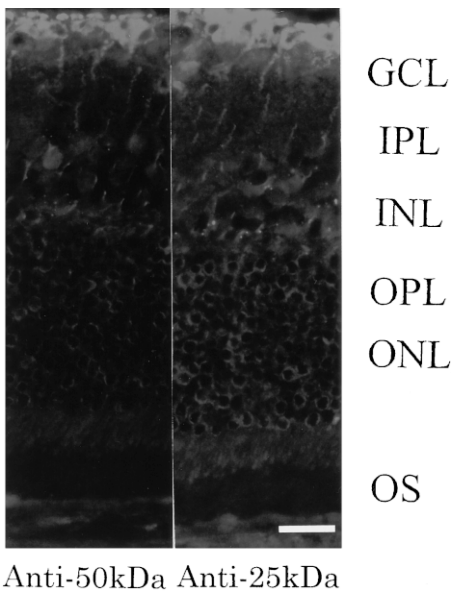

Figure 4. Representative photomicrograph showing immunofluoresence labeling of retinal ganglion cells by patient's IgG. GCL: ganglion cell layer; IPL: inner plexiform layer; INL: inner nuclear layer; OPL: outer plexiform layer; ONL: outer nuclear layer; OS: outer segment. Bar = 50 μm.

The identity of the antigens is not known at present. However, the retinal 25 kDa antigen has a molecular mass similar to α-crystallin and hsp 27, which have been identified as autoantigens in NTG.[11] In contrast, the 50 kDa antigen may be an entirely new antigen since its molecular mass does not correspond to any of the antigens identified previously. Wax et al reported that antibodies toward α-crystallin and hsp 27 cause apoptosis of retinal cells in a cultured system. Therefore, they suggested that these autoantibodies found in NTG may be involved in the pathogenesis of the apoptotic retinal ganglion cell death in glaucoma.[11] If this is true, we can reasonably speculate that the 50 kDa antigens may play roles similar to those of α-crystallin and hsp 27 in the etiology of glaucoma.

The details of the relationship between these autoantibodies and apoptosis of retinal ganglion cells is as yet unknown. However, this apoptotic process by autoantibodies may well be possible since there is a great deal of evidence that several paraneoplastic syndromes, such as cancer-associated retinopathy,[15] Eaton-Lambert syndrome,[16] and paraneoplastic cerebellar degeneration[17] are caused by some specific autoantibodies. In cancer-associated retinopathy, it was suggested that photoreceptor cell death (apoptosis) in vitro and in vivo might be caused by an autoimmune reaction against several retinal antigens including recoverin,[12,15,18] hsc 70,[12] enolase[19] and neurofilaments.[20]

In order to clarify the relationship between 25 kDa and 50 kDa antigens and glaucoma etiology, the following important questions remain to be elucidated: (1) How do serum antibodies get to the target cells (retinal ganglion cells) and cause apoptosis? (2) What are the retinal 25 kDa and 50 kDa antigens?

Jpn J Ophthalmol
Vol 44: 648–652, 2000

(3) What are the specificities of the serum antibodies among several retinal diseases and their controls? and (4) What is the relationship between anti-25 kDa and anti-50 kDa antibodies and disease progression? Therefore, a study with large numbers of patients and using protein chemistry to identify the retinal 25 kDa and 50 kDa antigens will be required for further investigation.

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