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Expression of Stress-Response Protein 60 in Lens Epithelial Cells in Atopic Cataract

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Purpose: To clarify the pathogenesis of atopic cataract, especially to determine if there is any relationship between autoimmunity and atopic cataract.

Methods: We investigated the lens epithelia obtained at surgery from 12 patients (12 eyes) with atopic cataract; from 8 patients (8 eyes) with nonatopic cataract (5 with senile cataract, 2 with juvenile cataract, and one with secondary cataract due to anterior uveitis); and from 4 autopsy eyes as controls.

Results: Histopathological findings in the lens epithelial cells from atopic and nonatopic cataract patients were essentially the same: atrophy of the cells, presence of the superimposed cells, migration of cells into the lens cortex, cytoplasmic vacuolation, and loss of cells. In an immunohistochemical study, the expression of stress-response protein 60 (srp 60), srp 27, and srp 72 was examined in the lens epithelial cells. In atopic cataract specimens, 71%–87% of the lens epithelial cells were stained with the antibody against srp 60, but the cells in nonatopic cataract and control specimens were not stained.

Conclusions: Srp 27 and srp 72 were not expressed in any observed epithelial cells. The expression of srp 60 may reflect a protective mechanism of the epithelial cells against injury triggered by immunorelated agents. These findings suggest that the pathogenesis of degeneration of the lens epithelial cells in patients with atopic cataract may be related to autoimmunity. **Jpn J Ophthalmol 1999;43:89–96**

Key Words: Atopic cataract, autoimmunity, histopathology, immunohistochemistry, lens epithelial cells, stress-response protein 60.

Introduction

The close association of atopic dermatitis with cataract has been well known since it was first described by Rothmund in 1868.[1] Patients with atopic dermatitis have increased worldwide, and the number of patients with atopic cataract is also on the rise.[2–6] Most patients with atopic cataract suffer from bilateral ocular lesions.[1] Taking into consideration the fact that many patients with atopic cataract are young people who require high-quality vision, atopic cataract is becoming a serious social problem.[5,6] Many hypotheses on the pathogenesis of atopic cataract have been proposed, but they are still controversial.[1,7] The histopathological findings of atopic cataract are almost the same as those of nonatopic cataract,[7–9] and ultrastructural studies have revealed an increased number of mitochondria as a typical finding in the cytoplasms of the lens epithelial cells in patients with atopic cataract.[7,9]

We are interested in the hypothesis that cataractogenesis is a result of an autoimmune response.[10–14] This hypothesis proposes that cataracts occur as a result of an immune response to proteins derived from the two main constituents of the lens: albuminoid and crystallin. Recent studies on human heat shock proteins have shown that the induced stress-response protein 60 (srp 60) itself is in the target tissues in various autoimmune diseases.[15–22]

In the present study, we report an immunohistochemical demonstration of the expression of srp 60 in the lens epithelial cells in patients with atopic cataract, which may relate to the pathogenesis of this

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disease. We also investigate the expression of other srp families, such as srp 27 and srp 72 in the lens epithelial cells as well as the histopathological findings in the lens materials from these patients.

Materials and Methods

Lens Materials

The study was carried out using anterior lens capsules obtained at surgery from patients with atopic and nonatopic cataract. Informed consent was obtained before surgery. Considering previous histopathological findings of atopic cataract,[7,9] each sample from the anterior subcapsular region was intentionally obtained from one eye of each patient during routine cataract surgery. A round piece of the central anterior capsule was removed with fine forceps following anterior capsulotomy by the can-opener technique or by continuous curvilinear capsulorhexis for extracapsular lens extraction.

The main clinical characteristics of the 12 patients with atopic cataract (cases No. A1–A12) and of the 8 patients with nonatopic cataract (cases No. nA1–nA8) are summarized in Table 1. The age at surgery of the 12 patients with atopic cataract ranged from 20–44 years (average: 30.8 years). Five of the patients showed an incipient type of anterior subcapsular cataract (cases No. A1–A5), 4 showed a type of anterior and posterior subcapsular cataract (cases No. A6–A9), and the other 3 showed a type of mature cataract (cases No. A10–A12). In the 8 patients with nonatopic cataract, anterior lens capsules were obtained from 5 eyes of 5 patients with senile cataract, 2 eyes of 2 patients with juvenile cataract, and 1 eye of 1 patient with secondary cataract due to anterior uveitis. The age at surgery of these patients ranged from 26–65 years (average: 47.0 years); the age of patients with senile cataract ranged from 42–65 years (average: 50.6 years). Two of the patients with senile cataract showed an incipient type of anterior subcapsular cataract (cases No. nA1, nA2); one showed a type of anterior and posterior subcapsular cataract (case No. nA3), and the other 2 showed mature cataract (cases No. nA4, nA5). The 2 patient with juvenile cataract showed a type of anterior and posterior subcapsular cataract (cases No. nA6, nA7) and the patient with secondary cataract had the type of anterior subcapsular cataract used in our study (case nA8).

As controls, 4 anterior lens capsules were obtained from clear, normal lenses from 4 autopsy cases without ophthalmic disorders (cases No. N1–N4); the causes of death were different (Table 2). The ages at death of these autopsy cases ranged

Table 1. Age, Sex, Diagnosis, and Type of Lens Opacity in Patients With Atopic or Nonatopic Cataract

Case No.	Age at Surgery (y)	Sex	Diagnosis	Type of Lens Opacity
A1	27	M	Atopic cataract	Anterior subcapsular cataract
A2	32	M	Atopic cataract	Anterior subcapsular cataract
A3	30	F	Atopic cataract	Anterior subcapsular cataract
A4	29	M	Atopic cataract	Anterior subcapsular cataract
A5	20	M	Atopic cataract	Anterior subcapsular cataract
A6	28	M	Atopic cataract	Anterior and posterior subcapsular cataract
A7	44	M	Atopic cataract	Anterior and posterior subcapsular cataract
A8	32	M	Atopic cataract	Anterior and posterior subcapsular cataract
A9	20	F	Atopic cataract	Anterior and posterior subcapsular cataract
A10	36	M	Atopic cataract	Mature cataract
A11	30	F	Atopic cataract	Mature cataract
A12	32	M	Atopic cataract	Mature cataract
nA1	50	M	Senile cataract	Anterior subcapsular cataract
nA2	42	F	Senile cataract	Anterior subcapsular cataract
nA3	44	F	Senile cataract	Anterior and posterior subcapsular cataract
nA4	52	M	Senile cataract	Mature cataract
nA5	65	M	Senile cataract	Mature cataract
nA6	35	M	Juvenile cataract	Anterior and posterior subcapsular cataract
nA7	35	M	Juvenile cataract	Anterior and posterior subcapsular cataract
nA8	26	M	Secondary cataract due to anterior uveitis	Anterior subcapsular cataract

F: female; M: male.

Table 2. Data in Controls (Autopsy Cases)

Case No.	Age (y)	Sex	Cause of Death
N1	58	F	Myocardial infarction
N2	62	F	Pneumonia
N3	68	F	Cerebral infarction
N4	75	F	Cerebral infarction

F: female.

from 58–75 years (average: 65.8 years). The procedures carried out in this study were performed with the consent of their immediate families.

Histopathology and Immunohistochemistry

The specimens were fixed in 10% buffered formalin, embedded in paraffin, and cut into 5-μm–thick sections using a Yung microtome (Yamato, Tokyo). One section was stained with hematoxylin and eosin (H&E) and the other sections were used in immunohistochemical studies. Representative sections were immunostained initially and subsequently stained with H&E: double-staining of immunostaining and H&E.

The lens epithelial cell changes were graded as follows, using a light microscope (Olympus, Tokyo) at a magnification of ×400: −: no pathological findings, +: pathological findings in 1–30 cells of 100 lens epithelial cells selected randomly from several serial specimens in our series, ++: similar findings in 31–80 cells of 100 randomly selected cells, and +++: similar findings in 81–100 cells of 100 randomly selected cells.

When the lens cortex was adjacent to the epithelia and histopathological tissue was obtained, degeneration of adherent lens fibers was rated as follows: +: mild, ++: moderate, and +++: severe degenerative changes.

The avidin–biotin–peroxidase complex (ABC, pH 7.4) method using Vectastain ABC kits (Vector Laboratories, Burlingame, CA, USA) was used for the detection of srp.[23] Sections were deparaffinized and endogenous peroxidase activity was quenched with 0.3% H_2O_2 for 30 minutes. Sections were then washed in phosphate-buffered saline (PBS, pH 7.4). Normal horse serum served as the blocking reagent. The following antibodies were used for immunohistochemistry: a mouse monoclonal antibody against srp 60 [Clone LK-1 (StressGen, Victoria, Canada), diluted 1:3,000 with bovine serum albumin (BSA)-PBS (pH 7.4)]; srp 27 [Clone G3.1 (BioGenex, San Roman, CA, USA), ready to use]; and srp 72 [Code RPNO1197 (Amersham International, Little Chalfont, UK), diluted 1:500 with BSA-PBS (pH 7.4)].

The specificity and utility of the antibodies for immunohistochemical demonstration in human paraffin blocks have been documented.[18,24–26] Sections were incubated with each primary antibody overnight at 4°C. Phosphate-buffered saline solution replaced the antibody in the control. The horse anti-mouse IgG Vectastain ABC kit (Vector Laboratories) was used to detect each bound antibody. As the final chromogen, 3, 3′-diaminobenzidine tetrahydrochloride (DAB; DAKO, Glostrup, Denmark) was used.

Each positive lens epithelial cell was identified by light microscopy at a magnification of ×400. The percentages of srp 60–, srp 27–, and srp 72–positive lens epithelial cells found among 100 randomly selected, labeled lens epithelial cells in representative serial sections of the atopic cataract, nonatopic cataract, and control specimens examined were adopted as the labeling index (LI, srp 60-LI, srp 27-LI, srp 72-LI), indicating the degree of immunohistochemical reaction determined in this study.

Results

Histopathological Findings

In control lenses from autopsy cases with no ophthalmic disorders, there was a layer of epithelial cells under the anterior lens capsule. These cells were cuboidal with large spherical nuclei. In the lens cortex adjacent to the epithelial cell layer, regularly arranged lens fibers were observed (Figure 1).

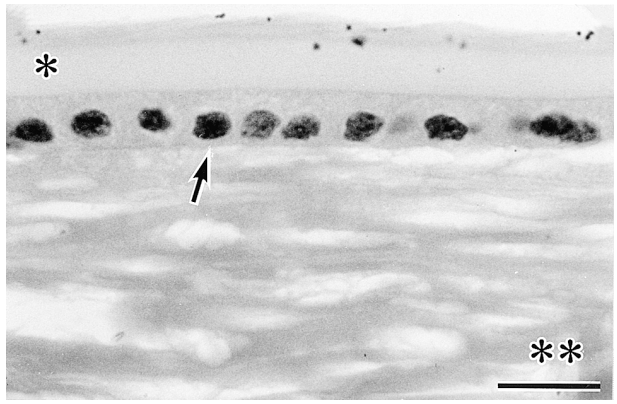

Figure 1. Light micrograph of control lens material from autopsy case. Layer of epithelial cells lies under anterior lens capsule (asterisk). These cells are cuboidal with large spherical nuclei (arrow). In lens cortex adjacent to epithelial cell layer, regularly arranged lens fibers (double asterisks) are seen. Hematoxylin and eosin staining. Bar = 20 μm.

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Table 3. Histopathological Findings of Lens Materials in Atopic and Nonatopic Cataract Cases

Case No.	Epithelial Lens Changes					Degeneration of Adherent Lens Fibers
	Degeneration			Proliferation		
	PCD	CV	FC	MUC	MIC	
A1	++	−	++	−	++	++
A2	+	−	+++	+	NA	NA
A3	+++	+	++	−	+++	+
A4	++	+++	+	++	NA	NA
A5	+++	−	++	+	NA	NA
A6	+	++	+	−	NA	NA
A7	++	−	+++	−	+	+++
A8	++	−	++	−	+++	+
A9	++	−	++	−	+	++
A10	++	−	+++	−	++	++
A11	+++	−	++	−	NA	NA
A12	++	−	++	+	+	+++
nA1	+	++	+++	−	NA	NA
nA2	+++	−	+	+	−	+
nA3	++	−	+++	−	NA	NA
nA4	++	−	++	+	+++	+++
nA5	+++	−	++	−	+	++
nA6	++	+	++	−	+	+
nA7	+++	−	+++	−	NA	NA
nA8	++	−	++	−	−	++

CV: cytoplasmic vacuolation; FC: flattened cell; MIC: migrating cell; MUC: multilayered cell; NA: not available; PCD: pyknosis and cell death.

Epithelial cell changes were graded as follows: −: no pathological findings, +: pathological findings in 1–30 cells of 100 lens epithelial cells selected randomly from several serial specimens in our series, ++: similar findings in 31–80 cells, and +++: similar findings in 81–100 cells. Degeneration of adherent lens fibers was rated as follows: +: mild, ++: moderate, and +++: severe degenerative changes.

Histopathological findings in lens materials from the atopic and nonatopic cataract cases are summarized in Table 3. In atopic cataract cases, the following findings were observed: the epithelial cells became flatter, occasionally had vacuoles in their cytoplasm, and showed epithelial degeneration. Their nuclei became oval to sausage-shaped and were pyknotic. Loss of some epithelial cells due to cell death was also observed (Figures 2A and 2B, Table 3). The flattened epithelial cells became multilayered in some cases, which indicated epithelial proliferation (Figure 2C, Table 3). Some epithelial cells were metaplastic and resembled fibroblasts. In all specimens, several epithelial cells migrated into the lens cortex, which was adjacent to the epithelial cells (Figure 2A, Table 3). The adherent lens fibers did not show a regular pattern and shrank in association with clefts or vacuoles (Figure 2A, Table 3).

Pathological alterations in cases of atopic and non-

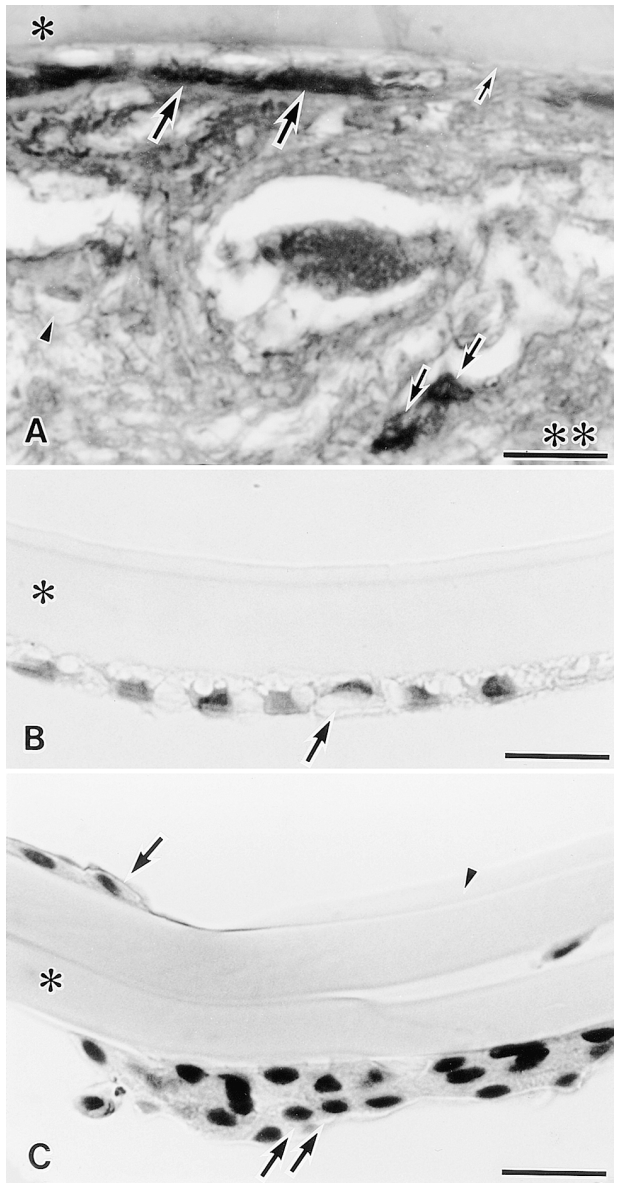

Figure 2. Light micrographs of lens materials in cases of atopic cataract. (**A**) Epithelial cells under anterior lens capsule (asterisk) show oval to sausage-shaped nuclei with pyknosis (large arrows). Loss of cells (small arrow) is observed. In lens cortex (double asterisks) adjacent to epithelial cell layer, migrating epithelial cells (medium-sized arrows) and irregular arrangement of adherent lens fibers are seen in association with clefts or vacuoles (arrowhead). (**B**) Many cytoplasmic vacuolations (arrow) are observed in epithelial cell layer under anterior lens capsule (asterisk). In this case, no data could be obtained concerning lens fibers due to absence of lens cortex adjacent to epithelium. (**C**) Flattened and multilayered epithelial cells (double arrows) are seen. Surgically removed anterior lens capsule artificially makes double fold on specimen; upper lens capsule (arrowhead) overlies original one (asterisk). Arrow indicates monolayered epithelial cells with upper lens capsule. Hematoxylin and eosin staining, A–C. Bar = 20 μm.

atopic cataract were almost the same as shown in Table 3, although these alterations did not necessarily correlate with the type of lens opacity and the clinical stages of cataract (Table 3).

Immunohistochemical Findings

In our immunohistochemical study, srp 60 was expressed intensively in the lens epithelial cells in all patients with atopic cataract (Figure 3A). Although the clinical stages of the atopic cataract varied from incipient to mature, srp 60 was demonstrated in all clinical stages. According to the srp 60-LI, 71%–87% of the lens epithelial cells were stained with the antibody against srp 60 (Table 4). The srp 60–positive cells showed strong cytoplasmic staining. On the contrary, none of the lens epithelial cells was stained with the antibody against srp 60 in the senile cataract, the secondary cataract, or the normal noncataractous lenses (Figure 4, Table 4). Srp 27 and srp 72 were not expressed in the epithelial cells in either atopic or nonatopic cataract lenses or in control lenses (Figures 3B and 3C, Table 4).

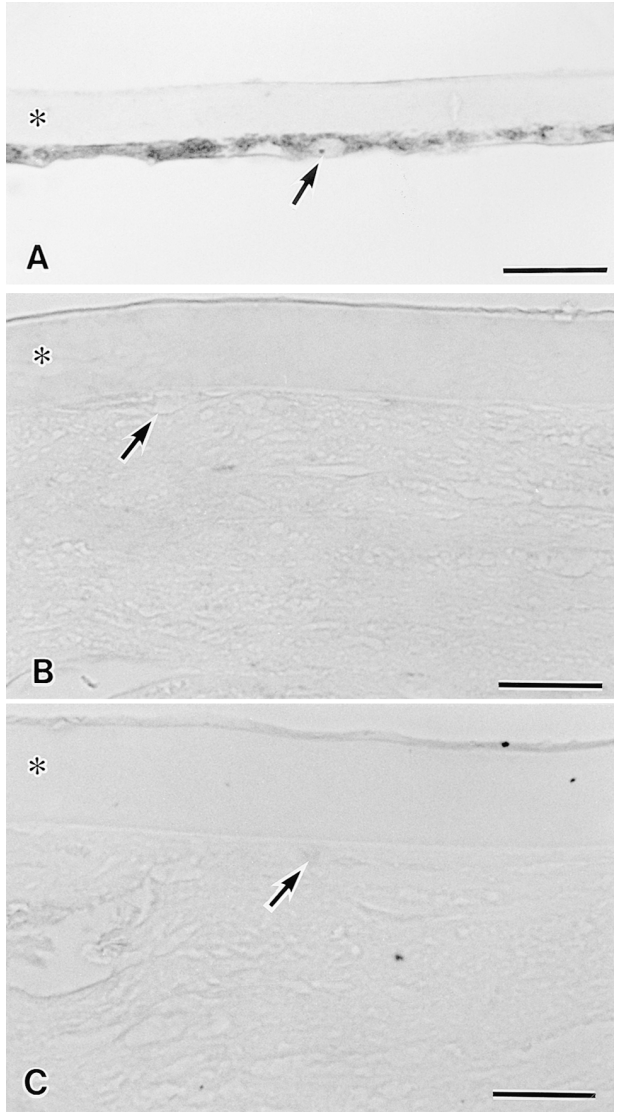

Figure 3. Immunohistochemical sections in cases of atopic cataract. Asterisk in each section indicates anterior lens capsule. (**A**) Lens epithelial cells are stained with antibody against stress response protein 60 (srp 60). Srp 60–positive cells (arrow) show strong cytoplasmic staining. Srp 60 immunostaining. (**B**) Srp 27 is not expressed in cytoplasms of lens epithelial cells (arrow). Srp 27 immunostaining. (**C**) Srp 72 also is not expressed in cytoplasms of lens epithelial cells (arrow). Srp 72 immunostaining. Bars = 20 μm.

Discussion

Atopic dermatitis has been reported to occur in 3.1%–19.3% of children ranging in age from 3 months to 12 years in Japan.[4] Some authors,[5,27,28] documented that atopic cataract occurred in about 20% of patients with atopic dermatitis. Recently, reports of atopic dermatitis are increasing globally.[2–4,6] Consequently, the number of patients with atopic cataract must also be increasing. In our study, the age at surgery of 12 patients with atopic cataract ranged from 20–44 years (average: 30.8 years), indicating that atopic cataract developed at a younger age.

The pathogenesis of atopic cataract is controversial at present.[1,7] The pathogenesis of senile cataract is also not yet established.[29] Considering the difference in the age of onset, the pathogenesis of atopic cataract may be different from that of senile cataract. Our histopathological findings of atopic cataract, however, demonstrated the degeneration of the lens epithelial cells (cytoplasmic vacuolation, pyknosis of nuclei, and cell death), the proliferation of the lens epithelial cells (cells becoming flattened and multilayered), and the metaplasia of the lens epithelial cells into fibroblasts. These pathological findings of atopic cataract are consistent with those of senile cataract, as previously reported.[7,8] There are no histopathological findings specific to atopic cataract.

In our immunohistochemical study, 71%–87% of the lens epithelial cell cytoplasms were intensively stained with the antibody against srp 60 in patients with atopic cataract. Although histopathological findings of nonatopic cataract were essentially the same as those of atopic cataract, srp 60 was not expressed in the cytoplasms of the lens epithelial cells in nonatopic cataract or control lenses. Srp 27 and srp 72 also were not expressed in the epithelial cells exam-

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Table 4. Labeling Indexes for Stress Response Protein (Srp) 60–, Srp 27–, and Srp 72–Positive Lens Epithelial Cells (Srp 60-LI, Srp 27-LI, and Srp 72-LI) in Atopic Cataract, Nonatopic Cataract, and Control Cases

Case No.	Srp 60-LI (%)	Srp 27-LI (%)	Srp 72-LI (%)
A1	74	0	0
A2	72	0	0
A3	75	0	0
A4	80	0	0
A5	78	0	0
A6	82	0	0
A7	83	0	0
A8	87	0	0
A9	71	0	0
A10	81	0	0
A11	82	0	0
A12	79	0	0
nA1	0	0	0
nA2	0	0	0
nA3	0	0	0
nA4	0	0	0
nA5	0	0	0
nA6	0	0	0
nA7	0	0	0
nA8	0	0	0
N1	0	0	0
N2	0	0	0
N3	0	0	0
N4	0	0	0

Srp 60-LI, srp 27-LI, and srp 72-LI indicate percentages of srp 60–, srp 27–, and srp 72–positive lens epithelial cells in 100 randomly selected labeled cells found in representative serial sections of atopic cataract, nonatopic cataract, and control specimens.

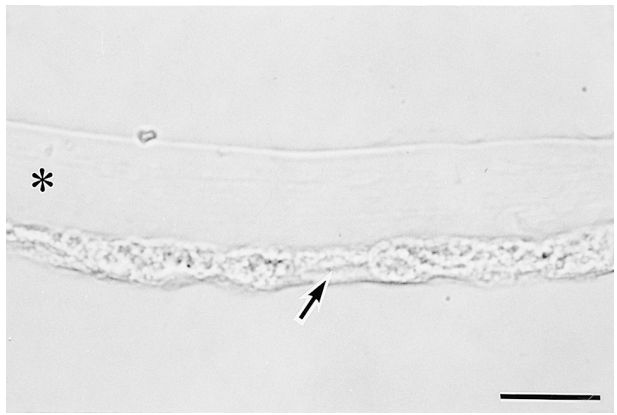

Figure 4. Immunohistochemical section in senile cataract case. Asterisk indicates anterior lens capsule. No immunoreaction with antibody against stress response protein 60 (srp 60) is observed in epithelial cells (arrow). Srp 60 immunostaining. Bar = 20 μm.

ined. Although the mean ages (30.8 years in patients with atopic cataract, 47.0 years in patients with nonatopic cataract, and 65.8 years in controls) of the three groups were different, there is no evidence that aging is a factor in inducing srp 60. Therefore, these findings suggest that srp 60 may develop in close association with an atopic condition.

The induction of srp 60 is known to be localized in the mitochondrial membrane.[20] An electron microscopic study reported an increased number of mitochondria in the cytoplasms of the lens epithelial cells in patients with atopic cataract, as described above.[7,9] These findings suggest that the immunohistochemical demonstration of srp 60 in the lens epithelial cells in our study may be correlated with an increased number of mitochondria in the cytoplasms of the lens epithelial cells. An increased number of mitochondria represents an acceleration of energy production in the lens epithelial cells. Thus, it is surmised that such long-standing accelerated energy may produce srp 60 on a large scale to protect the lens epithelia from harmful stress, or atopy.[9]

On the other hand, the expression of srp is induced in a cell for its protection from harmful stress,[30,31] such as heat shock,[32,33] infection,[32,34] ischemia,[33] or inflammation.[33] Among the stress-response proteins with well-preserved amino acid sequences from the prokaryote to the cells of mammals, srp 60 has strong immunogenicity to γδ T cells.[19,20,35] When srp 60 is induced to protect the self-structure from stress, the γδ T cells identify the srp 60 as an antigen.[36,37] The γδ T cells attack the cells producing srp 60, resulting in persistent inflammation, ie, an autoimmune inflammation.[16–20,32–34] Srp 60 is reported to develop in the target tissues of autoimmune diseases, such as synovial membranes in juvenile chronic arthritis,[18] hepatocytes in autoimmune hepatitis,[20] and oligodendrocytes in multiple sclerosis.[16,17,21] However, the srp 60 induced in lens epithelial cells cannot be the target of γδ T cells because the lens is an avascular tissue. The expression of srp 60 in lens epithelial cells may result from the srp inducers of humoral immunorelated agents, such as cytokines,[31] in the aqueous humor in patients with atopic cataract, although further biochemical studies are needed to reach a definite conclusion. The srp 60 induced in the lens epithelial cells may participate in the autoimmunity leading to the development of atopic cataract.

In conclusion, the presence of srp 60 immunoreactivity may reflect a protective mechanism of the epithelial cells in atopic cataract against the injury trig-

gered by humoral immunorelated agents. These findings suggest that the pathogenesis of degeneration of the lens epithelial cells in patients with atopic cataract may be related to autoimmunity.

In the epidermis of atopic dermatitis and in normal skin, which is ectomorphic as are the lens epithelial cells, recent immunohistochemical studies[38–43] have revealed that all stress-response proteins tested (srp 27,[42,43] srp 65[39] in srp 60 families, srp 70,[41] and srp 72[38,40]) are strongly expressed in the cytoplasms of keratinocytes as a protective mechanism in human skin from the various harmful outside stresses.[30–34,38–43] In the present study, however, srp 27 and srp 72 were not expressed in the lens epithelial cells in either atopic or nonatopic cataract specimens nor in control specimens. These findings were not equal to the extensive expression of srp in the atopic and normal epidermides.[38–43] Little is known about the immunoreactivity of srp 27 and srp 72, and further studies are requested to determine the cause for the lack of expression of these two stress-response proteins in the epithelial cells examined in this study.

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