INTRODUCTION

Diabetes, also referred to as diabetes mellitus (DM), is associated with a 5-fold higher prevalence of cataract, one of major causes of blindness in the world [1]. The adult-onset diabetic cataracts are readily formed in subjects with type 2 diabetes. It is well known that adult-onset diabetic cataracts may present with single cortex opacity, nuclear opacity, subcapsular opacity, or more than 2 kinds of opacities concurrently, which closely resemble age-related cataracts without diabetes. However, the nuclear and cortex hardness is different between adult-onset diabetic cataract and age-related cataract without diabetes even if the same opacities in nuclear or cortex are observed clinically. It suggests that adult-onset diabetic cataracts have more inherent alterations than age-related cataracts without diabetes in the progression of cataract.

Proliferating cell nuclear antigen (PCNA) is present in the late G1 and early S phases but not in the G0 phase of the cell cycle. PCNA expression level is a sensitive index of DNA synthesis and cell proliferation. In vivo and in vitro experiments have suggested that the decreased lens epithelial cell density observed in diabetes patients results from attenuated cell growth and induction of apoptosis [2–4].

Intercellular adhesion molecule-1 (ICAM-1), also known as CD54, is a 75–115-kDa cell surface glycoprotein. It is a member of the immunoglobulin super family of cell adhesion molecules, which preferentially mediate the attachment and transendothelial migration of circulating leukocytes, including monocytes, lymphocytes, and polymorphonuclear cells [5, 6]. ICAM-1 has been detected in LECs of cataract samples and is involved in the attachment and growth of LECs on collagen- or laminin-coated plates [7]. Recently, experimental studies have shown that fructose can directly increase the expression of ICAM-1 [8, 9].

Vimentin has been regarded as a marker of epithelial-mesenchymal transition (EMT). EMT is a central mechanism for diversifying the cells found in complex tissues. This dynamic process helps organize the formation of the body plan, and while EMT is well studied in the context of embryonic development, it also plays a role in the genesis of fibroblasts during organ fibrosis in adult tissues [10]. According to previous research, vimentin is low expressed in normal LECs; both vimentin and cytokeratin are over expressed in the LECs of nuclear cataract patients but only vimentin is over expressed in the LECs of anterior subcapsular cataract patients; the expression level of vimentin is higher in LECs of subcapsular cataract patients than that in LECs of nuclear cataract patients [11].

In our study, we hypothesized that the progression of cataract in patients with type 2 diabetes is because of abnormal proliferation, adhesion, migration, and transdifferentiation of LECs. Thus, we investigated the expression of PCNA, ICAM-1, and vimentin in the LECs of cataract patients with and without type 2 diabetes.

MATERIALS AND METHODS

Patients

Circular anterior capsules with attached LECs were...
Informed consent was obtained from all patients (PBS). The obtained products were observed with 1:400; Dako, Japan) for 60 min at RT or mouse monoclonal anti-human ICAM-1 (dilution 1:2000; Santa Cruz Biotechnology Inc., USA) or mouse monoclonal anti-human vimentin (dilution 1:8000; Dako, Japan). Hematoxylin was used as the counterstain.

The diagnosis of type 2 diabetes was made by a diabetologist at Tokai University according to the criteria of the American Diabetes Association (ADA) [13]. Informed consent was obtained from all patients enrolled in the study. The research followed the tenets of the Declaration of Helsinki. The study protocol was approved by the Clinical Trials Review Committee of Tokai University School of Medicine in 2010, before study activation.

Biopsy collection

One capsule was randomly selected if the patients underwent surgery of both eyes. The circular anterior capsules, approximately 5-6 mm in diameter, with attached LECs were obtained by performing continuous curvilinear capsulorhexis (CCC) with capsulorhexis forceps (Kawai Capsulorhexis Forceps; Asico, USA).

Immunohistochemistry staining

Paraffin-embedded anterior capsular tissues fixed with 4% paraformaldehyde (PFA; pH 7.4) were sliced into sections of 3 µm thickness, and 100 sections were obtained for each subject. The method used for immunohistochemistry (IHC) staining was as follows. After deparaffinization and rehydration, heat-mediated antigen retrieval was done with citrate buffer (0.01 M, pH 6.0) in a microwave oven (98ºC, 10 min). The sections were incubated with 3% hydrogen peroxide for 10 min to quench endogenous peroxidase activity and then blocked with 10% normal goat serum for 20 min at room temperature (RT). The sections were incubated with mouse monoclonal anti-human PCNA (dilution 1:400; Dako, Japan) for 60 min at RT or mouse monoclonal anti-human ICAM-1 (dilution 1:2000; Santa Cruz Biotechnology Inc., USA) or mouse monoclonal anti-human vimentin (dilution 1:8000; Dako, Japan) overnight at 4ºC. Finally, the sections were incubated with secondary antibody (Histofine Simple Stain MAX-PO/MULTI; Nichirei, Japan) for 30 min at RT after rinsing with 0.01 M phosphate-buffered saline (PBS). The obtained products were observed with 3', 3'-Diaminobenzidine Tetrahydrochloride (DAB, Liquid; Dako, Japan).

The number of PCNA-, ICAM-1– and vimentin-positive LECs was counted in each section. Positive cell rate was defined as the percentage of cells that showed expression of the antigens in any position of the cell membrane. The average positive cell rate obtained from 3 sections per stain was used for statistical analysis.

Immunolocalization of ICAM-1

ICAM-1 was immunolocalized on the surface, side, and basement of LECs. The study subject was considered as positive if the average positive cell rate of ICAM-1 expression in the sections analyzed was > 25%. The ratio of the number of positive subjects to the total number of subjects from 3 immunolocalization findings of ICAM-1 was obtained.

Quantitative analysis of PCNA, ICAM-1, and vimentin expression

The differences in the expression of PCNA, ICAM-1, vimentin, the severity of different types of cataracts, age, and the immunolocalization findings of ICAM-1 in cataract LECs between the DM and control groups were analyzed by Student’s t-test, Mann-Whitney U-test, and Pearson’s Chi-square test, as appropriate. The correlations between PCNA, ICAM-1 expression and clinical parameters in the DM group were analyzed by Spearman’s rank correlation test. The correlations among ICAM-1, PCNA, and vimentin expression were analyzed by Spearman’s rank correlation test. The receiver operator characteristic (ROC) curve was used to determine the optimal cut-off value of ICAM-1 expressed on the side and basement of LECs, according to the coordinates of the cut-off point of the ROC curve that was closest to the coordinate (0, 1). The odds ratio of the immunolocalization of ICAM-1 on the surface, side, and basement of LECs was also evaluated as a risk factor associated with the progression of cataract in type 2 diabetes between the DM and the control groups.

RESULTS

Clinical characteristics

Patient characteristics are summarized in Table 1. The classification of cataract, according to the LOCS III criteria, in DM and control groups is shown in Table 2 [12]. No significant difference in the severity
of different types of cataracts, i.e., cortical, nuclear, and posterior subcapsular cataract (P = 0.065, P = 0.924, and P = 0.347, respectively) and age (P = 0.756) between the DM and control groups was noted. Almost all the subjects under study had complex cataracts, with more than 2 kinds of opacities occurring concurrently.

PCNA expression in LECs

The positive cell rates of PCNA expression in the DM and control groups were 66.97% ± 15.92% and 77.75% ± 15.24%, respectively. PCNA antigen level was higher in the control group than in the DM group (P = 0.036; Fig. 1, Table 1).

ICAM-1 expression in LECs

The positive cell rate of ICAM-1 expression was 74.89% in the DM group and 38.30% in the control group. The level of ICAM-1 expression was significantly different between the 2 groups (P = 0.0001, Table 1). ICAM-1 was immunolocalized on the surface, side, and basement of LECs (Fig. 1). In the DM group, ICAM-1 expression was observed on each side of several LECs, while in the control group, it was mainly observed on the surface and partly on the side of LECs. The ratio of the number of positive subjects with ICAM-1 immunolocalization on the surface, side, and basement of LECs to the total number of subjects in the 2 groups is shown in Table 1. Compared to that in the control group, a significant increase in ICAM-1 expression (positive subject ratio) was found in the DM group, and this finding was consistent with ICAM-1 immunolocalization on the side and basement of LECs (P = 0.005 and P < 0.0001, respectively). However, no difference was found in ICAM-1 expression on the surface of LECs (P = 0.082).

Vimentin expression in LECs

Vimentin was expressed in almost all the cells in both DM and control groups (Fig. 1). No significant difference was noted in the positive cell rates between the 2 groups (95.65% ± 3.58% in the DM group and 91.41% ± 12.02% in the control group; P = 0.264; Table 1).

Correlations between PCNA, ICAM-1 expression in LECs and several clinical and immunohistochemical parameters

In the DM group, no significant correlation was noted between ICAM-1 expression and several clinical parameters, such as age, duration of diabetes, HbA1c level, severity of DR, and severity of different types of cataracts (Table 3); PCNA expression had a negative correlation with duration of diabetes (r = -0.478, P < 0.05) but a positive correlation with subcapsular opacity (r = 0.661, P < 0.001; Table 3). A negative correlation was found between PCNA and ICAM-1 expression (r = -0.561, P < 0.0001), but no correlation was found between PCNA and vimentin expression (r = 0.151, P = 0.341).

---

Table 1 Patient characteristics and results of immunohistochemistry staining

<table>
<thead>
<tr>
<th></th>
<th>DM group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Age (years; mean ± SD)</td>
<td>67.88 ± 16.26</td>
<td>70.84 ± 7.89</td>
</tr>
<tr>
<td>Gender (Male/Female)</td>
<td>14/11</td>
<td>10/15</td>
</tr>
<tr>
<td>HbA1c (%; mean ± SD)</td>
<td>6.72 ± 1.04</td>
<td>5.01 ± 0.98</td>
</tr>
<tr>
<td>Duration of diabetes (years; mean ± SD)</td>
<td>15.86 ± 15.59</td>
<td>0</td>
</tr>
<tr>
<td>Positive cell rate of PCNA (%; mean ± SD)</td>
<td>66.97 ± 15.92</td>
<td>77.75 ± 15.24</td>
</tr>
<tr>
<td>Positive cell rate of ICAM-1 (%; mean ± SD)</td>
<td>74.89 ± 18.16</td>
<td>38.30 ± 16.85</td>
</tr>
<tr>
<td>Positive cell rate of vimentin (%; mean ± SD)</td>
<td>95.65 ± 3.58</td>
<td>91.41 ± 12.02</td>
</tr>
<tr>
<td>ICAM-1 on the surface of LECs (positive subject ratio)</td>
<td>18/25</td>
<td>3/25</td>
</tr>
<tr>
<td>ICAM-1 on the side of LECs (positive subject ratio)</td>
<td>24/25</td>
<td>16/25 ***</td>
</tr>
<tr>
<td>ICAM-1 on the basement of LECs (positive subject ratio)</td>
<td>24/25</td>
<td>20/25</td>
</tr>
</tbody>
</table>

* (P<0.05), DM group vs. control group.
*** (P<0.0001), DM group vs. control group.
**** (P<0.0001), DM group vs. control group.

DM: diabetes mellitus; HbA1c: hemoglobin A1c; PCNA: proliferating cell nuclear antigen; ICAM-1: intercellular adhesion molecule-1; LECs: lens epithelial cells

Table 2 Classifications of cataracts in diabetes mellitus and control groups, according to the Lens Opacities Classification System III criteria

<table>
<thead>
<tr>
<th>Grade</th>
<th>DM</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortical opacity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(positive subjects/total subjects)</td>
<td>11/25</td>
<td>7/25</td>
</tr>
<tr>
<td>Nuclear opacity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(positive subjects/total subjects)</td>
<td>4/25</td>
<td>2/25</td>
</tr>
<tr>
<td>Posterior subcapsular opacity</td>
<td>10/25</td>
<td>16/25</td>
</tr>
</tbody>
</table>

DM: diabetes mellitus
ROC curve analysis and odds ratio of ICAM-1 expression in LECs

The ROC curve was analyzed, and the optimal cut-off values of ICAM-1 expression on the side and basement of LECs were 35.90% and 63.83%, respectively. Immunolocalization of ICAM-1 on the surface of LECs did not differ significantly (odds ratio = 6.000; 95% confidence interval, 0.647–55.661) between the 2 groups, while the odds ratios of the immunolocalization of ICAM-1 on the side and basement of LECs were higher in the DM group (13.500; 95% confidence interval, 1.556–117.137 and 18.857; 95% confidence interval, 4.254–83.592, respectively) than in the control group.

DISCUSSION

Studies on both animals and humans have highlighted the important contribution of increased AR activity, which can lead to multiple metabolic and signal-transduction changes, ultimately affecting transcriptional regulation and gene expression in LECs subject to diabetic cataract [1]. Several factors have been identified as risk factors for the onset of diabetic cataract [1]. Especially in elderly subjects, diabetic cataract onset has been shown to be associated with age, increased severity of retinopathy, diuretic usage, low intraocular pressure, smoking, and low diastolic blood pressure but not with duration of diabetes and...
glycosylated hemoglobin levels. In our study, there was no difference in the age and severity of the different types of cataracts between the 2 groups (Table 1).

LECs can undergo proliferation, migration, transdifferentiation, or apoptosis from cataract onset to maturation in different types of cataracts [14–17]. Bras et al. showed that there was a higher expression of PCNA in LECs from cataract patients with diabetes compared with normal patients and those with inherited cataracts, but they found no significant difference in DNA damage (Gadd45) or cell apoptosis in LECs from diabetic dogs with anterior subcapsular cataracts [18]. In our study performed on cataract patients without anterior subcapsular opacities, the PCNA expression in DM group had a positive correlation with subcapsular opacity, which suggested that the increased PCNA expression may aggravate the subcapsular opacity. PCNA was expressed in 77.75% of LECs in the control group and 65.08% of LECs in diabetes compared with normal patients and those with inherited cataracts, but found no significant difference in DNA damage (Gadd45) or cell apoptosis in LECs from diabetic dogs with anterior subcapsular cataracts [18]. In our study performed on cataract patients without anterior subcapsular opacities, the PCNA expression in DM group had a positive correlation with subcapsular opacity, which suggested that the increased PCNA expression may aggravate the subcapsular opacity. PCNA was expressed in 77.75% of LECs in the control group and 65.08% of LECs in diabetes compared with normal patients and those with inherited cataracts, but found no significant difference in DNA damage (Gadd45) or cell apoptosis in LECs from diabetic dogs with anterior subcapsular cataracts [18]. In our study performed on cataract patients without anterior subcapsular opacities, the PCNA expression in DM group had a positive correlation with subcapsular opacity, which suggested that the increased PCNA expression may aggravate the subcapsular opacity. PCNA was expressed in 77.75% of LECs in the control group and 65.08% of LECs in diabetes compared with normal patients and those with inherited cataracts, but found no significant difference in DNA damage (Gadd45) or cell apoptosis in LECs from diabetic dogs with anterior subcapsular cataracts [18].

In all kinds of ocular pathological situations, such as disciform herpes simplex virus (HSV) keratitis [19], allergic conjunctivitis [20], uveitis [21] and epiretinal membrane [22], ICAM-1, also known as an inflammatory molecule, is considered necessary for cell adhesion, migration, proliferation, apoptosis, and cell signal transmission. Inflammatory cytokines, such as interleukin (IL)-1 [23] and tumor necrosis factor (TNF)-alpha [24] have increased the expression of ICAM-1 on the side and basement of LECs. Nishi et al. indicated that cell adhesion molecules, including ICAM-1, may serve in the attachment process of cataractous LECs to extracellular matrix and may be involved in the formation and disruption of cell-to-cell and cell-to-posterior capsule interactions when LECs migrate onto the posterior capsule after surgery [7]. Consequently, we have paid special attention to the expression of ICAM-1 in LECs. In this study, we first reported the immunolocalization of ICAM-1 on the surface, side, and basement of LECs (Fig. 1). ICAM-1 was expressed only on the surface of LECs when the positive cell rate was < 35.90%. With an increase in the level of ICAM-1 expression (positive cell rate range, 35.90–63.83%), the protein was detected both on the surface and side of LECs. Finally, when the expression of ICAM-1 was higher than a certain level (positive cell rate, > 63.83%), the expression of ICAM-1 also extended from the side to the basement of LECs, especially in the DM group. Moreover, the odds ratios of the immunolocalization of ICAM-1 on the side and basement of LECs were 13.500-fold and 18.857-fold higher in the DM group, respectively, than in the control group. Thus, ICAM-1 may be an important risk factor during the progression of cataract in type 2 diabetic patients.

Tanaka et al. have demonstrated that ICAM-1-positive synovial cells were growth-arrested and underwent subsequent apoptosis, whereas ICAM-1-negative synovial cells were proliferative [25]. In the present study, PCNA expression was negatively correlated with ICAM-1 expression in all subjects, which indicated that during the progression of cataract, the proliferation ability of LECs is decreased when there is an over expression of ICAM-1. Therefore, it is possible that ICAM-1 expression and cell proliferation in the lens epithelium of cataract are correlated. Further research is needed to identify such relationships between ICAM-1 expression and proliferation in cataractous LECs in order to clarify cataract pathogenesis, especially in diabetes patients.

**ACKNOWLEDGMENTS**

We thank the members in the Department of Histological Science of Tokai University School of Medicine for their excellent technical assistance. We also thank all the members in the Department of Ophthalmology of Tokai University School of Medicine who provided the materials for the study.

**REFERENCES**

51–57.