Iris Damage Is Associated With Elevated Cytokine Levels in Aqueous Humor

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PURPOSE. To evaluate the association between iris damage and cytokine levels in the aqueous humor (AqH).

METHODS. A total of 201 AqH samples from 201 consecutive patients (mean age 73.7 ± 10.6) were collected at the beginning of corneal transplantation or cataract surgery. Iris damage of each case was assessed from preoperative slit-lamp findings based on its severity. The subjects were classified into three groups: eyes without iris damage (126 eyes), eyes with mild iris damage (51 eyes), and eyes with severe iris damage (24 eyes). The levels of cytokines (IL-1α, IL-1β, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, IL-17α, interferon gamma-induced protein [IP]-10, monocyte chemotactic protein [MCP]-1, IFN-γ, IFN-γ, macrophage inflammatory protein [MIP]-1α, MIP-1β, P-selectin, E-selectin, soluble intercellular adhesion molecule [sICAM]-1, TNF-α, and granulocyte-macrophage colony-stimulating factor [GM-CSF]) in AqH were measured by multiplex beads immunoassay.

RESULTS. The levels of aqueous protein, IL-1α, IL-1β, IL-4, IL-6, IL-8, IL-10, IL-17A, MCP-1, TNF-α, E-selectin, P-selectin, and sICAM-1 in eyes with mild and severe iris damage were higher than in those without iris damage (P < 0.035). Multivariate analyses of clinical factors revealed that iris damage was associated with the history of complicated glaucoma, and the number of previous intraocular surgeries. The levels of AqH IL-6, IL-8, IL-13, MIP-1α, TNF-α, and sICAM-1 were significantly elevated in eyes with mild and severe iris damage in phakic eyes, and the levels of AqH IL-8 and sICAM-1 were significantly elevated in eyes with severe iris damage in pseudophakic eyes, compared with the eyes without iris damage (P < 0.045).

CONCLUSIONS. Iris damage was associated with the elevation in the levels of aqueous protein and cytokines.

Keywords: iris, aqueous humor, cytokine, immune privilege, glaucoma
METHODS

This prospective consecutive study was performed in accordance with the Declaration of Helsinki, and approved by the Institutional Ethics Review Board of Tokyo Dental College, Ichikawa General Hospital (I-15-42). Written informed consent was obtained from all the participants.

Patients

A total of 201 consecutive patients who underwent corneal transplantation and cataract surgery at Tokyo Dental College in the period from October, 2015 to September, 2016 were included. The demographics of the participants are shown in Table 1. The diseases for which surgeries were performed included bullous keratopathy (72 eyes), cataract (61 eyes), corneal scar (28 eyes), Fuchs’ endothelial corneal dystrophy (FECD; 19 eyes), hereditary epithelial/stromal dystrophies (11 eyes; lattice corneal dystrophy, 5 eyes; macular corneal dystrophy, 3 eyes; granular corneal dystrophy, 3 eyes), and keratoconus (10 eyes). We did not perform corneal transplantation or cataract surgery in eyes with active inflammation of the cornea or anterior chamber, and such eyes were not included in the study. We confirmed that the anterior chamber did not contain cells, ciliary injections, or keratoprecipitates using slit-lamp microscopy before surgery. As for flare in the anterior chamber, we measured aqueous protein levels directly, using slit-lamp microscopy in the eyes with corneal opacity. We excluded 18 eyes with ocular comorbidities (8 eyes with a history of exfoliation syndrome, 3 eyes with chemical burn, 2 eyes with ocular cicatrical pemphigoid, 1 eye with aniridia, 1 eye with endotheliitis, 1 eye with Stevens-Johnson syndrome, 1 with exposure keratatis, and 1 eye with iritis), because previous studies showed that aqueous cytokine levels are elevated in eyes with exfoliation syndrome due to breakdown of the blood–aqueous barrier (BAB), and that tear cytokine levels are elevated in inflammatory ocular surface diseases, which can affect aqueous cytokine levels due to breakdown of the corneal epithelial barrier.22 In the eyes with cataract and bullous keratopathy, when we perform two-step surgery, we always perform cataract surgery first, followed by DSAEK, with an interval of more than 3 months. In the eyes with cataract after penetrating keratoplasty (PKP), we make it a rule to wait for an interval of more than 6 months after PKP to perform the cataract surgery.

AqH Samples

Aqueous humor was obtained under sterile conditions at the beginning of surgery after retrobulbar anesthesia in corneal transplantation or topical anesthesia in cataract surgery. First, paracentesis was placed at the clear cornea. The AqH samples (70–300 µL) were obtained using a 27-G needle, taking care not to touch the iris, lens, or corneal endothelium. The samples were centrifuged at 3000g for 5 minutes. The soluble fractions were collected and stored at −80 °C until cytokine levels were measured.

Measurement of Protein Concentration

The concentrations of protein in the AqH samples were determined using the DC protein assay kit (Bio-Rad, Hercules, CA, USA). The reactions were based on the Lowry assay, and were performed according to the manufacturer’s instructions. In brief, BSA in the concentration range of 0.23 to 1.37 mg/mL was used as a standard. Samples (5 µL of BSA and AqH were added to 96-well microplates; this was followed by an immediate addition of a mixture containing 25 µL of reagent A + S and 200 µL of reagent C. After 15-minutes incubation at room temperature in the dark, the microplates were read at 690 and 405 nm using a microplate reader. The concentrations of protein in the AqH samples were determined using the DC protein assay kit (Bio-Rad).

Measurement of Cytokine Levels

The levels of cytokines (IL-1α, IL-1β, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, IL-17A, IFN-α, IFN-γ, monocyte chemotactic protein [MCP]-1, TNF-α, E-selectin, P-selectin, soluble intercellular adhesion molecule [sICAM]-1, granulocyte-macrophage colony-stimulating factor [GM-CSF], macrophage inflammatory protein [MIP]-1α, MIP-1β, and interferon gamma-induced protein [IP]-10) in the AqH samples were measured using Luminex (ProCepTix kit; Luminex, San Antonio, TX, USA) beads-based multiplex immunoassay according to previous reports. Briefly, 50 µL of AqH samples were incubated with antibody-coated capture beads in an incubation buffer at room temperature. After 2-hours incubation, the beads were washed thrice using washing buffer and phycoerythrin-labeled streptavidin was added and allowed to bind with in the dark at room temperature for 30 minutes. After three washes with the washing buffer, 150 µL of the reading buffer was added to the plates, and the assays were performed using Luminex 200 system.

Definition of the Severity of Iris Damage

Iris damage was defined as iris depigmentation, laser iridotomy, or iris defect due to intraocular surgeries. The severity grade of iris damage for each case was determined based on its severity from slit-lamp findings. Briefly, healthy eyes are regarded as “no iris damage” (Fig. 1A), eyes with “mild iris damage” are...
Aqueous Cytokine Levels and Iris Damage

**RESULTS**

**Aqueous Protein Levels in Eyes With and Without Iris Damage**

The aqueous protein levels in eyes without iris damage were 0.51 ± 0.46 mg/mL, which significantly increased to 0.98 ± 0.14 mg/mL in eyes with mild iris damage (P = 0.0033) and 1.70 ± 0.29 mg/mL in eyes with severe iris damage (P < 0.0001). The aqueous protein level in eyes with severe iris damage was significantly higher than in eyes with mild iris damage (Fig. 2, P = 0.016).

**Increased Aqueous Cytokine Levels in Eyes With Iris Damage**

The mean cytokine levels in AqH, in terms of iris damage severity in all the subjects are shown in Table 2. The levels of IL-1α, IL-1β, IL-6, IL-8, IL-10, IL-17A, MCP-1, TNF-α, E-selectin, P-selectin, and sICAM-1 in eyes with mild and severe iris damage were higher than those without iris damage (P < 0.0033). The levels of IL-13, MIP-1α, IFN-α and IFN-γ in eyes with mild iris damage were higher than those without iris damage (P < 0.0008), and IP-10 in eyes with severe iris damage was higher than that of no iris damage (P = 0.0013). None of the cytokines in eyes with severe iris damage was lower than that in eyes without iris damage (P > 0.05), and only IP-10 was lower in eyes with mild iris damage, compared with that in eyes without iris damage (P < 0.05). Interferon-γ was higher in eyes with mild iris damage than in eyes with severe iris damage. There were no other significant differences in the cytokine levels between eyes with mild and severe iris damage.

**Associations Between Clinical Factors and the Severity of Iris Damage**

Table 3 shows the univariate and multivariate regression analyses of the association between iris damage severity and clinical factors. Univariate correlation analysis showed iris damage severity was correlated with the number of previous intraocular surgeries (ρ = 0.754, P = 0.0001), presence of IOL (ρ = 0.516, P = 0.0001), complicated glaucoma (ρ = 0.491, P = 0.0001), history of corneal transplantation (ρ = 0.371, P = 0.0001), and axial length (ρ = –0.170, P = 0.018). Multiple regression analyses showed that iris damage severity had significant correlations with the number of previous intraocular surgeries (β = 0.356, P = 0.0001) and history of complicated glaucoma (β = 0.355, P = 0.013).

**Aqueous Cytokine Levels Stratified With the Severity of Iris Damage in Phakic and Pseudophakic Eyes**

Having demonstrated that the levels of aqueous protein and cytokines were increased in eyes with iris damage, we assessed the levels of aqueous protein and cytokines by dividing the samples into two categories—phakic and pseudophakic eyes—because previous report has shown that specific cytokine levels alter after the cataract surgery. In phakic eyes (Table 4, total 142 eyes), the levels of IL-6, IL-8, IL-13, MIP-1α, TNF-α, and sICAM-1 in the eyes with mild and severe iris damage were significantly higher than those in the eyes without iris damage (P < 0.045). The levels of protein, IL-1β, IL-17A, GM-CSF, IFN-α, IFN-γ, and P-selectin in the eyes with mild iris damage were significantly higher than that in the eyes without iris damage (P < 0.045). The levels of IL-6 and MCP-1 in eyes with severe iris damage were significantly higher than that in the eyes without iris damage (P = 0.0007 and P = 0.0027, respectively).

In pseudophakic eyes (Table 5, total 59 eyes), the IL-8 levels in the eyes with mild and severe iris damage were significantly higher than that in eyes without iris damage (P = 0.0003 and P = 0.007, respectively).
Aqueous Cytokine Levels and Iris Damage

**Table 2. Aqueous Cytokine Levels in All Eyes**

<table>
<thead>
<tr>
<th>Aqueous Cytokine</th>
<th>No Iris Damage, N = 126</th>
<th>Mild Iris Damage, N = 51</th>
<th>Severe Iris Damage, N = 24</th>
<th>P Value*</th>
<th>P Value†</th>
<th>P Value‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1z</td>
<td>62.9 ± 7.2 (46.9)</td>
<td>102 ± 19.5 (71.6)</td>
<td>97.6 ± 17.7 (68.6)</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>IL-1β</td>
<td>2.7 ± 0.6 (1.1)</td>
<td>11.5 ± 3.1 (3.1)</td>
<td>24.3 ± 18.1 (1.6)</td>
<td>&lt;0.0001</td>
<td>0.46</td>
<td>0.0001</td>
</tr>
<tr>
<td>IL-6</td>
<td>26.3 ± 2.6 (20.9)</td>
<td>48.1 ± 5.9 (29.2)</td>
<td>40.5 ± 5.4 (30.8)</td>
<td>&lt;0.0001</td>
<td>0.95</td>
<td>0.0001</td>
</tr>
<tr>
<td>IL-8</td>
<td>380 ± 110 (94.9)</td>
<td>1497 ± 466 (335)</td>
<td>1185 ± 701 (210)</td>
<td>&lt;0.0001</td>
<td>0.50</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TNF-α</td>
<td>41.0 ± 9.4 (16.6)</td>
<td>70.7 ± 11.4 (39.5)</td>
<td>78.5 ± 19.9 (51.9)</td>
<td>&lt;0.0001</td>
<td>0.77</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>2.6 ± 0.2 (1.8)</td>
<td>8.5 ± 3.0 (3.7)</td>
<td>12.5 ± 5.5 (4.1)</td>
<td>&lt;0.0001</td>
<td>0.57</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>IL-12p70</td>
<td>7.9 ± 0.6 (6.5)</td>
<td>14.0 ± 1.7 (8.5)</td>
<td>11.8 ± 1.4 (9.0)</td>
<td>0.0001</td>
<td>0.95</td>
<td>0.0001</td>
</tr>
<tr>
<td>IL-13</td>
<td>8.4 ± 0.8 (7.2)</td>
<td>10.6 ± 1.0 (9.6)</td>
<td>9.6 ± 1.3 (9.2)</td>
<td>&lt;0.0001</td>
<td>0.41</td>
<td>0.11</td>
</tr>
<tr>
<td>IL-17A</td>
<td>5.6 ± 0.4 (4.6)</td>
<td>10.6 ± 2.0 (7.2)</td>
<td>8.3 ± 1.0 (7.2)</td>
<td>0.0089</td>
<td>0.84</td>
<td>0.0073</td>
</tr>
<tr>
<td>MIP-1α</td>
<td>11.2 ± 0.9 (9.2)</td>
<td>20.1 ± 3.2 (12.3)</td>
<td>14.5 ± 2.0 (11.8)</td>
<td>0.0081</td>
<td>0.67</td>
<td>0.07</td>
</tr>
<tr>
<td>MIP-1β</td>
<td>338 ± 42.9 (303.7)</td>
<td>384 ± 70.4 (323)</td>
<td>365 ± 75.7 (294)</td>
<td>0.661</td>
<td>0.99</td>
<td>0.75</td>
</tr>
<tr>
<td>MCP-1</td>
<td>627 ± 70.6 (466.7)</td>
<td>860 ± 146 (615)</td>
<td>756 ± 86.5 (694)</td>
<td>0.0056</td>
<td>0.57</td>
<td>0.0005</td>
</tr>
<tr>
<td>TNF-γ</td>
<td>110 ± 8.1 (80.9)</td>
<td>326 ± 157 (102)</td>
<td>139 ± 14.9 (122)</td>
<td>0.035</td>
<td>0.68</td>
<td>0.011</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>5.4 ± 1.1 (3.6)</td>
<td>8.0 ± 1.9 (5.1)</td>
<td>8.8 ± 2.8 (8.5)</td>
<td>0.16</td>
<td>0.52</td>
<td>0.068</td>
</tr>
<tr>
<td>IFN-α</td>
<td>4.5 ± 0.4 (3.9)</td>
<td>6.5 ± 0.8 (4.9)</td>
<td>4.1 ± 0.4 (4.0)</td>
<td>0.0003</td>
<td>0.035</td>
<td>0.95</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>62.7 ± 5.0 (51.9)</td>
<td>102.1 ± 13.0 (66.6)</td>
<td>68.9 ± 4.9 (62.6)</td>
<td>&lt;0.0001</td>
<td>0.26</td>
<td>0.95</td>
</tr>
<tr>
<td>E-selectin</td>
<td>2739 ± 256 (2295)</td>
<td>4326 ± 752 (2812)</td>
<td>3885 ± 512 (2785)</td>
<td>0.0004</td>
<td>0.83</td>
<td>0.002</td>
</tr>
<tr>
<td>P-selectin</td>
<td>5477 ± 432 (3895)</td>
<td>14147 ± 2625 (7540)</td>
<td>10026 ± 3409 (6850)</td>
<td>&lt;0.0001</td>
<td>0.21</td>
<td>0.004</td>
</tr>
<tr>
<td>sICAM-1</td>
<td>2093 ± 235 (1313)</td>
<td>4721 ± 737 (3100)</td>
<td>5279 ± 1054 (3242)</td>
<td>&lt;0.0001</td>
<td>0.30</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>IP-10</td>
<td>377 ± 160 (95.8)</td>
<td>302.9 ± 56.8 (151)</td>
<td>400 ± 115 (181.9)</td>
<td>&lt;0.0001</td>
<td>0.56</td>
<td>0.0013</td>
</tr>
</tbody>
</table>

Mean ± SE (median) (pg/mL).

* Compared between eyes without iris damage and eyes with mild iris damage, Mann-Whitney U-test.
† Compared between eyes with mild iris damage and eyes with severe iris damage, Mann-Whitney U-test.
‡ Compared between eyes without iris damage and eyes with severe iris damage, Mann-Whitney U-test.

higher than that in the eyes without iris damage (P = 0.0001 and P = 0.0031, respectively). The levels of protein and sICAM-1 in the eyes with severe iris damage were significantly higher than that in the eyes without iris damage (P = 0.03 and P = 0.034, respectively).

**Aqueous Protein/Cytokine Levels and Iris Damage Score (IDS)**

Having demonstrated that the aqueous protein level was correlated with the severity of iris damage, we sought to evaluate the association of iris damage and aqueous levels of protein and cytokines in detail. We assessed the aqueous protein and cytokine levels after classifying the iris damage into four grades as reported previously.12 In brief, IDS 0 indicates no iris damage (Fig. 1A); IDS 1 is defined as iris damage limited only to one quadrant (Fig. 1B); or no iris damage with laser iridotomy (Fig. 1D); IDS 2 is defined as iris damage in two quadrants (Fig. 1C); IDS 3 is defined as iris damage in three quadrants (Fig. 1E); and IDS 4 is defined as iris damage in four quadrants (Fig. 1F). The aqueous protein levels in the eyes with IDS 0 increased from 0.52 ± 0.05 mg/mL (Fig. 3) to 0.86 ± 0.18 mg/mL in IDS1 (P < 0.001), 1.22 ± 0.24 mg/mL in IDS2 (P = 0.024), 1.62 ± 0.30 mg/mL in IDS3 (P < 0.001), and 1.80 ± 0.52 mg/mL in IDS4 (P < 0.001). The protein concentration in the AqH significantly correlated with iris damage score (r = 0.469, P < 0.001). On the contrary, the aqueous cytokine levels were more complex (Table 6; Fig. 4). Although the levels of some aqueous cytokines were significantly elevated in eyes with IDS 1 to 4 compared with those in eyes with IDS 0, there were no statistically significant differences in aqueous cytokine levels among eyes with IDS 1, 2, 3, and 4 (Fig. 4). However, IDS was significantly positively correlated with the levels of IL-1α, IL-1β, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, IL-17A, IFN-α, IFN-γ, MCP-1, TNF-α, E-selectin, P-selectin, sICAM-1, and IP-10 (Table 6; Spearman’s correlation, P < 0.028).

**DISCUSSION**

In the current study, we demonstrated that the iris damage was associated with elevated cytokine levels in AqH. Furthermore,
TABLE 4.  Aqueous Cytokine Levels in Phakic Eyes

<table>
<thead>
<tr>
<th>Aqueous Cytokine</th>
<th>No Iris Damage, N = 110</th>
<th>Mild Iris Damage, N = 27</th>
<th>Severe Iris Damage, N = 5</th>
<th>P Value*</th>
<th>P Value†</th>
<th>P Value‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>0.44 ± 0.04 (0.33)</td>
<td>0.82 ± 0.17 (0.61)</td>
<td>0.52 ± 0.11 (0.31)</td>
<td>0.025</td>
<td>0.68</td>
<td>0.43</td>
</tr>
<tr>
<td>IL-1α</td>
<td>59.4 ± 7.7 (45.9)</td>
<td>76.7 ± 13.3 (63.9)</td>
<td>103 ± 23.6 (82.6)</td>
<td>0.085</td>
<td>0.24</td>
<td>0.0007</td>
</tr>
<tr>
<td>IL-1β</td>
<td>2.7 ± 0.6 (1.1)</td>
<td>9.7 ± 4.3 (3.1)</td>
<td>56.5 ± 54.1 (2.3)</td>
<td>0.0003</td>
<td>0.77</td>
<td>0.20</td>
</tr>
<tr>
<td>IL-4</td>
<td>25.4 ± 2.8 (20.3)</td>
<td>42.1 ± 5.8 (27.9)</td>
<td>42.9 ± 14.9 (27.9)</td>
<td>0.0001</td>
<td>0.89</td>
<td>0.07</td>
</tr>
<tr>
<td>IL-6</td>
<td>223 ± 86.2 (89.0)</td>
<td>960 ± 291 (335)</td>
<td>3525 ± 3310 (228)</td>
<td>&lt;0.0001</td>
<td>0.97</td>
<td>0.0024</td>
</tr>
<tr>
<td>IL-8</td>
<td>33.3 ± 8.8 (16.2)</td>
<td>51.1 ± 11.8 (34.8)</td>
<td>496 ± 12.0 (38.8)</td>
<td>0.0001</td>
<td>0.58</td>
<td>0.0031</td>
</tr>
<tr>
<td>IL-10</td>
<td>2.1 ± 0.2 (1.8)</td>
<td>4.5 ± 0.6 (3.3)</td>
<td>4.1 ± 0.7 (3.9)</td>
<td>&lt;0.0001</td>
<td>0.66</td>
<td>0.0019</td>
</tr>
<tr>
<td>IL-12p70</td>
<td>7.6 ± 0.6 (6.4)</td>
<td>12.1 ± 1.7 (9.5)</td>
<td>14.5 ± 3.8 (13.6)</td>
<td>&lt;0.0001</td>
<td>0.52</td>
<td>0.012</td>
</tr>
<tr>
<td>IL-13</td>
<td>8.1 ± 0.9 (6.9)</td>
<td>12.1 ± 1.7 (9.5)</td>
<td>14.5 ± 3.8 (13.6)</td>
<td>&lt;0.0001</td>
<td>0.52</td>
<td>0.012</td>
</tr>
<tr>
<td>IL-17A</td>
<td>5.6 ± 0.5 (3.9)</td>
<td>12.9 ± 3.6 (7.4)</td>
<td>9.9 ± 3.8 (7.4)</td>
<td>0.0041</td>
<td>0.99</td>
<td>0.082</td>
</tr>
<tr>
<td>MIP-1z</td>
<td>11.1 ± 1.0 (9.1)</td>
<td>18.0 ± 2.7 (12.8)</td>
<td>16.6 ± 3.9 (13.6)</td>
<td>0.0045</td>
<td>0.83</td>
<td>0.019</td>
</tr>
<tr>
<td>MIP-1β</td>
<td>332 ± 47.5 (304)</td>
<td>345 ± 65.9 (279)</td>
<td>424 ± 154 (392)</td>
<td>0.82</td>
<td>0.56</td>
<td>0.30</td>
</tr>
<tr>
<td>MCP-1</td>
<td>590 ± 77.3 (458)</td>
<td>634 ± 64.3 (595)</td>
<td>662 ± 72.5 (690)</td>
<td>0.058</td>
<td>0.84</td>
<td>0.0027</td>
</tr>
<tr>
<td>TNF-α</td>
<td>104 ± 8.2 (61.4)</td>
<td>214 ± 54.2 (74.1)</td>
<td>164 ± 26.6 (154)</td>
<td>0.012</td>
<td>0.63</td>
<td>0.0073</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>5.1 ± 1.1 (3.6)</td>
<td>9.9 ± 3.0 (5.1)</td>
<td>13.75</td>
<td>0.036</td>
<td></td>
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<tr>
<td>IFN-α</td>
<td>4.5 ± 0.4 (3.9)</td>
<td>5.8 ± 0.9 (5.0)</td>
<td>4.6 ± 1.1 (6.5)</td>
<td>0.04</td>
<td>0.49</td>
<td>0.86</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>61.2 ± 5.7 (52.0)</td>
<td>93.8 ± 12.1 (68.8)</td>
<td>71.6 ± 17.4 (56.8)</td>
<td>&lt;0.0001</td>
<td>0.31</td>
<td>0.27</td>
</tr>
<tr>
<td>E-Selectin</td>
<td>2728 ± 264 (2468)</td>
<td>3424 ± 475 (2677)</td>
<td>2783 ± 771 (2404)</td>
<td>0.043</td>
<td>0.66</td>
<td>0.51</td>
</tr>
<tr>
<td>P-Selectin</td>
<td>5256 ± 472 (3723)</td>
<td>10518 ± 1786 (7540)</td>
<td>6907 ± 1132 (8154)</td>
<td>&lt;0.0001</td>
<td>0.72</td>
<td>0.062</td>
</tr>
<tr>
<td>sICAM-1</td>
<td>1974 ± 248 (1301)</td>
<td>3721 ± 501 (2845)</td>
<td>3447 ± 1000 (2278)</td>
<td>&lt;0.0001</td>
<td>0.68</td>
<td>0.045</td>
</tr>
<tr>
<td>IP-10</td>
<td>406 ± 183 (94.1)</td>
<td>254 ± 82 (129)</td>
<td>422 ± 246 (147)</td>
<td>0.015</td>
<td>0.39</td>
<td>0.16</td>
</tr>
</tbody>
</table>

Mean ± SE (median), protein (mg/mL), cytokines (pg/mL).
* Compared between eyes without iris damage and eyes with mild iris damage, Mann-Whitney U-test.
† Compared between eyes with mild iris damage and eyes with severe iris damage, Mann-Whitney U-test.
‡ Only one sample could be measured using multiplex beads assay among the samples in each group.

Multivariate analysis showed that iris damage was correlated with complicated glaucoma surgery and number of previous intraocular surgeries. These results suggest that iris damage lead to elevation in the levels of aqueous inflammatory cytokines, which would be a useful clinical finding for increased cytokine levels in ApH; however, there was no significant difference in the aqueous cytokine levels between mild and severe iris damage.

Corneal transplants are among the most successful solid tissue transplants. The primary cause of graft failure is endothelial decompensation, even in eyes without any evidence of immunologic rejection. After corneal transplan-
Aqueous Cytokine Levels and Iris Damage

Figure 3. Protein level in AqH stratified based on iris damage score. Aqueous protein level increased with iris damage score.

The presence of complicated glaucoma leads to elevated levels of aqueous cytokines as reported previously.15,39 Furthermore, the study showed that the levels of aqueous protein and cytokine were elevated in the eyes with iris damage both in phakic and pseudophakic conditions. In phakic eyes, the causes of iris damage were LI and surgical iris damage during corneal transplantation and trabeculectomy, whereas the phakic eyes without iris damage included cataract eyes and eyes with FEDC. We previously reported the elevation of aqueous cytokines in eyes with a history of LI and past intraocular surgeries.13 To assess the association between iris damage and the cytokine levels in eyes after corneal transplantation, we compared the levels of aqueous cytokines among the different iris damage severity after selecting the eyes after corneal transplantation (Supplementary Table S1, 19 eyes after PKP, 6 eyes after DSAEK, and 2 eyes after anterior lamellar keratoplasty). The intraocular surgeries that caused the iris damage were performed 6 months to 10 years before the AqH collection. Although we have to evaluate the association among the kind of surgeries and the interval between surgeries and AqH collection after increasing the number of subjects, we postulated that iris damage during complicated intraocular surgery might induce the elevated levels of aqueous cytokines over the years in a chronic fashion.

The previous laboratory studies showed that the interaction among iris pigment epithelial cells (I-CB cells in the literature), and the components of the AqH might maintain the homeostasis of the immune system in the anterior chamber. The previous reports on the immunosuppressive effects of the iris pigment epithelium are shown in Table 7.8,9,12,40–48 Streilein et al.7 reported that the iris pigment epithelial cells have immunomodulatory properties. The elevated cytokine levels might be attributed to the breakdown of the BAB because the aqueous protein level correlated significantly with the iris damage severity ($r = 0.454$, $P < 0.001$). It is tempting to speculate that the results of this study suggest that the lack of immunomodulatory factors from the I-CB cells can change the microenvironment in the AqH, such as elevation in the levels of aqueous inflammatory cytokines, considering the results of previous laboratory reports. In general, cytokines are produced

<table>
<thead>
<tr>
<th>Aqueous Cytokine</th>
<th>Correlation Coefficient</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1α</td>
<td>0.276</td>
<td>0.0002</td>
</tr>
<tr>
<td>IL-1β</td>
<td>0.272</td>
<td>0.0004</td>
</tr>
<tr>
<td>IL-4</td>
<td>0.392</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.490</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>IL-8</td>
<td>0.459</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>IL-10</td>
<td>0.457</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>IL-12p70</td>
<td>0.316</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>IL-13</td>
<td>0.278</td>
<td>0.0006</td>
</tr>
<tr>
<td>IL-17A</td>
<td>0.294</td>
<td>0.0002</td>
</tr>
<tr>
<td>MIP-1α</td>
<td>0.161</td>
<td>0.055</td>
</tr>
<tr>
<td>MIP-1β</td>
<td>0.028</td>
<td>0.695</td>
</tr>
<tr>
<td>MCP-1</td>
<td>0.293</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TNF-α</td>
<td>0.279</td>
<td>0.0007</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>0.206</td>
<td>0.08</td>
</tr>
<tr>
<td>IFN-α</td>
<td>0.174</td>
<td>0.028</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>0.305</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>E-Selectin</td>
<td>0.289</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>P-Selectin</td>
<td>0.352</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>sICAM-1</td>
<td>0.454</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>IP-10</td>
<td>0.343</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Spearman’s correlation analysis.
Figure 4. Cytokine levels in aqueous humor stratified based on iris damage score. Levels of some cytokines were higher in the eyes with IDS 1-4 compared with the eyes with IDS 0.
in immune cells in response to specific stimuli, such as infection, trauma, or autoimmune diseases. Hence, further evaluation of the causes of chronic inflammation in the anterior chamber is needed in future studies.

Because cytokines are very sensitive, some systemic condition can affect the aqueous cytokine levels, as reported for the elevation of tear cytokine levels with deterioration of rheumatoid arthritis. Systemic inflammatory conditions can also affect AqH cytokine levels via elevation of serum cytokines or breakdown of the BAB. We thoroughly checked the clinical records of all patients regarding steroid use and systemic diseases. There were nine patients with systemic inflammatory diseases: three patients with rheumatoid arthritis, two patients with asthma, one patient with atop dermatitis, one patient with polymyositis, one patient with polyneuritis, and one patient with Sjogren syndrome (2 patients without iris damage, 3 patients with mild iris damage, and 4 patients with severe iris damage). We compared the aqueous protein and cytokine levels between these nine patients and 192 patients without systemic diseases and found that there were no statistically significant differences in the aqueous protein and cytokine levels, except IL-8 (39.7 ± 32.0 pg/mL) in the 9 patients with systemic inflammatory diseases and 54.0 ± 102 pg/mL in the 192 patients without systemic inflammatory diseases). Topical steroids can also potentially be used as a treatment modality to suppress AqH cytokine levels. Regarding steroid use, topical steroids were administered in the eyes after corneal transplantation (10 eyes), pseudophakic bullous keratopathy (PBK; eight eyes), and trabeculectomy (three eyes), when the AqH samples were collected. No patients received systemically administered steroids. We analyzed the differences in protein and cytokine levels between the eyes administered with topical steroids and the eyes that did not receive such treatment. However, we did not find any clinically relevant difference.

This study has some limitations. Firstly, this study included only patients who had undergone corneal transplantation and cataract surgery, and did not include patients with retinal or glaucoma diseases with normal ECD. All the eyes that underwent multiple complicated intraocular surgeries had severe iris damage and this study did not include eyes with a healthy iris, which underwent several intraocular surgeries. Such heterogenous selection of subjects can induce selection bias. In such eyes, the elevation in the levels of aqueous cytokines has been reported and we have to consider other reasons for the elevated levels of aqueous cytokines.

We will have to conduct comprehensive studies to evaluate the effect of different kinds of surgeries on aqueous cytokines in the future. Second, whereas the aqueous proteins level was directly correlated with the severity of iris damage (Figs. 3), there were no statistically significant differences in aqueous cytokine levels between the eyes with mild and severe iris damage (Supplementary Fig. S1). Iris damage was associated with various clinical factors and the measurement of cytokines in healthy iris, which underwent several intraocular surgeries. We need further studies to evaluate the effect of iris damage on the levels of aqueous cytokines in other races. Forth, the interval between the previous glaucoma surgery and AqH collection can affect the aqueous levels of cytokines. The mean interval was 14.4 ± 14.8 years, ranging from 2 to 54 years (total 15 eyes: 12 eyes after trabeculectomy and 3 eyes with multiple glaucoma surgeries). The aqueous levels of protein and cytokines were not significantly correlated with the interval between the glaucoma surgery and AqH collection, although we have to increase the number of subjects after glaucoma surgery. The other limitation is that we could not

<table>
<thead>
<tr>
<th>Author (y)</th>
<th>Cell Line/ Animal/ Human</th>
<th>Model/ Subjects</th>
<th>Molecules</th>
<th>Principal Source</th>
<th>Principal Cellular Targets and Biologic Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streilein, et al. (1990)⁸</td>
<td>Cell line</td>
<td>MLR</td>
<td>TGF-β</td>
<td>AqH</td>
<td>T cell; suppress proliferation</td>
</tr>
<tr>
<td>Streilein, et al. (1991)⁹</td>
<td>Cell line</td>
<td>MLR</td>
<td>NA</td>
<td>I-CB</td>
<td>Immune cells; increased cell infiltration in the iris and aqueous protein levels</td>
</tr>
<tr>
<td>Suzuma, et al. (1997)⁰¹</td>
<td>Rat</td>
<td>EIU</td>
<td>P-selectin</td>
<td>I-CB</td>
<td>Immune cells; increased cell infiltration in the iris and aqueous protein levels</td>
</tr>
<tr>
<td>Suzuma, et al. (1998)¹¹</td>
<td>Rat</td>
<td>EIU</td>
<td>E-selectin</td>
<td>I-CB, retina</td>
<td>Immune cells; increased cell infiltration in the iris and aqueous protein levels</td>
</tr>
<tr>
<td>Marle, et al. (1999)⁵²</td>
<td>Rat</td>
<td>EIU</td>
<td>TNF-α, IL-6, IL-13</td>
<td>I-CB</td>
<td>Immune cells; IL-13 suppresses cell infiltrates in the iris by downregulating TNF-α and IL-6</td>
</tr>
<tr>
<td>Yoshida, et al. (2000)⁵³</td>
<td>Cell line/ mouse</td>
<td>MLR/ACAID</td>
<td>TGF-β</td>
<td>I-CB/AqH</td>
<td>T cell; suppress proliferation</td>
</tr>
<tr>
<td>Ohta, et al. (2000)⁴⁴</td>
<td>Mouse</td>
<td>EIU/MLR</td>
<td>TGF-β</td>
<td>I-CB</td>
<td>Downregulate IFN-γ, IL-2, IL-4 and IL-10</td>
</tr>
<tr>
<td>Yoshida, et al. (2000)⁴⁵</td>
<td>Mouse</td>
<td>Cell line/ mouse</td>
<td>MLR</td>
<td>CD95</td>
<td>T cell; suppress proliferation</td>
</tr>
<tr>
<td>Lemaître, et al. (2001)⁴⁶</td>
<td>Mouse</td>
<td>EIU</td>
<td>IL-13</td>
<td>Exogenous IL-13</td>
<td>Exogenous IL-13 suppresses cell infiltrates in the iris by downregulating MCP-1 and MIP</td>
</tr>
<tr>
<td>Mo, et al. (2003)⁴⁷</td>
<td>Mouse</td>
<td>PDS/ACAID</td>
<td>CD95</td>
<td>I-CB</td>
<td>I-CB contribute to ACAID and suppress T cell proliferation</td>
</tr>
<tr>
<td>Sugita, et al. (2007)⁴⁸</td>
<td>Cell line</td>
<td>Treg assay</td>
<td>B7</td>
<td>I-CB</td>
<td>Iris damage was associated with endothelial cell loss, leading to graft failure</td>
</tr>
<tr>
<td>Ishii, et al. (2016)¹²</td>
<td>Human</td>
<td>EK</td>
<td>NE</td>
<td>I-CB</td>
<td>Iris damage was associated with elevated cytokine levels in AqH</td>
</tr>
<tr>
<td>The current study</td>
<td>Human</td>
<td>Cataracts/corneal disease</td>
<td>Cytokine</td>
<td>NE</td>
<td>Iris damage was associated with elevated cytokine levels in AqH</td>
</tr>
</tbody>
</table>

MLR, mixed lymphocyte reaction; NA, not available; EIU, endotoxin-induced uveitis; ACAID, anterior chamber associated immune deviation; PDS, pigment dispersion syndrome; Treg, regulatory T cell; NE, not evaluated; BK, bullous keratopathy.
Aqueous Cytokine Levels and Iris Damage

grade flare in the AqH owing to the presence of corneal opacities in some of the subjects.

In conclusion, we have shown that the iris damage is associated with elevated levels of aqueous protein as well as inflammatory cytokines, such as IL-1α, IL-1β, IL-4, IL-6, IL-10, IL-13, IL-17A, MCP-1, TNF-α, E-selectin, P-selectin, MIP-1α and sICAM-1. The multivariate analyses revealed that the iris damage severity is associated with the number of previous intraocular surgeries and with the presence of complicated glaucoma. Thus, the preexisting iris damage can be one of the useful clinical parameters for chronic breakdown of BAB and elevated inflammatory cytokines, although there was no direct correlation between the extent of iris damage and the level of cytokines.

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References


