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Cornea & External Disease

Role of Monocytes/Macrophages in the Etiology of Bullous Keratopathy After Argon Laser Iridotomy

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Purpose: The etiologic mechanisms of bullous keratopathy (BK) after argon laser iridotomy (ALI) are still unknown. Therefore, we investigated potential mechanisms on BK after ALI.

Methods: Corneal endothelial surface obtained in penetrating keratoplasty for BK after ALI was observed and analyzed immunohistochemically. We investigated how various leukocytes react to cultured human corneal endothelial cells in an inflamed condition and monocytes/macrophages respond to the iris treated by an argon and YAG laser or pigmented and nonpigmented iris treated by an argon laser.

Results: We detected infiltration of CD68- and CD11b-positive monocytes/macrophages in the posterior surface of trephined corneas obtained during penetrating keratoplasty for BK after ALI in three of the seven eyes with ALI. In vitro, monocytes/macrophages, but not T cells, B cells, neutrophils, or pan-leukocytes, removed many cultured human corneal endothelial cells in the medium stimulated with proinflammatory cytokines. Human pigmented iris tissues treated by the argon laser, but not those treated by the YAG laser, attracted many monocytes/macrophages and formed large, round colonies. Human monocytes/macrophages formed large colonies on the argon laser–treated pigmented iris from C3H mice but not nonpigmented iris from albino BALB/c mice.

Conclusions: Our results suggest that monocytes/macrophages, argon laser, and pigmented iris are all involved in the pathogenesis of BK after LI.

Translational Relevance: Etiology in BK after ALI has not been clear, but our findings based on clinical and experimental findings give a critical clue to explain possible mechanisms on BK after ALI.

Introduction

Laser iridotomy (LI) is an established technique for the treatment and prevention of acute angle-closure glaucoma.[1–3] In addition to photocoagulation with an argon laser alone, iridotomy is also performed by photodisruption with a neodymium:YAG laser alone and sequential argon/YAG laser.[4] Although argon laser LI (ALI) is an effective technique for iridotomies of dark irises with high levels of pigment, such as in the eyes of Asian people, YAG laser alone does not work effectively.[5] Compared with surgical peripheral iridotomy, ALI is a relatively safe, feasible procedure; however, after ALI, the density of corneal endothelial cells decreases and focal or generalized corneal endothelial decompensation (i.e., bullous keratopathy [BK]) can develop several years later in Asian countries, especially in Japan.[6–9] To date, the etiologic mechanisms of BK after ALI are unknown.

BK after LI has several unique characteristics. First, it occurs after LI by argon laser but not after LI by YAG laser. One study in Japan followed patients (n = 50) after laser treatment and found BK in 48 cases (96%) treated by argon laser only, in 1 case (2%) treated by sequential argon/YAG laser, and in 1 case (2%) treated by YAG laser only.[9] Sequential argon/YAG laser iridotomy is the standard procedure for LI in

Singapore,[10,11] and BK after LI is rare there compared with Japan, where argon laser without subsequent YAG laser was the treatment of choice at that time in Japan.[9] These findings suggest that LI with argon laser is associated with BK after LI, whereas YAG laser is not.[9] BK after ALI is an important problem in Japan but not necessarily in other countries (e.g., England).[8] For example, ALI-induced BK was 20% (39/195 eyes) of all cases of BK at Kyoto Prefectural University in Japan and 0.0% (0/245 eyes) of all cases at Queen's Medical Centre, in Nottingham, England.[8] After ALI in the superior iris, most patients present with diffuse edema of the whole cornea, but some have inferior corneal edema,[6–8] suggesting that argon laser does not directly damage the corneal endothelium. Although prophylactic ALI also decreases the number of corneal endothelial cells, BK occurs more frequently in the eyes with acute angle-closure glaucoma. These characteristics may help to identify the mechanism of BK after ALI.

We detected infiltration of many monocytes/macrophages on the corneal endothelial side of corneal buttons obtained during penetrating keratoplasty for BK induced by ALI. Therefore, we hypothesized that pigmented iris in Asian eyes burned with argon laser induces infiltration of monocytes/macrophages, which damage corneal endothelium after ALI. In this report, we present surprising findings from the endothelial side of corneal buttons obtained during penetrating keratoplasty for BK induced by ALI and subsequent in vitro experiments. On the basis of these findings and the clinical characteristics of BK after ALI, we suggest possible etiologic mechanisms of BK after ALI in Asian eyes.

Methods

This study using human samples was performed in accordance with the guidelines of the University of Tokyo and approved by the Institutional Review Board of the University of Tokyo. All methods used in this study were conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from patients in whom corneal transplantation was performed.

All experimental protocols using animals were approved by the University of Tokyo. Animals were treated in accordance with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research.

Examination of the Corneal Endothelium

We examined the trephined corneal button of BK after ALI during full-thickness corneal transplantation. After removal of the cornea by penetrating keratoplasty, we used fine forceps to peel off the corneal endothelium and Descemet's membrane from the trephined corneal button to expose the corneal stroma. Then, we placed the endothelial cell side down on the culture dish and examined it under an inverted phase-contrast microscope (ELWD 0.3; Nikon, Tokyo, Japan). After examining the corneal endothelial cells and Descemet's membranes, we embedded them in optimal cutting temperature compound. Then, we cut frozen cross sections (10 μm) on a cryostat and air-dried them for 10 minutes. We stained the cell nucleus with hematoxylin and observed it under a light microscope.

Immunohistochemistry

For immunohistochemistry, we stained frozen cross sections without fixation. The immunohistochemical investigations were performed with the following primary monoclonal antibodies (mAbs): anti-CD11b phycoerythrin (PE) (ICRF44 for natural killer [NK] cells, granulocytes, and monocytes/macrophages), CD56-PE (B159 for pan-NK cells), anti-CD66 fluorescein isothiocyanate conjugated (FITC) (B6.2/CD66 for granulocytes), anti–CD68-PE (Y1/82A for monocytes/macrophages, lymphocytes, fibroblasts, and endothelial cells), and anti-CD163 (GHI/61 for scavenger receptor, monocytes/macrophages). The following primary mAbs were obtained from BD Biosciences (San Diego, CA, USA): mouse IgG1, κ-FITC (MOPC-31C, isotype control) and mouse IgG2b, κ-PE (27-35, isotype control). Each FITC- or PE-conjugated mAb, or the FITC- or PE-conjugated isotype-matched control antibody, was applied for 30 minutes. After three washes in phosphate-buffered saline, the nucleus was stained with SYBR Green or DAPI. The sections were coverslipped with an antifading mounting medium T (Vector Laboratories, Burlingame, CA, USA) and examined under a fluorescent microscope (BH2-RFL-T3 or BX50; Olympus, Tokyo, Japan). All staining procedures were performed at room temperature.

Culture of Human Corneal Endothelial Cells

We obtained six donor corneas from the Rocky Mountain Lions' Eye Bank (Aurora, CO, USA) and SightLife (Seattle, WA, USA). The age of the

donors was 53 to 69 years. We used the human corneal endothelial cells (HCECs) for primary culture, as described previously.[12,13] The endothelial layer, including Descemet's membrane, was removed with forceps. The primary cultures and serial passaging of the HCECs were performed in growth medium consisting of low-glucose Dulbecco's modified Eagle's medium supplemented with 15% fetal bovine serum (FBS), 2.5 mg/L amphotericin B, 2.5 mg/L doxycycline, and 2 ng/mL basic fibroblast growth factor (Sigma, St. Louis, MO, USA). The explants from each cornea were placed endothelial cell-side down into 35-mm culture dishes coated with bovine extracellular matrix, which was obtained by incubating bovine corneal endothelial cells on culture dishes. After 3 days culture in the incubator, the medium was exchanged and then replaced every other day thereafter. Third- or fourth-passaged cells were used for experiments. HCECs were cultured in a T25 culture flask to confluence.

Leukocyte Separation

Mononuclear cells were harvested from the peripheral blood of healthy volunteers, and neutrophils and mononuclear cells were separated with sodium diatrizoate and polysaccharide (Lymphoprep; COSMO BIO, Inc., Tokyo, Japan) according to the manufacturer's instructions. Then, the mononuclear cells were separated into T cells (Pan T Cell Isolation Kit, human; Miltenyi Biotec, Bergisch Gladbach, Germany), B cells (Pan B Cell Isolation Kit, human; Miltenyi Biotec), and monocytes/macrophages (CD14 MicroBeads, human; Miltenyi Biotec) by magnetic cell sorting (MACS cell separation; Miltenyi Biotec) according to the manufacturer's protocol.

Effect of Activated Leukocytes on HCECs

In five T25 flasks with cultured HCECs, five kinds of human leukocytes (pan-leukocytes, T cells, B cells, neutrophils, and monocytes/macrophages; 4 million each) were placed in the RPMI 1640 medium of each flask, together with 10 ng/mL interleukin 1, 10 ng/mL tumor necrosis factor α, and 1% FBS. The flasks were attached to a magnetic stirrer with cellulose tape set up as shown in Figure 1 and kept in a 5% CO_2 incubator at 37°C. The stirrers were rotated in the flasks to create a current in the culture solution for 3 days, and we examined the cultured endothelium at the bottom of the flasks under an inverted phase-contrast microscope.

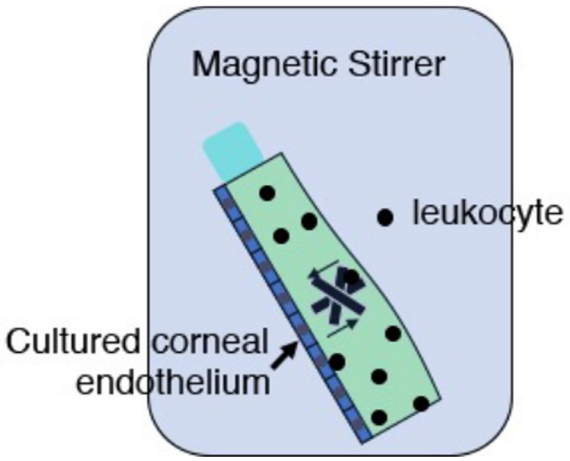

Figure 1. Schematic diagram of magnetic stirrer and T25 flask with cultured human corneal endothelial cells. Magnetic stirrer was set up vertically and a T25 flask with cultured human corneal endothelial cells was attached to magnetic stirrer with cellulose tape diagonally. The stirrer was turned slowly to create a stream of culture medium like warm current in the anterior chamber. Each T25 flask was inoculated with 4 million human leukocytes (total pan-leukocytes, T cells, B cells, neutrophils, or monocytes/macrophages). Magnetic stirrer and T25 flask were set in a humidified incubator at 37°C under 5% CO_2.

Reaction of Monocytes/Macrophages in Iris Treated with Argon/YAG Lasers and Pigmented/Nonpigmented Iris

On the basis of our findings of monocyte/macrophage infiltration, together with previous findings of BK after ALI but much lower rates after sequential argon/YAG, we hypothesized that the iris pigment of the Asian eye burned by the photocoagulation caused by the argon laser is recognized as a foreign body by monocytes/macrophages, leading to monocyte/macrophage infiltration. Therefore, we compared how monocytes/macrophages react to photocoagulation of the iris by argon laser and photo-disruption with YAG laser. The iris obtained at the trabeculectomy was bisected, and one half was pulverized into small particles with a YAG laser (power: 8 mJ; shot number: 400) while the other was photocoagulated with an argon laser (color: monogreen; spot size: 500 µm; duration: 0.5 seconds; power: 400 to 700 mW; shot number: 700). The iris fragments treated with both types of laser were placed separately in a flat-bottomed, 96-well microplate with RPMI 1640 medium containing 10 ng/mL interleukin 1, 10 ng/mL tumor necrosis factor α, and 1% FBS in a 5% CO_2 incubator at 37°C, and then 5×10^5 human monocytes/macrophages were added. After 12 hours, we observed the reaction under a phase-contrast microscope.

To estimate the area of any colonies that had formed, we assumed that the morphology of the colony was elliptical and calculated the area with the following formula:

$$\text{Area of the colony } (\text{mm}^2) = \text{major axis}/2\,(\text{mm}) \times \text{minor axis}/2\,(\text{mm}) \times 3.14.$$

In an additional experiment, we obtained pigmented iris from C3H mice and nonpigmented iris from albino BALB/c mice and treated the irises with an argon laser (color: monogreen; spot size: 500 µm; duration: 0.5 seconds; power: 700 mW; shot number: 800). We then incubated the iris fragments from both types of mice in a 96-well microplate with RPMI 1640 medium containing 10 ng/mL interleukin 1, 10 ng/mL tumor necrosis factor α, and 1% FBS in a 5% CO_2 incubator at 37°C and added 5×10^5 human monocytes/macrophages. After 12 hours, we observed the reaction under a phase-contrast microscope and used the same formula as above to calculate the area of any colonies. We used proinflammatory cytokine because immune reaction was not evident without them in our preliminary study.

Statistical Analysis

Statistical comparisons were performed with the unpaired Mann–Whitney U test. Analyses were performed with StatView software (SAS Institute Japan, Tokyo, Japan), and the level of significance was set at $P < 0.05$.

Results

Examination of the Corneal Endothelium and Immunohistochemistry

We examined the corneal endothelium obtained from corneal buttons in full-thickness corneal transplantations for BK after ALI ($n = 7$) and cataract surgery ($n = 7$). We detected CD68-positive cells on Descemet's membrane and the corneal endothelium in three of the seven eyes with ALI-induced BK and none of the seven eyes with BK after cataract surgery. In a case with prophylactic ALI 8 years previously for treatment of acute angle-closure glaucoma, the central cornea 1 month before corneal transplantation showed focal corneal edema in the center of the cornea with a slit-lamp microscope (Supplementary Fig. S1A). In the corneal button obtained during full-thickness corneal transplantation, a panoramic photograph showed massive cell infiltration on Descemet's membrane removed from the corneal button (Supplementary Fig. S1B), with numerous cells in the central area (Fig. 2A). Cell infiltrations were seen on the left side and residual corneal endothelial cells on the right side (Fig. 2B). Infiltrating cells were clustered in cells that were assumed to be fibrotic corneal endothelium, and the corneal endothelium surrounding the infiltrating cells had disappeared (Fig. 2C). In the histologic examination, hematoxylin staining showed multilayered massive cell infiltration on Descemet's membrane in the central area

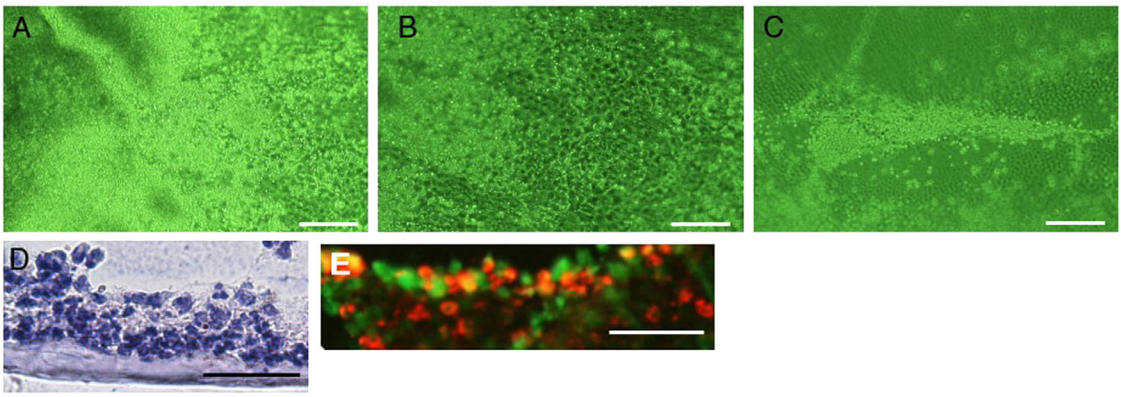

Figure 2. Phase-contrast microscopic and cross-sectional findings of the endothelial surface of the cornea obtained at full-thickness corneal transplantation for bullous keratopathy after laser iridotomy. (A) Numerous cell infiltrates are observed on the paracentral area of the corneal endothelium. (B) In addition to numerous cell infiltrations, remaining endothelial cells are found in the right half of the photo. (C) Infiltrating cells clustering in fibrotic tissue are observed. There is no endothelial cell around the cluster. Reprinted from *Atarashii Ganka* 24: 2007 with permission from Medical-Aoi Publications, Inc. (D) Histologic findings of the corneal endothelial cross section. Nuclear staining with hematoxylin. A large number of cells centered on mononuclear cells are stratified into Descemet's membrane. *Scale bar*: 50 µm. (E) The result of staining by CD68 of the macrophage marker (*red*). Nuclear staining is by SYBR Green (*green*). Many cells attached to the corneal endothelial surface are CD68-positive cells. *Scale bar*: 50 µm.

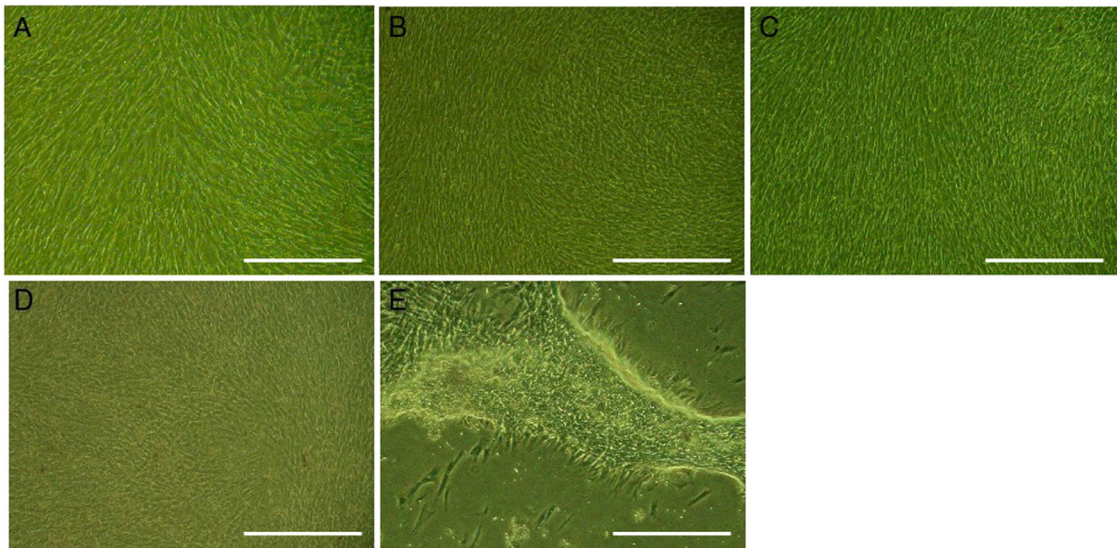

Figure 3. Effect of activated leukocytes on human corneal endothelial cells. Each T25 flask was inoculated with 4 million human leukocytes (total pan-leukocytes, T cells, B cells, neutrophils, or monocytes/macrophages). Three days later, the cultured endothelial cells were examined. Hexagonal shape of cultured corneal endothelial cells is changed to a fibroblastic shape due to the medium containing proinflammatory cytokine, and no apparent changes are detected in pan-leukocytes (A), T cells (B), B cells (C), and neutrophils (D). (E) In the flask containing monocytes/macrophages, cultured human corneal endothelial cells were locally removed and monocytes/macrophages had infiltrated the remaining endothelial cells. This finding was similar to the ex vivo findings on the endothelial surface of post–laser iridotomy bullous keratopathy shown in Fig. 1C. These experiments were repeated twice, with similar results. *Scale bar*: 100 µm.

of the corneal button (Fig. 2D). Almost all infiltrating cells were positive for CD11b (data not shown), CD68 (Fig. 2E), and scavenger receptor marker CD163 (Supplementary Fig. S1C) but almost negative for pan-NK cell marker, CD56, granulocyte marker, and CD66 (data not shown), indicating that these cells were mainly monocytes/macrophages. We detected CD68-positive cells on Descemet's membrane and the corneal endothelium in two of the six eyes (total three of the seven eyes) with ALI-induced BK and none of the seven eyes with BK after cataract surgery.

Effect of Activated Leukocytes on Cultured HCECs

Because immunohistochemistry showed monocyte/macrophage infiltration on the corneal endothelium of the corneal button obtained from the patient, we conducted additional experiments to study the interrelationship between argon laser treatment and monocyte/macrophage infiltration in the pigmented iris of the patient with BK after LI with argon laser in vitro. To investigate reasons for the monocyte/macrophage infiltration, we tested whether human leukocytes can affect HCECs in the inflamed condition. In five T25 flasks with cultured HCECs[12,13] from the six donors, five kinds of human leukocytes (pan-leukocytes, T cells, B cells, neutrophils, and monocytes/macrophages; 4 million each) were placed. The flasks were attached to a magnetic stirrer with cellulose tape set up as shown in Figure 1 and kept in a 5% CO_2 incubator at 37°C. The stirrers were rotated in the flasks to create a current in the culture solution for 3 days. The cultured HCECs, mixed with pan-leukocytes (Fig. 3A), T cells (Fig. 3B), B cells (Fig. 3C), and neutrophils (Fig. 3D), had become fibroblastic cells in the presence of proinflammatory cytokines/FBS in the T25 flasks, but no other changes were observed. In contrast, many of the cultured HCECs had been removed and monocytes/macrophages were clustered on the residual corneal endothelium, as shown in Figure 3E. The ex vivo finding of BK after ALI, as shown in Figure 2C, was well reproduced from the in vitro finding with monocytes/macrophages in Figure 3E.

Reaction of Monocytes/Macrophages in Iris Treated with Argon/YAG Lasers and Pigmented/Nonpigmented Iris

In the fragments from the iris obtained at the trabeculectomy, we saw that monocytes/macrophages had accumulated around the iris and begun to form colonies after 12 hours. Small numbers of

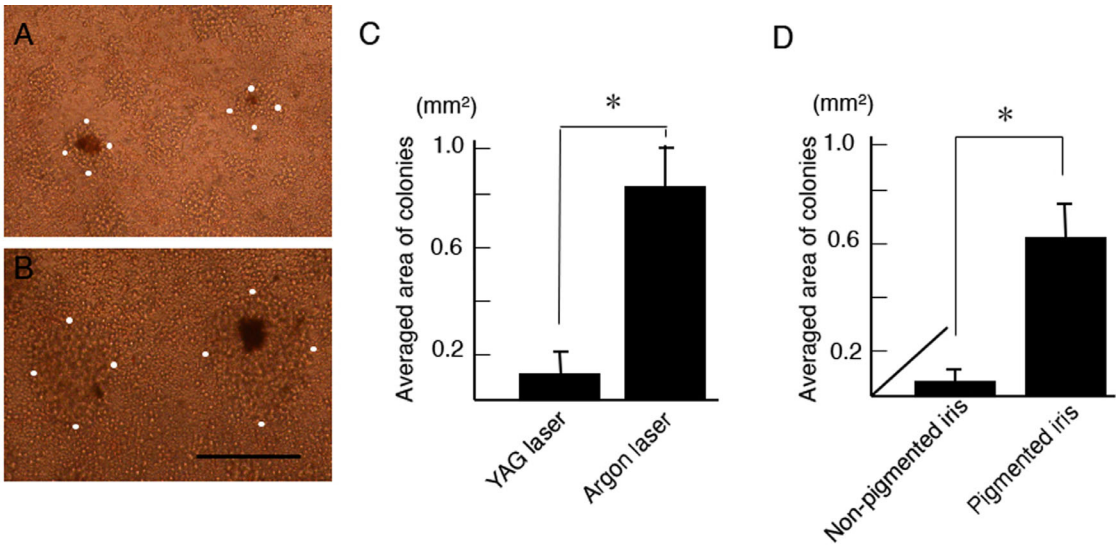

Figure 4. Monocytes/macrophages gathered around iris fragments and began to form colonies. Representative photographs are shown of colonies (A) of human iris sample treated with the YAG laser 24 hours after culturing and colonies (B) of human iris sample treated with the argon laser. The morphology of the colony was assumed to be elliptical, and the area of the colony was calculated on the basis of the *white dots*. The outside of the colony was plotted by the *white dots*. B colonies of iris treated with the argon laser showed a tendency to be larger than those of A colonies. (C) Calculation of the mean area of these colonies showed that the area of the colonies ($n = 12$) in the iris samples treated with the argon laser is significantly larger than the area of the colonies ($n = 11$) of the iris samples treated with the YAG laser. Data are shown as mean ± SD. (D) The mean area of the colonies in the argon laser–treated pigmented iris from the C3H mice ($n = 11$) is significantly larger than that in the argon laser–treated nonpigmented iris from the BALB/c mice ($n = 9$). These experiments were repeated twice and representative results are shown. *Scale bar*: 1 mm. *$P < 0.01$.

monocytes/macrophages were clustered around the iris pulverized with a YAG laser (Fig. 4A), whereas large colonies of monocytes/macrophages formed around the iris treated with the argon laser (Fig. 4B). The mean area of the colonies from the iris photocoagulated with the argon laser was significantly larger than that of those pulverized with the YAG laser (Fig. 4C).

We also observed colonies of monocytes/macrophages in the iris samples from the C3H and BALB/c mice treated with the argon laser. The mean area of the colonies in the pigmented iris from the C3H mice was significantly larger than that in the nonpigmented iris from the BALB/c mice (Fig. 4D).

Discussion

We detected CD68-positive cells on Descemet's membrane and corneal endothelium in three of the seven eyes with ALI-induced BK but none of the seven eyes with BK after cataract surgery and showed a representative case of a corneal endothelial side after ALI. Hematoxylin staining of corneal samples showed multilayered massive cell infiltration on Descemet's membrane in the central area of the corneal button. Subsequent immunohistochemical studies identified these cells as being mainly monocytes/macrophages. In our subsequent in vitro experiments with monocytes/macrophages and cultured HCECs, we found that monocytes/macrophages clustered on the cultured HCECs, but T cells, B cells, neutrophils, and pan-leukocytes did not in the inflamed condition. This in vitro experiment (Fig. 3E) reproduced the ex vivo findings in the corneal endothelium in the cornea from the patient with BK after ALI, as shown in Figure 2C. These findings suggest that only monocytes/macrophages have the potential to attack and remove cultured HCECs in the inflamed condition. Moreover, monocytes/macrophages reacted in human pigmented iris tissues treated by the argon laser but not in those treated by the YAG laser. Our findings indicate that monocytes/macrophages respond strongly after photocoagulation of the iris with an argon laser but not after photo-disruption with a YAG laser. Furthermore, monocytes/macrophages formed large colonies in pigmented iris samples from C3H mice but not in nonpigmented samples from albino BALB/c mice after photocoagulation with the argon laser. We suggest that treatment of pigmented iris with an argon laser triggers monocyte/macrophage infiltration in the anterior chamber of the eye in the inflamed condition.

These findings are consistent with the clinical characteristics that BK after ALI often occurs in argon laser–treated Japanese patients, who have a pigmented iris, but it does not occur in Singaporean patients, who also have a pigmented iris but are treated with the YAG laser, as well as in English patients, who have low pigmentation of the iris. Our results suggest that monocytes/macrophages, argon laser, and pigmented iris are all involved in the pathogenesis of BK after ALI in Asian patients.

The exact mechanisms that attract monocytes/macrophages into the anterior chamber are still unknown. Cultured HCECs can abundantly express a chemoattractant of monocytes/macrophages, monocyte chemoattractant protein 1 (MCP-1, CCL2).[14] CCL2 is detected in the anterior chamber of the human eye[15–19] and is regulated by transforming growth factor $\beta 2$ produced in the anterior chamber of the eye.[20] Human monocyte-derived macrophages can induce recruitment of monocytes from a peripheral blood mononuclear cell pool in a transwell migration model in vitro.[15] On the other hand, damage to the iris induces a higher amount of cytokines/chemokines, including CCL2 and macrophage inflammatory protein 1α (MIP-1α, CCL3).[21] Fluorescein leakage in the iris is seen at the site of ALI after prophylactic treatment and in a wide area of the iris in the eyes of people with a history of acute angle-closure glaucoma,[22] indicative of breakdown of the blood–aqueous barrier. These findings suggest that damage to the iris, whether by ALI or acute angle-closure glaucoma, induces a high amount of monocyte/macrophage chemoattractants and increases vascular permeability, contributing to enhancement of cell infiltration in the anterior chamber of the eye.

The findings that corneal endothelial cell density decreases faster in BK eyes treated with ALI than in BK eyes not treated with ALI[23] may be explained by the high amount of monocyte/macrophage chemoattractants and high vascular permeability of the anterior chamber after acute angle-closure glaucoma and ALI. Whatever the color of the normal iris, iris color did not affect the outcome of endothelial keratoplasty.[24] However, widespread iris damages due to severe and repeated surgical trauma and acute angle-closure glaucoma increase the protein level, cytokine receptor expression, and complement activities in the aqueous humor, leading to corneal endothelial cell loss.[25] This dynamic change of aqueous humor also can accelerate infiltration of immune cells (i.e., monocytes/macrophages). Actually, we have observed massive focal cell infiltration in the center of the corneal endothelium after ALI, and topical application of corticosteroid eye drops well suppressed cell infiltration to the corneal endothelium (unpublished observation). From a therapeutic point of view, corticosteroid eye drops should be a treatment option to suppress the anterior chamber inflammation in the eyes after ALI.

The question remains why monocytes/macrophages produced by a person's bone marrow damage their own tissues. Lung macrophages in the airways and from blood monocytes are essential immune effector cells in both innate and adaptive immune responses to foreign matter.[26] Lung macrophages have a pivotal role in the progression of the chronic inflammatory process of chronic obstructive pulmonary disease.[27] For example, macrophages accumulate in the lungs and contribute to cigarette smoke–induced emphysema, and matrix metalloproteinase-12 (MMP-12)–producing interstitial macrophages have resulted in the destruction of alveolar walls and led to the development of emphysema.[28] In the eye, high expression levels of cytokines/chemokines involving CCL2 in the aqueous humor are associated with a decrease in corneal endothelial cells after penetrating keratoplasty and Descemet's stripping automated endothelial keratoplasty,[16,18,19] suggesting involvement of monocytes/macrophages in the inflammation in the anterior chamber. Therefore, we hypothesize that if monocytes/macrophages recognize photocoagulated iris pigment in Asian eyes as a burned foreign body, activated monocytes/macrophages may accelerate inflammation and harm the tissues in the anterior chamber.

It is interesting that CD68-positive cells on Descemet's membrane and the corneal endothelium were detected in three of the seven eyes with ALI-induced BK and none of the seven eyes with BK after cataract surgery. Moreover, corneal endothelial cells on Descemet's membrane in BK after cataract surgery had many small gaps between cells, as shown in Supplementary Figure S2A, whereas the corneal endothelium on Descemet's membrane after ALI showed a large area of endothelial cell loss, and many endothelial cells remained in the island shape (Supplementary Fig. S2B). It makes sense to think that this widely exposed Descemet's membrane is explained by the state after ex vivo findings of BK after ALI in Figure 2C and in vivo findings of activated monocyte/macrophage attack in Figure 3E. These findings may also support the hypothesis that monocytes/macrophages strip corneal endothelial cells from Descemet's membrane.

In summary, we conducted in vitro experiments on the basis of clinical findings and the monocyte/macrophage infiltration detected on the endothelial side of BK after ALI. Monocytes/macrophages damaged a wide area of HCECs in the medium stimulated with proinflammatory cytokines,

and endothelial cells of BK after ALI remained in the island shape. Monocytes/macrophages reacted more strongly to human pigmented iris treated by an argon laser than to that treated by a YAG laser and to photocoagulated pigmented C3H mouse iris than to photocoagulated nonpigmented albino BALB/c iris. These findings are consistent with the clinical characteristics of BK after ALI and suggest that monocytes/macrophages, argon laser, and pigmented iris are all involved in the pathogenesis of BK after ALI in Asian patients.

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