Cytokine Levels in the Aqueous Humor Are Associated With Corneal Thickness in Eyes With Bullous Keratopathy

NATSUME SUZUKI, TAKEFUMI YAMAGUCHI, SHINSUKE SHIBATA, TOSHIHIRO NAGAI, HISASHI NOMA, KAZUO TSUBOTA, AND JUN SHIMAZAKI

• PURPOSE: We sought to investigate the association between the severity of bullous keratopathy and proinflammatory cytokine levels in the aqueous humor (AqH).
• DESIGN: Cross-sectional study.
• METHODS: This study included a total of 95 eyes: 62 with bullous keratopathy and 33 that underwent cataract surgery. Central corneal thickness (CCT) and central corneal volume within 4 and 6 mm (CCV_{4mm} and CCV_{6mm}, respectively) were determined using anterior segment optical coherence tomography. A total of 95 AqH samples were collected at the beginning of surgery. The levels of cytokines (interleukins [ILs]-1α, -1β, -4, -6, -8, -10, -12p70, -13, -17A, interferon [IFN]-α, IFN-γ, monocyte chemotactic protein [MCP]-1, E-selectin, P-selectin, and soluble intercellular adhesion molecule-1 [sICAM-1]) in the AqH were measured using multiplex beads immunoassay. We evaluated the correlation among AqH cytokine levels, CCT, CCV_{4mm}, and CCV_{6mm} in eyes with bullous keratopathy.
• RESULTS: The levels of protein, ILs-4, -6, -8, -10, -12p70, and -17A, MCP-1, IFN-γ, E-selectin, P-selectin, and sICAM-1 were significantly higher in eyes with bullous keratopathy compared with those of the normal control subjects (all $P < .0025$). CCT was significantly correlated with the levels of IL-13 ($r = 0.551$, $P = .0009$) and sICAM-1 ($r = 0.448$, $P = .0005$). CCV_{4mm} was significantly correlated with the levels of IL-13 ($r = 0.514$, $P = .0022$) and sICAM-1 ($r = 0.404$, $P = .0019$). CCV_{6mm} was significantly correlated with the level of sICAM-1 ($r = 0.459$, $P = .0003$).
• CONCLUSION: The severity of corneal edema in eyes with bullous keratopathy was associated with the levels of specific cytokines in the AqH. (Am J Ophthalmol 2019;198:174–180. © 2018 Elsevier Inc. All rights reserved.)

AJO.com Supplemental Material available at AJO.com.

Accepted for publication Oct 3, 2018.

From the Department of Ophthalmology (N.S., T.Y., K.T., J.S.), Tokyo Dental College, Ichikawa General Hospital, Chiba, Japan; Department of Ophthalmology (N.S., T.Y., K.T., J.S.), Keio University School of Medicine, Tokyo, Japan; Electron Microscope Laboratory (S.S., T.N.), Keio University School of Medicine, Tokyo, Japan; and the Department of Data Science (H.N.), The Institute of Statistical Mathematics, Tokyo, Japan.

Inquiries to Takefumi Yamaguchi, Department of Ophthalmology, Ichikawa General Hospital, Tokyo Dental College, 5-11-13, Sugano, Ichikawa, Chiba, 272-8513, Japan; e-mail: yama19770614@hotmail.com

ENDOTHELIAL CELL DENSITY (ECD) DECREASES WITH age and in various ocular conditions.[1-3] The reduction in ECD is exacerbated over time after intraocular surgery,[4-6] and is a serious complication because it can lead to bullous keratopathy and loss of vision.[7-9] It has been established that ECD ≤500 cells per mm^2 results in corneal edema. The extent of endothelial dysfunction is believed to determine the severity of corneal edema. However, the exact reasons behind the intraindividual difference in corneal edema remain poorly understood.

The corneal endothelium has a barrier function that prevents the aqueous humor (AqH) from entering the corneal stroma and maintains corneal clarity.[10] Histopathologic studies reported that corneal endothelial cells are degenerated, reduced, or absent with an acellular area of collagenous fibrils and bare Descemet membrane in eyes with bullous keratopathy.[11]

Recently, we reported an elevation in inflammatory cytokine levels in the AqH of eyes with bullous keratopathy,[12-15] which, in turn, caused a rapid loss of ECD after penetrating keratoplasty (PKP) and Descemet stripping automated endothelial keratoplasty (DSAEK).[16,17] Anatomically, AqH with elevated proinflammatory cytokine levels faces the corneal endothelium. Chronic higher levels of proinflammatory cytokines in the AqH can lead to the breakdown of tight junctions in corneal endothelium.[18,19] In addition, they can also induce inflammation in the corneal stroma and recruit immune cells. We hypothesized that elevated levels of inflammatory cytokines in the AqH are associated with severe corneal edema in eyes with bullous keratopathy. In the current study, we measured central corneal thickness (CCT), and quantified the central corneal volume (CCV) within 4 to 6 mm in diameter (CCV_{4mm} and CCV_{6mm}, respectively) in eyes with bullous keratopathy, using anterior segment optical coherence tomography (AS-OCT). We then evaluated correlations among CCT/CCV and AqH cytokine levels in eyes with bullous keratopathy.

0002-9394/$36.00
https://doi.org/10.1016/j.ajo.2018.10.008

METHODS

THIS PROSPECTIVE STUDY INCLUDED CONSECUTIVE patients with pseudophakic bullous keratopathy (PBK) or healthy control subjects who underwent corneal transplantation or cataract surgery at Tokyo Dental College (Chiba, Japan) between October 2015 and March 2018. We defined PBK as eyes with epithelial microcystic changes or bullae caused by decreased ECD after complicated cataract or intraocular surgeries. We excluded eyes with ocular surface diseases,[20] post-PKP, and active inflammation of the cornea, or anterior chamber. Control subjects were defined as patients who underwent cataract surgery without uveitis, diabetes mellitus, or inflammatory systemic diseases, such as ulcerative colitis or rheumatoid arthritis, and who had not undergone corneal or intraocular surgeries previously. All participants in the control group had an ECD >2000 cells/mm^2. The study was performed in accordance with the Declaration of Helsinki and approved by the institutional ethics review board of Tokyo Dental College (I-15-42R). Written informed consent was obtained from all participants.

• **AQH SAMPLES:** AqH samples were 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 sample was obtained using a 27-gauge needle taking care not to touch the iris, lens, or corneal endothelium. The samples were centrifuged at 3000 *g* for 5 minutes. The soluble fractions were collected and stored in silicon-coated Eppendorf tubes at −80°C until cytokine level measurement.

• **ASSESSMENT OF CCT AND CCV:** CCT was determined using AS-OCT (SS-1000, CASIA; Tomey, Nagoya, Japan). CCV was calculated using software installed on the AS-OCT (version 4.8.6). Briefly, 16 rotating AS-OCT scans were used to reconstruct 3-dimensional models of the entire corneal structure. The CASIA system corrected distortions in the AS-OCT images based on the refractive index of the anterior surface. One corneal specialist (N.S.) carefully verified all AS-OCT images to ensure that the surface digitalization recognized by the automated inbuilt software was correct. CCV was calculated using the following formula, where $CT_{i,j}$: corneal thickness at $P_{i,j}$, θ_i: angle ($0 \le i \le 16$), r_j: radius ($0 \le j \le 4$ or 6 mm):

$$CCV_{4\text{ or }6mm} = \sum_{j=0}^{4\ or\ 6\ mm} \sum_{i=1}^{16} \left(\frac{CT_{i,j}\theta_i r_j}{\pi} \right)$$

• **PROTEIN LEVEL MEASUREMENTS:** The protein levels of AqH samples were determined using the DC protein assay (Bio-Rad, Hercules, CA, USA). The reactions were based on the Lowry assay and were measured according to the manufacturer's instructions. In brief, bovine serum albumin was used as a standard in the range of 0.23 to 1.37 mg/mL. Samples (5 μL) of bovine serum albumin and AqH were added to 96-well microplates, followed by immediate addition of a mixture containing 25 μL reagent A and 200 μL reagent C. After 15 minutes of incubation at room temperature in the dark, the microplates were read at 690 and 405 nm using a microplate reader (Model 550; Bio-Rad). Protein levels were calculated by the subtraction method using the microplate manager system (BioRad).

• **CYTOKINE LEVEL MEASUREMENTS:** The cytokine levels of ILs-1α, -1β, -4, -6, -8, -10, -12p70, -13, and -17A, IFN-α, IFN-γ, MCP-1, tumor necrosis factor-α (TNF-α), E-selectin, P-selectin, soluble intercellular adhesion molecule-1 (sICAM-1), macrophage inflammatory proteins-1α and -1β, and IFN-γ–induced protein (IP)-10 in AqH samples were measured using Luminex (ProcaPlex kit; Luminex, San Antonio, TX, USA) beads-based multiplex immunoassay according to previous reports.[13,21] Briefly, 50 μL of AqH samples were incubated with antibody-coated capture beads in an incubation buffer at room temperature. After 2 hours of incubation, the beads were washed 3 times using washing buffer and phycoerythrin-labeled streptoavidin was added for 30 minutes in the dark at room temperature. After being washed 3 times with washing buffer, plates were resuspended in 150 μL of reading buffer and the assays were performed using a Luminex 200.

• **STATISTICAL ANALYSIS:** Data were analyzed using Prism for Windows software (version 6.04; Graphpad Software, Inc., La Jolla, CA, USA). The D'Agostino and Pearson omnibus normality test was used to assess whether the data showed a normal distribution. The Spearman correlation analyses were used to evaluate the correlations among protein and cytokine levels, CCT, CCV_{4mm}, and CCV_{6mm}. Mann-Whitney *U* tests were used to evaluate the difference in AqH protein and cytokine levels between the groups (control subjects and the bullous keratopathy group). Data are expressed as mean ± standard deviation (SD) or standard error (SE). $P < .05$ was considered statistically significant except for cytokine data, for which a Bonferroni correction was applied. There were 20 different comparisons (1 protein and 19 cytokines), and $P < .0025$ (ie, $P = .05/20$) was considered statistically significant after Bonferroni correction.

RESULTS

• **STUDY PARTICIPANTS:** Ninety-five consecutive patients with PBK (62 eyes) and cataract surgery (control group [n = 33 eyes]) were included in the study (Table 1). Twenty patients with bullous keratopathy were excluded from the study because of post-DSAEK

TABLE 1. Demographics of Patients

	Pseudophakic Bullous Keratopathy	Control	*P* Value
Eyes, n	62	33	
Sex, n (%)			
Male	25 (40.3)	16 (48.5)	.516[a]
Female	37 (59.7)	17 (51.5)	
Age, y (mean ± SD)	73.8 ± 10.5	75.9 ± 7.6	.438[b]
BSCVA, logMAR (mean ± SD)	1.50 ± 0.71	0.38 ± 0.36	<.001
IOP, mm Hg (mean ± SD)	12.3 ± 4.8	14.3 ± 2.6	.065[b]
Axial length, mm (mean ± SD)	23.92 ± 2.26	23.84 ± 1.58	.774[b]
ECD, cells/mm^2 (mean ± SD)	NA	2760 ± 343	
CCT, μm (mean ± SD)	763 ± 104	534 ± 23	<.001[b]
Previous intraocular surgeries, n ± SD	1.9 ± 1.4	0.0	<.001[b]

BSCVA = best spectacle-corrected visual acuity; CCT = central corneal thickness; DM = diabetes mellitus; ECD = endothelial cell; IOP = intraocular pressure; logMAR = logarithm of minimal angle resolution; NA = not available; SD = standard deviation.

[a]χ^2 test.

[b]Mann-Whitney *U* test.

failed grafts (15 eyes), failed graft after anterior lamellar keratoplasty, corneal scarring caused by previous corneal infection, ischemic optic neuropathy,[22] microphthalmia, and conjunctival epithelialization and limbal stem cell deficiency caused by long-standing bullous keratopathy (1 eye).

• **CCT AND CCV IN EYES WITH PSEUDOPHAKIC BULLOUS KERATOPATHY:** In eyes with PBK, CCT was 763 ± 104 μm (range 591-994 μm). The CCV_{4mm} was 9.66 ± 1.96 mm^3 (range 6.53-15.02 mm^3). The CCV_{6mm} was 23.00 ± 3.72 mm^3 (range 17.12-33.12 mm^3). CCT was significantly correlated with CCV_{4mm} and CCV_{6mm} (r = 0.736, $P < .0001$ and r = 0.741, $P < .0001$, respectively).

• **PROTEIN AND CYTOKINE LEVELS IN AQH:** Levels of protein, ILs-4, -6, -8, -10, -12p70, and -17A, MCP-1, IFN-γ, E-selectin, P-selectin, and sICAM-1 were significantly higher in eyes with PBK compared with the control group ($P < .0001$ for protein, ILs-4, -6, -12p70, and -17A, MCP-1, IFN-γ, E-selectin, P- selectin, and sICAM-1; $P = .0006$ for IL-8; $P = .0015$ for IL-10) (Table 2).

• **CORRELATION ANALYSES IN EYES WITH PSEUDOPHAKIC BULLOUS KERATOPATHY:** In eyes with PBK (Table 3, Figure 1), CCT was significantly correlated with levels of IL-13 (r = 0.551 [95% confidence interval {CI} 0.246-0.757], $P = .0009$) and sICAM-1 (r = 0.448 [95% CI 0.205-0.639], $P = .0005$). CCV_{4mm} was significantly correlated with levels of IL-13 (r = 0.514 [95% CI 0.197-0.734], $P = .0022$) and sICAM-1 (r = 0.404 [95% CI 0.152-0.606], $P = .0019$). CCV_{6mm} was significantly correlated with the level of sICAM-1 (r = 0.459 [95% CI 0.218-0.647], $P = .0003$).

DISCUSSION

BULLOUS KERATOPATHY IS ONE OF THE LEADING CAUSES OF visual loss among corneal diseases. Corneal edema is widely believed to be caused by loss of pump function or barrier function in the corneal endothelium. Regarding corneal endothelial barrier function, TNF-α and matrix metalloproteinase-9 lead to barrier dysfunction via p38 mitogen-activated protein kinase.[18,19] However, in the current study, severity of corneal edema was correlated with the AqH levels of protein and specific cytokines (IL-13 and sICAM-1) and not with the AqH level of TNF-α. Even in eyes with PBK, the AqH level of TNF-α was 200 pg/mL, which is 1/100th the level (20 ng/mL) of that used in previous in vitro studies.[18] Therefore, we postulate that assessment of cytokine levels in actual human samples is important to validate their pathological effects.

We recently reported that preoperative levels of IL-17A, MCP-1, IFN-γ, and sICAM-1 were associated with rapid reduction of ECD after DSAEK.[16] The results in the current study suggest that cytokines that are involved in exacerbating corneal edema (IL-13 and sICAM-1) can be different from those associated with corneal endothelial cell loss (IL-17A, MCP-1, IFN-γ, and sICAM-1); IL-13 may be a specific cytokine related to the severity of corneal edema. IL-13, a central T helper 2 cytokine, is a potent activator of inflammatory responses and fibrosis. In the cornea, exogenous IL-13 recruits immune cells by inducing

TABLE 2. Aqueous Protein/Cytokine Levels in Eyes With Pseudophakic Bullous Keratopathy

	Pseudophakic Bullous Keratopathy, Mean ± SE (Median)	Control, Mean ± SE (Median)	*P* Value[a]
Protein[b]	1.38 ± 0.16 (1.14)	0.31 ± 0.40 (0.25)	<.0001
IL-1α	70.3 ± 15.7 (44.5)	49.3 ± 4.4 (44.2)	.638
IL-1β	3.8 ± 1.1 (1.19)	3.1 ± 1.7 (1.1)	.497
IL-4	60.2 ± 11.0 (30.8)	20.5 ± 1.0 (20.8)	<.0001
IL-6	2127 ± 495 (864)	85 ± 69 (5.1)	<.0001
IL-8	86.0 ± 15.9 (39.8)	46.4 ± 25.3 (21.4)	.0006
IL-10	6.0 ± 2.1 (3.06)	1.9 ± 0.1 (1.7)	.0015
IL-12p70	14.0 ± 2.0 (8.19)	6.4 ± 0.2 (6.4)	<.0001
IL-13	9.0 ± 0.8 (9.18)	7.1 ± 0.2 (7.1)	.055
IL-17A	17.0 ± 2.6 (9.33)	4.0 ± 0.4 (3.5)	<.0001
MIP-1α	21.9 ± 10.0 (9.8)	9.4 ± 0.4 (8.7)	.538
MIP-1β	236 ± 37.4 (77.5)	345 ± 20.3 (321)	<.0001
MCP-1	982 ± 122 (847)	592 ± 84.4 (470)	<.0001
TNF-α	210 ± 119 (76.7)	76.4 ± 5.4 (70.2)	.610
IFN-α	3.9 ± 0.6 (3.7)	4.1 ± 0.1 (3.9)	.203
IFN -γ	179 ± 30.6 (74.9)	55.6 ± 2.1 (54.6)	<.0001
E-selectin	4621 ± 648 (3189)	2149 ± 46.0 (2145)	<.0001
P-selectin	10195 ± 2453 (5891)	3724 ± 131 (3581)	<.0001
sICAM-1	7050 ± 807 (4641)	2027 ± 450 (1304)	<.0001
IP10	195 ± 24.7 (123)	257 ± 89.4 (98.3)	.515

IFN = interferon; IL = interleukin; IP10 = interferon-gamma–induced protein 10; MCP = monocyte chemotactic protein; MIP = macrophage inflammatory protein; SE = standard error; sICAM = soluble intracellular adhesion molecule; TNF = tumor necrosis factor.

[a]Mann-Whitney *U* test.

[b]Proteins measured in mg/mL; cytokines measured in pg/mL.

ICAM-1 expression.[23] IL-13 shares a functional receptor with IL-4,[24] and exposure to IL-4 and IL-13 increases epithelial permeability by decreasing the expression of intercellular junction protein.[25,26] In addition, dupilumab, a monoclonal antibody that blocks IL-4 and IL-13, has been shown to improve atopic dermatitis.[27] Although we evaluated the levels of only 19 cytokines, we believe that specific pathways must be involved in the pathological processes of severe bullous keratopathy. More comprehensive studies, such as proteomic analysis of the inflamed AqH, will specify implicated pathways in the future.

This study included only classic PBK to avoid bias caused by heterogeneous etiologies. During the study period, there were a total of 160 eyes with bullous keratopathy caused by laser iridotomy (35 eyes), endothelial decompensation after PKP (25 eyes), Fuchs endothelial corneal dystrophy (FECD; 22 eyes), birth injury (6 eyes), endotheliitis (5 eyes), aphakic bullous keratopathy (ABK; 3 eyes), and others (1 irido-corneal-endothelial syndrome, and 1 Axenfeld–Rieger syndrome). To validate the results, we assessed the correlation between CCT/CCV and aqueous cytokine levels in all subjects (Supplementary Table 1; Supplemental Material available at AJO.com), in eyes with endothelial decompensation after PKP (Supplementary Table 2; Supplemental Material available at AJO.com), in eyes after laser iridotomy (Supplementary Table 3; Supplemental Material available at AJO.com), and in eyes with FECD (Supplementary Table 4; Supplemental Material available at AJO.com). Correlation analysis in all subjects and in the post-PKP group showed similar trends, whereas we could not find consistent trends in eyes after laser iridotomy and in those with FECD. Indeed, this can be attributed to the small number of subjects in the subgroups and to different cytokine elevation patterns observed in the groups.[13] However, careful interpretation is needed, because the heterogenous etiologies can introduce bias.

The present study, however, had a few limitations. First, the extent of corneal edema is sometimes dependent on the timing of AS-OCT measurement: more specifically, in eyes with early-stage bullous keratopathy, CCT increases in the morning and decreases in the evening. In the current study, AS-OCT was performed in the morning in most of the cases. We believe that the influence of the timing of AS-OCT measurement was minimal. Second, the results of the current study merely showed an association between corneal edema and cytokine levels in the AqH. However, an imaging analysis of corneal endothelium using an electron microscope showed exposure of collagen fibers in a hole-shaped pattern with degeneration of the Descemet membrane in bullous keratopathy (Supplementary Figure 1; Supplemental

TABLE 3. Correlations Among Protein/Cytokine Levels, Central Corneal Thickness and Corneal Volume in Eyes With Pseudophakic Bullous Keratopathy

	Central Corneal Thickness[a]			Central Corneal Volume (4 mm)[a]			Central Corneal Volume (6 mm)[a]		
	r	95% CI	*P* Value	r	95% CI	*P* Value	r	95% CI	*P* Value
Protein	0.176	-0.110 to 0.435	.211	0.170	-0.116 to 0.430	.229	0.220	-0.065 to 0.471	.117
IL-1α	0.286	0.001 to 0.529	.044	0.144	-0.149 to 0.413	.320	0.144	-0.149 to 0.413	.319
IL-1β	0.059	-0.285 to 0.388	.734	0.160	-0.188 to 0.472	.352	0.127	-0.220 to 0.445	.460
IL-4	0.315	0.040 to 0.545	.022	0.142	-0.141 to 0.404	.311	0.227	-0.054 to 0.475	.102
IL-6	0.230	-0.038 to 0.467	.083	0.087	-0.183 to 0.345	.516	0.102	-0.168 to 0.358	.447
IL-8	0.206	-0.065 to 0.449	.124	0.130	-0.143 to 0.384	.336	0.104	-0.168 to 0.362	.440
IL-10	0.167	-0.119 to 0.428	.237	0.008	-0.273 to 0.288	.953	0.018	-0.305 to 0.306	.900
IL-12p70	0.013	-0.293 to 0.317	.932	-0.026	-0.329 to 0.281	.865	0.001	-0.305 to 0.306	.997
IL-13	0.551	0.246 to 0.757	.0009	0.514	0.197 to 0.734	.0022	0.365	0.0142 to 0.636	.0368
IL-17A	0.257	-0.060 to 0.527	.101	0.122	-0.197 to 0.419	.440	0.195	-0.125 to 0.478	.217
MIP-1α	-0.093	-0.417 to 0.253	.591	-0.116	-0.437 to 0.230	.500	-0.195	-0.500 to 0.405	.254
MIP-1β	0.212	-0.062 to 0.456	.117	0.120	-0.155 to 0.378	.377	0.151	-0.124 to 0.405	.266
MCP-1	-0.003	-0.269 to 0.262	.979	-0.128	-0.381 to 0.142	.337	-0.092	-0.349 to 0.178	.493
TNF-α	0.100	-0.208 to 0.390	.514	-0.100	-0.389 to 0.209	.516	-0.017	-0.156 to 0.407	.559
IFN-α	0.102	-0.249 to 0.429	.560	-0.051	-0.386 to 0.504	.772	-0.017	-0.357 to 0.327	.924
IFN -γ	0.117	-0.175 to 0.390	.417	0.084	-0.207 to 0.361	.563	0.137	-0.156 to 0.407	.324
E-selectin	0.075	-0.207 to 0.346	.594	0.140	-0.144 to 0.402	.318	0.164	-0.119 to 0.423	.239
P-selectin	0.073	-0.196 to 0.332	.584	0.018	-0.249 to 0.282	.896	0.042	-0.226 to 0.304	.756
sICAM-1	0.448	0.205 to 0.639	.0005	0.404	0.152 to 0.606	.0019	0.459	0.218 to 0.647	.0003
IP10	-0.002	-0.267 to 0.264	.989	-0.044	-0.306 to 0.225	.746	-0.049	-0.311 to 0.219	.714

CI = confidence interval; IL = interleukin; MIP = macrophage inflammatory protein; MCP = monocyte chemotactic protein; TNF = tumor necrosis factor; IFN = interferon; sICAM = soluble intracellular adhesion molecule; IP10 = interferon-gamma–induced protein 10.

[a]Spearman correlation analysis. $P<0.0025$ was considered to be statistically significant after Bonferroni correction.

Material available at AJO.com), suggesting a complete loss of its barrier function. In such conditions, AqH with high levels of proinflammatory cytokines is expected to undergo influx into the corneal stroma because of barrier disruption of the endothelium in bullous keratopathy. Third, some of the patients included in the present study used topical steroids at the time of AqH collection, which may have decreased cytokine levels by suppressing inflammation and reduced corneal edema by preventing endothelial permeability.[28] Finally, a question arises as to why no corneal neovessels develop in bullous keratopathy despite such high levels of proinflammatory cytokines. Previous studies have shown that neovascularization is inhibited by the presence of the corneal nerves[29] and soluble anti–vascular endothelial growth factor proteins.[30] Future comprehesive study will be necessary to address this question.

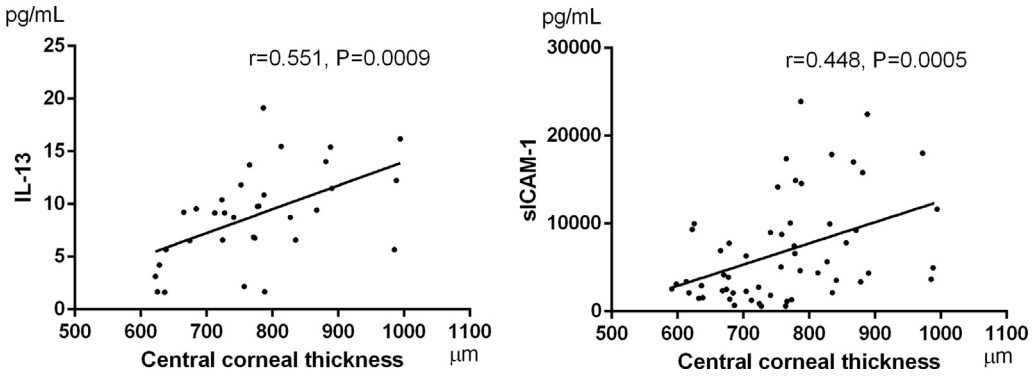

FIGURE 1. Correlation between central corneal thickness (CCT) and cytokine levels in the aqueous humor in eyes with bullous keratopathy. CCT was significantly correlated with the levels of interleukin-13 (left, r = 0.551, *P* = .0009), and sICAM-1 (right, r = 0.448, *P* = .0005).

In conclusion, we revealed that CCT and CCV were correlated with AqH levels of protein and specific cytokines (IL-13 and sICAM-1) in eyes with PBK, suggesting that proinflammatory cytokines in the AqH can be associated with severe corneal edema in eyes with PBK.

FUNDING/SUPPORT: THIS STUDY WAS SUPPORTED BY A GRANT-IN-AID FOR SCIENTIFIC RESEARCH (15K10906) FROM THE MINistry of Education, Culture, Sports, Science and Technology and by a Grant for Brain/MINDS by AMED (JP17dm0207002) (Shinsuke Shibata). The following authors have no financial disclosures: Natsume Suzuki, Toshihiro Nagai, Hisashi Noma, Kazuo Tsubota, Jun Shimazaki. All authors attest that they meet the current ICMJE criteria for authorship.

Other Acknowledgments: We thank Editage for English language editing.

REFERENCES

1. Bourne WM, Nelson LR, Hodge DO. Central corneal endothelial cell changes over a ten-year period. *Invest Ophthalmol Vis Sci* 1997;38(3):779–782.
2. Pillai CT, Dua HS, Azuara-Blanco A, Sarhan AR. Evaluation of corneal endothelium and keratic precipitates by specular microscopy in anterior uveitis. *Br J Ophthalmol* 2000;84(12):1367–1371.
3. Alfawaz AM, Holland GN, Yu F, Margolis MS, Giaconi JA, Aldave AJ. Corneal endothelium in patients with anterior uveitis. *Ophthalmology* 2016;123(8):1637–1645.
4. Ambrose VM, Walters RF, Batterbury M, Spalton DJ, McGill JI. Long-term endothelial cell loss and breakdown of the blood-aqueous barrier in cataract surgery. *J Cataract Refract Surg* 1991;17(5):622–627.
5. Numa A, Nakamura J, Takashima M, Kani K. Long-term corneal endothelial changes after intraocular lens implantation. Anterior vs posterior chamber lenses. *Jpn J Ophthalmol* 1993;37(1):78–87.
6. Bourne WM, Nelson LR, Hodge DO. Continued endothelial cell loss ten years after lens implantation. *Ophthalmology* 1994;101(6):1014–1022.
7. Patel SV, Hodge DO, Bourne WM. Corneal endothelium and postoperative outcomes 15 years after penetrating keratoplasty. *Am J Ophthalmol* 2005;139(2):311–319.
8. Cornea Donor Study Investigator Group, Lass JH, Gal RL, et al. Donor age and corneal endothelial cell loss 5 years after successful corneal transplantation. Specular microscopy ancillary study results. *Ophthalmology* 2008;115(4):627–632.e8.
9. Ing JJ, Ing HH, Nelson LR, Hodge DO, Bourne WM. Ten-year postoperative results of penetrating keratoplasty. *Ophthalmology* 1998;105(10):1855–1865.
10. Edelhauser HF. The balance between corneal transparency and edema: the Proctor Lecture. *Invest Ophthalmol Vis Sci* 2006;47(5):1754–1767.
11. Kenyon KR, Van Horn DL, Edelhauser HF. Endothelial degeneration and posterior collagenous proliferation in aphakic bullous keratopathy. *Am J Ophthalmol* 1978;85(3):329–336.
12. Ishii N, Yamaguchi T, Yazu H, Satake Y, Yoshida A, Shimazaki J. Factors associated with graft survival and endothelial cell density after Descemet's stripping automated endothelial keratoplasty. *Sci Rep* 2016;6(1):25276.
13. Yamaguchi T, Higa K, Suzuki T, et al. Elevated cytokine levels in the aqueous humor of eyes with bullous keratopathy and low endothelial cell density. *Invest Ophthalmol Vis Sci* 2016;57(12):5954–5962.
14. Yagi-Yaguchi Y, Yamaguchi T, Higa K, et al. Association between corneal endothelial cell densities and elevated cytokine levels in the aqueous humor. *Sci Rep* 2017;7(1):13603.
15. Aketa N, Yamaguchi T, Suzuki T, et al. Iris damage is associated with elevated cytokine levels in aqueous humor. *Invest Ophthalmol Vis Sci* 2017;58(6):BIO42–BIO51.
16. Yazu H, Yamaguchi T, Aketa N, et al. preoperative aqueous cytokine levels are associated with endothelial cell loss after Descemet's stripping automated endothelial keratoplasty. *Invest Ophthalmol Vis Sci* 2018;59(2):612–620.
17. Yagi-Yaguchi Y, Yamaguchi T, Higa K, et al. Preoperative aqueous cytokine levels are associated with a rapid reduction in endothelial cells after penetrating keratoplasty. *Am J Ophthalmol* 2017;181(1):166–173.
18. Shivanna M, Rajashekhar G, Srinivas SP. Barrier dysfunction of the corneal endothelium in response to TNF-alpha: role of p38 MAP kinase. *Invest Ophthalmol Vis Sci* 2010;51(3):1575–1582.
19. Rajashekhar G, Shivanna M, Kompella UB, Wang Y, Srinivas SP. Role of MMP-9 in the breakdown of barrier integrity of the corneal endothelium in response to TNF-alpha. *Exp Eye Res* 2014;122(1):77–85.
20. Aketa N, Yamaguchi T, Asato T, et al. Elevated aqueous cytokine levels in eyes with ocular surface diseases. *Am J Ophthalmol* 2017;184(1):42–51.
21. Yamaguchi T, Calvacanti BM, Cruzat A, et al. Correlation between human tear cytokine levels and cellular corneal changes in patients with bacterial keratitis by in vivo confocal microscopy. *Invest Ophthalmol Vis Sci* 2014;55(11):7457–7466.
22. Micieli JA, Lam C, Najem K, Margolin EA. Aqueous humor cytokines in patients with acute nonarteritic anterior ischemic optic neuropathy. *Am J Ophthalmol* 2017;177(1):175–181.
23. Berger RB, Blackwell NM, Lass JH, Diaconu E, Pearlman E. IL-4 and IL-13 regulation of ICAM-1 expression and eosinophil recruitment in Onchocerca volvulus keratitis. *Invest Ophthalmol Vis Sci* 2002;43(9):2992–2997.
24. Munitz A, Brandt EB, Mingler M, Finkelman FD, Rothenberg ME. Distinct roles for IL-13 and IL-4 via IL-13 receptor alpha1 and the type II IL-4 receptor in asthma pathogenesis. *Proc Natl Acad Sci U S A* 2008;105(20):7240–7245.
25. Wise SK, Laury AM, Katz EH, Den Beste KA, Parkos CA, Nusrat A. Interleukin-4 and interleukin-13 compromise the sinonasal epithelial barrier and perturb intercellular junction protein expression. *Int Forum Allergy Rhinol* 2014;4(5):361–370.
26. Omori-Miyake M, Yamashita M, Tsunemi Y, Kawashima M, Yagi J. In vitro assessment of IL-4- or IL-13-mediated changes

in the structural components of keratinocytes in mice and humans. *J Invest Dermatol* 2014;134(5):1342–1350.
27. Beck LA, Thaci D, Hamilton JD, et al. Dupilumab treatment in adults with moderate-to-severe atopic dermatitis. *N Engl J Med* 2014;371(2):130–139.
28. Macdonald JM, Geroski DH, Edelhauser HF. Effect of inflammation on the corneal endothelial pump and barrier. *Curr Eye Res* 1987;6(9):1125–1132.
29. Ferrari G, Hajrasouliha AR, Sadrai Z, Ueno H, Chauhan SK, Dana R. Nerves and neovessels inhibit each other in the cornea. *Invest Ophthalmol Vis Sci* 2013;54(1):813–820.
30. Albuquerque RJ, Hayashi T, Cho WG, et al. Alternatively spliced vascular endothelial growth factor receptor-2 is an essential endogenous inhibitor of lymphatic vessel growth. *Nat Med* 2009;15(9):1023–1030.