Role of Soluble Vascular Endothelial Growth Factor Receptors-1 and -2, Their Ligands, and Other Factors in Branch Retinal Vein Occlusion With Macular Edema

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PURPOSE. To evaluate the association between multiple factors in aqueous humor and the severity of macular edema in patients with branch retinal vein occlusion (BRVO).

METHODS. We measured the aqueous humor levels of 11 factors (including vascular endothelial growth factor receptors, growth factors, and inflammatory factors) in BRVO patients with macular edema and in cataract patients as controls. Aqueous humor samples were obtained from 40 patients (31 patients with BRVO and 9 with cataract). Then the levels of vascular endothelial growth factor (VEGF), soluble VEGF receptor (sVEGFR)-1, sVEGFR-2, placental growth factor (PlGF), soluble intercellular adhesion molecule (sICAM)-1, monocyte chemotactic protein (MCP)-1, platelet-derived growth factor (PDGF)-AA, interleukin (IL)-6, IL-8, IL-12(p70), and IL-13 were measured by the suspension array method. Macular edema was examined by optical coherence tomography, and its severity was determined from the central macular thickness (CMT), neurosensory retinal thickness (TNeuro), and subfoveal serous retinal thickness (SRT).

RESULTS. Aqueous humor levels of growth factors, sVEGFR-1, sVEGFR-2, and inflammatory factors were significantly higher in eyes with BRVO than in control eyes. Aqueous levels of sVEGFR-1 and -2 were significantly correlated with the SRT, as well as with the levels of growth factors (PIGF and PDGF-AA) and various inflammatory factors (sICAM-1, MCP-1, IL-6, and IL-8). Levels of the growth factors (VEGF, PIGF, and PDGF-AA) were also significantly correlated with each other.

CONCLUSIONS. These findings suggest the importance of the cytokine network in BRVO patients, and may contribute to understanding the mechanism of macular edema associated with BRVO and to development of new treatments.

Keywords: VEGF receptors-1, VEGF receptors-2, VEGF, PIGF, BRVO
controls, and then analyzed the association between each factor and the severity of macular edema.

METHODS

Subjects

This study was conducted at the Department of Ophthalmology at the Tokyo Medical University. Approval was obtained from the Ethics Committee of the Tokyo Medical University. The procedures used conformed to the tenets of the Declaration of Helsinki. All patients signed an informed consent form before inclusion.

Thirty-one eyes with BRVO that were scheduled to undergo intravitreal injection of 1.25 mg bevacizumab (0.05 mL; Avastin; Genentech and Hoffmann La Roche, Basel, Switzerland) were studied between January 2013 and July 2013. Aqueous humor was collected just before the injection of bevacizumab. Criteria for the intravitreal injection of bevacizumab were macular edema involving the fovea (retinal thickness > 300 μm), best-corrected visual acuity (BCVA) < 20/30. Patients were not included if spontaneous resorption of the macular edema and visual acuity improvement were observed within 4 weeks after the first ophthalmic evaluation. Best-corrected visual acuity was indicated as the logarithm of the minimum angle of resolution (logMAR).

Exclusion criteria were history of retinal diseases other than BRVO, glaucoma, uveitis, diabetes mellitus, rubecosis iridis, ocular infections, laser photocoagulation, and intraocular surgery including cataract surgery in the study eye within 6 months of the planned injection of bevacizumab.

Fundus Findings

A masked grader independently assessed ischemic retinal vascular occlusion by examining fluorescein angiograms. The ischemic region of the retina was measured with the public domain Scion Image program (Scion Corporation, National Institutes of Health), as reported previously.³,11 On digital fundus photographs, the disc area was outlined with a cursor and then measured, and the same was done for the non-perfused area. The severity of retinal ischemia was assessed as the non-perfused area divided by the disc area.

Optical coherence tomography (OCT) was performed in each subject within 1 week before intravitreal injection of bevacizumab, employing a spectral-domain OCT apparatus (Spectralis, Heidelberg Engineering, Heidelberg, Germany). The severity of macular edema was classified on the basis of the central macular thickness (CMT), the TNeuro, and the SRT.¹⁶,¹⁷ These parameters were measured as follows: (1) CMT was calculated as the distance from the inner limiting membrane to the basal membrane of the retinal pigment epithelium (including all compartments between); (2) TNeuro was the thickness of the subfoveal neurosensory retina; and (3) SRT was the subfoveal serous retinal thickness. Measurements were performed with calipers incorporated into the software of the OCT machine by two retinal specialists who were blinded to the BCVA status and cytokine levels of the subjects.

Sample Collection

All patients with BRVO were given intravitreal injections of 1.25 mg bevacizumab. Aqueous humor samples were taken at the same time that intravitreal injection was performed. A mean volume of 0.1 mL aqueous humor was collected by anterior chamber limbal paracentesis with a 50-gauge needle attached to an insulin syringe. The intravitreal injection of bevacizumab was then administered through the pars plana, 3.5 mm from the limbus. Antibiotic ointment was given after surgery for 7 days. Immediately after collection, aqueous humor samples were transferred to sterile plastic tubes and stored at –80°C until analysis. Control aqueous samples were collected from nine patients undergoing routine cataract surgery by limbal paracentesis, and the samples were stored frozen at –80°C. We believe that aqueous humor is a reliable control because we collected our samples before surgery, and aqueous humor turnover did not change postoperatively when Kondo et al.¹⁸ compared aqueous humor outflow between eyes undergoing cataract surgery and control eyes at 7 hours after surgery.

Measurement of Cytokines and Growth Factors

Samples were analyzed using suspension array technology (xMAP; Luminex Corp., Austin, TX, USA).¹⁴ Capture bead kits (Beadlyte; Upstate Biotechnology, Lake Placid, NY, USA) were employed for the detection of sVEGFR1, sVEGFR2, VEGF, placentonal growth factor (PIGF), soluble intercellular adhesion molecule (sICAM)-1, monocyte chemotactic protein 1 (MCP-1), platelet-derived growth factor (PDGF-AA), interleukin (IL)-6, IL-8, IL-12(p70), and IL-13. Samples of undiluted aqueous humor (25 μL) were incubated overnight (16–18 hours) for PIGF and sICAM1 or for 2 hours to measure the other factors. Kits were used according to the manufacturer’s instructions. Standard curves for each cytokine were generated (in duplicate) by using the reference set of cytokine concentrations supplied in each kit. All incubation steps were performed at room temperature and in the dark. Samples were read on the suspension array system. To avoid between-run imprecision, we measured cytokines in the samples from all patients in a single run. Control samples were included in all runs. The levels of these factors in the aqueous humor samples were within the detection ranges of the assays, with minimum detectable concentrations of 1.59 pg/mL for sVEGFR1, 44.81 pg/mL for sVEGFR2, 0.64 pg/mL for VEGF, 0.37 pg/mL for PIGF; 0.03 ng/mL for sICAM-1, 1.2 pg/mL for MCP-1, 0.64 pg/mL for PDGF-AA, 0.29 pg/mL for IL-6, 0.14 pg/mL for IL-8, 0.14 pg/mL for IL-12(p70), and 0.12 pg/mL for IL-13.

Statistical Analysis

Analyses were performed with SAS System 9.3 software (SAS Institute, Inc., Cary, NC, USA). Student’s t-test was employed to compare normally distributed unpaired continuous variables between the two groups, while the Mann-Whitney U test was used for other variables with a skewed distribution. The χ² test or Fisher’s exact test was used to compare discrete variables. Differences between the median aqueous levels were assessed with the Wilcoxon single-rank test. To examine relationships among the variables, Spearman’s rank-order correlation coefficients or Pearson’s correlation coefficients were calculated. Two-tailed P values less than 0.05 were considered to indicate statistical significance.

RESULTS

The BRVO group (18 men and 13 women) was aged 67.4 ± 11.0 years (mean ± SD), while the control group (3 men and 6 women) was aged 65.0 ± 4.9 years. There was no significant difference of age (P = 0.530) or sex (P = 0.191) between the control group and the BRVO group. The mean duration of macular edema was 1.9 ± 1.4 months (range, 1–6 months). The mean CMT, TNeuro, and SRT of the eyes with BRVO was 371 ± 216, 492 ± 186, and 180 ± 164 μm, respectively. Mean BCVA in the eyes with BRVO was 0.65 ± 0.32 logMAR units. Nineteen of the 31 BRVO patients (61%) had hypertension.
TABLE 1. Aqueous Humor Factors in the Control and BRVO Groups

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>BRVO</th>
<th>P Value</th>
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</thead>
<tbody>
<tr>
<td>sVEGFR-1 pg/mL</td>
<td>790 [327–1374]</td>
<td>2435 [1670–3142]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>sVEGFR-2 pg/mL</td>
<td>85.5 [44.8–230]</td>
<td>537 [431–661]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VEGF pg/mL</td>
<td>22.5 [0.64–87.7]</td>
<td>83.5 [31.9–137.9]</td>
<td>0.033</td>
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<tr>
<td>PGF pg/mL</td>
<td>0.37 [0.37–0.37]</td>
<td>1.69 [0.37–5.65]</td>
<td>0.004</td>
</tr>
<tr>
<td>PDGF-AA pg/mL</td>
<td>15.1 [8.95–21.6]</td>
<td>50.5 [22.9–33.3]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>sICAM-1 pg/mL</td>
<td>0.06 [0.03–0.31]</td>
<td>0.29 [0.19–1.77]</td>
<td>0.002</td>
</tr>
<tr>
<td>MCP-1 pg/mL</td>
<td>839 [710–1019]</td>
<td>1721 [1087–2337]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IL-6 pg/mL</td>
<td>2.73 [0.78–8.61]</td>
<td>8.17 [5.75–13.2]</td>
<td>0.013</td>
</tr>
<tr>
<td>IL-8 pg/mL</td>
<td>2.54 [1.19–4.20]</td>
<td>21.0 [10.8–30.3]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IL-12 pg/mL</td>
<td>0.14 [0.14–0.14]</td>
<td>2.48 [0.14–2.69]</td>
<td>0.001</td>
</tr>
<tr>
<td>IL-13 pg/mL</td>
<td>0.63 [0.12–1.67]</td>
<td>4.09 [0.12–4.53]</td>
<td>0.025</td>
</tr>
</tbody>
</table>

Values are the median [interquartile range].

Eleven of the 31 BRVO patients (35%) had hyperlipidemia. One of the 9 control patients (11%) had hypertension, and 2 control patients (22%) had hyperlipidemia. There was a significant difference in the prevalence of hypertension between the control group and the BRVO group (P = 0.008), but there was no significant difference for hyperlipidemia (P = 0.455) between the two groups.

Measurement of the aqueous humor cytokine levels showed significantly higher concentrations of sVEGFR-1, sVEGFR-2, VEGF, PGF, sICAM-1, MCP-1, PDGF-AA, IL-6, IL-8, IL-12(p70), and IL-13 in the BRVO group than in the control group in descending order (Table 1).

When the relationship between cytokine levels and the severity of retinal ischemia was assessed, three subjects were excluded because judgment of the nonperfused area was difficult due to severe retinal hemorrhage. Significant positive correlations were found between the levels of VEGF, PGF, PDGF-AA, sICAM-1, or IL-8 and the severity of retinal ischemia (Table 2). On the other hand, there were significant negative correlations between the levels of IL-12(p70) or IL-13 and the severity of retinal ischemia (Table 2).

Relationships between the cytokine levels in aqueous humor and the three OCT parameters (CMT, TNeuro, and SRT) were also assessed. Significant correlations were found between CMT and the levels of sVEGFR-2, VEGF, PGF, PDGF-AA, sICAM-1, MCP-1, and IL-8 in the BRVO group (Table 2). There were also significant correlations between TNeuro and the levels of VEGF and PGF (Table 2). Furthermore, there were significant correlations between the levels of sVEGFR-1, sVEGFR-2, PDGF-AA, sICAM-1, MCP-1, and IL-8 (Table 2). In contrast, there were significant negative correlations between CMT and the levels of IL-12(p70) and IL-13, as well as significant negative correlations between SRT and the levels of IL-12(p70) and IL-13 (Table 2).

In the BRVO group, there were significant correlations between the level of sVEGFR-1 and the levels of VEGF, PGF, PDGF-AA, sICAM-1, MCP-1, IL-6, and IL-8 (Table 3). There were also significant correlations between the level of VEGF and the levels of PDGF-AA, sICAM-1, MCP-1, IL-6, and IL-8 in the BRVO group (Table 3). Furthermore, there were significant correlations between the level of VEGF and the levels of PDGF-AA, sICAM-1, MCP-1, and IL-8 (Table 3), as well as a significant correlation between PDGF-AA and the levels of MCP-1 or IL-8 (Table 3). In addition, there was a significant correlation between the level of sICAM-1 and the levels of MCP-1 or IL-8 (Table 3), as well as a significant correlation between MCP-1 and IL-6 or IL-8 (Table 3). In contrast, there were significant negative correlations between the level of IL-12(p70) and the levels of VEGF, PGF, sICAM-1, and IL-8 in the BRVO group, as well as significant negative correlations between IL-13 and the levels of VEGF, PGF, PDGF-AA, sICAM-1, and IL-8 (Table 3). In addition, there was a significant correlation between the level of IL-12(p70) and that of IL-13 (Table 3).

We also assessed the relationship between each cytokine and three possible confounding factors, which were a history of hypertension, hyperlipidemia, and the disease duration. When each cytokine was compared between the subjects with or without hypertension or hyperlipidemia in the control group and the BRVO group, none of the cytokines showed a significant difference (data not shown). In addition, none of the cytokines were significantly correlated with disease duration (data not shown). Moreover, none of the cytokines were significantly correlated with age and sex in either group (data not shown).

TABLE 2. Correlations Between Aqueous Humor Factors and the Severity of Retinal Ischemia or OCT Parameters

<table>
<thead>
<tr>
<th></th>
<th>sVEGFR-1</th>
<th>sVEGFR-2</th>
<th>VEGF</th>
<th>PGF</th>
<th>PDGF-AA</th>
<th>sICAM-1</th>
<th>MCP-1</th>
<th>IL-6</th>
<th>IL-8</th>
<th>IL-12, p70</th>
<th>IL-13</th>
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<tbody>
<tr>
<td>Variable</td>
<td>r, P Value</td>
<td>r, P Value</td>
<td>r, P Value</td>
<td>r, P Value</td>
<td>r, P Value</td>
<td>r, P Value</td>
<td>r, P Value</td>
<td>r, P Value</td>
<td>r, P Value</td>
<td>r, P Value</td>
<td>r, P Value</td>
</tr>
<tr>
<td>Severity of</td>
<td>0.10</td>
<td>0.17</td>
<td>0.88</td>
<td>0.68</td>
<td>0.46</td>
<td>0.70</td>
<td>0.32</td>
<td>0.24</td>
<td>0.68</td>
<td>−0.62</td>
<td>−0.63</td>
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<td>retinal ischemia</td>
<td>0.601</td>
<td>0.365</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.011</td>
<td>&lt;0.001</td>
<td>0.075</td>
<td>0.187</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
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<td>CMT</td>
<td>0.34</td>
<td>0.37</td>
<td>0.42</td>
<td>0.47</td>
<td>0.56</td>
<td>0.54</td>
<td>0.51</td>
<td>0.31</td>
<td>0.59</td>
<td>−0.52</td>
<td>−0.53</td>
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<tr>
<td>TNeuro</td>
<td>−0.13</td>
<td>−0.04</td>
<td>0.062</td>
<td>0.043</td>
<td>0.018</td>
<td>0.007</td>
<td>0.002</td>
<td>0.003</td>
<td>0.005</td>
<td>0.092</td>
<td>0.004</td>
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<td>SRT</td>
<td>0.458</td>
<td>0.811</td>
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<td>0.002</td>
<td>0.489</td>
<td>0.059</td>
<td>0.377</td>
<td>0.317</td>
<td>0.061</td>
<td>0.148</td>
<td>0.109</td>
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r, correlation coefficient.
TABLE 3. Correlation Matrix for Aqueous Humor Factors

<table>
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<tr>
<th>Variable</th>
<th>sVEGFR-1</th>
<th>sVEGFR-2</th>
<th>VEGF</th>
<th>PlGF</th>
<th>PDGF-AA</th>
<th>sICAM-1</th>
<th>MCP-1</th>
<th>IL-6</th>
<th>IL-8</th>
<th>IL-12</th>
<th>IL-13</th>
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<td>sVEGFR-1</td>
<td>0.82</td>
<td>0.10</td>
<td>0.38</td>
<td>0.38</td>
<td>0.38</td>
<td>0.39</td>
<td>0.46</td>
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<td>-0.27</td>
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<tr>
<td></td>
<td>&lt;0.001</td>
<td>0.596</td>
<td>0.037</td>
<td>0.008</td>
<td>0.036</td>
<td>0.001</td>
<td>0.032</td>
<td>0.011</td>
<td>0.172</td>
<td>0.109</td>
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<td>sVEGFR-2</td>
<td>0.28</td>
<td>0.38</td>
<td>0.41</td>
<td>0.42</td>
<td>0.54</td>
<td>0.39</td>
<td>0.48</td>
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<td>0.132</td>
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<td>0.048</td>
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<td></td>
<td>0.011</td>
<td>&lt;0.001</td>
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<td>0.484</td>
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<td>PDGF-AA</td>
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<td>0.52</td>
<td>-0.31</td>
<td>-0.44</td>
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<td>0.012</td>
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<td>sICAM-1</td>
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<td>0.11</td>
<td>0.77</td>
<td>-0.78</td>
<td>-0.83</td>
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<tr>
<td>MCP-1</td>
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<td>0.66</td>
<td>-0.30</td>
<td>-0.31</td>
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<td>0.069</td>
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<td>IL-6</td>
<td>0.25</td>
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<td></td>
<td>0.169</td>
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<td>IL-8</td>
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<td></td>
<td>&lt;0.001</td>
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<td>IL-12</td>
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**DISCUSSION**

In the present study, we demonstrated that the aqueous humor levels of both sVEGFR-1 and sVEGFR-2 were significantly higher in eyes with BRVO than in control eyes with cataract and that there was a significant correlation between the levels of sVEGFR-1 and sVEGFR-2, suggesting that expression of the two receptors increases together. Soluble VEGFR-1 and -2 are produced by alternative mRNA splicing, which allows the same gene to encode transmembrane forms of VEGFR-1 and VEGFR-2 or soluble forms that are released from the cell surface. A correlation between the expression of sVEGFR-1 and transmembrane VEGFR-1 in human umbilical vein endothelial cells stimulated by phorbol 12-myristate 13-acetate or bFGF has been demonstrated with Northern blotting. Therefore, it is possible that expression of transmembrane VEGFR-1 and transmembrane VEGFR-2 is enhanced, as well as that of sVEGFR-1 and sVEGFR-2, in order to regulate vascular permeability. Previous studies have demonstrated that VEGF signaling in vivo is tightly regulated by VEGFR-1 and VEGFR-2. Vascular endothelial growth factor acts by binding to VEGFR-1 (also known as Flt-1) and VEGFR-2 (also known as kinase insert domain receptor [KDR] or Flk-1), which have tyrosine kinase activity and are expressed by vascular endothelial cells. Vascular endothelial growth factor receptor 1 is expressed not only by vascular endothelial cells but also by monocytes/macrophages at both the mRNA and protein levels, and VEGF-1 signaling plays a role in the recruitment of these cells by VEGF at sites of angiogenesis and inflammation. On the other hand, VEGFR-2 is exclusively expressed by endothelial cells, and VEGF signaling mediated via the membrane-bound isoform of VEGFR-2 is essential for normal endothelial cell function, influencing vascular permeability and angiogenesis. It has also been reported that binding of VEGF to VEGFR-2 induces inflammatory factors such as MCP-1 and ICAM-1 via nuclear factor kappa B (NF-kB). Thus, it is possible that these VEGF receptors have an important role in the pathogenesis of macular edema associated with BRVO.

Generally, soluble receptors inactivate their ligands by binding with them, since the soluble receptor does not possess the intracellular domain required to initiate signaling. That is, sVEGFR-1 acts as a decoy (endogenous antagonist) for VEGF and as a negative regulator of postischemic angiogenesis and reperfusion. Both in vitro and in vivo studies have shown that elevated levels of sVEGFR-1 impair the vasodilatory response, while sVEGFR-2 has antiangiogenic activity. A decrease of sVEGFR-2, the soluble form of the major proangiogenic signal transducer for VEGF, is a physiological response to ischemia that is designed to increase angiogenesis. It was reported that sVEGFR-2 contributes to vascular maturation by mediating interactions between endothelial cells and mural cells that lead to stabilization of vessels. The present study revealed a positive correlation between the severity of macular edema (CMT or SRT) and the levels of sVEGFR-1 and sVEGFR-2 in the aqueous humor, suggesting that elevation of sVEGFR-1 and sVEGFR-2 contributed to an increase of vascular permeability and the occurrence of macular edema, irrespective of whether sVEGFR-1 and sVEGFR-2 bound with VEGF to neutralize its effect on the vascular endothelium. Macular edema is reported to be promoted by an increase of VEGF that binds to these receptors expressed on vascular endothelial cells, monocytes, and macrophages. Interestingly, it has also been reported that sVEGFR-1 promotes inflammation. This is supported by our results showing that the aqueous humor level of sVEGFR-1 was significantly correlated with the levels of various inflammatory factors (sICAM-1, MCP-1, IL-6, and IL-8). In addition, we previously suggested that various inflammatory factors are induced via NF-kB because we found that the vitreous fluid level of sVEGFR-2 was significantly correlated with the levels of several inflammatory factors, including sICAM-1, MCP-1, and IL-6. The present study showed that the aqueous humor level of sVEGFR-2 was correlated significantly with the levels of various inflammatory factors (sICAM-1, MCP-1, IL-6, and IL-8), as was previously found for vitreous levels. Clinical and experimental evidence indicates that both sVEGFR-1 and sVEGFR-2 may influence vascular permeability in the inflammatory response. However, further investigations...
will be required to confirm the role of sVEGFR-1 and sVEGFR-2 in macular edema associated with BRVO.

We also found that the levels of PI GF and PDGF-AA were significantly higher in aqueous humor samples from the BRVO group than in controls. This result corresponds with previous reports that PDGF-AA and PI GF are upregulated in the vitreous humor of patients with retinal diseases (such as RVO, diabetic retinopathy, and age-related macular degeneration).23,29,30 Placental growth factor is a member of the VEGF family, and is a 25-kd dimeric protein that is highly homologous with VEGF.31,32 As a specific ligand for VEGFR-1, PI GF potentially promotes angiogenesis and also induces the growth and migration of endothelial cells.33 In addition to VEGF, PI GF is also a ligand for VEGFR-1, so both molecules could play a role in signaling under pathological conditions. In addition, PI GF modulates the inflammatory process in the following ways. Placental growth factor stimulates tissue factor production and chemotaxis in monocytes/macrophages.28 It also increases the production of IL-8, as well as MCP-1 by cultured monocytes after binding to VEGFR-1 via a calcineurin-dependent pathway.32,33 suggesting that it has a direct influence on the inflammatory reaction. This is supported by our finding that the aqueous humor level of PI GF was correlated with those of sVEGFR-1, MCP-1, and IL-8. Furthermore, it was reported that PI GF induces VEGF secretion by mononuclear cells.33 This is supported by our finding that the aqueous humor level of PI GF was correlated significantly with that of VEGF. In addition, the aqueous level of PI GF was correlated significantly with the CMT and the TNeuro, and there was also a correlation between VEGF and the CMT or TNeuro. These findings suggest that PI GF may act synergistically with VEGF during the development of macular edema associated with BRVO.

Platelet-derived growth factor is a growth factor that contributes to regulating the migration of mesenchymal cells (fibroblasts, smooth muscle cells, and glial cells), and it belongs to the PDGF/VEGF family. Platelet-derived growth factor-AA has been reported to show increased expression by endothelial cells when blood flow is decreased by arteriosclerosis.34 In the present study, the aqueous level of PDGF-AA was correlated with that of MCP-1 and IL-8, suggesting that PDGF-AA also contributes to the inflammatory pathologic state. Moreover, the aqueous level of PDGF-AA was correlated with that of VEGF, while the aqueous level of PDGF-AA was significantly correlated with the full CMT and SRT. It was reported that PDGF-AA acts on induction of the gap bond formation.35 These findings suggest that VEGF and PDGF-AA family members may act synergistically in BRVO patients with macular edema.

We previously reported that levels of three inflammatory factors (sICAM-1, MCP-1, and IL-6) were significantly increased in vitreous fluid samples from BRVO patients compared with controls, and that the inflammatory factors significantly correlated with each other, suggesting that these inflammatory factors play an important role in the development of macular edema associated with BRVO.12 Similarly, in this study, the levels of inflammatory factors (IL-6, IL-8, sICAM-1, and MCP-1) were significantly higher in aqueous humor samples from the BRVO group compared with the controls, and most of the inflammatory factors were significantly correlated with each other. Feng et al.56 reported that the aqueous humor concentrations of IL-6 and IL-8 were significantly higher in patients with retinal vein occlusion than in control patients. Their report is consistent with the results of the present study. They also reported that the IL-6 level was significantly associated with the CMT. However, we demonstrated that IL-8 was not only correlated with the CMT but was also more closely associated with the severity of retinal ischemia than IL-6. This finding is supported by Lee et al.57 who reported that the IL-8 level and was positively correlated with severity of macular edema and retinal ischemia in BRVO patients with macular edema. Interleukin-8 is a potent chemoattractant and an activator of neutrophils and T cells. Interleukin-8 production is induced by exposure of vascular endothelial cells to hypoxia and this cytokine then angiogenesis and tumor invasion.58 It has also been reported that PI GF stimulates vascular permeability.59 Taken together, these reports and our results suggest that IL-8 may be the main inflammatory factor involved in macular edema associated with BRVO.

On the other hand, our study revealed a significant negative correlation between the level of IL-12(p70) and the levels of VEGF, PI GF, sICAM-1, and IL-8 in the BRVO group, as well as a significant negative correlation between IL-13 and VEGF, PI GF, PDGF-AA, sICAM-1, and IL-8. Interleukin-12 is a key inducer of Th1-type cytokines and is strongly expressed in atherosclerotic plaques.60 Interleukin-12 inhibits endothelial cell cycle progression and adhesion, and it also triggers anti-inflammatory pathways.61,62 Kaneda et al.63 reported significant elevation of IL-12 in patients who were refractory to bevacizumab. They suggested that IL-12 has a distinct anti-VEGF role and may interfere with reperfusion to promote inflammatory responses. Interleukin-13 is a key inducer of Th2 cytokines and it reduces the production of inflammatory factors (such as TNF-α and IL-1β).64 Suzuki et al.64 reported that IL-13 is significantly correlated with VEGF in patients with diabetic retinopathy, and that the levels of anti-inflammatory cytokines like IL-13 are closely related to VEGF in the vitreous fluid. In our study, there was a significant correlation between the aqueous humor level of IL-12(p70) and the level of IL-13, while the aqueous humor level of IL-12 and IL-13 both showed a significantly negative correlation with CMT and SRT. These findings and our results suggest that IL-12 and IL-13 may act synergistically as anti-inflammatory cytokines in BRVO patients with macular edema.

Taken together, it seems that numerous different factors (growth factors, VEGF-R1, -2, and inflammatory factors) are involved in the pathogenesis of macular edema associated with BRVO. The upregulation of PI GF, PDGF, and various inflammatory factors may be dependent on sVEGFR-1/sVEGFR-2, because there were significant correlations between the aqueous humor levels of sVEGFR-1 and sVEGFR-2 and the aqueous humor levels of PI GF and PDGF or various inflammatory factors in our BRVO patients with macular edema. Accordingly, multiple factors could be inhibited by an antibody targeting VEGFR-1 and VEGFR-2, so it may be worth considering anti-VEGFR (-1 and -2) therapy to treat macular edema in BRVO patients. Indeed, it has been reported that VEGF inhibitors are effective in patients with diabetic macular edema and age-related macular degeneration.55,66 Furthermore, SRT was correlated not only with sVEGFR-1 and sVEGFR-2, but also with PDGF-AA, sICAM-1, MCP-1, and IL-8, suggesting that serous retinal detachment may contribute to inflammation. Therefore, intravitreal injection of aflibercept and/or VEGF inhibitors may be effective for serious retinal detachment. However, a prospective clinical trial would be required to determine the response to such therapy.

This study has several limitations. First, we were not able to collect enough vitreous fluid samples because intravitreal anti-VEGF therapy was performed more often than vitreous surgery. Our previous study revealed that the VEGF level in aqueous humor was associated with that in vitreous fluid, indicating that aqueous cytokine levels can reflect retinal levels,67 although Ecker et al.68 reported that the levels of proteins in the aqueous humor are not necessarily correlated with their vitreous levels. Second, we were unable to collect samples from BRVO patients without macular edema because we collected samples just before intravitreal injection of bevacizumab, and patients without macular edema did not receive such treatment. Third, we were not able to enroll patients who...
had a history of BRVO without macular edema and who had undergone cataract surgery. Further investigations will be required to conduct a study of aqueous humor samples collected from BRVO patients without macular edema as a control.

In conclusion, the aqueous humor levels of various factors (growth factors, sVEGFR-1, sVEGFR-2, and inflammatory factors) were significantly higher in eyes with BRVO than in control eyes. The levels of sVEGFR-1 and -2 were correlated with the SRT and with the levels of growth factors (PIGF and PDGF-AA) and various inflammatory factors. Levels of the growth factors (VEGF, PIGF; and PDGF-AA) were also significantly correlated with each other. These findings suggest the importance of investigating relations among the cytokine network, and may contribute to better understanding of the mechanism of macular edema in BRVO patients and to the development of new treatments.

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