Increased sCD200 Levels in Vitreous of Patients With Proliferative Diabetic Retinopathy and Its Correlation With VEGF and Proinflammatory Cytokines

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PURPOSE. The purpose of this study was to determine the levels of sCD200 expression in the vitreous of proliferative diabetic retinopathy (PDR) patients and to clarify its correlation with different vitreoretinal conditions, VEGF and its receptors, and proinflammatory cytokines.

METHODS. The expression of sCD200, VEGF and its receptors, and other proinflammatory cytokines were examined by using ELISA. Clinical stratification was performed on patients with different vitreoretinal conditions for correlation analysis.

RESULTS. The vitreous levels of sCD200 were significantly higher in the PDR group (182.2 ± 17.65 pg/mL) compared with those in the control group (56.86 ± 6.573 pg/mL; P < 0.0001). The venous blood levels of sCD200 were 26.71 ± 4.32 pg/mL in the PDR group and 19.94 ± 3.87 pg/mL in the control group (P = 0.2614). The vitreous levels of sCD200 were significantly elevated in PDR patients with diabetic macular edema (DME; 266.9 ± 28.82 pg/mL) or traction retinal detachment (TRD; 256.9 ± 34.50 pg/mL) compared with the PDR group without DME (136.9 ± 15.13 pg/mL; P < 0.0001) or TRD (146.9 ± 15.97 pg/mL; P = 0.0024). The vitreous levels of CCL2, CXCL4, CXCL9, CXCL10, VEGF, sVEGFR-1, sVEGFR-2, IL-6, IL-8, IL-10, and IL-18 were also elevated significantly in the PDR group. Statistical association was found between sCD200 levels and VEGF (r = 0.6566, P < 0.0001), sVEGFR-1 (r = 0.5774, P = 0.006), sVEGFR-2 (r = 0.3605, P = 0.0362), CCL-2 (r = 0.6001, P = 0.0002), IL-6 (r = 0.5704, P = 0.0004), IL8 (r = 0.5712, P = 0.0307), and IL-10 (r = 0.5618, P = 0.0355).

CONCLUSIONS. Expression of sCD200 may contribute to retinal angiogenesis by interacting with VEGF-mediated inflammatory response and represents a potential therapeutic target for the patients with PDR.

Keywords: proliferative diabetic retinopathy, sCD200, vascular endothelial growth factor, proinflammatory cytokines, vitreous samples

Chronic inflammation and ischemia-induced angiogenesis are the main clinical features of proliferative diabetic retinopathy (PDR). The complications of PDR, including tractional retinal detachment (TRD) and vitreous hemorrhage (VH), often lead to irreversible vision loss. Through up-regulating the production of VEGF in hypoxia, it appears to be a primary stimulus initiating angiogenesis in diabetic retinopathy (DR). Strong evidence indicates that increased VEGF production enhances vascular permeability and is the major angiogenic factor in PDR. In addition, low-grade chronic inflammation is another participating factor, which also contributes to PDR. As well as VEGF, multiple proinflammatory chemokines attract inflammatory cells to lesion sites, resulting in angiogenesis and fibrosis, which is implicated in the pathogenesis of PDR. However, the relationship between inflammation and angiogenesis in this disease has not been clarified.

A novel immuno-effective molecule, CD200 (previously known as OX-2), exists in two forms: cell membrane bound and soluble in serum (sCD200). It is widely expressed on myeloid cells and T and B lymphoid cells. sCD200 regulates inflammatory and acquired immune responses through interaction with cell-bound receptor CD200R. Many inflammatory cytokines, including TNF-α and IFN-γ, induce CD200 expression, which binds to CD200R. This interaction lessens the inflammatory response. Clinically, the CD200R activator has been used for the treatment of collagen-induced arthritis.

In the central nervous system, CD200 could activate CD200R, which exists on the surface of microglia. Recent studies detected extensive CD200 expression in the neuron and endothelium of retina. The interaction between CD200 and CD200R had the potential to suppress or limit immunogenic inflammatory damage resulting from activated retinal microglia. Inhibition of the CD200-CD200R axis can lead to microglia activation and significantly improve their phagocytic ability in injured retinal tissue. Moreover, recent research showed that CD200-related inflammatory response played a critical role in the pathogenesis and development of wet age-related macular degeneration (AMD). Increased expression of CD200 was detected in the surface of circulating CD11b+ monocytes from the patients with neovascular AMD compared with the control group. All the aforementioned studies
Increased sCD200 in the Vitreous Samples of PDR Patients

revealed that CD200 might be related to the regulation of inflammatory response in vitreous and retinal disease. However, its expression and possible function in the PDR are still not well understood.

In the present study, we hypothesized that CD200 might contribute to PDR development and progression. To elucidate this hypothesis, this study examined the levels of sCD200 in the vitreous of patients with PDR or nondiabetic patients and ascertained its correlation with different vitreoretinal conditions and other proinflammatory cytokines.

MATERIALS AND METHODS

Subjects

The study was approved by the ethic review committee of Zhongshan Ophthalmic Center in Sun Yat-sen University and was conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from each subject for all examinations and procedures.

The vitreoretinal conditions of DR were assessed on the fluorescein angiogram and the standardized fundus color photographs. If there was a dense cataract or VH resulting in an opacity of the ocular fundus, the vitreoretinal conditions of DR were assessed by ocular ultrasound.

Sample Collection

The vitrectomy samples in all cases were underwent a standard three-port pars plana vitrectomy (PPV). At the onset of vitrectomy, 0.5- to 1.0-mL vitreous samples were obtained by using the vitreous cutter. After transferred to a sterile tube, the vitreous samples were placed immediately on ice and then centrifuged at 15,000g for 5 minutes at 4°C for cells and debris ablation. For plasma determinations, venous blood samples were taken after an overnight fast, and the plasma was separated by centrifugation at 15,000g for 5 minutes at 4°C. The samples were frozen at -80°C until the assay was performed.

Measurement of Cytokines in Vitreous and Venous Blood Samples

A multiplex bead immunoassay system (Milliplex Human Cytokine kit; Millipore Corp., Billerica, MA, USA) was used to analyze cytokine concentrations. The results were analyzed by using the Bio-Plex suspension array system (Bio-Plex200; BioRad, Hercules, CA, USA) according to the manufacturer's instructions, vitreous or venous blood samples were diluted 2-fold to 10-fold, and 100 μL diluted sample was added into each of the ELISA plates for the analysis. Interleukin (IL)-1β (cat # MLB00C), IL-6 (cat # HS600B), IL-8 (cat # VAL103), IL-10 (cat # S1000B), IL-18 (cat # AF177), CCL1 (cat # BAF272), CCL2 (cat # SCP00), CCL3 (cat # BA1265), CCL4 (cat # AJ1481b), CXCL4 (cat # DY795), CXCL9 (cat # DCX900), CXCL10 (cat # SIP100), VEGF (cat # DVE00), VEGF-R1 (cat # DRV100B), and VEGF-R2 (cat # DVR200) were purchased from R&D Systems (Minneapolis, MN, USA); CCL7 (cat # 11926-H07E), CCL18 (cat # 11899-H21E), CXCL5 (cat # 10889-H01E), CXCL6 (cat # HG10612-M), TNF-α (cat # 10600-H01E), IFN-γ (cat # 10538-H05H), and sCD200 (cat # 10886-H08H) were purchased from Sino Biological, Inc. (Beijing, China). In each ELISA kit, the detection limits were 9.0, 3.5, 355, and 25 pg/mL. The FLUOstar Omega-Microplate reader (BMG Labtech, Offenburg, Germany) was used for the ELISA plate readings. The antibodies against each of cytokines conjugated to horseradish peroxidase were added to each well of the ELISA plate. After samples incubation into the wells of ELISA plates, substrate mix solution was added for color development. After the addition of 2 N sulfuric acid, the reaction was stopped, and optical density was read at 450 nm in a microplate reader. The actual concentration for each sample was calculated by using the four-parameter fit logistic (4-PL) curve equation. After the standard curve was obtained using 4-PL, the correction read was multiplied by the dilution factors to attain the actual reading for each sample.

Statistical Analysis

We used SPSS version 12.0 and program 3S (the BMDP 2007 Statistical Package; IBM, Chicago, IL, USA) for statistical analyses. The means of two independent groups were compared by Mann-Whitney test. Spearman’s correlation test was used to calculate the correlations between cytokine concentrations. All vitreous samples were tested in triplicate, and statistical significance was \( P < 0.05 \). For the correction of multigroup comparisons, \( P \) values of 0.0071 for the Spearman’s correlation test and 0.0167 for the Mann-Whitney \( U \) test were considered statistically significant. Based on Bonferroni’s methods, statistical significance was \( P < 0.05 \).

RESULTS

Clinical and Demographic Characteristics of the Patients in Vitreous Samples

Undiluted vitreous fluid samples (0.5–1 mL) were obtained from 34 eyes of 34 patients with PDR (the PDR group) and 37 eyes of 37 nondiabetic patients (the control group) with other conditions who required PPV. In the control group, the retina was not directly affected by neovascularization. The diagnoses of these samples included rhegmatogenous retinal detachment (n = 29), macular hole (n = 4), and epiretinal membrane (n = 4). The patients in both groups were enrolled consecutively from January 2014 to October 2014.

As shown in Table 1, the patients in the PDR group were 19 females and 15 males whose ages ranged from 34 to 69 years, with a mean of 52.12 ± 1.474 years. The duration of diabetes ranged from 1 to 32 years, with a mean of 9.49 ± 6.84 years. The patients in the control group were 15 females and 22 males (P = 0.08) whose ages ranged from 20 to 79 years, with a mean of 52.14 ± 2.063 years (P = 0.9957). The fasting blood glucose in the PDR group ranged from 7.4 to 18.4 mM, with a mean of 11.54 ± 0.47 mM. The fasting blood glucose in the control group ranged from 4.4 to 9.3 mM, with a mean of 6.39 ± 0.17 mM (P < 0.0001). Seven patients in the PDR group and five patients in the control group had hypertension (P > 0.05). In the PDR group, 16 patients had VH, 12 patients had diabetic macular edema (DME), 11 patients had TRD, and 12 patients received panretinal photocoagulation (PRP) in the preceding 6 months. In the control group, no patient had VH, DME, and TRD or received PRP in the preceding 6 months.

Clinical and Demographic Characteristics of the Patients in Venous Blood Samples

One to two mL venous blood samples were obtained from 21 eyes of 21 patients with PDR (the PDR group) and 17 eyes of 17 non-diabetic patients (the control group) with other conditions who required PPV. In the control group, the retina was not directly affected by neovascularization. The diagnoses of these samples included rhegmatogenous retinal detachment (n = 13) and macular hole (n = 4). The patients in both groups were enrolled consecutively from January 2014 to June 2015.
Panretinal photocoagulation history 12/22 0/37
Vitreoretinal condition
Fasting blood glucose, mM 10.17
Hypertension 2/19 0/17
Duration of diabetes, y 8.60
Age, y 51.43
Serum soluble CD200, pg/mL 26.71
Vitreoretinal condition
Vitreous hemorrhage 16/18 0/37
Diabetic macular edema 12/22 0/37
Traction retinal detachment 11/23 0/37
Panretinal photocoagulation history 12/22 0/37

As shown in Table 2, the patients in the PDR group were 11 females and 10 males whose ages ranged from 33 to 64 years with a mean of 51.43 ± 1.89 years. The duration of diabetes ranged from 0.5 to 20 years with a mean of 8.60 ± 1.47 years. The patients in the control group were 8 females and 9 males (P > 0.05), whose ages ranged from 26 to 73 years with a mean of 50.18 ± 3.27 years (P = 0.7307). The fasting blood glucose in the PDR group ranged from 7.8 to 18.4 mM with a mean of 10.17 ± 0.62 mM. The fasting blood glucose in the control group ranged from 4.9 to 7.5 mM with a mean of 6.49 ± 0.31 mM (P < 0.0001). Two patients in the PDR group and no patients in the control group have hypertension (P > 0.05). In the PDR group, nine patients have VH, five patients have diabetic macular edema (DME), five patients have traction retinal detachment (TRD) and seven patients received panretinal photocoagulation (PRP) in the preceding 6 months. In the control group, no patient has VH, DME, and TRD or receives PRP in the preceding 6 months.

Levels of sCD200 in Vitreous Samples
Expression of sCD200 was detected in all vitreous samples from the PDR group and the control group. When all the patients were considered, mean sCD200 levels in vitreous samples from the PDR group (182.2 ± 17.63 pg/mL) were significantly higher than in the control group (56.86 ± 6.57 pg/mL; P < 0.0001; Fig. 1).

Levels of sCD200 in Venous Blood Samples
Expression of sCD200 was detected in 18 (85.7%) venous blood samples from the PDR group and 12 (70.6%) venous blood samples from the control group. The concentrations of sCD200 were 26.71 ± 4.32 and 19.94 ± 3.87 pg/mL, respectively (P = 0.2614; Table 2). The concentrations of sCD200 in venous blood samples is lower than that in vitreous samples (data not shown).

Relationship Between sCD200 and Different Vitreoretinal Conditions
The patients in the PDR group were further divided into two groups: with or without VH, DME, TRD, or PRP. The mean sCD200 levels in venous samples were significantly different between PDR patients with or without DME or TRD. In the PDR with TRD group and PDR without TRD group, the concentrations of sCD200 were 256.9 ± 34.50 and 146.9 ± 15.97 pg/mL, respectively (P = 0.0024; Fig. 2b). In the PDR with DME group and PDR without DME group, the concentrations of sCD200 were 266.9 ± 28.82 and 136.9 ± 15.13 pg/mL, respectively (P < 0.0001; Fig. 2c). In contrast, there was no obvious difference in the mean levels of sCD200 between PDR patients with or without VH or PRP. In the PDR with VH group and PDR without VH group, the concentrations of sCD200 were 179.0 ± 28.53 and 185.1 ± 22.36 pg/mL,

### Table 1. Baseline Patient Characteristics From Vitreous Samples

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>PDR Group, n = 34</th>
<th>Control Group, n = 37</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>52.12 ± 1.47</td>
<td>52.14 ± 2.06</td>
<td>0.9957*</td>
</tr>
<tr>
<td>Female sex, n (%)</td>
<td>19 (55.9)</td>
<td>15 (40.5)</td>
<td>0.08†</td>
</tr>
<tr>
<td>Duration of diabetes, y</td>
<td>9.49 ± 6.84</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Hypertension</td>
<td>7/27</td>
<td>5/56</td>
<td>&gt;0.05†</td>
</tr>
<tr>
<td>Fasting blood glucose, mM</td>
<td>11.34 ± 0.47</td>
<td>6.39 ± 0.17</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Vitreoretinal condition</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitreous hemorrhage</td>
<td>16/18</td>
<td>0/37</td>
<td></td>
</tr>
<tr>
<td>Diabetic macular edema</td>
<td>12/22</td>
<td>0/37</td>
<td></td>
</tr>
<tr>
<td>Traction retinal detachment</td>
<td>11/23</td>
<td>0/37</td>
<td></td>
</tr>
<tr>
<td>Panretinal photocoagulation history</td>
<td>12/22</td>
<td>0/37</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as the mean ± SD or the median and range. P < 0.05 indicates statistically significant results compared to the corresponding data in the control group.

* Independent sample t-test.
† χ² test.

### Table 2. Baseline Patient Characteristics From Venous Blood Samples

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>PDR Group, n = 21</th>
<th>Control Group, n = 17</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>51.43 ± 1.89</td>
<td>50.18 ± 3.27</td>
<td>0.7307*</td>
</tr>
<tr>
<td>Female sex, n (%)</td>
<td>11 (52.4)</td>
<td>8 (47.1)</td>
<td>&gt;0.05†</td>
</tr>
<tr>
<td>Duration of diabetes, y</td>
<td>8.60 ± 1.47</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Hypertension</td>
<td>2/19</td>
<td>0/17</td>
<td>&gt;0.05†</td>
</tr>
<tr>
<td>Fasting blood glucose, mM</td>
<td>10.17 ± 0.62</td>
<td>6.49 ± 0.31</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Vitreoretinal condition</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitreous hemorrhage</td>
<td>9/12</td>
<td>0/17</td>
<td></td>
</tr>
<tr>
<td>Diabetic macular edema</td>
<td>5/16</td>
<td>0/17</td>
<td></td>
</tr>
<tr>
<td>Traction retinal detachment</td>
<td>5/16</td>
<td>0/17</td>
<td></td>
</tr>
<tr>
<td>Panretinal photocoagulation history</td>
<td>7/14</td>
<td>0/17</td>
<td></td>
</tr>
<tr>
<td>Serum soluble CD200, pg/mL</td>
<td>26.71 ± 4.32</td>
<td>19.94 ± 3.87</td>
<td>0.2614*</td>
</tr>
</tbody>
</table>

Data are expressed as the mean ± SD or the median and range. P < 0.05 indicates statistically significant results compared to the corresponding data in the control group.

* Independent sample t-test.
† χ² test.
respectively \( (P = 0.8669; \text{Fig. 2a}) \). In the PDR with PRP group and PDR without PRP group, the concentrations of sCD200 were \( 164.7 \pm 33.03 \) and \( 191.8 \pm 20.75 \) pg/mL, respectively \( (P = 0.4709; \text{Fig. 2d}) \).

Levels of VEGF and Its Receptors (sVEGFR-1 and sVEGFR-2) in Vitreous Samples

As shown in Table 3, VEGF, sVEGFR-1, and sVEGFR-2 were detected in all vitreous samples from PDR patients and nondiabetic control patients. Mean levels of VEGF, sVEGFR-1, and sVEGFR-2 in vitreous samples from PDR patients were \(3380 \pm 359\), \(35,510 \pm 3838\), and \(2465 \pm 161.2\) pg/mL, respectively, which were significantly higher than those in control group \( (497.8 \pm 183\) pg/mL, \(P < 0.0001\); \(372.5 \pm 54.46\) pg/mL, \(P < 0.0001\); \(533 \pm 70.02\) pg/mL, \(P = 0.0017\); and \(1491 \pm 167.1\) pg/mL, \(P < 0.0001\), respectively; Table 3).

Relationship of sCD200 With VEGF, sVEGFR-1, and sVEGFR-2 in Vitreous Samples

When the whole PDR group was considered, significant correlations were detected between sCD200 and VEGF \( (r = 0.6566, P < 0.0001; \text{Fig. 3a}) \), between sCD200 and sVEGFR-1 \( (r = 0.5574, P = 0.006; \text{Fig. 3b}) \), and between sCD200 and sVEGFR-2 \( (r = 0.3605, P = 0.0362; \text{Fig. 3c}) \).

Levels of the Angiogenic Chemokines in Vitreous Samples

Of all the angiogenic chemokines studied in Table 3, CCL1, CCL4, CCL8, CXCL5, and CXCL6 were not detected in all vitreous samples. Because CCL3 and CCL7 were detected in fewer than 50% of samples in both the PDR and control groups, they were not included in further analysis; CCL2, CXCL4, CXCL9, and CXCL10 were detected in all vitreous samples from the PDR and control groups. Mean levels of CCL2, CXCL4, CXCL9, and CXCL10 in vitreous samples from the PDR group were \(4560 \pm 248.0\), \(889.3 \pm 104.5\), \(1065 \pm 152.1\), and \(2565 \pm 180.1\) pg/mL, respectively, which were significantly higher than those in the control group \( (2233 \pm 222.4\) pg/mL, \(P < 0.0001\); \(372.5 \pm 54.46\) pg/mL, \(P < 0.0001\); \(533 \pm 70.02\) pg/mL, \(P = 0.0017\); and \(1491 \pm 167.1\) pg/mL, \(P < 0.0001\), respectively; Table 3).

Relationships of sCD200 With CCL2, CXCL4, CXCL9, and CXCL10 in Vitreous Samples

When the whole PDR group was considered, a significant correlation was detected between vitreous levels of sCD200 and CCL2 \( (r = 0.6001, P = 0.0002; \text{Fig. 4a}) \). No significant correlations were detected between sCD200 and CXCL4 \( (r = 0.3291, P = 0.0573; \text{Fig. 4b}) \), between sCD200 and CXCL9 \( (r = 0.1739, P = 0.3253; \text{Fig. 4c}) \), and between sCD200 and CXCL10 \( (r = 0.0573, P = 0.7475; \text{Fig. 4d}) \).

Levels of the Interleukin Family Members in Vitreous Samples

As shown in Table 3, IFN-\(\gamma\) was not detected in all vitreous samples; TNF-\(\alpha\) was detected in all vitreous samples from the PDR and control groups, but there was no significant difference between the PDR group \( (155.8 \pm 27.07\) pg/mL \(P = 0.5574, P = 0.006; \text{Fig. 3b}) \), and between sCD200 and sVEGFR-2 \( (r = 0.3605, P = 0.0362; \text{Fig. 3c}) \).
and the control group (106.7 ± 21.26 pg/mL; P = 0.1631). Of all the interleukin family members studied, IL-1β and IL-10 were detected in all vitreous samples from the control and PDR groups. Interleukin-6 and IL-8 were detected in all vitreous samples from the PDR group and 36 (97.3%) vitreous samples from the control group. Interleukin-6 was detected in 35 (97.1%) vitreous samples from the PDR group and 36 (97.3%) vitreous samples from control group. Mean levels of IL-6, IL-8, IL-10, and IL-18 in vitreous samples from the PDR group were 973.2 ± 115.4, 632.9 ± 76.65, 395.0 ± 41.50, and 569.1 ± 59.76 pg/mL, respectively, which were significantly higher than those in the control group (504.6 ± 59.66 pg/mL, P = 0.0004; 258.3 ± 36.29 pg/mL, P < 0.0001; 144.9 ± 22.99 pg/mL, P < 0.0001; and 249.0 ± 35.29 pg/mL, P < 0.0001, respectively; Table 3). There was no significant difference in mean level of IL-1β between the PDR group (39.43 ± 4.13 pg/mL) and the control group in Table 3 (35.29 pg/mL, P = 0.4603).

Relationships of sCD200 With IL-6, IL-8, IL-10, and IL-18 in Vitreous Samples

When the total patients in the PDR group were considered, there were significant correlations between sCD200 and IL-6 (r = 0.5704, P = 0.0004; Fig. 5a), between sCD200 and IL-8 (r = 0.3712, P = 0.0507; Fig. 5b), and between sCD200 and IL-10 (r = 0.3618, P = 0.0355; Fig. 5c). No significant correlation was detected between vitreous fluid levels of sCD200 and IL-18 (r = 0.0101, P = 0.9549; Fig. 5d).

DISCUSSION

For the first time, this study analyzed sCD200 expression levels in the vitreous of PDR patients and its relationships with different vitreoretinal conditions and other pro-inflammatory cytokines. The main findings were as follows: (1) sCD200 was significantly up-regulated in the vitreous of PDR patients; (2) there was no significant difference between the PDR group and control group, indicating that sCD200 could not leak into the vitreous from leaked blood vessels; (3) sCD200 levels were significantly elevated in PDR patient vitreous compared with those from PDR patients without DME or TRD; (4) the expressions of VEGF, VEGFR-1, and sVEGFR-2 in the vitreous fluid were found remarkably up-regulated from PDR patients (the expression of sCD200 was found to be positively correlated with those of VEGF, VEGFR-1, and sVEGFR-2 in vitreous fluid from PDR patients); (5) the angiogenic cytokines (CCL2, CXCL4, CXCL9, and CXCL10) in the vitreous of PDR patients were found significantly elevated (the vitreous levels of sCD200 and CCL2 were significantly correlated in PDR patients); and (6) the levels of interleukin family members (IL-8, IL-10, IL-6, and IL-18) were significantly elevated in the vitreous humor from PDR patients, and the interleukin expression was also found to be positively related with sCD200 in vitreous fluid from patients with PDR. These findings suggested that the sCD200-CD200R signaling axis might be a critical player in the pathogenesis and development of PDR.

CD200 is a novel immunosuppressive molecule that exists as a cell membrane-bound protein and also has a soluble form in serum (sCD200); it exerts pivotal function in adaptive immune responses. Recent studies have shown that CD200 is a membrane glycoprotein expressed in neurons that suppresses immune activity via interacting with CD200R, which is mainly located in macrophages/microglia.20 Interleukin-4 could regulate the expression of CD200 on neurons by interacting with CD200R and thus determine the activation state of microglia and consequently reduce the production of inflammatory mediators, such as IL-6 and IL-1β.21 In addition, IL-10 also could increase the expression of CD200 in neurons after endocannabinoid anandamide-induced pathologic neuro-inflammatory conditions, which is also involved in reducing the expression of IL-1β and IL-6.20

Recently, some preliminary clinical studies indicated that CD200 might be useful as a biomarker and therapeutic agent in autoimmune and inflammatory disorders.22 In bullosa pem-

![Figure 3](https://via.placeholder.com/150)

**Figure 3.** The correlations between sCD200 and VEGF (a), sVEGFR-1 (b), and sVEGFR-2 (c) in the PDR group. Each point represents a measurement from a single patient.
phigoid patients, the expression of sCD200 was found elevated in both serum and blister fluid, and anti-IgE monoclonal antibody therapy significantly suppressed sCD200 expression. In rheumatoid arthritis patients, significantly increased expression of CD200 was found in peripheral follicular helper T cells, which has a significantly positive correlation with the enhanced production of autoantibodies, disease severity, and the response to the treatment of biologic agents. In the cell membrane of bone marrow–derived CD11b\(^+\) monocytes from patients with wet AMD, CD200 expression was increased in the sickened eyes compared with the eyes from healthy controls.

The retina is an integral part of the neural system that shares the same principles of CD200/CD200R interaction to control neuroinflammation. Recent research described the participation of CD200R in the polarization of microglia in experimental autoimmune uveitis. Retinal microglia in CD200 knockout mice display normal morphology, but unlike the wild-type mice,
are present in increased expression of proinflammatory cytokines, such as nitric oxide synthase 2.25 Obtained data showed that CD200/CD200R activation may reduce retinal cell loss in experimental glaucoma.26 As of now, the underlying mechanisms in the development of PDR remain unclear. Accumulated evidence implicates that low-grade chronic inflammation is involved in the pathogenesis of PDR. This study showed that the intravitreal sCD200 expression was statistically significantly elevated in PDR patients compared with that in nondiabetic control patients. The CD200/CD200R interaction inhibits microglia accumulation in the central nervous system by reducing surface adhesion molecules expression. Moreover, CD200R activation by CD200Fc (CD200R1 agonist) is followed by down-regulated expression of proinflammatory cytokines (TNF-α, IL-1β, matrix metalloproteinase-13, and IFN-γ). To further explore the possible role of sCD200 in the pathogenesis of PDR, this study focused on the link between increased level of sCD200 and the activation of proinflammatory cytokines. The production of angiogenic chemokines (CCL2, CXCL4, CXCL9, and CXCL10) and interleukin family members (IL-6, IL-8, IL-10, and IL-18) was remarkably enhanced in the PDR group. A significant correlations were found regarding sCD200 levels and those of CCL2, IL-6, IL-8, and IL-10.

In PDR, ischemia-induced angiogenesis is dependent on several inflammatory molecules. A key player of this process is VEGF: VEGF exerts its functions on endothelial cells via interaction with VEGF-R1 (Flt-1) and VEGF-R2. A widely accepted theory is that VEGF-R1 activation influences the metabolism of angiogenesis molecules, while the endothelial cells migrate and proliferate via the activation of VEGF-R2.27 These receptors are the decisive signaling transducer for the abnormal neovascularization under pathologic conditions, such as cancer and diabetic retinopathy.28 Previous studies29,30 and this present study demonstrated significantly higher vitreous levels of VEGF, sVEGFR-1, and sVEGFR-2 in patients with PDR compared with nondiabetic patients. Moreover, this research also found that the vitreous levels of sCD200 and those of VEGF and their corresponding receptors in patients with PDR were significantly correlated. These findings of this present study and previous investigations suggested that CD200 might participate in inflammatory response in the pathogenesis of PDR.

Diabetic macular edema is the most prominent cause for vision loss in DR patients.31 The impairment of retinal microvessels lead to retinal ischemia and the collapse of the blood-retinal barrier. Consequently, DME occurs as a result of extravascular serous fluid accumulation.32,33 It is suggested that DME could be a result of a proinflammatory condition.34 Interleukin-6, VEGF, and other proinflammatory cytokines, synergistically working on intraocular tissues, are involved in the pathogenesis of DME.35 Previous studies showed that the aqueous levels of VEGF and IL-6 were significantly higher than their plasma levels and were associated significantly with the severity of DME.35 Those results are in accordance with this present study. In the current study, sCD200 levels in vitreous fluid from PDR patients accompanied with DME were significantly higher than those in PDR patients without DME. Moreover, a significantly positive correlation was observed between the vitreous levels of sCD200 and those of IL-6 and VEGF in patients with PDR. These results suggested that sCD200 might have a significant role in the pathogenesis of DME by modulating the release of IL-6, VEGF, and other proinflammatory cytokines.

This present study also showed that sCD200 levels in vitreous samples of PDR patients with TRD were significantly higher than those in PDR patients without TRD. However, there was no obvious difference in the mean levels of sCD200 between PDR patients with or without VH. These results indicated that there was a power correlation between sCD200 levels and clinical severity and sCD200 could of potential importance as a biomarker of PDR.

There were several limitations in this study. First, this study only collected a relatively small size sample. Second, preoperative use of antibiotic eye drops might have affected the vitreous concentrations of sCD200 and proinflammatory cytokines concentrations. Third, none of the vitreous samples from nonproliferative DR patients were collected, which could further show the correlation between sCD200 levels and clinical severity. Fourth, the findings of the vitreous levels of sCD200 and its positive correlations with proinflammatory cytokines and different vitreoretinal conditions do not allow us to draw the conclusions about a causal or temporal relationship between sCD200 and PDR. We do not know the exact mechanism of CD200 in disease initiation and/or progression.

**Conclusions**

In this study, sCD200 levels were found to be increased in the vitreous samples of PDR patients and correlated significantly with different vitreoretinal conditions (DME and TRD), VEGF and its receptors (sVEGFR-1 and sVEGFR-2), and other proinflammatory cytokines. The possible mechanisms by which the CD200-CD200R axis regulated inflammatory response in DR were showed in the following: Cytokines, such as IL-10 or IL-4, enhanced the intercellular interaction between CD200 and CD200R. The CD200R signaling acted to regulating the release of proinflammatory molecules, such as IL-6 and IL-8, which initiated microglial activation and subsequent neurodegeneration in DR. Further studies are needed to explore the possible molecular mechanisms of CD200 that contribute to inflammatory response and retinal angiogenesis in animal models of DR, which will help to determine the role of CD200 in the pathologic progression of PDR.

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**References**


