Distinct Profiles of Soluble Cytokine Receptors Between B-Cell Vitreoretinal Lymphoma and Uveitis

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Purpose. To determine the profiles of soluble cytokine receptors and cytokines, including mostly their ligands, in the vitreous humor of patients with B-cell vitreoretinal lymphoma (VRL) and uveitis.

Methods. Vitreous samples were collected from immunocompetent patients with VRL (n = 21), uveitis (n = 20), and idiopathic epiretinal membrane (n = 21) as controls. Cytometric beads assay were used to determine the vitreous concentrations of soluble receptors and cytokines.

Results. Vitreous levels of soluble IL-2 receptor α (sIL-2Rα), sIL-6R, soluble tumor necrosis factor receptor (TNFR) 1, sTNFR2, soluble vascular endothelial growth factor receptor (sVEGFR) 1, sVEGFR2, and IL-10 were higher in patients with VRL than in those with uveitis and controls, whereas those of sIL-1R1, sIL-1R2, and sIL-4R were higher in patients with uveitis than those with VRL and controls. In analyses in patients with VRL, elevation of sVEGFR1 and sVEGFR2 levels was more prominent in patients with systemic metastatic retinal lymphoma (SMRL) than in those with primary VRL/primary central nervous system lymphoma (PVRL/PCNSL). Furthermore, sIL-2Rα levels were increased in patients with VRL who developed subretinal lesions compared with in those who mainly had vitreous cavity opacity, positively correlated with the density of $CD3^+$ cells in the vitrectomy cell blocks.

Conclusions. The profiles of soluble cytokine receptors and cytokines in patients with VRL were different from those with uveitis. In addition, sVEGFR1 and sVEGFR2 levels may be differential diagnostic markers between PVRL/PCNSL and SMRL, and sIL-2Rα levels can anticipate infiltration of VRL cells into the subretina and/or retina.

Keywords: vitreoretinal lymphoma, systemic metastatic retinal lymphoma, subretinal infiltration, single-center study, noninfectious uveitis

The majority of vitreoretinal lymphoma (VRL) is related to a high-grade non-Hodgkin's lymphoma (NHL), which can be subtyped as a diffuse large B-cell lymphoma (DLBCL).[1] Vitreoretinal lymphoma often occurs in elderly immunocompetent patients and usually develops in the retina, vitreous chamber, and/or optic nerve.[2] Most VRL is primary or secondary to central nervous system (CNS) or may present simultaneously with it; however, it can also rarely be derived from systemic metastatic lymphoma.[1,3] After the onset of ocular symptoms, 60% to 90% of primary VRL patients develop CNS involvement, leading to a poor prognosis.[4,5] Because clinical features of VRL, termed as "masquerade syndrome," are often similar to chronic uveitis, a misdiagnosis of VRL sometimes leads to introducing anti-inflammatory agents, such as corticosteroids, resulting in a delay of the initial appropriate treatment and loss of visual acuity due to tissue destruction by tumor cells. Although morphologic analysis of cytology is necessary for a definitive diagnosis of VRL, the detection rate of malignant cytology is low due to necrosis and the fragility of VRL cells.[4] Further, vitreous samples of B-cell VRL contain not only B-cell lymphoma cells, but also a large numbers of reactive immune cells, including various subsets of benign T cells and macrophages, resulting in difficulty in VRL diagnosis.[4,6]

There are several complementary diagnostic techniques for VRL, including morphologic analysis of the vitreous samples in combination with additional immunohistochemistry or flow cytometry, molecular analysis using PCR for immunoglobulin-heavy chain (IgH) rearrangements to confirm monoclonality, and biochemical studies such as cytokine assays to determine an IL-10 to IL-6 (IL-10/IL-6) ratio.[4,7] Among several complementary diagnostic techniques, cytokine assays to assess an IL-10/IL-6 ratio greater than 1.0 in the vitreous samples are reported as the most sensitive test for the diagnosis of B-cell VRL.[5]

Several studies suggested that increased vitreous levels of soluble factors, including cytokines, in patients with primary VRL (PVRL) are involved in its pathogenesis of VRL.[8–10] However, the levels of some cytokines are sometimes barely detectable, as cytokines are easily degraded by calcium-dependent proteinases in the serum.[11] In addition, cytokines, such as IL-2 and TNF-α, are removed from the circulation rapidly.[12,13] Soluble cytokine receptors, which are generated in vivo, either by proteolytic cleavage of their membrane forms or

by alternative splicing, catch and stabilize their ligands to facilitate the interaction of cytokines with membrane-bound cytokines in an agonistic fashion.[14,15] However, it is also reported that soluble receptors can prevent cytokines from binding to their cognate membrane receptors as an antagonist.[16] Most soluble receptors are critical to the regulation of physiological and pathological responses, such as tumor growth and inflammation in cancers and autoimmune diseases.[17,18] Thus, the assessment of soluble receptors may lead to a better understanding of the pathogenesis of VRL and uveitis. In clinical analyses of patients with DLBCL, the levels of serum soluble receptors, such as soluble IL-2 receptor α (sIL-2Rα) and soluble tumor necrosis factor receptor 2 (sTNFR2), have been reported to be correlated with overall survival after treatment of chemotherapy,[19–21] suggesting that the assessment of soluble receptors is useful in the clinical use such as diagnosis markers and therapeutic targets for VRL and uveitis.

In this study, we determined the profiles of soluble receptors and cytokines in vitreous humor of patients with both VRL and uveitis. In addition, we assessed the association of several soluble receptors and cytokines with clinical parameters in patients with VRL.

Materials and Methods

Patients

This study was conducted in accordance with the guidelines of the Declaration of Helsinki and approved by the Kyushu University (Fukuoka, Japan) institutional review board for clinical research. We obtained written informed consent from all the participants before any study procedures or examinations were performed.

We examined 21 eyes of immunocompetent patients with VRL (all classified as DLBCL; 9 men and 12 women; mean age 66.0 ± 11.3 years), including 15 eyes of patients with PVRL/primary central nervous system lymphoma (PVRL/PCNSL) and 6 eyes of patients with systemic metastatic retinal lymphoma (SMRL), 20 eyes of patients with uveitis (6 men and 14 women; mean age 61.9 ± 9.49 years), and 21 eyes of patients with idiopathic epiretinal membrane (ERM; 7 men and 14 women; mean age 65.0 ± 6.30 years) as negative controls at the Department of Ophthalmology, Kyushu University Hospital between January 2008 and June 2015. All patients were Asian adults. The diagnosis of patients with VRL was based on the definitive identification of malignant lymphoid cells in the eye using either cytological smear or vitrectomy cell block technique. The patients with uveitis were diagnosed as having mostly noninfectious uveitis, 12 with ocular sarcoidosis, 7 with ocular Behçet disease, and 1 with uveitis of unknown etiology. The patients with preoperative trauma, pre-existing macular pathologies, vitreous hemorrhage, and diabetes mellitus, which are likely to affect the immune mediators in the vitreous humor, were excluded from the study. The following clinical data were extracted for VRL patients: age at diagnosis of VRL, sex, eye involved, main ocular lesions at initial diagnosis, primary organ involved, presence of brain involvement, relapses after initial diagnosis of malignant lymphoma, and current status (Table 1).

Cytology Specimens, Cytopathology, and Immunohistochemistry

Undiluted human vitreous samples (500–1000 μL) were collected during 23-G pars plana vitrectomy, prior to intraocular fluid infusion as previously described.[22] The samples were immediately placed in sterile tubes and were centrifuged at 800*g* for 10 minutes; and the supernatants were divided into aliquots for assays for soluble receptors and cytokines. The concentrated cells were immediately delivered at the department of pathology for cytological examination. The following classifications were used for cytological analysis as previously described.[5] "Negative" was defined as the absence of typical malignant cells, or the presence of abnormal cells or atypical cytology, but with no evidence of malignancy. "Suspicious" was defined as cytology suggestive of, but not conclusive for malignancy. "Positive" was defined as cytology strongly suggestive of, or conclusive for malignancy.

Furthermore, immunohistochemical diagnoses were also performed using vitrectomy cell block technique as previously described.[23] Vitreous fluid was transferred to one or two 50-mL tubes and centrifuged at 800*g* for 20 minutes. The pellets were suspended and fixed with 1 mL of 10% formalin solution. The final pellet was embedded in paraffin. Paraffin sections were then cut and deparaffinized with xylene and a graded ethanol series. The sections were stained with hematoxylin-eosin. For immunohistochemistry, the sections were incubated with 3% hydrogen peroxide for 5 minutes to inactivate endogenous peroxidase, and blocked with 10% normal goat serum for 10 minutes. In most cases, the sections were then incubated with primary antibodies against CD3, CD20, CD79a, leukocyte common antigen (LCA), and MIB-1 (Dako, Carpinteria, CA, USA) overnight at 4°C, washed with 0.05% Tween 20-containing phosphate buffered saline (T-PBS) three times, stained with the secondary antibody at room temperature for 30 to 60 minutes, and washed with T-PBS three times. The color was developed with diaminobenzidine, and the nuclei were counterstained with hematoxylin.

The ratios of T cells, represented by the density of $CD3^+$ cells/LCA^+ cells, were determined in three areas under a high-power field (×400) as previously described with some modification.[24] $CD3^+$ cells were counted by two blinded observers.

Measurement of Soluble Receptors

After diluting 5× the above-mentioned supernatants collected from the vitreous body with PBS, the concentration of each of the following soluble receptors and cytokines were measured using Milliplex MAP Human Soluble Cytokine Receptor Panel – Immunology Multiplex Assay (Millipore, St. Charles, MO, USA), Human Cytokine/Chemokine Panel 1, or Human Angiogenesis panel 1 with Luminex 100 (Luminex, Austin, TX, USA), according to the manufacturer's directions: human soluble CD30 (sCD30), sIL-1 receptor 1 (sIL-1R1); sIL-1R2; sIL-2Rα; sIL-4R; sIL-6R; sgp130; soluble receptor for advanced glycosylation end products (sRAGE); sTNFR1; sTNFR2; soluble vascular endothelial growth factor receptor (sVEGFR) 1; sVEGFR2; sVEGFR3; IL-6; IL-10; IL-1β; TNF-α; placenta growth factor (PlGF); VEGF-A; VEGF-C; and VEGF-D. Levels of soluble receptors and cytokines were obtained from the standard curve for each soluble receptors and cytokines. When the concentrations of the raw data were below the detection limit, they were coded as 0 and were included in statistical analysis.

Statistical Analysis

The data were analyzed using Jump version 10 software (Business Unit of SAS, Cary, NC, USA). If the data were normally and equally distributed, a one-way ANOVA was used to compare the vitreous concentrations of each cytokine among the groups, followed by Turkey–Kramer test to detect significant differences between VRL, uveitis, and controls. If the data were not distributed normally or equally distributed, the Kruskal–Wallis test was performed to compare the vitreous

Table 1. Clinical Data of Patients With VRL

Case No.	Sex	Age at Diagnosis of B-Cell Lymphoma, y	Primary Origin	Eye Involved	Main Ocular Lesions at Initial Diagnosis	Brain Involved	Relapse, mo After Initial Diagnosis	Outcome
1	F	51	Eye	OU	Subretina and vitreous	Yes	Brain; 16 mo	Died 38 mo
2	F	68	Eye	OU	Vitreous	No	No relapse	Alive
3	F	55	Eye	OU	Subretina and vitreous	No	No relapse	Alive
4	M	63	Brain	OU	Subretina and vitreous	Yes	Eye; 10 mo	Alive
5	M	61	Brain	OU	Vitreous	Yes	Eye; 20 mo	Alive
6	F	80	Eye	OS	Subretina and vitreous	Yes	Brain; 12 mo	Alive
7	F	80	Brain	OS	Subretina and vitreous	Yes	Eye; 17 mo	Died 44 mo by pancreatic ca.
8	M	60	Eye	OU	Vitreous	Yes	Brain; 25 mo	Lost to follow-up
9	M	45	Eye	OU	Subretina and vitreous	Yes	Brain; 48 mo	Died 70 mo
10	M	69	Brain	OU	Vitreous	Yes	Eye; 72 mo	Died 120 mo
11	F	73	Brain and eye	OU	Vitreous	Yes	Brain; 16 mo	Died 25 mo
12	M	59	Brain	OU	Vitreous	Yes	Eye; 33 mo	Lost to follow-up
13	M	38	Brain	OS	Subretina and vitreous	Yes	Eye; 6 mo	Alive
14	F	69	Brain	OU	Vitreous	Yes	Eye; 35 mo	Alive
15	F	67	Eye	OU	Vitreous	Yes	Brain; 4 mo	Alive
16	F	75	Abdominal LN and eye	OS	Subretina and vitreous	No	No relapse	Alive
17	F	73	Nose, Paranasal sinus	OU	Vitreous	Yes	Eye; 120 mo Brain; 102 mo	Alive
18	M	78	Chest wall	OU	Vitreous	Yes	Eye; 18 mo Brain; 24 mo	Alive
19	M	68	Testis	OS	Subretina and vitreous	Yes	Eye; 108 mo Brain; 96 mo	Died 132 mo
20	F	74	Breast	OU	Subretina and vitreous	No	Eye; 59 mo	Died 30 mo, but not due to ML
21	F	79	Intestine	OU	Vitreous	No	Eye; 19 mo	Alive

F, female; M, male; OU, both eyes; OS, left eye; ca., carcinoma; LN, lymph node; ML, malignant lymphoma.

levels of each soluble receptor and cytokine, followed by Steel–Dwass test to detect significant differences between the three groups. Two-group comparisons of numerical variables were performed using the Mann–Whitney *U* test. To determine a significant correlation existed between T-cell density and sIL-2Rα levels, Spearman correlation tests were used. A *P* value less than 0.05 was considered to be significant.

Results

Vitreous Levels of Soluble Receptors and Cytokines

The data of vitreous levels of soluble receptors and cytokines obtained from patients with controls, VRL, and uveitis are summarized in Table 2. Soluble IL-2Rα, sIL-6R, sgp130, sTNFR1, sTNFR2, sVEGFR1, sVEGFR2, IL-6, and IL-10 levels were significantly different among patients with controls, VRL, and uveitis (Table 2; $P < 0.001$, respectively). Soluble IL-2Rα, sIL-6R, sTNFR1, sTNFR2, sVEGFR1, sVEGFR2, and IL-10 levels were significantly higher in patients with VRL than those with uveitis and controls ($P < 0.05$), whereas sIL-1R1, sIL-1R2, and sIL-4R levels were higher in patients with uveitis than those with VRL and controls ($P < 0.01$). Interleukin-6 and sgp130 levels were increased in patients with VRL and uveitis compared with controls ($P < 0.01$), but comparable in patients between VRL and uveitis. Tumor necrosis factor-α, VEGF-A, PlGF, and VEGF-C levels were mostly below detection limit and were not significantly different between the three groups. Soluble CD30, sRAGE, sVEGFR3, IL-1β, and VEGF-D levels in each group were under the detection limit (data not shown).

Increased Vitreous Levels of sVEGFR1 and sVEGFR2 in Patients With SMRL

Although SMRL is a rare disease,[3] accumulation of patients with SMRL was observed at our institute because there have been specialists for ocular oncology, uveitis, or hematologic oncology as a foundation hospital in Kyushu region in Japan (Table 1). The phenotype of PCNSL cells is reported to differ from that of non-PCNSL cells.[25,26] Therefore, we compared sIL-2Rα, sIL-6R, sgp130, sTNFR1, sTNFR2, sVEGFR1, sVEGFR2, IL-6, and IL-10 levels in patients with SMRL to those with PVRL/PCNSL. Both sVEGFR1 and sVEGFR2 levels in patients with SMRL were significantly increased compared with those with PVRL/ PCNSL (Fig. 1A; $P < 0.001$ and $P = 0.0057$, respectively), whereas levels of sIL-2Rα, sIL-6R, sgp130, sTNFR1, sTNFR2, IL-6 (data not shown), and IL-10 (Fig. 1B) were not. Soluble VEGFR1 levels were increased in the conditioned media from human umbilical vein endothelial cells (HUVEC) or DLD-1 cells, a colon carcinoma cell line, as positive controls for sVEGFR1 (Supplementary Figs. S1A, S1B), whereas sVEGFR2 levels were not detected (data not shown).

Elevation of Vitreous sIL-2Rα Levels in Patients With VRL Who Developed Subretinal Lesions and Vitreous Opacity

In patients with VRL, we investigated the association of vitreous levels of sIL-2Rα, sIL-6R, sgp130, sTNFR1, sTNFR2, sVEGFR1, sVEGFR2, IL-6, and IL-10 with clinical parameters, including main lesions at initial diagnosis, brain involvement, or relapse. Soluble IL-2Rα levels were significantly higher in

Table 2. Concentrations of Soluble Receptors and Cytokines in the Vitreous Humor

	Control, $n = 21$	VRL, $n = 21$	Uvetis, $n = 20$	*P* Value
sIL-1R1 (ng/mL)	0 (0–0.826)	0 (0–0)¶	1.23 (0.986–1.37)§	<0.001*
sIL-1R2 (ng/mL)	0 (0–0)	0 (0–0)¶	2.66 (1.08–4.13)§	<0.001*
sIL-2Rα (ng/mL)	0 (0–0)	3.40 (1.35–11.1)§,||	0.630 (0.098–2.55)§	<0.001*
sIL-4R (ng/mL)	0 (0–0)	0 (0–0)¶	1.35 (1.17–1.63)§	<0.001†
sIL-6R (ng/mL)	2.01 (1.10–3.09)	5.94 (3.61–13.3)§	3.37 (1.85–5.13)	<0.001*
sgp130 (ng/mL)	42.8 (27.7–53.9)	74.7 (45.7–105.0)§	92.0 (75.8–112.3)§	<0.001*
sTNFR1 (ng/mL)	1.40 (0–2.80)	9.55 (5.45–16.0)§,||	3.22 (2.06–4.58)‡	<0.001*
sTNFR2 (ng/mL)	4.32 (2.51–5.95)	92.3 (28.8–184.8)§,¶	14.1 (7.41–32.9)§	<0.001*
sVEGFR1 (ng/mL)	153.5 (54.1–198.8)	404.3 (247.3–792.5)§,¶	118.5 (101.1–141.0)	<0.001*
sVEGFR2 (ng/mL)	17.3 (14.5–21.9)	41.4 (22.2–58.3)§,¶	14.4 (13.3–16.6)	<0.001*
IL-6 (ng/mL)	0 (0–0.177)	0.853 (0.344–1.65)§	1.32 (0.695–1.68)§	<0.001*
IL-10 (ng/mL)	0.020 (0–0.118)	57.2 (20.5–110.1)§,¶	0.089 (0.009–0.225)	<0.001*
TNF-α (pg/mL)	0 (0–0)	0 (0–0)	0 (0–0)	0.26*
VEGF-A (pg/mL)	0 (0–1036.6)	0 (0–1068.7)	0 (0–1098.3)	0.80*
PlGF (pg/mL)	0 (0–114.3)	145.7 (0–269.9)	155.6 (0–602.8)	0.064*
VEGF-C (pg/mL)	0 (0–41.1)	0 (0–1099.2)	0 (0–532.3)	0.28*

Soluble receptors and cytokines are expressed as median with interquartile range in parentheses. R, receptor.
* Kruskal–Wallis test.
† One-way ANOVA test.
‡ < 0.05 vs. control.
§ < 0.01 vs. control.
|| < 0.05 vs. uveitis.
¶ < 0.01 vs. uveitis: Steel–Dwass test or Turkey–Kramer test.

patients with VRL who had subretinal lesions and vitreous opacity than in those who mainly had vitreous opacity (Fig. 2A; $P = 0.0027$). In addition, to evaluate T-cell invasion in the vitreous body, the density of $CD3^+$ cells in vitrectomy cell blocks was compared between the two groups. The ratios of $CD3^+$ cells were significantly higher in patients with VRL who had subretinal lesions and vitreous opacity than in those who mainly had vitreous opacity (Fig. 2C; $P = 0.0021$). There was a strong correlation between the density of $CD3^+$ cells and the vitreous levels of sIL-2Rα in patients with VRL ($r = 0.7255$, $P = 0.0015$; Spearman correlation coefficient: Fig. 2D). In contrast, levels of sIL-6R, sgp130, sTNFR1, sTNFR2, sVEGFR1, sVEGFR2, IL-6 (data not shown), and IL-10 (Fig. 2B) were not different with or without subretinal lesions. There was no significant association of the levels of the factors with the other clinical parameters, such as brain involvement and relapse (data not shown).

Discussion

In this study, we identified differential profiles of soluble receptors and cytokines, including mainly their ligands, between patients with VRL and uveitis, providing a new insight for and a better understanding of the pathogenesis of both VRL and uveitis.

Vascular endothelial growth factor–A is reported to have potent mitogenic activity for DLBCL cells through VEGFR1 and VEGFR2 pathways.[27] Although VEGF-A is an important proangiogenic factor, the role of angiogenesis in the progression of lymphomas has not been established.[6] Recently, it was shown that sVEGFR1 is generated by tumor necrosis-α–converting enzyme (TACE)/a disintegrin and metalloproteinase (ADAM) 17–dependent proteolytic cleavage of VEGFR1 on the surface of lymphoma cell lines derived from DLBCL, leading to the trapping and inhibition of VEGF-A and PlGF function, including tumor proliferation and migration.[28] Additionally,

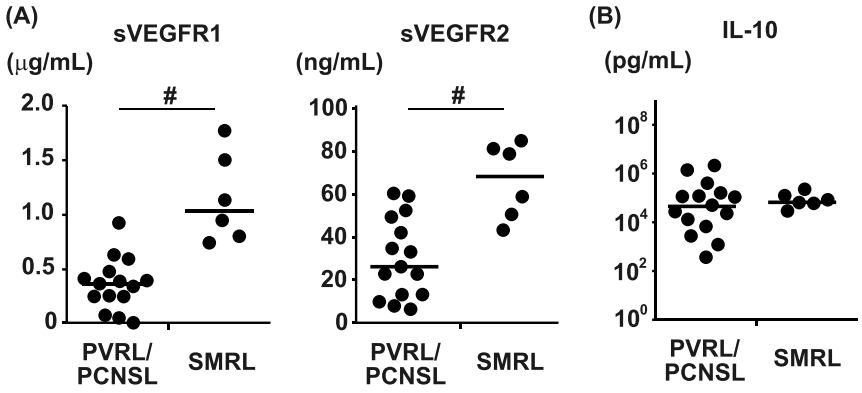

Figure 1. Vitreous levels of sVEGFR1 and sVEGFR2 in patients with SMRL. Shown are comparison of (**A**) sVEGFR1, sVEGFR2, and (**B**) IL-10 levels in the vitreous humor between patients with SMRL ($n = 6$) and PVRL/PCNSL ($n = 15$). The *horizontal lines* show the median concentration. The ordinate showed the concentrations of (**B**) IL-10 in the log scale. #$P < 0.01$; Mann–Whitney *U* test.

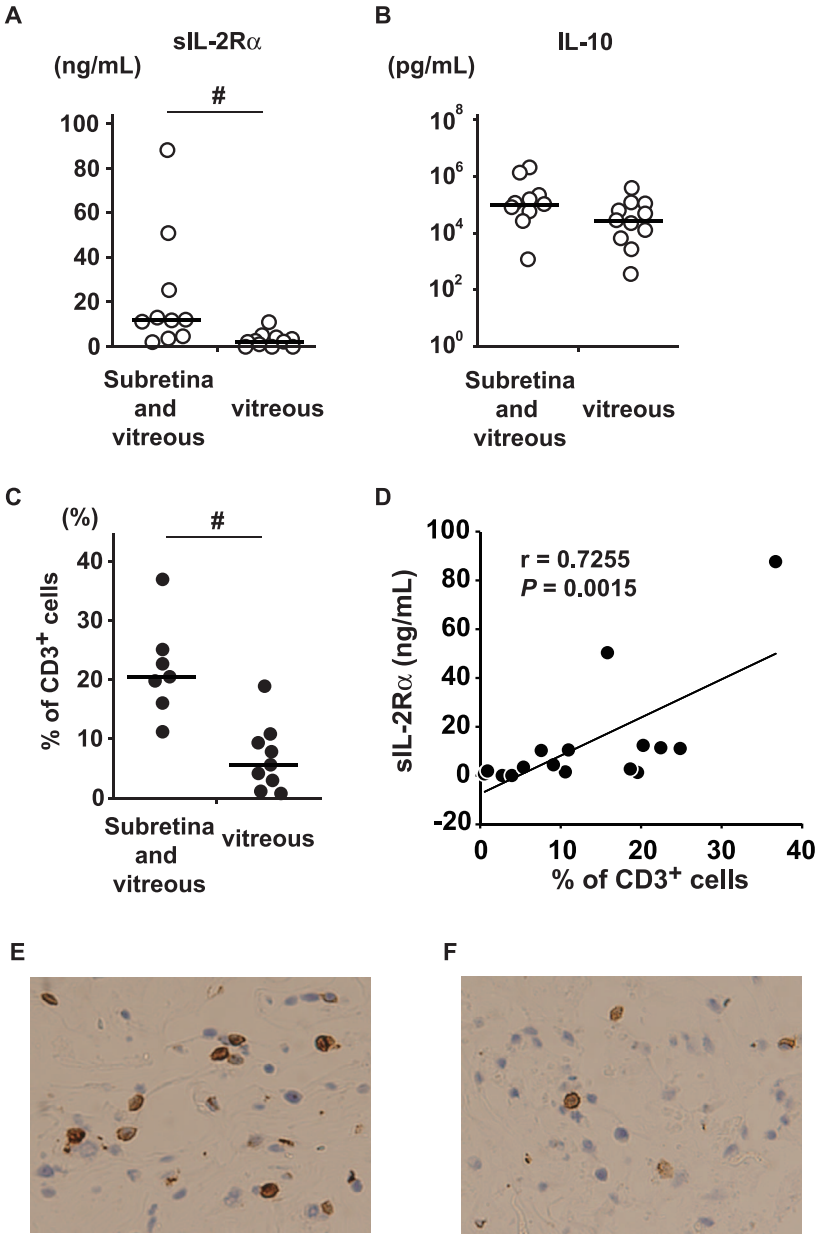

FIGURE 2. Vitreous levels of sIL-2Rα in patients with VRL who developed subretinal lesions. (**A**) sIL-2Rα and (**B**) IL-10 were compared in vitreous humor in patients with VRL who developed subretinal lesions and vitreous opacity ($n = 10$) and in those who predominantly did vitreous opacity ($n = 11$). The *horizontal lines* show the median concentration. The ordinate showed the concentrations of (**B**) IL-10 in the log scale. (**C**) Comparison of the percentages of T cells in the vitrectomy cell blocks in patients with VRL who developed subretinal lesions and vitreous opacity ($n = 7$) to in those who predominantly did vitreous opacity ($n = 9$). (**D**) Correlations between vitreous concentrations of sIL-2Rα and T-cell densities in vitrectomy cell blocks in patients with VRL ($n = 16$). (**E**, **F**) Representative images of immunohistochemistry of $CD3^+$ cells in vitrectomy cell blocks in a patient with VRL who developed subretinal lesions and vitreous opacity ([**E**], Case 3) and that who predominantly did vitreous opacity ([**F**], Case 18). $^{\#}P < 0.01$; Mann–Whitney *U* test.

elevated expression of VEGFR1 in DLBCL cells is reported to be associated with improved overall survival.[29] Therefore, we propose that increased sVEGFR1 levels partially contribute to the direct inhibition of DLBCL cell development in the eyes of VRL patients.

Soluble VEGFR2 is a natural antagonist for VEGF-C, which is involved in tumor invasion and metastasis.[30,31] Although elevation of serum sVEGFR2 levels is reported to be associated with an increased future risk of NHL,[32] the roles of sVEGFR2 have not been clearly defined in NHL. Immunohistochemical analysis of the samples in patients with DLBCL showed that the expression of VEGF-C was detected in lymphoma and endothelial cells in blood and lymphatic vessels,[33] indicating that sVEGFR2 might play a role in the infiltration of DLBCL cells into the retina. Recently, it is reported that a morpholino that shifts expression of membrane-bound VEGFR2 to sVEGFR2 by targeting alternative polyadenylation can inhibit VEGF-A–dependent ocular and tumor angiogenesis as well as lymphangiogenesis in murine models.[34,35] Thus, these results suggest that sVEGFR2 prevents VEGF-A–dependent DLBCL cell growth, although it is not clear whether expression of sVEGFR2 is mediated by an alternative VEGFR2 mRNA splicing

or proteolytic cleavage of VEGFR2 in B-cell VRL. Further studies are required to elucidate the mechanism of sVEGFR2 induction and roles of sVEGFR2 in B-cell VRL.

Several studies revealed that PCNSL and non-PCNSL phenotypes are distinct.[1,25,26] Jahnke et al.[36] found that expression patterns of chemokine receptors for B-cells, including CXCR4, CXCR5, and CCR7, are different between PCNSL and peripheral B-cell lymphoma because their expression of chemokine receptors is confined to the cytoplasm in PCNSL, whereas the expression in peripheral B-cell lymphoma, it is in both the membrane and the cytoplasm. Therefore, these results suggest that there are some differences in the expression patterns of sVEGFR1 and sVEGFR2 between PCNSL and non-PCNSL cells. Furthermore, the measurement of sVEGFR1 and/or sVEGFR2 levels in vitreous humor is helpful in differential diagnosis of SMRL other than PVRL/PCNSL.

Because the discovery of T-lymphocytes invasion into tumors is a common phenomenon, several studies have shown that the magnitude and composition of the intratumoral T-cell infiltrate can predict the disease course of several human tumors.[37] In patients with VRL, it is reported that vitreous specimens contain a mixture of reactive T cells as well as lymphoma cells.[38] Soluble IL-2Rα, which is generated by the enzymatic cleavage of IL-2Rα (CD25), competes with activated T cells for IL-2 binding, leading to the prevention of growth of T cells, including $CD8^+$ T cells, which possess antitumor activity.[39] Furthermore, the complex of sIL-2Rα and IL-2 is reported to promote IL-2-dependent $CD4^+CD25^+$ regulatory T-cell (Treg) development and function, which involves inhibition of $CD8^+$ T-cell action, resulting in DLBCL-cell growth.[15] In the analysis of tumor specimens in patients with DLBCL, not only an increased frequency of Treg cells, but also a low prevalence of T-helper (Th) 17 cells, were observed because IL-2 mediates reciprocal Treg and Th17 cell differentiation.[40,41] In murine models of B-cell VRL, it is reported that intravitreal injection of B-cell lymphoma cells induces infiltration of $CD4^+$ and $CD8^+$ T cells into the vitreous body, retina, and choroid, closely contacted with B-cell lymphoma cells.[42] Thus, these results suggest that increased vitreous levels of sIL-2Rα in patients with B-cell VRL reflect massive infiltration of reactive T cells and lymphoma cells into the retina and/or subretina with ongoing tissue destruction of neural retina. In addition, the assessment of sIL-2Rα levels in the vitreous humor contributes to early detection and early treatment of B-cell VRL cells in the retina and/or subretina to maintain visual acuity.

Tumor necrosis factor–α is a pleiotropic cytokine that contributes to the immune responses to protect a localized area from invasion or injury, but it is also involved in whether target cells live or die.[43] Both TNFR1 and TNFR2 can mediate TNF-α–dependent proinflammatory responses, although there are some difference in the structure, functions, and expression patterns between TNFR1 and TNFR2.[44] Both sTNFR1 and sTNFR2, which arise because of shedding through proteolytic processing in response to a variety of inflammatory stimuli, can inhibit TNF-α bioactivities.[14] In clinic, TNF inhibitors are reported to be associated with increased risk for lymphomas,[45] suggesting that both sTNFR1 and sTNFR2 play a suppressive role in not only antitumor immunity but also TNF-α–dependent DLBCL cell apoptosis in patients with VRL. However, another group reported that low but more than slightly elevated amounts of sTNFR1 and sTNFR2 act as agonists of TNF-α by stabilizing and facilitating with the interaction of TNF-α with membrane-bound TNF receptors.[14] Sugita et al.[46] found that low but more than slightly elevated levels of sTNFR1 and sTNFR2 could induce TNF-α production in ocular infiltrating T-cells in patients with active uveitis. Thus, either sTNFR1 or sTNFR2 can elicit TNF-α–dependent proinflammatory responses in VRL.

Even though the expression of IL-6R is limited in specific cells, the complex of IL-6/sIL-6R can transmit its signaling on IL-6R–negative and gp130-positive cells due to ubiquitous expression of gp130, termed as IL-6/sIL-6R transsignaling. In lymphoproliferative disorders, including DLBCL, the IL-6/sIL-6R complex is reported to be a potent growth factor for tumors as well as proinflammatory responses.[47] Interleukin-6 and sIL-6R levels were increased in patients with VRL, suggesting that the IL-6/sIL-6R complex promotes DLBCL cell growth and inflammation in patients with VRL. In addition, levels of sgp130, which act as an antagonist of IL-6/sIL-6R transsignaling,[48] were elevated in patients with uveitis and VRL to suppress IL-6–dependent not only proinflammatory responses but also VRL cell growth.

Th17 cell responses are reported to play a pivotal role in the pathogenesis of uveitis.[49,50] Several studies have revealed that IL-1β and TGF-β, but not IL-6, promotes human Th17 cell differentiation.[41] Soluble IL-1R2, which is also shed by TACE/ADAM17, is considered as a decoy receptor to neutralize IL-1β and pro–IL-1β,[51] indicating that elevation of IL-1R2 expression in patients with uveitis might reflect inhibition of excess of Th17 responses. In contrast, increased levels of sIL-1R1, which binds with IL-1 receptor antagonist (IL-1RA), a natural antagonist of IL-1β,[52] were observed in patients with uveitis, but not in those with VRL. Therefore, because sIL-1R1 neutralizes IL-1RA action, it is likely that sIL-1R1 could promote Th17 cell differentiation in uveitis. Moreover, sIL-4R, which is generated, either by proteolytic cleavage of their membrane forms or by alternative splicing, is reported as an inhibitor of IL-4, a key regulator for Th2 responses.[53,54] Interleukin-4 can inhibit Th17 cell differentiation and reactivation of committed Th17 cells, suggesting that sIL-4R blocks IL-4 action to elicit Th17 responses in the eyes of patients with uveitis.

From the multiplex bead analyses of vitreous samples in patients with VRL and uveitis, we determined that some soluble receptors act on tumor growth, and others are able to elicit and/or suppress immune responses in the eye. However, in this study, we were not able to clarify the association of soluble receptors with brain involvement and relapse, which is one of the most critical life-threatening events, due to the limitations, including smaller sample size and shorter observation period. Larger series with a longitudinal study are required to elucidate the further association of soluble receptors and cytokines with the pathogenesis and clinical outcome in patients with B-cell VRL and uveitis. The potential targeting of immune mediators such as soluble receptors and cytokines warrants further research to develop a new diagnostic and treatment strategies.

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