Immune Mediators in Vitreous Fluids from Patients with Vitreoretinal B-Cell Lymphoma

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Purpose. Various immune mediators are hypothesized to have important roles in the pathogenesis of vitreoretinal B-cell lymphoma, although the exact mechanisms remain unclear. We determined the immune mediator profile in the vitreous of eyes with vitreoretinal B-cell lymphoma.

Methods. We studied 28 eyes (23 patients) with vitreoretinal B-cell lymphoma, and 27 eyes (27 patients) undergoing vitrectomy for macular hole and epiretinal membrane served as controls. Undiluted vitreous samples were collected, and cytometric bead array and ELISA were used to determine the vitreous concentrations of 38 immune mediators, including 14 interleukins (IL); interferon (IFN)-γ; oncostatin M (OSM); IFN-γ-inducible protein (IP)-10; monocyte chemoattractant protein (MCP)-1; macrophage inflammatory protein (MIP)-1α; MIP-1β, regulated on activation, normal T-cell expressed and secreted (RANTES); monokine induced by IFN-γ (Mig); stromal cell-derived factor (SDF)-1α; B-cell-attracting chemokine (BCA)-1; basic fibroblast growth factor (bFGF); Fas ligand; granzyme A; and granzyme B.

Results. Vitreous levels of BCA-1, bFGF, Fas ligand, granzyme A, granzyme B, IFN-γ, IL-6, IL-8, IL-10, IP-10, MCP-1, Mig, MIP-1α, MIP-1β, OSM, RANTES, and SDF-1α were significantly higher in vitreoretinal B-cell lymphoma patients than in controls. A moderate-to-strong positive correlation was observed between granzyme A and BCA-1, IFN-γ, or MIP-1β; between IFN-γ and Mig or SDF-1α; between IL-6 and IL-8, IL-10, IP-10, or MCP-1; between IL-8 and MCP-1, Mig, or MIP-1β; between IL-10 and MCP-1 or MIP-1α; between Mig and IP-10 or Mig; and between MIP-1α and MIP-1β.

Conclusions. Our study suggested that elevated vitreous levels of various immune mediators inducing growth, migration, and apoptosis of B-cell lymphoma are involved possibly in the pathophysiology of vitreoretinal B-cell lymphoma. (*Invest Ophthalmol Vis Sci.* 2012;53:5395–5402) DOI:10.1167/iovs.11-8719

Vitreoretinal lymphoma usually is a form of non-Hodgkin's lymphoma of the diffuse large B-cell type (DLBCL),[1] but may be T-cell type in rare cases. Vitreoretinal lymphoma often occurs in elderly patients who present initially with an ocular lesion with or without concomitant central nervous system (CNS) involvement. Most vitreoretinal lymphomas are extranodal localized to intraocular structures, such as retinal or subretinal spaces and vitreous.[2] CNS involvement appears in 50–80% of vitreoretinal lymphoma patients several years after the onset of ocular symptoms.[3,4] Once CNS lymphoma occurs, if untreated, death generally occurs within 3 months as a result of progressive intracranial disease,[5] and a 5-year survival rate below 63% was reported in a clinical series in Japan.[6]

The pathogenesis of vitreoretinal lymphoma is understood poorly. In particular, it remains unclear why vitreoretinal lymphoma mainly manifests diffuse vitreous opacities and multiple retinal or subretinal white lesions. Currently, the underlying molecular mechanism by which malignant B-cells home to immune privileged sites, such as the brain, eye, and testis, where resident B-cells are not present under normal conditions, essentially is unknown. Furthermore, although the lymphoma spreads aggressively within the CNS, it is widely known that it rarely metastasizes elsewhere in the body. Chan et al. speculated that malignant B-cells acquire selective homing receptors for ligands expressed by ocular tissues, especially the RPE.[7] It also is postulated that the expression of B-cell-attracting chemokine (BCA)-1 and stromal cell-derived factor (SDF)-1 on RPE may enhance lymphoma cell migration and signal transduction into the malignant cells, promoting lymphoma growth and survival. Proof of this hypothesis will enhance our understanding of vitreoretinal B-cell lymphoma conditions.

An elevated interleukin (IL)-10:IL-6 ratio in ocular fluids is essential to establish a diagnosis of vitreoretinal B-cell lymphoma.[8] IL-10 is a pleiotropic cytokine produced by B- and T-cells as well as monocytes,[9] which acts as a growth factor for normal human B-cells and B-cell lymphoma cells. Besides its growth factor activity, IL-10 protects the tumor from the immune system.[10] With respect to the recruitment, homing, and proliferation of lymphocytes within the eye, many studies have shown that immune mediators, such as cytokines and chemokines, have a pivotal role.[1] Immune mediators are suspected to be important in the pathogenesis of vitreoretinal B-cell lymphoma via autocrine and/or paracrine mechanisms. Better understanding of the role of immune mediators may provide new clues to immunopathogenesis and effective treatment of vitreoretinal B-cell lymphoma.

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Recently, two-color flow cytometry has been used for simultaneous detection of many analytes in a very small sample volume.[11] Previous studies on cytokines or chemokines in vitreoretinal lymphoma have focused on quantification of a few cytokines or chemokines using ELISA.[7,12] However, single-assay measurements of immune mediators provide limited information, and to obtain a more comprehensive picture of vitreoretinal B-cell lymphoma, multiple immune mediators must be analyzed simultaneously. Moreover, Maier et al. compared the multiplex cytometric bead array (CBA) and ELISA, and reported that CBA technology is comparable to conventional ELISA assay for measurements of immune mediators in the vitreous.[11] To determine the levels of various immune mediators in vitreoretinal B-cell lymphoma, we simultaneously measured a broad spectrum of immune cell-mediated cytokines, chemokines, growth factors, and molecules that could contribute to cell death, by using a multiplex CBA system combined with traditional ELISA. With these methods, we investigated the protein expression levels of 38 different immune mediators in ocular fluid samples obtained during vitrectomy for vitreoretinal B-cell lymphoma.

Understanding the mechanisms that mediate B-cell migration, proliferation, and apoptosis, as well as immunologic interaction between tumor cells and the surrounding reactive inflammatory cells recruited to the eye are important goals in research on the biology of vitreoretinal B-cell lymphoma. Our study addresses the pattern of expression of a set of immune mediators in vitreoretinal B-cell lymphoma, which may indicate the potential roles of these molecules in the pathophysiology of vitreoretinal B-cell lymphoma. The difference between the type of vitreoretinal B-cell lymphoma with diffuse vitreous opacities only and the type with concomitant presence of multiple subretinal white lesions also was assessed.

Materials and Methods

Patients, Sample Collection, and Diagnosis

We studied 23 immunocompetent patients (9 males and 14 females, mean age 66.0 ± 11.7 years) with vitreoretinal B-cell lymphoma with or without CNS involvement at the time of diagnosis. Our study was approved by the Tokyo Medical University institutional review board. Informed consent was obtained according to the Declaration of Helsinki. All patients were Asian adults. In each patient, systemic lymphoma was excluded by clinical staging of the disease. All patients underwent magnetic resonance imaging (MRI) to evaluate the brain, as well as a standard 3-port 23-gauge vitrectomy. Vitreous samples were harvested from the mid vitreous region at the start of vitrectomy. The vitreous was removed by a vitreous cutter before intraocular infusion. Then, a complete vitrectomy with infusion of balanced salt solution was performed. Undiluted specimens and diluted vitreous specimens were delivered immediately to the Cytology and Molecular Laboratory. The samples were stored immediately at −80°C until assayed. Sample volumes ranged between 500 μL and 1 mL. All patients gave informed consent to the collection and analysis of samples.

The diagnosis of vitreoretinal B-cell lymphoma was established on the basis of clinical, morphologic, cytochemical, gene rearrangement, and immunologic features. Cytopathologic and immunohistochemical evaluations were performed on the undiluted vitreous specimen to evaluate features of the lymphoma. In brief, undiluted ocular fluid was centrifuged (200*g*, room temperature, 3 minutes) within 1 hour after sample collection onto a single glass slide, air-dried, and stained with Papanicolaou staining and/or anti-CD20 mAb. Two experienced cytopathologists (TN and TN) independently examined all cytospins. The specimens were diagnosed without knowledge of the findings of immunoglobulin heavy (IgH) chain gene rearrangement, and concentrations of IL-10 and IL-6. Undiluted vitreous specimens also were used in measurements of mediators. Diluted vitreous samples were prepared for PCR analyses. B-cell clonal expansion was detected by analysis of IgH chain gene rearrangement using PCR (SRL, Tokyo, Japan). Samples were subjected to amplification of the IgH chain gene using primers directed to the framework three and joining regions of the gene, as described previously.[13] The presence of 1 to 2 dominant bands was interpreted as monoclonal, and 3 to 4 dominant bands as oligoclonal. IL-2 was not detected in the vitreous, and the presence of gene rearrangement and/or presence of malignant B-cells in CNS and/or eye confirmed that all cases presented here were B-cell type lymphoma, as described previously.[14] Excluded from the study were patients with preoperative trauma, pre-existing macular pathologies (such as age-related macular degeneration), vitreous hemorrhage, immunodeficiency, and diabetes mellitus, all of which are likely to influence the vitreous levels of immune mediators. The following clinical data were extracted for each patient: sex, age at diagnosis of vitreoretinal B-cell lymphoma, ocular involvement, main ocular lesions at initial diagnosis, primary organ involved, presence of CNS involvement, pattern of spread, cytopathology, presence of IgH chain gene rearrangement, relapse after first diagnosis of vitreoretinal B-cell lymphoma, and current status (Table 1). The control group consisted of 17 patients with macular holes and 10 with epiretinal membranes (10 males and 17 females, mean age 68.0 ± 9.6 years); none of these patients had any associated vitreoretinopathy. Vitreoretinal B-cell lymphoma patients were divided into two groups according to clinical features: diffuse vitreous opacities with or without multiple subretinal white lesions.

Measurements of Immune Mediators

The CBA Flex immunoassay kit (BD Biosciences, San Jose, CA) was used to measure the concentrations of the following immune mediators, according to the methods recommended by the manufacturer: human IL-1α; IL-1β; IL-2; IL-3; IL-4; IL-5; IL-6; IL-8; IL-9; IL-10; IL-11; IL-12p70; IL-17A; IL-21; interferon-γ (IFN-γ); tumor necrosis factor-α (TNF-α); TNF-β; oncostatin M (OSM); IFN-γ-inducible protein (IP)-10; monocyte chemoattractant protein (MCP)-1; macrophage inflammatory protein (MIP)-1α; MIP-1β, regulated on activation, normal T-cell expressed and secreted (RANTES); monokine induced by interferon γ (Mig); IFN-inducible T-cell α-chemoattractant (I-TAC); fractalkine; eotaxin; VEGF; granulocyte-colony stimulating factor (G-CSF); granulocyte macrophage colony-stimulating factor (GM-CSF); basic fibroblast growth factor (bFGF); Fas ligand; CD40 ligand; granzyme A; and granzyme B. Two-color flow cytometric analysis was performed using a FACSCaliber flow cytometer (BD Biosciences). The concentrations of immune mediators were obtained from the standard curve for each cytokine.

Although BCA-1, and SDF-1α have been reported to have important roles in vitreoretinal B-cell lymphoma, CNS lymphoma, and DLBCL,[7,15,16] the CBA flex kit does not contain these two immune mediators. Therefore BCA-1 and SDF-1α in vitreous also were determined by ELISA using commercially available kits (R&D Systems, Minneapolis, MN). The immunoassays were performed and analyzed according to the manufacturer's instructions. 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

Statistical analyses were performed using JMP version 9 (Business Unit of SAS, Cary, NC). Two-group comparisons of numerical variables were done using Student's *t*-test or the Mann-Whitney *U* test, based on the pattern of data distribution. The nonparametric Mann-Whitney *U* test was used to analyze immune mediator levels because the data were not distributed normally. Correlations were determined by the Spearman's *rho* test. Differences were considered significant when *P* was less than 0.05.

Table 1. Clinical Data of All Patients with Vitreoretinal B-Cell Lymphoma

N	Sex	Age at Diagnosis of Vitreoretinal B-Cell Lymphoma (y)	Eye Involved	Main Ocular Lesions at Initial Diagnosis	Primary Organ	Brain Involvement	Pattern of Spread	Cytopathology	IgH	Relapse (Mos. after First Diagnosis)	Outcome
1	F	67	OD	Subretinal infiltration + vitreous	Eye	Yes	Eye→Brain	NA	Monoclonal	Brain relapse 2 mos.	Alive 43 mos.
2	F	63	OD	Vitreous	Brain	Yes	Brain→Eye	ML	Oligoclonal	Eye relapse 24 mos.	Died 37 mos.
3	M	55	OU	Vitreous	Eye	Yes	Eye→Brain	OD:, OS: Suspicious for ML	Monoclonal	Brain relapse 32 mos.	Alive 44 mos.
4	F	76	OU	Subretinal infiltration + vitreous	Eye	Yes	Eye→Brain	OD: ML, OS: Suspicious for ML	Monoclonal	No relapse	Alive 38 mos.
5	M	73	OD	Vitreous	Eye	Yes	Eye→Brain	Suspicious for ML	Monoclonal	Eye relapse 14 mos.	Alive 40 mos.
6	M	61	OU	Vitreous	Eye	Yes	Brain→Eye	OU: negative for malignancy	Monoclonal	Eye relapse 7 mos.	Alive 35 mos.
7	M	52	OS	Subretinal infiltration + vitreous	Eye	No	Eye	Suspicious for ML	Monoclonal	No relapse	Alive 35 mos.
8	F	65	OD	Subretinal infiltration + vitreous	Eye	Yes	Eye→Brain	Suspicious for ML	Monoclonal	Brain relapse 31 mos.	Alive 34 mos.
9	M	73	OD	Vitreous	Eye	Yes	Eye→Brain	Suspicious for ML	Oligoclonal	Brain relapse 11 mos.	Died 18 mos.
10	F	76	OS	Vitreous	Eye	Yes	Brain→Eye	Suspicious for ML	Monoclonal	Eye relapse	Lost to follow-up
11	F	41	OS	Vitreous	Brain	Yes	Brain→Eye	Suspicious for ML	Monoclonal	Eye relapse	Lost to follow-up
12	F	73	OS	Vitreous	Eye	No	Eye	Suspicious for ML	Monoclonal	Eye relapse	Alive 25 mos.
13	M	53	OS	Vitreous	Brain	Yes	Brain→Eye	Suspicious for ML	Monoclonal	Eye relapse 6 mos.	Alive 33 mos.
14	F	51	OS	Vitreous + subretinal infiltration	Eye	No	Eye	Suspicious for ML	Monoclonal	No relapse	Alive 26 mos.
15	F	88	OU	Vitreous	Brain	Yes	Brain→Eye	OU: ML	Monoclonal	Eye and Brain relapse 14 mos.	Alive 24 mos.
16	M	70	OS	Subretinal infiltration + vitreous	Eye	No	Eye	Negative for malignancy	Monoclonal	Eye relapse 13 mos.	Alive 49 mos.
17	F	76	OS	Subretinal infiltration + vitreous	Brain	Yes	Brain→Eye	Suspicious for ML	Oligoclonal	Brain relapse 11 mos.	Alive 28 mos.
18	F	63	OD	Vitreous	Eye	Yes	Brain→Eye	Suspicious for ML	Monoclonal	Eye & Brain relapse 7 mos.	Alive 54 mos.
19	F	76	OS	Vitreous + subretinal infiltration	Eye	No	Eye	Suspicious for ML	Monoclonal	No relapse	Alive 14 mos.
20	F	79	OD	Subretinal infiltration + vitreous	Eye	Yes	Eye→Brain	Suspicious for ML	Oligoclonal	Brain relapse 10 mos.	Alive 20 mos.
21	F	70	OD	Vitreous	Eye	Yes	Eye→Brain	Suspicious for ML	Monoclonal	No relapse	Alive 64 mos.
22	M	47	OU	Vitreous	Brain	Yes	Brain→Eye	OU: Suspicious for ML	Monoclonal	No relapse	Alive 17 mos.
23	M	71	OS	Subretinal infiltrate	Eye	No	Eye	Negative for malignancy	Monoclonal	No relapse	Alive 9 mos.

F, female; M, male; OU, both eyes; OD, right eye; OS, left eye; ML, malignant lymphoma; NA, not available; mos., months.

Table 2. Immune Mediator Levels in Vitreous Samples of Patients with Vitreoretinal B-Cell Lymphoma and Controls

	Vitreoretinal B-Cell Lymphoma ($n = 28$)	Control Subjects ($n = 27$)	*P* Values
Angiogenin (pg/mL)	3823.8 (2348.5–5004.7)	3260.1 (2242.8–4158.1)	0.4795
BCA-1 (pg/mL)	9949.0 (429.2–1664.9)	12.0 (2.8–18.1)	<0.0001
bFGF (pg/mL)	52.9 (0–114.8)	11.3 (0–17.5)	0.0416
CD40 ligand (pg/mL)	2.4 (0–0.9)	1.5 (0–0)	0.7876
Eotaxin (pg/mL)	2.9 (0–0)	0.6 (0–0)	0.9195
Fas ligand (pg/mL)	59.4 (6.4–80.3)	1.0 (0–0)	<0.0001
Fractalkine (pg/mL)	16.3 (0–4.6)	8.7 (0–0)	0.6374
G-CSF (pg/mL)	1.3 (0–0)	0.2 (0–0)	0.4795
GM-CSF (pg/mL)	0.2 (0–0)	0.1 (0–0)	0.8202
Granzyme A (pg/mL)	88.1 (26.4–148.3)	0.6 (0–0)	<0.0001
Granzyme B (pg/mL)	152.5 (0–67.3)	1.1 (0–0)	0.0004
IFN-γ (pg/mL)	5.6 (0–7.7)	0	0.0031
IL-1α (pg/mL)	3.3 (0–0)	0	0.3633
IL-1β (pg/mL)	0	0.2 (0–0)	0.3458
IL-2 (pg/mL)	0	0.5 (0–0)	0.7941
IL-3 (pg/mL)	0.7 (0–0)	0.3 (0–0)	0.6616
IL-4 (pg/mL)	1.1 (0–0)	0	0.6494
IL-5 (pg/mL)	0	0.1 (0–0)	0.9866
IL-6 (pg/mL)	128.6 (27.2–95.0)	24.8 (1.3–12.7)	<0.0001
IL-8 (pg/mL)	159.6 (26.5–155.8)	7.5 (1.5–11.0)	<0.0001
IL-9 (pg/mL)	0.5 (0–0)	0.2 (0–0)	0.9866
IL-10 (pg/mL)	2367.5 (474.4–2776.3)	0.1 (0–0)	<0.0001
IL-11 (pg/mL)	7.5 (0–0)	0	0.1727
IL-12p70 (pg/mL)	0.8 (0–0)	0.1 (0–0)	0.9866
IL-17A (pg/mL)	2.5 (0–0)	1.5 (0–0)	0.6862
IL-21 (pg/mL)	24.9 (0–0)	0	0.1727
IP-10 (pg/mL)	2026.9 (393.3–3522.9)	59.8 (20.5–64.8)	<0.0001
ITAC (pg/mL)	318.4 (0–10.0)	2.3 (0–0)	0.232
LT-α (pg/mL)	0	0.1 (0–0)	0.7941
MCP-1 (pg/mL)	8874.6 (2961.2–13,250.0)	367.8 (221.9–391.5)	<0.0001
Mig (ng/mL)	237.4 (0.4–8.6)	0	<0.0001
MIP-1α (pg/mL)	12.2 (2.9–16.4)	0.1 (0–0)	<0.0001
MIP-1β (pg/mL)	74.9 (33.4–108.5)	2.6 (0–2.0)	<0.0001
OSM (pg/mL)	8.3 (0–11.8)	0.8 (0–0)	0.0004
RANTES (pg/mL)	22.9 (0–27.3)	0.3 (0–0)	0.0001
SDF-1α (pg/mL)	816.8 (343.1–794.8)	220.6 (16.3–327.6)	<0.0001
TNF-α (pg/mL)	0.4 (0–0)	0	0.4953
VEGF (pg/mL)	180.0 (0–12.9)	15.1 (0–0)	0.192

Immune mediator levels are expressed as mean with interquartile range in parentheses. IP-10, IFN-γ-induced protein 10 kDa; LT-α, lymphotoxin-α.

Results

Vitreous Concentrations of Cytokines in Intraocular Lymphoma

The results of the study are summarized in Table 2. Of 23 patients with vitreoretinal B-cell lymphoma 21 had IL-10:IL-6 ratios >1.0 (mean 29.9 ± 25.4, range 0.4–92.6). The high ratios were diagnostic of the disease and consistent with previous reports.[17] Concentrations of BCA-1, bFGF, Fas ligand, granzyme A, granzyme B, IFN-γ, IL-6, IL-8, IL-10, IP-10, MCP-1, Mig, MIP-1α, MIP-1β, OSM, RANTES, and SDF-1α in the vitreous were elevated significantly in vitreoretinal B-cell lymphoma patients compared to controls ($P < 0.05$ for bFGF, $P < 0.01$ for all others). The levels of CD40 ligand, eotaxin, G-CSF, GM-CSF, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-9, IL-11, IL-12p70, IL-17A, LT-α, and TNF-α were mainly below detection limit and did not vary significantly compared to controls.

When comparing the various immune mediators that were upregulated significantly in vitreoretinal B-cell lymphoma patients, we found a low-to-moderate positive correlation between BCA-1 and Fas ligand ($r = 0.495$), IFN-γ ($r = 0.465$), MIP-1β ($r = 0.386$), and RANTES ($r = 0.391$); between bFGF and MIP-1β ($r = 0.402$); between granzyme A and Mig ($r = 0.396$) and MIP-1α ($r = 0.447$); between IFN-γ and IL-6 ($r = 0.433$), IP-10 ($r = 0.451$), MIP-1α ($r = 0.438$), and MIP-1β ($r = 0.405$); between IL-6 and Mig ($r = 0.447$), MIP-1β ($r = 0.429$), OSM ($r = 0.402$), and SDF-1α ($r = 0.400$); between IL-8 and IL-10 ($r = 0.441$), IP-10 ($r = 0.461$), and MIP-1α ($r = 0.449$); between IL-10 and IP-10 ($r = 0.423$), MIP-1β ($r = 0.466$), and MCP-1 ($r = 0.387$); and between MCP-1 and MIP-1β ($r = 0.446$). There was a moderate-to-strong positive correlation between BCA-1 and granzyme A ($r = 0.612$); between granzyme A and IFN-γ ($r = 0.586$) and MIP-1β ($r = 0.578$); between IFN-γ and Mig ($r = 0.528$) and SDF-1α ($r = 0.520$); between IL-6 and IL-8 ($r = 0.614$), IL-10 ($r = 0.571$), IP-10 ($r = 0.514$), and MCP-1 ($r = 0.64$); between IL-8 and MCP-1 ($r = 0.691$), Mig ($r = 0.61$), and MIP-1β ($r = 0.50$); between IL-10 and MCP-1 ($r = 0.561$), MIP-1α ($r = 0.509$), and Mig ($r = 0.774$); between MCP-1 and Mig ($r = 0.51$); and between MIP-1α and MIP-1β ($r = 0.679$). Interestingly, we found a moderate negative correlation between Fas ligand and bFGF ($r = –0.394$) (Table 3).

The vitreous levels of IP-10 were significantly ($P = 0.0087$) elevated in eyes with diffuse vitreous opacities and multiple subretinal white lesions (mean 3246.7 pg/mL, range 1172.3–

Table 3. Correlation Analyses among Upregulated Immune Mediators in the Vitreous of Vitreoretinal B-Cell Lymphoma Patients

		BCA-1	bFGF	Fas ligand	Granzyme A	Granzyme B	IFN-γ	IL-6	IL-8	IL-10	IP-10	MCP-1	Mig	MIP-1α	MIP-1β	OSM	RANTES	SDF-1α
BCA-1	r	-	0.136	0.495	0.612	-0.0582	0.465	0.129	0.135	0.232	0.126	0.23	0.349	0.329	0.386	-0.025	0.391	0.167
	p	-	0.4811	0.0101	0.0015	0.7625	0.0158	0.5038	0.4841	0.2279	0.5111	0.2317	0.07	0.0869	0.0447	0.8965	0.0422	0.3857
bFGF	r		-	-0.394	0.291	0.107	0.243	0.325	0.162	-0.0969	-0.031	0.109	0.165	0.202	0.402	-0.0636	-0.0129	-0.0412
	p		-	0.0408	0.1309	0.5778	0.2068	0.0914	0.3996	0.6145	0.872	0.572	0.3904	0.2949	0.0369	0.7408	0.9465	0.8303
Fas ligand	r			-	0.331	0.0797	0.331	-0.292	-0.0143	0.129	0.271	0	0.324	0.217	0.13	-0.0757	0.335	0.314
	p			-	0.085	0.6789	0.085	0.1294	0.9406	0.5024	0.1589	0.9977	0.0919	0.2595	0.4988	0.694	0.0821	0.1023
Granzyme A	r				-	0.359	0.586	0.149	0.304	0.123	0.2	0.189	0.396	0.447	0.578	-0.0941	0.374	0.34
	p				-	0.0623	0.0023	0.4392	0.1139	0.5219	0.2982	0.3261	0.0398	0.0201	0.0027	0.6248	0.0518	0.0772
Granzyme B	r					-	0.28	0.287	0.248	0.251	0.268	0.189	0.308	0.15	0.221	0.104	0.299	0.12
	p					-	0.146	0.1361	0.1978	0.1917	0.1634	0.3271	0.1092	0.4356	0.2513	0.5892	0.1208	0.534
IFN-γ	r						-	0.433	0.25	0.294	0.451	0.11	0.528	0.438	0.405	0.319	0.141	0.52
	p						-	0.0245	0.1932	0.126	0.0191	0.5674	0.0061	0.0228	0.0353	0.0977	0.463	0.0068
IL-6	r							-	0.614	0.571	0.514	0.64	0.447	0.319	0.429	0.402	0.027	0.4
	p							-	0.0014	0.003	0.0076	0.0009	0.0203	0.0977	0.0259	0.0366	0.8884	0.0375
IL-8	r								-	0.441	0.461	0.691	0.61	0.449	0.5	0.0217	0.107	0.33
	p								-	0.0219	0.0167	0.0003	0.0015	0.0196	0.0093	0.9102	0.5787	0.0869
IL-10	r									-	0.423	0.561	0.322	0.509	0.466	0.205	-0.0459	0.184
	p									-	0.0279	0.0036	0.0945	0.0082	0.0154	0.2859	0.8114	0.3378
IP-10	r										-	0.387	0.774	0.155	-0.0167	0	0.141	0.374
	p										-	0.0441	0.0001	0.4202	0.9309	0.9977	0.4625	0.0522
MCP-1	r											-	0.51	0.366	0.446	-0.0966	-0.0894	0.325
	p											-	0.0081	0.0572	0.0204	0.6158	0.6424	0.0916
Mig	r												-	0.213	0.202	-0.0929	0.137	0.334
	p												-	0.2694	0.294	0.6291	0.4759	0.0823
MIP-1α	r													-	0.679	0.209	0.231	0.0632
	p													-	0.0004	0.2771	0.2306	0.7424
MIP-1β	r														-	0.12	0.0721	0.3
	p														-	0.5341	0.708	0.1191
OSM	r															-	0.2209	0.299
	p															-	0.2538	0.1204
RANTES	r																-	0.0977
	p																-	0.6117
SDF-1α	r																	-
	p																	-

Significant correlation ($P < 0.05$) is colored in yellow, low-to-moderate correlation ($r < 0.5$) in blue, moderate-to-high correlation ($r \geq 0.5$) in green, and negative correlation in orange. r, Spearman's rank correlation coefficient; p, *P* value.

5354.9) than in those with diffuse vitreous opacities only (mean 1237.5 pg/mL, range 361.1–1213.2). There were no significant relations between the concentrations of these immune mediators and other clinical parameters, such as pattern of spread, relapse, and outcome.

Discussion

Our study demonstrated a pattern of immune mediator expression that may be involved in driving a broad spectrum of tumor and immune processes in vitreoretinal B-cell lymphoma. Since patients with vitreoretinal B-cell lymphoma suffer immune reactions against the "lymphoma," we used noninflammatory diseases, such as macular hole and epiretinal membrane cases, as disease controls. We identified a subset of immune mediators that differ significantly between controls and vitreoretinal B-cell lymphoma patients, as well as differentiated between subsets of patients with vitreoretinal B-cell lymphoma. The key immune mediators that discriminate between vitreoretinal B-cell lymphoma patients and controls were BCA-1, bFGF, Fas ligand, granzyme A, granzyme B, IFN-γ, IL-6, IL-8, IL-10, IP-10, MCP-1, Mig, MIP-1α, MIP-1β, OSM, RANTES, and SDF-1α. These findings supported the hypothesis that a broad spectrum of tumor processes are involved in the pathogenesis of vitreoretinal B-cell lymphoma, which affect lymphoma cells and immune cell responses, cell proliferation/growth, and cell death in the ocular sites.

On the other hand, vitreoretinal B-cell lymphoma is one of the masquerade syndromes, and differentiation from uveitis often is important. When we compared the immune mediator profile of vitreous samples from 26 uveitis patients (10 males and 16 females, aged 62.3 ± 14.3 years; 11 with ocular sarcoidosis, 3 ocular Behçet disease, 1 Vogt-Koyanagi-Harada disease, 1 Fuchs' heterochromic iridocyclitis, 2 ocular tuberculosis, 1 ocular toxoplasmosis, 1 ocular toxocariasis, and 6 uveitis of unknown etiology) measured by the same methods, vitreous levels of BCA-1, bFGF, IL-10, MCP-1, MIP-1α, MIP-1β, SDF-1α, and VEGF levels were significantly higher in patients with vitreoretinal B-cell lymphoma than in uveitis patients. In contrast, vitreous levels of angiogenin, fractalkine, granzyme A, IFN-γ, IL-6, and IP-10 levels were significantly higher in uveitis patients than in patients with vitreoretinal B-cell lymphoma (Supplemental Table, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.11-8719/-/DCSupplemental). Regarding the difference in vitreous immune mediator profile between vitreoretinal B-cell lymphoma and other intraocular solid malignant tumors, such as uveal melanoma and retinoblastoma, we were able to measure vitreous levels of immune mediators in 7 cases of malignant choroidal melanoma and 2 cases of retinoblastoma. IL-8, angiogenin, Fas L, RANTES, IP-10, and MIP-1β were significantly higher in intraocular solid malignant tumors compared to vitreoretinal B-cell lymphoma, while IL-10 was significantly higher in vitreoretinal B-cell lymphoma than in intraocular solid malignant tumors (data not shown). The immune mediators that differentiate between vitreoretinal B-cell lymphoma, and uveitis and other malignant intraocular tumors require further investigations.

It is well established that IL-10 is detected in the vitrectomy specimens of patients with vitreoretinal B-cell lymphoma, and serves as a valuable clue in the diagnosis.[8] The IL-10:IL-6 ratio (greater than one) also has been shown to be a useful diagnostic marker for intraocular lymphoma.[17] Our result confirms this finding. Furthermore, elevated serum IL-10 levels in patients with systemic DLBCL have been shown to correlate with poor prognosis.[18] To our knowledge, the role of IL-10 in vitreoretinal B-cell lymphoma has not been examined intensively; however, it has been reported commonly that IL-10 as an autocrine growth factor increases B-cell proliferation, differentiation, and survival.[19,20] Besides contributing to the proliferation of B-cell lymphoma, IL-10 is a pleiotropic cytokine also possessing immunosuppressive properties that constitute

one of the most useful immune escape mechanisms in vitreoretinal B-cell lymphoma.[10] Whether the IL-10 level in the vitreous is related to vitreoretinal B-cell lymphoma activity can be answered only by further studies to elucidate the exact roles of these mediators in the pathogenesis and clinical course of vitreoretinal B-cell lymphoma.

The mechanisms involved in homing of malignant B-cells to the eye and/or CNS remain unknown. Chemokines represent a group of molecules that regulate cell migration. Therefore, chemokines have been implicated in the metastatic spread of a wide variety of tumors.[21] Because the eye and brain contain no lymphoid tissues, a popular hypothesis of the pathogenesis of vitreoretinal B-cell lymphoma maintains that malignant B-cells home to the eye and/or brain,[1,7] although the putative homing signal has not been identified. How malignant B-cells could localize so specifically to the eye and CNS remains one of the most fascinating questions. There is growing evidence that CNS DLBCL cells have a different chemokine receptor profile compared to their morphologic counterparts in systemic DLBCL.[22] The data of Chan et al.[7] focus on SDF-1α and BCA-1, and their receptors CXCR4 and CXCR5, respectively, which affect the homing of B-cells to the eye and/or CNS. BCA-1 selectively drives the migration of B-cells via CXCR5. SDF-1α and BCA-1 are upregulated on cerebrovascular endothelial cells[23] and on RPE,[7,24] which suggests a chemokine-mediated regulation of B-cell homing to the eye and CNS, and their spread within eye and brain. However, in the setting of vitreoretinal B-cell lymphoma, it remains unknown whether B-cells enter the eye already as malignant B-cells while others are eliminated by the immune system in the periphery, or as non-transformed cells and undergo malignant transformation in the eye. In addition, SDF-1 and BCA-1 support CNS B-cell growth.[16] We observed expected increases in vitreous concentrations of BCA-1 and SDF-1α, which is consistent with previous observation.[7]

IL-8, a cytokine and chemokine that has an important role in inflammation, is a potent chemoattractant factor for neutrophilic granulocytes and T-cells, and also can activate B-cells.[25] Moreover, in a recent study, IL-8 has been shown to impact survival in B-cell chronic lymphocytic leukemia by inducing the transcription and translation of the antiapoptotic protein Bcl-2.[26] In systemic DLBCL patients, those with higher serum IL-8 levels respond less favorably to treatment.[25,27] Vitreous IL-8 levels were significantly higher in patients with vitreoretinal B-cell lymphoma compared to controls. Our results thus suggest that the pathophysiology of vitreoretinal B-cell lymphoma may be related to higher IL-8 levels, which led us to suspect that IL-8 might be a factor related to the development of vitreoretinal B-cell lymphoma.

MCP-1 has been reported to be upregulated in the vitreous of patients with uveitis and vitreoretinal diseases, such as proliferative diabetic retinopathy, proliferative vitreoretinopathy, and retinal detachment.[28,29] In addition, MCP-1 expression was found in and secreted by primary central nervous system B-cell lymphoma, which may provide important insights into the pathogenesis of recruitment of inflammatory cells, such as monocytes and T-cells.[30] The CXC chemokines IP-10 and Mig bind the CXCR3 receptor and are specifically chemotactic for activated lymphocytes.[31] Mig expression also is observed in MALT lymphoma and atypical B-cells of chronic lymphocytic leukemia.[32,33] Several articles have reported that malignant B-cells express CXCR3 and mediate migration and autocrine proliferation following the binding of IP-10 and Mig.[34–36] Moreover, cytokine-stimulated RPE cells are capable of producing IP-10, Mig, and IL-8.[37,38] In our study, IP-10 and Mig expression levels were higher in vitreoretinal B-cell lymphoma than in control subjects, and correlated strongly with each other ($r = 0.774$). In another study on uveitis, IP-10 levels correlate with tissue infiltration of T-cells.[39] This report is consistent with our finding that IP-10 was elevated in vitreoretinal B-cell lymphoma with subretinal infiltration, which frequently is accompanied by reactive T-cells in the vitreous of patients with vitreoretinal B-cell lymphoma of the subretinal infiltration type.[40–43] It remains unknown whether lymphoma cell or the infiltrating cells produce these mediators. Further studies are required.

Besides neoplastic cells, small reactive T-cells and monocytes usually are found within the CNS and eye.[30,40–44] Thus, it remains possible that some inflammatory cells other than malignant B-cells may be the source of some mediators of interest. MCP-1, Mig, MIP-1α, MIP-1β, IP-10, IL-8, and RANTES, which we found upregulated in vitreoretinal B-cell lymphoma, have been considered to be important chemokines specifically stimulating directional migration of T-cells and monocytes as well as B-cells that could form diffuse opacities in the vitreous. It is possible that these mediators are produced by lymphoma cells and the infiltrating inflammatory cells, and the mediators produced may stimulate further the lymphoma and inflammatory cells to produce more or other mediators. However, these possibilities remain unproven. The contribution of surrounding inflammatory cells probably is minor, but the presence of these non–B-cells in some instances may be functionally relevant to some aspects of the expression profile.[45] In fact, macrophages, dendritic cells, and T-cells support B-cell survival and proliferation.[46,47] Moreover, DLBCL recruit monocytes including dendritic cells, macrophages, and T-cells via RANTES to support B-cell survival and proliferation.[47] In our study, RANTES level in vitreoretinal B-cell lymphoma was significantly higher than in controls. Therefore, it can be expected that B-cell survival and proliferation are due, in part, to the infiltration of various leukocytes via RANTES. Further investigation is required to elucidate the relationship of vitreoretinal B-cell lymphoma with the ocular microenvironment including these chemokines.

Although to our knowledge, there is no information in the literature concerning the role of immune mediators in promoting apoptosis in vitreoretinal B-cell lymphoma, several studies have highlighted the issue of soluble Fas, granzyme A, and granzyme B having a role in promoting apoptosis in neoplastic cells.[48–50] Granzymes A and B are the most abundant cytolytic molecules of immune cells. However, these granzymes target distinct cell death pathways. Granzyme A activates caspase-independent programmed cell death, whereas granzyme B activates the caspase-dependent cell death pathway by initiating effector caspase cleavage and by cleaving directly some key caspase pathway substrates, such as Bid and inhibitor of caspase-activated DNase.[51,52] Furthermore, granzyme A triggers inflammation by inducing proinflammatory cytokines.[53] On the contrary, elevated levels of immune mediators promote immune cell infiltration and activation, which, in turn, promotes increased granzyme production in the inflamed tissue.[52] For this reason, immune-mediated, granzyme-induced apoptosis may have a causative role in the pathogenesis of vitreoretinal B-cell lymphoma. The protein Fas leads to apoptosis of immune cells by binding with the Fas ligand. The immune privilege of the eye is augmented by the constitutive expression of Fas L in ocular tissue. Ocular resident cells express Fas L, and Fas-expressing infiltrating inflammatory cells interact with Fas L and subsequently undergo apoptosis.[54] Little is known about the function of the Fas/Fas L signaling system in vitreoretinal B-cell lymphoma. We propose a hypothesis that the Fas/Fas L system within the eye may regulate the apoptosis of lymphoma cells and infiltrating inflammatory cells. Approximately 30% to 70% of patients with DLBCL have Fas expression on cell surface membranes.[55] Hara et al. reported that high soluble Fas

expression levels in patients with aggressive DLBCL indicate a poor prognosis and serve as a useful tool for selecting appropriate therapeutic strategies.[56] Clarification of these issue will require further investigation in a larger number of patients with vitreoretinal B-cell lymphoma.

In conclusion, various immune mediators were upregulated in the vitreous fluids in patients with vitreoretinal B-cell lymphoma. Vitreoretinal B-cell lymphoma is a rare malignancy, and this unusual series of 23 cases provided us a unique opportunity to study the expression of immune mediators related to the disease. The presence of malignant B-cells and infiltrating immune cells in the lymphomatous eye results in the production of various immune mediators that clearly differ from controls. However, the specific cells responsible for the secretion of immune mediators in the eye with vitreoretinal B-cell lymphoma remain unidentified. Furthermore, it is likely that a complex immune mediator network is formed in individual patients, so that the linkage between a single mediator and the clinical pattern is difficult to establish, even though abnormal overproduction of each immune mediator may cause pathophysiologic changes and influence clinical manifestations in vitreoretinal B-cell lymphoma patients. Identification of the cells present in the vitreous of patients with vitreoretinal B-cell lymphoma may provide a clue to which mediators are important. Additional studies of the potential functions of the detected immune mediators may provide important insights into the pathogenesis of vitreoretinal B-cell lymphoma, and targeting the production of immune mediators could be a useful strategy for the prophylaxis or treatment of vitreoretinal B-cell lymphoma.

References

1. Coupland SE, Chan CC, Smith J. Pathophysiology of retinal lymphoma. *Ocul Immunol Inflamm*. 2009;17:227–237.
2. Faia LJ, Chan CC. Primary intraocular lymphoma. *Arch Pathol Lab Med*. 2009;133:1228–1232.
3. Deangelis LM, Hormigo A. Treatment of primary central nervous system lymphoma. *Semin Oncol*. 2004;31:684–692.
4. Akpek EK, Ahmed I, Hochberg FH, et al. Intraocular-central nervous system lymphoma: clinical features, diagnosis, and outcomes. *Ophthalmology*. 1999;106:1805–1810.
5. Jellinger K, Radaskiewicz TH, Slowik F. Primary malignant lymphomas of the central nervous system in man. *Acta Neuropathol Suppl*. 1975;Suppl 6;95–102.
6. Kimura K, Goto H. The clinical findings and prognosis of 28 cases of intraocular lymphoma. *Nippon Ganka Gakkai Zasshi*. 2008;112:674–678.
7. Chan CC, Shen D, Hackett JJ, Buggage RR, Tuaillon N. Expression of chemokine receptors, CXCR4 and CXCR5, and chemokines, BLC and SDF-1, in the eyes of patients with primary intraocular lymphoma. *Ophthalmology*. 2003;110: 421–426.
8. Chan CC, Whitcup SM, Solomon D, Nussenblatt RB. Interleukin-10 in the vitreous of patients with primary intraocular lymphoma. *Am J Ophthalmol*. 1995;120:671–673.
9. de Waal Malefyt R, Abrams J, Bennett B, Figdor CG, de Vries JE. Interleukin 10(IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. *J Exp Med*. 1991;174:1209–1220.
10. Tuaillon N, Chan CC. Molecular analysis of primary central nervous system and primary intraocular lymphomas. *Curr Mol Med*. 2001;1:259–272.
11. Maier R, Weger M, Haller-Schober EM, et al. Application of multiplex cytometric bead array technology for the measurement of angiogenic factors in the vitreous. *Mol Vis*. 2006;12: 1143–1147.
12. Sugita S, Takase H, Sugamoto Y, Arai A, Miura O, Mochizuki M. Diagnosis of intraocular lymphoma by polymerase chain reaction analysis and cytokine profiling of the vitreous fluid. *Jpn J Ophthalmol*. 2009;53:209–214.
13. Diss TC, Peng H, Wotherspoon AC, Isaacson PG, Pan L. Detection of monoclonality in low-grade B-cell lymphomas using the polymerase chain reaction is dependent on primer selection and lymphoma type. *J Pathol*. 1993;169:291–295.
14. Bhat PV, Jakobiec FA, Papaliodis G, Sobrin L. Primary T-cell lymphoma of the retina and cerebellum: immunophenotypic and gene rearrangement confirmation. *Am J Ophthalmol*. 2009;148:350–360.
15. Smith JR, Braziel RM, Paoletti S, Lipp M, Uguccioni M, Rosenbaum JT. Expression of B-cell-attracting chemokine 1 (CXCL13) by malignant lymphocytes and vascular endothelium in primary central nervous system lymphoma. *Blood*. 2003;101:815–821.
16. Smith JR, Falkenhagen KM, Coupland SE, Chipps TJ, Rosenbaum JT, Braziel RM. Malignant B cells from patients with primary central nervous system lymphoma express stromal cell-derived factor-1. *Am J Clin Pathol*. 2007;127:633–641.
17. Buggage RR, Whitcup SM, Nussenblatt RB, Chan CC. Using interleukin 10 to interleukin 6 ratio to distinguish primary intraocular lymphoma and uveitis. *Invest Ophthalmol Vis Sci*. 1999;40:2462–2463.
18. Lech-Maranda E, Bienvenu J, Michallet AS, et al. Elevated IL-10 plasma levels correlate with poor prognosis in diffuse large B-cell lymphoma. *Eur Cytokine Netw*. 2006;17:60–66.
19. Gary-Gouy H, Harriague J, Bismuth G, Platzer C, Schmitt C, Dalloul AH. Human CD5 promotes B-cell survival through stimulation of autocrine IL-10 production. *Blood*. 2002;100: 4537–4543.
20. Burdin N, Rousset F, Banchereau J. B-cell-derived IL-10: production and function. *Methods*. 1997;11:98–111.
21. Kakinuma T, Hwang ST. Chemokines, chemokine receptors, and cancer metastasis. *J Leukoc Biol*. 2006;79:639–651.
22. Jahnke K, Coupland SE, Na IK, et al. Expression of the chemokine receptors CXCR4, CXCR5, and CCR7 in primary central nervous system lymphoma. *Blood*. 2005;106:384–385.
23. Krumbholz M, Theil D, Cepok S, et al. Chemokines in multiple sclerosis: CXCL12 and CXCL13 up-regulation is differentially linked to CNS immune cell recruitment. *Brain*. 2006;129:200–211.
24. Crane IJ, Wallace CA, McKillop-Smith S, Forrester JV. CXCR4 receptor expression on human retinal pigment epithelial cells from the blood-retina barrier leads to chemokine secretion and migration in response to stromal cell-derived factor 1 alpha. *J Immunol*. 2000;165:4372–4378.
25. Nacinovic-Duletic A, Stifter S, Dvornik S, Skunca Z, Jonjic N. Correlation of serum IL-6, IL-8 and IL-10 levels with clinicopathological features and prognosis in patients with diffuse large B-cell lymphoma. *Int J Lab Hematol*. 2008;30: 230–239.
26. Binsky I, Haran M, Starlets D, et al. IL-8 secreted in a macrophage migration-inhibitory factor- and CD74-dependent manner regulates B cell chronic lymphocytic leukemia survival. *Proc Natl Acad Sci U S A*. 2007;104:13408–13413.
27. Duletic-Naćinović A, Stifter S, Marijić B, et al. Serum IL-6, IL-8, IL-10 and beta2-microglobulin in association with International Prognostic Index in diffuse large B cell lymphoma. *Tumori*. 2008;94:511–517.
28. Banerjee S, Savant V, Scott RA, et al. Multiplex bead analysis of vitreous humor of patients with vitreoretinal disorders. *Invest Ophthalmol Vis Sci*. 2007;48:2203–2207.
29. Abu El-Asrar AM, Struyf S, Kangave D, Geboes K, Van Damme J. Chemokines in proliferative diabetic retinopathy and proliferative vitreoretinopathy. *Eur Cytokine Netw*. 2006;17:155–165.

30. Kitai R, Ishisaka K, Sato K, et al. Primary central nervous system lymphoma secretes monocyte chemoattractant protein 1. *Med Mol Morphol*. 2007;40:18–22.
31. Loetscher P, Pellegrino A, Gong JH, et al. The ligands of CXC chemokine receptor 3, I-TAC, Mig, and IP10, are natural antagonists for CCR3. *J Biol Chem*. 2001;276:2986–2991.
32. Trentin L, Agostini C, Facco M, et al. The chemokine receptor CXCR3 is expressed on malignant B cells and mediates chemotaxis. *J Clin Invest*. 1999;104:115–121.
33. Suefuji H, Ohshima K, Karube K, et al. CXCR3-positive B cells found at elevated frequency in the peripheral blood of patients with MALT lymphoma are attracted by MIG and belong to the lymphoma clone. *Int J Cancer*. 2005;114:896–901.
34. Trentin L, Cabrelle A, Facco M, et al. Homeostatic chemokines drive migration of malignant B cells in patients with non-Hodgkin lymphomas. *Blood*. 2004;104:502–508.
35. Ohshima K, Suefuji H, Karube K, et al. Expression of chemokine receptor CXCR3 and its ligand, mig, in gastric and thyroid marginal zone lymphomas. Possible migration and autocrine mechanism. *Leuk Lymphoma*. 2003;44:329–336.
36. Momose K, Makishima H, Ito T, et al. Close resemblance between chemokine receptor expression profiles of lymphoproliferative disease of granular lymphocytes and their normal counterparts in association with elevated serum concentrations of IP-10 and MIG. *Int J Hematol*. 2007;86:174–179.
37. Hooks JJ, Nagineni CN, Hooper LC, Hayashi K, Detrick B. IFN-beta provides immuno-protection in the retina by inhibiting ICAM-1 and CXCL9 in retinal pigment epithelial cells. *J Immunol*. 2008;180:3789–3796.
38. Elner SG, Delmonte D, Bian ZM, Lukacs NW, Elner VM. Differential expression of retinal pigment epithelium (RPE) IP-10 and interleukin-8. *Exp Eye Res*. 2006;83:374–379.
39. El-Asrar AM, Al-Obeidan SS, Kangave D, et al. CXC chemokine expression profiles in aqueous humor of patients with different clinical entities of endogenous uveitis. *Immunobiology*. 216:1004–1009.
40. Coupland SE, Bechrakis NE, Anastassiou G, et al. Evaluation of vitrectomy specimens and chorioretinal biopsies in the diagnosis of primary intraocular lymphoma in patients with Masquerade syndrome. *Graefes Arch Clin Exp Ophthalmol*. 2003;241:860–870.
41. Whitcup SM, de Smet MD, Rubin BI, et al. Intraocular lymphoma. Clinical and histopathologic diagnosis. *Ophthalmology*. 1993;100:1399–1406.
42. Cummings TJ, Stenzel TT, Klintworth G, Jaffe GJ. Primary intraocular T-cell-rich large B-cell lymphoma. *Arch Pathol Lab Med*. 2005;129:1050–1053.
43. Davis JL, Viciana AL, Ruiz P. Diagnosis of intraocular lymphoma by flow cytometry. *Am J Ophthalmol*. 1997;124:362–372.
44. Yeh S, Weichel ED, Faia LJ, et al. 25-Gauge transconjunctival sutureless vitrectomy for the diagnosis of intraocular lymphoma. *Br J Ophthalmol*. 94:633–638.
45. Tun HW, Personett D, Baskerville KA, et al. Pathway analysis of primary central nervous system lymphoma. *Blood*. 2008;111:3200–3210.
46. Craxton A, Magaletti D, Ryan EJ, Clark EA. Macrophage- and dendritic cell–dependent regulation of human B-cell proliferation requires the TNF family ligand BAFF. *Blood*. 2003;101:4464–4471.
47. Mueller CG, Boix C, Kwan WH, et al. Critical role of monocytes to support normal B cell and diffuse large B cell lymphoma survival and proliferation. *J Leukoc Biol*. 2007;82:567–575.
48. Markovic O, Marisavljevic D, Cemerikic V, et al. Clinical and prognostic significance of apoptotic profile in patients with newly diagnosed nodal diffuse large B-cell lymphoma (DLBCL). *Eur J Haematol*. 86:246–255.
49. Yamada M, Hirasawa A, Shiojima S, Tsujimoto G. Granzyme A mediates glucocorticoid-induced apoptosis in leukemia cells. *FASEB J*. 2003;17:1712–1714.
50. Grullich C, Friske V, Finke J. Ex vivo detection of primary leukemia cells resistant to granule cytotoxin-induced cell death: a rapid isolation method to study granzyme-B-mediated cell death. *Ann Hematol*. 2008;87:701–708.
51. Lieberman J. Granzyme A activates another way to die. *Immunol Rev*. 235:93–104.
52. Chowdhury D, Lieberman J. Death by a thousand cuts: granzyme pathways of programmed cell death. *Annu Rev Immunol*. 2008;26:389–420.
53. Metkar SS, Menaa C, Pardo J, et al. Human and mouse granzyme A induce a proinflammatory cytokine response. *Immunity*. 2008;29:720–733.
54. Griffith TS, Brunner T, Fletcher SM, Green DR, Ferguson TA. Fas ligand-induced apoptosis as a mechanism of immune privilege. *Science*. 1995;270:1189–1192.
55. Kojima Y, Tsurumi H, Goto N, et al. Fas and Fas ligand expression on germinal center type-diffuse large B-cell lymphoma is associated with the clinical outcome. *Eur J Haematol*. 2006;76:465–472.
56. Hara T, Tsurumi H, Goto N, et al. Serum soluble Fas level determines clinical outcome of patients with diffuse large B-cell lymphoma treated with CHOP and R-CHOP. *J Cancer Res Clin Oncol*. 2009;135:1421–1428.