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Quantitative Analysis of Interleukin-6 in Vitreous from Patients with Proliferative Vitreoretinal Diseases

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Purpose: To explore immunological mechanisms in the pathogenesis of proliferative vitreoretinal diseases, we measured the concentration of interleukin-6 in the vitreous body and serum from patients with proliferative diabetic retinopathy (PDR), proliferative vitreoretinopathy (PVR), and premacular fibrosis. To evaluate immunological etiology, interleukin-6 levels in each disease were compared with disease severity.

Methods: Clinical samples were obtained at the beginning of pars plana vitrectomy from 30 eyes of 26 patients with PDR, 12 eyes of 12 patients with PVR, and 10 eyes of 10 patients with premacular fibrosis. Interleukin-6 was quantitated with an enzyme-linked immunosorbent assay.

Results: The levels of detectable interleukin-6 in the vitreous specimens ranged from 22.8 to 666.4 pg/mL in the PDR patients and from 28.2 to 416.3 pg/mL in the PVR patients. No interleukin-6 was detected in the vitreous specimens from patients with premacular fibrosis or in any serum samples from patients. Interleukin-6 levels of vitreous specimens from PDR patients were higher than those from PVR patients ($P < .02$, Mann–Whitney U-test). There was no correlation between clinical severity and interleukin-6 levels in vitreous specimens from either PDR or PVR patients.

Conclusion: Our results indicated that cell-mediated immunity is involved in the pathogenesis of proliferative vitreoretinal diseases. **Jpn J Ophthalmol 2001;45:40–45** © 2001 Japanese Ophthalmological Society

Key Words: Interleukin-6, vitreous, vitreoretinal disease.

Introduction

Preretinal membranes in patients with proliferative vitreoretinal diseases, such as proliferative diabetic retinopathy (PDR) and proliferative vitreoretinopathy (PVR), are thought to be caused by migration and proliferation of various types of cells, including pigment epithelial cells, fibroblasts, glial cells, vascular endothelial cells, and macrophages.[1–6] It has been reported that these phenomena are modified by various cytokines and growth factors[7–10] that are released from both normal and damaged retina. When retinal ischemia or retinal detachment occurs, these damaged areas may release cytokines. Many studies have been performed to resolve the pathogenesis of proliferative retinal diseases. Autoimmune mechanisms have been thought to play a role in the pathogenesis of proliferative retinal diseases.[11–13] Rahi and Addison[14] have suggested a relationship between immunological mechanisms and the development of microangiopathy. Baudouin et al[15–21] found ocular deposits of immunogloblin, complement, and human leukocyte antigen in patients with PDR and PVR in a series of studies using an immunohistochemical method.

Interleukin-6 is known to be a potent cytokine in cell-mediated immunity that plays an important role in the regulation of immune response, acute phase reactions, and hematopoiesis. Abnormal production of interleukin-6 has been implicated in many kinds of diseases, including autoimmune diseases, chronic inflammation, and lymphoid malignancies. To explore immunological mechanisms in the pathogene-

Received: October 25, 1996

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Published by Elsevier Science Inc.

0021-5155/01/$–see front matter
PII S0021-5155(00)00290-2

sis of proliferative vitreoretinal diseases, we measured the concentration of interleukin-6 in the vitreous body and serum from patients with PDR, PVR, and premacular fibrosis and compared it with the severity of each disease.

Materials and Methods

Patients

Clinical samples were obtained at the beginning of pars plana vitrectomy from 30 eyes of 26 patients with PDR (Table 1), 12 eyes of 12 patients with PVR (Table 2), and 10 eyes of 10 patients with premacular fibrosis (Table 3). The PDR patients included 17 men and 9 women, ranging in age from 28 to 67 years (average: 52 years). The duration of diabetes in these patients ranged from 2 to 30 years. Of 26 PDR patients, 25 patients had non-insulin-dependent diabetes (type II). Only 1 patient was insulin-dependent (type I). The clinical severity of PDR was classified from 0 to 4, according to the grading of Abu el Asrar et al.[22] Grading was based on the presence and extent of active fibrovascular tissue, vitreous hemorrhage, iris neovascularization, and hyphema. Grade 0 indicated inactive residual fibrous tissue from proliferative diabetic retinopathy; grade 1+, vitreous hemorrhage without active neovascularization; grade 2+, vitreous hemorrhage with actively growing fibrovascular tissue; grade 3+, iris neovascularization in addition to the findings in grade 2+; and grade 4+, hyphema in addition to the findings in grade 3+. The PVR patients included 9 men and 3 women, ranging in age from 6 to 64 years (average: 40 years). The clinical severity of PVR was classified by the grading reported by the Retina Society Terminology Committee.[22] The premacular fibrosis patients included 5 men and 5 women, ranging in age from 13

Table 1. Interleukin-6 Levels in Vitreous from Proliferative Diabetic Retinopathy (PDR) Patients and Clinical Severity

Patient No.	Sex	Age (y)	Clinical Severity (Grade)*	IL-6† (pg/mL)	HbA1c‡ (mg/dL)
1	M	50	1	666.4	6.7
2	M	39	1	141.9	NA
3	F	62	2	223.4	5.3
			2	97.9	NA
4	M	57	2	167.3	NA
5	F	51	1	136.9	7.8
6	F	45	1	229.9	10.5
7	F	46	1	50.5	5.8
8	F	43	2	ND	7.1
9	F	49	2	73.1	7.1
			2	77.1	NA
10	M	56	2	ND	7.7
11	M	67	1	116.7	6.9
12	M	43	2	58.5	7.1
13	F	56	3	51.1	7.4
			3	43.2	NA
14	M	61	1	81.9	8.1
15	M	52	1	207.2	5.6
16	M	55	1	103.7	6.7
17	M	57	1	96.6	7.1
18	M	52	1	134.7	NA
19	M	60	1	53.4	6.7
20	M	41	0	45.4	9.8
21	M	51	2	24.9	7.1
22	M	54	2	171.9	9.2
23	M	28	3	208.6	6.1
24	F	59	3	171.6	11.8
25	F	65	1	22.8	6.7
26	M	50	2	73.5	NA
			2	73.5	NA

*Clinical severity according to method of Abu el Asrar et al.
†ND: Not detectable.
‡NA: Not available.

Table 2. Interleukin-6 Levels in Vitreous from Proliferative Vitreoretinopathy (PVR) Patients

Patient No.	Sex	Age (y)	Clinical Severity*	IL-6 (pg/mL)†
1	M	57	D1	111.9
2	F	64	C2	ND
3	M	19	D1	65.8
4	M	44	D1	92.7
5	M	24	C1	ND
6	F	63	B	ND
7	M	18	D1	ND
8	F	62	D1	28.2
9	M	23	D3	86.8
10	M	48	C2	416.3
11	M	6	C2	ND
12	M	58	B	36.7

*Clinical severity according to Retina Society Terminology Committee.
†ND: Not detectable.

Table 3. Interleukin-6 Levels in Vitreous from Premacular Fibrosis (PMF) Patients

Patient No.	Sex	Age (y)	PMF Diagnosis	IL-6 (pg/mL)*
1	F	72	Idiopathic	ND
2	F	59	Idiopathic	ND
3	M	42	Idiopathic	ND
4	F	50	Idiopathic	ND
5	F	76	Idiopathic	ND
6	M	58	Secondary	ND
7	M	60	Secondary	ND
8	M	65	Secondary	ND
9	M	13	Secondary	ND
10	F	50	Secondary	ND

*ND: Not detectable.

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to 75 years (average: 60 years). Five of these patients had idiopathic premacular fibrosis, while the conditions of the others were secondary to cryoretinopexy and scleral encircling procedures. Patients who had previous vitrectomy or inflammatory diseases were excluded from this study. Informed consent was obtained from each patient before surgery.

Collection of Vitreous and Serum Specimens

Collection of vitreous and serum specimens was performed at the beginning of pars plana vitrectomy, as described previously.[23] In brief, vitreous specimens were collected in a glass bottle that had an airtight rubber cap and arms. The bottle was connected via the arms to the spliced suction tube of a vitreous cutter (Alcon, Ft. Worth, TX, USA). The infusion line was first filled with air, and then core vitrectomy was carefully done. Air was infused at the pressure level of 40 to 55 mm Hg. Thus, undiluted vitreous fluid was collected in the bottle. After a sufficient amount of vitreous was obtained safely, the vitreous cutter was removed from the eyeball, and the air in the vitreous cavity was replaced by irrigating solution (BSS; Alcon). The collecting bottle was disconnected from the suction line and was placed immediately in ice-cold water. After the vitreous specimen was obtained, venous blood was sampled and serum was prepared by centrifugation. The vitreous specimen and serum were aliquoted in micro test tubes and frozen at −70°C until the enzyme assay.

Quantification of Interleukin-6

Interleukin-6 was quantitated with an enzyme-linked immunosorbent assay kit (Serotec; Blackthorn, UK). Duplicate aliquots of 100 μL of undiluted vitreous and serum were tested. The standard curve was obtained with recombinant human interleukin-6, ranging from 12.5 to 800 pg/mL. The detection limit was 16 pg/mL.

Statistical Analysis

Data were analyzed by using the Mann–Whitney *U*-test. A *P*-value $<.05$ was considered significant. Values were expressed as mean ± SEM.

Results

Interleukin-6 levels could be measured in 28 of 30 vitreous specimens (93%) from patients with PDR and in 7 of 12 specimens (58%) from patients with PVR (Tables 1 and 2). In the vitreous specimens from patients with premacular fibrosis, no interleukin-6 was detected (Figure 1). The levels of detectable interleukin-6 in the vitreous specimens ranged from 22.8 to 666.4 pg/mL (136.1 ± 26.1 pg/mL; mean ± SEM) in the PDR patients and from 28.2 to 416.3 pg/mL (69.9 ± 33.7 pg/mL; mean ± SEM) in the PVR patients. In none of the serum samples was interleukin-6 detected. Interleukin-6 levels of vitreous specimens from PDR patients were significantly higher than those from PVR patients ($P < .02$, Mann–Whitney *U*-test) (Figure 2). There was no correlation between interleukin-6 levels in the vitreous specimen from PDR patients and the grade of clinical severity (Figure 3). There also was no correlation in PVR patients between interleukin-6 levels in vitreous specimens and clinical severity (Figure 4).

Discussion

Preretinal membranes of PDR patients contain newly formed vessels and extracellular matrix that were affected by liberated angiogenic factors produced by the ischemic retina, so-called nonperfusion areas. The characteristics of these angiogenic factors involved in retinal angiogenesis have not yet been clarified.

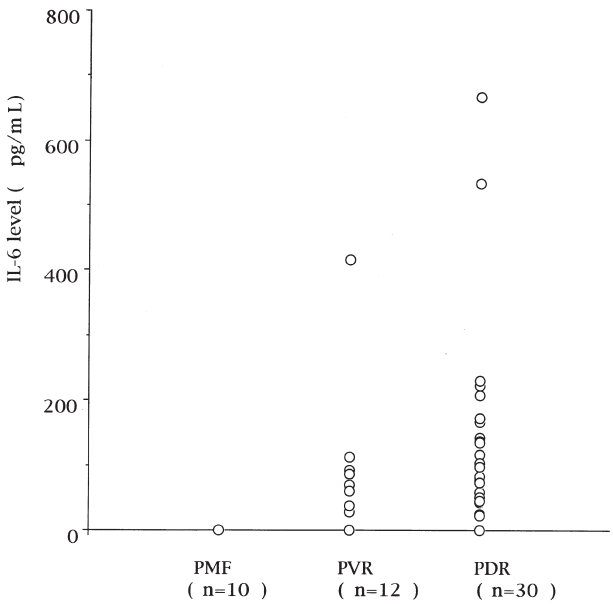

Figure 1. Interleukin-6 levels in vitreous from patients with proliferative diabetic retinopathy (PDR), proliferative vitreoretinopathy (PVR), and premacular fibrosis (PMF). No interleukin-6 levels could be detected in any vitreous samples from premacular fibrosis patients, nor in 5/12 samples from PVR patients, or in 2/30 samples from PDR patients.

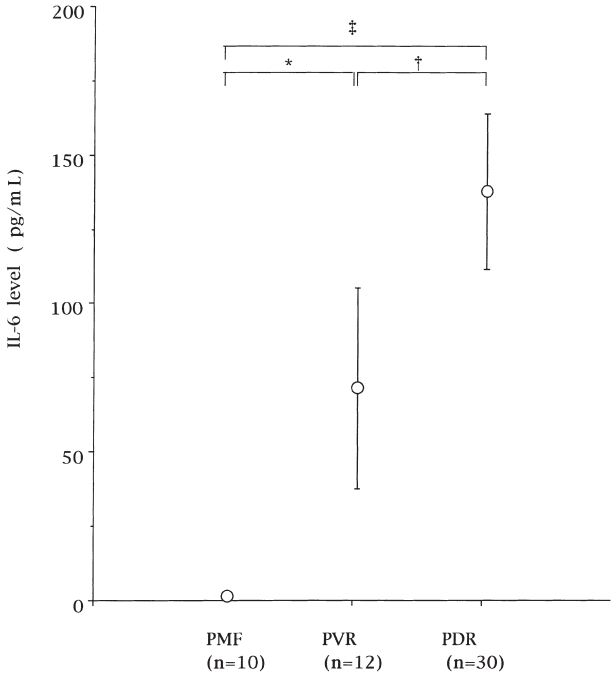

Figure 2. Interleukin-6 levels in vitreous from patients with proliferative diabetic retinopathy (PDR), proliferative vitreoretinopathy (PVR), and premacular fibrosis (PMF). Each point represents mean ± SE. Interleukin-6 levels in vitreous from PDR patients were higher than those from PVR patients. $^{*}P < .05$, $^{\dagger}P < .02$, $^{\ddagger}P < .0001$ (Mann–Whitney *U*-test)

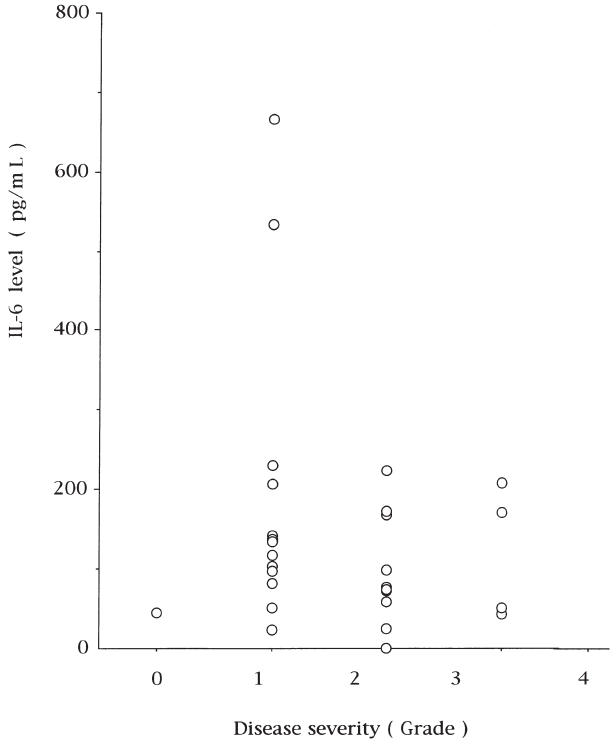

Figure 3. Interleukin-6 levels in vitreous from patients with proliferative diabetic retinopathy (PDR). There was no correlation between interleukin-6 levels in 30 vitreous samples from PDR patients and grade of clinical severity.

The main complication of rhegmatogenous retinal detachment is PVR. This complication results from migration and proliferation of various types of cells, including those derived from pigment epithelium, glial cells, fibroblast-like cells, and macrophages.[1–6] Although the pathogenesis of these two diseases are different in many aspects, both PDR and PVR have similar cellular processes; for example, cell activation, migration, proliferation, and synthesis of extracellular matrix. All these phenomena are recently reported to be induced by various cytokines and growth factors,[7–10] but the roles of these mediators in these two diseases remain speculative.

Interleukin-6 is a multifunctional cytokine; its functions include induction of the final differentiation of B cells to antibody-producing cells; induction of T cells to killer T cell activation; interleukin-2 receptor expression; proliferation of IL-6 itself; induction of acute phase protein production; and suppression of albumin production. This cytokine also is known to be a mediator in cell-mediated immunity, which increases in the synovial fluid or in the serum of patients with autoimmune disease, such as rheumatoid arthritis.[24–26] Interleukin-6 levels correlate with autoimmune disease activity.[26]

In recent studies,[11–13] cell-mediated immunity has played an important role in the pathogenesis of proliferative retinopathy. Biopsies of the pars plana in PDR and PVR patients have demonstrated the presence of immunogloblin, deposits of complement, and human leukocyte antigen expression at the level of the pigmented epithelium. These deposits and expression have been detected in the preretinal membranes in the connective stroma and within the vascular walls.[15–20] Under normal conditions, these antigens are restricted to immunocompetent cells and play regulatory roles in the interactions among the various cells of the immune system. Deviant expression of class II antigen has been found on nonlymphoid cells in various autoimmune diseases.[27,28] From the present study, it has been speculated that ischemic or detached retina in proliferative vitreoretinal disease has autoimmunity that causes an excessive wound-healing process. This excessive process may be involved in the pathogenesis of proliferative vitreoretinal diseases.

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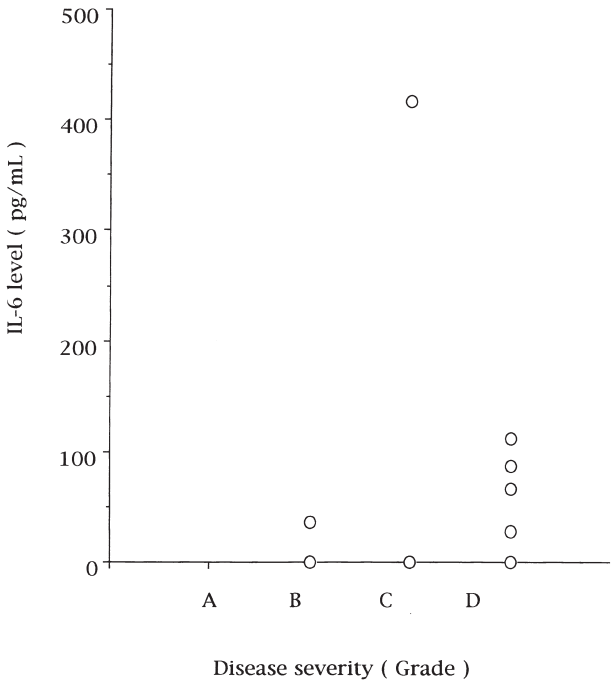

Figure 4. Interleukin-6 levels in vitreous from patients with proliferative vitreoretinopathy (PVR). There was no correlation between interleukin-6 levels in 12 vitreous samples from PVR patients and grade of clinical severity.

Cell-mediated immunity is thought to be involved in the pathogenesis of acute retinal necrosis (ARN). We measured the concentration of interleukin-6 in the vitreous from 3 eyes of 3 patients with ARN. The levels of interleukin-6 were high, ranging from 602.4 to 1142.8 pg/mL (864.2 ± 156.2 pg/mL; mean ± SEM). We think that these levels may reflect intense cell-mediated immunity in inflammatory disease.

Interleukin-6 levels in the vitreous of patients with proliferative retinopathy have been reported by Abu el Asrar et al.[24] They reported that interleukin-6 levels in the vitreous from PDR patients increased, and they found correlations between the level of this cytokine in the vitreous and the grade of clinical severity. Kauffmann et al[29] reported that interleukin-6 levels correlated with clinical severity in PVR patients. In this study, interleukin-6 levels in the vitreous from both PDR and PVR patients increased. The PDR patients had higher interleukin-6 levels in the vitreous than did PVR patients.

Our results were somewhat different from those of Abu el Asrar et al[21] and Kauffmann et al.[29] We collected not only the soluble fraction of the vitreous but also the gel fraction by using the vitreous cutter. It is, however, unclear what difference the sampling method may have on interleukin-6 levels in the vitreous. But it is a fact that interleukin-6 levels were increased in PDR and PVR patients using our method. The results of our current study indicate that cell-mediated immunity may be involved in the pathogenesis of proliferative vitreoretinal diseases.

Our results showed that ischemic retinal damage in PDR may be much more severe than detached retinal damage in PVR from the viewpoint of stimulation of interleukin-6 production. There was no correlation between interleukin-6 levels in the vitreous and clinical ocular severity in both PDR and PVR patients. We think the present grading of clinical severity for PDR and PVR does not reflect the intensity of cell-mediated immunity. A new classification for these diseases may be necessary to evaluate the activation level of intraocular immunity.

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