Chemokine Levels in Subretinal Fluid Obtained during Scleral Buckling Surgery after Rhegmatogenous Retinal Detachment

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Purpose. Interleukin (IL)-6, a multifunctional cytokine with regulatory functions in wound healing, and several chemokines have been implicated in the pathogenesis of proliferative vitreoretinopathy (PVR) after rhegmatogenous retinal detachment (RRD). The exact role of these chemokines, their correlation with IL-6 after primary RRD, and their association with the future development of PVR are not yet known.

Methods. A multiplex immunoassay was used to determine levels of 15 different chemokines and IL-6 in subretinal fluid samples obtained during scleral buckling surgery for primary RRD. Samples from patients with preoperative uveitis, preoperative trauma, or preoperative vitreous hemorrhage were excluded. Patients who developed a redetachment due to postsurgical PVR within 2.5 months ($n = 21$) were compared with control subjects who had an uncomplicated retinal detachment during the overall follow-up period ($n = 54$). Control subjects were matched for sex, age, and storage time.

Results. Levels of IL-6 ($P = 0.001$), MIF ($P = 0.016$), CCL2 ($P = 0.041$), CCL11 ($P = 0.012$), CCL17 ($P = 0.003$), CCL18 ($P = 0.007$), CCL19 ($P < 0.001$), CCL22 ($P < 0.001$), CXCL8 ($P = 0.027$), CXCL9 ($P = 0.007$), and CXCL10 ($P = 0.002$) were significantly higher in patients who developed postoperative PVR after primary RRD than in patients with uncomplicated retinal detachment. A significant positive correlation was observed between IL-6 and both CCL22 ($r = 0.538$; $P < 0.0001$) and CXCL8 ($r = 0.645$; $P < 0.0001$).

Conclusions. Various chemokines and IL-6 are upregulated in patients in whom fibrotic membranes develop after primary RRD repair and may therefore be involved in the future development of postoperative PVR. (*Invest Ophthalmol Vis Sci.* 2010;51:4143–4150) DOI:10.1167/iovs.09-5057

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Failure of the retina to reattach after surgical repair of rhegmatogenous retinal detachment (RRD) is mostly due to proliferative vitreoretinopathy (PVR).[1] This eyesight-threatening disease is characterized by the formation of fibrotic membranes that usually form within 3 months after reattachment surgery. The biological processes that lead to the development of these contractile membranes show many similarities with the normal wound-healing response, in which inflammation plays a pivotal role.[2]

Abundant evidence is available showing that inflammatory cytokines and inflammatory cells may underlie the pathologic changes that ultimately lead to the development of PVR. Several immunohistochemical studies have demonstrated the presence of macrophages and lymphocytes in PVR membranes.[3–6] A possible role of these immune cells in the PVR process is supported by cytologic studies showing their presence in vitreous specimens from patients with PVR.[7,8] Taken together, chemokines must be operative to elicit a vast influx of immune cells into the subretinal space after RRD.

Chemokines are small proteins that regulate migration of various types of leukocytes to sites of inflammation.[9] Based on their chemotactic activity and the arrangement of cysteine residues, chemokines can be divided into two major subfamilies. Generally, CC chemokines potently attract monocytes, T lymphocytes, eosinophils, and basophils, whereas CXC chemokines are known to recruit neutrophils and activated T lymphocytes to the site of injury.[10] Two of the most extensively studied chemokines in PVR specimens include CCL2 and CXCL8.[11–16] Both chemokines have been detected in PVR vitreous, and levels of these chemokines were shown to be higher than in vitreous specimens from patients with proliferative diabetic retinopathy or idiopathic epiretinal membranes.[17] In the same study, many other inflammatory mediators were detected including the chemokines CCL4 and CCL11, showing that a complex mix of chemokines may play a role in the pathogenesis of PVR. Interleukin (IL)-6, a multifunctional cytokine with regulatory functions in wound healing, has also been shown to be elevated in PVR specimens[16,18–20] and has been implicated in the induction of several chemokines.[21]

We hypothesized that chemokines play an important role in the pathogenesis of PVR, in particular in the very early stages when no fibrotic membranes have formed yet. In most studies, a limited number of chemokines were measured per sample, and samples were obtained when PVR had already developed.[12–16] Recently developed multiplex bead-based immunoassays allow for the simultaneous detection of many analytes in a very small sample volume.[22] Using this technique, we investigated protein expression of 15 different chemokines in subretinal fluid samples obtained during routine scleral buckling surgery for primary RRD. The results showed that several

chemokines as well as IL-6 may play a role in the development of PVR.

Methods

Subjects

In our department, subretinal fluid samples are routinely collected during scleral buckling surgery for primary RRD. Based on a medical record study, we made a selection of subretinal fluid samples stored in the BioBank Maastricht. Samples from patients who had preoperative uveitis, preoperative trauma, preoperative vitreous hemorrhage, or preoperative cryotherapy were excluded from the study. After exclusion, we were able to identify 21 samples from patients in whom a redetachment developed due to PVR within 2.5 months after scleral buckling surgery for primary RRD. This disease group, defined as the PVR-positive group, was compared with patients who did not have a redetachment during the overall follow-up period. Samples from these patients served as control specimens and were defined as the PVR-negative group. To improve statistical power, we selected two to three age-, sex-, and storage-time–matched control subjects without a redetachment during follow-up. In all, 54 patients with uncomplicated retinal detachment were included. All subretinal fluid samples were collected between 2001 and 2008. The study adhered to the tenets of the Declaration of Helsinki and was performed with the agreement of the University Hospital ethics committee. All patients gave written informed consent before the surgical procedure and after the nature of the study was explained.

Sample Collection

Undiluted subretinal fluid samples were obtained during scleral buckling surgery for primary RRD by making a small incision through the sclera and choroid. Scleral and choroidal vessels were carefully cauterized before the incision. Any macroscopic blood surrounding the incision opening was removed with a cotton tip. Subretinal fluid was collected from the surface of the sclera with a 25-gauge bent needle. Samples showing macroscopic hemorrhage were discarded. All samples were collected in sterile polypropylene tubes, immediately stored at −80°C, and thawed immediately before analysis. Sample volumes ranged between 50 and 250 μL.

Multiplex Bead–Based Immunoassay

Chemokines were measured at a commercial facility (Luminex Core Facility; Utrecht, The Netherlands) with expertise in the simultaneous measurement of proteins in a wide range of biological fluids including aqueous humor, human plasma, and synovial fluid, with a multiplex immunoassay (Luminex, Austin, TX).[23,24] Immunoassays were validated for the detection of proteins with in-house–developed assays. In summary, the antibody-coated microspheres were incubated for 60 minutes with standards or subretinal fluid (50 μL) in 96-well, 1.2-μm filter plates (Millipore, Amsterdam, The Netherlands). Plates were washed, and a cocktail of biotinylated detection antibodies was added for 60 minutes. After repeated washings, streptavidin–phycoerythrin was added for an additional 10 minutes. Beads were washed twice, and the fluorescence intensity was measured. Measurements and analysis of the data from all assays were performed (Bio-Plex system in combination with Bio-Plex Manager software, ver. 4.1; Bio-Rad, Hercules, CA), by using five-parameter curve fitting. The concentrations of IL-6 and the following chemokines were measured: the chemokine-like macrophage migration inhibitory factor (MIF); the CC chemokines CCL2, CCL3, CCL5, CCL11, CCL17, CCL18, CCL19, CCL21, CCL22; the CXC chemokines CXCL8, CXCL9, CXCL10, CXCL12; and CX_3CL1. This set represented all chemokines present in the chemokine package (Luminex Core Facility).

Clinical Variables

For all patients, we collected demographic variables, potential clinical risk factors for the development of PVR (Table 1), and the following clinical variables: follow-up time, occurrence of a redetachment, postoperative PVR grade, and preoperative and final postoperative best corrected Snellen visual acuity. PVR was graded according to the Classification of Retinal Detachment with PVR.[25] Data were collected according to the following scale: 0 (no PVR), 1 (grade A), 2 (grade B), 3 (grade C), and 4 (grade D). The duration of retinal detachment was defined as the interval between the onset of symptoms and surgery and was estimated according to a precise history of each patient's symp-

Table 1. Demographics and Potential Clinical Risk Factors for PVR

Risk Factor	Postoperative PVR Negative (n = 54)	Postoperative PVR Positive (n = 21)	Univariate Testing
Age, y			
Median (range)	61 (43–79)	62 (43–76)	NS
Sex, %			
Female	26	29	NS
Male	74	71	
Size of retinal detachment (quadrants)			
Median (range)	2 (1–3)	2 (1–4)	NS
Total size of retinal defects in optic disc diameters			
Median (range)	2 (0–5.5)	1 (0–4)	NS
Macular detachment, %	64	86	NS
Preoperative logMAR VA			
Median (range)	0.75 (0.05–2.52)	1.77 (0.10–2.52)	P = 0.045
Detachment duration, days			
Median (range)	5 (1–75)	6 (1–90)	NS
Diabetes mellitus, %	11	10	NS
Preoperative myopia >5 D, %	17	24	NS
Preoperative lens status, %			
Pseudophakia	19	33	NS
Aphakia	0	0	NS
Preoperative uveitis, %	0	0	NS
Preoperative vitreous hemorrhage, %	0	0	NS
Preoperative cryotherapy, %	0	0	NS
Preoperative trauma, %	0	0	NS

toms. For statistical analysis, Snellen visual acuity was transformed into logMAR (logarithm of minimal angle of resolution) visual acuity. Net visual outcome was calculated by subtracting logMAR visual acuity at final follow-up from logMAR visual acuity before surgery.

Statistical Analysis

Patients in whom a redetachment developed due to PVR within 2.5 months after reattachment surgery were compared with patients with an uncomplicated follow-up. Further, we investigated whether chemokine levels were different in patients who had development of PVR soon after surgery (within 1 month) and whether differences in chemokine expression were related to PVR grade. The nonparametric Mann-Whitney U test was used for ordinal variables such as chemokine levels, since data were not normally distributed. The χ^2 test was used to compare nominal variables such as diabetes mellitus. Correlations were determined by the Spearman's rho test. Differences were considered significant at $P < 0.05$, with two-tailed testing (SPSS ver. 16.0; SPSS for Windows; SPSS, Chicago, IL).

Results

Subretinal fluids from 75 patients who were undergoing scleral buckling surgery for primary RRD were analyzed for chemokine content. Twenty-one patients who developed postoperative PVR were compared with 54 patients who had an uncomplicated follow-up. In the PVR-positive group, 10 patients were classified with PVR grade B, 10 with PVR grade C, and 1 with PVR grade D. The median time interval between the scleral buckling procedure and redetachment due to PVR was 37 days (range, 7–80) and the median follow-up time was 21 months (range, 3–80). The group consisted of 6 (29%) women and 15 (71%) men with a median age of 62 years (range, 43–76). The PVR-negative patients included 14 (26%) women and 40 (74%) men with a median age of 61 years (range, 43–79). Their median follow-up time was 6 months (range, 2–80). Potential clinical risk factors for the development of postoperative PVR were available for all 75 patients included in this study. Except

Table 2. IL-6 and Chemokine Levels and the Development of Postoperative PVR

Analyte (pg/mL)	Postoperative PVR Negative (n = 54)	Postoperative PVR Positive (n = 21)	Univariate Testing
IL-6			
Median (range)	59.5 (8.33–1,211)	149 (36.3–2,656)	$P = 0.001$
Mean (SD)	139 (202)	483 (712)	
MIF			
Median (range)	3,618 (997–15,020)	6,691 (1,691–12,900)	$P = 0.016$
Mean (SD)	4,484 (2,880)	6,682 (3,638)	
CCL2			
Median (range)	849 (442–1,139)	930 (629–1,134)	$P = 0.041$
Mean (SD)	822 (153)	900 (132)	
CCL3			
Median (range)	359 (237–491)	391 (268–662)	NS
Mean (SD)	362 (53.6)	398 (89.3)	
CCL5			
Median (range)	151 (49.0–630)	186 (50.4–619)	NS
Mean (SD)	179 (96.3)	205 (151)	
CCL11			
Median (range)	8.58 (5.32–12.0)	9.18 (6.90–15.0)	$P = 0.012$
Mean (SD)	8.49 (1.45)	10.0 (2.16)	
CCL17			
Median (range)	2.04 (1.41–4.31)	2.59 (1.76–5.84)	$P = 0.003$
Mean (SD)	2.16 (0.48)	2.97 (1.24)	
CCL18			
Median (range)	4,305 (184–14,344)	6,897 (434–14,967)	$P = 0.007$
Mean (SD)	4,791 (3,091)	7,049 (3,480)	
CCL19			
Median (range)	115 (21.4–705)	309 (76.8–570)	$P < 0.001$
Mean (SD)	144 (117)	290 (160)	
CCL21			
Median (range)	752 (454–2,453)	777 (614–2,221)	NS
Mean (SD)	822 (286)	917 (371)	
CCL22			
Median (range)	18.2 (11.7–44.1)	31.9 (10.9–92.5)	$P < 0.001$
Mean (SD)	20.4 (6.75)	34.3 (18.5)	
CXCL8			
Median (range)	83.5 (28.4–728)	131 (42.4–1,324)	$P = 0.027$
Mean (SD)	155 (166)	259 (296)	
CXCL9			
Median (range)	15.1 (8.47–91.1)	17.3 (12.3–55.0)	$P = 0.007$
Mean (SD)	17.0 (11.1)	22.2 (11.0)	
CXCL10			
Median (range)	206 (42.6–875)	377 (128–1,002)	$P = 0.002$
Mean (SD)	239 (166)	425 (275)	
CXCL12			
Median (range)	12,254 (5,773–16,759)	10,991 (7,005–17,286)	NS
Mean (SD)	11,610 (2,775)	11,376 (2,897)	
CX_3CL1			
Median (range)	229 (140–318)	228 (147–465)	NS
Mean (SD)	231 (35.2)	231 (63.4)	

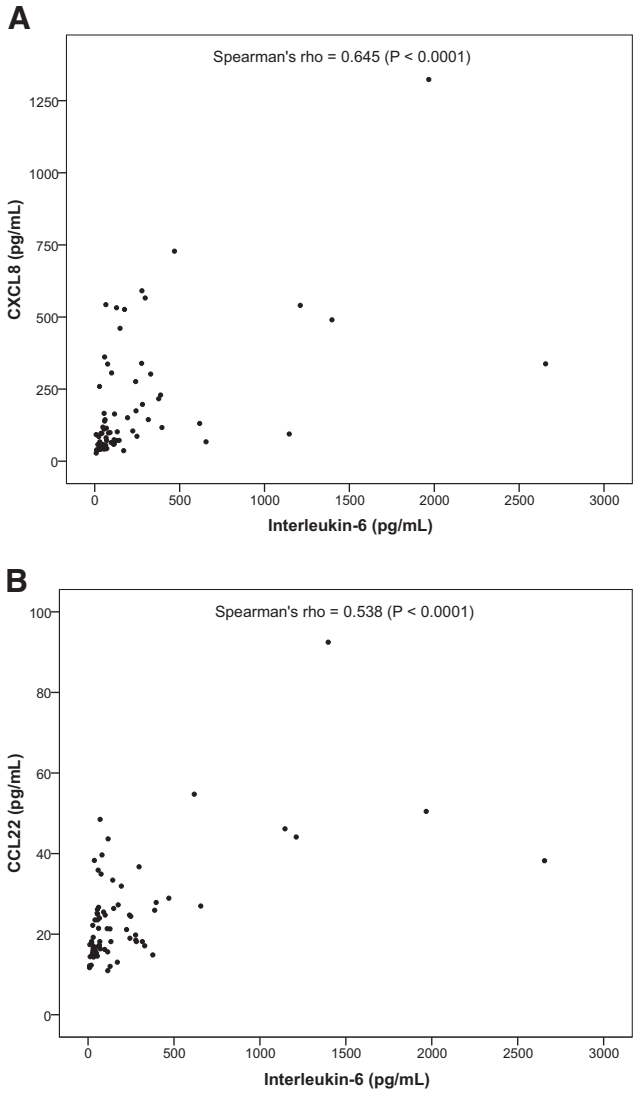

FIGURE 1. Correlation of IL-6 with chemokine levels in subretinal fluid after primary rhegmatogenous retinal detachment. (**A**) Correlation of IL-6 with CXCL8. (**B**) Correlation of IL-6 with CCL22.

for a worse preoperative visual acuity in the PVR-positive group ($P = 0.045$), there were no statistically significant differences between the two groups (Table 1).

Median and mean subretinal fluid IL-6 levels were approximately three times higher in patients with redetachment due to postoperative PVR compared with those with an uncomplicated follow-up ($P = 0.001$). Simultaneous measurement of 15 different chemokines in every sample with a multiplex bead-based immunoassay revealed the following results (Table 2): Univariate testing showed that subretinal fluid levels of MIF ($P = 0.016$), CCL2 ($P = 0.041$), CCL11 ($P = 0.012$), CCL17 ($P = 0.003$), CCL18 ($P = 0.007$), CCL19 ($P < 0.001$), CCL22 ($P < 0.001$), CXCL8 ($P = 0.027$), CXCL9 ($P = 0.007$), and CXCL10 ($P = 0.002$) were significantly higher in the PVR-positive group than in the PVR-negative group. Median levels of CCL3, CCL5, and CCL21 were higher in the PVR-positive group but did not reach statistical significance ($P > 0.05$).

There was a low to moderate positive correlation between IL-6 and CCL2 ($r = 0.232$), CCL11 ($r = 0.326$), CCL17 ($r = 0.428$), CCL18 ($r = 0.460$), CCL19 ($r = 0.340$), CXCL9 ($r = 0.267$), and CXCL10 ($r = 0.322$) ($P < 0.05$ for all), whereas the correlation between IL-6 and both CCL22 and CXCL8 was moderate to strong ($r = 0.538$ and $r = 0.645$, respectively) ($P < 0.0001$ for both; Fig. 1). When comparing the various chemokines that were significantly upregulated after univariate testing, we found several moderate to strong correlations ($r > 0.5$; $P < 0.001$; Table 3). Significant correlations between biochemical variables and clinical variables were low ($r < 0.4$). For example, we found a negative correlation between the total size of retinal defects and levels of IL-6 ($r = -0.289$), CCL11 ($r = -0.296$), CCL18 ($r = -0.384$), CXCL9 ($r = -0.369$), and CXCL10 ($r = -0.253$; $P < 0.05$ for all).

Preoperative visual acuity correlated moderately but significantly with final and net visual acuity ($r = 0.428$ and $r = 0.633$, respectively; $P < 0.001$ for both). As expected, the postoperative and net visual acuities of the PVR-positive group were significantly worse than those of the PVR-negative group at final follow-up ($P < 0.001$ and $P = 0.003$, respectively). Correlations between clinical variables and final and net visual acuities were very low ($r < 0.3$). However, we found moderate correlations between final visual acuity and both CCL19 ($r = 0.418$; $P < 0.001$) and CXCL10 ($r = 0.475$; $P < 0.001$).

In eight patients, redetachment developed from PVR within 1 month after reattachment surgery. We compared chemokine levels in these patients with those in patients who had a redetachment due to postoperative PVR between 1 and 2.5 months after ocular surgery ($n = 13$). Except for a lower median level of CXCL10 in the latter group ($P = 0.036$), we were not able to find any significant differences between both groups. To find out whether there were differences in chemokine expression between patients with different PVR grades, we compared chemokine levels in 10 patients with PVR grade B with those in 11 patients with PVR grade C or higher. IL-6 ($P = 0.024$) and CCL3 ($P = 0.017$) were significantly lower in patients with higher PVR grades, whereas no significant differences were detected between both groups concerning the other analytes.

DISCUSSION

The present results show that levels of MIF, CCL2, CCL11, CCL17, CCL18, CCL19, CCL22, CXCL8, CXCL9, and CXCL10 were significantly higher in patients with retinal redetachment due to postoperative PVR than in patients with an uncomplicated detachment. IL-6, with a threefold increase in the PVR-positive group, was positively correlated with several chemokines. Demographic variables and potential clinical risk factors for the development of PVR, except for preoperative visual acuity, were not significantly different between the two groups. Moreover, patients with preoperative uveitis and those with conditions that may induce an inflammatory response such as preoperative trauma and vitreous hemorrhage, were excluded from this study. Therefore, our findings indicate that there is an association between a wide range of chemokines

TABLE 3. The Spearman Correlation Coefficients for Significantly Upregulated Chemokines

Chemokine	Correlation Coefficient (r)
MIF × CCL2	0.596
CCL11 × CCL17	0.693
CCL11 × CCL22	0.513
CCL17 × CCL22	0.668
CCL17 × CXCL9	0.515
CCL18 × CCL19	0.594
CCL18 × CXCL9	0.502
CCL18 × CXCL10	0.592
CCL19 × CXCL10	0.628

All correlations are significant at $P < 0.001$.

TABLE 4. Summary of Mean Values of Various Chemokines in Patients with Proliferative Vitreoretinopathy

	Old Classification[10]	Receptor[10]	Target Cell(s)[10]	Ricker et al.	Banerjee et al.[17]*	Abu El-Asrar et al.[11]	Capeans et al.[12]	Elner et al.[13]	Abu El-Asrar et al.[14]	Mitamura et al.[15]	El-Ghrably et al.[16]
Specimen				SF	VF	VF	VF	VF	VF	VF	VF
Patients with PVR				$n = 21$	$n = 8$	$n = 32$	$n = 20$	$n = 13$	$n = 43$	$n = 29$	$n = 15$
Established PVR				No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Chemokine											
CCL1	I-309	CCR8	T, NK			ND					
CCL2	MCP-1	CCR2, CCR10	M, m, T, NK, b	900 ± 132	6,101 (128–8,777)	2,740 ± 2,460	890 (286–1,806)	7,700 ± 6,250	9,100 ± 6,900	1,761 ± 471	
CCL3	MIP-1α	CCR1, CCR5	M, m, T, NK, N, b, e	398 ± 89.3	ND	ND	ND				
CCL4	MIP-1β	CCR5	M, m, T, NK		22 (0–133)	ND	ND				
CCL5	RANTES	CCR1, CCR3, CCR5	M, m, T, NK, N, b, e	205 ± 151	ND						
CCL7	MCP-3	CCR1, CCR2, CCR3	M, m, T, NK, N, b, e			ND					
CCL8	MCP-2	CCR1, CCR2, CCR3	M, m, T, NK, N, b, e			ND					
CCL11	Eotaxin	CCR3	T, b, e	10.0 ± 2.16	6 (0–23)						
CCL17	TARC	CCR4, CCR8	T, NK, b	2.97 ± 1.24							
CCL18	PARC/MIP-4	?	T	7,049 ± 3,480							
CCL19	MIP-3β	CCR7	T, B, D	290 ± 160							
CCL21	SLC/6Ckine	CCR7	T, B, D	917 ± 371							
CCL22	MDC	CCR4, CCR8	T, NK, b	34.3 ± 18.5							
CXCL5	ENA-7	CXCR2	N			ND					
CXCL6	GCP-2	CXCR1, CXCR2	N, M			ND					
CXCL8	IL-8	CXCR1, CXCR2	N, M	259 ± 296	63.5 (0–977)			11,900 ± 15,200	ND†		63.6‡
CXCL9	Mig	CXCR3	T, NK	22.2 ± 11.0							
CXCL10	IP-10	CXCR3	T, NK	425 ± 275		500 ± 550					
CXCL11	I-TAC	CXCR3	T, NK			ND					
CXCL12	SDF-1	CXCR4	T, B, D, m	11,376 ± 2,897							
CX_3CL1	fractalkine	CX_3CR1	T, NK	231 ± 63.4							

Levels are expressed as the mean ± SD or the mean (range) in picograms per milliliter, unless otherwise indicated. SF, subretinal fluid; VF, vitreous fluid; ND, not detected; M, macrophages; m, monocytes; T, T cells; NK, natural killer cells; B, B cells; D, dendritic cells; N, neutrophils; b, basophils; e, eosinophils.

* Study did not compare PVR vs. RRD.

† Not detected in 41 of 43 cases.

‡ Median level only.

that are elevated briefly after the onset of RRD and the future development of postsurgical PVR.

Previous studies in which chemokine content in PVR specimens has been investigated have focused particularly on a few chemokines using enzyme-linked immunosorbent assays (ELISAs). Two of the most extensively studied chemokines are CCL2 and CXCL8. Several investigations have shown an upregulation of these chemokines in PVR patients.[11–16] We confirmed the findings of these earlier studies in our subretinal fluid samples, although we were able to show only a slight but significant increase in CCL2 levels briefly after the onset of RRD. For example, Elner et al.[13] demonstrated a six-fold increase in CCL2 levels in PVR vitreous compared with that in samples from patients with uncomplicated RRD. These results imply that CCL2 has a more profound role in later stages after RRD and when PVR membranes have already developed.

Our study, however, differed from previous reports on some crucial points. Most studies have dealt with vitreous samples, whereas we studied chemokine content in subretinal fluid. Study of subretinal fluid may be more appropriate, since it is the ocular fluid that surrounds the resident retinal cells after initial retinal detachment. Another important difference from earlier studies is the time point at which the ocular samples were obtained. Sampling at a time close to the onset of the primary RRD may provide clues to the local triggers initiating the PVR process.[26] Our patient population served this purpose, since the median overall delay between reported onset of RRD and surgery was only 5 to 6 days. Moreover, the multiplex-bead–based immunoassay allowed us to detect a large number of chemokines simultaneously, with comparable performance in sensitivity, accuracy, and reproducibility to ELISAs performed in previous studies.[27] Consequently, detection of a whole spectrum of chemokines briefly after the onset of RRD may provide new insight into the pathologic mechanisms leading to the formation of PVR membranes and may thus be important in future prophylaxis or treatment purposes. Differences between our study and previous studies, chemokine function, and chemokine levels are summarized in Table 4. Before the present study, 13 different chemokines had been analyzed in ocular fluids in relation to PVR development. Our data set expanded the number to 21, and it is evident that several novel chemokines will be discovered in the near future. The precise role of each of these chemokines after the onset of RRD remains to be elucidated. It has been postulated that many CC and CXC chemokines share a common function and tend to act on a broad range of leukocytes, including monocytes, lymphocytes, and neutrophils (Table 4).[10] This concept is further supported by redundancy and binding affinity between many chemokine ligands and receptors.[28]

For some chemokines, however, a specific role in the development of PVR has been suggested. CCL2 is mainly known for its induction of monocyte chemotaxis in areas of injury.[29] It has been shown that CCL2 is critical in the infiltration of macrophages/microglia to the subretinal space after retinal detachment.[30] Although a wide variety of cells can express CCL2, local production by resident ocular cells including retinal pigment epithelial (RPE) cells has been suggested. For example, in vitro studies have shown that IL-1 or TNF-α stimulated RPE cells may express CCL2.[31,32] Moreover, CCL2 has been shown to stimulate RPE cell migration,[33] suggesting a role for this chemokine in PVR development. Further, monocytes/macrophages have been shown to have a potentiating effect on RPE cell proliferation.[34] Similarly, CXCL8 may be secreted by many different cell types in response to inflammatory stimuli.[35] CXCL8 exerts many functions depending on cell type and tissue. Nevertheless, its main function is the recruitment of neutrophils to inflammatory sites in response to injury.[35] Goczalik et al.[36] showed that microglial cells may contribute to increased intraocular levels of CXCL8 in retinal detachment and PVR, and CXCL8 receptor expression in glial cells of PVR retinas and PVR membranes suggests a role for CXCL8 in the initiation of reactive gliosis.

Some chemokines can exhibit high cell and receptor specificity. CXCL9 and CXCL10 are highly specific for T lymphocytes.[37] CXCR3, the receptor for CXCL9 and CXCL10, is preferentially expressed on T lymphocytes, which are responsible for several T-lymphocyte-mediated diseases, including autoimmune uveitis and vernal keratoconjunctivitis.[38–40] Moreover, cytokine-stimulated RPE cells are capable of producing CXCL9 and CXCL10, whereas pretreatment of RPE cells with the anti-inflammatory cytokine IFN-β resulted in the elimination of CXCL9 production.[41] The CC chemokines CCL17, CCL18, and CCL22 have also been shown to play a role in trafficking and activation of T lymphocytes.[42–45] Together with the presence of T lymphocytes in subretinal fluid, vitreous fluid,[7] and epiretinal membranes[4,6] from patients with PVR, these findings warrant further investigation concerning the role of T lymphocytes in the pathogenesis of PVR.

IL-6 is a cytokine with a wide range of functions in inflammation and wound healing. For instance, IL-6 was found to induce chemokines and to recruit leukocytes in an animal model.[21] On the other hand, IL-6 may be produced by inflammatory cells invading the subretinal space after RRD due to chemotactic signaling.[46] In previous studies, increased levels of IL-6 in RRD patients[47,48] and PVR patients[16,18–20] have been shown, whereas others have demonstrated a correlation between IL-6 and several chemokines.[14,47] Our study confirmed the findings of these previous studies. Whether IL-6 plays a role in the induction of chemokines or is secreted by cells invading the subretinal space after RRD remains to be elucidated.

So far, it is not clear which cells are responsible for the secretion of chemokines into the subretinal space after retinal detachment. Previous studies have demonstrated that increased chemokine levels are most likely due to the intraocular production by resident ocular cells.[13,15] The RPE cell is believed to be an important candidate cell type, since RPE cells are able to produce many chemokines after an appropriate stimulus. Contact with vitreous[49] or monocytes,[50] stimulation by proinflammatory cytokines,[51] and mechanical injury[52] have all been shown to be a trigger for the production and secretion of chemokines by RPE cells. Other resident cell types or inflammatory cells, however, may also contribute to the subretinal fluid content of chemokines after retinal detachment. Correlations between some chemokines in our study may indicate that a common pathway is involved.

To conclude, our findings indicate that several chemokines are upregulated briefly after the onset of RRD in patients in whom postoperative PVR develops after primary RRD repair. Increased chemotactic signaling in these patients may be the underlying phenomenon leading to a vast and immediate influx of inflammatory cells after the onset of RRD and may cause an inflammatory response that is associated with the future development of PVR. Moreover, the results identify some specific chemokines as potential therapeutic targets for patients who are at risk of redetachment due to PVR.

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