Soluble Apoptotic Factors and Adhesion Molecules in Rhegmatogenous Retinal Detachment

Lukas J. A. G. Ricker,1,2 Raffaele Altara,3 Fleur Goezinne,1 Fred Hendrikse,1 Aize Kijlstra,1 and Ellen C. La Heij1,4

PURPOSE. To investigate the association between soluble apoptosis and adhesion molecules and the development of proliferative vitreoretinopathy (PVR) after reattachment surgery for rhegmatogenous retinal detachment (RRD).

METHODS. A multiplex immunoassay was used to measure soluble Fas (sFas), sFas ligand (sFasL), soluble intercellular adhesion molecule (sICAM)–1, and soluble vascular cell adhesion molecule (sVCAM)–1 levels in 55 subretinal fluid samples collected during scleral buckling surgery for primary RRD. Seventeen patients who developed a redetachment due to postoperative PVR after reattachment surgery (PVR group) were compared with age-, sex-, and storage-time–matched RRD samples from 38 patients with an uncomplicated postoperative course (RRD group). Ten vitreous samples from patients with macular hole and ten vitreous samples from eye bank eyes served as additional controls.

RESULTS. A 2- to 3-fold increase in levels of sFas, sFasL, sICAM-1, and sVCAM-1 was found in the PVR group compared with those of the RRD group (P < 0.05 for all analytes), as well as a 5- to 20-fold increase in the PVR group compared with those of additional control groups (P < 0.001 for all analytes). Significant associations (P < 0.001) were found between sICAM-1 (r = 0.84) and sVCAM-1 (r = 0.93) and between sFasL and both sICAM-1 (r = 0.82) and sVCAM-1 (r = 0.85). In addition, sFas, sFasL, and sVCAM-1 were significantly correlated (P < 0.05) with the extent and duration of retinal detachment.

CONCLUSIONS. These findings indicate that an increased expression of soluble apoptosis and adhesion molecules at the time of primary retinal detachment surgery is associated with the future development of PVR. (Invest Ophthalmol Vis Sci. 2011; 52:4256–4262) DOI:10.1167/iovs.10-6892

Proliferative vitreoretinopathy (PVR), the primary cause of failure of retinal detachment surgery, is an eye-sight-threatening condition that is characterized by intraretinal gliosis and the formation of cellular membranes on both sides of the retina.1,2 The retinal pigment epithelial (RPE) cell is considered to be a critical cell type in the formation of PVR membranes. It has been shown that already shortly after onset of rhegmatogenous retinal detachment (RRD) RPE cells beneath a detached retina may leave the monolayer and start proliferating and migrating.3,4 The exact mechanism by which only in a small minority of patients with RRD does the growth of RPE cells lead to postoperative PVR is unknown. We have previously suggested that PVR-affected patients lack a mechanism that stimulates apoptosis of uncontrolled RPE cells shortly after RRD onset.5 Ligation of the cell surface receptor Fas by its ligand FasL has been shown to induce apoptosis in proliferating RPE cells, whereas nonproliferating RPE cells also express Fas but were resistant to Fas ligation.6 The Fas/FasL system may thus play a role in the removal of excessive RPE cells after RRD onset, and it therefore seems plausible that a defective Fas/FasL system may predispose to PVR development.

So far, previous work has focused on the identification of apoptosis in patients with established PVR. Apoptotic cell nuclei were detected in epiretinal membranes of patients with PVR,7 and Fas expression has been reported in traction membranes in traumatic PVR.8 Furthermore, vitreous Fas and FasL mRNA were detected in patients with retinal detachment with and without PVR.9 Interestingly, both Fas and FasL also exist in soluble forms, sFas and sFasL. sFas inhibits Fas-mediated apoptosis,10 whereas the function of sFasL is controversial. It has been demonstrated that sFasL is a functional molecule that is capable of inducing apoptosis in preneoplastic cells.11 On the other hand, there is also abundant evidence that sFasL is antiapoptotic by binding the receptor protein Fas, thereby interfering with the induction of apoptosis by the membrane-bound form of FasL.12–15 Whether sFasL is a death promoter or death inhibitor probably depends on various factors such as the microenvironmental context and trimerization of the ligand.11

Intraocular levels of sFas and sFasL have been demonstrated to be significantly elevated in patients with active uveitis,16 suggesting that both molecules may also have proinflammatory properties. Moreover, in vitro studies have shown that the ligation of Fas resulted in increased expression of intercellular adhesion molecule (ICAM)–1 and vascular cell adhesion molecule (VCAM)–1.17 Both ICAM-1 and VCAM-1 mediate the recruitment of inflammatory cells to sites of injury, and their soluble forms have been shown to be increased in patients with PVR.18 The Fas/FasL system may thus also be indirectly involved in the exaggerated inflammatory response that underlies PVR development.

In this study, we have detected and quantified the expression of sFas, sFasL, sICAM-1, and sVCAM-1 in subretinal fluid samples of patients who underwent scleral buckling surgery for primary RRD. Patients who developed a redetachment due to PVR were compared with age-, sex-, and storage-time–matched control patients who had an uncomplicated postoperative course during the overall follow-up period. We found...
that significantly increased levels of sFas, sFasl, sICAM-1, and sVCAM-1 were associated with the future development of PVR.

**METHODS**

**Patients**

In our department subretinal fluid samples are routinely obtained during scleral buckling surgery for primary RRD and transferred to the BioBank Maastricht, where they are aliquoted in 50 μL portions. All samples in this study were collected between 2003 and 2008. In this time frame, a total of 232 samples were collected. Of these, 32 samples represented patients who developed a redetachment due to PVR later on during the postoperative course. Of these, we excluded 3 patients with preoperative vitreous hemorrhage, 3 patients with preoperative trauma, and 3 patients because of late PVR development (>2½ months after reattachment surgery). Furthermore, 6 patients were excluded due to low sample volumes (<50 μL) or contamination with blood. Finally, 17 samples from patients who developed a redetachment due to PVR within 2½ months after scleral buckling surgery for primary RRD were included in the study (defined as the PVR group). These were compared with 38 samples from patients who did not develop a redetachment during the total follow-up period, i.e., patients with an uncomplicated postoperative course (defined as the RRD group). The samples from both groups were matched for age, sex, and storage time. None of the included patients had preoperative uveitis or autoimmune disease, whereas none of the diabetics (n = 5) had diabetic retinopathy.

For all these patients, we collected demographic variables and potential clinical risk factors for the development of postoperative PVR (Table 1), and the following clinical variables: follow-up time, occurrence of a redetachment, postoperative PVR grade, and preoperative potential clinical risk factors for the development of postoperative 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. Retinopathy by intraoperative cryotherapy was performed in 64% of patients. In the majority of patients cryotherapy was applied two to three times around the retinal break. For statistical analysis, Snellen visual acuity was transformed into logMAR (logarithm of minimal angle of resolution) visual acuity. PVR was graded according to the Classification of Retinal Detachment with PVR.19 Data were collected as 0 (no PVR), 1 (grade A), 2 (grade B), 3 (grade C), and 4 (grade D). Duration of retinal detachment was defined as the interval between the onset of symptoms and reattachment surgery and was estimated according to a precise history of patients’ symptoms.

Accordingly, we included 10 vitreous samples from patients who underwent pars plana vitrectomy for macular hole and 10 vitreous samples obtained from 10 eye bank eyes with consent for research. The study was performed with the agreement of the University Hospital Maastricht Medical Ethics Committee. All patients gave their informed consent before inclusion in the study and after the nature of the study was explained. The study adhered to the tenets of the Declaration of Helsinki.

**Specimens**

Undiluted subretinal fluid samples were obtained during scleral buckling surgery for primary RRD as described previously.20,21 Before the incision, scleral and choroidal vessels were carefully cautetured. A cotton tip was used to remove any macroscopic blood that surrounded the incision opening. Subretinal fluid samples were collected from the surface of the sclera with the use of a 25-gauge bent needle. Undiluted vitreous samples were obtained by conventional three-port, closed vitrectomy by manual suction at the start of the surgical procedure before opening the infusion line. Samples were taken from the core of the vitreous body.

Vitreous samples from eye bank eyes were obtained from the Cornea Bank Amsterdam and were isolated within 24 hours after death. The donors did not have any known eye disease and deceased as the result of trauma, cerebrovascular event, or cardiac or respiratory failure. All samples were collected in sterile tubes, immediately stored at our BioBank at −80°C, and thawed directly before analysis. Samples that were contaminated with macroscopic hemorrhage were discarded. Sample volumes ranged between 50 and 300 μL for subretinal fluid and between 50 and 1300 μL for vitreous fluid.

**Multiplex Immunoassay**

sFas, sFasl, sVCAM-1, and sICAM-1 were measured with diagnostic process software (Luminex-100 device; Luminex, Austin, TX) using a commercial multiplex immunoassay kit (Millipore, Billerica, MA). The assay was run according to the instructions of the manufacturer. In summary, the premixed beads coated with the target antibodies were incubated for 120 minutes at room temperature with premixed standards or with subretinal fluid or vitreous fluid (25 μL). After repeated washings, biotinylated detection antibodies were added for an addi-

### Table 1. Demographics and Potential Clinical Risk Factors for PVR

<table>
<thead>
<tr>
<th>Potential Clinical Risk Factor</th>
<th>RRD (n = 38)</th>
<th>PVR (n = 17)</th>
<th>Univariate Testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years, median (range)</td>
<td>60 (43–72)</td>
<td>59 (43–72)</td>
<td>NS</td>
</tr>
<tr>
<td>Sex, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>29</td>
<td>29</td>
<td>NS</td>
</tr>
<tr>
<td>Male</td>
<td>71</td>
<td>71</td>
<td>NS</td>
</tr>
<tr>
<td>Size of retinal detachment in quadrants, median (range)</td>
<td>2 (1–3)</td>
<td>2 (1–4)</td>
<td>NS</td>
</tr>
<tr>
<td>Number of retinal defects, median (range)</td>
<td>1.5 (1–5)</td>
<td>1 (0–4)</td>
<td>NS</td>
</tr>
<tr>
<td>Macular detachment, %</td>
<td>70</td>
<td>82</td>
<td>NS</td>
</tr>
<tr>
<td>Preoperative logMAR visual acuity, median (range)</td>
<td>0.75 (0.05–2.52)</td>
<td>1.77 (0.10–2.52)</td>
<td>P = 0.046</td>
</tr>
<tr>
<td>Detachment duration in days, median (range)</td>
<td>5 (1–75)</td>
<td>7 (1–58)</td>
<td>NS</td>
</tr>
<tr>
<td>Preoperative PVR grade Median (range)</td>
<td>1 (0–3)</td>
<td>2 (0–3)</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>11</td>
<td>6</td>
<td>NS</td>
</tr>
<tr>
<td>Preoperative myopia &gt; 5D, %</td>
<td>17</td>
<td>31</td>
<td>NS</td>
</tr>
<tr>
<td>Preoperative lens status, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pseudophakia</td>
<td>16</td>
<td>35</td>
<td>NS</td>
</tr>
<tr>
<td>Aphakia</td>
<td>0</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>Preoperative uveitis, %</td>
<td>0</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>Preoperative vitreous hemmorhage, %</td>
<td>0</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>Preoperative cryotherapy, %</td>
<td>0</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>Preoperative trauma, %</td>
<td>0</td>
<td>0</td>
<td>NS</td>
</tr>
</tbody>
</table>

D, Diopters; NS, not significant.
tional 60 minutes. Subsequently, streptavidin-phycocerythrin was added to the wells. After incubation for 30 minutes, the wells were washed twice, the beads were resuspended in 100 μL sheath fluid, and fluorescence intensity was measured. Data collection and analysis of the data from all assays were performed (software from Bioplex Manager 4.1.1, Bio-Rad, Hercules, CA) using five-parameter curve fitting. Levels of sFasL below the detection limit were assigned the lowest value from the standard curve, whereas sICAM-1 levels above the detection limit were assigned the highest value from the standard curve because remeasurements were not possible, given the limited amount of sample. For statistical analysis, concentrations below or above the detection limit were converted to a value 0.5-fold the lowest value or 2.0-fold the highest value of the calibration curve, respectively.

Statistics

Samples were divided into four groups: patients who developed a redetachment due to PVR within 2½ months after scleral buckling surgery (the PVR group), patients with an uncomplicated postoperative course after reattachment surgery (the RRD group), patients who underwent pars plana vitrectomy for macular hole, and eye bank eyes. The latter two groups served as additional controls. Since data were not normally distributed, nonparametric tests were used for statistical analysis. Analyte levels between more than two groups were compared using the Kruskal–Wallis test, and levels between two groups were analyzed using the Mann–Whitney U test. Correlations were determined by the Spearman ρ test. To compare clinical variables between the PVR group and the RRD group, the χ² test and the Mann–Whitney U test were used as appropriate. Statistical analysis was performed using an algorithm, input–output module software (SPSS for Windows, version 16.0; SPSS, Chicago, IL). Differences were considered significant at P < 0.05, with two-tailed testing.

RESULTS

Demographics and Clinical Results

sFas, sFasL, sVCAM-1, and sICAM-1 levels were quantified by multiplex immunoassay in subretinal fluid samples of 55 patients who underwent scleral buckling surgery for primary RRD. Initial reattachment was achieved in all 55 cases. Seventeen patients who developed a redetachment due to postoperative PVR were compared with 38 patients with an uncomplicated postoperative course. In the PVR group, there were 5 women (29%) and 12 men (71%) with a median age of 59 years (range, 43–72 years). Of these 17 patients with postoperative PVR, 9 patients were classified with PVR grade B, 7 with PVR grade C, and 1 with PVR grade D. The median time interval between reattachment surgery and redetachment due to postoperative PVR was 35 days (range, 7–80 days) and the median follow-up time was 21 months (range, 3–80 months). The RRD group consisted of 11 women (29%) and 27 men (71%) with a median age of 60 years (range, 43–72 years). Their median follow-up time was 21⁄2 months after scleral buckling surgery (the PVR group), patients with an uncomplicated postoperative course (the RRD group), patients who underwent pars plana vitrectomy for macular hole, and eye bank eyes. The latter two groups served as additional controls. Since data were not normally distributed, nonparametric tests were used for statistical analysis. Analyte levels between more than two groups were compared using the Kruskal–Wallis test, and levels between two groups were analyzed using the Mann–Whitney U test. Correlations were determined by the Spearman ρ test. To compare clinical variables between the PVR group and the RRD group, the χ² test and the Mann–Whitney U test were used as appropriate. Statistical analysis was performed using an algorithm, input–output module software (SPSS for Windows, version 16.0; SPSS, Chicago, IL). Differences were considered significant at P < 0.05, with two-tailed testing.

sFas and sFasL Levels in Subretinal Fluid

According to Kruskal–Wallis tests, significant differences between groups were found for both sFas (P < 0.0001) and sFasL (P < 0.0001). Detectable levels of sFas were determined in all samples investigated. Subretinal fluid levels of sFas from patients who developed a redetachment due to PVR were approximately 2.5-fold higher than those from eyes with RRD and an uncomplicated postoperative course (Mann–Whitney U test; P = 0.0396). In addition, subretinal fluid sFas levels of both the PVR group and the RRD group were significantly elevated compared with the macular hole group (P < 0.001 for both comparisons) and the eye bank eye group (P < 0.001 for both comparisons) (Fig. 1A).

sFasL was detected in 16/17 (94%) patients in the PVR group, in 32/38 (84%) patients in the RRD group, in 0/10 (0%) patients with macular hole, and in 3/10 (30%) vitreous fluids from eye bank eyes. Median subretinal fluid sFasL concentrations were approximately twofold higher in the PVR group than those in the RRD group. This twofold difference between both groups at this early stage of investigation, i.e., at the time of primary RRD when no PVR has manifested yet, was highly significant (Mann–Whitney U test; P = 0.0057). Moreover, levels in samples from both retinal detachment groups were significantly higher compared with those in both the macular hole group and the eye bank eye group (P < 0.001 for all comparisons) (Fig. 1B).

sVCAM-1 and sICAM-1 Levels in Subretinal Fluid

sVCAM-1 and sICAM-1 levels were significantly different between the four groups (Kruskal–Wallis test; P < 0.0001 for both analytes). For sICAM-1, 2/17 (12%) PVR samples and 2/38 (5%) RRD samples were above the upper detection limit, whereas sVCAM-1 was detected in all samples investigated.
TABLE 2. Summary of sFas, sFasL, sICAM-1, and sVCAM-1 Levels in Retinal Detachment, Macular Hole, and Eye Bank Eyes

<table>
<thead>
<tr>
<th>Analyte (pg/mL)</th>
<th>PVR (SRF; n = 17)</th>
<th>RRD (SRF; n = 38)</th>
<th>Macular Hole (VF; n = 10)</th>
<th>Eye Bank Eyes (VF; n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>sFas Median (range)</td>
<td>3688* (271–17677)</td>
<td>1524* (446–22600)</td>
<td>370 (104–482)</td>
<td>576 (321–1864)</td>
</tr>
<tr>
<td>sFasL Median (range)</td>
<td>40* (&lt;9.5–347)</td>
<td>19* (&lt;9.5–293)</td>
<td>&lt;9.5 (&lt;9.5–9.5)</td>
<td>&lt;9.5 (&lt;9.5–15)</td>
</tr>
<tr>
<td>sICAM-1 Median (range)</td>
<td>27540* (3707–245000)</td>
<td>11434* (1629–245000)</td>
<td>1336 (635–2523)</td>
<td>2149 (777–7297)</td>
</tr>
<tr>
<td>sVCAM-1 Median (range)</td>
<td>36436* (6883–114189)</td>
<td>19477 (7252–149754)</td>
<td>3252 (2471–6622)</td>
<td>5613 (2836–21862)</td>
</tr>
</tbody>
</table>

SRF, subretinal fluid; VF, vitreous fluid.

* PVR vs. RRD, P < 0.05; PVR vs. macular hole, P < 0.001.

** PVR vs. RRD, P < 0.05; PVR vs. macular hole, P < 0.001; PVR vs. eye bank eyes, P < 0.001.

Figure 2. Box-and-whisker plots of (A) sICAM-1 and (B) sVCAM-1 levels in subretinal fluid samples from patients with primary RRD. Patients who developed a retadetachment as a result of postoperative PVR (n = 17, PVR group) were compared with controls who had an uncomplicated retinal detachment during the overall follow-up period (n = 38, RRD group). Vitreous fluids from patients with MH (n = 10, MH group) and EBE (n = 10, EBE group) served as additional controls. **PVR versus RRD, P < 0.05; PVR versus MH, P < 0.001; PVR versus EBE, P < 0.001; *RRD versus MH, P < 0.01; RRD versus EBE, P < 0.001. Box: lower and upper quartiles; horizontal line: the median.

We found a two- to threefold increase in both sICAM-1 and sVCAM-1 levels in the PVR group compared with levels in the RRD group (Mann–Whitney U test; P = 0.0056 and P = 0.0414, respectively). Furthermore, levels of both adhesion molecules in the PVR group and the RRD group were significantly elevated (up to 20-fold) compared with levels in the macular hole group and the eye bank eye group (P < 0.001 for all comparisons) (Figs. 2A, 2B). The levels of all soluble factors investigated (sFas, sFasL, sICAM-1, sVCAM-1) are summarized in Table 2.

Correlations between Soluble Factors and Clinical Variables

There was a strong positive correlation between sFas and sFasL (Spearman rho test; r = 0.89; P < 0.0001) and sVCAM-1 and sICAM-1 (r = 0.75; P < 0.0001) (Figs. 3A, 3B). Moreover, we found significant associations (P < 0.0001) between sICAM-1 and both sFas (r = 0.84) and sFasL (r = 0.82) and between sVCAM-1 and both sFas (r = 0.93) and sFasL (r = 0.85) (Figs. 3C-F). When comparing the soluble factors with clinical variables, we found that sFas, sFasL, and sVCAM-1 levels were significantly correlated (P < 0.05) with detachment duration (r = 0.45, r = 0.31, and r = 0.39, respectively), duration of macular detachment (r = 0.55, r = 0.37, and r = 0.54), and the number of quadrants involved (r = 0.34, r = 0.30, and r = 0.32). Both sFasL and sICAM-1 were not associated with postoperative PVR, whereas its correlations with sFas and sVCAM-1 were low (r = 0.31 and r = 0.32, respectively) (P < 0.05). There were no significant correlations between sICAM-1 and other preoperative clinical variables. Final visual acuity was significantly better in the RRD group (median logMAR visual acuity, 0.22; range, −1–1.30) than that in the PVR group (median logMAR visual acuity, 1.10; range, 0–2.52) (Mann–Whitney U test; P < 0.0001). Preoperative visual acuity (r = 0.41; P = 0.002) and sICAM-1 (r = 0.52; P = 0.017) were significantly associated with final visual outcome, whereas sFas, sFasL, and sVCAM-1 were not (r = 0.21, r = 0.21, and r = 0.16, respectively; P > 0.05).

Discussion

In the present study, subretinal fluid levels of sFas, sFasL, sICAM-1, and sVCAM-1 were significantly increased in patients who developed postoperative PVR compared with patients with an uncomplicated follow-up after primary retinal detachment surgery. To illustrate, we found a two- to threefold increase in levels of all soluble factors investigated in the PVR group compared with that in the RRD group, and even a 5- to 20-fold increase compared with the additional control groups (Figs. 1, 2; Table 2). Furthermore, strong associations (r ≥ 0.75) were found between sFas, sFasL, sICAM-1, and sVCAM-1.

Previous studies have implicated an abnormal regulation of apoptosis in the pathogenesis of proliferative vitreoretinal disorders. Research has focused on the detection of apoptotic cells in epiretinal membranes of patients with established PVR.7–9,22,23 Most apoptotic cells appeared to be from RPE cell origin,22,23 and the Fas/FasL system has been assigned an important role in RPE cell death.9 Shifting the balance toward a proapoptotic environment following trauma may lead to the uncontrolled proliferation of cells in this disorder.

In contrast to these reports, the time point at which samples were obtained and the sampling specimen (subretinal fluid instead of vitreous) differed in the present study. In retinal detachment, it was shown that within 24 hours after loss of contact between the pigment epithelium and the photorecep-
tor layer, RPE cells begin to proliferate and dedifferentiate. Therefore, we believe that sampling at a time close to the onset of primary RRD and in the near vicinity of the RPE may provide clues as to which local factors may initiate the uncontrolled growth of cells that lead to PVR membrane formation. Therefore, a comparison was made between subretinal fluids from patients in whom PVR develops after reattachment surgery and from patients with an uncomplicated follow-up. We used vitreous fluids from donated eyes and from patients with macular hole as additional controls for our subretinal fluid samples, as described earlier. Although vitreous differs in composition from subretinal fluid, and thus cannot function as an ideal control specimen, it has been suggested that vitreous is the main source of subretinal fluid in recent onset RRD.

Since our goal was to identify soluble mediators that might contribute to the initial stages of PVR development, it was of utmost importance to have similar baseline characteristics between those patients who developed PVR and those who did not. Therefore, we decided to match both groups for age, sex, and storage time of the sample. Although other preoperative variables may also be considered for matching, our subretinal fluid database was not large enough to allow us to do so. Nevertheless, no significant differences with respect to important preoperative clinical characteristics such as extent and duration of retinal detachment were detected between both groups, except for preoperative visual acuity. This may be explained in part by the higher percentage of patients with a detached macula in the RRD group compared with the RRD group that, however, did not reach statistical significance ($P = 0.347$). Moreover, the difference in logMAR preoperative visual acuity is less evident when mean levels instead of median levels are taken into account (PVR group, 1.62; RRD group, 1.10) (data not shown). The introduction of bias was also minimized by exclusion of patients with preoperative conditions known to induce PVR, such as preoperative vitreous hemorrhage and preoperative trauma. Furthermore, none of the patients had uveitis or autoimmune disease, and none of the diabetics had signs of diabetic retinopathy. The correlations between increased levels of soluble apoptotic molecules as well as adhesion molecules and PVR development may thus suggest their involvement in the initial stages of this fibrotic eye condition. Of note, the percentage of patients with postoperative PVR in our database (13.7%) was somewhat higher than numbers reported in the literature. This higher incidence may be explained by the fact that we did not collect subretinal fluid in patients with small or shallow detachments, i.e., retinal detachments at low risk for developing PVR.

Fas is expressed on a wide variety of cells such as inflammatory cells, which are known to infiltrate the subretinal space after retinal detachment, and resident ocular cells. Because all these cells have the potential to transcribe Fas, the source of sFas in our subretinal fluid samples remains to be clarified. It has been postulated that increased shedding of the receptor protein Fas from the cell membrane, which leads to the secretion of the antiapoptotic sFas, may downregulate the killing activity of FasL-bearing cells; however, the surface expression of FasL is limited to resident cells in immune privileged sites and activated immune cells. In the retina, FasL is expressed on RPE cells and throughout the retina. Moreover, RPE cells have been shown to upregulate FasL in areas with subretinal inflammatory infiltrates, whereas cultured RPE cells were capable of releasing both the membrane form of

![Figure 3. Correlations between various analytes in subretinal fluid samples after primary RRD. Correlation between (A) sFas and sFasL, (B) sICAM-1 and sVCAM-1, (C) sICAM-1 and sFas, (D) sICAM-1 and sFasL, (E) sVCAM-1 and sFas, and (F) sVCAM-1 and sFasL.](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933461/)
FasL (mFasL) in vesicles and sFasL. Although FasL expression on epithelial cells appears to be an important mechanism for the maintenance of immune privilege in the eye by inducing apoptosis of infiltrating Fas-positive immune cells, the exact role of its soluble form remains to be clarified. In contrast to studies that have demonstrated the apoptosis-inducing properties of sFas, there is abundant evidence that its killing capacity is greatly reduced or even may counteract the induction of apoptosis by mFasL. Taken together, the source and function of both sFas and sFasL in the subretinal space after the neurosensory retina separates from the underlying RPE are unclear and should be determined in future studies.

Because the relationship between increased soluble mediators and PVR development may represent only a correlation, alternative explanations for our findings must be considered. There is a large body of evidence that the separation of photoreceptors from the RPE leads to disruption of normal photoreceptor homeostasis, and the Fas-mediated extrinsic death pathway and the mitochondrial intrinsic death pathway have both been assigned an important role in photoreceptor loss after retinal detachment. In addition, these studies have shown that both pathways are activated in a time-dependent fashion after retinal detachment. Even though the correlations between sFas/sFasL and the extent and duration of retinal detachment in our study were weak, these findings may point to a protective role in the survival of photoreceptors after RRD. In line with this hypothesis, the intrinsic production of the antiapoptotic sFas after hypoxic-ischemic events in the brain was suggested to protect central nervous system cells from further damage.

Both sFas and sFasL have also been attributed proinflammatory properties in addition to their roles in apoptosis. The hypothesis that the Fas/FasL system merely confers immune privilege has been challenged by several studies demonstrating that sFasL may induce a granulocytic response. Furthermore, sFas has been found in a number of conditions of acute and chronic inflammation, including cardiovascular, respiratory, and autoimmune disease. In the eye, the levels of sFas and sFasL in the vitreous of patients with active uveitis were significantly higher than levels in those with inactive uveitis, whereas in patients who underwent penetrating keratoplasty the incidence of detectable sFasL was higher in those who had immune reactions than that in those without immune reactions.

Next to the increased levels of sFas and sFasL, we observed significantly elevated levels of the proinflammatory adhesion molecules sICAM-1 and sVCAM-1 in the PVR group compared with the RRD group (Fig. 2). Both adhesion molecules have been shown to be elevated in vitreous of patients with PVR compared with that in patients with macular hole in a prior study, whereas ICAM-1 expression was found on PVR membranes. Since ICAM-1 and VCAM-1 have a role in leukocyte adhesion and migration into the injured tissue, both molecules may be involved in the early events that elicit an inflammatory response that is associated with the future development of PVR. Elevated sICAM-1 levels in the present study confirm the results of a noncommercial sICAM-1 multiplex assay that we performed elsewhere in a similar population (data submitted). The local production of adhesion molecules by resident ocular cells has been suggested since serum levels in patients with PVR were not increased. Retinal endothelial cells and RPE cells are possible sources of sICAMs in patients with retinal detachment; in vitro studies have demonstrated the increased expression of both ICAM-1 and VCAM-1 in these cell types in the presence of inflammatory cytokines. Moreover, retinas separated from the RPE in experimental retinal detachment showed increased ICAM-1 and VCAM-1 gene transcription.

Interestingly, we found strong correlations between sFas/sFasL and both adhesion molecules (Fig. 3). There is scarce evidence that Fas activation may lead to the upregulation of adhesion molecules. In vitro studies have shown that the ligation of Fas resulted in the increased expression of ICAM-1 and VCAM-1 on endothelial cells. The Fas/FasL system may thus be indirectly involved in the recruitment of inflammatory cells to sites of injury, although in vitro studies are needed to investigate whether resident ocular cells are capable of secreting ICAM-1 and VCAM-1 after activation of the Fas/FasL system. Alternatively, the strong correlations between apoptotic and adhesion molecules may also be explained by the activation of resident ocular cells after RRD onset, leading to the simultaneous secretion of sFas, sFasL, sICAM-1, and sVCAM-1.

To summarize, our findings show that levels of sFas, sFasL, sICAM-1, and sVCAM-1 are significantly higher in patients who developed PVR compared with levels in patients with an uncomplicated RRD. The elevated levels of soluble apoptotic factors and soluble adhesion molecules may point to their involvement in the cellular processes that occur in the subretinal space after retinal detachment. The exact roles of the soluble forms of Fas/FasL as death promoters, death inhibitors, or inducers of inflammation need further clarification.

Acknowledgments

The authors thank Elisabeth Pels and colleagues of the Cornea Bank Amsterdam for providing the vitreous fluids from donor eyes.

References

38. Dupont PJ, Warrens AN. Fas ligand exerts its pro-inflammatory effects via neutrophil recruitment but not activation. *Immunology.* 2006;120:153–159.
42. Chen DY, Lan JL, Lin FJ, Hsieh TY. Elevated levels of soluble Fas (APO-1, CD95), soluble Fas ligand, and matrix metalloproteinase-3 in sera from patients with active untreated adult onset Still’s disease. *Clin Rheumatol.* 2007;26:393–400.