Soluble TNF Receptors in Vitreoretinal Proliferative Disease

G. Astrid Limb,¹ Robert D. Hollifield,² Lynne Webster,³ David G. Charteris,¹ and Anthony H. Chignell²

PURPOSE. To measure vitreous levels of soluble TNF-receptors (sTNF-Rs) types I and II in eyes with rhegmatogenous retinal detachment (RRD), uncomplicated or complicated with proliferative vitreoretinopathy (PVR), and in eyes with proliferative diabetic retinopathy (PDR). To examine whether there is any relationship between vitreous levels of sTNF-Rs and clinical features of these conditions and between vitreous sTNF-Rs and TNFα levels and serum levels of sTNF-Rs.

METHODS. Vitreous levels of sTNF-Rs and TNFα were measured by enzyme-linked immunosorbent assay in 30 eyes with PVR, 30 eyes with uncomplicated RRD, and 29 eyes with PDR. Vitreous from eyes of 10 deceased donors and 9 eyes with macular holes served as control specimens. Serum levels of sTNF-Rs were measured in 17 patients with PDR and 21 patients with PVR.

RESULTS. Vitreous levels of sTNF-Rs I and II were increased in eyes with PVR, PDR, and PDR when compared with control eyes (P < 0.002). However, vitreous levels of sTNF-Rs I and II were higher in eyes with PVR than in eyes with RRD (P < 0.01) or PDR (P < 0.03). This contrasted with the findings that serum sTNF-Rs were higher in PDR than in PVR (P < 0.016) and that vitreous levels of TNFα were higher in eyes with PVR than in eyes with PDR (P < 0.005). In PVR, vitreous sTNF-Rs levels were associated with the duration of retinal detachment, number of previous external operations, and grade of severity, whereas in PDR these levels were not related to the type or duration of diabetes or its complication with traction retinal detachment.

Conclusions. These observations suggest the existence of TNF inhibitory mechanisms within the eye during retinal processes of inflammation and angiogenesis. High vitreous levels of sTNF-Rs relate to severity of retinopathy suggests that these molecules may constitute reactive products of inflammation. Effective control of TNFα activity by sTNF-Rs within the retinal microenvironment may determine the outcome and severity of retinal proliferative conditions. (Invest Ophthalmol Vis Sci. 2001;42:1586–1591)

The term proliferative retinopathy is applied to distinct conditions characterized by cellular proliferation and matrix deposition within the retina. Proliferative vitreoretinopathy (PVR) is a complication of rhegmatogenous retinal detachment (RRD) in which fibrocellular membranes form on the retina, whereas proliferative diabetic retinopathy (PDR) is a complication of diabetes mellitus, characterized by neovascularization of the retina and vitreous with formation of fibrovascular membranes at the vitreoretinal interface. Although PVR and PDR have different causes and clinical characteristics, retinal membranes from both conditions share the features of fibroplasia, excessive matrix protein deposition, and cellular infiltration. They differ in that PDR membranes are highly vascular due to the angiogenic activity that takes place within the diabetic retina, whereas PVR membranes are relatively avascular and are not regarded as complications of systemic disease.

Tumor necrosis factor (TNFα) is a cytokine that plays a pivotal role in inflammation, and high levels of these molecules in fluids and serum have been associated with inflammatory processes such as rheumatoid arthritis, Crohn disease, and multiple sclerosis. Although very low levels of this cytokine are detected in vitreous from eyes with PVR and PDR, it constitutes the predominant proinflammatory cytokine observed within the extracellular matrix of PVR membranes and also within the extracellular matrix and luminal abluminal surface of infiltrating vessels in PDR membranes. That this cytokine predominates in retinal tissues, but that only low levels are detected in vitreous, suggests that TNF biological activity may be abrogated by inhibitors present in the vitreous.

Production of TNFα is associated with synthesis and secretion of specific receptors, known as TNF-R1 (55 kDa) and TNF-R2 (75 kDa). Both receptors are expressed on nearly all nucleated cell types, and, after cell activation by TNFα itself, they are cleaved by metalloproteinases and are found as soluble forms in serum and body fluids. These receptors are thought to protect cells from TNFα and to block the activity of this cytokine once the cytokine is released into the circulation. Positive correlation between serum levels of TNF-Rs and disease status has been shown in systemic lupus erythematosus, rheumatoid arthritis, cancer, meningococccia, sepsis, and human immunodeficiency virus (HIV), among others. Although the presence of these receptors has been demonstrated in normal human vitreous, at present it is not known whether abnormal levels of these molecules may be associated with retinal proliferative disease.

In view of this evidence and of the important biological role of these molecules, we measured the vitreous levels of sTNF-R1 and sTNF-R2 in eyes with RRD complicated or uncomplicated by PVR and in eyes with PDR. We also examined whether high levels of these soluble receptors were associated with the clinical history of vitreoretinal disease and with the vitreous levels of TNFα.

PATIENTS AND METHODS

Vitreous samples were obtained from 98 patients at the time of vitrectomy for treatment of uncomplicated RRD (30 patients), PVR (30 patients), PDR (29 patients), or macular holes (9 patients). Presence of PVR was determined at the time of surgery by the criteria of Machemer

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et al.\textsuperscript{20} Accordingly, nine patients had PVR grade B, seven had anterior PVR grade C, six had posterior PVR grade C, and eight had anterior and posterior PVR grade C. In this PVR group, 13 eyes had undergone none or one scleral buckling procedure for RRD, and 17 had undergone two to four similar procedures. Of the patients with PDR, 22 had insulin-dependent diabetes mellitus (IDDM) and 7 had non-insulin-dependent diabetes mellitus (NIDDM). The known duration of diabetes was 18 months to 40 years (median, 18 years). Cadaveric control vitreous was obtained within 7 to 18 hours after death from eye donors who had no known ocular or systemic inflammatory disease. Undiluted vitreous samples (approximately 0.5 ml) were centrifuged for 5 minutes at 600 g to remove contaminating cells and then transferred to cryotubes for storing at \(-70^\circ\text{C}\) until use. Serum was also obtained from 21 patients with PVR and 17 patients with PDR at the time of surgery. Vitreous and blood specimens were obtained by consent and approval of the ethics committee of the local health authority, and the study was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

**Measurement of TNF\(\alpha\), sTNF-RI, and sTNF-RII Levels in Vitreous and Serum**

Levels of sTNF-RI and sTNF-RII were determined with our established methods\textsuperscript{21} by using commercial enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Oxford, UK) as follows: Microtiter well plates coated with specific antibodies to individual sTNF-Rs were incubated with 100 \(\mu\)l of a 1:10 dilution of vitreous, together with 100 \(\mu\)l of the respective anti-TNF-R antibody. After 2 hours’ incubation at room temperature, antibodies and test samples were removed and the plates washed six times with phosphate-buffered saline (PBS) containing 0.05% Tween-20. The amount of conjugated antibodies was detected by addition of 100 \(\mu\)l tetramethylbenzidine (substrate) and incubation for 30 minutes at room temperature. The enzymatic reaction was stopped by addition of 100 \(\mu\)l of 1 M \(\text{H}_2\text{SO}_4\) and the absorbance read at 450 nm, with a correction wavelength of 620 nm, in a plate reader (Dynatech MR5000; Dynex Technologies, Ashford, UK). Levels of specific sTNF-Rs and TNF\(\alpha\) present in vitreous samples were interpolated from specific calibration curves prepared with the standard reagents supplied.

**Statistical Analysis**

The significance of difference between corresponding groups of observations was evaluated by the nonparametric Mann-Whitney test. Acceptable significance was recorded at \(P < 0.05\).

**RESULTS**

**Vitreous Levels of sTNF-RI and sTNF-RII in Eyes with PVR, RRD, and PDR**

The levels of sTNF-RI and sTNF-RII in vitreous from eyes with uncomplicated RRD, PVR, PDR, and macular holes are shown in Figure 1. Vitreous from the uncomplicated RRD group contained significantly higher concentrations of sTNF-RI (range, 226-5901 pg/ml) and sTNF-RII (range, 128-4522 pg/ml) than control cadaveric vitreous (range, 101-836 and 96-551 pg/ml, respectively; \(P < 0.005\)). Vitreous from eyes with RRD complicated by PVR also exhibited significantly higher levels of sTNF-RI (range, 244-4290 pg/ml) and sTNF-RII (range, 128-4429 pg/ml) than did control vitreous (\(P < 0.0003\)). Similarly, vitreous levels of sTNF-RI and sTNF-RII were higher in the PDR group (range, 261-3013 pg/ml and 121-2505 pg/ml, respectively) than in cadaveric vitreous (\(P < 0.005\)). Comparison of the vitreous levels of sTNF-Rs between the different groups of patients showed that eyes with PVR contained higher concentrations of both sTNF-RI and sTNF-RII than did eyes with uncomplicated RRD (\(P < 0.01\) and \(P < 0.0044\), respectively) or PDR (\(P < 0.05\) and \(P < 0.05\), respectively; Fig. 1). It was interesting to note that vitreous from eyes with macular holes exhibited marked lower levels of these molecules than did cadaveric vitreous and eyes with PVR, RRD, or PDR (\(P < 0.005\) versus cadaveric controls and \(P < 0.0004\) versus PVR, PDR, and RRD).

**Vitreous Levels of sTNF-Rs in Relation to the Clinical Features of PVR**

Figure 2 shows the vitreous levels of sTNF-Rs in relation to the duration of retinal detachment in eyes with RRD complicated by PVR. Vitreous levels of both sTNF-RI and sTNF-RII were significantly increased in eyes with retinal detachment of more than 12 weeks’ duration (ranges, 766-4290 and 676-4429 pg/ml, respectively; \(P < 0.005\) and \(P < 0.0002\), respectively) when compared with eyes with retinal detachment of less than 12 weeks’ duration (ranges, 244-2596 and 128-1989 pg/ml, respectively).

As illustrated in Figure 3, vitreous levels of sTNF-RI in eyes with PVR that had been subjected to two to four scleral buckling operations contained higher, but not significant, lev-
Vitreous Levels of sTNF-Rs in Relation to the Clinical Features of RRD

Table 1 shows that vitreous from eyes with uncomplicated RRD of less than 4 weeks' duration contained levels of both sTNF-RI and sTNF-RII similar to those in eyes with RRD of more than 4 weeks' duration \( (P > 0.05) \). In addition, there were no differences between the levels of sTNF-RI and sTNF-RII in eyes with uncomplicated RRD that had undergone no or one scleral buckling operation, when compared with eyes that had undergone two to four similar external operations \( (P > 0.05) \).

Vitreous Levels of sTNF-Rs in Relation to the Clinical Features of Patients with PDR

Table 2 shows that there were no differences between the vitreous levels of sTNF-RI and sTNF-RII in patients with IDDM complicated by PDR and those in patients with NIDDM who had this complication. Vitreous levels of both sTNF-RI and sTNF-RII were similar in patients with diabetes of less than 10 years' duration and those with diabetes of more than 10 years' duration. Vitreous levels of sTNF-RI and sTNF-RII did not differ

![Figure 2. Levels of sTNF-RI and sTNF-RII in vitreous from eyes with RRD complicated by PVR, in relation to the duration of the detachment. *Mann-Whitney test, \( P < 0.005 \) versus less than 12 weeks; **Mann-Whitney test, \( P < 0.00002 \) versus less than 12 weeks. Lines: median values.](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933588/)

![Figure 3. Levels of sTNF-RI and sTNF-RII in vitreous from eyes with RRD complicated by PVR, in relation to the number of previous conventional external operations for scleral buckling. *Mann-Whitney test, \( P = 0.062 \) versus no or one operation; **Mann-Whitney test, \( P < 0.01 \) versus no or one operation. Lines: median values.](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933588/)
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Table 2. Vitreous Levels of sTNF-RI and sTNF-RII in Relation to the Clinical Features of Patients with PDR

<table>
<thead>
<tr>
<th>Type of diabetes</th>
<th>Levels of sTNF-RI</th>
<th>Levels of sTNF-RII</th>
</tr>
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<tbody>
<tr>
<td>IDDM [22]</td>
<td>730 (261–2562)</td>
<td>681 (121–2342)</td>
</tr>
<tr>
<td>Duration of diabetes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;10 years [23]</td>
<td>748 (298–3013)</td>
<td>688 (121–2505)</td>
</tr>
<tr>
<td>Complication with TRD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No [13]</td>
<td>534 (261–3013)</td>
<td>674 (228–2505)</td>
</tr>
<tr>
<td>Yes [16]</td>
<td>784 (361–1961)</td>
<td>688 (121–2450)</td>
</tr>
</tbody>
</table>

Data are the median ± SEM sTNF-RI and sTNF-RII levels in picograms per milliliter with ranges in parentheses. Numbers in square brackets indicate the number of eyes investigated in each group.

whereas levels of sTNF-RI and sTNF-RII were significantly increased in eyes with PVR when compared with eyes with PDR ($P = 0.026$ and 0.049, respectively). In contrast, serum levels of sTNF-RI and sTNF-RII were significantly higher in patients with PDR than in patients with PVR ($P = 0.016$ and 0.0032, respectively). There was no relationship between the vitreous levels of sTNF-RI and sTNF-RII and those of TNFα ($P > 0.2$) in patients with PVR or PDR.

**Discussion**

The present results show that levels of sTNF-RI and sTNF-RII were significantly higher in vitreous from eyes with PVR, uncomplicated RRD, and PDR than in normal cadaveric vitreous and eyes with macular holes. In PVR, levels of both sTNF-RI and sTNF-RII were markedly higher than in RRD alone and PDR and were related to the duration of retinal detachment, number of previous conventional external operations (scleral buckling), and grade of PVR. In contrast, vitreous levels of these molecules in uncomplicated RDR did not relate to the duration of detachment or surgical history, and in PDR, these were not influenced by the type (IDDM or NIDDM) or duration of diabetes or its complication with TRD. Although both PVR and PDR have different causes and clinical characteristics, they share similarities in the formation of fibrocellular retinal tissue. An increase in vitreous levels of sTNF-RI and sTNF-RII may constitute an important feature of clinical and biological significance, in that sTNF-Rs are responsible for the control of TNFα, which has been implicated in the pathogenesis of these conditions.

Both sTNF-RI and sTNF-RII are released from TNFα-producing cells in response to TNFα itself. These are shed from the cell membrane by proteolytic cleavage into surrounding tissues and fluids, where they serve as a marker of disease activity, and TNFα is the predominant proinflammatory cytokine found in the extracellular matrix of PVR and PDR membranes and in the luminal and abluminal surfaces of vessels infiltrating PDR membranes, and it is possible that this cytokine may induce synthesis and release of sTNF-Rs by local retinal cells. This is supported by the demonstration that RPE and glial cells express and release TNF-RI and TNF-RII and by observations in our laboratory that vascular endothelium from inflamed retina expresses these molecules (our unpublished observations, 1998). Basal serum levels of sTNF-Rs are normally found in healthy individuals, and it is also possible that high vitreous levels of these molecules may derive from the circulation during breakdown of the blood-retinal barrier. However, vitreous levels of sTNF-Rs did not differ between eyes...
with PDR complicated by vitreous hemorrhage alone and eyes with PDR complicated by TRD without vitreous hemorrhage ($P = 0.07$, data not shown), suggesting that sTNF-Rs found in vitreous are locally produced.

This view is further supported by our present observations that although patients with PDR exhibited higher serum levels of sTNF-Rs than patients with PVR, the vitreous levels of both receptors were significantly higher in PVR than in PDR. That vitreous sTNF-Rs levels are higher in eyes with PVR than in eyes with PDR indicates that local release of these molecules may be potentiated by inflammatory reactions, as observed with vascular cell adhesion molecules, which appear to be locally produced as a result of inflammation caused by retinal detachment.4,25 Although the presence of sTNF-Rs in normal vitreous has been documented, we, to our knowledge, there are no studies that demonstrate an increase in vitreous levels of these molecules in eyes with proliferative retinopathies or their association with various clinical features of these conditions.

We view the presence of high vitreous levels of sTNF-Rs in proliferative retinopathy as a marker of TNFα activity, because their production and release reflect a process of retinal cell activation during the development of these complications. That high vitreous levels of these receptors in eyes with PVR are associated with the duration of retinal detachment, surgical history, and severity of PVR indicate that TNFα may be persistently produced throughout this process and that they reflect the chronicity of inflammation that characterizes this condition. Our findings that vitreous from eyes with macular holes contained lower levels of sTNF-Rs than cadaveric vitreous suggest that either sTNF-Rs may be spontaneously released by dying cells into the vitreous or that there is an impairment of TNF-R production in eyes with macular holes. This merits further investigation.

In PVR it is well recognized that inflammation is caused by the trauma of retinal detachment, whereas in PDR it is not generally accepted that inflammation plays a role in its pathogenesis. However, the features that characterize the inflammatory process are those that promote extracellular matrix deposition leading to angiogenesis and fibrocellular proliferation, the main characteristics of PDR. In this condition, inflammation may not be the primary trigger for retinal fibrovascular proliferation, but it may constitute an important response to retinal hypoxia, abnormal levels of glucose metabolites, and increased retinal blood flow, all of which precede the development of PDR.5,25 This is supported by the demonstration that hypoxia and methylglyoxal-modified proteins induce expression of mRNA coding for TNFα and that increased shear stress promotes the upregulation of vascular adhesion molecules, whose induction is highly dependent on TNFα.22 Several functions have been attributed to sTNF-Rs—among them, stabilization of TNFα, cell protection against the effect of TNFα by reducing cell sensitivity to this cytokine, and inhibition of TNFα’s biological activity. In this context, it is of interest that in comparison with PVR, eyes with PDR exhibited higher vitreous levels of TNFα but lower levels of sTNF-Rs. This suggests that in PVR vitreous sTNF-Rs may be abrogating TNFα activity, making it possible that in severe proliferative retinopathy there is a failure in the mechanisms that control TNFα activity by these receptors within the eye. However, it is feasible that a combination of the described functions may control the pathogenic capability of TNFα during retinal proliferative disorders. Investigation of the mechanisms that control the regulatory activity of sTNF-Rs on TNFα within the eye may aid in the development of new therapeutic approaches to treat and prevent proliferative retinopathy.

**Acknowledgments**

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**References**


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**TABLE 3. Comparison between Vitreous and Serum Levels of TNFα sTNF-RI, and sTNF-RII in Patients with PVR and PDR**

<table>
<thead>
<tr>
<th></th>
<th>Vitreous</th>
<th></th>
<th>Blood</th>
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<tbody>
<tr>
<td></td>
<td>TNFα</td>
<td>sTNF-RI</td>
<td>sTNF-RII</td>
</tr>
<tr>
<td>PVR</td>
<td>2.5 (2.0–22.4)† [17]*</td>
<td>1419 (244–4290) [30]†</td>
<td>1320 (128–4429) [30]†</td>
</tr>
<tr>
<td>PDR</td>
<td>4.8 (3.0–19.3) [25]</td>
<td>750 (201–3013) [29]</td>
<td>676 (121–2505) [29]</td>
</tr>
<tr>
<td>PVR</td>
<td>ND</td>
<td>1169 (257–2151) [21]§</td>
<td>2926 (1522–4283) [21]</td>
</tr>
<tr>
<td>PDR</td>
<td>ND</td>
<td>1412 (945–6630) [17]</td>
<td>4218 (1749–5218) [17]</td>
</tr>
</tbody>
</table>

Data are the median ± SEM of TNFα levels in vitreous from eyes with PVR and PDR and of sTNF-RI and sTNF-RII levels in vitreous and serum from the same groups of patients, with ranges in parentheses. Data are in picograms per milliliter. Numbers in square brackets indicate the number of samples examined in each group. ND, not determined.

* $P = 0.0005$ versus PDR vitreous.
† $P = 0.026$ versus PDR vitreous.
‡ $P = 0.049$ versus PDR vitreous.
§ $P = 0.016$ versus PDR serum.
|| $P = 0.0032$ versus PDR serum.
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