Cytokine profiles of phakic and pseudophakic eyes with primary retinal detachment

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ABSTRACT.

Purpose: To compare the cytokine profiles of phakic (p) and pseudophakic (ps) eyes with primary rhegmatogenous retinal detachment (RD) to eyes with macular holes (MH) and to identify differences in the specific cytokine profiles.

Methods: Aqueous humour (AH) and vitreous fluid (VF) were obtained from patients with primary RD without proliferative vitreoretinopathy undergoing vitrectomy. AH and VF of patients with macular holes (MH) served as controls. Forty-three different cytokines were quantified using multiplex cytokine analysis. Intergroup and intragroup comparisons were performed. To control for multiple comparisons, Holm’s correction was applied.

Results: VF and AH samples of 71 eyes with RD (pRD N = 38; psRD N = 33) and 26 eyes with MH were included. Cytokine levels in psRD and pRD were similar (none with >10-fold difference). The levels of 39 of 43 cytokines in the VF were significantly higher in eyes with RD than in those with MH (>10-fold: CXLC5, CCL26, CCL1, IL-6, CXCL11, CCL7, CCL13, MIG/CXCL9, CCL19 and TGF-β1). In the AH, 23 of 43 cytokines were significantly higher compared to MH (>10-fold: CXCL5, IL-4, IL-6, IL-8/CXCL8 and CCL7).

Conclusion: A complex, but nonspecific cytokine environmental response seems to initiate immunological and profibrotic processes following RD. Relevant differences in the cytokine profiles of eyes with pRD and psRD were not identified, whereas cytokine differences between AH and VF in RD could be explained by upregulation in the vitreous, a higher turn around in the anterior chamber, or differences in inflammatory cascades in both compartments.

Key words: biomarkers – chemokines – cytokines – interleukins – macular – hole – rhegmatogenous retinal detachment

Introduction

Cytokines are involved in the regulation of inflammatory processes, wound healing and scar formation as cell-signalling mediators (Zaja-Milatovic & Richmond 2008). In eyes with retinal tears, in particular, those with retinal detachment (RD), the upregulation of a variety of cytokines has been shown to mediate a wound-healing response involving retinal pigment epithelial (RPE) and glial cells, fibroblasts, and inflammatory cells (Hollborn et al. 2008). This leads to a breakdown of the uveo vascular barrier, resulting in an influx of inflammatory cells, and damage to the inner limiting membrane (Hollborn et al. 2008). Consequently, a cascade of migration, proliferation, and prolonged survival of involved cells, their production of extracellular matrix proteins and vitreal membranes, and finally the contraction of the involved cells is triggered (Hollborn et al. 2008; Lei et al. 2010). In such a situation, high cytokine levels have been found not only in the vitreous fluid (VF), but also in the subretinal space (Hollborn et al. 2008). The upregulation of certain cytokines may also be the stimulus leading to the development of proliferative vitreoretinopathy (PVR) (Roldán-Pallarés et al. 2008; Ricker et al. 2010; Roldán-Pallarés et al. 2010). For example, higher vitreal levels of IL-6, MIF (macrophage inhibitory factor), and the chemokine ligands CCL2, CCL11, CCL17, CCL18, CCL19, CCL22, CXCL8, CXCL9, and CXCL10 have been reported in eyes with RD and PVR (Ricker et al. 2010). Cytokine measurements in the anterior chamber and/or the vitreous have been published for patients with RD (Ciprian 2015); however, no conclusive results are available regarding systematic changes in the cytokine profile, based on parallel
investigation of a large number of cyto- and chemokines, in both the anterior chamber and in the vitreous in primary RD without relevant PVR. This is, however, necessary to estimate which cytokines are markedly up- and downregulated in response to RD, and, also, to better understand if, and how, the subsequent cascade of profibrotic and anti-inflammatory processes could be influenced by targeting specific cytokines. Differences in cytokine levels have previously been reported in phakic compared to pseudophakic eyes for selected cytokines (Jakobsson et al. 2015).

The aim of this study was to assess not only single cytokines, but the proinflam- matory and profibrotic cytokine profiles in the aqueous humour (AH) and VF of eyes with uncomplicated primary RD, to determine potential differences to a control group of eyes with macular hole (MH), and to compare phakic and pseudophakic primary RD.

Patients and Methods

Patients

The investigation was designed as a prospective study involving a consecu- tive case series of patients undergoing pars plana vitrectomy due to primary RD. Control groups included samples from otherwise healthy patients under- going MH surgery (AH and VF sam- ples), collected in parallel. Patients with systemic or ocular comorbidities, or any topical and/or systemic treatments that may potentially influence ocular cytokine levels, were excluded (i.e. patients with diabetes mellitus, rheuma- tologic and immunoregulatory diseases, vitreous haemorrhaging, proliferative vitreoretinopathy, uveitis, glaucoma, or any concomitant retinal pathology, and local or systemic immunomodula- tory or antiproliferative therapies including corticosteroids). If both eyes were affected, only the first operated eye was included. All surgeries were per- formed at the Berner Augenklinik am Lindenhofspital, Bern, Switzerland.

The study was approved by the Ethical commission of the University of Bern (KEK no. 152/08), was under- taken with the informed written consent of each of the participants and was fully compliant with the tenets of the Declaration of Helsinki.

Handling of VF and AH samples

AH samples were collected by anterior chamber tap at the beginning of surgery (200–250 μl), and undiluted VF samples (approximately 500 μl) were collected after placement of ports prior to opening the infusion cannula. After collection, the samples were stored at −20° Celsius for up to 2 months, and, thereafter, at −80° Celsius until the time of the analysis, which was performed for all samples in parallel.

Cytokine analyses

The samples were analysed using a multiplex system (Bio-Plex 100 array reader with Bio-Plex Manager soft- ware version 6.1; Bio-Rad, Hercules, CA, USA). Using this system, multiple analytes can be detected and quanti- fied in parallel in a small, single sample volume. In this study, the concentrations of 43 cytokines in each aqueous and vitreous sample (Table S1) were quantified. All analy- lytic procedures were performed according to the manufacturer’s guidelines. In short, magnetic microspheres tagged with a fluorescent label were coupled to specific capture antibodies and mixed with samples containing unknown quantities of the cytokines. Biotinylated detection antibodies and Streptavidin-R-Phycocerythin were then introduced. The mixture was analysed by flow cytometry. The instrument’s two lasers identified microsphere type and quantified the amount of bound antigen. A concentra- tion standard was run in parallel on each test plate. Measurements were performed in a blinded manner by a laboratory technician who was exper- ienced in the execution of this tech- nique.

Statistical analyses

Data below the working range of quantification of the multiplex assay were substituted with half of the lowest level of quantification (LLOQ) provided by the manufacturer which regularly lay above the internal concentra- tion standard run in parallel. The Shapiro–Wilk test was used to test whether the data were normally distributed. Since the criteria for a normal distribution were not satisfied, the intergroup (pRD versus psRD versus MH) and intragroup (AH versus VF) comparisons were conducted using the nonparametric Mann–Whitney U test and Kruskal–Wallis H test. A p < 0.05 was considered to be significant. As we made a number of hypotheses, comparing two or more groups, for a number of different outcome variables, and since such multiple comparisons increase the risk of introducing a Type-I error, we applied the Holm correction to control for this type of error, but without simultaneously, drastically driving up Type II errors (Holm 1979; Lehmann & Romano 2005). Cytokine upregu- lation of >10-fold was defined as poten- tially clinically relevant. Statistical evaluation was performed using the R statistical package (version 3.2.4; R: A language and environment for sta- tistical computing, R Foundation for Statistical Computing, Vienna, Aus- tria, 2016).

Results

Patients

A total of 71 eyes with primary RD were included in this study (Fig. 1). Eyes with any signs of PVR, and any other potentially confounding ocular or systemic disease, were excluded. Of the included eyes, 38 were phakic and 33 eyes pseudophakic. The control group comprised AH and VF samples of 26 phakic eyes with MH. The mean age was similar (p > 0.05) between the two RD groups (pRD: 59.0 ± 14.6 years; psRD: 66.9 ± 12.0 years) and the control group (66.7 ± 9.1 years). The mean time interval between cataract surgery and vitrectomy in eyes with psRD was 3.9 ± 3.8 years (range 0.1–14.2 years), three of them had under- gone cataract surgery within 6 months before the development of RD. The duration of symptoms (pRD 7.0 ± 6.5 days; psRD 9.9 ± 14.5 days; p = 0.83), the number of retinal breaks (pRD 1.5 ± 1.1; psRD 1.5 ± 0.9; p = 0.69), the portion of patients with retinal breaks with a diameter of more than one clock hour (pRD 33.3%; psRD 21.9%; p = 0.53) and the location of the retinal breaks (superior: pRD 64.9%; psRD 71.0%; p = 0.90) were similar between the groups. The postoperative outcome was similar
Comparisons of cytokine profiles between RD and MH
A total of 39 of the tested 43 cytokines were significantly higher in the vitreous of the RD group compared to the MH group (Table 1). Out of these, a ≥10-fold upregulation in the RD group was observed for CXLC5, CCL26, CCL1, IL-6, CXCL11, CCL7, CCL13, MIG/CXCL9, CCL19, and TGF-β1 (Fig. 4A).

On the other hand, there were no differences in the levels of CCL27, CXCL16, CCL17 and TGF-β2 between the eyes of subjects with RD and MH. Significantly higher levels were reported for 23 cytokines in the AH of the eyes of subjects with RD compared to those with MH (with a ≥10-fold upregulation in CCL5, IL-4, IL-6, IL-8/CXCL8 and CCL7; Table 2, Fig. 4B), whereas significantly lower levels were reported for GM-CSF and TGF-β2 (difference <10-fold, each).

Intragroup comparison of VF and AH
In patients with RD, the cytokines IL-16 and CCL7 were significantly higher in the VF than in the AH, whereas CCL24, GM-CSF, TGF-β1 and TGF-β2 were significantly lower in the VF. However, none of these differences was found to be ≥10-fold.

In eyes with MH, in comparison, a significant difference in the levels of most cytokines was found between the VF and the AH (i.e. in the AH, significantly higher levels of CCL21, CXCL5, CCL11, CCL24, CCL26, CCL31, GM-CSF, CXCL1, CCL1, IL-1β, IL-6, IL-8/CXCL8, IL-10, IL-16, CXCL11, CCL8, CCL13, MIF, MIG/CXCL9, CCL3, CCL25, TNF-α, TGF-β1 and TGF-β2 were found, thereof CXCL5, CCL1, CXCL11, CCL13 and TGF-β1 were >10-fold higher than in the VF). On the other hand, CXCL16 was lower in the AH compared to the VF in eyes with MH (Fig. 2).

Comparisons of cytokine profiles in RD: pseudophakic versus phakic eyes
Similar cytokine profiles were observed in the VF of phakic and pseudophakic eyes, with slightly, but not significantly, higher cytokine levels in the pseudophakic RD group for the majority of cytokines (Table S2, Figs 2 and 3A). Only IL-6 was significantly higher (6.7-fold) in the pseudophakic RD group. On the other hand, the levels of GM-CSF, CCL3, and TGF-β2 were

regarding redetachment rate (pRD 10.5% versus psRD 15.1%, p = 0.83) and PVR development (pRD versus psRD: PVR-grade ≤B: 2 versus 0 patients; PVR-grade C1 and C2: 2 versus 2 patients; PVR-grade ≥C3: 0 versus 3 patients; p = 0.22). The measured cytokine levels in the VF and AH of all groups are shown in Figs 2 (heatmap), 3A, B (cytokine profiles displayed as curves to depict the cytokine environment).

Fig. 1. Cytokine levels in the vitreous and aqueous humour of phakic (N = 38) and pseudophakic (N = 33) eyes of patients with primary retinal detachment (N = 71) were compared to eyes with macular holes (N = 26).

Fig. 2. Heatmaps for all cytokines measured in the aqueous humour and vitreous of eyes with phakic (pRD) and pseudophakic (psRD) retinal detachment and macular holes.
slightly, but not significantly, lower in the pseudophakic RD group. Similarly, in the AH of both groups, slightly, but not significantly, higher cytokine levels were observed in the pseudophakic RD group for 41 cytokines (Table S3, Fig. 3B). The levels of only two cytokines (CXCL6 and CCL20) were significantly higher in the pseudophakic RD group and that of only one cytokine (TGF-β2) was slightly, but not significantly, lower; however, none of them showed a more than 10-fold difference.

**Discussion**

The finding that 39 of the 43 tested cytokines in our study were significantly different between the RD group and the control group indicates a broad and nonspecific environmental change and strongly argues against drawing conclusions from differences in single cytokines. A significant upregulation had been reported for the majority of these cytokines (Abu el-Asrar et al. 1992; de Boer et al. 1993; Elner et al. 1995; Banerjee et al. 2007; Yoshimura et al. 2009). Beyond application of the Holm correction, we therefore introduced the ‘upregulation factor’ as a marker of biological relevance beyond significant cytokine differences. A 10-fold upregulation in the vitreous was still present in more than 23% (10 of 43) of the tested cytokines. Our 10-fold cut-off level was a cautious assumption (in comparison with the calculation of statistical significance), also considering the high variance of measurements. From systemic and ocular inflammatory disease it is known that two- to threefold differences in TNFα levels are found between healthy and diseased and even lower differences are linked to relevant clinical differences in the disease activity (Mesquida et al. 2014; Chen et al. 2015; Lopalco et al. 2017).

We think that our multiplex approach allowed us to identify the most abundant cytokines in a complex cytokine environmental change, suggesting their potential biological role in the evolution of RD, thus making them deserve of closer attention. The role of immune mediators in RD has also been studied in animal models (Jo et al. 2003; Nakazawa et al. 2007, 2011; Yang et al. 2007; Chong et al. 2008). Whereas in the majority of those publications, an arbitrary selection of few cytokines was analysed, we intended to reveal a whole environmental cytokine change by analysing a broad set of cytokines previously reported as abundant. Moreover, we based our analysis on maximally possible homogenous groups of patients after exclusion not only of ocular, but also systemic, comorbidities and their treatments (Zandi et al. 2016). In this study, we also aimed to evaluate a potential influence of the lens status on the cytokine milieu in eyes with RD, but failed to detect relevant differences in the biologic milieu between phakic and pseudophakic states. This is in line with recent publications, where other clinical factors (e.g. number, size and location of retinal breaks, and presence of PVR) and not primarily the lens status had a relevant influence on the clinical outcome (Lumi et al. 2016; Takkar et al. 2017).
No relevant clinical differences in the reattachment rates between eyes with phakic and pseudophakic RD have been reported by (Christensen & Villumsen 2005). In our study, clinical characteristics of RD and also postoperative outcome were similar between the pRD and psRD group, which might explain the similarity of the cytokine profiles.

The RD-evoked changes may thus represent an undirected, acute response to tissue trauma, which cannot readily be attributed to one single biological activation factor for fibroblasts or inflammatory cells (Asaria & Charteris 2006; Garweg et al. 2013). Such nonspecific upregulation is observed in a variety of tissues and organs during wound-healing processes (Pastor et al. 2002; Ricker et al. 2010). However, in the eye, this response may have disastrous consequences on the maintenance of visual function due to the development of PVR and tractional redetachment after primarily successful reattachment surgery. Cytokine concentrations in the blood in ten of our patients were about 500-times higher than in the vitreous of eyes with retinal detachment (data not shown). This is well in the range of our and published experience from other fields, namely antibody investigations in uveitis (Garweg et al. 2005). Clearly, any minimal impact onto the uveovascular barrier would be expected to have a major impact on cytokine concentrations. The high variability in cytokine levels between eyes with RD (even after excluding eyes with intravitreal bleeding) thus probably indicates a wide

### Table 1. Cytokine levels (pg/ml; mean) in the vitreous (VF) of eyes with retinal detachment (RD) versus macular holes (MH) revealed a significant upregulation of 39 out of 43 cytokines.

| Cytokines | VF RD | SD  | VF MH | SD  | p value | Holm correction |
|-----------|-------|-----|-------|-----|---------|-----------------|
| CCL21     | 2112.1| 4277.9 | 341.3 | 190.5 | 8.75E-08 | Sig.            |
| CXCL13    | 2.0   | 3.2  | 0.4   | 0.2  | 2.28E-08 | Sig.            |
| CCL27     | 7.5   | 24.3 | 2.4   | 1.9  | 0.04051  | n.s.            |
| CCL5      | 165.1 | 203.0 | 12.3  | 7.2  | 1.91E-08 | Sig.            |
| CCL11     | 12.9  | 15.9 | 1.4   | 1.2  | 9.46E-11 | Sig.            |
| CCL24     | 19.8  | 20.8 | 6.5   | 6.6  | 7.33E-07 | Sig.            |
| CCL26     | 9.1   | 13.1 | 0.5   | 0.3  | 1.38E-10 | Sig.            |
| CX3CL1    | 60.4  | 56.9 | 22.1  | 14.4 | 5.94E-05 | Sig.            |
| CXCL6     | 2.4   | 4.0  | 0.4   | 0.0  | 6.00E-05 | Sig.            |
| GM-CSF    | 44.5  | 18.6 | 30.0  | 12.7 | 0.000713 | Sig.            |
| CXCL1     | 65.5  | 66.8 | 7.2   | 9.8  | 5.46E-11 | Sig.            |
| CXCL2     | 24.0  | 50.4 | 4.9   | 2.2  | 0.003174 | Sig.            |
| CCL1      | 34.7  | 59.0 | 0.9   | 0.0  | 2.14E-10 | Sig.            |
| IFN-γ     | 8.8   | 12.2 | 1.2   | 0.0  | 3.21E-06 | Sig.            |
| IL-1β      | 1.4   | 1.9  | 0.3   | 0.2  | 4.21E-08 | Sig.            |
| IL-2      | 1.5   | 1.8  | 0.4   | 0.0  | 7.84E-07 | Sig.            |
| IL-4      | 3.0   | 4.8  | 0.8   | 0.5  | 0.004736 | Sig.            |
| IL-6      | 121.9 | 343.5 | 9.5  | 20.2 | 4.35E-10 | Sig.            |
| IL-8/CXCL8 | 37.0 | 52.9 | 9.4   | 4.9  | 9.71E-10 | Sig.            |
| IL-10     | 3.2   | 5.7  | 1.1   | 1.1  | 5.22E-10 | Sig.            |
| IL-16     | 56.4  | 45.7 | 9.8   | 19.5 | 1.07E-09 | Sig.            |
| CXCL10    | 381.8 | 1631.4 | 52.9 | 31.0 | 2.53E-07 | Sig.            |
| CXCL11    | 4.9   | 8.0  | 0.2   | 0.1  | 2.04E-10 | Sig.            |
| CCL2      | 1469.4 | 1270.9 | 792.7 | 591.6 | 6.36E-09 | Sig.            |
| CCL8      | 13.0  | 32.5 | 1.7   | 1.2  | 2.09E-10 | Sig.            |
| CCL7      | 21.7  | 24.6 | 1.0   | 0.0  | 6.92E-10 | Sig.            |
| CCL13     | 2.2   | 2.4  | 0.2   | 0.0  | 5.69E-10 | Sig.            |
| CCL22     | 12.8  | 12.9 | 3.6   | 3.3  | 3.74E-06 | Sig.            |
| MIF       | 98575.4 | 90280.5 | 27059.8 | 37164.6 | 9.58E-08 | Sig.            |
| MIG/CXCL9 | 327.0 | 2405.0 | 11.0  | 11.1 | 1.65E-08 | Sig.            |
| CCL3      | 3.8   | 8.0  | 0.6   | 0.3  | 5.47E-11 | Sig.            |
| CCL15     | 777.0 | 799.7 | 419.2 | 413.7 | 0.0006062 | Sig.             |
| CCL20     | 10.2  | 13.3 | 4.1   | 3.4  | 0.000531 | Sig.            |
| CCL19     | 40.3  | 65.9 | 2.7   | 2.0  | 4.44E-10 | Sig.            |
| CCL23     | 16.5  | 19.1 | 7.3   | 7.3  | 0.000597 | Sig.            |
| CXCL16    | 808.8 | 301.1 | 659.8 | 239.5 | 0.03421 | n.s.            |
| CXCL12    | 163.6 | 158.3 | 48.9  | 30.7 | 1.95E-07 | Sig.            |
| CCL17     | 5.9   | 19.6 | 0.9   | 0.0  | 0.03315 | n.s.            |
| CCL25     | 368.8 | 421.9 | 45.9  | 43.2 | 5.02E-10 | Sig.            |
| TNF-α     | 13.7  | 18.9 | 3.4   | 2.2  | 8.42E-09 | Sig.            |
| TGF-β1    | 100.4 | 220.7 | 9.3   | 32.1 | 0.00202 | Sig.            |
| TGF-β2    | 1257.7 | 874.2 | 998.0 | 529.8 | 0.388 | n.s.            |
| TGF-β3    | 10.5  | 21.8 | 2.2   | 3.4  | 0.003675 | Sig.            |

n.s. = not significant after application of the Holm correction; Sig. = significant (p < 0.0016).
range in the dimension of trauma to the blood–retinal barrier most likely associated with the number and size of retinal breaks, the acuity and extension of the retinal detachment as well as its duration. Further studies with higher sample numbers would be necessary to evaluate an impact of these parameters after correction for any underlying local and systemic comorbidity and their corresponding therapies. More importantly, this would not bear any therapeutic consequences since none of these factors adding to the severity of uveovascular barrier disruption can be influenced except by the surgery itself. That, on the other hand, single cytokines were specifically upregulated cannot be traced to the breakdown of the uveovascular barrier, but advocates a tissue-specific response.

Interestingly, adjuvant treatments with intraocular or systemic corticosteroids during vitreoretinal surgery for RD have broadly been used, though they have not been found to correlate with improvement of the clinical outcome (Koerner et al. 1982; Weller et al. 1990; Berger et al. 1996; Cheema et al. 2007). Therefore, a complete, and nonspecific, dampening of cytokine upregulation may not be the ideal approach. A more targeted therapeutic approach, applied as early as possible, might possibly slow down this process at a stage before loss of retinal stability and further functional loss have occurred; however, little data regarding potential targets are available. Ranibizumab, for example, has been shown to reduce the bioactivity of vitreous from patients and experimental animals with PVR, and protected rabbits from developing PVR (Pennock et al. 2013). A strategy to identify cytokines

Fig. 4. (A) Cytokines with significant and relevant (>10-fold) upregulation in the vitreous of eyes with retinal detachment (RD) versus macular holes (MH). (B) Cytokines with significant and relevant (>10-fold) upregulation in the aqueous humour of eyes with RD versus MH.
with a biological role, indicated by relevant changes in their concentration, may not be as simple to identify promising targets for anti-inflammatory or antifibrotic treatment. Whether our strategy of assessing a broad cytokine environment and identifying the most abundant of these factors is able to deliver these promising targets will be addressed subsequently.

Cytokine levels in eyes with psRD and pRD in our study were quite similar in the vitreous as well as in the AH, as was the severity and duration of retinal detachment. In a previous study by (Jakobsson et al. 2015), a total of 14 cytokines (eotaxin, IP-10, MCP-1, MDC, MIP-1α, MIP-1β, TARC, IL-12p40, IL-15, IL-16, IL-7, VEGF, IL-6, IL-8) were found to be significantly upregulated in pseudophakic compared to phakic eyes of patients with MH, epiretinal membranes, vitreous macular traction, or vitreous floaters, but corresponding data pertaining to RD have not been available. IL-6, IL-8, IL-15 and IL-16 revealed a significant trend of decreasing concentration over time (Jakobsson et al. 2015). Based on the absolute cytokine concentrations reported by Jakobsson et al., it has to be assumed that in the presence of RD – as in our study – the manifold greater upregulation of cytokines outweighs potential differences between pseudophakic and phakic state. This hypothesis is strengthened by our findings that more than 90% of cytokines were upregulated in patients with RD compared to MH. Moreover, higher IL-6 levels in pseudophakic eyes have not only been

### Table 2. Cytokine levels (pg/ml; mean) in the aqueous humour (AH) of eyes with retinal detachment (RD) versus macular holes (MH) revealed a significant upregulation of 23 out of 43 cytokines.

| Cytokines | AH RD | AH MH | p value | Holm correction |
|-----------|-------|-------|---------|----------------|
| CCL21     | 1313.1| 374.5 | 0.01628 | n.s.           |
| CXCL13    | 4.5   | 0.5   | 2.229e-08| Sig.           |
| CCL27     | 3.3   | 1.4   | 0.06004 | n.s.           |
| CXCL5     | 2384.9| 178.2 | 0.06488 | n.s.           |
| CCL1      | 9.7   | 5.1   | 0.01211 | n.s.           |
| CCL24     | 44.3  | 26.9  | 0.7323  | n.s.           |
| CCL26     | 7.3   | 3.5   | 0.00168 | Sig.           |
| CX3CL1    | 82.2  | 43.4  | 0.04425 | Sig.           |
| CXCL6     | 2.6   | 0.7   | 0.00282 | n.s.           |
| GM-CSF    | 91.7  | 152.0 | 2.37E-07| Sig.           |
| CXCL1     | 107.6 | 38.7  | 5.63E-05| Sig.           |
| CXCL2     | 18.1  | 4.1   | 0.06299 | n.s.           |
| CCL1      | 15.4  | 11.4  | 0.1388  | n.s.           |
| IFN-γ     | 12.2  | 1.6   | 5.69E-06| Sig.           |
| IL-1β     | 2.9   | 1.0   | 0.05999 | n.s.           |
| IL-2      | 1.7   | 0.6   | 2.04E-05| Sig.           |
| IL-4      | 13.1  | 0.8   | 0.04502 | n.s.           |
| IL-6      | 822.9 | 27.5  | 2.76E-07| Sig.           |
| IL-8/CXCL8| 51.8  | 5.0   | 5.43E-10| Sig.           |
| IL-10     | 8.7   | 3.3   | 1.93E-05| Sig.           |
| IL-16     | 25.5  | 14.1  | 0.02427 | n.s.           |
| CXCL10    | 350.3 | 41.3  | 3.73E-06| Sig.           |
| CXCL11    | 1.9   | 0.9   | 0.001688| Sig.           |
| CCL2      | 1592.1| 415.9 | 3.18E-08| Sig.           |
| CCL8      | 10.9  | 2.8   | 2.79E-05| Sig.           |
| CCL7      | 12.0  | 1.1   | 7.42E-05| Sig.           |
| CCL13     | 3.3   | 1.3   | 0.03097 | n.s.           |
| CCL22     | 20.8  | 6.8   | 1.51E-05| Sig.           |
| MIF       | 77741.9| 37135.4| 0.0006832| Sig.           |
| MIG/CXCL9 | 68.0  | 15.0  | 0.06706 | n.s.           |
| CCL3      | 2.1   | 0.9   | 9.01E-05| Sig.           |
| CCL15     | 812.4 | 520.1 | 5.04E-13| n.s.           |
| CCL20     | 17.2  | 3.3   | 1.21E-06| Sig.           |
| CCL19     | 21.2  | 4.2   | 2.70E-05| Sig.           |
| CCL23     | 24.5  | 11.4  | 0.02369 | n.s.           |
| CXCL16    | 829.4 | 446.1 | 8.0001381| Sig.           |
| CXCL12    | 223.8 | 94.5  | 0.003502| n.s.           |
| CCL17     | 4.2   | 1.1   | 0.2377  | n.s.           |
| CCL25     | 164.3 | 105.9 | 0.006887| n.s.           |
| TGF-β1     | 388.3 | 456.9 | 0.009605| n.s.           |
| TGF-β2     | 2454.2| 3496.0| 0.001195| Sig.           |
| TGF-β3     | 30.0  | 4.2   | 4.98E-06| Sig.           |

n.s. = not significant after application of the Holm correction; Sig. = significant (p < 0.0016).
found in our study, but have also been reported in pseudophakic versus phakic eyes of patients with vitreoretinal pathologies, excluding RD (Jakobsson et al. 2015).

Though the source of this cytokine production has not yet been identified, the similarity between the cytokine profiles in the AH and VF in eyes with RD fits well with a washout of cytokines from the posterior to the anterior segment. The differences between the AH and VF for single cytokines may result from a gradient from the retina to the vitreous, from the higher turn around in the anterior chamber, or from differences in the inflammatory cascade induced in both compartments in response to RD. The similarity between cytokine levels in eyes with phakic and pseudophakic RD reveals that the presence of an intraocular lens (IOL) does not change the amount of this washout and, furthermore, that differences in clinical outcomes and PVR incidence may not be attributed to lens-state-related biological differences, which in turn advocates for the assumed role of differences in mechanical forces.

Our finding of upregulation of profibrotic and proinflammatory cytokines in 39 of 43 cytokines in eyes with RD compared to MH is consistent with results of previous studies, which showed such changes for IL-6 and IL-8 (Yoshimura et al. 2009; Takahashi et al. 2016); MCP1, MIP-1β, and IP10 (Takahashi et al. 2016); and, in RD with PVR, for IL-6, CXCL8/IL-8, CCL2, and, in some samples, also for IL-10, TNF-α, TNF-γ, CCL3, CCL4, CCL5, G-CSF, and FGF (Kaufmann et al. 1994; Banerjee et al. 2007; Rasier et al. 2010). Our study confirms the outstanding >10-fold upregulation of IL-6 in the vitreous of RD eyes compared to eyes with MH, but reveals also the upregulation of CXCL5, CCL26, CCL1, CXCL11, CCL7, CCL13, MIG/CXCL9, CCL19 and TGF-β1 (>10-fold, each) compared to eyes with MH. Interestingly, any of the here identified, most abundant cytokines have been identified as key players in the inflammatory response (Shinkai et al. 1999; Chen et al. 2004; Radeke et al. 2007; Hooks et al. 2008; Turner et al. 2014; Wermuth & Jimenez 2015). IL-6 is a proinflammatory cytokine that amplifies inflammatory responses and is involved in wound-healing and leukocyte recruitment (Romano et al. 1997; Wu et al. 2010). Elevated IL-6 levels have been found in RD and other vitreoretinal diseases, such as diabetic retinopathy and retinal vein occlusion (Yoshimura et al. 2009). Increased levels of IL-6 have also been reported in PVR (El-Ghrably et al. 2001), with invading cells being postulated as their source. Human RPE cells increase CXCL11 production in an inflammatory milieu, presumably contributing to the inflammation and angiogenesis in the retina, retinal pigment epithelium and choroid complex (Shi et al. 2008; Juel et al. 2012). This is consistent with our hypothesis that these cytokines with >10-times upregulation are potentially suitable as targets for PVR prophylaxis and treatment.

The strengths of this study are its strict selection criteria, resulting in homogenous cohorts, and a relatively large sample size with the possibility of subgroup analysis (e.g. phakic versus pseudophakic RD). The use of a multiplex device allowed analysis of a large set of cytokines, in parallel, and after confirming these results by repeated measurements in a subset of samples, with a high sensitivity and reproducibility for measurements of cytokine concentrations in the picogram range. Unfortunately, but understandably, no VF for healthy eyes could be observed. Based on an average interval of 3.8 years between cataract surgery and RD fits well with a washout of cytokines from the retina, retinal pigment epithelium and choroid complex (Shi et al. 2008; Juel et al. 2012). This is consistent with our hypothesis that these cytokines with >10-times upregulation are potentially suitable as targets for PVR prophylaxis and treatment.

In conclusion, our results show that the majority of single cytokines are significantly upregulated indicating substantial changes in the proinflammatory and profibrotic cytokine environment early in RD. We strongly believe that the correction for multiple comparisons and identification of cytokines with an at least 10-fold upregulation allows to more specifically identify only the biologically relevant changes. That single cytokines were specifically more abundant than the majority might indicate a local response which could specifically be targeted. Lens status does not seem to play a relevant role in this process.

References

Abu el-Azwar AM, Maimone D, Morse PH, Gregory S & Reder AT (1992): Cytokines in the vitreous of patients with proliferative diabetic retinopathy. Am J Ophthalmol 114: 731–736.

Asaria RHY & Charteris DG (2006): Proliferative vitreoretinopathy: developments in pathogenesis and treatment. Compr Ophthalmol Update 7: 170–185.

Banerjee S, Savant V, Scott RAH, Curnow SJ, Wallace GR & Murray PI (2007): Multiplex bead analysis of vitreous humor of patients with vitreoretinal disorders. Invest Ophthalmol Vis Sci 48: 2203–2207.

Berger AS, Cheng CK, Pearson PA, Ashton P, Crooks PA, Cynkowski T, Cynkowska G & Jaffe GJ (1996): Intravitreal sustained release corticosteroid-5-fluorouracil conjugate in the treatment of experimental proliferative vitreoretinopathy. Invest Ophthalmol Vis Sci 37: 2318–2325.

de Boer HJ, Hack CE, Verhoeven AJ et al. (1993): Chemointrattractant and neutrophil degranulation activities related to interleukin-8 in vitreous fluid in uveitis and vitreoretinal disorders. Invest Ophthalmol Vis Sci 34: 3376–3385.

Cheema RA, Peyman GA, Fang T, Jones A, Lukaris AD & Lim K (2007): Triamcinolone acetonide as an adjuvant in the surgical treatment of retinal detachment with proliferative vitreoretinopathy. Ophthalmic Surg Lasers Imaging 38: 365–370.

Chen J, Vistica BP, Takase H et al. (2004): A unique pattern of up- and down-regulation of chemokine receptor CXCR3 on inflammation-inducing Thy1 cells. Eur J Immunol 34: 2885–2894.

Chen W, Zhao B, Jiang R, Zhang R, Wang Y, Wu H, Gordon L & Chen L (2015): Cytokine expression profile in aqueous humor and sera of patients with acute anterior uveitis. Curr Mol Med 15: 543–549.

Chong DY, Boelhke CS, Zheng Q-D, Zhang L, Han Y & Zacks DN (2008): Interleukin-6 as a photoreceptor neuroprotectant in an experimental model of retinal detachment. Invest Ophthalmol Vis Sci 49: 3193–3200.

Christensen U & Villumsen J (2005): Prognosis of vitreoretinopathy. Br J Ophthalmol 89: 731–736.

Cipriani D (2015): The pathogenicity of proliferative vitreoretinopathy. Rom J Ophthalmol 59: 88–92.

El-Ghrably IA, Dua HS, Orr GM, Fischer D & Tighe PJ (2001): Intravitreal invading cells contribute to vitreal cytokine milieu in proliferative vitreoretinopathy. Br J Ophthalmol 85: 461–470.

Elner SG, Elner VM, Jaffe GJ, Stuart A, Kunkel SL & Strieer RM (1995): Cytokines in proliferative diabetic retinopathy and proliferative vitreoretinopathy. Curr Eye Res 14: 1045–1053.
Garweg JG, Ventura ACS, Halberstadt M, Silveira C, Muccioli C, Belfort RJ & Jaquyer P (2005): Specific antibody levels in the aqueous humor and serum of two distinct populations of patients with ocular toxoplasmosis. Int J Med Microbiol 295: 287–295.

Garweg JG, Tappeiner C & Halberstadt M (2013): Pathophysiology of proliferative vitreoretinopathy in retinal detachment. Surv Ophthalmol 58: 321–329.

Holländer M, Francke M, Jandiev I et al. (2008): Early activation of inflammatory and immune response-related genes after experimental detachment of the porcine retina. Invest Ophthalmol Vis Sci 49: 1262–1273.

Holen S (1979): A simple sequentially repetitive multiple test procedure. Scand J Stat 6: 65–70.

Hooks JJ, Nagemini CN, Hooper LC, Hayashi K & Detrick B (2008): IFN-beta provides immunomodulation in the retina by inhibiting ICAM-1 and CXCL9 in retinal pigment epithelial cells. J Immunol 180: 3789–3796.

Jakobsson G, Sundelin K, Zetterberg H & Zetterberg M (2015): Increased levels of inflammatory immune mediators in vitreous from pseudopapillary eyes. Invest Ophthalmol Vis Sci 56: 3407–3414.

Jo N, Wu G-S & Rao NA (2003): Upregulation of chemokine expression in the retinal vasculature in ischemia-reperfusion injury. Invest Ophthalmol Vis Sci 44: 4054–4060.

Juel HB, Faber C, Udén MS, Folkerken L & Nissen MH (2012): Chemokine expression in retinal pigment epithelial ARPE-19 cells in response to coculture with activated T cells. Invest Ophthalmol Vis Sci 53: 8472–8480.

Kauffmann DJ, van Meurs JC, Mertens DA, Peperkamp E, Master C & Gerritsen ME (1994): Cytokines in vitreous humor: interleukin-6 is elevated in proliferative vitreoretinopathy. Invest Ophthalmol Vis Sci 35: 900–906.

Koerner F, Merz A, Glor B & Wagner E (1982): Postoperative retinal fibrosis—a controlled clinical study of systemic steroid therapy. Graefes Arch Clin Exp Ophthalmol 219: 268–271.

Lehmann EL & Romans JP (2005): Generalizations of the familywise error rate. Ann Stat 33: 1138–1154.

Lei H, Rhaume M-A & Kazlauskas A (2010): Recent developments in our understanding of how platelet-derived growth factor (PDGF) and its receptors contribute to proliferative vitreoretinopathy. Exp Eye Res 89: 376–381.

Lopaloce G, Lucherini OM, Lopaloce A et al. (2017): Cytokine signatures in macuocutaneous and ocular Behçet’s disease. Front Immunol 8: 200.

Lumi X, Luzhik Z, Petrovski G, Petrovski B & Hawlina M (2016): Anatomical success rate of pars plana vitrectomy for treatment of complex rhegmatogenous retinal detachment. BMC Ophthalmol 16: 216.

Mesquida M, Molins B, Llorenç V, Sainz de la Maza M, Hernandez MV, Espinosa G & Adán A (2014): Proinflammatory cytokines and C-reactive protein in uveitis associated with Behçet’s disease. Mediators Inflamm 2014: 396204–396208.

Nakazawa T, Hisatomi T, Nakazawa C et al. (2007): Monocyte chemotactant protein 1 mediates retinal detachment-induced photoreceptor apoptosis. Proc Natl Acad Sci USA 104: 2425–2430.

Nakazawa T, Kayama M, Ruyu M et al. (2011): Tumor necrosis factor-alpha mediates photoreceptor death in a rodent model of retinal detachment. Invest Ophthalmol Vis Sci 52: 1384–1391.

Pastor JC, la Rúa de ER & Martín F (2002): Proliferative vitreoretinopathy: risk factors and pathobiology. Prog Retin Eye Res 21: 127–144.

Pennock S, Kim D, Muki S et al. (2013): Ranibizumab is a potential prophylaxis for proliferative vitreoretinopathy, a nonangiogenic blinding disease. Am J Pathol 182: 1659–1670.

Radeke MJ, Peterson KE, Johnson LV & Anderson DH (2007): Disease susceptibility of the human macula: differential gene transcription in the retinal pigmented epithelium/chorioid. Exp Eye Res 85: 366–380.

Rasier R, Gormus U, Artanay O, Yuzbasioğlu E, Oncel M & Bahcecióglu H (2010): Vitreous levels of VEGF, IL-8, and TNF-alpha in retinal detachment. Curr Eye Res 35: 505–509.

Ricker LJAG, Kijistra A, de Jager W, Liem ATA, Hendriks F & La Heij EC (2010): Chemokine levels in subretinal fluid obtained during sceral buckling surgery after rhegmatogenous retinal detachment. Invest Ophthalmol Vis Sci 51: 4143–4150.

Roldán-Pallarés M, Sadiq-Musa A, Rollin R, Bravo-Llatas C, Fernández-Cruz A & Fernández-Durango R (2008): Retinal detachment: visual acuity and subretinal immunoreactive endothelin-1. J Fr Ophtalmol 31: 36–41.

Roldán-Pallarés M, Musa A-S, Bravo-Llatas C & Fernández-Durango R (2010): Preoperative duration of retinal detachment and subretinal immunoreactive endothelin-1: a repercussion on logarithmic visual acuity. Graefes Arch Clin Exp Ophthalmol 248: 21–30.

Romano M, Siromi M, Tomiatti C et al. (1997): Role of IL-6 and its soluble receptor in induction of chemokines and leukocyte recruitment. Immunity 6: 315–325.

Shi G, Minaminishik A, Banzon T, Jalicke S, Li R, Hammer J & Miller SS (2008): Control of chemokine gradients by the retinal pigment epithelium. Invest Ophthalmol Vis Sci 49: 4620–4630.

Shinkai A, Yoshihise H, Kosuke M et al. (1999): A novel human CC chemokine, eotaxin-3, which is expressed in IL-4-stimulated vascular endothelial cells, exerts potent activity toward eosinophils. J Immunol 163: 1602–1610.

Takahashi S, Adachi O, Suzuki Y, Maeno A & Nakazawa M (2016): Profiles of inflammatory cytokines in the vitreous fluid from patients with rhegmatogenous retinal detachment and their correlations with clinical features. Biomed Res Int 2016: 4256183–4256183.

Takkar B, Azad S, Shashni A, Pujari A, Bhatia I & Azad R (2016): Missed retinal breaks in rhegmatogenous retinal detachment. Int J Ophthalmol 9: 1629–1633.

Turner MD, Nedijs B, Hurst T & Pennington DJ (2014): Cytokines and chemokines at the crossroads of cell signalling and inflammatory disease. Biochem Biophys Acta 1843: 2563–2582.

Weller M, Wiedemann P & Heimann K (1990): Proliferative vitreoretinopathy—is anything more than wound healing at the wrong place? Int Ophthalmol 14: 105–117.

Wermuth PJ & Jimenez SA (2015): The significance of macrophage polarization subtypes for animal models of tissue fibrosis and human fibrotic diseases. Clin Transl Med 4: 2.

Wu-W-C, Hu-D-N, Gao-H-X, Chen M, Wang D, Rosen R & McCormick SA (2010): Suboxic levels hydrogen peroxide-induced production of interleukin-6 by retinal pigment epithelial cells. Mol Vis 16: 1864–1873.

Xu K, Chiu EK, Bennett SR et al. (2018): Predictive factors for proliferative vitreoretinopathy formation after uncomplicated primary retinal detachment repair. Philadelphia, PA: Retina 1.

Yang L-P, Zhu X-A & Tso MO (2007): A possible mechanism of microglia-photoreceptor crosstalk. Mol Vis 13: 2084–2087.

Yoshimura T, Sonoda K-H, Sugahara M et al. (2009): Comprehensive analysis of inflammatory immune mediators in vitreoretinal diseases. PLoS ONE 4: e8158.

Zaja-Milatovic S & Richmond A (2008): CXCl chemokines and their receptors: a case for a significant biological role in cutaneous wound healing. Histol Histopathol 23: 1399–1407.

Zandi S, Tappeiner C, Pfister IB, Despont A, Rieben R & Garweg JG (2016): Vitreal cytokine profile differences between eyes with epiretinal membranes or macular holes. Invest Ophthalmol Vis Sci 57: 6320–6326.

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Supporting Information
Additional supporting information may be found online in the Supporting Information section at the end of the article:

Table S1. Overview of all 43 cytokines analyzed with a multiplex system.

Table S2. Mean values (±SD) for vitreous fluid (VF) samples of eyes with phakic retinal detachment (pRD), pseudophakic retinal detachment (pSRD), and macular hole (MH).

Table S3. Mean values (±SD) for aqueous humor (AH) samples of eyes with phakic retinal detachment (pRD), pseudophakic retinal detachment (pSRD) and, macular hole (MH).