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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

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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-Pallarès 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 proinflammatory 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 consecutive case series of patients undergoing pars plana vitrectomy due to primary RD. Control groups included samples from otherwise healthy patients undergoing MH surgery (AH and VF samples), 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, rheumatologic and immunoregulatory diseases, vitreous haemorrhaging, proliferative vitreoretinopathy, uveitis, glaucoma, or any concomitant retinal pathology, and local or systemic immunomodulatory or antiproliferative therapies including corticosteroids). If both eyes were affected, only the first operated eye was included. All surgeries were performed 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 undertaken 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 software version 6.1; Bio-Rad, Hercules, CA, USA). Using this system, multiple analytes can be detected and quantified in parallel in a single, small sample volume. In this study, the concentrations of 43 cytokines in each aqueous and vitreous sample (Table S1) were quantified. All analytic 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-Phycoerythrin 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 concentration standard was run in parallel on each test plate. Measurements were performed in a blinded manner by a laboratory technician who was experienced in the execution of this technique.

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 concentration 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 upregulation of >10-fold was defined as potentially clinically relevant. Statistical evaluation was performed using the R statistical package (version 3.2.4; R: A language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria, 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 undergone 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

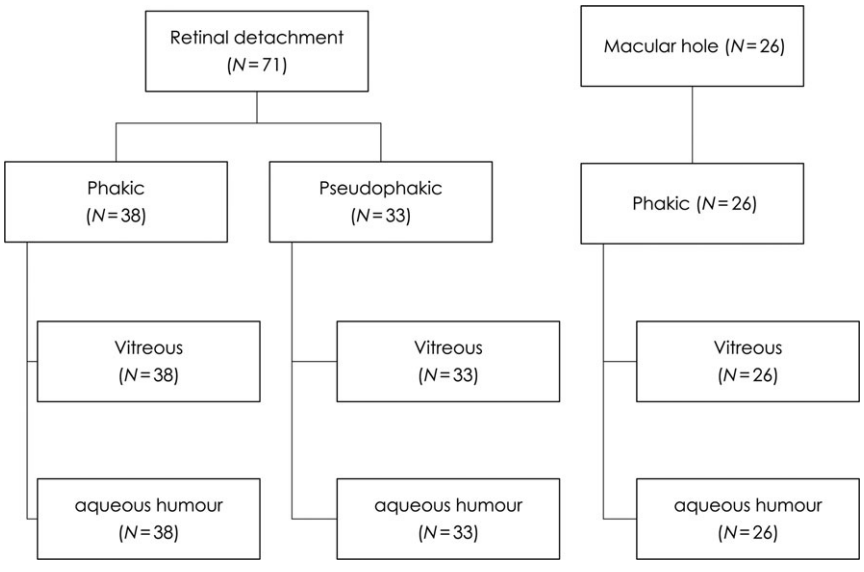

Fig. 1. Cytokine levels in the vitreous and aqueous humour of phakic (N = 38) and pseudophakic (N = 33) eyes of patients with primary retinal detachment (N = 71) were compared to eyes with macular holes (N = 26).

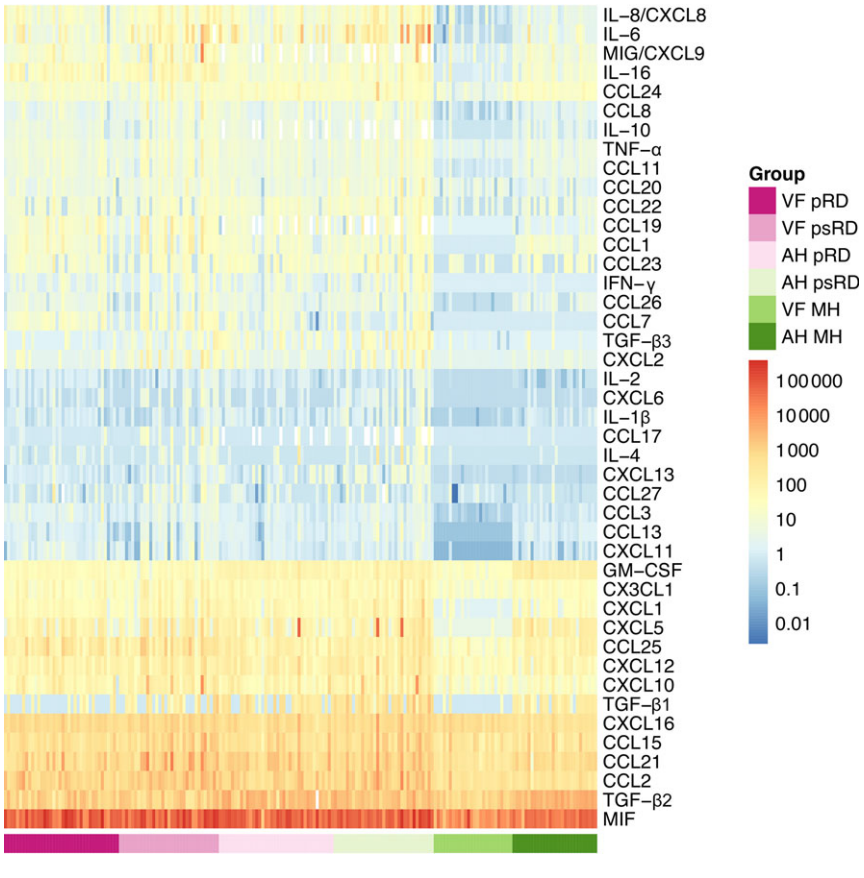

Fig. 2. Heatmaps for all cytokines measured in the aqueous humour and vitreous of eyes with phakic (pRD) and pseudophakic (psRD) retinal detachment and macular holes.

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).

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 CXCL5, 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 and TGF-β3 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, CX3CL1, 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

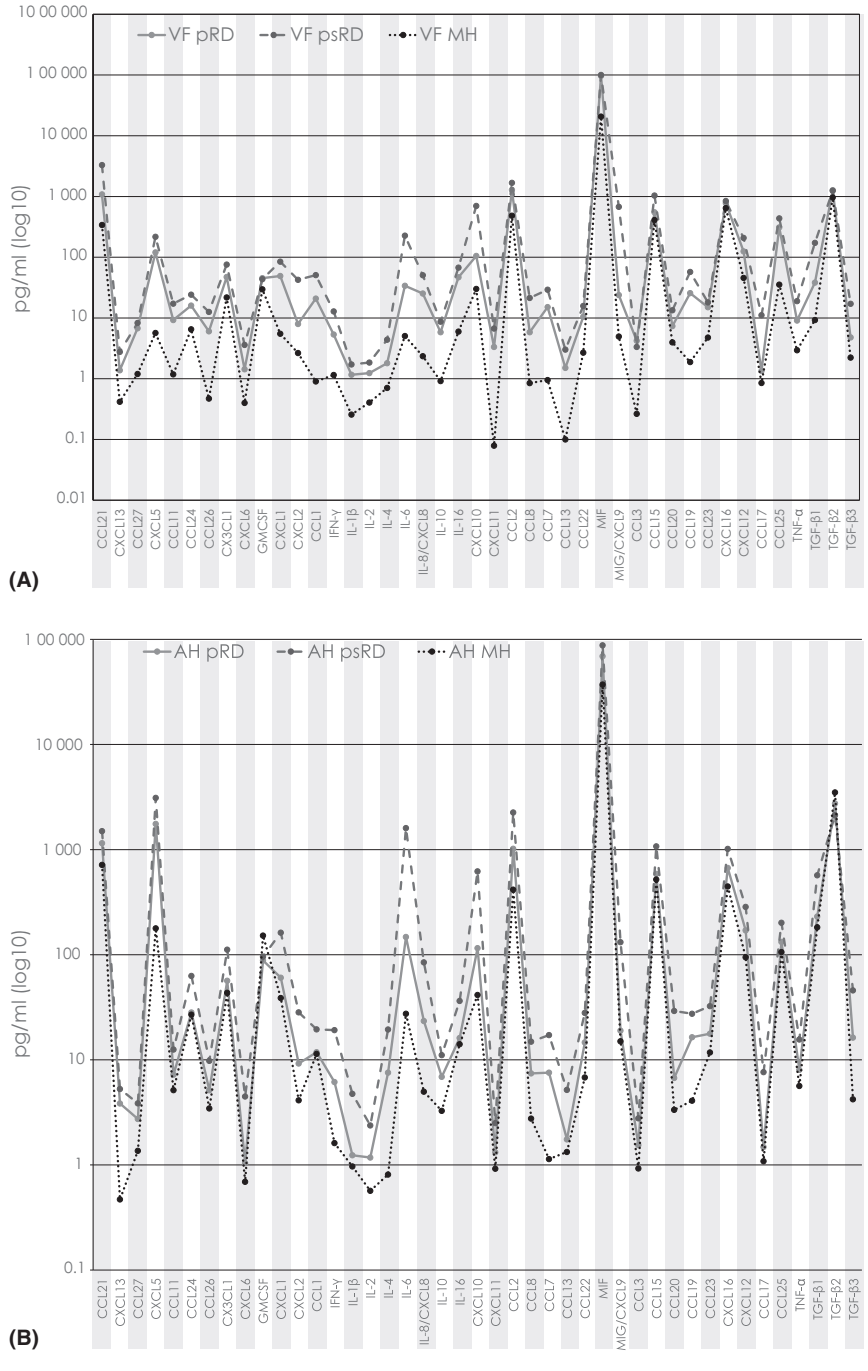

Fig. 3. (A) Profile (curves) of vitreal cytokine levels in eyes with phakic (pRD) and pseudophakic (psRD) retinal detachment and macular holes (MH) to illustrate the cytokine environment. (B) Profile (curves) of aqueous humour (AH) cytokine levels in eyes with pRD and psRD retinal detachment and MH to illustrate the cytokine environment.

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.

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.

	VF RD		VF MH			
Cytokines	Mean	SD	Mean	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.
CXCL5	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	0.0003174	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	4.8	9.71E-10	Sig.
IL-10	7.2	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	12.9	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	0.0005531	Sig.
CCL19	40.3	65.9	2.7	2.0	4.44E-10	Sig.
CCL23	16.5	19.1	7.3	7.3	0.0005097	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 ($p < 0.0016$).

2016; Xu et al. 2018). 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 non-specific 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

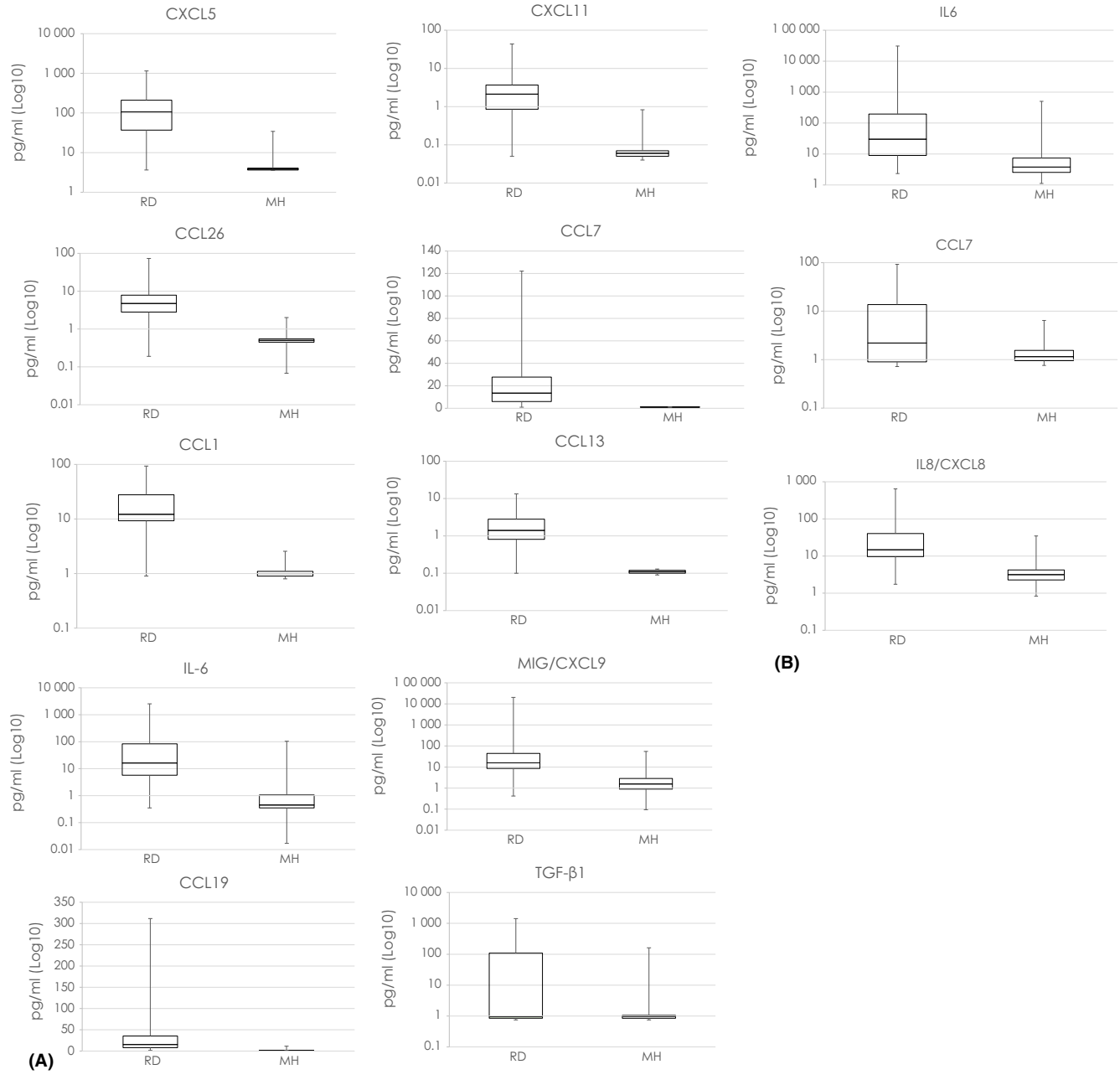

Fig. 4. (A) Cytokines with significant and relevant (>10-fold) upregulation in the vitreous of eyes with retinal detachment (RD) versus macular holes (MH). (B) Cytokines with significant and relevant (>10-fold) upregulation in the aqueous humour of eyes with RD versus MH.

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

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.

	AH RD		AH MH			
Cytokines	Mean	SD	Mean	SD	p value	Holm correction
CCL21	1313.1	1327.1	714.5	380.6	0.01628	n.s.
CXCL13	4.5	4.7	0.5	0.6	2.229e-08	Sig.
CCL27	3.3	6.5	1.4	2.9	0.06004	n.s.
CXCL5	2384.9	10941.8	178.2	143.3	0.06488	n.s.
CCL11	9.7	11.7	5.1	2.7	0.01211	n.s.
CCL24	44.3	136.7	26.9	14.8	0.7323	n.s.
CCL26	7.3	7.9	3.5	3.1	0.00168	Sig.
CX3CL1	82.2	120.9	43.4	19.0	0.04425	n.s.
CXCL6	2.6	5.1	0.7	0.8	0.002821	n.s.
GM-CSF	91.7	56.4	152.0	101.5	2.37E-07	Sig.
CXCL1	107.6	206.3	38.7	26.2	5.63E-05	Sig.
CXCL2	18.1	36.3	4.1	3.1	0.06299	n.s.
CCL1	15.4	16.3	11.4	9.3	0.1388	n.s.
IFN-γ	12.2	22.8	1.6	1.5	5.69E-06	Sig.
IL-1β	2.9	6.1	1.0	0.8	0.05999	n.s.
IL-2	1.7	2.1	0.6	0.6	2.04E-05	Sig.
IL-4	13.1	51.1	0.8	0.8	0.04502	n.s.
IL-6	822.9	3764.6	27.5	97.9	2.76E-07	Sig.
IL-8/CXCL8	51.8	108.5	5.0	6.6	5.43E-10	Sig.
IL-10	8.7	10.4	3.3	3.1	1.933e-05	Sig.
IL-16	25.5	29.9	14.1	11.6	0.02427	n.s.
CXCL10	350.3	1512.2	41.3	35.2	3.73E-06	Sig.
CXCL11	1.9	2.3	0.9	0.9	0.001688	Sig.
CCL2	1592.1	2431.5	415.9	231.1	1.58E-08	Sig.
CCL8	10.9	15.9	2.8	1.9	2.79E-05	Sig.
CCL7	12.0	19.8	1.1	1.0	7.423e-05	Sig.
CCL13	3.3	6.9	1.3	0.6	0.03057	n.s.
CCL22	20.8	26.6	6.8	5.6	1.51E-05	Sig.
MIF	77741.9	66286.8	37135.4	20113.5	0.0006832	Sig.
MIG/CXCL9	68.0	324.3	15.0	19.3	0.06706	n.s.
CCL3	2.1	1.8	0.9	0.5	9.01E-05	Sig.
CCL15	812.4	898.4	520.1	564.6	0.04013	n.s.
CCL20	17.2	43.2	3.3	3.3	1.21E-06	Sig.
CCL19	21.2	27.0	4.1	4.2	2.70E-05	Sig.
CCL23	24.5	35.8	11.7	11.4	0.02369	n.s.
CXCL16	829.4	894.5	446.1	189.4	0.0001381	Sig.
CXCL12	223.8	334.9	94.5	70.7	0.003502	n.s.
CCL17	4.2	11.0	1.1	0.9	0.2377	n.s.
CCL25	164.3	142.9	105.9	67.5	0.06687	n.s.
TNF-α	11.5	13.1	5.6	4.7	0.000754	Sig.
TGF-β1	388.3	456.9	182.0	149.9	0.06905	n.s.
TGF-β2	2454.2	1565.7	3496.0	1427.5	0.001195	Sig.
TGF-β3	30.0	45.6	4.2	9.7	4.98E-06	Sig.

n.s. = not significant after application of the Holm correction; Sig. = significant ($p < 0.0016$).

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

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 out 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 (Kauffmann 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 CXLC5, 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 leucocyte 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 harvested due to ethical reasons. All samples were harvested in the same fashion (see methods section). Although storage conditions and times might theoretically have an impact on absolute cytokine concentrations, we assume that this would have affected all groups in the same way. No relevant interference with intergroup comparisons of such a potential bias is expected, especially none explaining the >10-fold differences that we have observed. Based on an average interval of 3.8 years between cataract surgery and retinal detachment, we are not able to address a potential timely association between recent cataract surgery, the development of RD and cytokine concentrations.

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.

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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).