Cytokines and Chemokines Involved in Acute Retinal Necrosis

Lenneke de Visser,[*,1,2] Joke H. de Boer,[1] Ger T. Rijkers,[3,4] Karin Wiertz,[†,1] Henk-Jan van den Ham,[5] Rob de Boer,[6] Anton M. van Loon,[2] Aniki Rothova,[5] and Jolanda D. F. de Groot-Mijnes[1,2]

[1]Department of Ophthalmology, Utrecht University, Utrecht, The Netherlands
[2]Department of Medical Microbiology, Utrecht University, Utrecht, The Netherlands
[3]Department of Science, University College Roosevelt, Middelburg, The Netherlands
[4]Department of Medical Microbiology and Immunology, St. Antonius Hospital, Nieuwegein, The Netherlands
[5]Department of Viroscience, Erasmus MC, Rotterdam, The Netherlands
[6]Theoretical Biology & Bioinformatics, Utrecht University, Utrecht, The Netherlands

Correspondence: Jolanda D. F. de Groot-Mijnes, Department of Medical Microbiology, University Medical Center Utrecht, Heidelberglaan 100, Room G04. 614, 3584 CX Utrecht, The Netherlands; J.D.F.deGroot@umcutrecht.nl.

Current affiliation: *Eye Clinic Heuvelrug, Zeist, The Netherlands.

†Department of Ophthalmology, St. Antonius Hospital, Nieuwegein, The Netherlands.

Submitted: September 21, 2016
Accepted: March 1, 2017

Citation: de Visser L, de Boer JH, Rijkers GT, et al. Cytokines and chemokines involved in acute retinal necrosis. *Invest Ophthalmol Vis Sci*. 2017;58:2139–2151. DOI:10.1167/iovs.16-20799

Purpose. To investigate which cytokines and chemokines are involved in the immunopathogenesis of acute retinal necrosis (ARN), and whether cytokine profiles are associated with clinical manifestations, such as visual outcome.

Methods. Serum and aqueous humor (AH) samples of 19 patients with ARN were analyzed by multiplex immunoassay. Infectious controls consisted of 18 patients with rubella virus–associated Fuchs' uveitis and 20 patients with ocular toxoplasmosis all confirmed by intraocular fluid analyses. The control group consisted of seven paired AH and serum samples from seven noninflammatory control patients with age-related cataract. In each sample, 4 anti-inflammatory, 12 proinflammatory, 2 vascular, and 4 other immune mediators were measured. In addition, various clinical characteristics were assessed.

Results. In ARN, 10 of the 22 mediators, including most proinflammatory and vascular mediators such as IL-6, IL-8, IL-18, MIF, MCP-1, Eotaxin, IP-10, IL-15, sICAM-1, and sVCAM-1, were significantly elevated when compared to all controls. In addition, one anti-inflammatory mediator (IL-10) was significantly elevated in ARN as compared to the controls. No association was found between the time of sampling and the extent and levels of immune mediator expression.

Conclusions. The pathogenesis of ARN is characterized by the presence of predominantly proinflammatory cytokines and chemokines with high expression levels as compared to other infectious causes of uveitis. There are no indications for an obvious Th-1 or Th-17 pathway. The combined data suggest that immune mediator expression is related to severity of disease, which is more fulminant in ARN, rather than to a specific uveitis entity.

Keywords: acute retinal necrosis, cytokine, aqueous humor

Acute retinal necrosis (ARN) represents an intraocular herpetic infection characterized by severe inflammatory symptoms, including progressive retinitis and choroiditis with occlusive vasculitis and papillitis. Acute retinal necrosis has a poor visual prognosis, due to extensive retinal inflammation with necrosis, occlusive vasculitis, frequent development of retinal detachment, and optic disc atrophy.[1,2] Varicella zoster virus (VZV) is the most frequent cause of ARN in adults,[3] but it may also be caused by other members of the herpes virus family, including herpes simplex virus (HSV) types 1 and 2, and rarely, cytomegalovirus.[4–8] To date, the pathogenesis of extensive inflammation responsible for visual loss in ARN is unknown. The necrotic process has been described to be driven by $CD4^+$T cells, macrophages, polymorphonuclear cells, B cells, and by the inflammatory cytokines TNF-α and IFN-γ.[9,10] Which (other) cytokines and chemokines are involved in the process of ARN has not been fully defined. Only a few studies[11–13] have shown changes in levels of specific cytokines and chemokines in the aqueous humor (AH) of patients with ARN. Additionally, most of these studies have investigated a limited number of patients and/or the quantity of AH available from each patient has allowed analysis of only a limited number of mediators. To study the mediators involved in this devastating inflammatory reaction, a comprehensive spectrum of immune mediators needs to be measured, in a large cohort of patients.

The aim of this study was to investigate which cytokines and chemokines are involved in the immunopathogenesis of ARN, caused by HSV or VZV, and whether specific cytokine profiles are associated with clinical manifestations. To this end, cytokine and chemokine expression patterns in the AH and serum were determined by multiplex immunoassay (Luminex, Austin, Texas, USA) technology, highly suited for high-throughput analysis in microvolumes, including ocular fluids.[11,14–16]

Materials and Methods

Patients

In this study, paired AH and serum samples of 19 patients with ARN who fulfilled the clinical criteria of the International

Uveitis Study Group, and confirmed by intraocular fluid analysis, were included.[17] Results were compared to mediator profiles detected in paired AH and serum samples of 18 patients with rubella virus–associated Fuchs' uveitis (RV-FU) and 20 patients with ocular toxoplasmosis (OT). Noninflammatory controls consisted of seven paired AH and serum samples from patients with age-related cataract.

Of the 19 patients with ARN, 4 patients had an HSV infection, 14 had a VZV infection, and 1 patient had an HSV/VZV double infection, all confirmed by PCR and by establishing specific intraocular antibody production with the Goldmann-Witmer coefficient (GWC). The infectious cause of OT and RV-FU uveitis was also determined by PCR and GWC, as described previously.[18–21] All samples were collected for diagnostic purposes and their remainders were used for the current examinations. This study was conducted according to the tenets of the Declaration of Helsinki. None of the patients objected to the use of the remnants of their samples for further research and this study was approved by the institutional review board of the University Medical Center Utrecht.

The following clinical data were assessed for each patient: sex, age at the time of sampling, time interval between onset of uveitis and sample collection, and (systemic) treatment at the time of sampling. All ARN patients were treated with intravenous acyclovir and none of the patients used steroids at the time of sampling. One OT patient was on antimicrobial medication for 4 days at time of sampling.

Aqueous humor and serum samples were collected as described previously, stored at −80°C in sterile screw-cap tubes within 5 hours of collection, and thawed directly before analysis to preserve the sample.[22]

Multiplex Immunoassay

Twenty-five microliters of AH and 50 μL serum sample were analyzed by multiplex immunoassay essentially as described previously.[14] In each sample, 22 immune mediators were analyzed: 4 anti-inflammatory cytokines (interleukin [IL]-4, IL-5, human-specific IL-10, and IL-13); 12 proinflammatory mediators (cytokines IL-1β, IL-2, IL-6, IL-12p70, IL-17, IL-18, interferon-γ [IFN-γ], tumor necrosis factor-α [TNF-α], and chemokines IL-8 [CXCL8], interferon-inducible 10-kDa protein [IP-10; CXCL10], monocyte chemotactic protein-1 [MCP-1; CCL2], macrophage inflammatory protein-1-α [MIP1α; CCL3]); 2 vascular mediators (soluble vascular cell adhesion molecule [sVCAM-1] and soluble intracellular adhesion molecule [sICAM-1]); and 4 additional mediators (cytokines IL-15 and macrophage migration inhibitory factor [MIF], and chemokines RANTES [regulated on activation, normal T-cell expressed and secreted; [CCL5] and Eotaxin [CCL11]). Concentrations above or below the detection limit were assigned as the highest or the lowest value from the respective standard curve. For statistical analysis, concentrations below the detection limit were converted to a value of 0.5×, the lowest value of the standard curve.[14] The assay sensitivities for each mediator are listed in the footnotes of Tables 1 and 2.

Statistical Analysis

Principal component analysis (PCA) was performed on log-transformed mediator concentrations. Cluster analysis was performed by using 1-Pearson's correlation as a distance measure and Ward's method for hierarchical clustering as before.[23] The PCA and the cluster analysis were performed and visualized in R, a language for statistical computing (http://www.r-project.org; in the public domain).

Additional statistical analyses of the data were performed by using SPSS version 21 (SPSS, Inc., Chicago, Illinois, USA). Kruskal-Wallis and Mann-Whitney *U* test were used for nonparametric comparison of the different groups. An overview of the data and initial Kruskal-Wallis test for AH and serum are shown in Tables 1 and 2, respectively. In Mann-Whitney *U* tests, Bonferroni correction was used to compensate for multiple comparisons. Correlations were determined by the Spearman's ρ test. Visual acuity was converted into the logMAR (logarithm of the minimum angle of resolution) visual acuity. *P* values of less than 0.05 were considered to be statistically significant.

Results

Cluster Analysis Identifies a Distinct Group of Highly Expressed Cytokines That Associates With ARN

As a first step in the analysis, we applied cluster analysis to obtain a global overview of cytokine expression in the AH. Contrary to conventional analysis, this analysis method does not divide the samples into a priori groups but aggregates them on the basis of similarity to each other (i.e., nonsupervised analysis; Fig. 1A). Application of this method to our data shows that the samples can be divided into three principal groups: one consisting primarily of ARN (Fig. 1A, cluster A) and two mixed groups (Fig. 1A, clusters B and C). The noninflammatory controls fall primarily in a subgroup of the bottom compartment in cluster C, while OT and RV-FU samples do not separate clearly from each other or from the controls. This analysis was confirmed by using PCA (Fig. 1B), a technique that uses all AH measurements to evaluate the extent to which samples are alike and projects this into a 2D plot. Principal component analysis data showed that ARN samples appear to contain different cytokines when compared to the other disease categories, as these segregated from the other samples on the first principal component that accounts for two-thirds of all variability in the data (66%) (Fig. 1B).

Cluster analysis further showed that the three clusters segregate primarily by the expression of a subset of cytokines, including IP-10, sVCAM-1, sICAM-1, MCP-1, Eotaxin, MIF, IL-8, IL-6, RANTES, IL-10, and IL-18, ranging from high, intermediate, and low levels in clusters A, B, and C, respectively. The control subgroup in cluster C segregated primarily on cytokines IL-12p70, MIP1α, TNF-α, and IL-13; a similar subgroup based on these cytokines was observed in the ARN-dominated cluster A.

Statistical Analysis of Differences in Cytokine Expression Between ARN and Controls

Further statistical analysis by Mann-Whitney *U* testing confirmed the cluster analysis observations described above. Similarly, in the ocular fluids of the ARN patients, IP-10, sVCAM-1, sICAM-1, MCP-1, Eotaxin, MIF, IL-8, IL-6, IL-10, and IL-18 were significantly elevated as compared to the noninflammatory controls (Fig. 2; Table 3). Proinflammatory mediators IL-6, IL-8, IL-18, IP-10, and MCP-1, as well as Eotaxin and vascular chemokines, were significantly elevated as compared to all controls (Table 1). Only one anti-inflammatory mediator, IL-10, was significantly elevated.

Acute retinal necrosis samples contained significantly higher levels of IFN-γ than RV-FU samples ($P = 0.039$; Mann-Whitney *U* test with Bonferroni correction), but not compared to OT ($P = 0.114$) (Fig. 2). Proinflammatory mediator IL-12 was not detected in ARN patients but was significantly elevated in OT ($P = 0.003$; Mann-Whitney *U* test with Bonferroni correction) and RV-FU ($P = 0.039$; Mann-Whitney *U* test with Bonferroni correction).

Table 1. Immune Mediator Expression in Aqueous Humor Samples

Mediator	ARN ($n = 19$)	RV-FU ($n = 18$)	OT ($n = 20$)	NIC ($n = 7$)	*P* Value*
IL-1β					
Mean, pg/mL†	1	n.a.	<1	n.a.	0.068
Range or *values*, pg/mL‡	<1.5 to 17	n.a.	*6.2*	n.a.	
No. detected (%)§	4 (21)	0	1 (5)	0	
IL-2					
Mean, pg/mL†	n.a.	3	n.a.	n.a.	0.158
Range or *values*, pg/mL‡	n.a.	*35.3; 70.5*	n.a.	n.a.	
No. detected (%)§	0	2 (11)	0	0	
IL-4					
Mean, pg/mL†	2	n.a.	<1	n.a.	**0.017**
Range or *values*, pg/mL‡	<1.2 to 35.4	n.a.	*2.8; 12.8*	n.a.	
No. detected (%)§	6 (32)	0	2 (10)	0	
IL-5					
Mean, pg/mL†	4	2	2	n.a.	0.072
Range or *values*, pg/mL‡	<1.2 to 74.7	*36.4*	*49.6*	n.a.	
No. detected (%)§	5 (26)	1 (6)	1 (5)	0	
IL-6					
Mean, pg/mL†	3246	27	32	458	**0**
Range or *values*, pg/mL‡	169 to >10,016	<2.4 to 9299	<2.4 to >10,016	<2.4 to 3191	
No. detected (%)§	19 (100)	11 (61)	14 (70)	1 (14)	
IL-10					
Mean, pg/mL†	51	<1	1	n.a.	**0**
Range or *values*, pg/mL‡	<2.3 to 559	*3.7*	<2.3 to 20.7	n.a.	
No. detected (%)§	18 (95)	1 (6)	5 (25)	0	
IL-12p70					
Mean, pg/mL†	2	7	6	n.a.	**0**
Range or *values*, pg/mL‡	*11.1*	<4.3 to 68.4	<4.3 to 81.1	n.a.	
No. detected (%)§	1 (5)	9 (50)	13 (65)	0	
IL-13					
Mean, pg/mL†	1	1	1	n.a.	0.321
Range or *values*, pg/mL‡	<1 to 50.0	<1 to 21.9	<1 to 28.3	n.a.	
No. detected (%)§	6 (32)	7 (39)	6 (30)	0	
IL-15					
Mean, pg/mL†	26	25	45	16	**0.038**
Range or *values*, pg/mL‡	<1.4 to 93.8	<1.4 to 171	12.3 to 112	<1.4 to 50.3	
No. detected (%)§	16 (84)	16 (89)	20 (100)	4 (57)	
IL-17					
Mean, pg/mL†	2	n.a.	2	n.a.	0.727
Range or *values*, pg/mL‡	*20*	n.a.	*8.7*	n.a.	
No. detected (%)§	1 (5)	0	1 (5)	0	
IL-18					
Mean, pg/mL†	28	n.a.	2	2	**0**
Range or *values*, pg/mL‡	<1.2 to 155	n.a.	<1.2 to 110	*9.2*	
No. detected (%)§	17 (89)	0	4 (20)	1 (14)	
IFN-γ					
Mean, pg/mL†	15	n.a.	3	n.a.	**0.001**
Range or *values*, pg/mL‡	<9.1 to 1246	n.a.	<9.1 to 294	n.a.	
No. detected (%)§	9 (47)	0	4 (20)	0	
TNF-α					
Mean, pg/mL†	2	2	2	n.a.	0.338
Range or *values*, pg/mL‡	<1.2 to 60.7	<1.2 to 30.0	<1.2 to 21.9	n.a.	
No. detected (%)§	6 (32)	6 (33)	8 (40)	0	
sICAM-1					
Mean, pg/mL†	34,668	1458	2038	9644	**0**
Range or *values*, pg/mL‡	4750 to >81,387	161 to 23,999	254 to >81,387	<26.4 to 51,603	
No. detected (%)§	19 (100)	18 (100)	20 (100)	6 (86)	

Table 1. Continued

Mediator	ARN ($n = 19$)	RV-FU ($n = 18$)	OT ($n = 20$)	NIC ($n = 7$)	*P* Value*
sVCAM-1					
Mean, pg/mL†	36,846	3041	2579	13,800	**0**
Range or *values*, pg/mL‡	6169 to >95,705	248 to 28,986	<25.0 to >95,705	<25.0 to 47,451	
No. detected (%)§	19 (100)	18 (100)	19 (95)	5 (71)	
RANTES					
Mean, pg/mL†	47	6	9	24	**0.001**
Range or *values*, pg/mL‡	<4.6 to 634	*433*	<4.6 to 262	*138*	
No. detected (%)§	12 (63)	1 (6)	4 (20)	1 (14)	
Eotaxin					
Mean, pg/mL†	332	4	5	2	**0**
Range or *values*, pg/mL‡	9 to 3975	<1.3 to 328.9	<1.3 to 1218.5	*8.7*	
No. detected (%)§	19 (100)	8 (44)	10 (50)	1 (14)	
IL-8					
Mean, pg/mL†	784	10	9	11	**0**
Range or *values*, pg/mL‡	24 to >2483	<1.3 to 63.9	<1.3 to 878.9	*17.4; 51.0*	
No. detected (%)§	19 (100)	12 (67)	12 (60)	2 (29)	
IP-10					
Mean, pg/mL†	2102	103	169	338	**0**
Range or *values*, pg/mL‡	228 to >2468	<1.0 to >2468	<1.0 to >2468	*32.7; 731; 1598*	
No. detected (%)§	19 (100)	13 (72)	17 (85)	3 (43)	
MIP1α					
Mean, pg/mL†	46	15	14	n.a.	**0.022**
Range or *values*, pg/mL‡	<9.3 to 805	*180; 241; 288*	*72.9; 249; 414*	n.a.	
No. detected (%)§	9 (47)	3 (17)	3 (15)	0	
MCP-1					
Mean, pg/mL†	2738	625	420	181	**0**
Range or *values*, pg/mL‡	759 to >4513	26.6 to 3919	72.0 to 3120	22.3 to 412	
No. detected (%)§	19 (100)	18 (100)	20 (100)	7 (100)	
MIF					
Mean, pg/mL†	1967	90	80	138	**0**
Range or *values*, pg/mL‡	240 to >10,000	<18.0 to 1962	<18.0 to 1089	*175; 268; 451*	
No. detected (%)§	19 (100)	10 (56)	11 (55)	3 (43)	

Assay sensitivities in pg/mL are 1.5 for IL-1β, 1.8 for IL-2, 1.2 for IL-4, 1.2 for IL-5, 2.4 for IL-6, 2.3 for IL-10, 4.3 for IL-12p70, 1.0 for IL-13, 1.4 for IL-15, 1.1 for IL-17, 1.2 for IL-18, 9.1 for IFN-γ, 1.2 for RANTES (CCL5), 1.3 for Eotaxin (CCL11), 5.3 for IL-8 (CXCL8), 1.0 for IP-10 (CXCL10), 1.2 for TNF-α, 4.7 for MIF, 1.2 for MCP-1 (CCL2), 9.3 for MIP1α (CCL3), 25.0 for sVCAM-1 (CD106), and 26.4 for sICAM-1 (CD54) (de Jager et al.,[14] 2005). IP, interferon-inducible protein; n.a., not applicable; NIC, noninflammatory controls.

* *P* value calculated by using the Kruskal-Wallis test.

† Geometric mean concentration (pg/mL).

‡ Range of measured concentrations (pg/mL). For fewer than four detectable samples, the values are given.

§ Number of samples above the lower limit of detection.

Further correlation analyses of mediators in the AH of ARN patients showed that IFN-γ expression correlated with that of TNF-α ($r = 0.71$, $P = 0.001$) and IL-18 ($r = 0.46$, $P = 0.050$), but not with that of IL-12 (Table 4). Expression of IL-8 correlated with that of MCP-1 ($r = 0.59$, $P = 0.008$), but not with that of IP-10. Soluble ICAM-1 and sVCAM-1 expression correlated with that of IP-10 ($r = 0.51$, $P = 0.026$ for both), as well as with that of IL-8 ($r = 0.74$, $P = 0.000$ and $r = 0.79$, $P = 0.000$, respectively), but not with that of MCP-1 (Table 4). Interleukin 10, the only significantly elevated anti-inflammatory cytokine, correlated with IL-6 ($r = 0.60$, $P = 0.007$), IL-8 ($r = 0.46$, $P = 0.049$), IP-10 ($r = 0.53$, $P = 0.019$), IFN-γ ($r = 0.82$, $P = 0.000$), MCP-1 ($r = 0.71$, $P = 0.001$), and Eotaxin ($r = 0.70$, $P = 0.001$), but not with IL-12, IL-18, sICAM-1, sVCAM-1, and RANTES.

Analysis of the interval between the onset of symptoms and time of sampling did not reveal an association with the level and extend of cytokine and chemokine production.

Analysis of the serum levels demonstrated that all cytokine and chemokine levels, except for RANTES, were lower than those found in the ocular fluids (Tables 1, 2). In contrast, the levels of the vascular mediators sVCAM-1 and sICAM-1 were in almost all cases higher in the serum than in the ocular fluid samples.

Discussion

In this study, analysis of AH samples of ARN patients revealed an increased production of mainly proinflammatory cytokines and chemokines as compared to AH samples of the infectious and noninflammatory controls. Also, vascular mediators, such as sICAM-1 and sVCAM-1, appeared to be more prevalent in ARN than in the other uveitis entities. Acute retinal necrosis AH samples contained a wider spectrum of mediators and these were produced in higher quantities. Most notably, the levels of proinflammatory mediators IL-6, IL-8, IP-10, and MCP-1, attracting neutrophils, T lymphocytes, and monocytes, are significantly higher in ARN than in the other uveitis

Table 2. Immune Mediator Expression in Serum Samples

Mediator	ARN ($n = 19$)	RV-FU ($n = 18$)	OT ($n = 20$)	NIC ($n = 7$)	*P* Value*
IL-1β					
Mean, pg/mL†	<1	n.a.	n.a.	n.a.	0.5
Range or *values*, pg/mL‡	*13.5*	n.a.	n.a.	n.a.	
No. detected (%)§	1 (5)	0	0	0	
IL-2					
Mean, pg/mL†	3	n.a.	n.a.	n.a.	0.5
Range or *values*, pg/mL‡	*118*	n.a.	n.a.	n.a.	
No. detected (%)§	1 (5)	0	0	0	
IL-4					
Mean, pg/mL†	<1	n.a.	n.a.	n.a.	0.5
Range or *values*, pg/mL‡	*37.5*	n.a.	n.a.	n.a.	
No. detected (%)§	1 (5)	0	0	0	
IL-5					
Mean, pg/mL†	2	n.a.	2	n.a.	0.44
Range or *values*, pg/mL‡	*58.9; 902*	n.a.	*15.4; 135*	n.a.	
No. detected (%)§	2 (11)	0	2 (10)	0	
IL-6					
Mean, pg/mL†	5	3	3	n.a.	0.336
Range or *values*, pg/mL‡	2.4 to 3737	*41.3*	*13.6; 23.4*	n.a.	
No. detected (%)§	4 (21)	1 (6)	2 (10)	0	
IL-10					
Mean, pg/mL†	<1	n.a.	n.a.	n.a.	**0.003**
Range or *values*, pg/mL‡	*79.4*	n.a.	n.a.	n.a.	
No. detected (%)§	1 (5)	0	0	0	
IL-12p70					
Mean, pg/mL†	1	n.a.	n.a.	n.a.	0.5
Range or *values*, pg/mL‡	*79.2*	n.a.	n.a.	n.a.	
No. detected (%)§	1 (5)	0	0	0	
IL-13					
Mean, pg/mL†	1	n.a.	1	n.a.	0.727
Range or *values*, pg/mL‡	*134*	n.a.	*28.2*	n.a.	
No. detected (%)§	1 (5)	0	1 (5)	0	
IL-15					
Mean, pg/mL†	1	n.a.	2	7	0.203
Range or *values*, pg/mL‡	*4.5; 92.5*	n.a.	*15.2; 30.4; 39.2*	*18.1; 23.6*	
No. detected (%)§	2 (11)	0	3 (15)	2 (29)	
IL-17					
Mean, pg/mL†	2	1	2	2	0.817
Range or *values*, pg/mL‡	<1.1 to 71.0	<1.1 to 14.3	<1.1 to 57.0	*11.1*	
No. detected (%)§	5 (26)	4 (22)	6 (30)	1 (14)	
IL-18					
Mean, pg/mL†	26	24	27	31	0.361
Range or *values*, pg/mL‡	8.0 to 316	13.4 to 63.9	8.4 to 68.8	3.1 to 121	
No. detected (%)§	19 (100)	18 (100)	20 (100)	7 (100)	
IFN-γ					
Mean, pg/mL†	<1	n.a.	n.a.	n.a.	0.5
Range or *values*, pg/mL‡	*180*	n.a.	n.a.	n.a.	
No. detected (%)§	1 (5)	0	0	0	
TNF-α					
Mean, pg/mL†	1	n.a.	n.a.	n.a.	0.5
Range or *values*, pg/mL‡	*30.7*	n.a.	n.a.	n.a.	
No detected (%)§	1 (5)	0	0	0	
sICAM-1					
Mean, pg/mL†	86,360	53,183	82,689	80,718	0.321
Range or *values*, pg/mL‡	75,860 to >86,985	28.7 to >86,985	31,590 to >86,985	48,848 to >86,985	
No. detected (%)§	19 (100)	17 (94)	20 (100)	7 (100)	

Table 2. Continued

Mediator	ARN ($n = 19$)	RV-FU ($n = 18$)	OT ($n = 20$)	NIC ($n = 7$)	*P* Value*
sVCAM-1					
Mean, pg/mL†	110,562	62,549	100,216	112,484	0.214
Range or *values*, pg/mL‡	78,474 to >112,820	41.0 to >112,820	10,555 to >112,820	110,472 to >112,820	
No. detected (%)§	19 (100)	17 (94)	20 (100)	7 (100)	
RANTES					
Mean, pg/mL†	4737	1704	5541	5091	**0.017**
Range or *values*, pg/mL‡	1486 to >5541	<4.6 to >5541	>5541	3805 to >5541	
No. detected (%)§	19 (100)	16 (89)	20 (100)	7 (100)	
Eotaxin					
Mean, pg/mL†	33	6	43	23	0.073
Range or *values*, pg/mL‡	<1.3 to 367	<1.3 to 115	3.0 to 217	7.8 to 45.1	
No. detected (%)§	18 (95)	10 (56)	20 (100)	7 (100)	
IL-8					
Mean, pg/mL†	58	12	5	5	**0.003**
Range or *values*, pg/mL‡	<1.3 to 2806	2.6 to 102	<1.3 to 827	1.8 to 14.4	
No. detected (%)§	17 (89)	18 (100)	12 (60)	7 (100)	
IP-10					
Mean, pg/mL†	20	6	18	21	**0.026**
Range or *values*, pg/mL‡	<1.0 to 222	<1.0 to 186	<1.0 to 72.7	2.7 to 84.6	
No. detected (%)§	18 (95)	8 (44)	19 (95)	7 (100)	
MIP1α					
Mean, pg/mL†	33	15	14	47	0.116
Range or *values*, pg/mL‡	<9.3 to 4682	*70.6; 92.9; 180*	*54.9; 79.6; 149*	*73.2; 208*	
No. detected (%)§	9 (47)	3 (17)	3 (15)	2 (29)	
MCP-1					
Mean, pg/mL†	97	72	76	55	0.145
Range or *values*, pg/mL‡	36.5 to 312	19.8 to 212	21.0 to 215	10.8 to 131	
No. detected (%)§	19 (100)	18 (100)	20 (100)	7 (100)	
MIF					
Mean, pg/mL†	178	136	62	147	0.141
Range or *values*, pg/mL‡	<13.8 to 5733	<13.6 to 2243	<13.6 to 1814	<13.8 to 505	
No. detected (%)§	18 (95)	13 (72)	10 (50)	4 (57)	

Assay sensitivities in pg/ml are 1.5 for IL-1β, 1.8 for IL-2, 1.2 for IL-4, 1.2 for IL-5, 2.4 for IL-6, 2.3 for IL-10, 4.3 for IL-12p70, 1.0 for IL-13, 1.4 for IL-15, 1.1 for IL-17, 1.2 for IL-18, 9.1 for IFN-γ, 1.2 for RANTES (CCL5), 1.3 for Eotaxin (CCL11), 5.3 for IL-8 (CXCL8), 1.0 for IP-10 (CXCL10), 1.2 for TNF-α, 4.7 for MIF, 1.2 for MCP-1 (CCL2), 9.3 for MIP1α (CCL3), 25.0 for sVCAM-1 (CD106), and 26.4 for sICAM-1 (CD54) (de Jager et al.,[14] 2005).

* *P* value calculated by using the Kruskal-Wallis test.

† Geometric mean concentration (pg/mL).

‡ Range of measured concentrations (pg/ml). For fewer than four detectable samples, the values are given.

§ Number of samples above the lower limit of detection.

entities.[24–27] Interestingly, the typical proinflammatory mediators IL1-β, IL-12, IFN-γ, TNF-α, and IL-17 were detected in a minority of patients. Apparently, the pathogenesis of ARN is not generally characterized by a typical Th-1 or Th-17 pathway.

High level expression of IL-6, IL-8, IP-10, and MCP-1 has been previously reported for herpetic and noninfectious uveitis entities.[11,12,28–31] Low expression levels of IL1-β, IL-2, IL-12, TNF-α, and IL-17 are reported for other infectious and noninfectious uveitis entities.[11–13,28–33] Contrary to our study, others have reported high IFN-γ expression in both infectious and noninfectious uveitis, although Ongkosuwito et al.[12] and Lahmar et al.[31] also have found IFN-γ in only about half of their viral uveitis cases.[11–13,28–31,33] Low level expression for the Th-2–type cytokines IL-4, IL-5, and IL-13 has also been reported for infectious and noninfectious uveitis.[11–13,31,33] Moreover, high IL-10 expression has been previously reported for herpetic uveitis and is mostly low in noninfectious uveitis entities.[11,28,29,31,34] The expression of IL-15, RANTES, and Eotaxin is consistent with other reports for both infectious and noninfectious uveitis.[11,28,32,34]

Interferon-γ was detected in 47% of ARN patients. It serves as an antiviral cytokine by inhibiting viral replication or eliminating viruses from infected cells[35] and has been described to be one of the factors that plays an important role in the clinical course of ARN.[36] Also, the level of IFN-γ has been shown to affect the severity of clinical signs of some viral infectious diseases.[37,38] The IFN-γ response is enhanced by TNF-α, which is a proinflammatory cytokine involved in systemic inflammation.[39] In this study, TNF-α was detected in approximately 30% of the aqueous of the ARN patients and it correlated with IFN-γ. Similar to our findings, Ongkosuwito et al.[12] and Lahmar et al.[31] have found significantly increased IFN-γ levels in AH samples of 41% and 57% of ARN patients, respectively. The differences in IFN-γ detection between ARN patients may be attributed to the timing of sampling, which varies in our study from 1 day up to 4 weeks after initiation of complaints. Levels may have dropped to undetectable levels

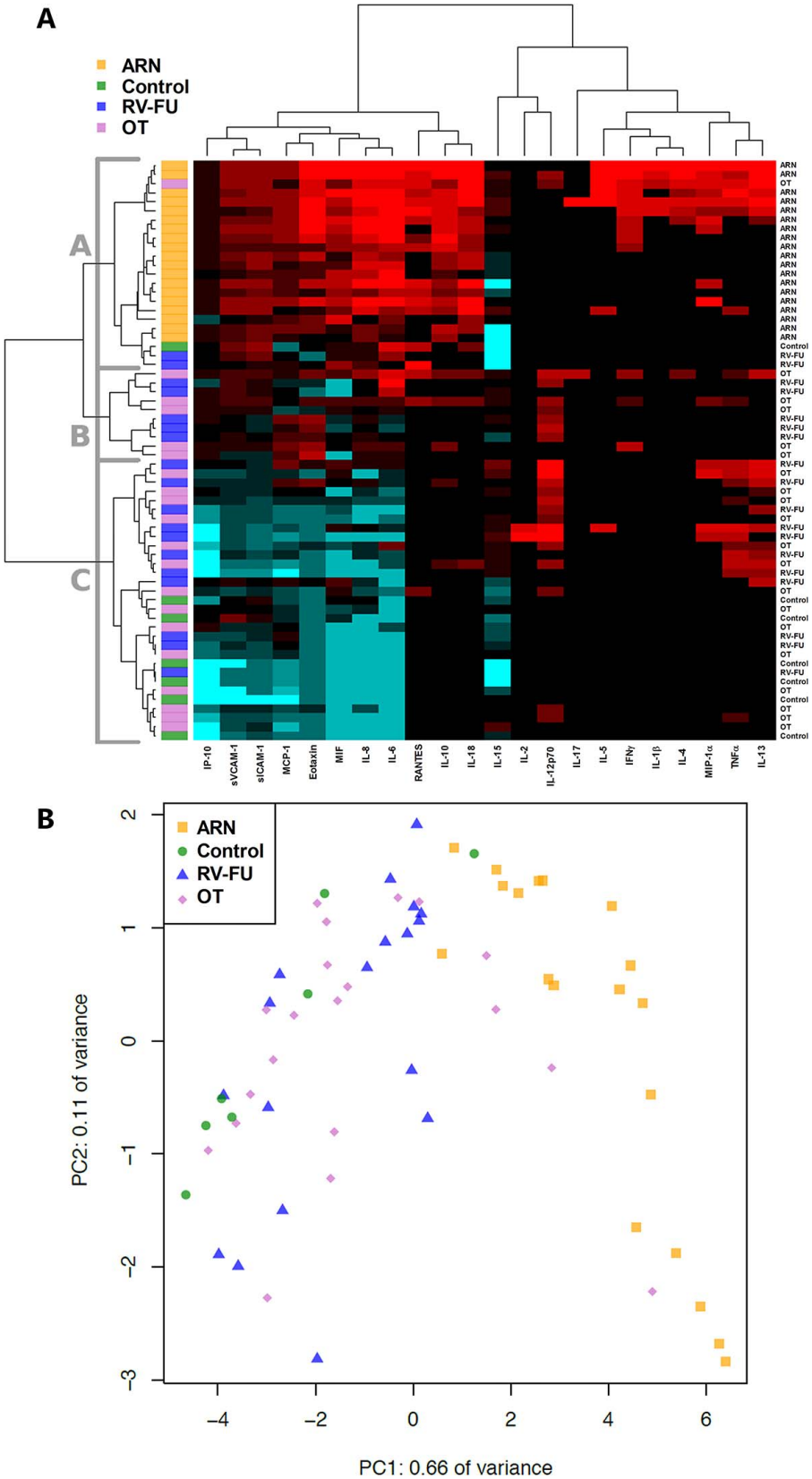

Figure 1. (**A**) Heat map of aqueous humor cytokine concentrations. Cytokine concentrations are depicted as colors ranging from *cyan* to *black* to *red*, indicating low, intermediate, and high concentration, respectively, relative to the mean of that cytokine. The phenotype of the samples is indicated by the label on the *right* as well as by the *colored bar* on the *left*. Samples are separated in one of three clusters (A, B, and C) indicated in *gray*. (**B**) Principal component analysis of aqueous humor samples, with the *x*- and *y*-axes representing the first and second principal components. The fraction of variation that is explained by each axis is noted in its label. The ARN samples are segregated from the other samples on the first principal component (PC1) that accounts for two-thirds of all variability in the data (66%). The phenotype of the individual samples is color indicated. Control, noninflammatory control.

Investigative Ophthalmology & Visual Science

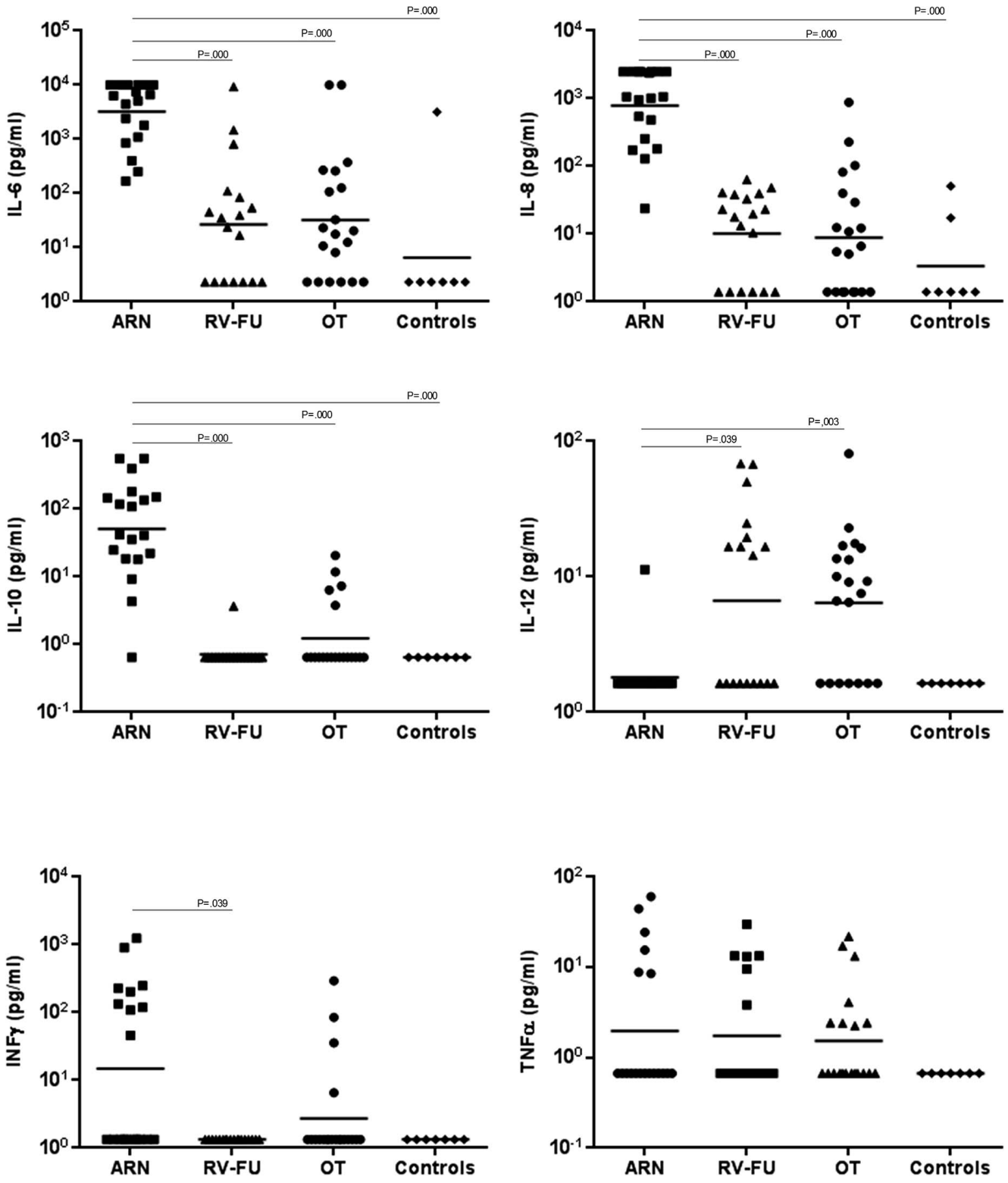

Figure 2. Dot plots of immune mediators significantly elevated in the ocular fluids of patients with ARN as compared to immune mediators in the ocular fluids of patients with RV-FU, OT, and noninflammatory controls (Controls). Significant *P* values are noted in the *upper part* of each graph. Statistical analysis was done with Mann-Whitney *U* tests followed by Bonferroni correction to compensate for multiple comparisons. A P value < 0.05 was considered significant.

within the clinical course of the disease as described by Abe et al.[36]

The ARN samples contained high levels of IL-18, whereas this cytokine was undetectable in RV-FU and only detectable in four OT samples. In mice, high levels of IL-18 have been associated with damage to the retinal pigment epithelium by inducing apoptosis.[40,41] Possibly, IL-18 contributes to the extent of retinitis in ARN patients, although no correlation

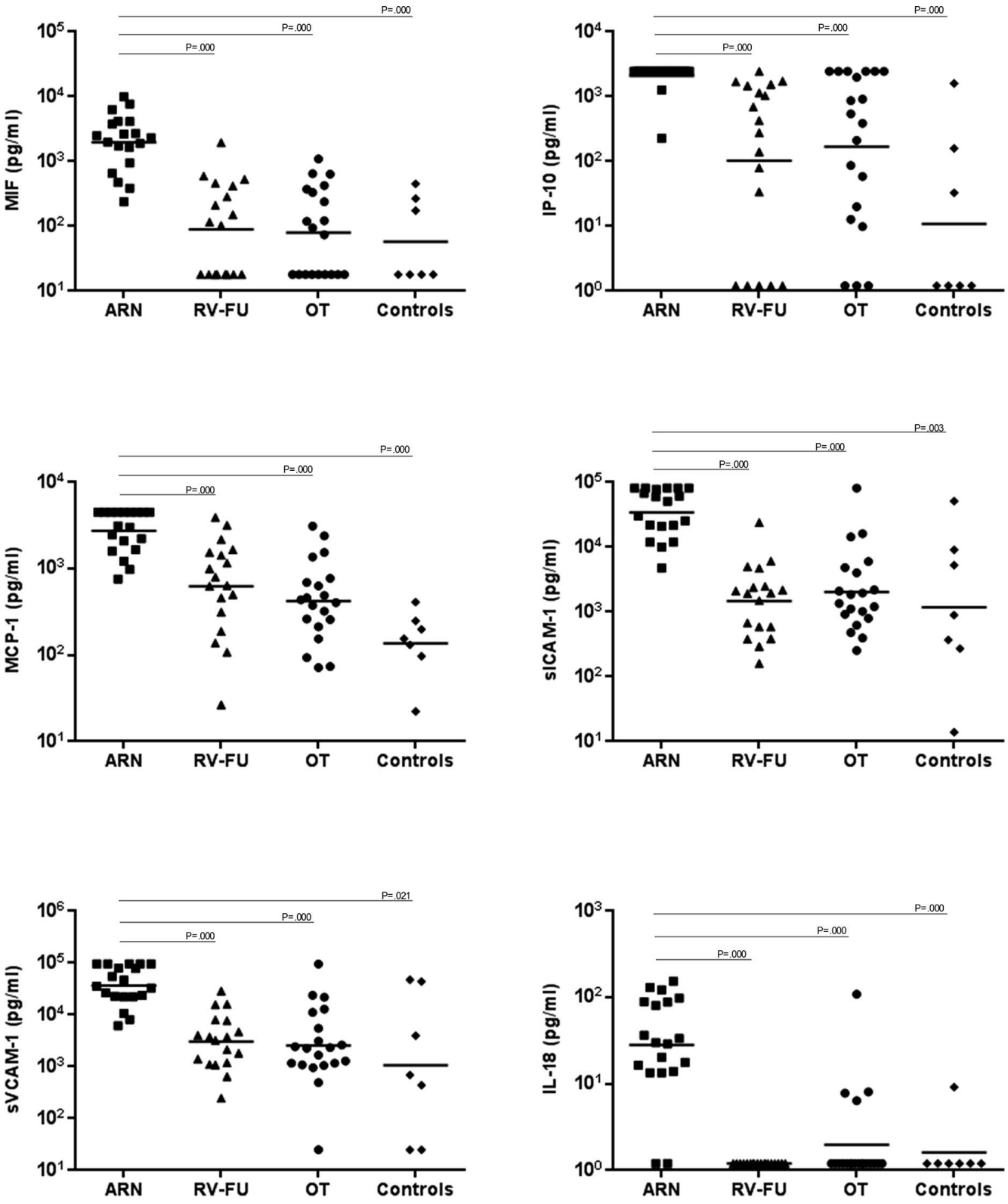

Figure 2. Continued.

was found between IL-18 expression and the presence of ablatio retinae.

Proinflammatory cytokine IL-12, while virtually absent in ARN, was elevated in RV-FU and OT, which is in concordance with other publications.[11,27,31,42] The lack of IL-12 in ARN samples may reflect negative feedback on IL-12 expression by IL-10, which was significantly increased.[43] Moreover, as IL-12 is a potent inducer of IFN-γ, the lack of IL-12 may explain why INF-γ was not significantly increased in our ARN cohort compared to the controls, contrary to other reports.[11,27,42] The

Table 3. Statistical Data of Immune Mediator Expression in Ocular Fluids of Patients With Acute Retinal Necrosis, Other Infectious Uveitis Entities, and Noninfectious Controls

Classification	Name	ARN vs. RV-FU	ARN vs. OT	ARN vs. NIC
Anti-inflammatory	IL-4	0.315	0.672	0.693
	IL-5	0.651	0.741	0.334
	IL-10	**0.000** ↑	**0.000** ↑	**0.000** ↑
	IL-13	1.000	1.000	0.693
Proinflammatory	IL-1β	0.852	1.000	1.000
	IL-2	1.000	1.000	1.000
	IL-6	**0.000** ↑	**0.000** ↑	**0.000** ↑
	IL-8	**0.000** ↑	**0.000** ↑	**0.000** ↑
	IL-12p70	**0.039** ↓	**0.003** ↓	1.000
	IL-17	1.000	1.000	1.000
	IL-18	**0.000** ↑	**0.000** ↑	**0.000** ↑
	IP-10	**0.000** ↑	**0.000** ↑	**0.000** ↑
	IFN-γ	**0.039** ↑	0.252	0.219
	TNF-α	1.000	1.000	0.693
	MCP-1	**0.000** ↑	**0.000** ↑	**0.000** ↑
	MIP1α	0.315	1.000	0.219
Vascular	sVCAM-1	**0.000** ↑	**0.000** ↑	**0.021** ↑
	sICAM-1	**0.000** ↑	**0.000** ↑	**0.003** ↑
Other	IL-15	1.000	1.000	0.105
	Eotaxin	**0.000** ↑	**0.000** ↑	**0.000** ↑
	RANTES	**0.012** ↑	**0.027** ↑	0.189
	MIF	**0.000** ↑	**0.000** ↑	**0.000** ↑

Represented are *P* values from pairwise comparison between ARN and the other three groups, using the Mann-Whitney *U* test followed by Bonferroni correction for multiple testing. Significant differences ($P < 0.05$) are in bold. ↑, significant increase compared to controls, ↓, significant decrease compared to controls.

observed correlation between IFN-γ and IL-18 in ARN and OT and the lack of correlation between IFN-γ and IL-12 in all three entities may indicate that in AH, IFN-γ is predominantly under regulatory control of IL-18 rather than of IL-12.

IP-10, which is under the control of IFN-γ, was expressed to high levels in all ARN patients, despite the presence of IFN-γ in only 47% of ARN patients. Maybe, IP-10 can be induced by another yet to be identified cytokine, for example, IL-18, which was expressed in 89% of ARN cases.

In our study, the cell adhesion molecules sICAM-1 and sVCAM-1 were elevated in the AH of all uveitis entities; however, they were more elevated in ARN than in the controls and both molecules correlated with IL-8. Soluble ICAM-1 mediates tight adhesion between all leukocytes and vascular endothelium, whereas sVCAM-1 mediates tight adhesion between lymphocytes, monocytes, and vascular endothelium before migration into the tissues.[39,44] Possibly, in case of viral infection of the retina, proinflammatory cytokines and chemoattractants, such as IL-8, are upregulated at the site of inflammation.[39] Concomitantly, infiltration of inflammatory cells occurs by adhesion of leukocytes to the vascular endothelium, mediated by VCAM-1– and ICAM-1–mediated receptor–ligand interactions, causing occlusive vasculitis.[39] During inflammation, adhesion molecules on endothelial cells are induced by vascular endothelial growth factor (VEGF). VEGF plays a major role in the development of intraocular neovascularizations, induces blood–retina barrier breakdown, and consequently increases vascular permeability. It is described as being highly elevated in the ocular fluids of patients with ARN, especially in patients with extensive retinitis.[2] This proinflammatory process, reminiscent of the so-called cytokine storm described for many inflammatory diseases including those of viral origin, will lead to extensive inflammation resulting in severe tissue damage.[45]

Our results showed that ARN is characterized by mainly a proinflammatory response. However, all but one ARN patient intraocularly expressed high levels of anti-inflammatory IL-10, which was significantly elevated as compared to the controls. Increased IL-10 production in herpes uveitis has been reported previously. Interleukin 10 is an immunosuppressive regulatory cytokine that enhances B-lymphocyte survival and antibody production.[39] Interleukin 10 can be produced by T lymphocytes, and it has been proposed that IL-10 downregulates the immune responses in order to dampen T-cell–mediated immune reactions and thereby control the extent of tissue damage.[46–50] The high IL-10 levels observed in the ocular fluids of the ARN patients seem paradoxal regarding the severe course of the disease. Possibly, the levels of IL-10 are not sufficient to dampen the harmful immune responses. Alternatively, immune responses other than those downregulated by IL-10 or other mechanisms all together may contribute to the damage, such as oxidative stress. Both infiltrating leukocytes and ocular resident cells, such as retinal pigmented epithelium and glial cells, can produce cytokines.[10,51] Therefore, the results presented here most likely represent the combined mediator expression from lymphocytes and ocular resident cells, possibly in response to each other. Limitations of our study included the differences in sampling timing within the cohort, which may be overcome by performing longitudinal follow-up studies in individual ARN patients. This may provide valuable insight into the course of the inflammatory process in ARN.

The question arises whether cytokine profiles might be discriminative for the diagnosis of various infectious uveitis entities. Our hierarchical cluster analysis and PCA might suggest that ARN is characterized by the intraocular expression of a specific set of immune mediators. Lahmar et al.[31] have suggested that cytokines may be used as diagnostic markers, as they have found IL-5 and IL-12 to be specific for OT, and IL-1β for viral uveitis. In our study, we also found IL-12 expression in OT and not in ARN; however, the reported association with IL-5 expression was not observed, and IL-1β was detected in only 4 of 19 ARN patients. In addition, the expression of other markers also differed between the study of Lahmar et al.[31] and ours. Moreover, the hierarchical cluster analysis and PCA (Fig. 1) showed that among ARN patients, clear differences in mediator expression levels may exist, as observed in the data by Lahmar et al.[31] In addition, in both studies, OT patients were observed who had a mediator profile similar to that of some ARN/viral uveitis patients. These inconsistencies might be explained by the fact that cytokine and chemokine expression patterns are part of an intricate regulatory network, which depends on many environmental stimuli, and may therefore vary considerably during the course of disease, which limits their use for diagnostic purposes. This is also reflected by the similarities in cytokine profiles between RV-FU and OT, while the causative agent of these ocular diseases is notably different: virus versus parasite, respectively. Possibly, in these patients inflammation has reached the chronic state such that the cytokine/chemokine profile has become similar in both ocular diseases. Therefore, immune mediator profiles in AH seem to provide more insight in severity and stage of the inflammatory process than etiologic clues.

In conclusion, we found an increased production of predominantly proinflammatory cytokines and chemokines in the ocular fluids of patients with ARN. The higher levels of mainly proinflammatory cytokines and chemokines in ARN might be a reflection of the more severe inflammation in ARN rather than a disease-specific cytokine pattern, although IL-10 and IL-18, and to a lesser extent IFN-γ, appear to play a more important role in ARN than in other infectious uveitis entities.

Table 4. Correlations of Relevant Mediators in Ocular Fluids of Patients With Acute Retinal Necrosis Determined by Spearman's ρ Test

	IL-6	IL-8	IL-10	IL-12	IL-18	IFN-γ	TNF-α	MIF	MIP1α	MCP-1	Eotaxin	IP10	IL-15	sICAM-1	sVCAM-1
IL-8															
Correlation coefficient	**0.839****														
Sig. (2-tailed)	**0.000**														
IL-10															
Correlation coefficient	**0.597****	**0.457***													
Sig. (2-tailed)	**0.007**	**0.049**													
IL-12															
Correlation coefficient	0.265	0.265	0.344												
Sig. (2-tailed)	0.273	0.273	0.149												
IL-18															
Correlation coefficient	**0.558***	**0.851****	0.360	0.301											
Sig. (2-tailed)	**0.013**	**0.000**	0.130	0.210											
IFN-γ															
Correlation coefficient	**0.748****	**0.587****	**0.819****	0.419	**0.456***										
Sig. (2-tailed)	**0.000**	**0.008**	**0.000**	0.074	**0.050**										
TNF-α															
Correlation coefficient	**0.460***	**0.567***	0.349	0.313	**0.624****	**0.713****									
Sig. (2-tailed)	**0.047**	**0.011**	0.143	0.192	**0.004**	**0.001**									
MIF															
Correlation coefficient	**0.697****	**0.614****	0.319	0.344	0.415	**0.587****	0.440								
Sig. (2-tailed)	**0.001**	**0.005**	0.183	0.149	0.077	**0.008**	0.059								
MIP1α															
Correlation coefficient	**0.725****	**0.860****	**0.525***	0.326	**0.763****	**0.649****	**0.527***	**0.709****							
Sig. (2-tailed)	**0.000**	**0.000**	**0.021**	0.174	**0.000**	**0.003**	**0.021**	**0.001**							
MCP-1															
Correlation coefficient	**0.719****	**0.592****	**0.712****	0.227	**0.470***	**0.747****	0.389	**0.551***	**0.753****						
Sig. (2-tailed)	**0.001**	**0.008**	**0.001**	0.349	**0.042**	**0.000**	0.100	**0.015**	**0.000**						
Eotaxin															
Correlation coefficient	**0.766****	**0.707****	**0.696****	0.301	**0.634****	**0.799****	**0.487***	**0.625****	**0.853****	**0.931****					
Sig. (2-tailed)	**0.000**	**0.001**	**0.001**	0.210	**0.004**	**0.000**	**0.034**	**0.004**	**0.000**	**0.000**					
IP-10															
Correlation coefficient	0.267	0.395	**0.533***	0.081	**0.466***	0.304	0.227	0.194	0.304	0.195	0.247				
Sig. (2-tailed)	0.270	0.094	**0.019**	0.743	**0.044**	0.205	0.349	0.426	0.205	0.424	0.308				
IL-15															
Correlation coefficient	0.458*	**0.579****	**0.482***	0.388	**0.588****	**0.697****	**0.694****	0.439	0.554	**0.535***	**0.592****	0.122			
Sig. (2-tailed)	0.049	**0.009**	**0.037**	0.101	**0.008**	**0.001**	**0.001**	0.060	0.014	**0.018**	**0.008**	0.619			
sICAM-1															
Correlation coefficient	0.414	**0.742****	0.112	0.174	**0.869****	0.163	0.444	0.264	**0.590****	0.133	0.326	**0.508***	0.216		
Sig. (2-tailed)	0.078	**0.000**	0.649	0.477	**0.000**	0.506	0.057	0.275	**0.008**	0.588	0.174	**0.026**	0.374		

Table 4. Continued

		IL-6	IL-8	IL-10	IL-12	IL-18	IFN-γ	TNF-α	MIF	MIP1α	MCP-1	Eotaxin	IP10	IL-15	sICAM-1	sVCAM-1
sVCAM-1																
	Correlation coefficient	**0.530***	**0.791****	0.372	0.304	**0.870****	0.380	**0.476***	0.336	**0.657****	0.337	**0.501***	**0.508***	0.427	**0.902***	
	Sig. (2-tailed)	**0.020**	**0.000**	0.117	0.206	**0.000**	0.109	**0.039**	0.159	**0.002**	0.159	**0.029**	**0.026**	0.068	**0.000**	
RANTES																
	Correlation coefficient	**0.592****	**0.670****	0.363	0.221	**0.597****	**0.535***	**0.576****	**0.518***	**0.735****	**0.485***	**0.565***	0.385	**0.476***	**0.485***	0.452
	Sig. (2-tailed)	**0.008**	**0.002**	0.126	0.364	**0.007**	**0.018**	**0.010**	**0.023**	**0.000**	**0.035**	**0.012**	0.104	**0.039**	**0.035**	0.052

Sig., significance. Significant correlations are in bold.
* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.001 level (2-tailed).

Future studies of intraocular immune mediators will help to further understand the immunopathogenesis of ARN.

Acknowledgments

The authors thank Nathalie van Uden of the Immunology Laboratory at the Wilhelmina Children's Hospital, University Medical Center Utrecht, The Netherlands, for technical assistance.

Supported by Dr. F. P. Fischer Foundation, Amersfoort, The Netherlands; and Foundation for Dutch Ophthalmic Research (SNOO), Rotterdam, The Netherlands.

Disclosure: **L. de Visser**, None; **J.H. de Boer**, None; **G.T. Rijkers**, None; **K. Wiertz**, None; **H.-J. van den Ham**, None; **R. de Boer**, None; **A.M. van Loon**, None; **A. Rothova**, None; **J.D.F. de Groot-Mijnes**, None

References

1. Verjans GM, Dings ME, McLauchlan J, et al. Intraocular T cells of patients with herpes simplex virus (HSV)-induced acute retinal necrosis recognize HSV tegument proteins VP11/12 and VP13/14. *J Infect Dis*. 2000;182:923–927.
2. Wiertz K, De Visser L, Rijkers G, De Groot-Mijnes J, Los L, Rothova A. Intraocular and serum levels of vascular endothelial growth factor in acute retinal necrosis and ocular toxoplasmosis. *Retina*. 2010;30:1734–1738.
3. Gaynor BD, Margolis TP, Cunningham ET Jr. Advances in diagnosis and management of herpetic uveitis. *Int Ophthalmol Clin*. 2000;40:85–109.
4. Ganatra JB, Chandler D, Santos C, Kuppermann B, Margolis TP. Viral causes of the acute retinal necrosis syndrome. *Am J Ophthalmol*. 2000;129:166–172.
5. Hellinger WC, Bolling JP, Smith TF, Campbell RJ. Varicella-zoster virus retinitis in a patient with AIDS-related complex: case report and brief review of the acute retinal necrosis syndrome. *Clin Infect Dis*. 1993;16:208–212.
6. Lewis ML, Culbertson WW, Post JD, Miller D, Kokame GT, Dix RD. Herpes simplex virus type 1: a cause of the acute retinal necrosis syndrome. *Ophthalmology*. 1989;96:875–878.
7. Silverstein BE, Conrad D, Margolis TP, Wong IG. Cytomegalovirus-associated acute retinal necrosis syndrome. *Am J Ophthalmol*. 1997;123:257–258.
8. Thompson WS, Culbertson WW, Smiddy WE, Robertson JE, Rosenbaum JT. Acute retinal necrosis caused by reactivation of herpes simplex virus type 2. *Am J Ophthalmol*. 1994;118:205–211.
9. Archin NM, Atherton SS. Infiltration of T-lymphocytes in the brain after anterior chamber inoculation of a neurovirulent and neuroinvasive strain of HSV-1. *J Neuroimmunol*. 2002;130:117–127.
10. Zheng M, Atherton SS. Cytokine profiles and inflammatory cells during HSV-1-induced acute retinal necrosis. *Invest Ophthalmol Vis Sci*. 2005;46:1356–1363.
11. Curnow SJ, Falciani F, Durrani OM, et al. Multiplex bead immunoassay analysis of aqueous humor reveals distinct cytokine profiles in uveitis. *Invest Ophthalmol Vis Sci*. 2005;46:4251–4259.
12. Ongkosuwito JV, Feron EJ, van Doornik CE, et al. Analysis of immunoregulatory cytokines in ocular fluid samples from patients with uveitis. *Invest Ophthalmol Vis Sci*. 1998;39:2659–2665.
13. Takase H, Futagami Y, Yoshida T, et al. Cytokine profile in aqueous humor and sera of patients with infectious or noninfectious uveitis. *Invest Ophthalmol Vis Sci*. 2006;47:1557–1561.
14. de Jager W, Prakken BJ, Bijlsma JW, Kuis W, Rijkers GT. Improved multiplex immunoassay performance in human

plasma and synovial fluid following removal of interfering heterophilic antibodies. *J Immunol Methods*. 2005;300:124–135.

15. Oliver KG, Kettman JR, Fulton RJ. Multiplexed analysis of human cytokines by use of the FlowMetrix system. *Clin Chem*. 1998;44:2057–2060.
16. Vignali DA. Multiplexed particle-based flow cytometric assays. *J Immunol Methods*. 2000;243:243–255.
17. Jabs DA, Nussenblatt RB, Rosenbaum JT; Standardization of Uveitis Nomenclature Working Group. Standardization of uveitis nomenclature for reporting clinical data: results of the First International Workshop. *Am J Ophthalmol*. 2005;140: 509–516.
18. de Groot-Mijnes JDF, de Visser L, Rothova A, Schuller M, van Loon AM, Weersink AJ. Rubella virus is associated with fuchs heterochromic iridocyclitis. *Am J Ophthalmol*. 2006;141: 212–214.
19. De Groot-Mijnes JDF, Rothova A, Van Loon AM, et al. Polymerase chain reaction and Goldmann-Witmer coefficient analysis are complimentary for the diagnosis of infectious uveitis. *Am J Ophthalmol*. 2006;141:313–318.
20. Goldmann H, Witmer R. Antibodies in the aqueous humor. *Ophthalmologica*. 1954;127:323–330.
21. Kijlstra A, Luyendijk L, Baarsma GS, et al. Aqueous humor analysis as a diagnostic tool in toxoplasma uveitis. *Int Ophthalmol*. 1989;13:383–386.
22. Van der Lelij A, Rothova A. Diagnostic anterior chamber paracentesis in uveitis: a safe procedure? *Br J Ophthalmol*. 1997;81:976–979.
23. van den Ham HJ, de Jager W, Bijlsma JW, Prakken BJ, de Boer RJ. Differential cytokine profiles in juvenile idiopathic arthritis subtypes revealed by cluster analysis. *Rheumatology (Oxford)*. 2009;48:899–905.
24. Crane IJ, McKillop-Smith S, Wallace CA, Lamont GR, Forrester JV. Expression of the chemokines MIP-1alpha, MCP-1, and RANTES in experimental autoimmune uveitis. *Invest Ophthalmol Vis Sci*. 2001;42:1547–1552.
25. Holtkamp GM, De Vos AF, Peek R, Kijlsta A. Analysis of the secretion pattern of monocyte chemotactic protein-1 (MCP-1) and transforming growth factor-beta 2 (TGF-beta2) by human retinal pigment epithelial cells. *Clin Exp Immunol*. 1999;118: 35–40.
26. Rollins BJ. Chemokines. *Blood*. 1997;90:909–928.
27. Sijssens KM, Rijkers GT, Rothova A, Stilma JS, Schellekens PA, de Boer JH. Cytokines, chemokines and soluble adhesion molecules in aqueous humor of children with uveitis. *Exp Eye Res*. 2007;85:443–449.
28. Abu El-Asrar AM, Struyf S, Kangave D, et al. Cytokine and CXC chemokine expression patterns in aqueous humor of patients with presumed tuberculous uveitis. *Cytokine*. 2012;59:377–381.
29. Ang M, Cheung G, Vania M, et al. Aqueous cytokine and chemokine analysis in uveitis associated with tuberculosis. *Mol Vis*. 2012;18:565–573.
30. El-Asrar AM, Al-Obeidan SS, Kangave D, et al. CXC chemokine expression profiles in aqueous humor of patients with different clinical entities of endogenous uveitis. *Immunobiology*. 2011;216:1004–1009.
31. Lahmar I, Abou-Bacar A, Abdelrahman T, et al. Cytokine profiles in toxoplasmic and viral uveitis. *J Infect Dis*. 2009; 199:1239–1249.
32. Ahn JK, Yu HG, Chung H, Park YG. Intraocular cytokine environment in active Behcet uveitis. *Am J Ophthalmol*. 2006;142:429–434.
33. Ooi KG, Galatowicz G, Towler HM, Lightman SL, Calder VL. Multiplex cytokine detection versus ELISA for aqueous humor: IL-5, IL-10, and IFNgamma profiles in uveitis. *Invest Ophthalmol Vis Sci*. 2006;47:272–277.
34. El-Asrar AM, Struyf S, Kangave D, et al. Cytokine profiles in aqueous humor of patients with different clinical entities of endogenous uveitis. *Clin Immunol*. 2011;139:177–184.
35. Novelli F, Casanova JL. The role of IL-12, IL-23 and IFN-gamma in immunity to viruses. *Cytokine Growth Factor Rev*. 2004; 15:367–377.
36. Abe T, Sato M, Saigo Y, Tamai M. Interferon gamma expression and clinical features in patients with acute retinal necrosis syndrome. *Graefes Arch Clin Exp Ophthalmol*. 2003;241: 982–987.
37. Aberle JH, Aberle SW, Dworzak MN, et al. Reduced interferon-gamma expression in peripheral blood mononuclear cells of infants with severe respiratory syncytial virus disease. *Am J Respir Crit Care Med*. 1999;160:1263–1268.
38. Fuji A, Kakumu S, Ohtani Y, Murase K, Hirofuji H, Tahara H. Interferon-gamma production by peripheral blood mononuclear cells of patients with chronic liver disease. *Hepatology*. 1987;7:577–581.
39. Roitt I, Brostoff J, Male D. Cytokines and cytokine receptors. In: Roitt I, Brostoff J, Male D, eds. *Immunology*. London: Mosby; 2001:119–129.
40. Doyle SL, Ozaki E, Brennan K, et al. IL-18 attenuates experimental choroidal neovascularization as a potential therapy for wet age-related macular degeneration. *Sci Transl Med*. 2014;6:230ra.
41. Ijima R, Kaneko H, Ye F, et al. Interleukin-18 induces retinal pigment epithelium degeneration in mice. *Invest Ophthalmol Vis Sci*. 2014;55:6673–6678.
42. el-Shabrawi Y, Livir-Rallatos C, Christen W, Baltatzis S, Foster CS. High levels of interleukin-12 in the aqueous humor and vitreous of patients with uveitis. *Ophthalmology*. 1998;105: 1659–1663.
43. Daftarian PM, Kumar A, Kryworuchko M, Diaz-Mitoma F. IL-10 production is enhanced in human T cells by IL-12 and IL-6 and in monocytes by tumor necrosis factor-alpha. *J Immunol*. 1996;157:12–20.
44. van Kooij B, Rothova A, Rijkers GT, de Groot-Mijnes JDF. Distinct cytokine and chemokine profiles in the aqueous of patients with uveitis and cystoid macular edema. *Am J Ophthalmol*. 2006;142:192–194.
45. Tisoncik JR, Korth MJ, Simmons CP, Farrar J, Martin TR, Katze MG. Into the eye of the cytokine storm. *Microbiol Mol Biol Rev*. 2012;76:16–32.
46. Awasthi A, Carrier Y, Peron JP, et al. A dominant function for interleukin 27 in generating interleukin 10-producing anti-inflammatory T cells. *Nat Immunol*. 2007;8:1380–1389.
47. Gazzinelli RT, Wysocka M, Hieny S, et al. In the absence of endogenous IL-10, mice acutely infected with Toxoplasma gondii succumb to a lethal immune response dependent on CD4+ T cells and accompanied by overproduction of IL-12, IFN-gamma and TNF-alpha. *J Immunol*. 1996;157:798–805.
48. Hunter CA, Abrams JS, Beaman MH, Remington JS. Cytokine mRNA in the central nervous system of SCID mice infected with Toxoplasma gondii: importance of T-cell-independent regulation of resistance to T. gondii. *Infect Immun*. 1993;61: 4038–4044.
49. McGeachy MJ, Bak-Jensen KS, Chen Y, et al. TGF-beta and IL-6 drive the production of IL-17 and IL-10 by T cells and restrain T(H)-17 cell-mediated pathology. *Nat Immunol*. 2007;8: 1390–1397.
50. Trinchieri G. Interleukin-10 production by effector T cells: Th1 cells show self control. *J Exp Med*. 2007;204:239–243.
51. Holtkamp GM, Kijlstra A, Peek R, de Vos AF. Retinal pigment epithelium-immune system interactions: cytokine production and cytokine-induced changes. *Prog Retin Eye Res*. 2001;20: 29–48.

Investigative Ophthalmology & Visual Science