Ocular Immunology and Inflammation

ISSN: 0927-3948 (Print) 1744-5078 (Online) Journal homepage: http://www.tandfonline.com/loi/ioii20

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To cite this article: Z. Fazil, J.C. Ten Berge, A.W. Langerak, A. Rothova & W.A. Dik (2018): An Intraocular Inflammatory Profile of Rubella Associated Uveitis, Ocular Immunology and Inflammation, DOI: 10.1080/09273948.2017.1421671

To link to this article: https://doi.org/10.1080/09273948.2017.1421671

Published online: 25 Jan 2018.

Ocular Immunology & Inflammation, 2018; 00(00): 1–6
Published with license by Taylor & Francis
ISSN: 0927-3948 print / 1744-5078 online
DOI: 10.1080/09273948.2017.1421671

ORIGINAL ARTICLE

An Intraocular Inflammatory Profile of Rubella Associated Uveitis

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ABSTRACT

Purpose: To analyze intraocular cytokine levels and cell profiles in patients with rubella virus-associated uveitis (RVU).

Methods: We collected intraocular fluid samples from patients with RVU (n = 10), uveitis of other causes (n = 27), and cataract (n = 22). Levels of 15 cytokines (IL-1β, IL-1ra, IL-2, IL-6, IL-6rα, IL-7, IL-8, IL-10, IL-17A, IL-23, TARC, MCP-1, TNF-α, PlGF, and VEGF) were measured using multiplex assay, and intraocular cell populations were determined by multiparameter flowcytometry. Clinical characteristics of RVU patients were collected and compared to laboratory outcomes.

Results: RVU patients exhibited high intraocular levels of MCP-1, IL-6rα, and TARC, whilst patients with noninfectious uveitis were characterized by high levels of PlGF. Cataract patients showed high levels of IL-2 and IL-23. Intraocular cell population of RVU patients disclosed mainly T-cells and monocytes/macrophages and B-cells were scarcely detected.

Conclusion: RVU patients exhibit a cytokine profile distinct from noninfectious uveitis and cataract.

Keywords: Cytokines, Fuchs uveitis syndrome, immunopathology, rubella-virus associated uveitis, intraocular fluid

Rubella virus-associated uveitis (RVU) was first reported by Quentin and Reiber as a major cause of Fuchs uveitis syndrome (FUS).[1,2] Multiple subsequent studies confirmed this association.[3–5] However, other causes of FUS were also repeatedly reported, and CMV has been identified as a potential cause of FUS in Asia.[6] Previous studies on cytokine profiles and intraocular inflammatory cells were predominantly performed in patients with clinical characteristics of FUS or in general uveitis cohorts. Studies of RVU patients addressing intraocular cytokine profiles and immune cell infiltration are mostly lacking.[7–15]

Herein, we investigate specific cytokine-, chemokine-, and growth factor levels in intraocular fluid samples from patients with RVU and compare these to noninfectious uveitis and cataract. In addition, we study the intraocular cell population of two RVU patients.

METHODS

Sample Collection

Remainders of intraocular fluid samples from a total of 59 patients, including RVU (n = 10), noninfectious uveitis (n = 27), and cataract (n = 22) were obtained from the biobank at the Erasmus University Medical

Received 9 November 2017; revised 20 December 2017; accepted 21 December 2017

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Center, Rotterdam, the Netherlands. All intraocular fluid samples were stored at −80°C.The study was approved by the local ethical committee and adhered to the tenets of the Declaration of Helsinki. All subjects gave consent and signed a biobank Informed Consent.

Patients and Data Collection

The diagnosis of RVU was based on positive anterior chamber fluid analysis for rubella virus using polymerase chain reaction (PCR) and/or Goldmann-Witmer Coefficient (GWC). The uveitis group consisted of patients with different types of noninfectious uveitis: birdshot retinopathy (n = 4), HLA-B27 acute anterior uveitis (n = 6), multiple sclerosis-associated uveitis (n = 5), sarcoidosis-associated uveitis (n = 10), and lastly patients with rubella-virus negative FUS (n = 2). Patients with age-related cataract had no other ocular co-morbidities. Basic characteristics including age, gender and disease duration were gathered for all patients. Clinical ocular characteristics were collected of all RVU patients and uveitis controls using electronic patient files. These characteristics included location of uveitis, activity of uveitis, presence of vitritis and various complications such as cataract, presence of cystoid macular edema (CME), posterior synechiae and glaucoma, and the use of systemic immunosuppressive medications, systemic corticosteroids, as well as local steroid treatments.

Laboratory Analyses

Measurement of interleukins (IL-1β, IL-1ra, IL-2, IL-6, IL-6rα, IL-7, IL-8, IL-10, IL-17A, IL-23), thymus- and activation-regulated chemokine (TARC), monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor-alpha (TNF-α), placental growth factor (PlGF), and vascular endothelial growth factor (VEGF) was performed in all patients, with the exception of one patient with RVU. Selection of the cytokine panel was based on potential relevance according to previous reports and/or possible targets for treatment options. Analysis was performed using a Luminex multiplex bead immunoassay system (R&D Systems Europe, Ltd; UK) according to the manufacturer's instructions with exception of one additional dilution step for the standard curve. Briefly, 50 µL of undiluted intraocular fluid samples were transferred to the plate, with exception of intraocular fluid samples with insufficient amount of material (n = 13), which were diluted to a total volume of 50 µL. Measurements were performed on a Bio-Plex MAGPIX instrument and data was analyzed using Bio-Plex Manager MP software. Cellular composition using flowcytometric immunophenotyping was performed in intraocular fluid of two RVU patients, as described previously.[16]

Statistical Analysis

Data from the Luminex immunoassay were analyzed both as continuous and categorical data. For continuous analyses, values below the lower limit of detection were replaced by the lowest value of the reference curve. For categorical analyses, the lower limit of detection was used for every individual cytokine as a cut-off for those cases not showing expression. The lowest value of the reference curve served as cut-off point. Continuous variables were presented using medians, and categorical variables were presented using percentages. Logistic and linear regressions with age, gender and diagnosis in the model were performed to compare laboratory outcomes between diagnosis groups. A p-value of <0.05 was considered as statistically significant. Statistical analyses were performed using IBM SPSS Statistics, version 21.

RESULTS

Patient Characteristics

The three separate groups showed similar gender distribution, but differed in age ($p < 0.001$); specifically, RVU patients were younger and patients with cataract were older (Table 1).

Prevalence of Intraocular Cytokines

Some cytokines were prevalent in all samples (IL-2, IL-6rα, and MCP-1), while other cytokines were never detected (IL-1 β and IL-17A). Presence or absence of cytokine, chemokine or growth factor showed no association with any of the three included groups.

TABLE 1. Patient characteristics.

	Rubella virus uveitis	Uveitis of other origin	Cataract	p-value
Total number	N = 10	N = 27	N = 22	
Age in years (median, range)	40 (27–71)	53 (25–79)	66 (18–80)	<0.001
Gender (males)	N = 5 (50%)	N = 9 (33%)	N = 8 (36%)	n.s.
Disease duration in years (median, range)	4 (1–35)	4 (1–33)	1 (0–5)	

Abbreviation: n.s. = not significant.

Levels of Intraocular Cytokines

The measured levels of intraocular cytokines showed no association with gender, however levels of IL-6, IL-7 and IL-23 increased with age ($p = 0.012$, $p = 0.039$, and $p = 0.015$, respectively). The intraocular levels of cytokines show distinct profiles for different groups (Table 2, Figure 1). RVU cases displayed higher MCP-1, IL-6rα and IL-23 levels than the uveitis control population, whereas the level of PlGF was lower. Moreover, RVU patients exhibited higher levels of IL-6, IL-6rα, MCP-1, and TARC than intraocular fluid samples of cataract patients, whilst IL-23 and PlGF were lower. Interestingly, IL-23 levels were much higher in samples of cataract patients compared to samples of patients with RVU and uveitis of other origin. The cytokine profiles of RVU patients differed from the profile of two patients with RV-negative FUS; in RVU levels of MCP-1, IL-23, and IL-2 were higher and levels of IL-7 were lower (significances were not determined because of the small sample size).

Associations between Clinical Features and Cytokines Levels

We assessed associations between multiple clinical features and cytokine levels for all our uveitis patients (N = 36; 9 RVU patients and 27 noninfectious uveitis controls; Table 3).

Secondary glaucoma was noted in 4/9 (44%) of RVU patients and in 13/36 (36%) of all uveitis patients, and was associated with higher levels of MCP-1. The use of systemic immunosuppressive medications as well as the use of corticosteroid drops were not associated with any changes of intraocular cytokines levels. Relationships between the clinical features and the levels of cytokines within the group of 10 RVU patients were not found.

Intraocular Cell Population in RVU Patients

Flow cytometric analysis of two intraocular RVU samples disclosed an infiltration of mainly T-cells and monocytes/macrophages, whereas B-cells were hardly detected. Within the T-cell fraction, in one case a clear dominance of CD8 + T-cells was seen, whereas in the other case, both CD4+ and CD8 + T-cells were identified.

DISCUSSION

Our study shows that RVU patients have an intraocular cytokine profile distinct from patients with uveitis of non-RVU origin and cataract controls; specifically, MCP1, IL-6rα and IL-23 levels were higher, and PlGF lower than in uveitis controls.

Inflammatory ocular diseases are predominantly characterized by high intraocular IL-6 levels, which were also observed in this study.[17–19] The high levels of MCP-1 and IL-6 in RVU are consistent with the results measured in other infectious uveitis.[7,20–22] One recent study included RVU patients as controls while studying intraocular profile of cytokines in patients with acute retinal necrosis.[15] This study disclosed cytokine results similar to us (elevated MCP-1 and IL-6). Intraocular IL-6rα, IL-23, and PlGF in RVU have not yet been investigated in other infectious uveitis, and

TABLE 2. Levels of intraocular cytokines/chemokines/growth factors in patients with rubella virus associated uveitis, uveitis of other origin and cataract.

	Cytokines	RVU (N = 9) Median (range)	Uveitis of other origin (N = 27) Median (range)	Cataract (N = 22) Median (range)	p-value RVU vs cataract	p-value RVU vs uveitis
Cytokines	**IL-1β**	N.A.*	5 (5–7)†	N.A.*	n.s.	n.s.
	IL-1ra	405 (187–1633)	148 (9–2279)†	124 (9–2357)†	n.s.	n.s.
	IL-2	232 (131–623)	235 (92–539)	275 (212–1101)	n.s.	n.s.
	IL-6rα	387 (122–3517)	228 (23–567)†	87 (25–264)	<0.001	0.003
	IL-6	20 (5–522)	10 (2–350)†	2 (2–152)†	0.002	n.s.
	IL-7	N.A.*	3 (1–18)†	2 (1–9)†	n.s.	n.s.
	IL-10	4 (4–37)†	N.A.*	4 (4–8)†	n.s.	n.s.
	IL-17A	N.A.*	N.A.*	N.A.*	n.s.	n.s.
	IL-23	279 (78–454)†	136 (26–367)†	2592 (144–3494)	0.011	0.028
	TNF-α	N.A.*	3 (3–10)†	3 (3–3)†	n.s.	n.s.
Chemokines	**IL-8**	47 (8–912)	18 (1–334)†	6 (1–17)†	n.s.	n.s.
	TARC	34 (29–41)†	29 (29–154)†	N.A.*	0.002	n.s.
	MCP-1	1472 (267–4526)	581 (14–4225)	618 (250–1920)	0.006	0.029
Growth factors	**PlGF**	1 (1–4)†	6 (1–14)	5 (3–13)	<0.001	0.008
	VEGF	10 (3–68)†	57 (3–326)†	64 (7–102)	n.s.	n.s.

Abbreviation: RVU = rubella associated uveitis; n.s. = not significant, n.a. = not applicable.

* All values were measured below the detection limit.

† Includes values measured below the detection limit.

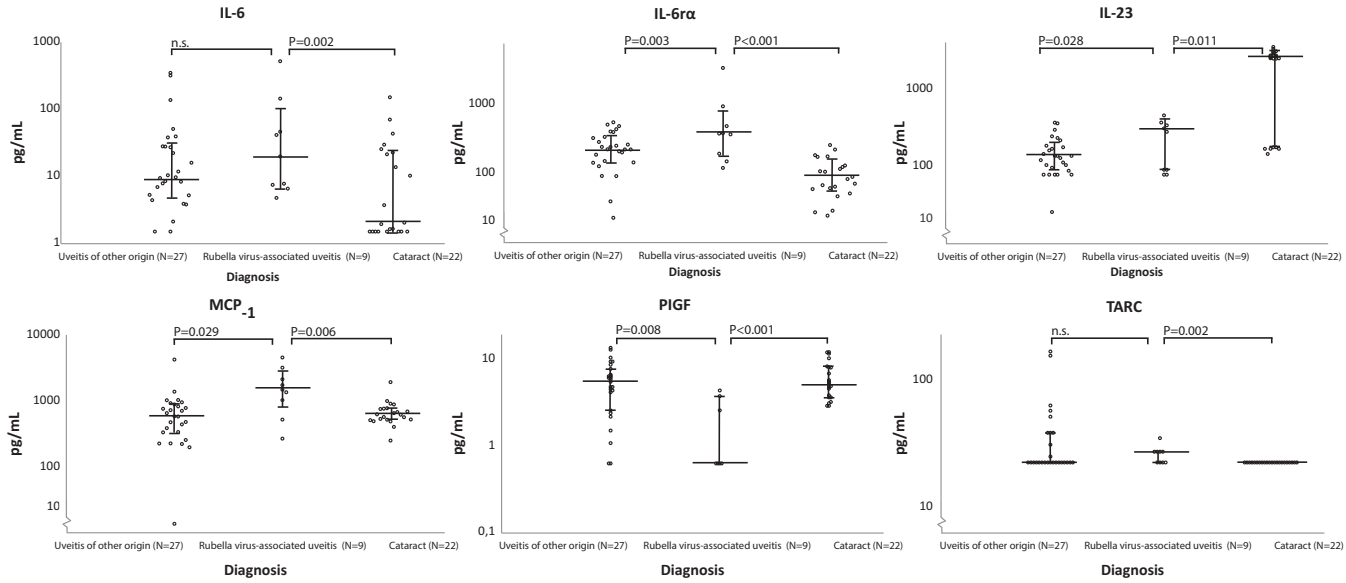

FIGURE 1. Levels of cytokines in rubella virus associated uveitis, uveitis of other origin and cataract. *p*-values were determined by linear regression with adjustment for age and gender.

TABLE 3. Associations between clinical features of uveitis patients and cytokine levels.

Clinical features	Significant cytokine level changes*
Location	
– Anterior	High VEGF (p=0.001)
– Intermediate	None
– Posterior	None
– Panuveitis	None
Activity of uveitis	None
Vitritis	Low VEGF (p=0.002)
Complications	
– Posterior synechiae	None
– Cataract	None
– Cystoid macular edema	None
– Secondary glaucoma	High MCP-1 (p=0.003)
Treatment	
– Systemic immunosuppressive	None
– Local corticosteroid drops	None

*All results were corrected for multiple testing by Bonferroni correction, and therefore only *p*-values ≤0.003 were considered significant. *p*-values were determined by linear regression with adjustment for age and gender.

their possible distinctive character could therefore not be established. Interestingly, IL-6rα can induce IL-6 trans-signaling by the IL-6/sIL-6Rα complex, which subsequently could enhance IL-6 activity.[23]

The higher levels of IL-6 in RVU cases compared to noninflammatory controls is similar to previous data on intraocular cytokines in FUS (without information on RV presence or absence).[14] However, when comparing levels of intraocular IL-6 in RVU with inflammatory uveitis controls, our study shows more elevated levels in RVU (not significant), whereas previously they were measured lower in FUS.[17] This difference might be due to rubella-virus status, composition of the control uveitis group and/or higher activity of inflammation in our RVU patients. It is feasible that the FUS samples in previous studies were obtained predominantly during the cataract extraction while on anti-inflammatory treatment whilst our samples were mainly obtained during the active period of inflammation for diagnostic purposes. The included rubella virus-negative FUS cases in our study had a cytokine profile which was not consistent with RVU samples, supporting that there is a distinction between rubella virus-negative FUS and RVU in pathophysiology.[3,4]

RVU was associated with high intraocular MCP-1. MCP-1 is a main chemotactic cytokine to control migration and infiltration of monocytes/macrophages during inflammation which also contributes to antiviral responses.[24] In line with this finding, we observed clear monocyte infiltration in two of the RVU cases examined. The high MCP-1 levels in RVU may thus reflect the viral nature of this disease. Responses of monocytes/macrophages to viral (and other) infections are controlled largely by activation of specific toll-like receptors (TLR) inducing production of pro-inflammatory mediators involved in activation of both innate and adaptive immune responses. Remarkably, RVU had lower PlGF than other types of uveitis. PlGF was recently found to enhance the inflammatory response of monocytes upon TLR7/8 activation, TLRs typically activated by single stranded RNA viruses.[25] The low PlGF in RVU may represent a mechanism to hamper excessive inflammation to control ocular damage, which is in agreement with the immune privileged site of the eye.

Our study includes measurements of intraocular fluids without simultaneous analyses of cytokines in serum. However, such studies were previously performed and revealed that sera of patients with uveitis roughly did not differ from controls (with the exception of patients with systemic inflammatory involvement).[15,26,27] Comparison of intraocular and serum levels might indicate which cytokines are produced within the eye and worth further exploration.

A previous study on intraocular cell populations of two FUS cases reported a predominance of intraocular CD8 + T cells with indications of clonogenic activation; in these specific cases however the RV involvement was not known.[28] Our results revealed clear T-cell infiltration, with a slight predominance of CD8 + T-cells above CD4 + T-cells in one patient, but equal distribution between CD4+ and CD8 + T-cells in the other. This indicates that RVU may not necessarily be associated with a predominance of CD8 + T-cells; possibly the stage of the disease can play a role in the intraocular cellular distribution.

In conclusion, our study shows that RVU patients had a distinct intraocular cytokine profile compared to noninfectious uveitis entities and cataract and were characterized by high levels of MCP-1 and IL-6rα, low levels of PlGF, and intraocular infiltration with CD8+ and CD4 + T-cells.

DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

FUNDING

This work was supported by the Stichting Lijf en Leven.

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