Cytokine Profile in Aqueous Humor and Sera of Patients with Infectious or Noninfectious Uveitis

Hiroshi Takase, Yuri Futagami, Tomoko Yoshida, Koji Kamo, Sunao Sugita, Yasubisa Imai, and Manabu Mochizuki

PURPOSE. To determine the cytokine expression profile at the protein level in aqueous humor (AqH) and sera of patients with uveitis.

METHODS. Patients with various clinical entities of strictly diagnosed infectious or noninfectious uveitis were tested. AqH and sera were collected from patients with uveitis. AqH was also collected during surgery from patients with cataract, as control specimens. Interferon (IFN)-γ, tumor necrosis factor (TNF)-α, and interleukin (IL)-2, -4, -5, and -10 were measured from nondiluted samples simultaneously, with microparticle-based flow cytometric analysis.

RESULTS. In AqH IFN-γ was the most abundant cytokine in both infectious (mean, 3240.5 pg/mL) and noninfectious (mean, 115.6 pg/mL) uveitis, and IL-10 was the second (mean, 402.1 pg/mL, infectious uveitis; 7.5 pg/mL, noninfectious uveitis). There was no remarkable difference, however, in the cytokine expression pattern in the levels were lower than that of IL-10. There was no remarkable variability among different types of infectious or noninfectious uveitis, the data showed that, in infectious uveitis, there is a high amount of cytokine–producing cells are polarized and sensitized in the peripheral lymphoid organ. Such Th1 cells accumulate in the target organ, the eye.1,2 It is still unclear, however, whether human noninfectious uveitis is driven by the same mechanisms as EAU.

In both types of uveitis, the immunopathology is controlled by numerous inflammation-related molecules. Overexpression or imbalance of one such molecule, the cytokine, controls the disease.2,3 In gaining a better understanding of the immunopathogenic mechanisms of uveitis, analysis of intraocular fluids, aqueous humor (AqH) or vitreous fluid, could be used as a powerful tool for the study of the involvement of cytokines in the local ocular inflammatory site.4 Because uveitis is composed of various clinical entities, immunopathogenic mechanism of each clinical entity may differ according to the pathogens or autoantigens. To study the differences in the mechanism of different types of uveitis, it is important to analyze the cytokine profile among the different clinical entities of the disease.

To determine the difference in the cytokine expression profile of the various clinical entities of uveitis, we analyzed cytokine concentration in AqH and sera of patients with uveitis. Because almost half of patients with uveitis have an idiopathic form of the disease that cannot be classified as either infectious or noninfectious, we carefully chose only patients with strictly diagnosed uveitis, to avoid confusing the different clinical entities, especially infectious and noninfectious uveitis. On the other hand, only ~100 μL AqH was obtainable per eye, and measuring multiple cytokines in AqH by conventional enzyme-linked immunosorbent assay (ELISA) in a single sample requires dilution to perform all assays. A newly developed technology, microparticle-based flow cytometric analysis, has made it possible to measure levels of six cytokines simultaneously from a small volume,5,6 so that the AqH does not have to be diluted before the assay.

Although there was not a remarkable variability among different types of infectious or noninfectious uveitis, the data showed that, in infectious uveitis, there is a high amount of locally expressed interferon (IFN)-γ and interleukin (IL)-10 in the eye, whereas noninfectious uveitis showed IFN-γ in both AqH and serum. These data suggest that infectious uveitis is characterized by cytokines produced in the eye, whereas noninfectious uveitis is characterized by the Th1 cytokine produced both in the eye and the peripheral blood.

METHODS

Patients and Samples

This study was performed in accordance with the Declaration of Helsinki. All patients were recruited from the Eye Clinic of Tokyo Medical and Dental University Hospital. The study protocol was approved by the ethics committee of Tokyo Medical and Dental University, and informed consent was obtained from each patient.

AqH and serum samples were obtained at the same time from patients with herpetic anterior uveitis (n = 3), acute retinal necrosis (ARN, n = 5), Vogt-Koyanagi-Harada (VKH) disease (n = 3), HLA-B27-associated acute anterior uveitis (AAU, n = 2), and ocular sarcoidosis

Uveitis is a syndrome of human intraocular inflammation characterized by accumulation of leukocytes in ocular tissues (e.g., iris, ciliary body, retina, and choroid). It can be of either infectious or noninfectious etiology. Infectious uveitis is caused by immune responses against exogenous pathogens present in the eye. Such pathogens involve viruses, fungi, parasites, and bacteria. Although the cause of noninfectious uveitis remains unknown, it is assumed that autoreactive T cells against self-antigens (Ags) mediate immune responses. Analysis of animal models of autoimmune uveitis, such as experimental autoimmune uveitis (EAU), revealed that Th1-cytokine-producing cells are polarized and sensitized in the peripheral lymphoid organ. Such Th1 cells accumulate in the target organ, the eye.1,2 It is still unclear, however, whether human noninfectious uveitis is driven by the same mechanisms as EAU.

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### Table 1. Details of Clinical Features and Cytokine Levels in Patients with Uveitis

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<th>Diagnosis</th>
<th>Age (y)</th>
<th>Sex</th>
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<td>*1.8 ± 4.4</td>
<td>0.5 ± 1.1</td>
<td>*1.8 ± 4.4</td>
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AC, anterior chamber; Day, day of sampling after onset of the disease; IFN, interferon; TNF, tumor necrosis factor; IL, interleukin; AqH, aqueous humor; ARN, acute retinal necrosis; VKH, Vogt-Koyanagi-Harada; HLA-B27 AAU, HLA-B27 associated acute anterior uveitis.

* Data are shown as the mean cytokine concentration (pg/mL) ± SD.
Cytokine Profile in Infectious or Noninfectious Uveitis

**RESULTS**

**Profile of the Patients**

Profile of the patients is summarized in Table 1. In the infectious uveitis group, the age of the patients ranged from 28 to 80 years (mean ± SD, 52.5 ± 19.2; male-female, 6:2), whereas patients with noninfectious uveitis ranged in age from 2 to 58 years (mean ± SD, 36.7 ± 13.6; male-female, 3:6). Sampling after onset was performed at 17.6 ± 17.6 days for infectious uveitis and 33.1 ± 37.2 days for noninfectious uveitis.

### Cytokine Level in AqH

As shown in Table 1, the most abundant cytokine detected was IFN-γ in both infectious and noninfectious uveitis. There was a notable difference in the level of IFN-γ between the two groups. The mean level of IFN-γ in infectious uveitis was 3240.5 pg/mL, almost 30 times higher than that in noninfectious uveitis (mean, 115.6 pg/mL; *P* = 0.00289). IL-10 was the second most abundant cytokine in both infectious (mean, 402.1 pg/mL) and noninfectious (mean, 7.5 pg/mL) uveitis. The difference in the IL-10 level in AqH between the two groups was as great as that of IFN-γ (*P* = 0.0062). IL-5 was detectable in all patients with acute retinal necrosis and in two of three patients with herpetic anterior uveitis, whereas no patients with noninfectious uveitis showed IL-5 expression. The levels of TNF-α, IL-2, and IL-4 were low but detectable in some cases of patients with infectious uveitis and were positive in fewer cases of noninfectious uveitis. Control AqH samples obtained from six patients with cataract contained only slight amounts or less than detectable levels of tested cytokines (Table 1).

In summary, IFN-γ was the most abundant cytokine in both infectious and noninfectious uveitis, and the expression level of cytokines was generally higher in infectious uveitis. There was little difference, however, in the cytokine expression pattern in AqH in the different clinical entities of uveitis (Fig. 1).

### Cytokine Level in Serum

IFN-γ and IL-10 was detected in the sera of half of the patients with infectious uveitis and IL-2 in three of eight, although the concentrations were close to the detection limit. The other cytokines were under detectable levels, except IL-5 in a patient with herpetic anterior uveitis (Table 1). Serum samples from noninfectious uveitis showed similar cytokine levels with an exception of IFN-γ. IFN-γ in serum was at a higher than detectable level in six of nine patients and was even greater than...
that in AqH in two patients (one patient with VKH disease and another patient with sarcoidosis). Figure 2 graphically describes the level of cytokines in both AqH and the serum of infectious and noninfectious uveitis.

The mean IFN-γ levels in serum was close to that in AqH in noninfectious but not in infectious uveitis, despite the high IFN-γ level in the AqH. The ratios of AqH to serum for IFN-γ concentration in each patient were higher in infectious (range, 12.9–5712.4) than in noninfectious (range, <1–108.1) uveitis (Fig. 3).

DISCUSSION
We have analyzed the cytokine profile in AqH and sera of patients with infectious or noninfectious uveitis. Cytokines were measured with a microparticle-based flow cytometric analysis that allowed measurement of six cytokines simultaneously in a small volume of nondiluted aqueous samples. Samples were collected only from patients who had a strictly diagnosed type of uveitis having inflammation in the anterior chamber and for whom this was the first episode. In addition, samples were collected in a relatively early stage of the disease, and none of the patients had been treated systemically. Therefore, all patients in each clinical group can be regarded as being in similar condition (i.e., first episode, nonsystemically treated, and newly diagnosed active disease).

IFN-γ serves as an antiviral cytokine by inhibiting viral replication or eliminating viruses from infected cells. High level of IFN-γ detected in AqH of herpetic anterior uveitis and ARN may be produced against the viruses. Findings similar to our data have been reported by Ongkosuwito et al., in which they detected IFN-γ and IL-10 in ocular fluids of patients with early-stage ARN. The level of IFN-γ declined in the later stages.
of the disease, which may imply that IFN-γ is induced by the presence of virus in the eye, but declines as the virus is eliminated.3

The etiology of noninfectious uveitis is not fully understood. Therefore, noninfectious uveitis is classified according to its epidemiology, clinical features, histopathology, and laboratory data. VKH disease is a multisystem disorder characterized by granulomatous panuveitis with exudative retinal detachments, often associated with neurologic and cutaneous manifestations. It is associated with several human leukocyte antigens (HLA) including HLA-DR4, HLA-DR53, and HLA-DQ4,11 and is a probable autoimmune disease against melanocytes.12–16 HLA-B27 positivity is strongly associated with acute recurrent unilateral fibrinous anterior uveitis. This form of uveitis frequently is associated with systemic diseases such as ankylosing spondylitis or reactive arthritis.17,18 Sarcoïdosis is a multifocal granulomatous inflammatory disease of unknown etiology. The eye is one of the organs frequently involved in sarcoidosis as are the lung, thoracic lymph nodes, and skin.19

Despite different clinical entities, most AqH samples from patients without noninfectious uveitis showed a cytokine expression pattern similar to that in infectious uveitis. Our data are in line with a recent study by Curnow et al.,20 in which they tested a larger panel of cytokines and chemokines by micro-particle-based flow cytometric analysis in AqH from patients with idiopathic uveitis, Fuchs’ heterochromic cyclitis (FHC), herpes viral uveitis, or Behçet’s uveitis. They detected high levels of IFN-γ and IL-10 in AqH of patients with infectious uveitis and a high level of IFN-γ but not of the other Th1 and Th2 cytokines in noninfectious uveitis.20 Moreover, it is of note that even different entities of noninfectious uveitis from our data (i.e., FHC and Behçet’s uveitis) showed similar expression patterns of Th1 and Th2 cytokines in AqH.20 These data may suggest that noninfectious uveitis of different diagnoses is driven by common immunopathologic mechanisms.

A study comparing the cytokine level in AqH and serum of patients with uveitis has previously been reported by Lacomba et al.,21 in which they showed higher levels of IFN-γ and IL-4 in sera than in AqH from patients with uveitis. Their findings, however, were contradicted by our data showing that cytokine levels are higher in AqH than in serum. It is possible that factors related to the ELISA system are responsible, at least in part, for the difference in the results of the two studies. It is also noteworthy that Lacomba et al. did not describe the duration of the disease, and the type of disease were different from those in our study. Such factors may have caused the difference in observed cytokine levels in AqH and serum.

By comparing cytokine levels in AqH and serum, our data suggest that, in infectious uveitis, cytokines are produced only in the infected organ—that is, the eye. In contrast, IFN-γ may be produced in both the peripheral blood and the eye in patients with noninfectious uveitis. Ooi et al. (JOVS 2005;46: ARVO E-Abstract 2813) tested AqH from patients with noninfectious uveitis by the same system we used in our study and reported a decreased level of the Th2 cytokine IL-5 in the uveitis group, in addition to an increased level of IFN-γ. Taken together, these data may suggest that human noninfectious uveitis is characterized by systemically and locally produced Th1 but not Th2 cytokines.

Analysis of intraocular fluids is often necessary to determine the presence of infectious agents in the eye in cases of severe uveitis in which prompt treatment seems to be necessary. PCR or the Goldman-Wittert coefficient is the common strategy used for the diagnosis, by examining intraocular fluids,22–24 but sometimes those assays produce false-negative results, especially in the early stage of the disease because of the small amount of antigens or poor production of Abs. Measurement of cytokines in both AqH and serum may be an additional option for the diagnosis of infectious uveitis, by identifying a high level of IFN-γ, even in a small amount of AqH, but further study with a larger sample group is needed.

References