Cytokine profiles in aqueous humor of patients with different clinical entities of endogenous uveitis

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Vogt–Koyanagi–Harada disease;
HLA-B27;
Uveitis;
Cytokines

Abstract We assayed aqueous humor (AH) samples from patients with Behçet's disease (BD), Vogt–Koyanagi–Harada (VKH) disease, and HLA-B27-associated uveitis and control patients for the proinflammatory cytokines IL-15, IL-17, interferon-γ and tumor necrosis factor-α and the immunosuppressive cytokine IL-10. Cytokine levels were significantly higher in the three disease groups than in controls. In patients with similar disease activity, levels of IL-15 and IFN-γ were significantly higher in BD patients than in VKH and HLA-B27-associated uveitis groups. Logistic regression identified a significant negative correlation between BD and high levels of IL-10 and a significant positive correlation between VKH disease and high levels of IL-10. The proinflammatory cytokines versus IL-10 ratios were significantly higher in BD compared with other groups. These data suggest that both T helper (Th) 1 and Th17 cells are involved in endogenous uveitis immunopathogenesis. BD is characterized by extensive Th1 polarization, severe proinflammatory conditions and a low immunosuppressive status.

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Various forms of human endogenous uveitis, such as Behçet's disease, Vogt–Koyanagi–Harada (VKH) disease, and HLA-B27-associated uveitis, are common vision-threatening intraocular inflammatory diseases. Specific CD4+ T helper (Th) cell-mediated immune responses are increasingly being considered to play a central role in the pathogenesis of uveitis [1]. CD4+ Th cells play a crucial role in regulating immune responses by orchestrating the function of other immune cell types. When activated by pathogens, naive CD4+ T cells differentiate in a specific cytokine environment into different subsets with distinct effector functions aimed at activation and mobilization of other cell types to effectively clear invading pathogens. Based on cytokine profiles, initially the existence of two distinct effector Th subsets

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was proposed: Th1 and Th2 [2]. Th1 cells that produce interferon (IFN)-γ activate macrophages and are responsible for cell-mediated immunity against intracellular pathogens and are associated with the pathogenesis of many organ-specific autoimmune diseases including endogenous uveitis. In contrast, Th2 cells, which produce interleukin (IL)-4, IL-5, IL-6, IL-10, IL-13, and IL-25, induce strong antibody responses by B cells and eosinophil activation and are associated with allergic reactions.

Recently, this paradigm has been updated following the observations that IFN-γ-deficient mice were not resistant but highly susceptible to many organ-specific autoimmune diseases, raising the possibility that effector T cells other than Th1 cells were responsible for inducing autoimmunity. This notion was supported by the discovery of IL-17-producing Th17 cells which represent a subset distinct from Th1 and Th2 cells and exhibit distinct effector functions [3,4]. Emerging data support a fundamental role for Th17 cells in mediating a wide range of autoimmune disorders in experimental animal models and in humans, such as rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, inflammatory bowel disease, and psoriasis [5–10]. At present, it is unclear whether endogenous uveitis is a Th1- and/or Th17-mediated disease.

Cytokines are crucially involved in the regulation of the normal human immune response. Dysregulation of cytokine expression has been shown to play a role in the pathogenesis of autoimmune diseases [11]. Analyzing the expression of cytokines has enabled a better understanding of the pathogenesis of various diseases. Because uveitis is composed of various clinical entities, the immunopathogenic mechanism of each clinical entity may differ according to the pathogen or autoantigen. The magnitude and the pattern of the cytokine response in different types of uveitis, however, remains unclear. Successful therapeutic strategies in uveitis might require characterization of the immune response in a given patient to design effective treatment protocols on an individual basis. To study the differences in the mechanism of different types of uveitis and identify markers and potential therapeutic targets, we measured the levels of the proinflammatory cytokines IL-15, IL-17, IFN-γ, and tumor necrosis factor (TNF)-α, and the immunosuppressive cytokine IL-10 in the aqueous humor from patients with active uveitis associated with Behçet’s disease, VKH disease, and HLA-B27-related intraocular inflammation.

1. Patients and methods

1.1. Patients and control subjects

Forty patients with active uveitis seen at the outpatient clinic of King Abdulaziz University Hospital were included in the study. All the patients were examined to determine the nature of their disease and associated systemic illnesses. Sixteen patients had Behçet’s disease, 16 had VKH disease, and 8 had human leukocyte antigen (HLA)-B27-associated acute anterior uveitis. Diagnosis of Behçet’s disease was based on the International Study Group for Behçet’s Disease Criteria [12]. Diagnosis of VKH disease was based on the Revised International Diagnostic Criteria [13]. HLA-B27-associated acute anterior uveitis was diagnosed based on HLA typing of peripheral blood cells and typical ocular manifestations of unilateral fibrinous acute anterior uveitis. The patients with Behçet’s disease were 12 males and 4 females, with a mean age of 25.8 ±8.5 (range, 14–44 years). The patients with VKH disease were 2 males and 14 females, with a mean age of 26.9 ±7.8 (range, 15–38 years). The patients with HLA-B27-associated uveitis were seven males and one female, with a mean age of 32.9 ±11.5 (range, 14–52 years). Twelve patients, who had undergone cataract extraction at King Abdulaziz University Hospital with no prior history of uveitis, served as a control group.

Patients were examined using slit-lamp biomicroscopy, indirect ophthalmoscopy, and fluorescein angiography. In each patient the uveitis activity was graded according to the criteria of the Standardization of Uveitis Nomenclature Working Group grading scheme [14]. Anterior chamber cell counts were clinically graded on a 0–5 scale as follows: 0 = <1 cell/field, 0.5+ = 1–5 cells/field, 1+ = 6–15 cells/field, 2+ = 16–25 cells/field, 3+ = 26–50 cells/field, 4+ = >50 cells/field. None of the patients was on topical or systemic therapy on presentation.

Aqueous humor (100–200 μl) was aspirated from each patient by means of limbic paracentesis with the use of 27 gauge needle attached to a tuberculin syringe after the application of topical local anesthetic oxypurine hydrochloride 0.4% (Benoxinate, Chauvin Pharmaceuticals Ltd., Kingston, United Kingdom). The procedure was performed under a surgical microscope. The samples were snap frozen and maintained at −70 °C until use. Aqueous humor samples from all patients with uveitis were obtained before therapy. In six patients with Behçet’s disease, repeated aqueous humor samples were obtained 7 days after treatment initiation.

All procedures followed the tenets of the Declaration of Helsinki, and informed consent was obtained from all patients and the control subjects. The study was approved by the Research Center, College of Medicine, King Saud University.

1.2. Cytokine assays

The cytokine expression profile in the aqueous humor samples was determined using a cocktail of antibody-coated non-magnetic beads concomitantly measuring the levels of IL-10, IL-15, IL-17, IFN-γ and TNF-α (Bio-plex pro-cytokine assay, BIORAD, Hercules, CA, USA). This technology allows measurement of multiple analytes in a single 50-μl sample. The analysis was performed following the manufacturer’s instructions, and results were generated using the Bio-Plex 200 system and software.

1.3. Statistical analysis

The Mann−Whitney test was used to compare means from two independent groups. Pearson correlation coefficients were computed to investigate correlations between variables. One-way ANOVA and post-ANOVA pairwise comparisons of means were conducted using the Kruskal–Wallis test. For three groups, the critical Z-value for post-ANOVA pairwise mean comparisons was Z = 2.39 at a 5% level of significance. Stepwise logistic regression analysis was conducted to identify the cytokines that related importantly to each disease. Cytokine levels before and after treatment were compared using the Wilcoxon test. SPSS version 15 and
programs 3S and LR from the BMDP 2007 Statistical Package were used for the statistical analyses.

2. Results

2.1. Cytokine levels in aqueous humor

Cytokines were detected in all aqueous humor samples from patients with uveitis and control patients. When the whole patient group was considered, all cytokine levels were significantly higher in the aqueous humor of patients than in controls (Table 1). The same phenomenon persisted for each disease group except for IL-17 in patients with HLA-B27-associated uveitis (Fig. 1).

Comparison of mean cytokine levels among the three disease groups was conducted with the use of Kruskal–Wallis test, and the results are shown in Table 2. Mean IL-17, IFN-γ, and TNF-α levels in aqueous humor samples differed significantly between patients with Behçet’s disease, VKH disease, and HLA-B27-associated uveitis (p=0.0405; p=0.0034; p=0.0322, respectively; ANOVA). Post-ANOVA pairwise comparisons of means indicated that mean IL-17 and IFN-γ levels in patients with Behçet’s disease were significantly higher than the mean levels in patients with VKH disease (Z=2.41; Z=2.82, respectively) and that mean IFN-γ levels in patients with Behçet’s disease were significantly higher than the mean levels in patients with HLA-B27-associated uveitis (Z=2.83). Post-ANOVA pairwise comparisons of means did not attain statistical significance for TNF-α. Patients with VKH disease had the highest mean IL-10 levels, and patients with Behçet’s disease had the highest mean IL-15 levels, but the differences were marginally statistically significant (p=0.0532; p=0.0521, respectively; ANOVA).

2.2. Effect of disease activity

When only patients with the same disease activity (≥3+) were taken into account, mean levels of cytokines differed significantly between the three disease groups for IL-17 (p=0.0308), and IFN-γ (p=0.007), but not for IL-10, IL-17 and TNF-α (Table 3). Post-ANOVA pairwise comparisons indicated that for IL-15, the mean for patients with Behçet’s disease was significantly higher than that for patients with VKH disease (Z=2.94). For IFN-γ, the mean for patients with Behçet’s disease was significantly higher than that for patients with VKH disease (Z=3.10) and that for patients with HLA-B27-associated uveitis (Z=2.88).

2.3. Evaluation of proinflammatory cytokines versus IL-10 ratios

The mean proinflammatory cytokines (IL-15, IL-17, IFN-γ, and TNF-α) versus IL-10 ratios differed significantly between patients with Behçet’s disease, VKH disease and HLA-B27-associated uveitis (p<0.001; p=0.005; p=0.004; p=0.007, respectively; ANOVA) (Table 4). Post-ANOVA pairwise comparisons indicated that the mean (IL-15 versus IL-10) and (IL-17 versus IL-10) ratios in patients with Behçet’s disease were significantly higher than the mean ratios in patients with VKH disease (Z=3.98; Z=3.45, respectively). The mean (IFN-γ versus IL-10) and (TNF-α versus IL-10) ratios in patients with Behçet’s disease were significantly higher than the mean ratios in patients with VKH disease (Z=3.05; Z=3.12, respectively) and in patients with HLA-B27-associated uveitis (Z=2.91; Z=2.74, respectively).

2.4. Stepwise logistic regression analysis

Stepwise logistic regression analysis was conducted to identify the cytokines that tended to relate most importantly to each disease, and the results are presented in Table 5. Disease activity and the levels of cytokines were used as the independent variables in this analysis. Behçet’s disease was positively associated with high levels of IFN-γ and negatively associated with high levels of IL-10. VKH disease was positively associated with high levels of IL-10 and IFN-γ.

Table 1: Comparison of mean cytokine levels between patients and controls with the use of Mann–Whitney test.

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Study group</th>
<th>Mann–Whitney test p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All patients (n=40)</td>
<td>Controls (n=12)</td>
</tr>
<tr>
<td>IL-10</td>
<td>26.4 ± 30.7 (pg/ml)</td>
<td>2.0 ± 0.0 (pg/ml)</td>
</tr>
<tr>
<td>IL-15</td>
<td>186.2 ± 138.5 (pg/ml)</td>
<td>20.3 ± 18.1 (pg/ml)</td>
</tr>
<tr>
<td>IL-17</td>
<td>58.7 ± 29.1 (pg/ml)</td>
<td>19.6 ± 3.7 (pg/ml)</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>473.9 ± 300.9 (pg/ml)</td>
<td>17.4 ± 16.8 (pg/ml)</td>
</tr>
<tr>
<td>TNF-α</td>
<td>151.0 ± 78.7 (pg/ml)</td>
<td>14.2 ± 3.6 (pg/ml)</td>
</tr>
</tbody>
</table>

IL = interleukin; IFN = interferon; TNF = tumor necrosis factor.
* Statistically significant at 5% level.
a Values are mean ± standard deviation.
and negatively associated with high levels of IL-15. HLA-B27-associated uveitis was positively associated with high levels of IL-15 and negatively associated with high levels of IFN-γ.

### 2.5. Effect of treatment

One week after topical and systemic corticosteroid treatment combined with systemic cyclosporine A therapy in six patients with Behçet's disease, a marked clinical improvement was observed. This was associated with a statistically significant reduction in aqueous humor levels of all cytokines except IL-10 (Table 6).

### 2.6. Correlation between cytokine levels and disease activity

The levels of IL-10 in aqueous humor samples correlated significantly with the disease activity in all patients, in patients with Behçet's disease, in patients with VKH disease, and in patients with HLA-B27-associated uveitis. The levels of IL-15, IL-17, and TNF-α correlated significantly with the disease activity in all patients and in patients with VKH disease. The levels of IFN-γ correlated significantly with disease activity in patients with VKH disease (Table 7).

### 3. Discussion

To determine the differences in the cytokine expression profiles of the various clinical entities of uveitis, we analyzed cytokine concentrations in aqueous humor from patients with active uveitis associated with specific diagnoses. Our findings suggest that both Th17 and Th1 subsets are involved in endogenous uveitis immunopathogenesis. IFN-γ-driven immune responses are more potent in Behçet's disease compared with VKH disease and HLA-B27-associated uveitis. In addition, severe proinflammatory conditions and a low immunosuppressive status were unique features in the aqueous humor from patients with Behçet's disease. To the best of our knowledge, this is the first report of cytokine profiles in the aqueous humor from three patient groups with specific clinical entities of endogenous uveitis.

In the present study, IL-17 levels in the aqueous humor from patients with uveitis were higher than those in the aqueous humor from normal controls. Furthermore, IL-17 levels in the aqueous humor from patients with uveitis significantly correlated with clinical disease activity. Several studies demonstrate a pathogenic role of Th17-associated cytokines and Th17 cells in inducing autoimmune tissue inflammation both in experimental animals and in humans [5–10]. IL-17 has proinflammatory capacities exerted through its ability to induce secretion of proinflammatory cytokines, chemokines, prostaglandin E2, intercellular adhesion molecule-1, and matrix metalloproteinases in various tissues and cell types. As a result, it recruits neutrophils, monocytes, and Th1 cells to the target tissues (5). Additionally, IL-17 acts synergistically with other cytokines, particularly IL-1β, and TNF-α [5].

The relative role of Th1 and Th17 cells in autoimmune diseases was examined in an animal model of experimental autoimmune encephalomyelitis, an autoimmune disease of the central nervous system (CNS). It was observed that both Th1 and Th17 cells infiltrate the CNS, but Th17 cells in the CNS peaked earlier than the Th1 cells. It is suggested that Th17 cells are generated and expand faster in response to antigen challenge and constitute the first wave of effector T cells.

### Table 3  Comparisons of mean cytokine levels in patients with the same disease activity (≥3+) with the use of ANOVA.

<table>
<thead>
<tr>
<th>Disease</th>
<th>IL-10 (pg/ml)</th>
<th>IL-15 (pg/ml)</th>
<th>IL-17 (pg/ml)</th>
<th>IFN-γ (pg/ml)</th>
<th>TNF-α (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Behçet’s disease (n=10)</td>
<td>20.3 ± 25.2</td>
<td>307.9 ± 160.3</td>
<td>78.0 ± 23.7</td>
<td>645.8 ± 179.2</td>
<td>202.9 ± 50.0</td>
</tr>
<tr>
<td>VKH disease (n=13)</td>
<td>43.1 ± 32.2</td>
<td>140.4 ± 68.8</td>
<td>51.1 ± 21.6</td>
<td>430.9 ± 183.1</td>
<td>151.2 ± 67.2</td>
</tr>
<tr>
<td>HLA-B27-associated uveitis (n=7)</td>
<td>31.1 ± 41.2</td>
<td>194.8 ± 155.8</td>
<td>54.4 ± 42.8</td>
<td>330.1 ± 298</td>
<td>117.2 ± 100.5</td>
</tr>
<tr>
<td>ANOVA p-value</td>
<td>0.2724</td>
<td>0.0308</td>
<td>0.1471</td>
<td>0.0070</td>
<td>0.0597</td>
</tr>
</tbody>
</table>

IL= interleukin; IFN= interferon; TNF= tumor necrosis factor.

* Statistically significant at a 5% level.

* Values are mean ± standard deviation.
transitional cell that expresses both IFN-γ and IL-17 can consistently be detected in vivo in the inflamed CNS. This observation may indicate the existence of a considerable population of cells secreting both IL-17 and IFN-γ can play a role in the recruitment of further waves of effector T cells migrating to the CNS. Consequently, Th17 cells may appear to be susceptible to regulatory T cells, which prevent tissue inflammation and mediate self-tolerance, whereas Th17 cells are resistant.[5].

Our findings of increased levels of both IFN-γ and IL-17 in the aqueous humor from patients with Behçet’s disease, VKH disease, and HLA-B27-associated uveitis, support the involvement of both Th1 and Th17 subsets in endogenous uveitis immunopathogenesis rather than an exclusive role of these subsets. This is in contrast to an earlier paper by Luger et al. [15] who showed, using the animal model of experimental autoimmune uveitis (EAU), that conditions of disease induction affect either a dominant Th17 or Th1 effector category. Several other studies demonstrated the involvement of IL-17 and IL-17-producing Th17 cells in the pathogenesis of EAU.[16–18]. Honki et al. [16] showed increased frequency of Th17 cells rather than Th1 cells in the early stage and increased Th1 cells in the late stage of EAU. In contrast, Yoshimura et al. [17] demonstrated the differential requirement of the two responses – Th1 at the early induction phase and Th17 at the later maintenance phase of EAU.

In the present study, IFN-γ levels were significantly higher in patients with Behçet’s disease than in patients with VKH disease and patients with HLA-B27-associated uveitis. Moreover, multivariate analysis identified a significant association between Behçet’s disease and high levels of IFN-γ. These results are consistent with those of Ahn et al. [19] who reported that levels of IFN-γ in the aqueous humor were significantly higher in patients with Behçet’s disease than in uveitis patients without Behçet’s disease. In a previous study we demonstrated that the levels of the Th1 chemoattractant IP-10/CXCL10, induced by IFN-γ, in the aqueous humor were significantly higher in patients with Behçet’s disease than in patients with VKH disease.[20]. Therefore, it is tempting to speculate that Th1-type immune responses are more potent in patients with Behçet’s disease compared with patients with VKH disease and patients with HLA-B27-associated uveitis.

IL-15, a pleiotropic proinflammatory cytokine, was first described as a T-cell activating and proliferating factor with structural homology to IL-2.[21]. IL-15 and IL-2 share a common receptor subunit and biological functions, including the initial stimulation of the proliferation of activated T and B cells as well as the maintenance and activation of NK (natural killer) cells. However, IL-2 is pivotal in the maintenance of CD4+ CD25+ T-regulatory cells and in activation-induced cell death (AICD) – a process that leads to the elimination of self-reactive T cells and the induction of peripheral tolerance. By contrast, IL-15 inhibits IL-2 induced AICD. Furthermore, IL-15 stimulates the maintenance of CD8+ memory-phenotype T cells, including those that are self-directed, whereas IL-2 inhibits their persistence in vivo.[22]. Therefore, IL-15 favors the persistence of lymphocytes that are of value in long-lasting specific immune responses to foreign pathogens. However, the uncontrolled expression of IL-15 carries with it the risk to the organism of the survival of foreign pathogens. In addition, IL-15 induces TNF-α, IL-1β, and inflammatory chemokines.[21,22].

<table>
<thead>
<tr>
<th>Disease</th>
<th>Cytokine</th>
<th>Coef S.E (coef.)</th>
<th>Odds ratio</th>
<th>95% C.I.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Behçet’s disease</td>
<td>IFN-γ</td>
<td>0.0053</td>
<td>2.86</td>
<td>1.01</td>
</tr>
<tr>
<td></td>
<td>IL-10</td>
<td>−0.04435</td>
<td>−2.11</td>
<td>0.957</td>
</tr>
<tr>
<td>VKH disease</td>
<td>IL-10</td>
<td>0.0978</td>
<td>2.66</td>
<td>1.10</td>
</tr>
<tr>
<td></td>
<td>IFN-γ</td>
<td>0.00909</td>
<td>2.16</td>
<td>1.01</td>
</tr>
<tr>
<td></td>
<td>IL-15</td>
<td>−0.04951</td>
<td>−2.55</td>
<td>0.952</td>
</tr>
<tr>
<td>HLA-B27-associated</td>
<td>IL-15</td>
<td>0.03093</td>
<td>2.67</td>
<td>1.03</td>
</tr>
<tr>
<td>uveitis</td>
<td>IFN-γ</td>
<td>−0.04823</td>
<td>−2.25</td>
<td>0.953</td>
</tr>
</tbody>
</table>

Coef = coefficient; S.E = standard error; C.I. = confidence interval; VKH=Vogt–Koyanagi–Harada; IL=interleukin; IFN=interferon; TNF=tumor necrosis factor.
study, aqueous humor IL-15 levels were increased in patients with Behçet’s disease, VKH disease and HLA-B27-associated uveitis. In addition, IL-15 levels were significantly higher in patients with Behçet’s disease than in patients with other causes of endogenous uveitis when patients with the same disease activity were evaluated. These findings suggest a role for IL-15 in the development of endogenous uveitis, particularly in Behçet’s disease and imply that antagonists to IL-15 action may have therapeutic potential in these diseases [22].

TNF-α, a proinflammatory cytokine, plays a central role in both the induction and maintenance of inflammation in autoimmune reactions [23]. In the present study, TNF-α levels in the aqueous humor of uveitis were significantly enhanced compared to controls and were higher in patients with Behçet’s disease than in patients with VKH disease and in patients with HLA-B27-associated uveitis. These findings support the clinical evidence regarding the favorable effect of anti-TNF-α therapy for refractory uveitis associated with Behçet’s disease [24].

The main biological function of IL-10 seems to be the limitation and termination of inflammatory responses and the regulation of differentiation and proliferation of several immune cells such as T cells, B cells, natural killer cells, antigen presenting cells, and granulocytes. IL-10 controls inflammatory processes by suppressing the expression of proinflammatory cytokines such as TNF-α, IFN-γ and IL-1β, chemokines, adhesion molecules, as well as antigen presenting and costimulatory molecules in monocytes/macrophages, dendritic cells, neutrophils, and T cells. In addition, it enhances the production of anti-inflammatory mediators, such as IL-1 receptor antagonists and soluble TNF-α receptors. The synthesis of cyclooxygenase-2 as well as the production of prostaglandin E2 are also inhibited by IL-10. It also inhibits the development of Th1 responses and IFN-γ production by Th1 cells via inhibition of the production of proinflammatory cytokines such as IL-12 by dendritic cells and macrophages. Moreover, the presence of IL-10 during the activation of CD4+ T cells results in the development of a regulatory phenotype of these cells [25,26]. Several studies demonstrated that exogenous IL-10 has a protective role in EAU [27], and endotoxin-induced uveitis [28]. IL-10 was also sufficient to suppress Th1 effector development and function [27]. Furthermore, adenovirus-mediated IL-10 gene therapy effectively reduced inflammation in experimental autoimmune anterior uveitis [29]. Mashimo et al. [30] demonstrated that continuous high expression of IL-10 in the eye and the reduction of peripheral blood neutrophil chemotaxis play significant roles in the mechanism of lipopolysaccharide tolerance in a model of endotoxin-induced uveitis.

In the current study, aqueous humor IL-15 levels were increased in all three patient groups with uveitis. Similarly, previous studies reported increased levels of IL-10 in the aqueous humor in animal models of endotoxin-induced uveitis and autoimmune anterior uveitis [29–31] and in the aqueous humor from patients with uveitis [32,33]. Furthermore, our analysis identified a significant positive correlation between IL-10 levels in the aqueous humor from patients with uveitis and clinical disease activity suggesting that an immunoregulatory response is occurring with the upregulation of IL-10 in an attempt to control the inflammation. IL-10 levels in aqueous humor samples from patients with Behçet’s disease were lower compared with VKH disease and HLA-B27-associated uveitis. Multivariate analysis demonstrated a significant negative correlation between Behçet’s disease and high levels of IL-10 and a significant positive correlation between VKH disease and high levels of IL-10. In agreement with our findings, recent studies suggested that genetic

### Table 6

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Mean level a</th>
<th>Wilcoxon test p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-10</td>
<td>22.1 ± 33.5</td>
<td>7.4 ± 3.9</td>
</tr>
<tr>
<td>IL-15</td>
<td>342.5 ± 187.7</td>
<td>133.0 ± 123.8</td>
</tr>
<tr>
<td>IL-17</td>
<td>76.7 ± 2.7</td>
<td>27.4 ± 12.1</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>859.3 ± 353.1</td>
<td>234.3 ± 161.7</td>
</tr>
<tr>
<td>TNF-α</td>
<td>207.7 ± 18.7</td>
<td>70.2 ± 46.1</td>
</tr>
</tbody>
</table>

IL= interleukin; IFN= interferon; TNF = tumor necrosis factor.

* Statistically significant at a 5% level.

### Table 7

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>All patients (n=40)</th>
<th>Behçet’s disease (n=16)</th>
<th>VKH disease (n=16)</th>
<th>HLA-B27-associated uveitis (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-10</td>
<td>r=0.569 p=0.001</td>
<td>r=0.621 p=0.010</td>
<td>r=0.731 p=0.016</td>
<td>r=0.805 p=0.016</td>
</tr>
<tr>
<td>IL-15</td>
<td>r=0.527 p=0.001</td>
<td>r=0.492 p=0.053</td>
<td>r=0.772 p=0.080</td>
<td>r=0.651 p=0.054</td>
</tr>
<tr>
<td>IL-17</td>
<td>r=0.385 p=0.014</td>
<td>r=0.296 p=0.266</td>
<td>r=0.671 p=0.159</td>
<td>r=0.549 p=0.195</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>r=0.308 p=0.053</td>
<td>r=0.232 p=0.388</td>
<td>r=0.741 p=0.383</td>
<td>r=0.349 p=0.349</td>
</tr>
<tr>
<td>TNF-α</td>
<td>r=0.344 p=0.030</td>
<td>r=0.269 p=0.314</td>
<td>r=0.755 p=0.383</td>
<td>r=0.001 p=0.349</td>
</tr>
</tbody>
</table>

* Statistically significant at 5% level.
variants contributing to low IL-10 expression may be a risk factor for Behçet’s disease [34,35]. In the present study, the ratios of proinflammatory cytokines to IL-10 were significantly higher in patients with Behçet’s disease compared with VKH disease and HLA-B27-associated uveitis, which would correlate with the clinical picture of Behçet’s disease presenting with severe panuveitis, which is often resistant to conventional immunosuppressive therapy.

In summary, our findings suggest that both Th17 and Th1 cells are implicated in the immunopathogenesis of endogenous uveitis, as elevated levels of both Th17- and Th1-associated cytokines are detected in the aqueous humor from these patients. However, it remains to be elucidated how Th17 and Th1 cells interact among themselves and with the other regulatory effector T cell subsets. Our findings also suggest that the intraocular levels of cytokines differ depending on the cause of uveitis. Compared with VKH disease and HLA-B27-associated uveitis, the intraocular cytokine environment in Behçet’s uveitis showed an extensive Th1 polarization, severe proinflammatory conditions, and a low immunosuppressive status.

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