Aqueous and Serum Interferon γ, Interleukin (IL) 2, IL-4, and IL-10 in Patients With Uveitis

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Objective: To determine the cytokine profile in aqueous humor and peripheral blood from patients with uveitis.

Methods: Cytokines (interferon [IFN]–γ, interleukin [IL] 2, IL-4, and IL-10) were measured in aqueous humor and peripheral blood samples from 23 patients with uveitis and 16 patients undergoing operation for uncomplicated cataracts (control group) by means of an enzyme-linked immunosorbent assay.

Results: Aqueous and serum samples from patients with uveitis showed higher levels of IFN-γ and IL-2 than those of controls (P < .001). Similarly, serum IL-10 levels were slightly higher in the uveitis group (P = .002). No differences were found between uveitis and control groups for aqueous and serum IL-4 or aqueous IL-10 levels. In patients with uveitis, IFN-γ levels were significantly higher in serum than aqueous (P < .001), whereas IL-2 levels were higher in aqueous than in serum (P < .001). Serum IFN-γ levels correlated with severe visual damage (r = 0.44; P = .03).

Conclusions: Aqueous and serum levels of IFN-γ and IL-2 were elevated in patients with uveitis, suggesting a predominantly type 1 response (high IFN-γ and low IL-4 levels). Elevated serum IFN-γ level seems to predispose the patient to more serious loss of vision.

Arch Ophthalmol. 2000;118:768-772

Although immunological disturbances have been involved in the pathogenesis of uveitis, the precise mechanisms by which tissue damage is mediated by the immune system are still unknown. During the past few years, a coherent view of the role of cytokines in this inflammatory disorder has begun to emerge as a result of human and animal studies. Thus, cytokines (interferon [IFN]–γ and interleukin [IL] 2) have been demonstrated in ocular tissue or in aqueous humor (IFN-γ and IL-6) obtained from patients with uveitis. Similarly, some cytokines (tumor necrosis factor, IL-1, IL-2, IL-6, and IFN-γ) have been shown to induce inflammation in experimental animals after intraocular injection. Moreover, the magnitude of the cytokine response in these assays appears unclear, and specific cytokine secretion patterns have not been determined.

Understanding of the role of cytokines in inflammatory eye disease may provide new clues in the treatment of uveitis. Measurements of cytokines also may play a role in predicting relapses and in monitoring disease activity in patients with uveitis. Therefore, an accurate method to determine the size and patterns of cytokine secretion in uveitis would be clinically important and might provide fundamental information about the immunopathogenesis of uveitis.

We examined aqueous humor and peripheral blood samples from patients with uveitis for the presence of IFN-γ, IL-2, IL-4, and IL-10. These studies were performed to determine the profile of soluble cytokines and its correlation with clinical characteristics.

RESULTS

Aqueous humor and serum levels of IFN-γ and IL-2 were elevated in patients with uveitis, suggesting a predominantly type 1 response (high IFN-γ and low IL-4 levels). Elevated serum IFN-γ level seems to predispose the patient to more serious loss of vision.

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The mean values for IFN-γ in aqueous humor (P < .001) and peripheral blood (P < .001) from patients with uveitis were higher than those of controls, as shown in Table 1. On the other hand, when we compared the serum with the aqueous levels of IFN-γ (39.26 ± 34.11 vs 13.86 ± 1.68 pg/
PATIENTS, MATERIALS, AND METHODS

PATIENTS AND MATERIALS

We studied 23 patients referred to us with a diagnosis of uveitis as primary process. Patients were included in the study if they had a minimum of 5 to 10 cells per field on slitlamp examination and had a clinical history or characteristics that made it necessary or possible to remove aqueous humor for diagnostic purposes. Pregnant women, patients with human immunodeficiency virus, intravenous drug users, and patients receiving local or systemic immuno-suppressive treatment were not included. The patients with uveitis were divided between those with disease associated and not associated with systemic diseases. The first group included 12 patients. Five of these had ankylosing spondylitis; 3, Behc¸et disease; 1, chronic juvenile iridocyclitis; 1, graft vs host disease; 1, systemic toxoplasmosis; and 1, Candida septicemia. The second group included 11 patients. Two of these had Fuchs uveitis syndrome; 1, birdshot retinochoroidopathy; 1, lens-induced uveitis; and 7, idiopathic uveitis. Sixteen patients who had undergone operation for uncomplicated cataracts, matched for age and sex, served as a control group.

The uveitis group was classified according to the recommendations proposed by the International Group for the Study of Uveitis.11 We thus took into account laterality, onset (insidious or sudden), duration (short, ie, ≤3 months, or chronic, ie, >3 months), patterns (single or repeated episode), visual damage (mild if visual loss is <50% of pre-disease vision, or severe if visual loss is ≥50%), location (anterior, intermediate, posterior, or panuveitis), and cellular and protein inflammatory activity (all cases with activity of ≤2+ were considered mild, and those with an activity of >2+ were considered severe). We also took into consideration other factors such as association with systemic disease, previous ocular surgery, and association with the B27 histocompatibility antigen.

Aqueous (0.2-0.3 mL) and peripheral blood (10 mL) samples were taken from each patient. The aqueous humor was extracted under a surgical microscope via limbal paracentesis with the use of a 27-gauge needle. Peripheral blood was extracted at the time of collection of the ocular specimen. The aqueous humor was deposited in a tube (Eppendorf, Hamburg, Germany) for subsequent processing. The cells were isolated from the supernatant by centrifugation at 3500 rpm, and both components were stored at −70°C until assay. The supernatant and cells were isolated from the peripheral blood by centrifugation at 3500 rpm and stored at −70°C until assay.

The tenets of the Declaration of Helsinki were followed. The Research Standards Committee of our center approved this project (resolution 68/95). All patients and controls gave their informed consent after the nature of the study had been fully explained to them.

CYTOKINE DETECTION

Levels of the cytokines IFN-γ, IL-2, IL-4, and IL-10 were determined in the supernatant of the aqueous humor and serum samples by means of an enzyme-linked immunosorbent assay. We used commercially available kits for cytokine quantification (Bender Medsystems, Vienna, Austria). Each experiment was duplicated, and the means of the pairs of determinations were used to give the final results. The results of the cytokine determinations are expressed as picograms per milliliter. The lowest detectable concentration of cytokines were: 1.5 pg/mL for IFN-γ, 10 pg/mL for IL-2, 2 pg/mL for IL-4, and 1.5 pg/mL for IL-10.

STATISTICAL ANALYSIS

Statistical analyses were performed using SPSS software, version 7.5 (SPSS Inc, Chicago, Ill). Categorical variables were compared by using χ² or Fisher exact tests. Two continuous variables were compared by using t test for independent and related samples when the variables fit a normal distribution; when they did not, we used the nonparametric Mann-Whitney test for independent samples and the Wilcoxon test for related samples. Association between 2 continuous variables was assessed with Pearson rank correlation coefficient. Multiple linear regression analysis for successive steps was used to assess the relationships among different clinical characteristics of uveitis and the various cytokines studied. All statistical tests were 2-tailed. Unless otherwise indicated, data are given as mean±SD.

mL) from patients with uveitis, the difference was statistically significant (P<.001). In the control group, no differences were observed between the aqueous and the serum levels (4.25±3.89 vs 1.75±3.83 pg/mL) (Figure 1).

INTERLEUKIN 2

Levels of IL-2 in aqueous humor and peripheral blood from patients with uveitis were significantly higher (P<.001) compared with those in the controls (Table 1). When we compared aqueous humor with serum levels of IL-2 (38.13±6.71 vs 20.65±14.57 pg/mL) in patients with uveitis, we found that the aqueous levels of IL-2 were significantly higher (P<.001) than those in the blood samples. In contrast, in the control group, serum levels (11.43±1.99 pg/mL) were significantly higher (P<.001) than those in the aqueous humor (3.25±1.14 pg/mL) (Figure 1).
In the aqueous humor, we found a close direct relationship between serum levels of IL-2 and IL-4 ($r = 0.83; P = .001$) and the aqueous humor ($0.39 \pm 1.30$ and $0.68 \pm 1.88$ pg/mL), which were higher ($7.06 \pm 1.28$ pg/mL) than those in patients with uveitis ($8.82 \pm 2.42$ pg/mL) and controls ($4.25 \pm 3.89$ pg/mL) ($P = .002$) from patients with uveitis. These observations support our present findings and add further evidence to the theory of disturbed immune homeostasis in this group of conditions. However, other authors did not confirm the raised IFN-γ levels in patients with uveitis $^{10}$ or IL-2 levels in patients with Vogt-Koyanagi-Harada syndrome. $^{11,12}$ It is difficult to interpret these discrepancies; perhaps several factors may account for the rise in cytokine levels in patients with uveitis. The wide variety of uveitis syndromes, the distinct degrees of inflammatory activity, and IL-2 and IFN-γ levels in patients with uveitis ($8.82 \pm 2.42$ pg/mL) and controls ($4.25 \pm 3.89$ pg/mL) ($P = .005$) and a weak inverse correlation between IL-2 and IL-4 levels ($r = -0.30; P = .03$) (Table 3).

**COMMENT**

Our study shows that the aqueous and serum samples from patients with uveitis have higher levels of IFN-γ and IL-2 than those from controls. Previous reports have demonstrated that the aqueous humor from patients with uveitis contain IFN-γ and IL-2, and that vitreous from patients with acquired immunodeficiency syndrome-related retinitis contains IL-2 and IFN-γ. Likewise, other studies have detected IFN-γ and IL-2 in peripheral blood from patients with clinical uveitis. These observations support our present findings and add further evidence to the theory of disturbed immune homeostasis in this group of conditions.
and the small number of subjects included in these studies as well as the different assays used may explain these contradictory results. Therefore, further investigations for selected cytokines are needed in more specific subtypes of uveitis, eg, acute (sudden onset) anterior uveitis, Bechet disease, or juvenile chronic arthritis.

Our patients showed a significantly higher IFN-γ level in blood than in aqueous humor. This suggests a leakage from the blood to the aqueous humor rather than local production. A number of investigations involving experimental models have distinguished between the protective effect of systemic IFN-γ and the inflammatory effect of local IFN-γ, suggesting that the protective effect might be explicable in terms of the generalized expression of adhesion molecules provoked by IFN-γ, which would avoid the local inflow of activated uveitogenic lymphocytes. The significance of this relationship remains to be confirmed, although just as in experimental uveitis, it may be related to the initiation of a protective response at systemic level. On the other hand, IL-2 behaved differently. Thus, increased IL-2 levels were found in aqueous samples from patients with uveitis, whereas serum samples obtained at the same time showed a significantly lower level of IL-2. The higher level of IL-2 in the aqueous suggests local production.

Slightly higher IL-10 levels were detected in the peripheral blood of our patients when compared with controls, and this increase correlated with uveitis not associated with systemic disease. Our results agree with those reported by Ongkosuwito et al, who detected IL-10 in 10 of 17 patients with acute retinal necrosis and in 13 of 27 samples from patients with Toxoplasma chorioretinitis. However, our data disagree with those of el-Shabrawi et al, who detected IL-10 in only 3 of their 22 patients with uveitis. It appears that our patients exhibited a low production of IL-10, and it is possible that this response was delayed. Therefore, sequential measurements of this cytokine are needed during clinically active uveitis to confirm this hypothesis.

In contrast to the increased IFN-γ and IL-2 levels reported herein, no increased IL-4 levels were found in aqueous and serum samples from patients with uveitis. The findings are in accord with a recent study that showed no detectable IL-4 levels in patient or control samples.

From the analysis of the relationship between cytokine levels (IFN-γ, IL-2, IL-4, and IL-10) and clinical variables of uveitis (latency, onset, patterns, visual damage, localization, inflammatory activity, association with systemic disease, previous ocular surgery, and association with the B27 histocompatibility antigen), we wish only to draw attention to the high levels of serum IFN-γ in the uveitis group with the most loss of vision, a finding that could be of prognostic value, although confirmation of this will require further study with longitudinal measurements.

Several investigators have shown that the integrated study of cytokines is more important than that of
individual cytokines. Based on their pattern of cytokine secretion, at least 2 functionally distinct subpopulations of CD+ T-helper cells (Th1) were characterized. The Th1 clones, which produce IL-2 and IFN-γ, are involved in the generation of a delayed type of hypersensitivity response, whereas the Th2 clones produce IL-4, IL-10, and IL-13 and play a key role in humoral immunity, including the IgE response.21 Although Th1 and Th2 cells are major sources of their respective cytokines, many other cells (γ-δ, CD8, natural killer, and mast cells; macrophages; and more) within and outside the immune system also produce these cytokines. Thus, several cell types may contribute to an overall Th1 or Th2 cytokine pattern, and it has been suggested that these responses should be described as type 1 or 2.22

Despite the large number of experimental studies that implicate the type 1 response in the pathogenesis of experimental autoimmune uveoretinitis,23 the type of immune response that participates in human uveitis has not been determined.

In aqueous humor, Ongkosuwito et al19 studied the type of immune response in a group of patients with acute retinal necrosis and Toxoplasma chorioretinitis without decisive conclusions; el-Shabrawi et al20 investigated the role of IL-12 (type 1 response) and IL-10 (type 2 response) in ocular fluid of patients with uveitis and found higher levels of IL-12. Our results show a predominately type 1 response (high IFN-γ and low IL-4 levels), confirming the data of the experimental studies and in agreement with the hypothesis that the delicate intracellular type1/type 2 balance, with an initial predominance of type 1, breaks down. The close correlation observed between IFN-γ and IL-2 reinforces the idea that the behavior of both cytokines is similar.

In the peripheral blood, other authors have studied the type of immune response that takes place during uveitis. Rahi et al14 studied the type 1 response (IFN-γ and IL-2) in a group of patients with uveitis and found elevated levels of these cytokines. Sugi et al13 studied types 1 and 2 immune responses in patients with Behçet disease and found a predominantly type 1 response in patients with active uveitis. Although the immune response at systemic level is not the main interest of this study, our results confirm the data of both studies.

Accepted for publication November 27, 1999.

This study was supported in part by the Department of Experimental Medicine, Reina Sofia University Clinic, Cordoba, Spain.

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