

Elevated Soluble Fas in Aqueous Humor of Patients With Behçet's Uveitis: Correlation With Uveitis Severity

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Purpose: To examine the role of soluble Fas (sFas) in patients with Behçet's uveitis.

Methods: We measured the sFas levels in both sera and aqueous humor (AH) of patients (n = 40) with uveitis and of non-uveitis controls (n = 27) using an enzyme-linked immunosorbent assay. The patients with uveitis comprised 24 with Behçet's disease, 6 pan-uveitis, 5 anterior uveitis, 2 lens-induced uveitis, 1 Vogt-Koyanagi-Harada disease, 1 sarcoidosis, and 1 retinal vasculitis. The severity of uveitis was determined by the Hogan grading method (0–4 grade) at the time of sampling.

Results: The concentration of aqueous sFas in uveitis patients was significantly higher than that in non-uveitis controls, while there was no difference in the serum concentration of sFas between the two groups. In the paired samples of serum and AH, obtained simultaneously, the aqueous sFas levels were higher than serum Fas levels in patients with uveitis, whereas the non-uveitis controls displayed significantly lower sFas levels in AH than in the serum. The sFas levels in AH or serum were not different between Behçet's uveitis patients and non-Behçet's uveitis patients. However, in patients with Behçet's uveitis, circulating sFas strongly correlated with aqueous sFas, which was not so in those with non-Behçet's uveitis. Patients (n = 29) with more active (grade ≥ 2) uveitis had significantly higher levels of aqueous sFas than those (n = 11) with less active (grade < 2) uveitis. After treatment with steroids and/or immunosuppressive agents, aqueous sFas levels decreased in parallel with a reduction in the number of inflammatory cells.

Conclusions: The levels of sFas were elevated in patients with Behçet's uveitis and correlated well with the uveitis severity in these patients. **Jpn J Ophthalmol 2002;46:18–23** © 2002 Japanese Ophthalmological Society

Key Words: Behçet's uveitis, disease severity, soluble Fas.

Introduction

Multicellular organisms maintain their integrity by complex mechanisms that ensure a balance between cell proliferation, cell differentiation, and cell death. The death of useless or unwanted cells during embryogenesis, tissue remodeling, immune system development, tumor regression, and normal tissue turnover is called programmed cell death. This is because programmed cell death involves the activation of a suicide machinery that is under genetic control.¹

Cell death can be elicited by a number of stimuli, such as growth factor deprivation, ionizing radiation, or the triggering of specific cellular receptors, such as the tumor necrosis factor (TNF) receptor 1 or Fas/APO-1.²

The Fas/Apo-1 molecule is a cell surface receptor belonging to the TNF- α family of apoptosis-signaling molecules and is constitutively expressed in various tissues.^{3,4} The triggering of Fas by its ligand results in rapid induction of apoptosis in susceptible cells. Fas ligand (FasL) is a 40-kDa type II integral membrane protein and a member of the TNF family. It is expressed constitutively in a few cells such as CD8 (+) T cells, dendritic cells, and NK cells.^{5–7} The tissue distribution of Fas and FasL suggests that the Fas/

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FasL system plays an important role in the homeostasis of the immune system. The Fas/FasL system has also been addressed as a mediator of the immune privilege in a variety of tissues. The expression of FasL by testicular Sertoli cells and by parenchymal cells of the anterior chamber of the eye enables these cells to kill invading activated Fas-expressing T cells, conferring a state of immune privilege to these sites.^{8,9}

Fas and FasL can occur in both membrane-bound and soluble forms. The soluble Fas (sFas), which is generated by alternative splicing of the primary Fas transcript, competes for FasL and is able to prevent FasL binding to Fas, thereby blocking Fas-mediated apoptosis.^{10,11} When Fas-mediated apoptosis is inhibited, autoreactive cells may escape apoptosis and provoke an autoimmune response resulting in tissue destruction. In several autoimmune diseases, it has been suggested that elevated sFas levels may inhibit apoptosis of autoreactive cells, and eventually lead to the progression of many diseases.^{12–15} Although the immunopathogenesis of noninfectious uveitis is still controversial, it is believed to have a putative autoimmune component.¹⁶ The aim of this study is to determine the levels of sFas in patients with uveitis and then to correlate the levels with uveitis severity.

Materials and Methods

Patients

This study was conducted in accordance with the principles embodied in the Declaration of Helsinki, and informed consent was obtained from all patients and healthy controls. Forty patients with uveitis (25 male and 15 female, mean age = 45.8 years, ranging from 13–68 years) being treated at the Kangnam St. Mary's Hospital between January 1998 and February 1999 were enrolled in this study. The patients with uveitis comprised 24 with Behçet's disease, 6 pan-uveitis, 5 anterior uveitis, 2 lens-induced uveitis, 1 Vogt-Koyanagi-Harada-disease, 1 sarcoidosis, and 1 retinal vasculitis. Patients with Behçet's uveitis consisted of posterior uveitis (66.7%), pan-uveitis (12.5%), and anterior uveitis (20.8%). Patients with trauma, infection, and history of intraocular surgery were excluded. Comparisons were made with 27 non-uveitis controls (14 male and 13 female, mean age = 48.6 years, ranging from 20–74 years) who were undergoing surgery for cataract, predominantly anterior subcapsular opacity. No difference was found in age and sex between uveitis patients and non-uveitis controls.

Determination of Soluble

Fas in Serum and Aqueous Humor

The sFas levels in both sera and aqueous humor (AH) were determined using an enzyme-linked immunosorbent assay kit (MBL, Nagoya) according to the manufacturer's instruction. The AH was obtained by anterior chamber paracentesis with a 30-gauge needle. If the patients were taking corticosteroids or immunosuppressive agents, the medications were temporarily withdrawn for 48 hours prior to sampling. The AH (volume of 0.1–0.2 mL) was centrifuged immediately after collection at 23 g for 10 minutes and frozen to -70°C until the assay. Human recombinant sFas was used as a calibration standard, and a plot of the optical density versus the log of the recombinant sFas concentration showed a standard curve with good linear correlation in all determinations ($r > 0.98$, data not shown). The sFas levels in both sera and AH, diluted 1:1, were determined by a direct comparison with the standard curve. The sensitivity limit was 10 pg/mL.

Assessment of Uveitis Activity

At the time of sampling, disease severity of the uveitis patients was evaluated using the Hogan-Kimura method (0–4 grade).¹⁷ The grading was as follows; Grade 0: complete absence of cells, Grade 1: 5 to 10 cells per field, Grade 2: 10 to 20 cells per field, Grade 3: 20 to 50 cells per field, Grade 4: over 50 cells per field.

Statistical Analysis

The data was expressed as the mean \pm SEM. Comparisons of numerical data between groups were performed using either the Mann-Whitney *U*-test or the Wilcoxon signed rank test. Any correlation between two variables was performed using the Spearman rank correlation coefficient. *P* values less than .05 were considered significant.

Results

Levels of sFas in Serum and Aqueous Humor

Figure 1 shows that the sFas concentration in the AH of uveitis patients ($n = 40$) was significantly higher than that in the non-uveitis controls ($n = 27$) (416 ± 54 versus 219 ± 25 pg/mL, $P < .001$). However, the serum concentration of sFas was not significantly different between the uveitis patients and non-uveitis controls (301 ± 17 versus 308 ± 21 pg/mL, respectively). In the paired samples of serum and AH, obtained simultaneously, the aqueous sFas in patients with uveitis was

significantly higher than the serum sFas ($P = .003$), whereas non-uveitis controls displayed significantly lower sFas levels in the AH than in serum ($P = .008$) (data not shown). The serum sFas did not show any correlation with the aqueous sFas in both uveitis patients and non-uveitis controls.

Association of sFas With Uveitis Type and Severity

The sFas levels in AH or serum were not significantly different between Behçet's uveitis ($n = 24$) and non-Behçet's uveitis ($n = 16$) (aqueous: 412 ± 57 versus 422 ± 48 pg/mL, serum: 307 ± 16 versus 292 ± 19 pg/mL, respectively). However, in patients with Behçet's uveitis, circulating sFas strongly correlated with aqueous sFas, which was not so in those with non-Behçet's uveitis (Figure 2) ($r = 0.537$ and $P = .007$ for Behçet's uveitis, $P = .88$ for non-Behçet's uveitis). The patients with uveitis were also divided into two groups according to disease severity, more active (grade ≥ 2) and less active (grade < 2) patients, and the sFas levels were then compared between the two groups. The comparison showed that patients ($n = 29$) with more active uveitis had significantly higher levels of aqueous sFas than those ($n = 11$) with less active uveitis (473 ± 63 versus 285 ± 32 pg/mL, $P = .008$) (Figure 3). Circulating sFas also tended to be higher in patients with more active uveitis, but the results were not statistically significant (310 ± 32 versus 246 ± 32 pg/mL, respectively).

Sequential Measurement of Aqueous sFas

To examine the effect of therapeutic agents on sFas concentration, we monitored the aqueous sFas

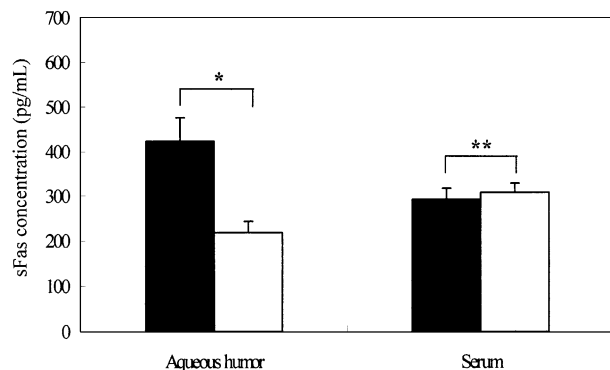


Figure 1. Levels of soluble Fas in serum and aqueous humor from patients with uveitis ($n = 40$) and non-uveitis controls ($n = 27$). Black bars show mean and SEM of soluble Fas concentration in uveitis patients; white bars, that in non-uveitis controls. * $P < .001$. **Not significant.

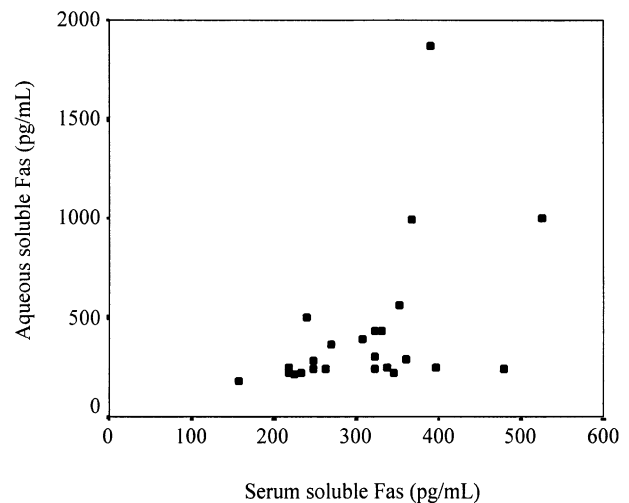


Figure 2. Correlation of circulating soluble Fas with aqueous soluble Fas in patients with Behçet's uveitis. $r = 0.537$. $P = .007$.

concentration sequentially in the 11 patients with uveitis who had not taken any medications after treatment with high dose steroids (> 30 mg/day) and/or immunosuppressive agents including azathioprine and cyclosporine. These patients consisted of 8 with Behçet's disease, 1 anterior uveitis, and 2 pan-uveitis (Table 1). The mean grade of uveitis decreased from 2.5 to 0.9 three to six months after treatment with the above drugs. The aqueous sFas levels decreased in all patients with improvement in their condition (mean levels of sFas: pretreatment

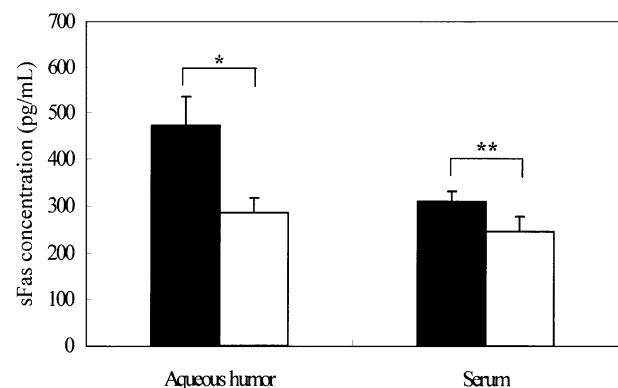


Figure 3. Correlation of aqueous soluble Fas with uveitis severity. Black bars indicate mean (\pm SEM) level of soluble Fas in more active patients, and white bars, in less active patients. More active patients ($n = 29$) were defined as those who had uveitis of grade 2 or greater by Hogan grading method; less active patients ($n = 11$) were defined as those with a grade of less than 2. * $P = .008$. **Not significant.

Table 1. Characteristics of Patients Sequentially Monitored for Grade of Uveitis and Aqueous Soluble Fas

| Patient No. | Age (years) | Sex | Diagnosis | Time Interval (months) | Pre-treatment Uveitis Grade | Post-treatment Uveitis Grade | Immunosuppressive Agents* |
|-------------|-------------|-----|------------------|------------------------|-----------------------------|------------------------------|---------------------------|
| 1 | 61 | F | Behçet | 6 | 4 | 1 | CSA+AZP |
| 2 | 37 | F | Behçet | 3 | 2 | 0 | CSA |
| 3 | 21 | M | Behçet | 4 | 3 | 1 | CSA |
| 4 | 39 | M | Behçet | 5 | 4 | 2 | CSA+AZP |
| 5 | 50 | M | Behçet | 4 | 1 | 0 | CSA |
| 6 | 47 | M | Behçet | 6 | 2 | 0 | CSA |
| 7 | 42 | M | Behçet | 3 | 1 | 0 | AZP |
| 8 | 51 | F | Behçet | 6 | 2 | 1 | CSA |
| 9 | 69 | F | Anterior uveitis | 4 | 2 | 1 | None |
| 10 | 33 | M | Pan-uveitis | 3 | 3 | 2 | CSA |
| 11 | 68 | M | Pan-uveitis | 4 | 3 | 2 | CSA |

*Immunosuppressive agents administered during follow-up periods. CSA: cyclosporine A, AZP: azathioprine.

511 ± 143 pg/mL, posttreatment 211 ± 26 pg/mL, $P = .003$) (Figure 4). Moreover, the degree of sFas reduction after treatment was greater in patients ($n = 5$) who showed conspicuous remission (≥ 2 grade reduction in uveitis grade) than the other patients (< 2 grade reduction, $n = 6$). The difference in sFas levels between pre- and posttreatment was 508 ± 272 versus 88 ± 30.4 pg/mL, $P = .045$. Serum sFas levels tended to decrease after treatment, but the results were not statistically significant (data not shown).

Discussion

The eye is a representative organ of an immune-privileged site. One of the mechanisms contributing to this immune privilege may be Fas-FasL-mediated apoptosis occurring within the eye.⁹ FasL is constitutively expressed in murine ocular tissue such as the iris ciliary body and corneal epithelium. Therefore, inflammatory cells entering the anterior chamber undergo apoptosis and thus produce no tissue damage. In contrast, immune tolerance was not observed in the animal model with a defect in either Fas or FasL, and it was required that the lymphoid cells should be Fas (+) and the eye be FasL (+).¹⁸

In the experimental uveitis model, the kinetics of the expression of FasL corresponded with the kinetics of apoptotic cells in the infiltrating cells, suggesting that Fas-FasL-mediated apoptosis may play a role in the immunopathogenic mechanisms to eliminate infiltrating cells in uveitis.¹⁹ In patients with anterior uveitis, FasL was functional and FasL-mediated apoptosis contributed to the local immune regulation of ocular inflammation and to a self-limiting clinical course for uveitis.^{20,21} Therefore, it is conceivable that a Fas-mediated apoptosis defect might result in the destruction of immune privilege within

the eye and, thus, in uveitis progression. This assumption is also supported by the recent finding that the continuous high expression of Bcl-2 over Bax in the eyes was found in the ocular inflammatory cells in the experimental model of uveitis, contributing to chronic recurrent inflammation.²²

A recent study has demonstrated that intraocular levels of sFasL and sFas are significantly increased in uveitis, particularly in active uveitis, suggesting that

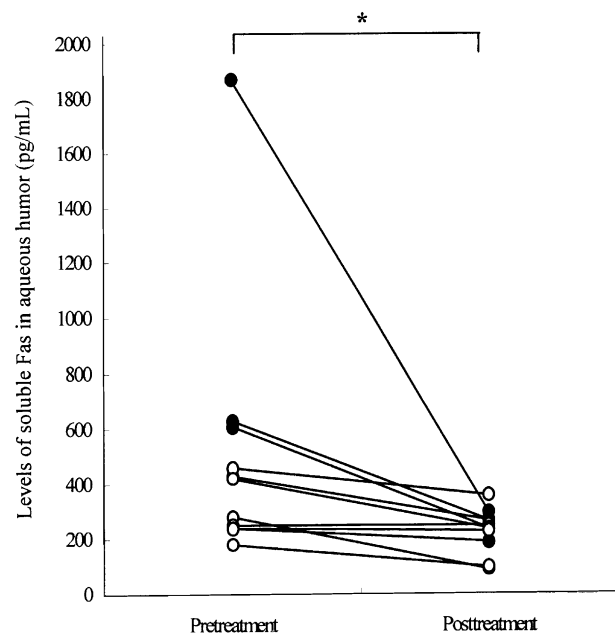


Figure 4. Changes in aqueous soluble Fas levels 3 to 6 months after treatment with steroids and/or immunosuppressive agents when compared to pretreatment. ● Patients who showed conspicuous remission (≥ 2 grade reduction in uveitis grade). ○ Patients who showed less remission. * $P = .003$.

intraocular sFasL and sFas may have a regulatory role in uveitis.²³ In the present study, we also demonstrated the presence of a high concentration of sFas, which is able to block Fas-mediated apoptosis, *in vitro*,^{10,11} in the AH of uveitis patients compared to non-uveitis controls. However, there was no difference in serum concentrations of sFas between the two groups, suggesting that sFas could be insufficiently removed or overproduced predominantly within the anterior chamber of uveitis patients, the actual site of immune reactions. It is likely that accumulation of sFas in AH is not unique in Behçet's disease because the levels of aqueous sFas were not different between Behçet's and non-Behçet's uveitis.

Nevertheless, in patients with Behçet's uveitis characterized by systemic vasculitis, aqueous sFas correlated well with circulating sFas. Of note, most patients with non-Behçet's uveitis except one sarcoidosis patient did not have any systemic symptom on uveitis attack, whereas all patients with Behçet's uveitis had several extraocular manifestations including recurrent oral/genital ulcer, skin lesions, and arthritis. Thus, it is possible that blood—ocular barrier breakdown is more prominent in the systemic form of uveitis (eg, Behçet's disease, sarcoidosis) compared to the localized form. Interestingly, healthy controls displayed significantly lower levels of sFas in paired AH than in the serum. If a Fas-mediated apoptosis might play a role in immune privilege, it is not surprising that the sFas, an apoptosis inhibitor, should be kept at a lower level within the normal eye to ensure the sufficient action of FasL.

Clinically, our findings may have implications for uveitis patients. The concentration of aqueous sFas correlated well with the degree of inflammatory cell infiltration in the anterior chamber, as assessed by a slit-lamp examination. Furthermore, the sFas levels decreased in parallel with a reduction in the number of inflammatory cells following treatment with steroids and/or immunosuppressive agents. This observation suggests that the aqueous sFas may reflect disease severity in pathologic lesions and thus may be useful in assessing uveitis severity. However, this may not be a specific finding in Behçet's disease because an elevated sFas level has been associated with disease activity in other autoimmune diseases, including systemic lupus erythematosus, rheumatoid arthritis, and multiple sclerosis.^{12–14} It is therefore likely that sFas accumulation may play a role in the progression of a variety of autoimmune diseases.

In summary, aqueous sFas levels were elevated in the patients with uveitis including Behçet's uveitis and correlated well with circulating sFas in patients

with Behçet's uveitis. The sFas concentration in AH reflected the degree of inflammatory cell infiltration within the anterior chamber. After treatment with steroids and/or immunosuppressive agents, aqueous sFas levels decreased along with clinical improvement. Our data suggests that sFas might play a role in the perpetuation of uveitis. Although further *in vitro* studies are required to determine the effect of sFas on apoptosis in the eye, this is the first report showing that elevated sFas is associated with uveitis severity.

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