Soluble Fas ligand and soluble Fas in ocular fluid of patients with uveitis

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Abstract

Aims—To investigate the presence of soluble Fas ligand (sFasL) and soluble Fas (sFas) in ocular fluid of patients with uveitis.

Methods—Samples of aqueous humour (AH, n=17), vitreous fluid (n=9), and serum (n=60) were collected from patients with uveitis which included Behçet’s disease, Vogt–Koyanagi–Harada disease, sarcoidosis, human T lymphotropic virus type 1 (HTLV-I) uveitis, sympathetic ophthalmia, HLA-B27 associated acute anterior uveitis, and ocular toxoplasmosis. The AH of patients with age related cataract without uveitis obtained during cataract surgery was used as controls (n=20). The amounts of sFasL and sFas were measured by enzyme linked immunosorbent assay.

Results—Significant amounts of sFasL was also detected in AH of patients with uveitis, though the amounts were slightly lower than those in the non-uveitis group. On the other hand, the levels of sFas in AH of patients with uveitis were significantly higher than those in controls. As for the disease activity, the levels of sFasL and sFas in the vitreous fluid of patients with active uveitis were significantly higher than those in inactive uveitis. sFasL in the serum of healthy donors and patients with uveitis was below detectable levels, except for patients with HTLV-I uveitis who had significant amounts of sFasL in the serum. The levels of sFas in the serum of patients with Behçet’s disease, sarcoidosis, and HTLV-I uveitis were significantly higher than those of healthy donors.

Conclusions—sFasL is present in the AH of non-uveitic eyes with age related cataract. Intraocular levels of sFasL and sFas are significantly increased in uveitis, particularly in active uveitis. These data suggest that intraocular sFasL and sFas may have a regulatory role in uveitis.

Materials and methods

AH samples (100–200 µl) were collected from patients with age related cataract (n=20) during cataract surgery, and from patients with various types of uveitis (n=17). The cataract patients had no clinical history of uveitis or systemic diseases, and the group was designated as a non-uveitis group in this study. The patients with uveitis had active intraocular inflammation, but were not treated with topical and systemic therapy at the time of aqueous humour sampling. The patients with uveitis included Behçet’s disease (n=2), Vogt–Koyanagi–Harada disease (VKH; n=3), sarcoidosis (n=4), human T lymphotropic virus type 1 infection (n=4) and systemic lupus erythematosus (n=4).
type 1 (HTLV-I) uveitis (n=3), sympathetic ophthalmia (SO; n=3), and HLA-B27 associated acute anterior uveitis (AAU; n=2).

The amounts of sFasL were determined by a sandwich ELISA as follows. A 96 well plate was coated with anti-human FasL antibody (5 µg/ml) and incubated for 1 hour at room temperature. After washing with PBS, biotinylated NOK-1 anti-human FasL antibody (5 µg/ml containing 5% mouse serum) was added and incubated for 1 hour at room temperature. The wells were washed five times with PBS and incubated with 50 µl avidin-peroxidase (Sigma Chemical Co, St Louis, MO, USA) for 1 hour at room temperature. Peroxidase activity was detected using a peroxidase detecting kit (Sumitomo Co, Tokyo, Japan). The absorbance was measured at 450 nm using an automated ELISA reader.

We also tested the serum samples from the following different sets of patients: Behcet's disease (n=10), VKH (n=10), sarcoidosis (n=10), HTLV-I uveitis (n=10), AAU (n=10), ocular toxoplasmosis (n=10), and healthy donors (n=10). The patients except for healthy donors had active uveitis and had no systemic therapy when their serum samples were collected.

The average age of the cataract patients was 76.5 years, and that of all uveitis patients was 44.0 years.

Informed consent was give by each patient before sampling. The research followed the tenets of the Declaration of Helsinki. This study was approved by the institutional ethics committee of Kurume University School of Medicine.

After the aqueous humour and the serum samples were centrifuged at 3000 rpm for 5 minutes and the vitreous fluid was centrifuged at 10 000 rpm for 5 minutes, the supernatant was collected and stored at −80°C until use. The samples used in this study were collected between 1995 and 1997, and assayed in 1998. The samples were thawed only once, and used for assay.

ELISA FOR SFASL AND SFAS

The amounts of sFasL were determined by a sandwich enzyme linked immunosorbent assay (ELISA) using two anti-human FasL monoclonal antibodies, NOK-1 (mouse IgG1, κ) and NOK-3 (mouse IgM, κ). The antibodies were provided by Dr Hideo Yagita, Department of Immunology, Juntendo University School of Medicine. The measurement of sFasL was carried out by a sandwich ELISA as follows. A 96 well plate was coated with NOK-3 anti-human FasL antibody (10 µg/ml) diluted with phosphate buffer saline, PBS overnight at 4°C. After washing the plate with PBS containing 0.05% Tween 20 (T-PBS), the wells were blocked by skimmed milk solution (Block Ace; Snow Brand Milk Co, Sapporo, Japan) for 2 hours at 37°C. Ocular fluid and serum samples were diluted twice with 1% bovine serum albumin (BSA)/PBS. Fifty µl of the samples and standard sFasL (diluted with 1% BSA/PBS) were added to the well and incubated for 1 hour at room temperature. After washing with T-PBS, biotinylated NOK-1 anti-human FasL antibody (5 µg/ml containing 5% mouse serum) was added and incubated for 1 hour at room temperature. The wells were washed five times with T-PBS and incubated with 50 µl avidin-peroxidase (Sigma Chemical Co, St Louis, MO, USA) for 1 hour at room temperature. Peroxidase activity was detected using a peroxidase detecting kit (Sumitomo Co, Tokyo, Japan). The absorbance was measured at 450 nm using an automated ELISA reader.

The specificity of the sandwich ELISA methods used in the study was evaluated as follows. The human FasL cDNA transfectant cells (hFasL/L5178Y) were cultured in RPMI-1640 medium containing 10% FCS, 100 U/ml penicillin G, and 50 µg/ml streptomycin. The supernatant of the culture was collected and used as positive controls. Commercial recombinant human TNF-α (Endogen, MA, USA) and the culture supernatants of human CD70 (CD27 ligand)/BCMGShyg in 300–19 cells and human CD154 (CD40 ligand)/BCMGShyg in 300–19 cells which contained CD70 and CD154, respectively, were used as negative controls. The CD70/BCMGShyg in 300–19 cells and human CD154/BCMGShyg in 300–19 cells were provided by Dr Tetsuji Kobata, Department of Immunology, Juntendo University School of Medicine. The present sandwich ELISA methods were able to detect sFasL from the culture medium of the hFasL/L5178Y cells, but not other members of TNF family—CD70 and CD154 (data not shown).

The amounts of sFas in the supernatant of samples measured by ELISA using a commercial assay kit (MBL, Nagoya, Japan).

STATISTICAL ANALYSIS

Statistical analysis was performed by the Mann–Whitney test. The difference between the two groups (controls versus uveitis) compared was determined to be statistically significant, when the p value was less than 0.05.

RESULTS

The levels of sFasL and sFas in ocular fluid of the non-uveitis group and the patients with uveitis were quantified by ELISA. Among the non-uveitis group of 20 patients with age related cataract without ocular inflammation, 11 patients had detectable levels of sFasL in aqueous humour ranging from 223 to 1343 pg/ml and the other nine patients had undetectable levels of sFasL. The mean level of sFasL in aqueous humour was 273 (SD 341) pg/ml. This indicates that more than one half of the non-uveitis group had significant amounts of sFasL in aqueous humour. As for
the patients with uveitis, significant amounts of sFasL were detected in six of 17 patients and the mean level of sFasL in aqueous humour was 132 (202) pg/ml. There was no statistically significant difference in the sFasL levels in aqueous humour between the two groups (Fig 1A).

sFas in aqueous humour in the majority of non-uveitis patients was below detectable levels (Fig 1B). In contrast, all patients with uveitis had high levels of sFas in the aqueous humour (Fig 1B). The mean values of sFas in aqueous humour of non-uveitis patients and uveitis patients were 67 (129) and 1132 (881), respectively. The difference between the two groups was statistically significant (p< 0.0005).

The levels of sFasL and sFas in the vitreous fluid were examined in relation to the activity of uveitis. When the eye had no signs and symptoms of uveitis for the past 3 months, the eye was considered to be inactive. On the other hand, if the eye had inflammation in the anterior segment, vitreous or the posterior segment at the time of vitreous surgery, it was classified as having active uveitis. Significant amounts of sFasL were detected in the vitreous in all four patients with active uveitis, while only one of five patients with inactive uveitis had detectable levels of sFasL (Fig 2A). The difference between the two groups was statistically significant (p<0.05). Similar to sFasL, the levels of sFas in the vitreous were significantly higher in patients with active uveitis than in those with inactive uveitis (p< 0.05) (Fig 2B).

The serum levels of sFasL and sFas were examined in healthy donors and patients with various types of uveitis (Fig 3A and B). The serum levels of sFasL in healthy donors were undetectable or very low. Similarly, all uveitis entities except for HTLV-I uveitis exhibited undetectable or low levels of sFasL in the

Figure 1 (A) Soluble Fas ligand (sFasL) in the aqueous humour. Non-uveitis indicates samples from patients with age related cataract (n=20). Uveitis indicates samples obtained from patients with the following (n=17): Behçet’s disease, Vogt–Koyanagi–Harada disease, sarcoidosis, sympathetic ophthalmia, HLA-B27 associated acute anterior uveitis, and HTLV-I uveitis. Bar indicates average values. (B) Soluble Fas (sFas) in the aqueous humour. Non-uveitis and uveitis indicate samples from patients with age related cataract (n=20) and those with uveitis (n=17), respectively, as in (A). Bar indicates average values and an asterisk indicates statistical significance (p <0.0005).

Figure 2 (A) Soluble Fas ligand (sFasL) in the vitreous fluid. Active uveitis (n=4) and inactive uveitis (n=5) indicate samples obtained from patients with the following: Behçet’s disease, HTLV-I uveitis, sarcoidosis, and sympathetic ophthalmia. Bar indicate average values and an asterisk indicates statistical significance (p<0.05). (B) Soluble Fas (sFas) in the vitreous fluid. Active uveitis (n=4) and inactive uveitis (n=5) indicate samples obtained from patients as in (A).
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The levels of sFasL in the serum of patients with HTLV-I uveitis were significantly higher than those of healthy donors (p<0.05). In contrast, significant amounts of sFas were determined in the serum of healthy donors and patients with uveitis. The levels of sFas in the serum of patients with Behçet's disease, sarcoidosis, and HTLV-I uveitis were significantly higher than those of healthy donors (p<0.05).

Discussion

The present study demonstrated for the first time significant amounts of sFasL in aqueous humour of patients with age related cataract who had no history of ocular inflammation. This indicates that aqueous humour of the human eye in normal condition contains significant amounts of sFasL. It has been well established that the interaction between FasL and Fas positive cells causes apoptotic cell death of Fas positive cells. Although the capacity of naturally processed sFasL to induce apoptotic cell death is considered to be much lower than that of membrane bound FasL, sFasL was shown to have biological activities similar to membrane bound FasL. Thus, sFasL in aqueous humour might be one of the factors which plays a part in inducing apoptotic cell death of Fas positive infiltrating cells in the eye, thereby regulating ocular inflammation. As for the source of sFasL in aqueous humour, Behçet's disease, sarcoidosis, and HTLV-I uveitis were significantly higher than those of healthy donors. In contrast with sFasL, sFas in aqueous humour in non-uveitic patients was undetectable or at minimal levels. These results are consistent with the fact that immunocytes are absent in aqueous humour of normal eyes.

The present study showed that in aqueous humour of patients with uveitis sFasL was relatively low but sFas was very high. As mentioned above, sFasL is considered to interact with Fas positive inflammatory cells in aqueous humour. The interaction between sFasL and Fas positive inflammatory cells in aqueous humour of patients with uveitis patients may cause apoptotic cell death of the inflammatory cells and participate in the resolution of ocular inflammation. Therefore, it is likely that sFasL detected in aqueous humour in non-uveitic patients may be a result of consumption of sFasL in these processes. On the other hand, sFas was reported to protect Fas mediated apoptosis. Therefore, the high amounts of sFasL found in aqueous humour of patients with uveitis can be a result of consumption of sFasL in these processes. As for the serum levels of sFasL, they were very low in healthy donors as well as in patients with uveitis except for those with HTLV-I uveitis. Although a few studies have determined the serum levels of sFasL, available data indicate that significant levels of sFasL in the serum were detected in the patients with specific types of leukaemia. HTLV-I uveitis is not leukaemia, but significant amounts of HTLV-I infected lymphocytes were detected in the peripheral blood of patients with HTLV-I uveitis. Therefore, the high amounts of sFasL detected in the serum of patients with uveitis may counteract the apoptosis mediated downregulation of intraocular inflammation.

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significantly higher than those of controls. This is in accord with the present results. The present study further determined the serum levels of sFas in many other entities of uveitis, and sarcoidosis and HTLV-I uveitis exhibited significantly larger amounts of sFas in the serum than in healthy donors. Serum levels of sFas in VKH were also much higher than those of healthy donors, although not statistically significant.

Recent studies have shown high levels of sFas in the serum in some autoimmune diseases, such as systemic lupus erythematosus and rheumatoid arthritis, probably reflecting continuous activation of immunocytes in these patients. The present results of high levels of the serum sFas in Behçet’s disease, sarcoidosis, and HTLV-I uveitis were in accord with these previous results, suggesting a systemic nature of these entities of uveitis with persistent activation of the immune system. In contrast with these systemic diseases, low levels of sFas in the serum were found in uveitis localised in the eye, such as ocular toxoplasmosis and AAU.

In conclusion, sFasL is present in the aqueous humour of the eyes of non-uveitic patients. Intraocular levels of sFasL and sFas are significantly increased in uveitis, particularly in active uveitis. These data suggest that intraocular sFasL and sFas may have a regulatory role in uveitis.
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Br J Ophthalmol 2000 84: 1130-1134
doi: 10.1136/bjo.84.10.1130

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