Fas–Fas Ligand–Mediated Apoptosis within Aqueous during Idiopathic Acute Anterior Uveitis

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PURPOSE. Despite ocular immune privilege, (auto)immune-mediated acute anterior uveitis (AAU) is relatively common. However, although relapses of AAU are usually self-limiting, possible regulatory mechanisms remain undefined in humans. Experimentally, Fas–Ligand (FasL)–mediated apoptosis of Fas+ inflammatory cells contributes to the immune privilege within the anterior chamber and provides an explanation for the success of corneal allograft transplantation. Therefore, whether such mechanisms regulate the immune response in AAU was investigated.

METHODS. Aqueous and peripheral blood samples from consecutive patients presenting with idiopathic AAU were obtained with consent. Leukocytic phenotype was analyzed by flow cytometry, and apoptosis was determined by both flow cytometry and TdT-dUTP terminal nick-end labeling analysis. Presence of soluble Fas and FasL was determined by western blot analysis and enzyme-linked immunosorbent assay and compared with control aqueous from patients undergoing cataract surgery. The ability of the aqueous to induce apoptosis in a Fas+ Jurkat cell line was also determined.

RESULTS. During AAU aqueous-infiltrating Fas+ cells included CD3+ T cells and granulocytes, whereas FasL+ cells comprised predominantly of non–CD3+ T cells. Higher levels of functional soluble FasL were found in aqueous of AAU patients than in normal aqueous, capable of inducing apoptosis in 68.9% ± 7.6% of Fas+ lymphoid cells. Compared with peripheral blood, the CD4+ T cells infiltrate within aqueous showed significantly increased CD69 and CD25(IL-2r) expression. Flow cytometric analysis of aqueous showed that 9.32% ± 1.2% of infiltrating non–granulocyte CD45+ cells were apoptotic, confirmed as T cells on subsequent three-color flow cytometric analysis.

CONCLUSIONS. Taken together with published experimental data, the present study provides evidence for FasL-mediated apoptotic cell death contributing to the local immune regulation of ocular inflammatory disease and provides a mechanism to account for the self-limiting clinical course of AAU. (Invest Ophthalmol Vis Sci. 1999;40:2258–2267)

The eye, like the central nervous system, is regarded as an immune privileged site (principally because of the high success of corneal allograft transplantation), yet intraocular inflammation remains not an uncommon occurrence. Traditionally, this immune privilege was thought to be due to physical blood–ocular and blood–retinal barriers, the absence of lymphatics, and the paucity of antigen-presenting cells within ocular tissues. However, it has been documented that first a network of major histocompatibility complex (MHC) class II–positive cells,1,2 some of which behave functionally as dendritic cells existing within the uveal tract of the eye.3 Second, a form of lymphatic drainage exists as antigen-specific T-cell expansion in the submandibular lymph node occurs after intraocular antigen administration.4 The phenomenon of anterior chamber–associated immune deviation (ACAID),5,6 in which foreign antigens and tissues when administered into the anterior chamber fail to elicit an immune response and furthermore induce suppression of antigen-specific delayed-type hypersensitivity (DTH) responses, have prompted investigation into the cellular and molecular bases of the regulation of immune responses within the eye.7 Since the observation that Fas-Ligand (FasL) is constitutively expressed in murine ocular tissue such as iris ciliary body and corneal endothelium,8 experiments have shown that Fas/FasL-dependent apoptosis is induced in inflammatory cells entering the eye in response to a viral infection, in which the ability to initiate apoptosis and interleukin (IL)-10 appears critical for the induction of immune privilege.9,10 Furthermore, without Fas/FasL, splenocytes prime for DTH rather than induce ACAID.11 Although the molecular mechanisms of immune deviation are still not fully understood, and despite recent work in autoimmune diabetes that has cast doubt on the theory of FasL-induced lymphocyte apoptosis as a damage-limiting mechanism,1,12 present experimental evidence within the eye strongly supports an important role for Fas-L not only in controlling intraocular inflammation but also in the induction of immune deviation and the accep-
tance of corneal allografts. If Fas-FasL-mediated interactions are important during immune-mediated ocular inflammatory disease, such as acute anterior uveitis (AAU), experimental data would suggest that cell death within the anterior chamber would require infiltrating mononuclear cells to be Fas+ and resident ocular cells to be FasL− or that Fasl is induced on infiltrating cells by ocular resident cell products.

AAU is pathogenetically distinct from posterior uveitis, although animal models show that CD4+ T cells are intimately involved in the immunopathogenesis of both diseases. Clinically, AAU is an acute self-resolving condition and is frequently associated with HLA-B27 MHC class I antigen. Most previous studies of cellular infiltrate and immune mechanisms in uveitis have been limited because they have included a wide spectrum of uveitis conditions, including posterior uveitis associated with systemic disease. Despite this, all studies in common show that CD4+ T cells are found within the aqueous humor in greater numbers than CD8+ cells. Studies have also shown that apoptosis of infiltrating mononuclear cells within the aqueous humor occurs during noninfectious intraocular inflammation such as Vogt–Koyanagi–Harada (VKH) syndrome. In addition to apoptosis, many of the CD4+ T cells within the aqueous and the cerebral spinal fluid in VKH were Fas+ CD29+ CD45RA+ T (memory) cells, and regulation of the inflammatory response by Fas-Fasl-mediated apoptotic cell death was therefore implicated. However, no study of human ocular inflammatory disease to date has shown that Fasl-Fasl interactions are functionally important in regulating immune responses in the eye. The present study further examined the phenotype of cells infiltrating the aqueous humor (particularly with respect to CD4+ T-cell activation, Fas/Fasl expression, and levels of soluble Fasl/Fasl during AAU) and demonstrates that the aqueous contains soluble Fasl (sFasl) capable of inducing apoptosis in Fas+ cells.

METHODS

Patients, Diagnosis, and Aqueous Sampling

In this prospective study, patients who presented to the emergency rooms of the Eye Department of the Aberdeen Royal Infirmary (Aberdeen, Scotland) with a clinical diagnosis of AAU were enrolled after informed consent and local ethical committee approval in accordance with the tenets of the Declaration of Helsinki. Patients with AAU had no clinical evidence or laboratory findings to suggest posterior uveitis or anterior uveitis associated with systemic disease. At the time of presentation, patients were taking no immunosuppressive agents or topical dexamethasone therapy for the treatment of their uveitis. Aqueous sampling was taken as previously described, with no complications observed as a result of the paracentesis. Samples (volumes of 0.1–0.2 ml) were placed immediately into an Eppendorf vial on ice, and after centrifugation (1000 rpm for 10 minutes) cells were further processed as described below for each assay. Residual aqueous was analyzed for soluble Fas (sFas) and sFasl by enzyme-linked immunosorbent assay (ELISA), western blot analysis, and induction of apoptosis in Jurkat cells (see below). Simultaneously, 5 ml of peripheral venous blood was collected into a sodium heparin evacuated tube (Vacutainer) for leukocyte flow cytometric phenotype analysis and determination of HLA-B27 status. Aqueous and peripheral blood samples of patients undergoing routine cataract surgery (patients without any signs or history of uveitis or other ocular disease) were used as controls.

Flow Cytometric Analysis

Two- and three-color immunophenotyping of peripheral blood leukocytes were performed using mouse monoclonal antibody (mAB) specific for human cell surface markers, which were obtained from Becton-Dickinson unless otherwise stated. These included CD45 (leukocyte common antigen), CD14 (LPS cell surface receptor on monocytes), CD3 (T cells), CD8, CD4, HLA DR (MHC class II antigen), CD69 (activated T-cell blasts), CD19 (B cells), CD16+CD56 (natural killer cells [NK]), CD95 (Fas; Calbiochem), NOK-1bio (Fas-L; Pharmingen), and HLA B7/B27 (SeroTec, Oxford, UK). Aliquots of 100 μl of peripheral blood were added to round-bottomed polystyrene tubes (Falcon), and directly conjugated mAb (conjugated to fluorescein isothiocyanate [FITC], phycoerythrin [PE], or Perchlorophyll protein [PerCP]) were added at predetermined optimal dilutions. After a 30-minute incubation, aliquots were lysed with FACSlyse (Becton-Dickinson) as per manufacturer’s instructions and then washed twice in FACS buffer (phosphate-buffered saline [PBS])/0.02% bovine serum albumin). Unconjugated mAb was detected with rat absorbed FITC-conjugated sheep F(ab′)2, anti-mouse immunoglobulin (Sigma), and biotinylated mAb with streptavidin–PE (Caltag). Cells were then fixed in 1% paraformaldehyde and kept at 4°C until analysis. Acquisition was performed on FACS Calibur flow cytometer and analyzed using CellQuest acquisition and analysis software. Leukocyte gates and instrument variables were set according to forward and side scatter characteristics and using appropriate unconjugated, biotinylated, or directly conjugated isotype immunoglobulin controls. Analysis of fluorescence was performed after further back gating to exclude dead cells and aggregates. Aqueous cells (1500–3000 cells per sample) were similarly prepared, without lysis, and distributed equally into polystyrene tubes for cell surface labeling.

Estimation of sFas and sFasl

sFas and sFasl were estimated in aqueous of AAU patients and control cataract patients by commercial ELISA. One hundred microliters of sample diluent (1:2) was used. sFas (APO-1) was determined using a commercial capture ELISA (Bender Medsystems) and standardized against recombinant sFas. Detection for reading at 450 nm was performed with streptavidin–peroxidase. Similarly, sFasl was determined using a standard commercial capture ELISA, using purified capture and peroxidase-conjugated detector antibody pairs (Medical and Biological Laboratories, Nagoya, Japan), and standardized against recombinant sFasl supplied by the manufacturer. For further identification of sFasl, western blot analysis was performed after 8% to 25% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE; Phast system; Pharmacia, Uppsala, Sweden). In addition to noncellular aqueous, a peptide of sFasl (PP61; Calbiochem Ltd) was run on the gel as a negative control because G247+4 mAb (see below) did not detect peptide by ELISA or western blot analysis. Gels then were stained routinely with Coomassie blue. After protein separation, gels were blotted onto a nitrocellulose membrane (0.45 μm) at 70°C for 30 minutes for chemiluminescence detection of sFasl. Incubation with primary mAb anti-Fasl (G247-4 clone; Pharmingen) was performed at room temperature for 1 hour on a
shaker, and after rinsing in Tris-buffered saline, biotinylated rabbit anti-mouse (Dako) mAb was added for a further 1 hour at room temperature. After further washes, development of blot was achieved with streptavidin-biotinylated complex (Dako) and placed in development solution (Amersham) for 1 to 5 minutes as per manufacturer’s instructions before x-ray detection.

Apoptosis Assay

Apoptosis of mononuclear cells from aqueous samples obtained from AAU patients was detected by flow cytometry and terminal deoxynucleotidyl transferase (TdT)-dUTP terminal nick-end label (TUNEL) staining of slide preparations. For flow cytometry, cells were fixed for 15 minutes in 1% paraformaldehyde in PBS (pH 7.4); after resuspending in PBS, the sample was further centrifuged and pellet resuspended in 70% ethanol at −20°C and stored until analysis. For analysis, ApopTag Plus (Oncor) was used according to manufacturer’s instructions to detect apoptosis by determining the increase in liberated 3′OH DNA ends localized in apoptotic bodies. TdT was used to catalyze the addition of digoxigenin-nucleotide residues to DNA ends generated by fragmentation, which were then detected using a FITC-conjugated anti-digoxigenin mAb and propidium iodide. Further flow cytometric analysis was performed by staining aqueous cells with CD3FITC, Annexin V-PE, and Viaprobe (Pharmingen) as per manufacturer’s instructions, to determine the percentage of apoptotic CD3+ T-cells (see legend; Fig. 5). In brief, after initial staining with CD3FITC and Annexin-V-PE, cells were stained with Viaprobe in calcium–PBS/bovine serum albumin and read on FACS caliber under voltage settings and background fluorescence was set with appropriate isotype controls. TUNEL staining23 was also performed on standard prepared ethanol-fixed slide preparations of cellular aqueous. Before fixation and staining, the slides were treated (1 minute) with collagenase (75 µg/ml) and hyaluronidase (5 U/ml) to prevent protein precipitation and cross-linking from the inhibiting staining procedure. TUNEL procedure was performed using a commercial kit (Trevigen), including standard control slides. Any further protein present was digested with 20 µg/ml of proteinase K. 3′OH end labeling was again performed using Tdt-digoxigenin labeling, and apoptotic cells were detected using streptavidin-peroxidase and DAB substrate.

Induction of Apoptosis in Jurkat Cells

Jurkat cells (2 × 10⁶/ml) were cultured in triplicate in 96-well plates (200 µl) with an optimal concentration of mouse IgM mAb to Fas (clone CH-11; Upstate Biotechnology) or recombinant human Fas-L in the presence or not of anti-Fas mAb (Clone ZB4 [anti-CD95; apo-1]; Immunotech) to block surface Fas on Jurkat cells and thus induction of apoptosis. Test noncellular component of aqueous from both AAU and control samples was added to cell cultures with and without anti-Fas mAb. Cells were grown in RPMI containing 10% fetal calf serum for 4 and 24 hours, and cells then were processed for apoptosis using Annexin-V/propidium iodide (PI) assay24 by flow cytometry (FACScalibur; Becton-Dickinson). Apoptotic cells are characterized by Annexin-V+ PI- phenotype. To assess any contribution toward induction of apoptosis of tumor necrosis factor (TNF)-α present in aqueous, parallel triplicate cultures were set up with optimal concentration (10 ng/ml) of recombinant human TNF-α (R+D Systems, Europe Ltd) or test aqueous with and without blocking with mAb anti-human TNF-α at 10 µg/ml (R+D Systems).

RESULTS

Fas-Positive Mononuclear Cells and Activated CD4+ T Cells in Aqueous Humor during AAU

Cells isolated from aqueous samples of AAU patients were analyzed by two- and three-color flow cytometry and compared with peripheral blood phenotype. Twenty-seven peripheral blood and 12 aqueous samples were analyzed for phenotype. No patients had evidence of systemic disease or concomitant infection at the time of sampling or as a possible cause of their uveitis. Twenty patients were HLA-B27 positive. Figures 1A and 1B present data of the phenotype of infiltrating cells within the aqueous compared with that of peripheral blood. Results indicated a relative increase in the percentage of T-cells in the aqueous compared with that in peripheral blood (25.9% ± 8.7% versus 15.4% ± 1.5% T cells, respectively), whereas the percentage of granulocytes in aqueous was reduced from that seen in peripheral blood (21.25% ± 5.92% versus 61.3% ± 1.2%, respectively). The CD4-positive T-cell proportion within the aqueous was comparable to that in peripheral blood (45.5% ± 10.1% and 47.7% ± 9.1% of CD3+ gate in aqueous and peripheral blood, respectively). B cells were of a lower percentage in the aqueous than in peripheral blood (1.2% ± 0.68% and 12% ± 1.2%, respectively). The majority of CD4+ T-cells within the aqueous was activated (as determined by three-color flow cytometric analysis of percentage of CD4+ CD69 or IL-2R+ expression on gated CD3+ T-cells), expressing CD69 (73.3% ± 6.9%) and IL-2R (54.1% ± 6.4%). There was no difference in aqueous cell phenotype and T-cell activation between HLA-B27–positive or –negative AAU patients (data not shown). Leukocyte subsets do not make up 100% of the cells analyzed because of the damage to cells during processing (paracentesis), and these have been excluded from analysis by scatterplot gate, or cells were fragmented as a result of necrosis/end stage apoptosis. In addition, iris pigment epithelial cells are liberated during the inflammatory process and do not express on their cell surface any leukocyte markers. Figure 1C shows the percentage Fas and Fasl cell surface expression on aqueous-infiltrating CD4+ mononuclear cells. The majority of peripheral T-cells expressed Fas (55.6% ± 11.02%), comparable with the number of CD4+ Fas+ cells within the aqueous (68.6% ± 4.2%). Back gating the Fas+ cells within aqueous to determine scatter characteristics of cells showed that the Fas+ cells were present within characteristic lymphocyte scatter profile, although Fas expression on NK cells or the small number of B cells present within the infiltrate cannot be excluded. Compared with peripheral leukocytes (0.78% ± 0.5%), a high percentage (58% ± 20%) of leukocytes within the aqueous expressed Fasl, and on back gating this population demonstrated a characteristic scatter profile consistent with non-T-cell population. We subsequently performed three-color flow cytometric analysis for CD3, Fas, and Fasl expression, which confirmed our interpretation of the scatterplots and showed that 98% of infiltrating CD3+ T-cells expressed Fas of which only 15% were also Fasl, positive.
FIGURE 1. Comparison of cell surface phenotype and activation markers between leukocytes infiltrating aqueous and peripheral blood. (A) Percentages of lymphocytes, monocytes, and granulocytes were calculated according to CD45/CD14 expression, NK cells according to CD56<sup>+</sup>CD16<sup>+</sup> expression, and B cells according to CD19 expression (10,000 events and 1,500–3,000 events were collected from peripheral blood and aqueous samples, respectively). (B) CD<sup>4+</sup> T-cell activation was calculated by three-color analysis by gating on CD3<sup>+</sup> cells and then subsequently calculating CD69 or IL-2R expression on CD4<sup>+</sup> cells. (C) Fas and FasL was expressed as a percentage of infiltrating CD45<sup>+</sup> cells. *Denotes statistically significant differences of P < 0.02 (Mann-Whitney U test).
Increased Levels of sFasL and Mononuclear Apoptotic Cell Death Present in Aqueous Humor during AAU

ELISA estimation of Fas and sFasL levels in the supernatant of aqueous is shown in Figure 2. The protein concentration (Fig. 2), not unexpectedly because of the concurrent breakdown of the blood-ocular barrier during ocular inflammation, was increased in AAU patients. Samples of vitreous were also included in patients with noninfectious autoimmune posterior uveitis as positive controls to compare protein concentrations in other intraocular inflammatory conditions. In 8 of 16 patients with AAU sFasL was detected within the aqueous by ELISA (25.96 ± 13.9 pg/ml; P < 0.039; Fig. 2), compared with undetectable levels in control patients with cataract. In addition, there was no difference in protein concentration between FasL⁺ and FasL⁻ AAU patients, which may account for sFasL⁻ status. sFas was detected in only 4 AAU patients, whereas no sFas was detected by ELISA in controls. Increased levels of sFasL were also seen in vitreous samples of patients with idiopathic noninfectious posterior uveitis compared with controls, although there was no significant difference in sFasL levels between aqueous (AAU) and vitreous (posterior uveitis) samples. Noncellular aqueous was also analyzed by SDS–PAGE and western blot analysis. Electrophoresis of the aqueous humor from AAU and control cataract patients showed a detectable band of molecular weight (MW) similar to that of sFasL (approximately 26 kDa; Fig. 3A). Western blot analysis confirmed that sFasL was present in the aqueous from AAU patients (Fig. 3B). SDS–PAGE protein bands were also of MW comparable to that of TNF-α in aqueous and cataract control patients, with the presence of TNF-α was confirmed by western blot analysis (data not shown). Levels of apoptotic cell death, as detected by ApopTag Plus (see the Methods section) in the aqueous humor of AAU patients (n = 6), was 9.32% ± 1.25% of infiltrating lymphocytes (as defined by cell scatter profile; Fig. 4A). Less than 2% of granulocyte scatter was apoptotic. In a separate specimen, three-color flow cytometric analysis confirmed that 55.6% of CD3⁺ T cells were apoptotic (Annexin V⁺ Viaprobe⁻; Fig. 4C). Histochemical confirmation of apoptosis was obtained with cytospin TUNEL preparations (data not shown).

sFasL in Aqueous of AAU Patients and Apoptotic Cell Death in Fas⁺ Jurkat Cells

Jurkat cells are greater than 90% Fas⁺ (data not shown), and recombinant human sFasL induces apoptosis in 40.5% ± 2.92% of Jurkat cells after only a 4-hour incubation compared with 13.54% ± 3.4% in medium alone (Fig. 5). Also we confirmed the active induction of apoptosis in Jurkat cells with CH-11 mAb and confirmed the inhibition of CH-11-induced apoptosis with ZB4 clone, which was therefore used throughout the remainder of the experiments (CH-11-induced apoptosis in 21.25% ± 5% of cells in 4 hours and was inhibited by prior incubation with ZB4 clone to basal levels of apoptosis of 12.9% ± 1% of cells). Although the increase in the percentage of apoptotic cell death after 4 hours’ incubation of Fas⁺ Jurkat cells with aqueous from AAU patients was not significant, by 24 hours a significant increase in apoptotic cell death was recorded (69.8% ± 7.6%; P < 0.001). Apoptosis was effectively blocked with prior incubation with anti-Fas mAb (25.9% ± 7.6%; P < 0.01). Jurkat cells do not express quantifiable levels

![Figure 2. ELISA quantification of sFasL and sFas in aqueous. Comparison of protein concentration (top), sFasL (middle), and sFas (bottom) in aqueous of AAU patients (AAU) and cataract patients (controls) or vitreous from patients with idiopathic posterior uveitis undergoing vitrectomy. There were statistically significantly increased levels of sFasL in aqueous of AAU patients compared with that in controls (P < 0.059). Levels of sFasL within aqueous were not accounted for by differences in protein concentration.](https://iovs.arvojournals.org/ on 11/23/2017)
of membrane tumor necrosis factor receptor (TNFr) (data not shown), and therefore, not surprisingly, the addition of recombinant human TNF-α did not induce apoptosis in Jurkat cells after 24 hours of incubation.

**DISCUSSION**

Several pathways of apoptotic regulation of inflammation and tolerance have been proposed, but the relative contribution to and importance of these in human disease remains unclear. In the present study we attempted to evaluate characteristics, both functional and phenotypical, of the inflammatory infiltrate within the immune privileged anterior chamber of the eye by investigating aqueous samples from a cohort of patients with idiopathic AAU. AAU is characterized by a brisk, unpredictable, self-resolving yet recurrent inflammation of the iris and ciliary body within the anterior chamber of the eye, of which 50% of cases are HLA-B27. Recent experimental evidence suggests that within the anterior chamber of the eye Fas/FasL-induced death is the mechanism by which cells are killed, and furthermore it is the apoptotic signal that remains critical to induce immunoregulation. We therefore hypothesized that regulation of the inflammatory response in AAU is mediated via Fas/FasL apoptotic cell death of infiltrating mononuclear cells.

Previous studies have shown that the aqueous leukocytic infiltrate during a range of uveitis conditions comprises, in addition to monocytes and CD8+ T cells, CD45+ CD29+ CD45RO+ Fas+ CD4+ T cells. Furthermore, in a cohort of patients with AAU CD4+ T cells are found to be associated with high levels of IL-12 and IL-10 as well as interferon-γ within the aqueous, although the precise cell origin of cytokine production remains undefined. Despite obvious limitations to any study of leukocytes in the aqueous because of the small sample size routinely obtained, we have still confirmed experimental data that Fas-FasL interactions are active during AAU. In addition, the data are confirmatory and show that the aqueous leukocytic infiltrate consists of an increased proportion of T cells compared with blood and that, although monocyte percentage was less than T cells within the aqueous, they occurred in greater proportion than in peripheral blood (Fig. 1). Infiltrating CD4+ T cells are activated, expressing high levels of CD69 and IL-2R. Whether CD4+ T cells are antigen-specific or not is not known, because putative autoantigens in AAU have not been confirmed to test this. Extrapolation from animal models suggests that AAU has an integral T-cell component and that no one cytokine is requisite to the pathogenesis of AAU. During AAU, the aqueous contains leukocytes, particularly T cells expressing Fas (CD95) and the non-T-cell population expressing its ligand (CD95L); and moreover, sFasL and Fas are detected in greater quantities than...
Later experiments showed that the aqueous could induce apoptotic FasL-dependent cell death in Fas\(^+\) Jurkat cells (Fig. 5). Normal aqueous also contains functional sFasL and induces apoptosis of Fas\(^+\)-dependent cell line, albeit at a lower level, suggesting a constitutive role for Fas/FasL signaling in the anterior chamber.

FasL is a type II integral membrane protein homologous with TNF.\(^{30,31}\) Membrane-bound FasL (mFasL) is released as a 26-kDa soluble form, like TNF,\(^{32}\) by matrix metalloproteinases (MMPs),\(^{33}\) resulting in an equally functional active form. Recent data suggest that with certain cell lines and under certain conditions, in which mFasL is cleaved, the resultant sFasL does not induce apoptosis and therefore is downregulatory\(^{34,35}\) (see below). MMPs have been recorded in the normal aqueous humor as well as raised levels in the aqueous during ocular inflammation,\(^{36}\) which may account for the increase in functional sFasL in AAU samples. Although TNF-\(\alpha\) is also found in increasing amounts early in the course of endotoxin-induced uveitis,\(^{37,38}\) the neutralization of TNF-\(\alpha\) activity resulted in disease exacerbation.\(^{39}\) Our data do not support or deny a role for TNF-mediated activation–induced apoptosis\(^{40}\) in regulation of the inflammatory response. In AAU TNF-\(\alpha\) may play a dual role. First, during the initial inflammatory response as a proinflammatory cytokine, but secondly as a result of the chronic production of low levels of TNF-\(\alpha,^{41}\) suppressing T-cell activation,\(^{42}\) and thus contributing to the immunoregulatory environment of the anterior chamber. TNF-\(\alpha\) may also act by inducing inactivation and apoptosis of the CD69\(^+\) CD16\(^+\) CD56\(^-\) NK cells,\(^{43}\) which we observed infiltrating the anterior chamber during AAU (data not shown).
Evidence suggests that cleaved trimeric sFasL re-identifying the CD3 within the scatter profile of T cells, which was confirmed by analysis showed that apoptotic events occurred predominantly after incubation over 24 hours with aqueous from both AAU patients and controls. Apoptosis was blocked by anti-Fas mAb. TNF-alpha FasL-induced apoptosis is necessary for corneal graft survival.13 Apoptosis has been proposed as a regulatory mechanism pivotal to generation of ACAID.6 In ACAID, TGF-beta can preferentially induce antigen presenting cells to secrete IL-10, which itself is central to the induction of ACAID.46 Interleukin-10 is present in greater quantities than interferon-gamma within the aqueous during AAU19 and therefore IL-10 may suppress DTH reactivity and antigen-specific responsiveness47 and direct the inflammatory response toward Th2 as has been shown experimentally.48 Additionally, antigen presenting cells may traffic from the eye and induce Th2 responses when antigen presentation occurs within the local drainage lymph nodes.5 Alternatively, other mechanisms that preserve Th2 responses may be secondary to the increased susceptibility of Th1 cells to FasL-mediated apoptosis and, thus, their preferential deletion when entering the eye.49 Although we have been unable to identify which subset of T cells is undergoing apoptosis (because of the low numbers of cells for analysis), we postulated that it is predominantly CD4+ T cells because these cells are highly activated and express Fas. Moreover, CD8+ T cells are less susceptible to FasL-mediated apoptosis and may indeed themselves contribute to immune regulation within the anterior chamber first by cytotoxic killing of Th1 cells and second as regulatory cells via the production of IL-10.50 Conversely, however, as we have mentioned, Fas-FasL interactions may be proinflammatory such as via activation of Fas+ granulocytes. During AAU, IL-8 levels within the aqueous are increased51 contributing toward neutrophil recruitment. In addition, neutrophil apoptosis is central to the resolution of acute inflammatory responses, and IL-8 impairs proapoptotic function of Fas-FasL.52 In this study, the majority of apoptotic events within the aqueous was noted within T-cell populations, and the percentage of apoptosis within granulocytes was low.

Although previous studies have investigated the ocular inflammatory infiltrate during uveitis, none have studied either a pure cohort of noninfectious anterior uveitis or potential regulatory mechanisms within the immunoprivileged anterior chamber environment. The role of Fas-FasL interactions is diverse, not only acting as a signal for apoptosis and thus regulating the immune response but also proinflammatory, principally by inducing Fas cytotoxicity. These data report on the role of FasL-mediated apoptosis of lymphocyte populations during AAU and provide a possible explanation for the self-limiting course of the disease and

![Figure 5](https://iovs.arvojournals.org/)
the maintenance of ocular immune privilege as inferred by the ability of sFasL within the control aqueous to also induce apoptosis. Knowledge of the nature of sFasL and MMP activity is required to elucidate why sFasL is proapoptotic and not blocking as previously shown under certain conditions.34,35 Further investigation is also required to investigate the role of Fas-FasL and TNF-α interaction on other cell types (e.g., neutrophils and NK cells and their contribution to the inflammatory response within the anterior chamber).

References


