Low Mature TGF-β2 Levels in Aqueous Humor During Uveitis

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Purpose. To investigate whether transforming growth factor-β2 (TGF-β2), a strong immunosuppressive factor normally present in aqueous humor, is involved in the inflammatory process of clinical uveitis.

Methods. Mature TGF-β2 levels were determined in aqueous humor samples of 9 patients with Fuchs' heterochromic cyclitis, aqueous humor samples of 21 patients with other uveitis entities, and vitreous fluid samples of 19 patients with uveitis by using a commercially available sandwich ELISA. Total TGF-β2 levels in ocular fluids were measured after heat activation. Aqueous humor samples from patients with cataract and glaucoma and vitreous fluid samples from eye bank eyes were tested as controls. Albumin levels, determined by radial immunodiffusion, were used as a measure of the disruption of the blood aqueous barrier.

Results. Significantly lower mature TGF-β2 levels were detected in aqueous humor samples of patients with uveitis, compared to the two control groups without intraocular inflammation. Samples of patients with uveitis without detectable mature TGF-β2 did contain latent TGF-β2 levels (504 to 6024 pg/ml). In aqueous humor, there was a significant negative correlation between mature TGF-β2 and albumin levels. No mature TGF-β could be detected in vitreous fluid. Total TGF-β2 levels in vitreous fluid were significantly lower in samples from patients with uveitis than in samples from eye bank eyes.

Conclusion. These results indicate that the mature TGF-β2 levels in aqueous humor and the total TGF-β2 levels in vitreous fluid are reduced during ocular inflammation. In aqueous humor, this might be caused by binding of mature TGF-β to serum proteins, for instance, α2-macroglobulin, or by a disturbance in the activation process of latent TGF-β2. Invest Ophthalmol Vis Sci. 1994;35:3702–3710.

Transforming growth factor-β (TGF-β) is a multifunctional peptide with immunosuppressive properties.1,2 These properties include inhibition of B- and T-cell proliferation,3,4 adherence of granulocytes,5 respiratory burst of macrophages,6 and HLA gene expression.7 In addition to immunosuppressive properties, TGF-β is also a promoting factor in the wound-healing process and fibrogenesis.8,9 It is suggested that the ability of TGF-β to stimulate fibroblasts and to suppress T and B cells may protect the host from prolonged inflammatory events and promote tissue repair. TGF-β can be synthesized by many different cells and is secreted in a latent complex that needs to be transformed into mature TGF-β to become biologically active.10 There are several isoforms of TGF-β described in humans: three homodimers (TGF-β1, TGF-β2, and TGF-β3), and one heterodimer (TGF-β1,2).11

Several studies have demonstrated the presence of TGF-β in aqueous humor (AH) and have shown that a portion of TGF-β is present in the mature form.12-14 TGF-β in AH contributes to the fluid’s immunosuppressive properties, and it is suggested that TGF-β is important for the maintenance of the immune-privileged environment of the anterior cham-

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TGF-β2 in Ocular Fluids in Uveitis

TGF-β has also been detected in vitreous fluid (VF) of patients with retinal detachment or proliferative vitreoretinopathy, and the level of TGF-β was reported to correlate with the degree of proliferative vitreoretinopathy. Therefore, TGF-β seems to be involved in the pathogenesis of intraocular fibrosis. The predominant isoform of TGF-β in AH and VF is TGF-β2.

Because of the immunosuppressive properties of TGF-β and its ability to inhibit T-cell proliferation, we were interested in whether TGF-β levels in the eye were altered during ocular inflammation. Uveitis is thought to be a T cell-mediated disease, and T cells are the predominant infiltrating cells in the eye during inflammation. One experimental animal study reported that the total TGF-β level decreased during acute intraocular inflammation. In humans, no data are available concerning the TGF-β levels in ocular fluids in uveitis. To investigate this issue, the mature TGF-β2 levels in AH and VF of patients with uveitis were measured with a specific-capture ELISA. Aqueous humor of patients with glaucoma or cataract and VF of eye bank eyes were served as controls.

This study demonstrates that significantly lower levels of mature TGF-β2 are present in AH of patients with uveitis than in AH of patients with no signs of ocular inflammation. In VF, no mature TGF-β was detectable, whereas total TGF-β2 levels were significantly lower during ocular inflammation.

MATERIALS AND METHODS

Samples

Twenty-one AH samples of patients with uveitis with anterior, intermediate, posterior, and panuveitis of different origin, and nine AH samples of patients with Fuchs' heterochromic cyclitis (FHC; n = 9) were used for this study (Table 1). Uveitis was diagnosed according to the criteria of the International Uveitis Study Group. The majority of the AH samples (n = 15) had been collected for diagnostic purposes and were obtained by performing a paracentesis during the active stage of the disease, characterized by cells and flare in AH or VF. Four other AH samples were obtained during cataract surgery in a less active stage of the disease, and no information was available concerning the disease activity during ocular fluid sampling for two AH samples. All the AH samples of patients with FHC (n = 9) were obtained during cataract surgery. Vitreous fluid samples of patients with uveitis (n = 19) were obtained during a therapeutic or diagnostic vitrectomy. Ten patients had active ocular inflammation at the time of vitrectomy, four patients were operated on during a less active stage of the disease, and for three patients no information was available concerning the disease activity. The VF samples were collected undiluted before the infusion line was opened. The indications for therapeutic vitrectomy in these patients were visual deterioration caused by vitreous opacities and progressive inflammation despite medical treatment. The indication for a diagnostic vitrectomy was the suspicion of an ocular lymphoma.

Aqueous humor of patients with glaucoma (n = 33) or cataract (n = 13) and with no evidence of inflammatory eye diseases, and VF of eye bank eyes (n = 17) were tested as controls. The AH samples were collected during trabeculectomy or cataract surgery, respectively. All patients were informed about these procedures, and their informed consent was obtained. No complications were seen after sampling. Vitreous fluid from eye bank eyes was obtained between 5 and 19 hours after death (mean time 13 hours). The samples were stored at −20°C until use.

TGF-β2 ELISA

The TGF-β2 levels were assessed with a commercially available specific-capture ELISA kit (R & D systems, Minneapolis, MN). This immunoassay will only detect mature TGF-β2 in the unbound, biologically active form. Latent TGF-β2 complexes and TGF-β2 in association with α2-macroglobulin are not recognized by antibodies used in this ELISA. There is no cross-reactivity with TGF-β1, but there is 14% cross-reactivity with the heterodimer TGF-β1,2. The 96-well plates provided in the kit are coated with a monoclonal anti-TGF-β2 antibody. After washing, a 200-μl sample dilution was incubated for 2 hours at room temperature. After washing, enzyme-linked polyclonal anti-TGF-β2 was added for detection and incubated for 2 hours. After washing, the substrate solution was added, and the reaction was stopped with 2 M H2SO4.

Recombinant human TGF-β2, provided in the kit, was used as a standard, and results were expressed in picograms per milliliter. All AH and VF samples were diluted 1:2.5 in assay diluent directly in the wells. The concentration of TGF-β2 was calculated by comparing the absorption of samples with that of serial dilutions of human recombinant TGF-β2. The detection limit of the assay was 62.5 pg/ml. In view of the ocular fluid sample dilution (dilution factor 2.5), therefore, the lowest detectable level was 157 pg/ml.

To determine whether latent TGF-β2 was present in AH and VF, some of the AH samples (uveitis, n = 5; FHC, n = 4; cataract, n = 3; glaucoma, n = 20) and VF samples (uveitis, n = 17; eye bank eyes, n = 16) were also tested after heating (8 minutes, 80°C). This is known to activate latent TGF-β. Eighty μl of AH or VF was supplemented with 20 μl of a human serum
TABLE 1. Diagnosis of Uveitis Patients

<table>
<thead>
<tr>
<th>Anatomical Diagnosis</th>
<th>Specific Diagnosis</th>
<th>Number of Aqueous Humor Samples</th>
<th>Number of Vitreous Fluid Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior uveitis</td>
<td>Fuchs' heterochromic cyclitis</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Chronic cyclitis</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Keratouveitis</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Intermediate uveitis</td>
<td>Unknown origin</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Posterior uveitis</td>
<td>Toxoplasma chorioretinitis</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>CMV retinitis</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Toxocara</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Serpiginous uveitis</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Unknown origin</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Retinal vasculitis</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Panuveitis</td>
<td>Crohn's disease</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>VZV infection</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Phacogenic uveitis</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Masquerade syndrome*</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Unknown origin</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Endophthalmitis</td>
<td>Unknown origin</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Aspergillosis</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>30</td>
<td>19</td>
</tr>
</tbody>
</table>

* Specific diagnosis: lymphoma.

albumin solution (165 μg/ml) in a plastic tube. This solution was heated at 80°C for 8 minutes and tested by ELISA. Testing of the human serum albumin solution alone (heated or untreated) by TGF-β2 ELISA did not give a significant result above the buffer controls.

Controls, including incubation of rec-TGF-β2 and activated AH and VF samples with an irrelevant enzyme-linked polyclonal antibody as a conjugate, did not result in a positive signal. To investigate whether ocular fluids interfere with the ELISA, AH and VF samples were spiked with rec-TGF-β2 before testing. The ELISA was not inhibited by either of the ocular fluids.

Serum Proteins

The albumin levels were determined in ocular fluids as a measure for the breakdown of the blood ocular barrier. The α2-macroglobulin levels in AH (uveitis, n = 9; cataract, n = 5; glaucoma, n = 5) and VF (uveitis, n = 4; eye bank eyes, n = 5) were also determined because of the ability of α2-macroglobulin to bind mature TGF-β. Albumin and α2-macroglobulin levels were measured by radial immunodiffusion (LC-Partigen and NOR-Partigen, respectively: Behring, Marburg, Germany). The concentrations were calculated by comparing the results with those of serial dilutions of a standard serum (H00041; Central Laboratory of The Netherlands' Red Cross Blood Transfusion Service; Amsterdam, The Netherlands). The detection limit of the α2-macroglobulin assay was 0.22 mg/ml.

Analysis of Data

Statistical analysis of the data was assessed with the nonparametric Kruskal–Wallis test for multiple patient groups. Differences between two patient groups were estimated with the nonparametric Mann–Whitney test using the Bonferroni method to correct for multiple comparison. Spearman correlation coefficient was determined between two parameters.

RESULTS

Mature TGF-β2 (>157 pg/ml) could be detected in almost all AH samples from patients with glaucoma (97%) or cataract (100%), but only in 6 of the 9 patients with FHC (67%) and 4 of the 21 AH samples of patients with uveitis with different uveitis entities (19%) (Table 2). The levels of TGF-β2 were significantly lower in AH of patients with uveitis with different uveitis entities and patients with FHC than in AH of patients with glaucoma or cataract (uveitis: P = 0.001 and P = 0.001, respectively; FHC: P = 0.001 and P = 0.01, respectively) (Fig. 1). The diagnosis of the four patients with uveitis with detectable mature TGF-β2 levels in AH were toxoplasma chorioretinitis (325 pg/ml), toxocara (296 pg/ml), masquerade syndrome (lymphoma; 265 pg/ml), and intermediate uveitis of unknown origin (257 pg/ml). The level of mature TGF-β2 in AH seems not to be influenced by the anatomic localization of the inflammation, because it was present in AH of patients with anterior, intermediate, and posterior uveitis, and panuveitis. Mature TGF-β2
TABLE 2. Mature TGF-β2 Levels and Albumin Levels in AH

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Mature TGF-β 2 in AH (Median [pg/ml], Range [pg/ml])</th>
<th>Albumin Level in AH (Median [mg/ml], Range [mg/ml])</th>
</tr>
</thead>
<tbody>
<tr>
<td>Different uveitis entities</td>
<td>4/21 (19%)</td>
<td>1.95 (0.07--44.3)</td>
</tr>
<tr>
<td>Fuchs' heterochromic cyclitis</td>
<td>6/9 (67%)</td>
<td>0.26 (0.12--0.95)</td>
</tr>
<tr>
<td>Cataract</td>
<td>13/13 (100%)</td>
<td>0.22 (&lt;0.001--0.44)</td>
</tr>
<tr>
<td>Glaucoma</td>
<td>32/33 (97%)</td>
<td>0.12 (0.02--0.88)</td>
</tr>
</tbody>
</table>

could be detected in only 2 of the 15 AH samples obtained during active ocular inflammation, compared to 8 of the 13 samples (FHC, n = 9; different uveitis entities, n = 4) obtained during cataract surgery in a less active stage of the disease (chi-square analysis, P = 0.01). Therefore, low mature TGF-β2 levels in AH seem to be related to active ocular inflammation. There was no relation between mature TGF-β2 level and age or sex of the patients. No information was available to investigate the relation between TGF-β2 levels in AH and the occurrence of posterior synechiae, because TGF-β may promote fibrosis.

Mature TGF-β (>157 pg/ml) was not detectable in VF samples obtained either from patients with uveitis or from eye bank eyes.

To determine whether latent, biologically inactive TGF-β is present in AH and VF, some of the samples were also tested after heat activation. TGF-β2 could be detected in all nine AH samples of patients with ocular inflammation (uveitis, n = 5; FHC, n = 4). In eight of these samples, no mature TGF-β2 was observed before heat activation, and, in the other sample of a patient with FHC, the mature TGF-β2 level was 236 pg/ml (Table 3). The total TGF-β2 level rose after heat activation in the majority of the AH samples of patients with glaucoma (median, 531 pg/ml; range, 300 to 1223 pg/ml) and cataract (median, 491 pg/ml; range, 445 to 767 pg/ml) (Fig. 2). In 1 of the 3 samples of patients with cataract and in 4 of the 20 samples of patients with glaucoma, the TGF-β2 level was not affected by heating. Therefore, all the TGF-β2 was present in the mature form.

After heat activation, TGF-β2 could be detected in all the VF samples of eye bank eyes (100%; median, 1364 pg/ml; range, 231 to 5061 pg/ml) and in 7 of the 17 VF samples of patients with uveitis (41%; median, 332 pg/ml; range, 225 to 2879 pg/ml) (Fig. 3). The difference in the TGF-β2 levels after heat activation between VF samples of patients with uveitis and those of eye bank eyes was significant (P = 0.000086).

The two patients with the highest total TGF-β2 concentrations in VF (2897 and 1104 pg/ml) had retinal vasculitis. The diagnosis of the other patients with uveitis with latent TGF-β2 in VF were intermediate uveitis of unknown origin (332 pg/ml), posterior uveitis of unknown origin (309 pg/ml), and masquerade syndrome (lymphoma: 229 pg/ml). No relation was observed between the presence of total TGF-β2 in VF and the activity of ocular inflammation, because latent TGF-β could be detected in 5 of the 10 VF samples obtained during active ocular inflammation and in 2 of the 4 samples obtained during a less active stage of the disease. However, the number of samples is too small to make a reliable conclusion on this issue.

The influence of immunosuppressive therapy on the intraocular TGF-β2 levels was also investigated. Corticosteroid treatments at the time of paracentesis varied from eye drops (n = 8), periocular injection (n = 1), and systemic (n = 1) to no corticosteroid therapy (n = 13) or unknown treatment (n = 7) (Table 4). Mature TGF-β2 could be detected in 2 of the 8 AH samples from patients treated with corticosteroid eye drops (uveitis, 1 of the 6 samples; FHC, 1 of the 2 samples), versus 6 of the 13 samples from patients...
TABLE 3. Mature and Total TGF-2 Level in Aqueous Humour of Uveitis Patients

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Mature TGF-β2 (pg/ml)</th>
<th>Total TGF-β2 (pg/ml)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Panuveitis uveitis of unknown origin</td>
<td>&lt;157</td>
<td>6024</td>
</tr>
<tr>
<td>Intermediate uveitis of unknown origin</td>
<td>&lt;157</td>
<td>1910</td>
</tr>
<tr>
<td>Phacogenic uveitis</td>
<td>&lt;157</td>
<td>1327</td>
</tr>
<tr>
<td>Toxoplasma chorioretinitis</td>
<td>&lt;157</td>
<td>671</td>
</tr>
<tr>
<td>Masquerade syndrome†</td>
<td>&lt;157</td>
<td>504</td>
</tr>
<tr>
<td>Fuchs’ heterochromic cyclitis</td>
<td>236</td>
<td>1051</td>
</tr>
<tr>
<td>Fuchs’ heterochromic cyclitis</td>
<td>&lt;157</td>
<td>706</td>
</tr>
<tr>
<td>Fuchs’ heterochromic cyclitis</td>
<td>&lt;157</td>
<td>671</td>
</tr>
<tr>
<td>Fuchs’ heterochromic cyclitis</td>
<td>&lt;157</td>
<td>447</td>
</tr>
<tr>
<td>Human serum albumin</td>
<td>&lt;157</td>
<td>&lt;157</td>
</tr>
</tbody>
</table>

* Total TGF-β2 level was determined after heat activation.
† Specific diagnosis: lymphoma.

without treatment (uveitis, 3 of the 8 samples; FHC, 3 of the 5 samples) (chi-square analysis, P = 0.3). There seems to be no relation between the presence of mature TGF-β2 in AH and local corticosteroid treatment; however, the number of patients might be too small to show a difference. At the time of vitrectomy, three patients were systemically treated with corticosteroids, and all three patients had latent TGF-β2 in VF.

α2-Macroglobulin

In the search for an inhibitor of mature TGF-β in ocular fluids, the α2-macroglobulin level was determined, because this protein is able to bind mature TGF-β. In all except one ocular fluid sample, the α2-macroglobulin level was below the detection limit of the assay. In one AH sample of the uveitis group with severe breakdown of the blood ocular barrier, the α2-macroglobulin level was 0.22 mg/ml. There was no mature TGF-β2 detectable in this AH sample.

Albumin

The albumin levels were determined as a measure of the breakdown of the blood aqueous barrier, because several serum proteins are able to bind mature TGF-β. The median albumin levels are provided in Table 2. The albumin levels in AH of patients with different uveitis entities were significantly higher than those of the three other patient groups (FHC, P = 0.01; glau-

![Figure 2](image1.png)

**FIGURE 2.** Mature and total TGF-β2 levels in AH of patients with different uveitis entities. FHC, cataract, or glaucoma. The total TGF-β2 levels were measured after heat activation of the samples (8 minutes, 80°C). All the AH samples of patients with different uveitis entities and FHC without detectable mature TGF-β2 appeared to contain latent TGF-β2.

The broken line represents the detection limit of the assay.

![Figure 3](image2.png)

**FIGURE 3.** Mature and total TGF-β2 levels in vitreous fluid obtained from patients with different uveitis entities and eye bank eyes. No mature TGF-β2 could be detected in vitreous fluid in both groups. Significantly lower total TGF-β2 levels were detected in vitreous fluid obtained from patients with uveitis than in vitreous fluid from eye bank eyes (P = 0.0000086). The broken line represents the detection limit of the assay.
TABLE 4. Number of Patients With Mature TGF-β2 in AH and Steroid Treatment

<table>
<thead>
<tr>
<th>Corticosteroid Therapy</th>
<th>Different Uveitis Entities</th>
<th>Fuchs Heterochromic Cyclitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eye drops</td>
<td>1/6</td>
<td>1/2</td>
</tr>
<tr>
<td>Periocular injection</td>
<td>0/1</td>
<td>—</td>
</tr>
<tr>
<td>Systemic treatment</td>
<td>0/1</td>
<td>—</td>
</tr>
<tr>
<td>No treatment</td>
<td>3/8</td>
<td>3/5</td>
</tr>
<tr>
<td>Treatment unknown</td>
<td>0/5</td>
<td>2/2</td>
</tr>
</tbody>
</table>

 coma, \( P = 0.001 \); cataract, \( P = 0.001 \). The albumin levels in AH of patients with FHC were just significantly higher than those in patients with glaucoma (\( P = 0.05 \)) but not in patients with cataract. There was a weak but significant negative correlation between the mature TGF-β2 and albumin levels in AH (\( r = -0.62; P = 0.000001 \)).

**DISCUSSION**

This study demonstrates that significantly lower levels of mature TGF-β2 are present in AH of patients with uveitis than those in AH of patients with no signs of ocular inflammation. The mature and total TGF-β2 levels in AH of patients with cataract or glaucoma measured by ELISA are comparable to the levels measured with the CCL-64 bioassay by other investigators.\(^{12}\) Because mature TGF-β is one of the immunosuppressive factors normally present in AH, a decrease in the TGF-β level might affect the immunosuppressive environment of the anterior chamber.\(^{14,22}\) Previous studies have demonstrated that various cytokines are involved in the inflammatory processes in uveitis.\(^{23}\) In experimental animal studies, the cytokines IL-1, IL-6, IL-8, TNF-α, and IFN-γ have been shown to induce uveitis after injection into the eye.\(^{31-35}\) The cytokines IL-2, IL-6, IL-8, and IFN-γ have been detected in the human eye during ocular inflammation.\(^{36-38}\) TGF-β can regulate the effects of several of these cytokines. For instance, it can inhibit the IL-1β-induced production of IL-6 by monocytes,\(^{33}\) regulate the production of IL-6 by IL-1-stimulated fibroblasts,\(^{36}\) and inhibit the IL-2-induced T-cell proliferation,\(^{37}\) and it has the opposite effect of TNF-α on the development of cytotoxic T cells.\(^{34}\) Therefore, a decrease in mature TGF-β levels in the eye during ocular inflammation might cause a disturbance of the regulatory or feedback mechanisms of various cytokines. This could maintain or even exacerbate the inflammatory processes in the eye.

Aqueous humor samples of patients with uveitis without detectable mature TGF-β2 levels contained various amounts of latent TGF-β2, whereas in experimentally induced uveitis in rabbits, it was observed that the total TGF-β level decreased during active uveitis.\(^{19}\) Our observations indicate that during ocular inflammation, the regulation of the TGF-β2 activation, rather than the TGF-β2 production itself, is disturbed. TGF-β2 is produced and secreted in a latent complex that is unable to bind to the receptor.\(^{10}\) The mechanism of latent TGF-β activation in the eye is still not clear. It is thought that activation in vivo takes place by exogenous proteases, such as cathepsin-D or plasmin, which are able to activate latent TGF-β in vitro.\(^{35}\) In the posterior part of the eye, it has been suggested that retinal pigment epithelial cells play a role in the activation of latent TGF-β, because these cells contain acid hydrolases such as cathepsin and tissue plasminogen activator.\(^{36-38}\)

In AH, there was a significant negative correlation between the TGF-β2 levels and the albumin concentration. Breakdown of blood ocular barrier during ocular inflammation might affect the mature TGF-β2 levels in several ways. It causes an influx of protease inhibitors that might subsequently disturb the proteolytic cleavage of the latent TGF-β complex. Another possibility is that mature TGF-β2 levels in AH bind to certain blood proteins, such as α2-macroglobulin; these complexes cannot be detected by the assay we used. α2-Macroglobulin is a serum protein that regulates the distribution and activity of several cytokines, including TGF-β.\(^{39}\) It is not yet clear whether TGF-β bound to α2-macroglobulin remains biologically active or whether this complex functions as a clearance mechanism. Normal levels of α2-macroglobulin in AH are unknown, but a concentration of 10 μg/ml would be sufficient to bind the mature TGF-β2 in AH.\(^{40}\) This concentration of α2-macroglobulin is less than the detection limit of the assays we and others used.\(^{41}\) Other soluble factors present in the serum that can bind TGF-β are proteoglycans also called “betaglycans.”\(^{42}\) However, the significantly lower mature TGF-β2 levels in AH of patients with FHC cannot be explained by breakdown of the blood ocular barrier, because the albumin levels in AH did not differ from those of patients with cataract and differed only slightly from patients with glaucoma.

No mature TGF-β2 could be detected in VF obtained from irriminated eyes and eye bank eyes. Other investigators already demonstrated the presence of TGF-β in VF, after activation of the samples.\(^{16}\) These observations might indicate that in VF, TGF-β immediately binds to proteins or receptors after it is transformed into the mature form. In this study, we investigated whether total TGF-β2 levels in VF were altered during ocular inflammation, and it seemed that the
levels were decreased. Treatment with corticosteroids did not seem to be responsible for the decreased total TGF-β2 levels in VF, because three patients with detectable total TGF-β2 in VF were systemically treated with corticosteroids at the time of vitrectomy. The lower levels of latent TGF-β2 levels in VF might be caused by a reduced synthesis or by an increased consumption during ocular inflammation. Several resident ocular cells have been implicated as the sources of TGF-β2 in the eye. Diffusion from the circulation is less likely for TGF-β2, because only small amounts of this isoform are present in plasma.\textsuperscript{45} Immunohisto logic studies of the posterior part of the eye showed the presence of TGF-β2 in the connective tissue of long ciliary arteries in the choroid and in hyalocytes. Hyalocytes also contained other isoforms of TGF-β.\textsuperscript{36} Conner et al found that retinal pigment epithelial cells can synthesize and secrete TGF-β in vivo.\textsuperscript{14} Immunolocalization studies of the anterior segment of the human eye demonstrated the presence of TGF-β1 and TGF-β2 in the ciliary body and on the outer surface of the eye, but there was no immunoreactivity with anti-TGF-β3 in the anterior segment.\textsuperscript{15} An indication for local TGF-β production in porcine eyes is the isolation of TGF-β1 mRNA from trabecular meshwork cells and of TGF-β2 mRNA from the ciliary body, from the iris and corneal epithelium, and from the cultured trabecular meshwork cells.\textsuperscript{16,17} Whether the aforementioned tissues and cells are responsible for the production of TGF-β in AH and VF and whether the production by these cells is affected during uveitis need further investigation.

The physiological function of TGF-β in the tissues of the eye is not fully understood. Disturbance of the TGF-β regulation might lead to several pathologic processes. For instance, TGF-β seems to participate in the process of proliferative vitreoretinopathy, and it has been suggested that it is involved in the pathogenesis of glaucoma.\textsuperscript{16,15} In this study, low mature TGF-β2 levels were observed in AH during uveitis. T cells are generally thought to play an important role in this disease.\textsuperscript{17} Compared to cyclosporin A, a T cell-specific immunosuppressive drug frequently used in uveitis, TGF-β is a much stronger suppressant for T lymphocytes on a molar basis.\textsuperscript{38,39} Therefore, there might be a therapeutic implication for TGF-β as an antiinflammatory or immunosuppressant agent in uveitis. In experimental autoimmune uveitis, intravitreal application of TGF-β showed a significant reduction in the severity of the disease.\textsuperscript{50} Still another area of potential therapeutic importance of TGF-β is its contribution to the wound-healing process. The benefit of intravitreal administration of TGF-β has already been demonstrated in the surgical management of full-thickness macular holes.\textsuperscript{51} Because TGF-β seems to be involved in different physiological and pathologic processes in the eye, insight into the local regulatory mechanisms of TGF-β might offer several therapeutic applications for various ocular diseases in the future.

**Key Words**

TGF-β2, aqueous humor, vitreous fluid, uveitis, Fuchs’ heterochromic cyclitis

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