Effect of Transforming Growth Factor Beta-1 in Endotoxin-Induced Uveitis

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Purpose. Transforming growth factor-beta 1 (TGFβ-1) can modulate inflammation. Endotoxin-induced uveitis (EIU) is characterized by acute uveal inflammation related to the release of cytokines, including interleukin (IL)-6. The authors investigated the effect of TGFβ-1 on EIU in mice.

Methods. Three independent experiments were performed. Endotoxin-induced uveitis was induced in C3H/HeN mice by an injection of 200 μg of lipopolysaccharide (LPS). Two micrograms of TGFβ-1 in 0.1 ml phosphate-buffered saline (PBS) or 0.1 ml PBS alone was administered intraperitoneally at 8 hours after LPS injection. Twenty-four hours after LPS injection, the aqueous humor of the right eyes was collected for leukocyte count, protein concentration, and IL-6 assay. Left eyes were processed for routine histology.

Results. TGFβ-1-treated mice showed less ocular inflammation histologically than to the animals that were given PBS. This was confirmed by decreases in leukocyte count, protein concentration, and IL-6 level in the aqueous humor.

Conclusions. TGFβ-1 inhibits the development of EIU. TGFβ-1 may be useful for the modulation of uveitis in humans. Invest Ophthalmol Vis Sci. 1997;38:257-260.

Transforming growth factor-beta 1 (TGFβ-1) has diverse functions that affect cellular development and immunologic processes.1 TGFβ-1 can modulate immune responses by initiation and resolution of inflammatory events and has potent regulatory effects on other cytokines.2 The administration of TGFβ-1 has been shown to suppress inflammation in several animal models, including experimental arthritis, experimental allergic encephalomyelitis, and experimental autoimmune neuritis.3,4 We also have reported that systemic TGFβ-1 inhibits recurrent inflammation in experimental melanin protein-induced uveitis.5

Endotoxin-induced uveitis (EIU) is an experimental model for acute anterior uveitis in humans. It can be induced in various species. In mice, iridocyclitis and posterior vitritis occur 8 hours after the subcutaneous injection of a sublethal dose of lipopolysaccharide (LPS), and they peak at 24 hours.5 Although the mechanism for EIU remains unknown, cytokines such as interleukin (IL)-1, IL-6, tumor necrosis factor alpha (TNFα), and interferon γ have been shown to play an important role in the development of EIU in the rat.6,7 During the course of EIU, TGFβ-1 mRNA levels in the rat are relatively stable.7 In a preliminary study,
TABLE 1. Inflammatory Cells in the Endotoxin-induced Uveitis Mice Received Systemic TGFβ-1 of PBS

<table>
<thead>
<tr>
<th>Experiment Group</th>
<th>TGFβ1-treated</th>
<th>PBS-treated</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>41.75 ± 5.74*</td>
<td>170.75 ± 20.34</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>12.88 ± 3.13</td>
<td>42.05 ± 28.25</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>35.00 ± 6.96</td>
<td>170.00 ± 52.07</td>
</tr>
</tbody>
</table>

*Mean ± SEM per histologic section of the left eye.
TGFβ1 = transforming growth factor β-1; PBS = phosphate-buffered saline.

however, we observed that the effect of TGFβ-1 on EIU was accomplished only when TGFβ-1 was given at 8 hours, not at the peak of inflammation, after injection of LPS in the mouse. In the current study, we investigated the effect of systemic TGFβ-1 on mice with EIU.

MATERIALS AND METHODS. Female C3H/HeN mice, 6 to 8 weeks of age, were obtained from the National Cancer Institute (Frederick, MD) and were treated according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Endotoxin-induced uveitis was induced by injecting 0.2 μg Salmonella typhimurium endotoxin (DIFCO, Detroit, MI) suspended in 0.1 ml of sterile phosphate-buffered saline (PBS) into one hind footpad. Mice were administered intraperitoneally either 2 μg recombinant human TGFβ-1 (rhTGFβ-1, kindly provided by Genentech, San Francisco, CA) in 0.1 ml PBS or 0.1 ml PBS alone at 8 hours after endotoxin administration. The dosage of TGFβ-1 was selected according to its effect on other animal models.

Paracentesis of the right eye was performed 24 hours after endotoxin injection. Then the mice were killed, and the left eyes were enucleated for histology. The collected aqueous fluid from the right eye of each mouse was analyzed for cell count and pooled for protein concentration and IL-6 assay. Briefly, 1 μl of aqueous fluid was placed on a silanized glass slide, air dried, and stained with Giemsa. The total number of

FIGURE 1. Photomicrographs of eyes with endotoxin-induced uveitis reveal fewer inflammatory cells in the anterior and posterior chambers and in the posterior vitreous of the mice that were given TGFβ-1 (A,B) than the mice that received phosphate-buffered saline (C,D). Hematoxylin and eosin, ×200.
the cells on the slide was counted and recorded. The rest of the aqueous from the same treatment group of animals was pooled. Pooled aqueous obtained from the right eyes of each group was measured for protein concentration using Coomassie colorimetric assay by reference to an albumin standard (Pierce, Rockford, IL) and for IL-6 level by ELISA (PerSeptive Diagnostics, Cambridge, MA). The enucleated left eyes were fixed, embedded in methacrylate, and sectioned vertically for histologic study as previously described.3

Three independent experiments were performed. In the first experiment, the collected aqueous humor of the right eye of each mouse was used to calculate for cell counts, and the rest of the aqueous of each group (six mice per group) was pooled and measured for protein concentration. In the second experiment, the pooled aqueous humor of the right eyes obtained from each group (10 mice per group) was assayed for IL-6 level only. In the third experiment, the collected aqueous humor of the right eyes (20 mice per group) was used for determining cell counts of each mouse and for measuring protein and IL-6 levels of each group. In all three experiments, the left eyes were processed for routine histology, and the total number of infiltrating cells in the anterior chamber and the posterior vitreous were counted by a masked observer (CCC).

The effect of TGF/β-1 on EIU between the TGFβ-1-treated and the control PBS groups were compared using Spreadware statistics for the Microsoft Excel program (Apple, Cupertino, CA). Because these data were not distributed normally, the nonparametric Mann–Whitney test was used, and the means were calculated only for descriptive purpose. The null hypothesis was rejected when the calculated P value was less than 0.05.

RESULTS. Three independent experiments demonstrated similar results. All mice given LPS developed EIU. Ocular infiltrating cells of the left eyes per histologic section, along with statistical comparisons, were recorded in Table 1. The EIU mice that received TGFβ-1 showed significantly less inflammation than the controls (Fig. 1). Figure 2 show data of individual animals in the second experiment.

Numbers of leukocytes (mean ± SEM per microliter) in the anterior chamber of the right eyes obtained by paracentesis of the first (n = 6 per group) and the third (n = 20 per group) experiments were 101 ± 14.01 and 89 ± 36.58 for the TGFβ-1 treated mice and 191 ± 11.52 and 399 ± 73.54 for the controls, respectively. P values were 0.031 and 0.039, respectively. Protein concentrations (mg/ml) measured from the pooled aqueous of these two experiments were 2.70 and 2.37 for the TGFβ-1-treated groups and 8.00 and 7.35 for the controls, respectively. Aqueous

![Graph](https://via.placeholder.com/150)

**FIGURE 2.** Histogram of the second experiment shows a lower number of infiltrating cells in eyes with endotoxin-induced uveitis in mice that received transforming growth factor beta-1 than in control mice.

IL-6 levels (pg/ml) from the second (n = 10 per group) and the third (n = 20 per group) experiments were 1,200 and 8,300 for the TGFβ-1-treated mice and 5,000 and 10,600 for the control animals, respectively.

DISCUSSION. We have demonstrated that systemic delivery of TGFβ-1 inhibits EIU. Less ocular inflammation and lower aqueous protein concentration are found in TGFβ-1-treated mice. The suppressive mechanism may involve several immunologic regulatory functions of TGFβ-1. de Vos and colleagues have studied the cytokine profile of EIU and reported that elevations of IL-6 and TNFα in the aqueous occur and initially peak 2 to 6 hours after LPS challenge; then they peak 18 to 20 hours later. The early release of IL-6 and TNFα in aqueous humor suggests that these cytokines may serve as initial mediators of ocular inflammation. Results of this study may reflect the interaction between TGFβ-1 and other cytokines, involving a cytokine network in eyes with EIU.

The current study detected lower IL-6 levels in aqueous humor of the mice treated with TGFβ-1. Less IL-6 in the anterior chamber would have dampened the development of EIU in these animals. TNFα in eyes with EIU peaks initially at 2 to 6 hours and later rises concomitantly, with maximal uveitis at 18 to 24 hours after injection of LPS. TGFβ-1 has been demonstrated to suppress the induction of TNFα expression at the protein and the mRNA levels of astrocytes by inhibiting TNFα gene transcription. Systemic administration of TGFβ-1 in mice with EIU could repress TNFα and improve ocular inflammation.
The suppressive effect of TGFβ-1 on EIU may be speculated further by its interaction with inducible nitric oxide synthase (iNOS). TGFβ-1 reduces iNOS mRNA translation and increases degradation of iNOS protein in macrophages. Inhibition of EIU has been achieved by inhibition of NOS.10

In summary, the current study has demonstrated that systemic TGFβ-1 is able to suppress acute uveitis in the mouse. The mechanism involves the TGFβ-1 effect on multiple cytokines and inflammatory mediators that are produced during the course of EIU. TGFβ-1 may become a potential therapeutic cytokine for patients with uveitis.

Key Words
cytokine, endotoxin-induced uveitis (EIU), interleukin-6, ocular inflammation, transforming growth factor beta-1 (TGFβ-1)

References

Modeling Spatial Integration and Contrast Invariance in Visual Pattern Discrimination
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Purpose. Human pattern discrimination performance has been reported to be largely independent of stimulus contrast but to depend on stimulus area. The authors propose a model that combines the effects of spatial integration and contrast. The model is based on the computation of similarity between pattern templates in memory and signals to be discriminated using normalized correlation. There are also two sources of additive noise, one before and one after the computation of correlation. The model was compared with human observers in an orientation discrimination task.

Methods. Orientation discrimination thresholds of human observers were measured for sinuoidal gratings of various areas, contrasts, and spatial frequencies. A two-interval, forced-choice method was used. The performance of the model was determined by using computer simulations.

Results. It was found that the effects of contrast and grating area were interrelated: The decrease of orientation thresholds as a function of grating area was considerably larger at low than at high contrast. On the other hand, orientation thresholds decreased clearly as a function of contrast at the smallest grating areas but hardly at all at the largest grating areas. The model accounted well for the experimental findings.

Conclusions. Because the invariance of orientation discrimination with respect to stimulus contrast depended on area, the cause of the invariance appeared to occur after spatial integration. The model explains this so that, with increasing contrast or area, the normalized correlation gradually approached a constant value. The proportion of pretemplate noise became negligible compared to the constant posttemplate noise. Thus,