Expression of Multiple Cytokines and IL-1RA in the Uvea and Retina During Endotoxin-Induced Uveitis in the Rat

Alex F. de Vos,* Vincent N. A. Klaren,* and Aize Kijlstra†

Purpose. Ample evidence is available demonstrating that cytokines play a pivotal role in the pathogenesis of uveitis. Because little is known concerning the site of cytokine synthesis in the eye, cytokine mRNA expression was analyzed in the uvea, retina, and cornea during endotoxin-induced uveitis (EIU) in the rat.

Methods. RNA was isolated from the iris, ciliary body, choroid-sclera, retina, and cornea at different points in time after foot-pad injection of 200 µg lipopolysaccharide (LPS) in Lewis rats. Reverse-transcription polymerase chain reaction analysis was used to determine tumor necrosis factor α (TNF-α), interleukin 1β (IL-1β), IL-6, IL-10, interferon γ (IFN-γ), monocyte chemotactic protein 1 (MCP-1), macrophage inflammatory protein 2 (MIP-2), and IL-1 receptor antagonist (IL-1RA) mRNA expression.

Results. Maximal mRNA expression of all cytokines examined already was observed in the uvea 4 hours after systemic LPS injection, before the onset of clinical uveitis. Elevated expression of TNF-α, IL-1β, IL-6, IFN-γ, MCP-1, and MIP-2 was also observed concomitant with maximal uveitis, at 22 to 24 hours. Except for IL-10 and IFN-γ, all cytokines investigated were induced in the retina, with maximal expression at 22 to 24 hours. Expression of IL-1RA was detected in the uvea and retina at 4 hours and remained elevated up to 48 hours, when the clinical uveitis started to decline. LPS did not induce cytokine expression in the cornea. Strikingly, a considerable expression of IL-1RA was found in normal corneas, suggesting an inherent control mechanism for IL-1-mediated responses.

Conclusions. Systemic LPS injection induces elevated mRNA expression of multiple cytokines and IL-1RA in the uvea and retina during various stages of EIU. This suggests that these mediators may contribute to the development and recovery of this intraocular inflammation.

to diminish 48 hours after injection and resolves within 1 week.

Because LPS is a potent inducer of various cytokines, it is plausible that these mediators play a role in the pathogenesis of EIU. This is supported by the fact that EIU in the rat is associated with systemic and intraocular release of TNF-\(\alpha\) and IL-6.\(^{17,18,21}\) Both TNF-\(\alpha\) and IL-6 were detected in aqueous humor 4 hours after foot-pad injection of LPS and at the moment of maximal uveitis. Analysis of uveal mRNA revealed that IL-6 expression coincided with intraocular IL-6 activity,\(^{18}\) which indicates local production of IL-6 during EIU. Furthermore, intraocular injection of TNF-\(\alpha\) or IL-6 in rats causes an acute uveitis that largely resembles the response to LPS.\(^ {17,22}\) Together, these observations imply that TNF-\(\alpha\) and IL-6 may contribute to the pathogenesis of EIU. Other cytokines, including IL-1\(\alpha\), IL-1\(\beta\), IL-8, and IFN-\(\gamma\), are likely to be involved in EIU because intraocular injection of these mediators also induces acute uveitis in animals.\(^{22}\) Moreover, others recently showed elevated expression of several cytokines, including IL-1\(\alpha\), IL-1\(\beta\), IFN-\(\gamma\), TNF-\(\alpha\), and IL-6, in the iris–ciliary body during the initiation of EIU, using reverse-transcription polymerase chain reaction (RT-PCR) analysis.\(^{23,24}\)

In the present study, we have expanded the investigations on intraocular cytokine mRNA expression during EIU. By RTPCR analysis, we examined the expression of TNF-\(\alpha\), IL-1\(\beta\), IL-6, and IFN-\(\gamma\), as well as IL-10, MCP-1, MIP-2 (which is functionally related to IL-8),\(^{25}\) and IL-1RA in the iris, ciliary body, choroid–sclera, retina, and cornea.

**MATERIALS AND METHODS**

**Experimental Design**

Inbred male Lewis rats (Harlan Sprague-Dawley, Indianapolis, IN), weighing 150 to 200 g (6 to 8 weeks of age), were used in this study. All animals were treated according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Rats were injected with 200 \(\mu\)g of LPS (Salmonella minnesota LPS, lot 89F4007; Sigma Chemical, St. Louis, MO) divided over the two hind foot-pads, as described previously.\(^ {17}\) At different points in time (0, 2, 4, 22, 24, and 48 hours after LPS injection), rats were anesthetized by intraperitoneal injection of 36 mg pentobarbital (0.6 ml Nembutal; Algin, Maassluis, The Netherlands) and perfused with 200 ml sterile, pyrogen-free saline, after which the eyes were immediately enucleated. Eyes were obtained from two independent experiments.

**RNA Isolation and cDNA Synthesis**

The eyes were quickly dissected using an operation microscope, fine surgical scissors, and forceps. Extraocular muscles were removed first; then the cornea, iris, ciliary body, retina, and choroid with sclera (designated choroid–sclera) were isolated. The retina was always found detached from the choroid and could only be cut free at the optic nerve head. The isolated tissues of both eyes of each rat were pooled and frozen immediately in liquid nitrogen. Total RNA was isolated by a single-step extraction method,\(^ {26}\) using RNAzol (Cinna Biotecx Laboratories, Houston, TX), and dissolved in 10 \(\mu\)l water. Initial experiments revealed that the amount of total RNA isolated from the different tissues was very small (0.5 to 5 \(\mu\)g). Furthermore, a considerable amount of RNA was lost by measurement of the concentration. Therefore, 9 \(\mu\)l of the RNA solution was used for reverse transcription; the remaining 1 \(\mu\)l was used as a control in the \(\beta\)-actin PCR to ensure that cDNA was not contaminated with genomic DNA. cDNA was synthesized using oligo(dT)\(^ {12-18}\) as a primer and 200-U superscript RNaseH reverse transcriptase (Gibco–BRL, Eggenstein, Germany), according to the manufacturer’s instructions. After incubation for 1 hour at 42°C, the reaction was terminated by heating the mixture at 68°C for 5 minutes.

**PCR Primers and Internal Control Probes**

The sense and antisense PCR primers for \(\beta\)-actin or different cytokines were designed to be located in separate exons of the appropriate rat genes. The control probes for Southern hybridization were designed to cross exon–exon boundaries. Oligonucleotides were synthesized at Pharmacia Biotech (Woerden, The Netherlands) or Applied Biosystems (Maarsen, The Netherlands). Sequences of the primers and probes are shown in Table 1. Specific PCR and hybridization conditions for each oligonucleotide set were selected using positive control cDNA, which consisted of a cDNA pool obtained from rat spleen cells that had been cultured for 1, 2, or 4 hours in the presence of 1 \(\mu\)g/ml LPS.

**PCR**

PCR amplification was performed using an automated thermocycler (Perkin Elmer Cetus, Norwalk, CT). The PCR reaction mixture (50 \(\mu\)l) contained 10-mM Tris–HCl, pH 9.0, 50-mM KCl, 0.1% Triton X-100, 1.5-mM MgCl\(_2\), 0.2-mM dNTPs, 0.2-\(\mu\)M sense and antisense oligonucleotide primers, and 1-U Taq DNA polymerase (Promega, Madison, WI). Because the amount of RNA was insufficient for measurement before cDNA synthesis, the cDNA concentration of the different samples was normalized to yield equivalent amounts of \(\beta\)-actin PCR product. Differences in \(\beta\)-actin cDNA concentration were determined as described by Mohler and Butler.\(^ {27}\) Briefly, cDNAs were diluted (1:20, 1:80, 1:320, and 1:1280), and 5 \(\mu\)l of each dilu-
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### TABLE 1. DNA Sequences of Oligonucleotide PCR Primers and Control Probes

<table>
<thead>
<tr>
<th>Cytokine*</th>
<th>Primer</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-actin²⁸</td>
<td>Sense</td>
<td>CTGGAGAAGAGCTATGAGCTG</td>
</tr>
<tr>
<td></td>
<td>Anti-sense</td>
<td>AATCTCCTTCTGCCATGCCTGTC</td>
</tr>
<tr>
<td></td>
<td>Internal</td>
<td>GCCTTTCCCTGCTGGGTATGGAATCCTGTGG</td>
</tr>
<tr>
<td>TNF-α²⁹</td>
<td>Sense</td>
<td>TCGAGTGACAGCCGGTAG</td>
</tr>
<tr>
<td></td>
<td>Anti-sense</td>
<td>CAGAAGCCATGCAGAATAAGAC</td>
</tr>
<tr>
<td></td>
<td>Internal</td>
<td>CACGTCGTAGCAAACCACCAAGG</td>
</tr>
<tr>
<td>IL-1β†</td>
<td>Sense</td>
<td>TTCCCATAGACAGCTGAC</td>
</tr>
<tr>
<td></td>
<td>Anti-sense</td>
<td>TGGTTGCGATCCACACCTGG</td>
</tr>
<tr>
<td></td>
<td>Internal</td>
<td>CATAAGCCAAACAGTGATATTTCTCCATGAC</td>
</tr>
<tr>
<td>IL-1RA³⁰</td>
<td>Sense</td>
<td>ACCACGCTTGTGCTGGTAG</td>
</tr>
<tr>
<td></td>
<td>Anti-sense</td>
<td>GACAGGCAAGTGTTCGAAAG</td>
</tr>
<tr>
<td></td>
<td>Internal</td>
<td>GCCTCACCTGGAGAGGTCAACAC</td>
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<tr>
<td>IL-6³¹</td>
<td>Sense</td>
<td>AAAATCTGCTCTGCTCTTGTGG</td>
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<tr>
<td></td>
<td>Anti-sense</td>
<td>GGTGGGCGAGTCAAGCCC</td>
</tr>
<tr>
<td></td>
<td>Internal</td>
<td>CTCGTGCCATTCAATCTTCATAGAAGGAGAAG</td>
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<tr>
<td>IL-10³²</td>
<td>Sense</td>
<td>AAGACACGCTGAGGACACAT</td>
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<td></td>
<td>Anti-sense</td>
<td>AGACAGCTTTTGTCTGGAGGTTA</td>
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<td></td>
<td>Internal</td>
<td>CACGTCGAAGCCTGCTACGATTTTCCTCCC</td>
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<tr>
<td>MCP-1³³</td>
<td>Sense</td>
<td>CTGTCCTGACCCAGATGCAGT</td>
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<tr>
<td></td>
<td>Anti-sense</td>
<td>CTACAGAATTGCTGGAGGTTG</td>
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<tr>
<td></td>
<td>Internal</td>
<td>AAGAAGCTGATAGTATTTGGTCAACAGAAGGAG</td>
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<tr>
<td>MIP-2‡</td>
<td>Sense</td>
<td>ACCATACGGAATGAGGGTT</td>
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<td></td>
<td>Anti-sense</td>
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<td></td>
<td>Internal</td>
<td>GATAGTCGATAGGGGACTGAC</td>
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<tr>
<td>IFN-γ³⁴</td>
<td>Sense</td>
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<tr>
<td></td>
<td>Anti-sense</td>
<td>TGACAGACCTTCGCGTGGGAT</td>
</tr>
<tr>
<td></td>
<td>Internal</td>
<td>CATGCCAAGCTTACGGGAGGAG</td>
</tr>
</tbody>
</table>

The sequences of all oligonucleotides are shown in 5' to 3' direction.

* Reference; † based on sequences derived from the NewGenBank database.

...The relative amount of β-actin PCR product was determined after Southern hybridization by assessment of the radioactive signal using a PhosphorImager (Molecular Dynamics, Sunnyvale, CA). Samples with a low β-actin cDNA concentration were excluded from further cytokine analysis.

Five microliters of normalized cDNA was subjected to various numbers of PCR cycles with cytokine specific primers: IL-1β, 25 PCR cycles; IL-6, IL-10, TNF-α, IFN-γ, MCP-1, and MIP-2, 30 PCR cycles; and IL-1RA, 40 PCR cycles. Each cycle consisted of 1 minute at 94°C, 1 minute at 55°C (except TNF-α: 1 minute at 50°C), and 1 minute at 72°C. Polymerase chain reactions were performed in duplicate with comparable results. In addition to the experimental samples, each PCR run included a titration (1:20, 1:40, 1:80) of positive control cDNA (from LPS-stimulated spleen cells) and a negative control, which consisted of all reagents without template, to ensure that none of the reagents was contaminated with cDNA or previous PCR products; no PCR product was detected under this condition. To confirm that our RT-PCR method was able to demonstrate semiquantitative differences in the amount of cytokine mRNA, serial dilutions of positive control cDNA were subjected to PCR with the primers. As an example, Figure 1 shows the linear relationship between the amount of input cDNA and the yield of PCR product for IFN-γ. Similar results were obtained with other cytokine primers.

### Analysis of PCR Products

An aliquot of PCR product (10 μl) was electrophoresed on 2% agarose gels and visualized by ethidium bromide staining. To verify the specificity of the PCR results, gels were blotted to Genescreen-plus membranes (NEN-DuPont, Hertogenbosch, The Netherlands), and the filters were hybridized with specific oligonucleotide probes, complementary to sequences within the region flanked by the PCR primers. These internal control probes were labeled at the 5' end with [γ³²P]ATP, using T4 polynucleotide kinase (New England Biolabs, Beverly, MA). Hybridization was car...
FIGURE 1. Semiquantitative RTPCR analysis of IFN-γ expression in rat spleen cells. RNA was extracted from LPS-stimulated rat spleen cells as described in Materials and Methods. A serial dilution of spleen cDNA was subjected to 30 cycles of polymerase chain reaction with rat IFN-γ specific primers. Polymerase chain reaction results were verified by Southern hybridization with a 32P-labeled IFN-γ internal control probe. The autoradiograph is shown in the insert. The radioactive signal on the blot was quantified as described in the text. The number of counts per minute from the samples was used to calculate the linear regression line.

ried out in 6 X SSC, 1% SDS, 5 X Denhardt’s, and 100 µg/ml sheared herring sperm DNA for 4 hours at 65°C. The membranes were washed with 2 X SSC, 0.1% SDS at 65°C and exposed to x-ray film (X-Omat; Kodak, Rochester, NY).

RESULTS
Semiquantitative RT-PCR analysis was used to determine cytokine mRNA expression in the iris, ciliary body, choroid-sclera, retina, and cornea of Lewis rats at different points in time after systemic LPS injection. The samples from normal (uninjected) and LPS-treated rats were normalized for expression of β-actin mRNA.

Cytokine Expression in the Uvea
The pattern of LPS-induced cytokine mRNA expression in the iris, ciliary body, and choroid-sclera showed considerable similarities (Figs. 2A, 2B, 2C), although differences were observed among rats at equal time points. The uvea of normal rats, slight expression of MCP-1 and IFN-γ was detected. After 2 to 4 hours, the expression of TNF-α, IL-1β, IL-6, IL-10, IFN-γ, MCP-1, MIP-2, and IL-1RA was already markedly raised in the iris, ciliary body, and choroid-sclera. At this time, clinical signs of uveitis were absent. Except for IL-10, elevated expression of all cytokines tested was detected after 22 to 24 hours, concomitant with maximal uveitis. A variable expression of IL-6, MCP-1, MIP-2, and IFN-γ was seen in the iris at these time points. One of the rats analyzed at 22 hours (rat 8) did not show detectable expression of IL-6, MIP-2, or IFN-γ, despite clinical signs of uveitis. A strong IFN-γ signal in the ciliary body and choroid-sclera at these time points was observed in one of the rats (rat 9). The expression of IL-6 in the ciliary body alone and TNF-α in all parts of the uvea appeared to be slightly decreased after 22 to 24 hours, compared to that after 2 to 4 hours. Forty-eight hours after LPS injection, when clinical signs of the inflammatory response started to diminish, the expression of IL-1β, IL-6, TNF-α, MCP-1, and MIP-2 in the uvea declined, whereas IFN-γ was still observed in the iris and ciliary body but not in the choroid-sclera. These results indicate that initiation and the acute stage, but not the recovery, of EIU are associated with elevated expression of multiple cytokines in the uvea.

LPS injection also induced the expression of IL-1RA in the uvea. Elevated expression of IL-1RA was
FIGURE 2. Induction of multiple cytokine and IL-1RA mRNA expression in the uvea during endotoxin-induced uveitis. Total RNA was isolated from the iris (A), ciliary body (B), and choroid-sclera (C) at different points in time after systemic lipopolysaccharide (LPS) injection, reverse transcribed into cDNA, and normalized for β-actin expression. Polymerase chain reaction and Southern blot analysis were performed as described in the text. Time after LPS injection are indicated on top. The negative control (−) lacks template cDNA, and the positive control is a dilution (1:20, 1:40, and 1:80) of spleen cDNA. On the bottom is indicated the number of each animal.
detected early after LPS injection and during maximal uveitis, similar to the expression of IL-1β. In contrast to IL-1β, raised expression of IL-1RA was still detectable in the iris and ciliary body after 48 hours.

Cytokine Expression in the Retina

Cytokine mRNA expression was also investigated in the retina, because systemic LPS injection causes a mild inflammation in this ocular tissue. In the normal retina, a minor expression of TNF-α was detected (rat 2, Fig. 3). Systemic LPS injection resulted in increased expression of TNF-α, IL-6, MCP-1, and MIP-2 after 4 hours. The expression of these cytokines reached a maximum at 22 to 24 hours. Expression of IL-1β and IL-1RA was detected first at 22 to 24 hours. The expression of TNF-α, IL-1β, IL-6, MCP-1, and MIP-2 was markedly decreased after 48 hours compared to that at 22 to 24 hours, whereas IL-1RA expression remained elevated. Expression of IL-10 and IFN-γ was not detected in the retina during EIU. Thus, a restricted set of cytokines was expressed in the retina during EIU, displaying kinetics different from those of the uvea.

Cytokine Expression in the Cornea

Because corneal endothelium is exposed to aqueous humor containing various inflammatory mediators during EIU, we further analyzed mRNA expression of cytokines in the cornea. A significant expression of TNF-α and MIP-2 was observed in one of the normal rats (rat 2) 2 hours after LPS injection, whereas at later time points, only a slight expression was found (Fig. 4). Although initially not detectable, a slight expression of MCP-1 was observed at 4 hours and 22 hours. Faint signals were also observed for IL-10 at 0 hours (rat 2) and for IFN-γ at 48 hours (rat 11). Expression of IL-1β or IL-6 was not detected in the cornea during EIU. These results suggest that the cornea does not contribute to intraocular levels of these cytokines during EIU. Interestingly, in corneas of normal rats, a considerable expression of IL-1RA was found, which was not altered by LPS injection. The constitutive expression of IL-1RA implies that the cornea contains a control mechanism for IL-1-mediated responses.

DISCUSSION

The results presented here demonstrate that systemic injection of LPS into young Lewis rats induces mRNA expression of multiple inflammatory mediators, including TNF-α, IL-1β, IL-6, IL-10, MCP-1, MIP-2, IFN-γ, and IL-1RA, in the uvea and retina but not in the cornea. All samples were normalized for β-actin expression. It should be noted that it is not known whether β-actin expression remains constant during the course of EIU. In the present study, it seems unlikely that fluctuations in β-actin expression have affected the results, in view of the “all or none” induction of the cytokines investigated. Expression of all cytokines examined was already detected in the uvea 2 to 4 hours after LPS injection, when clinical signs of uveitis or protein extravasation into the aqueous humor were not yet present.18 Expression of IL-1RA and all cytokines tested, except IL-10, was still elevated in the uvea 22 to 24 hours after LPS injection, when the intraocular inflammation was maximal. During this stage of EIU, expression of most of these cytokines was also observed in the retina. The pattern of TNF-α, IL-1β, IL-6, and IFN-γ mRNA expression in the iris and ciliary body was largely consistent with the results of others.35,36 Moreover, Yoshida et al showed that IL-1α, IL-1β, and TNF-α mRNA levels in the iris-ciliary
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**Table 1.** Expression of cytokines in the corneas at different times after systemic lipopolysaccharide (LPS) injection. Reverse-transcription polymerase chain reaction and Southern blot analyses were performed as described in the text. Time points after LPS injection are indicated on top. The negative control (−) lacks template cDNA, and the positive control is a dilution (1:20, 1:40, and 1:80) of spleen cDNA. On the bottom is indicated the number of each animal.

- **β-Actin**
- **IL-1β**
- **IL-1RA**
- **IL-6**
- **IL-10**
- **TNF-α**
- **MCP-1**
- **MIP-2**
- **IFN-γ**

**Figure 4.** Expression of β-actin, TNF, MIP-2, MCP-1, and IL-1RA in the cornea at different times after systemic lipopolysaccharide (LPS) injection. Reverse-transcription polymerase chain reaction and Southern blot analyses were performed as described in the text. Time points after LPS injection are indicated on top. The negative control (−) lacks template cDNA, and the positive control is a dilution (1:20, 1:40, and 1:80) of spleen cDNA. On the bottom is indicated the number of each animal.

body peaked 6 hours and 24 hours after systemic LPS injection had declined, suggesting a biphasic synthesis of these mRNAs during EIU.24 We expanded these studies by including the two chemokines MCP-1 and MIP-2, as well as IL-10 and IL-1RA, which may be involved in the downregulation of the response.

Expression of cytokine mRNA does not necessarily mean that an active protein is released into the eye. In case of IL-1, for example, it has been shown that the translation of mRNA and the processing and secretion of protein are complexly regulated.35 Previously, we have found that EIU is accompanied by high levels of TNF-α and IL-6 in aqueous humor during the initial stage and concomitant with maximal uveitis.18 In this study, we also showed, by Northern blot analysis, that IL-6 was produced locally. Our present results show that transcription of multiple cytokines and IL-1RA is initiated in the eye during EIU and imply that these mediators are involved in the pathogenesis of this inflammatory disorder.

TNF-α and IL-1 appear to be key mediators in many LPS-induced models, because treatment with specific antibodies to these cytokines reduced the deleterious effects of LPS and the release of subsequent cytokines.8,10-30 Systemic and intraocular TNF-α and IL-1 may also play a pivotal role in the induction of EIU. Systemic administration of TNF-α or IL-1 in animals induced profound vascular effects, including vascular leakage.37 During EIU in the rat, high levels of TNF-α were found in serum and aqueous humor early after LPS injection.18 In addition, IL-1 was detected in aqueous humor after intravitreal injection of endotoxin in rabbits.38 Furthermore, intraocular injection of TNF-α or IL-1 in animals induced severe uveitis, which greatly resembled the response to LPS.22 Systemic treatment with anti-TNF-α antibodies during EIU, however, was not effective in reducing the disease.22,39 This lack of benefit may be due to the fact that blocking the activity of a single cytokine is circumvented by the effects of other inflammatory cytokines released in the eye early after LPS injection, as shown earlier16 and suggested by the present study. Previous studies indicate that IL-6 may act also as a proinflammatory mediator in EIU. Intraocular IL-6 production during EIU was detected early after LPS injection and at the moment of maximal uveitis.17,18,31 The levels of IL-6 in aqueous humor 24 hours after LPS injection were correlated with the severity of uveitis.17 Furthermore, intraocular injection of IL-6 in EIU-susceptible rats caused severe uveitis, with protein extravasation into the anterior chamber and infiltrating leukocytes.17

EIU is characterized by a massive cellular infiltrate in the anterior segment, consisting predominantly of PMNs and a few monocytes-macrophages.19 Recruitment of these cells from the circulation requires intraocular production of chemotactic factors, such as the complement-activation fragments C3a and C5a, or leukotriene B4, which has been detected in aqueous humor of rats during EIU.40 In addition, chemotactic cytokines such as MIP-2 and MCP-1 are likely to be involved in this process. MIP-2 is a potent chemoattractant for neutrophils, both in vivo and in vitro.41 Furthermore, MIP-2 induced PMN degranulation with release of lysozyme.41 In addition, intraocular injection of human IL-8, a functional equivalent of rat MIP-2, triggered the influx of neutrophils in the anterior chamber of rats.42 Our finding of MIP-2 expression during an experimental model of uveitis is concordant

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with the detection of IL-8 in the vitreous humor of patients with uveitis.\(^4\) MCP-1 has been shown specifically to induce monocyte–macrophage infiltration after subcutaneous injection into rats.\(^4\) MCP-1 is also a strong activator of monocytes and macrophages, triggering the respiratory burst.\(^4\) The localized induction of both MIP-2 and MCP-1 during EIU strongly suggests that these mediators contribute to the cellular infiltration of various parts of the eye.

The expression of IFN-γ mRNA in the uvea at all points in time after LPS injection, observed by us and others,\(^2\) suggests involvement of this cytokine in various stages of EIU. Intraocular IFN-γ may be involved in the induction of this model, because IFN-γ causes acute anterior uveitis in rats when injected intravitreally.\(^4\) Moreover, IFN-γ may also contribute to the recovery of EIU because treatment with anti-IFN-γ during EIU in mice results in an exacerbation of the uveitis.\(^4\) However, intravitreal administration of IFN-γ was not effective in reducing BSA-induced uveitis in mice.\(^4\)

IL-10 was initially described as a Th2 cell-derived factor, with cytokine synthesis inhibitory activity on Th1-cells.\(^4\) However, IL-10 also inhibits the LPS-induced release of monocytes of several cytokines, including TNF-α, IL-1α, IL-6, and IL-8, and stimulates the expression of IL-1RA.\(^5\) Recent data indicate that IL-10 may have important downregulatory effects in LPS-induced responses.\(^6\) Systemic injection of LPS results in the release of IL-10 into the blood, with maximal levels at 6 hours.\(^6\) Furthermore, administration of IL-10 protected mice against a lethal dose of LPS and reduced LPS-induced TNF-α release in the circulation.\(^5\) Our results demonstrate a slight expression of IL-10 in the uvea only, 4 hours after LPS injection. Further studies are required to determine the moment of maximal IL-10 expression in the uvea and whether intraocular IL-10 release is associated with a decreased level of other cytokines. Expression of IL-10 was not detected 24 or 48 hours after LPS injection, when the expression of all other cytokines in the uvea decreased. The involvement of IL-10 in the downregulation of cytokines in the eye during the acute stage of EIU deserves further investigation.

A striking observation was the intraocular expression of IL-1RA during various stages of EIU. The sole activity of this receptor antagonist is to block IL-1 activity by binding to the same receptors as IL-1 without stimulating the cell.\(^5\) Two forms of the human IL-1RA have been described, a secreted form and an intracellular form, which share 152 C-terminal amino acids but differ in their N-terminus because of the use of a different first exon.\(^5\) Until now, only one form of rat IL-1RA has been cloned,\(^8\) analogous to the secreted form of human IL-1RA. Because the sense primer used in our study is located on the second exon, which in humans is transcribed for both the secreted and intracellular forms of IL-1RA, mRNA coding for the intracellular or secreted form of rat IL-1RA may not be distinguished. Systemic administration of large amounts of IL-1RA has been shown to reduce LPS-induced mortality in rabbits.\(^1\) Intraocular treatment with IL-1RA, although significantly suppressing IL-1-induced uveitis, did not inhibit ocular inflammation in rabbits injected intravitreally with LPS.\(^5\) This lack of efficacy may be due to an insufficient excess of IL-1RA over IL-1 in the eye or to other inflammatory mediators induced by LPS. The sustained expression of IL-1RA in the uvea and retina after systemic LPS injection is in accordance with the release of circulating IL-1RA in human volunteers after endotoxin administration.\(^4\) The intraocular expression of IL-1RA suggests that IL-1 receptors in the eye may be blocked during EIU, which may contribute to the recovery of the disease.

Involvement of the retina in EIU in the rat has been described previously.\(^9\) Histologic analysis revealed vasodilation 12 hours after LPS injection and infiltration of different retinal layers by PMNs at 24 hours after injection. Our findings show induction of multiple cytokines in the retina, with maximal expression 22 to 24 hours after LPS injection. The delay between maximal cytokine expression in the uvea and the retina suggests that the retinal response may be a secondary reaction induced by inflammatory mediators released in other ocular tissues.

In contrast to the uvea and retina, elevated cytokine expression was not found in the cornea during EIU. This is surprising, because the cornea is exposed to aqueous humor containing various inflammatory mediators, including TNF-α and IL-6, during EIU.\(^1\) Corneal involvement during EIU is evidenced by the observation of MHC class II and ELAM-1 expression on the corneal endothelium.\(^5\) In the present study, cytokine mRNA expression by corneal endothelial cells may not be detected, because RNA from whole corneas was analyzed. We are currently studying the various layers of the cornea to address this issue.

Because our RT-PCR study only provides information about cytokine expression at the tissue level, further studies are needed to determine their exact cellular source. It is plausible that the inflammatory process starts after binding of LPS or circulating cytokines to vascular endothelial cells in the uvea. These endothelial cells may produce several cytokines, such as IL-1 or IL-6, in response to LPS or TNF-α. After disruption of the blood-ocular barrier, LPS, as well as TNF-α or IL-1, may trigger other ocular resident cells in the uvea, such as epithelial cells, mast cells, or tissue macrophages, to produce subsequent cytokines, including...
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MCP-1, MIP-2, and IFN-γ. Cultured ciliary epithelial cells have been shown to produce IL-1 in response to LPS. Retinal pigment epithelial cells, which may have been present in the retina and choroid–sclera fractions, have been shown to produce a variety of cytokines in vitro, including IL-1β, IL-6, IL-8, and MCP-1. Moreover, T lymphocytes, which are a major source of IFN-γ, have recently been demonstrated to play an important role in the development of EIU in mice. Finally, infiltrating cells, like PMNs and monocytes–macrophages, may contribute to the production of various cytokines during later stages of EIU.

In summary, elevated mRNA expression of multiple cytokines in the uvea is one of the initial events in the development of EIU. These cytokines may be involved in numerous inflammatory reactions in the eye, leading to disruption of the blood–ocular barrier, influx of PMNs, monocytes, and macrophages, and other features of the disease, which reach a maximum 24 hours after LPS injection. Furthermore, systemic LPS injection induced intraocular expression of IL-1RA, which may play an important role in the downregulation of IL-1-mediated responses during EIU.

Key Words
endotoxin-induced uveitis, rat, cytokines, mRNA, polymerase chain reaction

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