Interleukin-6 Does Not Mediate Endotoxin-Induced Uveitis in Mice: Studies in Gene Deletion Animals

James T. Rosenbaum,1–5 Paul Kievit,2 Young Bok Han,4 Jong Moon Park,5 and Stephen R. Planck1–3

PURPOSE. Interleukin-6 (IL-6) has been strongly implicated in anterior uveitis based on its presence in aqueous humor from infected eyes and its inflammatory effects when injected intravitreally into rats. We used IL-6-deficient mice to test further the hypothesis that IL-6 contributes to the development of endotoxin-induced uveitis.

METHODS. Uveitis was scored by histologic analysis of C3H/HeN mice 24 hours after intravitreal injections of up to 200 ng of recombinant murine IL-6. Uveitis was similarly measured in IL-6-deficient mice and congenic controls 24 hours after intravitreal injection of 250 ng of *Escherichia coli* endotoxin. Reverse transcription-polymerase chain reaction was used to detect mRNAs for several cytokines at 3 hours postinjection. The IL-6 concentration in aqueous humor samples was determined with a bioassay using the murine B9 plasmacytoma cell line.

RESULTS. Direct injection of IL-6 did not induce uveitis. Mice genetically deficient in IL-6 developed endotoxin-induced uveitis that was comparable or more severe than congenic control mice. Compensatory changes in the expression of mRNA for other cytokines were not detected in irises from the IL-6-deficient mice. In IL-6-competent mice that received bilateral endotoxin injections, no correlation was found between the number of infiltrating cells in one eye and the IL-6 concentration in the aqueous humor of the contralateral eye.

CONCLUSIONS. In marked contrast to previous conclusions with rats, IL-6 was not sufficient for inducing uveitis in mice. Additionally, IL-6 was not necessary for the development of uveitis subsequent to intravitreal injection of endotoxin in mice. (Invest Ophthalmol Vis Sci. 1998;39: 64–69)
tious agents. We have tested the ability of these animals to respond to endotoxin as an inducer of anterior uveitis.

METHODS

Animals

Male IL-6-deficient mice (B6.129-IL-6<sup>tm1Koe</sup> ) and congenic controls (B6129F2) were purchased from The Jackson Laboratory (Bar Harbor, ME). Male C3H/HeN mice were obtained from Simonsen Laboratories (Gilroy, CA). All mice were provided food and water ad libitum and were kept on a 12-hour light-dark cycle. All animals were treated according to the tenets of the ARVO Statement on the Use of Animals in Ophthalmic and Vision Research.

Uveitis was induced in 6- to 8-week-old IL-6-deficient mice and congenic controls by bilateral intravitreal injection of 250 ng of Escherichia coli 055:B5 endotoxin (List Biologies, Campbell, CA) in 2 μl of saline with 0.25% human serum albumin (Baxter Healthcare, Glendale, CA). In most experiments, the mice were killed after 24 hours and one eye per animal was fixed in 10% neutral-buffered formalin, embedded in paraffin, and sectioned for histologic analysis. Aqueous humor (3 to 5 μl) was collected from the contralateral eye and centrifuged briefly at 10,000g. Two-microliter aliquots of the supernatants were diluted in 60 μl of saline containing 0.25% human serum albumin and kept frozen until they were assayed for IL-6 bioactivity. In some experiments, mice were killed 3 hours postinjection and their iris-ciliary bodies were quickly dissected and kept in liquid nitrogen until RNA was extracted.

Reverse transcription-polymerase chain reaction detection of cytokine mRNA was done as previously described. For detection of IL-10 and interferon-γ mRNAs a touch-down polymerase chain reaction procedure was also used. The Mann-Whitney rank sum test was used to determine the statistical significance of differences in the median values of two experimental groups. Spearman rank order correlation analysis was used to determine the statistical significance of relationships between two variables.

RESULTS

Effect of Intravitreal IL-6

Recombinant murine IL-6 was injected into the vitreous bodies of C3H/HeN mice to determine intracocular effects of IL-6. Groups of four to five mice received 0, 1, 4, 10, 20, or 200 ng of IL-6 in 2 μl of saline plus 0.25% human serum albumin. At 24 hours postinjection, the eyes were collected and processed for histologic evaluation of inflammatory cell infiltration. Injection of IL-6, at any dose tested, had no effect on the number of infiltrating cells counted (Fig. 1). IL-6 alone was not capable of inducing an inflammatory response. These results are in contrast to reports that intravitreal injection of 1 to 10 ng of recombinant human IL-6 causes uveitis in rats.
IL-6 is not required for endotoxin-induced uveitis and it might actually have a protective role (Fig. 2). Although there was substantial overlap in the cell counts for the two groups, eyes from the IL-6-deficient mice had significantly more infiltrating cells (median = 42) than eyes from the congenic controls (median = 15; P = 0.014).

**IL-6 Levels in Aqueous Humor**

IL-6 bioactivity was measured in IL-6-deficient and control mouse aqueous humor samples collected 24 hours after intravitreal endotoxin injection. As expected, no IL-6 activity was detected in samples from the genetically altered mice (<400 pg/ml). Congenic control mice IL-6 levels ranged from undetectable to more than 10 ng/ml. Spearman rank order correlation analysis indicated no significant relationship (P > 0.05) between the infiltrating cell count in one eye and the IL-6 bioactivity in the contralateral eye of mice that received bilateral endotoxin injections (Fig. 3).

A neutralizing antibody protocol was used to verify that the observed bioactivity was indeed due to IL-6. The murine B9 plasmacytoma cells might also have been stimulated by residual endotoxin or by interleukin-11. The stimulatory activity in three aqueous humor samples collected as above was completely blocked by the addition of 500 ng/ml of a neutralizing antibody specific for mouse IL-6 (MP5-20F3; Pharmingen, San Diego, CA) (Table 1). The specificity and lack of toxicity of the antibody were demonstrated by its lack of effect on the stim-

**TABLE 1. Specific Inhibition of Bioactivity by a Neutralizing Antibody for Murine Interleukin-6**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Control</th>
<th>Neutralizing Antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recombinant mouse IL-6</td>
<td>3.58</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td>Recombinant human IL-6</td>
<td>1.46</td>
<td>1.45</td>
</tr>
<tr>
<td>Aqueous humor 1</td>
<td>1.37</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td>Aqueous humor 2</td>
<td>0.67</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td>Aqueous humor 3</td>
<td>0.95</td>
<td>&lt;0.2</td>
</tr>
</tbody>
</table>

Aqueous humor samples were collected from three mice 24 hours after intravitreal endotoxin injection. The bioactivities of these samples and recombinant IL-6 controls were measured in the absence and presence of 500 ng/ml anti-IL-6. The values represent the means of triplicate assays expressed in terms of pg/ml murine IL-6 in the assay medium.
**Endotoxin-Induced Cytokine mRNA in IL-6-Deficient Mice**

Reverse transcription-polymerase chain reaction was used to verify that the IL-6-deficient mice were indeed lacking functional IL-6 mRNA and to investigate the transcript levels for several other gene products likely to be involved in endotoxin-induced uveitis. As expected, 3 hours after endotoxin injection, IL-6 mRNA was not detected in iris-ciliary bodies from IL-6-deficient mice but was detectable in congenic controls (Fig. 4). The apparent variability of IL-6 mRNA levels in the controls is consistent with the interanimal variability seen in the bioassays. The mRNA levels for IL-1α, IL-1 receptor antagonist, TNFα, and IL-10 were all increased after endotoxin treatment, but no consistent difference was detected between the samples from IL-6-deficient mice and controls. In these assays, IL-1β and IFNγ transcripts were detectable in positive control mRNA from lung and blood, respectively, but not in the experimental samples. There was no evidence that the IL-6-deficient mice had changes in level of any assayed cytokine mRNA resulting from compensation for the lack of IL-6 activity. Likewise, the levels of iNOS mRNA indicated increased expression of this gene, and the lack of IL-6 did not alter these aspects of the inflammatory process.

**DISCUSSION**

Although IL-6 has many potentially inflammatory effects, its role in inflammation has been questioned. It is one of the most readily detectable cytokines after endotoxin treatment, but its presence alone should not be accepted as an indicator that it is actively enhancing the local inflammatory response. IL-6 also has potential anti-inflammatory effects. For example, IL-6 may provide negative feedback to inhibit the production of IL-1 or TNFα and may also stimulate the secretion of anti-inflammatory corticosteroids.

The availability of gene-deficient mice allowed us the opportunity to test the role of IL-6 in a mouse model of anterior uveitis. Our results suggest that IL-6 is not a mediator of endotoxin-induced uveitis in the mouse. Inflammation in mice that lack the ability to synthesize IL-6 was comparable to or greater than inflammation in otherwise genetically identical control mice.

The use of gene-deficient mice has some major advantages over trying to eliminate cytokine activity by a pharmacologic method. Pharmacologic inhibitors may be only partially effective and may fail to obtain the critical concentration in a site such as the anterior chamber of the eye. On the other hand, a gene-deficient mouse may have compensated for its lack of a specific protein by increasing the synthesis of another protein or a receptor such that the biologic consequences of the gene deficiency are minimized. Although our studies did not demonstrate a compensatory increase in any cytokine, reverse transcription-polymerase chain reaction should be regarded as a qualitative technique that could miss quantitative differences and would not detect effects resulting from translational or post-translational differences. Cytokines not studied or other mediators, such as free radicals, platelet-activating factor, or arachidonic acid metabolites, might also account for the increased inflammation that is suggested in the IL-6-deficient mice.

Because nitric oxide has been implicated in endotoxin-induced uveitis, we assessed mRNA levels for iNOS, the enzyme that controls the inducible synthesis of nitric oxide. Expression of iNOS was upregulated by endotoxin in control and IL-6-deficient mice.

The differences between our observations and those of prior uveitis studies may relate to species differences, although one would not anticipate major differences between mouse and rat. The route of endotoxin exposure could also affect the role of IL-6 in anterior uveitis. In theory, systemically injected endotoxin might be IL-6 dependent in inducing anterior uveitis because of a source of IL-6 arising outside of the eye, whereas the local injection of endotoxin is IL-6 independent. The intensity of inflammation could also impact the role of IL-6 such that mild inflammation might require IL-6 for amplification, whereas severe inflammation proceeds without a need for this cytokine.

IL-6 appears to be produced as part of a generalized stress response induced by endotoxin and other tissue insults. In vitro and in vivo studies suggest that this IL-6 enhances antibody production and boosts neutrophil responses. Arguments that these responses are beneficial to a host combating an infection are supported by correlations with increased susceptibility of IL-6-deficient mice to vesicular stoma-
matritis virus, 

Escherichia coli, Listeria monocytogenes, or Candida albicans. However, an antibody response is not likely to be an essential component of acute endotoxin-induced uveitis. Furthermore, endotoxin activates many cell types to produce a variety of cytokines which may circumvent possible roles for IL-6. For example, IL-6-deficient mice with systemic endotoxin produce three times as much TNFα as congeneric controls. Other studies with IL-6-deficient mice indicate that endotoxin-induced shock or endotoxin-induced acute-phase responses have moderate to no dependence on IL-6. In contrast, IL-6-deficient mice are incapable of evoking fever responses to endotoxin or IL-1β.

Our studies show that IL-6 is not essential for the model of anterior uveitis that we have used. Just as the presence of albumin at a site of inflammation should not be taken as definitive proof that albumin is playing a regulatory role in the inflammatory process, so the detectability of a cytokine is not necessarily proof that it has played an active role in the local inflammatory process.

Acknowledgments

The authors thank Leslie O’Rourke, Xiao-Na Huang, and Yuzhen Pan for their technical assistance.

References


