IL-1 and TNF-α Are Important Factors in the Pathogenesis of Murine Recurrent Herpetic Stromal Keratitis

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PURPOSE. To better understand the role of interleukin (IL)-1 and tumor necrosis factor (TNF)-α in recurrent herpetic stromal keratitis (HSK), the cytokine content and the effects of anti-cytokine antibodies on mouse corneas with the disease were examined.

METHODS. Competitive reverse transcription–polymerase chain reaction and enzyme-linked immunosorbent analyses of IL-1α and TNF-α content were performed on corneas removed 3, 5, 7, 10, 14, and 21 days after latently infected NIH mice were irradiated with UV-B light to reactivate herpes simplex virus (HSV). In separate experiments, mice were injected with anti-IL-1 or anti-TNF-α antibodies 1 day before and 7 days after reactivation.

RESULTS. UV-B irradiation stimulated an increase in corneal IL-1α mRNA in reactivated (virus shedding) mice. This increase persisted longer and was higher than in UV-B irradiated uninfected control animals. IL-1α and TNF-α protein in corneas of reactivated mice was significantly elevated on days 3 to 10 compared with day 0 levels, and exceeded levels in control corneas on the same days. Anti-IL-1 and anti-TNF-α antibody administration both resulted in significantly decreased virus-induced corneal opacity between 7 and 21 days after UV-B exposure.

CONCLUSIONS. IL-1α and TNF-α are upregulated in corneas in mice experiencing recurrent HSK. Abrogation of virus-induced corneal disease by anti-cytokine antibodies suggests that these cytokines play important roles in the pathogenesis of recurrent disease. Therefore, neutralization of specific proinflammatory cytokines may have potential therapeutic value. (Invest Ophthalmol Vis Sci. 2000;41:96–102)

Herpes simplex virus (HSV) is a leading infectious cause of blindness in humans and accounts for 3% of corneal transplants performed in the United States each year.¹ The virus may damage the cornea during primary infection or subsequent ocular virus recrudescence after disruption of latency within the trigeminal ganglia. In humans, primary ocular HSV infection is often mild or subclinical.¹ Recurrent infection inflicts greater corneal damage through the accumulation over time of virus-induced lesions, such as stromal opacification and neovascularization. As a result, recurrent herpetic ocular infections have the greatest potential to impair sight.

The presence of HSV within the cornea stroma stimulates an inflammatory response or keratitis. Herpetic stromal keratitis (HSK) and attendant corneal lesions have been attributed to an immunopathologic process that is orchestrated by T cells.² Although T cells may play a key role in both tissue damage and repair, their activity is modulated by the actions of other cells and cytokines. Accordingly, the release of interleukin (IL)-1 and tumor necrosis factor (TNF)-α from damaged corneal cells and infiltrating cells may comprise an initial response to virus infection that complements later T-cell–mediated events.³–⁵

IL-1 is a potent proinflammatory cytokine produced by a variety of cells including monocytes, macrophages, and corneal cells.⁶,⁷ Among its functions are mediation of acute-phase response, chemotaxis and activation of inflammatory and anti-gen-presenting cells, upregulation of adhesion molecules, enhancement of neovascularization, and the ability to serve as a cofactor in lymphocyte activation.⁶,⁸ TNF-α is produced mainly by activated macrophages and T cells but may also be made by resident corneal cells.⁹,¹⁰ Many of its actions are similar to and synergistic with that of IL-1.⁶,¹¹ In the eye, IL-1 and TNF-α activity has been associated with uveitis and the corneal response to various types of injury.⁷–¹⁰,¹² Recently, IL-1α expression was correlated with corneal opacity after acute infection of the mouse eye with HSV.³ The exact relationship between TNF-α and corneal HSV infection has not been defined, but HSV-induced expression of both TNF-α and its receptors by ocular cells and infiltrating inflammatory cells suggests a role for this cytokine in HSK.⁴,⁵,¹³

We have characterized a murine model of recurrent HSK that mimics the human disease.¹⁴ In this model, corneas of latently infected mice exposed to UV-B irradiation experience
recurrent viral shedding and disease. As in humans, recurrent ocular disease in mice is characterized by the appearance of microdendrites, focal stromal opacification, endotheliitis, and neovascularization. In the present work, we quantitated IL-1α and TNF-α levels in corneas of mice experiencing recurrent HSK and examined the effect of anti-cytokine treatment on corneal opacity. We found that IL-1 and TNF-α may play important parts in the immunopathogenesis of recurrent corneal HSV infection.

**MATERIALS AND METHODS**

**Virus and Cells**

The virus used in these studies was the human isolate HSV-1 McKrae strain. A plaque-purified stock was grown and assayed on VERO cells, as previously described. Material from eye swabs was similarly cultured on VERO cells and subsequently monitored for cytopathic effects for 48 to 96 hours at 37°C in a humidified incubator containing 5% CO₂.

**Mice and Primary Infection**

All investigations with mice conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Three-week-old female NIH inbred mice were obtained from Harlan Olac (Oxford, UK) and infected at 4 to 8 weeks of age. The eyes of all mice were examined for corneal opacity before infection, and only animals with clear corneas were used. Mice were infected with 10⁶ plaque-forming units of HSV-1 McKrae strain, as previously described. Concurrent with infection, each mouse received an intraperitoneal injection of pooled human serum (Chemicon, Temecula, CA; known to have anti-HSV reactivity with an effective dose for 50% viral neutralization). For each given day after UV-B irradiation, the eyes of mice were swabbed with surgical spears (Weckcel; Xomed–Treace, Jacksonville, FL), and the swab material was cultured on VERO cells to detect recurrent virus shedding. PCR was performed in a 50-μl reaction mixture containing 1× PCR buffer (60 mM Tris-HCl [pH 8.5], 15 mM (NH₄)₂SO₄, 3.5 mM MgCl₂; Invitrogen: San Diego, CA), 400 μM dNTP mixture (Perkin Elmer–Roche), 5% dimethyl sulfoxide (Sigma, St. Louis MO), 6 to 8 picomoles of primers, and 10 μl cDNA (from RT reaction), with or without competitor plasmid. The PCR protocol consisted of 35 cycles of denaturation at 95°C for 30 seconds, annealing at 57°C for 30 seconds, and extension at 72°C for 2 minutes. The reaction mixture was prepared as a master mixture to minimize reaction variation.

**Reverse Transcription–Polymerase Chain Reaction**

The total cellular RNA for each day was reverse transcribed (RT) according to RNA polymerase chain reaction (PCR) kit instructions (GeneAmp; Perkin Elmer, Foster City, CA). Control reactions for each day were performed in the absence of reverse transcriptase to ensure purity of RNA preparations. The cDNA prepared from corneas was frozen at −20°C and diluted as needed on the day of quantitation. PCR was performed in a 50-μl reaction mixture containing 1× PCR buffer (60 mM Tris-HCl [pH 8.5], 15 mM (NH₄)₂SO₄, 3.5 mM MgCl₂; Invitrogen: San Diego, CA), 400 μM dNTP mixture (Perkin Elmer–Roche, Branchburg, NJ), 0.5 U Taq DNA polymerase (AmpliTaq, Perkin Elmer–Roche), 5% dimethyl sulfoxide (Sigma, St. Louis MO), 6 to 8 picomoles of primers, and 10 μl cDNA (from RT reaction), with or without competitor plasmid. The PCR protocol consisted of 35 cycles of denaturation at 95°C for 30 seconds, annealing at 57°C for 30 seconds, and extension at 72°C for 2 minutes. The reaction mixture was prepared as a master mixture to minimize reaction variation.

**Qualitative PCR Analysis of Cytokine mRNA Expression**

Part of the cDNA preparation for each experimental sample was used initially in a qualitative PCR to analyze the expression pattern of TNF-α, IL-1α, and the constitutively expressed peripheral benzodiazepine receptor (BzR). For each primer set, undiluted cDNA from every sample day was evaluated by PCR, as described, without competitor plasmid. Results were used for the establishment of latency.

**UV-B Irradiation and Virus Reactivation**

The eyes of all mice were examined for corneal opacity before irradiation, and only animals with clear corneas were used. At least 5 weeks after primary infection (9–13 weeks of age), the eyes of latently infected and control mock-infected mice were exposed to 170 mJ/cm² UV-B light (TM20 Chromato-Vu transilluminator; UVP, San Gabriel, CA; peak UV-B wavelength of 302 nm). Before (day 0), and on days 1 to 7 after UV-B irradiation, the eyes of mice were swabbed with surgical spears (Weckcel; Komed–Trecce, Jacksonville, FL), and the swab material was cultured on VERO cells to detect recurrent virus shedding from the cornea. Reactivation was defined as the finding of any HSV-positive eye swab on days 1 to 7 after UV-B exposure, with day 0 swabs serving as a control. In this system, approximately 70% of UV-B irradiated corneas of latently infected mice shed virus and characteristic corneal lesions develop, including opacity. Virus-induced corneal opacification typically peaks from 7 to 14 days after the reactivation stimulus. Because previous work has established that non–virus-shedding mice may nevertheless harbor HSV in deep layers of the corneal stroma, data for latentently infected, nonreactivated mice are not included. Uninfected eyes serve as a control for the transient effects of UV-B on corneal opacity (peaking 3 to 7 days post irradiation) and cytokine induction (clinical observation).

On the designated days after UV-B irradiation, eyes were evaluated for clinical disease by a masked observer using a dissecting microscope. Stromal opacification was rated on a scale of 0 to 4, where 0 indicates clear stroma, 1 indicates mild stromal opacification, 2 indicates moderate opacity with discernible iris features, 3 indicates dense opacity with loss of defined iris detail except pupil margins, and 4 indicates total opacity with no posterior view.

**Enzyme-Linked Immunosorbent Assays**

On specified days, corneas were removed from mice and frozen individually at −80°C in media. On the day of assay, corneas were thawed on ice, minced, sonicated for 30 seconds (Sonifer 450, Branson Ultrasonics, Danbury, CT) and clarified by centrifugation to produce a corneal lysate. For a given day after UV-B exposure, lysates from reactivated mice (confirmed by virus-positive eye-swab data) were pooled. Corneal lysates obtained from uninfected, UV-B-irradiated mice were included as controls. The cytokine assays performed for IL-1α and TNF-α used sandwich enzyme-linked immunosorbent assay (ELISA) kits (Genzyme, Cambridge, MA) with a sensitivity of 15 pg/mL.
to determine the presence or absence of a given mRNA species on each day and to gauge the amount of competitor to be used for quantitative PCR. The expression level of IL-1α mRNA present in each cDNA sample was subsequently quantified using a competitor plasmid in quantitative PCR. RNA from concanavalin A–stimulated T cells was used as a positive control.

**PCR Primers and Competitor Plasmids for Quantitative PCR**

A competitor plasmid (pMUS) contained a multispecific primer cassette with primer binding sites for IL-1α, TNF-α, BzR, and other cytokines.16 Primer sequences were: IL-1α sense, 5′-CAGTTCTGCCATTGACCATC-3′; IL-1α antisense, 5′-CTTCAC TGAACATCGCCGT-3′; TNF-α sense, 5′-TCTCATCAGTTCC TATGCCC-3′; TNF-α antisense, 5′-GGGAGTACAAAGTTAC AACA-3′; and BzR sense, 5′-TCTGGAAAA GAGCTGGGAGG-3′; BzR antisense, 5′-AAGGCCAGCCAGGCAGG3′. Primers were designed to span introns. Amplified products from cDNA (218 bp for IL-1α, 212 bp for TNF-α, 237 bp for BzR) and competitor templates (320 bp) differed in size by approximately 100 bp. Using these primers, the detection limit of mRNA molecules at 35 cycles was less than 100 molecules, as determined in preliminary experiments.

**Quantitative PCR**

The level of IL-1α transcripts was determined by competitive PCR as described using the multispecific competitor plasmid pMus.16,17 Briefly, a constant amount of the sample cDNA was coamplified with the IL-1α primers in 6 to 10 reactions along with varying amounts of the competitor. In these reactions, specific primers compete for annealing and amplification of the competitor plasmid and cellular cDNA, resulting in products that differ by approximately 100 bp. Amplification was performed in the exponential phase as determined in preliminary experiments. After amplification, the competitor and the cytokine products were separated on a 12% polyacrylamide gel and stained with ethidium bromide. Band intensities were measured by video densitometry, and the amount of mRNA present in the initial volume of cDNA was calculated as described in the Methods section. For a given set of corneas, analysis of IL-1α and BzR levels was performed in the same PCR run. M, marker.

**Anti-Cytokine Treatment**

Hamster antibody to murine TNF-α (TN3.19.12), IL-1α (ALF 161.1), IL-1 β (B122), and IL-1 R (JAMA 147) were kindly provided by Robert Schreiber, Washington University School of Medicine (St. Louis, MO). Hamster immunoglobulin (IgG) whole molecule control was obtained from Accurate Chemical and Scientific (Westbury, NY). Antibodies were administered to latently infected mice subconjunctivally (40 μg total protein: 13.33 μg of each antibody for IL-1; 40 μg total protein for TNF-α) and intraperitoneally (690 μg total protein: 230 μg of each antibody for IL-1; 230 μg total protein for TNF-α) on day 0 and intraperitoneally (450 μg total protein: 150 μg of each antibody for IL-1; 150 μg total protein for TNF-α) on day 7 after UV-B exposure. Doses of antibody were based on the recommendation and experience of R. Schreiber. Recurrent virus shedding (reactivation) and corneal opacity was assessed, as described.

**Statistical Analysis**

Linear regression used for mRNA quantitation was performed with the aid of Sigmaplot 4.0 (Jandel, Corte Madera, CA). Student’s t-tests and the Mann-Whitney rank sum test were used as appropriate to analyze ELISA and opacity data.

**RESULTS**

**IL-1 Transcription after UV-B–Induced HSV Reactivation**

Previous studies have suggested that IL-1 plays an important role in corneal disease. A on the basis of such work, we
initially examined corneal IL-1α expression in a mouse model of recurrent HSK. Table 1 presents quantitative data for IL-1α mRNA in reactivated and uninfected corneas before (day 0), and up to 21 days after exposure to 170 mJ/cm² UV-B irradiation. Although present in both groups, IL-1α mRNA per cornea of reactivated mice surpassed that of control animals from day 5 onward in each experiment, reaching up to two- to threefold higher levels. In addition, the UV-B increase in IL-1α message persisted longer in reactivated corneas (days 5, 7, and 14) than in uninfected control corneas (day 3 or 5). When IL-1α message for each day was normalized to the expression of the constitutive peripheral benzodiazepine receptor (BzR), similar results were obtained (Table 1), with even greater disparity between reactivated and control corneas (differences more than fourfold) on days 5 to 21. Although not quantitated, strong TNF-α mRNA bands were observed in control and reactivated corneas on qualitative PCR gels.

**IL-1α and TNF-α Expression after UV-B–Induced HSV Reactivation**

In parallel experiments, we performed ELISAs on protein extracts from corneas of NIH inbred mice with recurrent HSK on days 0, 1, 3, 5, 7, 10, and 14 after UV-B irradiation (Figs. 2 and 3). Results demonstrate that there was a only a slight increase in IL-1α protein in uninfected control animals, whereas corneas of reactivated mice demonstrated a significant increase in IL-1α from days 3 to 10 (*P < 0.01 for all time points) that returned to control levels by day 14 (Fig. 2). Similarly, corneas of reactivated mice contained significantly more TNF-α protein than did uninfected controls from day 3 to day 10 (*P = 0.006–0.030) and returned to control levels by day 14 (Fig. 3).

**Effect of IL-1 and TNF-α Neutralization on Recurrent HSK**

To assess the functional importance of increased levels of IL-1 and TNF-α in the pathogenesis of recurrent HSK, we administered specific antibodies directed against these cytokines to latently infected mice before reactivation. Antibodies (anti-TNF-α or a cocktail of anti-IL-1α, anti-IL-1β, and anti-IL-1 receptor) were injected into mice on day 0 and day 7 relative to UV-B exposure. As Figure 4 indicates, both antibody preparations demonstrated therapeutic efficacy when compared with mice receiving the isotype-matched hamster antibody control. Significantly reduced corneal opacity was noted for the anti-IL-1α and anti-TNF-α treatments compared with control animals (Fig. 4).

**Table 1.** Corneal IL-1α mRNA Expression in Reactivated and Control Mice

<table>
<thead>
<tr>
<th>Days after UV-B Irradiation</th>
<th>0</th>
<th>3</th>
<th>5</th>
<th>7</th>
<th>14</th>
<th>21</th>
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<tr>
<td><strong>Experiment 1</strong></td>
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<tr>
<td>IL-1α R</td>
<td>1025.7 (0.130)</td>
<td>1138.1 (0.120)</td>
<td>2015.9 (0.283)</td>
<td>2559.2 (0.190)</td>
<td>3396.8 (0.449)</td>
<td>ND</td>
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<tr>
<td>IL-1α C</td>
<td>1028.9 (0.076)</td>
<td>2421.1 (0.157)</td>
<td>961.0 (0.078)</td>
<td>915.5 (0.045)</td>
<td>1719.8 (0.102)</td>
<td>ND</td>
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<tr>
<td><strong>Experiment 2</strong></td>
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<tr>
<td>IL-1α R</td>
<td>1916.9 (0.278)</td>
<td>3339.8 (0.556)</td>
<td>4563.1 (0.609)</td>
<td>4068.4 (0.499)</td>
<td>2443.8 (0.241)</td>
<td>1537.7 (0.292)</td>
</tr>
<tr>
<td>IL-1α C</td>
<td>1672.3 (0.169)</td>
<td>567.3 (0.027)</td>
<td>3896.5 (0.263)</td>
<td>2079.4 (0.117)</td>
<td>980.9 (0.098)</td>
<td>1390.4 (0.099)</td>
</tr>
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</table>

Data are in mRNA copies (×1000) per cornea, with IL-1α mRNA/BzR mRNA shown in parentheses. Eyes of latently infected mice were exposed to UV-B irradiation and recurrent viral shedding was determined by tear film swabs. Uninfected control mice received the same UV-B exposure. Day 0 corneas were harvested before UV-B exposure. Cytokine mRNA was quantified in two independent experiments using from 6 to 11 corneas for a given day. Levels of constitutive BzR mRNA expression ranged from 5269 to 13,492 in reactivated mice and 9911 to 21,074 for control mice. R, reactivated mice; C, control mice; ND, not done.
cocktail on days 7 ($P = 0.011$) and 21 ($P = 0.05$) and on days 14 ($P = 0.016$) and 21 ($P = 0.045$) for the anti-TNF-α antibody. The number of mice shedding virus after UV-B irradiation was 7 of 10 in the anti-cytokine groups and 10 out of 10 in the hamster antibody control group. Thus, although we did not rule out anti-cytokine antibody effects on virus titer in tear films or tissues, there was no significant change ($P = 0.44$) in the ability of HSV to reactivate after UV-B irradiation.

**DISCUSSION**

Corneal disease of HSK is thought to be a byproduct of the immune cell response to virus rather than direct virus-induced damage. Guided by T cells and their products, the inflammatory process may be directed against viral antigens or newly exposed, cross-reactive autoantigens. Additionally, proinflammatory cytokines and chemokines present in infected corneas may initiate proposed bystander activation of non-HSV-specific T cells, which further drive inflammation in the absence of virus. Thus, much study and speculation relate to the pivotal role of T-cell derived cytokines in disease pathogenesis, whereas relatively little is known about the part played by proinflammatory cytokines such as IL-1 and TNF-α in primary or recurrent HSK.

The present work demonstrates that recurrent HSV infection induced expression of IL-1α and TNF-α in murine corneas (Table 1, Figs. 2 and 3). In comparison, uninfected, UV-B irradiated corneas did not substantially increase levels of these cytokines. Research in primary ocular HSV infection models supports our findings for IL-1, but results for TNF-α have been variable. In one study, IL-1α protein was readily detected in corneal lysates after acute infection with HSV, whereas significant amounts of TNF-α could not be found. In contrast, work by other researchers has demonstrated TNF-α production by HSV-primed ocular cells in response to HSV in vitro and TNF-α mRNA induction in HSV-infected corneas. Although IL-1α

![Figure 3](image3.png)  
**FIGURE 3.** TNF-α in corneas of latently infected and uninfected mice after UV-B irradiation. Five weeks after primary infection, mouse eyes were UV-B irradiated, and corneas were harvested before (day 0) and on days 3, 5, 7, 10, and 14 after exposure. Corneal lysates were prepared and examined by ELISA for TNF-α expression. Data are presented as mean picograms per cornea ± SEM. Four separate experiments were performed using 6 to 10 corneas per given day. *$P = 0.006–0.03$ compared with UV-B irradiated, uninfected control corneas.

![Figure 4](image4.png)  
**FIGURE 4.** Effect of anti-cytokine treatment on recurrent HSK. Antibodies (anti-TNF-α or a cocktail of anti-IL-1α, anti-IL-1β, and anti-IL-1 receptor) were injected into latently infected mice on day 0 and day 7 relative to UV-B exposure. Corneal opacity of reactivated mice was scored as in the Methods section. Data are reported as mean maximum corneal opacity ± SEM; $n = 10$ mice per group. *Anti-IL-1 group: $P = 0.011$ and 0.05 on days 7 and 21; anti-TNF-α group: $P = 0.016$ and 0.045 on days 14 and 21 compared with the hamster IgG control group.
protein was detected in primary HSK in relatively high amounts up to 20 days after infection, levels of the cytokine in recurrent HSK returned to baseline by 14 days after UV-B exposure (Fig. 2). Apart from mouse and virus strain differences, this inequity may reflect the fact that recurrent ocular HSV infection in mice and humans is a focal disease that does not characteristically progress to necrotizing keratitis.

In addition to resident corneal cells, likely sources of TNF-α and IL-1α in recurrent HSK are the inflammatory cells that invade the cornea in response to the renewed presence of HSV. Indeed, earlier work in our laboratory and others, has shown that macrophages, Langerhans’ cells, T cells and polymorphonuclear cells (PMNs) infiltrate the corneas of mice experiencing recurrent HSK and may serve as potential sources of IL-1 and TNF-α. Thus, our previous report of centripetal migration of macrophages into the central cornea starting within 3 days of the reactivation stimulus correlates well with the increases in IL-1α and TNF-α that we observed on day 3 in the present study (Figs. 2 and 3). Similarly, large numbers of PMNs invading the corneal stroma of virus-shedding mice within 4 days of UV-B irradiation could be a significant source of the two cytokines. Levels of IL-1 and TNF-α would be further augmented by the influx of cytokine-producing Langerhans’ cells and T cells occurring by 7 days after reactivation. Fewer infiltrating cells in uninfected UV-B control corneas may account for the relatively small amounts of the proinflammatory cytokines detected (Figs. 2 and 3).

IL-1 and TNF-α may contribute to the corneal disease of recurrent HSK in numerous ways based on known properties of the two cytokines. After virus-associated tissue damage, local release of IL-1 and/or TNF-α would trigger a series of events including an increase in the activity of inflammatory mediators such as prostaglandins, leukotrienes, and nitric oxide. At the same time, upregulation of cell adhesion molecules and chemotraction by IL-1 and TNF-α would promote the accumulation of PMNs, macrophages, Langerhans cells, and lymphocytes at sites of viral recurrence. Enhanced production of IL-1 and TNF-α by infiltrating cells could boost synthesis of chemokines, such as IL-8 and macrophage inflammatory protein-1α, which have been associated with recruitment and activation of PMNs and T cells in HSK. Neutrophils, the most prominent infiltrating cell type seen in HSK, are thought to play a crucial role in tissue damage and may perpetuate corneal inflammation by exposing neoantigens subject to T-cell responses. With regard to T cell function, IL-1 and TNF-α could enhance virus-specific or nonspecific activation.

Studies of corneal allografting and primary ocular HSV infection have recently illustrated the importance of IL-1 in mediating corneal disease. Thus, grafted corneal buttons treated with IL-1 receptor antagonist are less opaque and more frequently accepted by recipients. Similarly, treatment of mice with protective anti-glycoprotein D antibodies at the time of initial ocular infection with HSV-1 diminishes both corneal disease and the presence of IL-1α in infected corneas. Here we have directly examined the importance of IL-1 and TNF-α in recurrent HSK by using specific neutralizing antibodies. We found that treatment with anti-IL-1 or anti-TNF-α antibodies decreased postreactivation corneal opacity up to 2 weeks after the last injection (Fig. 4, day 21). This protective effect may result from decreased IL-1α and TNF-α activity as well as the loss of synergistic interaction between the two cytokines. Thus, although IL-1 was dominant in the absolute quantity present in reactivated corneas (Fig. 2), TNF-α could indirectly influence disease outcome by boosting IL-1 effects. Loss of this cooperative interaction would manifest as a decrease in corneal opacity in anti-TNF-α-treated mice (Fig. 4). Although there are many possibilities (as outlined earlier), diminished inflammatory cell infiltration and function may account for a large part of the decline in corneal disease seen in antibody-treated mice. Given the hypothesized importance of T cells and PMNs in the immunopathogenesis of HSK, such a limitation could easily diminish downstream corneal disease.

In summary, we examined the role of IL-1 and TNF-α in a mouse model of recurrent HSK with many similarities to the human disease. For both mice and humans, these cytokines are among the first to be produced or released when the cornea is damaged. In the context of recrudescent ocular HSV infection, IL-1 and TNF-α may directly contribute to corneal damage and stimulate or amplify later cell-mediated events. Amelioration of corneal opacity by blocking antibodies underscores the importance of these cytokines in at least the initiation of corneal lesions. The role of T cells in mediating recurrent HSK is currently under investigation in this laboratory.

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References

6. Dinarello CA. The biological properties of interleukin-1. Eur Cyto-

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