The Effect of Cellular Immune Tolerance to HSV-1 Antigens on the Immunopathology of HSV-1 Keratitis

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Previous studies have revealed that herpes simplex virus type 1 (HSV-1) corneal stromal lesions do not develop in the absence of a cell-mediated immune (CMI) response to HSV-1 antigens. HSV-1 glycoprotein C (gC) has been shown to play an important role in the induction of the cytotoxic T lymphocyte (CTL) response to HSV-1 infections at anatomical sites other than the eye. Here we report that a deletion mutant lacking gC (gC-39) when used to infect the corneas of A/J mice was a poor inducer of both CTL and delayed type hypersensitivity (DTH) responses. We have also followed histologically and immunohistochemically the course of HSV-1 stromal disease in A/J mice following topical corneal (TC) infection with wild type (WT) HSV-1, TC infection with gC-39 HSV-1, and simultaneous TC and anterior chamber (TC + AC) infection with WT HSV-1. The latter type of infection has been shown to induce a profound state of DTH and CTL tolerance of HSV-1 antigens. Following TC infection with WT HSV-1, stromal disease progressed to severe ulcerative keratitis with neovascularization by day 21. Histologic and immunohistochemical analysis revealed a predominantly mononuclear infiltrate consisting of numerous plasma cells as well as L3T4+ (T helper/inducer) and Lyt-2+ (T suppressor/cytotoxic) T lymphocytes. Following TC infection with gC-39, or simultaneous TC + AC infection with WT HSV-1, the severity of stromal disease did not progress beyond day 7. On day 21, there was at most a mild stromal cellular infiltrate consisting predominantly of polymorphonuclear neutrophils. These findings indicate that early stromal disease consists of a nonspecific inflammatory response, but severe stromal disease involves a CMI response to HSV-1. AC injection of HSV-1 inhibits the CMI response, thereby halting the progression of stromal disease. Similarly, gC-39, a poor inducer of CMI responses, is also a poor inducer of stromal disease. Invest Ophthalmol Vis Sci 30:105–115, 1989

The link between an immunopathologic response to herpes simplex virus type 1 (HSV-1) in the cornea and HSV-1 stromal keratitis is supported by both clinical and experimental observations. Clinical evidence for an immunopathogenic mechanism is of three types: first, it has been difficult to impossible to isolate infectious HSV-1 directly from stromal lesions; second, stromal lesions do not generally respond well to drugs that inhibit HSV-1 replication; and third, in most cases HSV-1 stromal lesions respond favorably to corticosteroids and immunosuppressive therapy. Experimental evidence for the involvement of the immune system in the pathogenesis of HSV-1 corneal stromal lesions was first provided by Metcalf et al. They reported that congenitally athymic nude mice failed to develop stromal lesions following corneal infection with HSV-1, while their euthymic litter mates did develop lesions. Russell et al. extended these findings by showing that nude mice could be made susceptible to stromal lesions following adoptive transfer of T lymphocytes from HSV-1 immune euthymic mice. The adoptively transferred cells were shown to express HSV-specific cytotoxic T lymphocyte (CTL) activity. Using another approach, Ksander and Hendricks demonstrated that A/J mice, a strain that is normally highly susceptible to HSV-1 corneal lesions, could be rendered resistant by the induction of cell-mediated immune (CMI) tolerance to HSV-1 antigens. In the latter study, tolerance to HSV-1 antigens was induced by injection of HSV-1 into the anterior chamber (AC) of the eye. AC injection of HSV-1 prior to or simultaneously with topical corneal (TC + AC) infection resulted in abrogation of the cytotoxic and delayed type hypersensitivity (DTH) responses, as well as virtually complete

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protection from the corneal stromal lesions that would normally result from TC infection alone.

The above studies suggested that some aspect of the CMI response to HSV-1 antigens contributed to the destruction of the corneal stroma that results from TC HSV-1 infection in this murine model. In a recent follow-up to that study, Hendricks and Glorioso9 demonstrated that AC infection of a mutant strain of HSV-1 with a deletion of the gene coding for glycoprotein C (gC- HSV-1) induced cytotoxic unresponsiveness to HSV-1 while permitting the DTH response to develop normally. This unique pattern of immune reactivity was shown to be associated with significantly reduced susceptibility to stromal disease following TC infection with wild type (WT) HSV-1. These studies ruled out an immunopathogenic role for DTH and suggested the possible involvement of HSV-specific CTL in this murine model of HSV-1 stromal keratitis.

Studies by Glorioso et al7 and Rosenthal et al8 have demonstrated a dominant role for HSV-1 gC both in the induction of HSV-1-specific CTL and as target antigens recognized by these CTL. Based on these observations and those described above, one would predict that TC infection with gC- HSV-1 would not effectively induce stromal disease in A/J mice.

In the current study, we have followed clinically, histologically and immunohistochemically the development of corneal stromal lesions in A/J mice following TC infection with WT HSV-1 or gC- HSV-1, and in mice receiving simultaneous TC + AC infection with HSV-1. Our findings are consistent with the hypothesis that severe stromal keratitis requires the expression of gC and a CMI response to HSV-1. All three groups of mice developed an inflammatory response in the corneal stroma consisting of a heavy granulocytic infiltrate and edema by day 7. However, in the TC + AC infected mice, and in mice receiving only TC infection with gC- HSV-1, the inflammation quickly subsided and the corneas appeared normal by day 21. This was in marked contrast to the mice receiving only TC infection with WT HSV-1. In these mice, the corneal inflammation observed on day 7 progresses to corneal neovascularization, conversion to a predominantly mononuclear cell infiltrate, and massive destruction of corneal stromal tissue by day 21.

Materials and Methods

Ocular HSV-1 Infections

These studies were performed with 4- to 12-week-old female A/J mice (Jacksons Labs, Bar Harbor, ME) and the KOS 321 strain of HSV-1 or a deletion mutant (gC- 39) derived from KOS 321. The gC- 39 mutant has a complete deletion of the gene coding for gC. The HSV-1 was grown in HEp-2 cells, and intact virions were purified on a Percoll gradient, as previously described.10 Prior to infection, the mice were anesthetized with 2 mg of ketamine hydrochloride (Vetalar, Parke-Davis, Morris Plains, NJ) and 0.04 mg of acepromazine malelate (Avco Co., Inc., Fort Dodge, IA) in 0.1 ml of calcium and magnesium-free Hank’s balanced salt solution injected intramuscularly. The anesthetized mice were then inoculated with HSV-1 using a 33 gauge needle and an automatic Hamilton diluter (model 100004, Hamilton Co., Reno, NV). TC infection was achieved by scarifying the central cornea ten times with a 33 gauge needle in a crisscross pattern. Three microliters of HSV-1 suspension (5.0 X 10^5 PFU) was applied topically to the scarified cornea and rubbed in with the eyelid.

AC HSV-1 inoculation was accomplished by inserting the 33 gauge needle parallel and anterior to the iris. The needle was removed, and the aqueous humor was expressed by applying slight pressure on the cornea with a sterile swab. The needle was then reimerted to the original puncture wound, and 3 μl (5.0 X 10^4 PFU) followed by a small volume of air was injected. The resulting small air bubble prevented leakage of the virus suspension when the needle was retracted and dissipated within 12 hr. In these experiments, AC inoculation was accomplished within 15 to 20 min after the TC infection.

The treatment of experimental animals in this study was in compliance with the ARVO Resolution on the Use of Animals in Research.

DTH Assay

Seven days after TC infection with WT HSV-1 or gC- 39 HSV-1, DTH was elicited by injecting 5.0 X 10^5 PFUs of ultraviolet light-inactivated WT HSV-1 (UV-WT HSV-1) or UV-gC- HSV-1 in a volume of 10 μl into the dorsal side of the mouse ear pinna. Ear swelling was measured 24 hr later using an engineer’s micrometer (Mitutoyo, Tokyo, Japan). The amount of ear swelling (ie, postchallenge minus prechallenge ear thickness) in immunized mice was compared to that of similarly challenged but unimmunized mice.

Cytotoxicity Assay

Effector cells: Eight days after TC HSV-1 infection, the regional (preauricular) lymph nodes (RLNs) were excised and single-cell suspensions were prepared. The RLN cells were restimulated with UV-WT
HSV-1 or UV-gC. HSV-1 for 72 hr in vitro as previously described.

**Target cells:** HSV-1 infected L929 (L929-HSV) cells (clone CCL 1, American Type Culture Collection, Rockville, MD) were used as targets. The L929 cells (obtained from mice compatible with A/J at the H-2K locus) were infected with WT HSV-1 or gC-

HSV-1 at a multiplicity of infection of 5.0 for 1 hr. All targets were labeled with ³¹Cr as described previously. At least 80% of the L929-HSV targets expressed HSV antigens as assessed by immunofluorescent staining with a fluorescein-conjugated antiserum to HSV (M.A. Bioproducts, Walkersville, MD).

**Chromium release (Cr Rel) assay:** After 72 hr of in vitro restimulation with UV HSV-1, titrated numbers of RLN effector cells and 5 x 10⁵ Cr-labeled targets were mixed in round-bottomed microtiter plates at effector to target (E/T) ratios of 100, 50, 25 and 10. Cytotoxic activity was measured in a 4 hr chromium release assay as previously described.

The data were expressed as lytic units (LUs), defined as the number of effector cells required for 10% Cr Rel. LUs were calculated using a nonlinear least-squares fitting program as previously described.

**Clinical Examination**

The eyes of infected mice were observed and photographed at various times after infection using a specially constructed, vertically mounted slit lamp modified for use with laboratory animals (Marco Equipment Corp., Jacksonville, FL). A fluorescein solution was prepared by dipping fluorescein ophthalmic strips (Fluorets, Smith and Nephew Pharmaceuticals Ltd., Romford, England) in 2 ml of phosphate-buffered saline (PBS) for 1 min. Three microcentrators (Amicon Co., Danvers, MA).

**Histologic Examination**

The infected eyes were enucleated and immediately fixed in 10% neutral buffered formalin, and 5 μm sections were prepared. Sections were stained with hematoxylin-eosin, mounted with Permount, and cover-slipped for microscopic examination.

**Immunohistochemical Examination**

For immunohistochemical staining, the eyes were embedded in O.C.T. (Tissue Tek, Miles, Naperville, IL) and snap-frozen in an isopentane dry ice bath. Six micron sections were cut at -20°C with a microtome-cryostat (model CTF, International Equipment Co., Needham Heights, MA) and placed on polylysine hydrobromide precoated slides (Polysciences, Inc., Warrington, PA). They were quickly fixed by dipping in acetone for 5 sec. Prior to staining the sections were fixed with cold acetone at 4°C for 10 min. Immunohistochemical staining was performed using a slight modification of the procedure of Wang et al. After two 15 min washes in 0.05 M TRIS-saline buffer, pH 7.6, the sections were incubated for 20 min with 1:5 diluted normal rabbit serum (NRS) in TRIS-saline containing BGEN (3% bovine serum albumin, 0.25% gelatin, 5 mM edetic acid (EDTA), and 0.025% NP-40). Excess serum was blotted from the sections, and 50 μl of appropriately diluted primary antibody was added and incubated overnight at 4°C in a moisture chamber. The specificity, concentration, and source of the primary antibodies used in these studies are listed in Table 1. The antibody containing supernatant from each hybridoma cell culture was pooled and concentrated using Centriprep Concentrators (Amicon Co., Danvers, MA).

The sections were then stained using the streptavidin-biotin complex (S-ABC) immunoperoxidase staining procedure (Zymed Laboratories, South San Francisco, CA). The sections were washed for 10 min in TRIS-saline, and incubated for 30 min with biotinylated rabbit anti-rat IgG (H + L), adsorbed with mouse serum protein (Zymed Laboratories) and diluted with a mouse skin extract. These slides were again washed two times for 5 min with TRIS-saline. They were incubated for 10 min in 3% H₂O₂ in methanol and washed two times for 10 min in TRIS-saline. The sections were then incubated for 10 min with streptavidin biotinylated peroxidase complex (Zymed Laboratories). Following two 10 min washes in TRIS-saline, they were incubated for 5 min in AEC (3-amin-9-ethyl-carbazole, Sigma Chemical Co., St. Louis, MO) solution, which was made from 1 ml of AEC (4 mg/ml) in N,N-dimethylformamide, 14 ml of 0.1 M acetate buffer, pH 5.2, and 150 μl of 3% H₂O₂. The slides were washed in distilled water, counterstained with Mayer's hematoxylin for 6 min,

<table>
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<th>Hybridoma clone*</th>
<th>Specificity</th>
<th>Concentration (μg/ml)</th>
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<tr>
<td>30-H12</td>
<td>Thy 1.2 (all T cells)</td>
<td>0.21</td>
</tr>
<tr>
<td>53-6-72</td>
<td>Lyt-2 (T&lt;sub&gt;ac&lt;/sub&gt;)†</td>
<td>1.2</td>
</tr>
<tr>
<td>Gk 1.5</td>
<td>L3T4 (T&lt;sub&gt;ad&lt;/sub&gt;)‡</td>
<td>0.42</td>
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* American Type Culture Collection, Rockville, MD.
† T suppressor/cytotoxic.
‡ T helper/inducer.
The gC- HSV-1 was compared to WT HSV-1 for the capacity to induce a cytotoxic response in the RLN following TC infection. In two experiments, groups of four mice received TC infections with 5.0 X 10^6 PFUs of gC- or WT HSV-1. Eight days later, RLN cells were prepared and restimulated in vitro with UV-WT HSV-1 or UV-gC- HSV-1 for 72 hr. The cells were then tested for their capacity to lyse WT or gC- infected H2-compatible targets. TC infection with gC- 39 HSV-1 induced a significantly lower cytotoxic response than that induced by TC infection with WT HSV-1 when measured on both WT and gC- HSV-1-infected targets (Fig. 1). In addition, the RLN cells from WT HSV-1-infected mice exhibited significantly less cytotoxic activity against target cells infected with gC- as compared with targets infected with WT HSV-1. These findings are in agreement with those of Glorioso et al following footpad infection of C57BL/6 mice.

The role of gC in the induction of the DTH response has not been tested. Therefore, the capacity of gC- and WT HSV-1 to induce a DTH response to HSV-1 antigens was also compared. Groups of mice received TC infections with gC- or WT HSV-1. Seven days later all mice were challenged in the ear pinna with UV-WT HSV-1 or UV-gC- HSV-1. Although both virus preparations induced a DTH response, the response of the gC- infected mice was significantly lower than that induced by WT HSV-1 when challenged with WT HSV-1 (Table 2).

The above results demonstrate an important role for gC in the induction of the DTH as well as the cytotoxic response to HSV-1 following TC infection. It should be noted, however, that both responses were significantly higher in gC- infected mice than in uninfected mice, suggesting that other HSV-1 antigens also contribute to the induction of the DTH and cytotoxic responses to HSV-1.

### Clinical and Histopathologic Examination of Infected Corneas

Groups of twenty A/J mice received unilateral ocular infections as follows: TC infection with WT HSV-1, simultaneous TC + AC infection with WT HSV-1, TC infection with gC- HSV-1. The groups were coded and the corneas were examined with a slit lamp on alternate days after infection. The study was only partially masked because the group receiving simultaneous TC + AC infection developed severe

### Table 2. Delayed type hypersensitivity induction following TC infection WT and gC- HSV-1

<table>
<thead>
<tr>
<th>TC infection*</th>
<th>Challenge</th>
<th>Mean change in ear thickness (x10^-2 inches) ± SEM</th>
<th>Significance†</th>
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<tbody>
<tr>
<td>WT</td>
<td>WT</td>
<td>31.52 ± 3.04</td>
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</tr>
<tr>
<td>WT</td>
<td>gC-</td>
<td>16.58 ± 2.01</td>
<td></td>
</tr>
<tr>
<td>gC-</td>
<td>WT</td>
<td>22.62 ± 1.03</td>
<td>P = 0.015</td>
</tr>
<tr>
<td>gC-</td>
<td>gC-</td>
<td>16.18 ± 1.52</td>
<td>P = 0.908</td>
</tr>
<tr>
<td>None</td>
<td>WT</td>
<td>6.45 ± 2.74</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>gC-</td>
<td>6.58 ± 0.97</td>
<td></td>
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</table>

*Groups of six mice received topical corneal infections with wild type (WT HSV-1) or a deletion mutant of HSV-1 lacking the gene for glycoprotein C (gC-). Seven days later, all mice were challenged in the ear pinna with UV-WT HSV-1 or UV-gC- HSV-1. Six uninfected mice were similarly challenged as a control. Ear thickness was measured before and 24 hr after challenge.

†The significance of differences in the response of similarly challenged mice infected with gC- HSV-1 compared to those infected with WT HSV-1 (ANOVA).
AC opacity by day 3 after infection and were, therefore, readily identifiable. The infected corneas of mice from each of the three groups were examined histologically or immunohistochemically on day 7 (during the early stages of stromal disease) and on day 21 when the disease was most severe. Slit-lamp photos were taken of each eye just prior to enucleation and sectioning.

All mice receiving only TC infection developed dendritic epithelial lesions by day 3. Seven days after TC infection with WT HSV-1 a mild stromal opacity was observed in ten of ten eyes. Histologic examination of these corneas (Fig. 2) revealed a significant cellular infiltration of the corneal stroma consisting primarily of polymorphonuclear neutrophils (PMNs). PMN infiltration of the AC was also seen. The PMNs infiltrating the AC adhered preferentially to the central cornea where they ultimately destroyed the corneal endothelium underlying the site of infection but did not penetrate Descemet’s membrane.

In five of ten mice receiving TC infection with WT HSV-1 the severity of disease progressed through day 21. Slit-lamp examination on postinfection day 21 (Fig. 3) revealed severe ulceration in the central cornea. The corneas were highly vascularized with vessels going into and around the lesions. Histologic examination on postinfection day 21 of eyes with stromal disease from mice receiving TC infection with WT HSV-1 showed destruction of the stromal and epithelial layers of the central cornea (Fig. 4). Numerous blood vessels were observed in the central stroma, and the stromal infiltrate was now predominantly mononuclear with a preponderance of plasma cells. Immunohistochemical examination on day 21 of the corneas of mice receiving TC infection with WT HSV-1 revealed the presence of a Thy-1.2+ T cell infiltrate (not shown), the majority belonging to the L3T4+ (class II restricted, helper/inducer) subpopulation (Fig. 5A). Some Lyt-2+ (class I restricted suppressor/cytotoxic population) cells were also found in the corneal stroma (Fig. 5B). The relative frequency of the L3T4 to Lyt-2 T cell subsets that were present in the cornea (4:1) approximated their proportions in the peripheral blood. These ratios...
Fig. 3. At 21 days following TC infection with WT HSV-1, corneas show central stromal opacity with corneal vessels (arrow) surrounding the site of infection.

Fig. 4. Micrograph of a cornea 21 days after infection with WT HSV-1 showing ulcerative keratitis with a predominantly mononuclear leukocytic stromal infiltrate and neovascularization (H & E, original magnification x82).
Fig. 5. Immunohistochemical staining of corneas 21 days after TC infection with WT HSV-1. (A) Stained with monoclonal anti-L3T4 (T helper/inducer) antibody. (B) Stained with monoclonal anti-Lyt-2 (T suppressor/cytotoxic) antibody. Arrows indicate positive reactions (original magnification ×82).
Fig. 6. Micrograph of a cornea 21 days after TC infection with the deletion mutant gC-39 strain of HSV-1. Note the mild posteriorly localized PMN stromal infiltrate (arrow) and the absence of corneal vessels (H & E, original magnification x82).

were rough estimates since the two subsets were quantitated in different sections of the same eye. There was, however, no evidence of preferential accumulation of one of the T cell subsets.

The mice receiving TC infection with gC- HSV-1 also developed mild stromal opacities by day 7. Histologically the corneas of these mice resembled eyes receiving TC infection with WT HSV-1 (not shown). However, none of these eyes developed severe stromal disease, and corneal neovascularization was not observed by slit-lamp or histologic examination on postinfection day 21. In the most severe cases (Fig. 6) a moderate, posteriorly localized PMN infiltrate was observed with no neovascularization.

No stromal or epithelial disease was revealed by slit-lamp examination of mice receiving simultaneous TC + AC infection with WT HSV-1. Histologic examination did reveal significant PMN infiltration of the cornea on day 7 (Fig. 7A). By day 21 after infection the corneas of these mice showed little if any inflammatory cell infiltrate and the structure of the cornea remained intact, although a substantial inflammatory infiltrate was present in the anterior chamber (Fig. 7B). Immunohistologic analysis of these corneas revealed a few Thy 1.2+ and L3T4+ cells in the stroma (not shown).

Discussion

Our study provides additional evidence for the involvement of the CMI response to HSV-1 antigens in the pathogenesis of HSV-1 corneal stromal disease. Metcalf et al. demonstrated that congenitally athymic mice do not develop stromal disease when their corneas are infected with HSV-1. These mice were, however, highly susceptible to disseminated disease and most succumbed to HSV-1 encephalitis following corneal infections. In contrast, their euthymic littermates were much more susceptible to stromal disease but less susceptible to disseminated disease following corneal infection. These observations suggested that the CMI response to HSV-1 can prevent the dissemination of an HSV-1 corneal infection while at the same time causing the tissue destruction in the cornea that is the manifestation of stromal disease. Russell et al. extended these findings by demonstrating that nude mice could be made susceptible
Fig. 7. Micrograph of corneas following simultaneous TC and AC infection with WT HSV-1. (A) Seven days after infection revealing a prominent PMN stromal infiltrate. (B) By day 21, only a mild stromal infiltrate remains. The epithelium is intact, and the architecture of the cornea is largely preserved. The anterior chamber, however, had a substantial chronic inflammatory infiltrate (H & E, original magnification ×82).
to corneal lesions by adoptive transfer of T lymphocytes from HSV-1 immune euthymic mice. Such T cell preparations have also been shown to protect the athymic mice from disseminated HSV-1 infections. Our previous studies have demonstrated that the induction of specific CMI tolerance to HSV-1 antigens significantly reduces the susceptibility of A/J mice to stromal disease following corneal infection. We have also demonstrated that mice exhibiting CTL tolerance to HSV-1 antigens but a normal DTH response are also less susceptible to stromal disease. This latter observation suggested that corneal damage does not result from a DTH response and further implicates the CTL response in the pathogenesis of this disease.

Recent studies have revealed that one of the major HSV-1 cell surface glycoproteins (gC) plays an important role in the inductive phase as well as the recognition phase of the CTL response following infection at anatomical sites outside of the eye. Our results extended these findings by demonstrating that gC plays a prominent role in the induction and recognition (as a target antigen) phase of the CTL response induced by ocular infections as well. This role was evidenced by the reduced capacity of gC- HSV-1 when compared with WT HSV-1 to induce a CTL response following corneal infection. Corneal infection with gC- HSV-1 was also found to induce a significantly lower DTH response than that induced by WT HSV-1 infection of the cornea.

Based on the above observations we hypothesized that gC- HSV-1 would be a poor inducer of stromal disease in A/J mice. We therefore investigated the disease that develops in the cornea following corneal infection with WT or gC- HSV-1 alone, or following corneal infection with WT HSV-1 in mice rendered tolerant to HSV-1 antigens through simultaneous AC infection. Our results revealed that all three routes of infection resulted in a mild stromal opacity, consisting of PMN infiltration of the central cornea by day 7 following TC infection with WT and gC- HSV-1.

Our failure to detect stromal opacities in mice that received TC + AC infection was probably related to our inability to see mild opacities superimposed on an opaque AC and lens. It was interesting to note the apparently directed migration of PMNs infiltrating the AC of TC infected mice. These cells appeared to migrate directly to the central cornea as if responding to a chemotactic stimulus. They attached to and ultimately destroyed the endothelium underlying the site of the infection. However, Descemet’s membrane appears to provide a barrier to the anterior migration of PMNs from the AC into the corneal stroma.

Between days 7 and 21 after infection, the stromal disease took a divergent course among the three groups. In mice that received TC infections with WT HSV-1 stromal disease progressed to severe ulcerative keratitis with neovascularization. This progression was accompanied by a shift to a predominantly mononuclear stromal infiltrate. Severe stromal disease also occurred in concert with the development of a CMI response to HSV-1 antigens in these mice. Progression to severe stromal disease was not observed either in mice receiving TC infection with gC- HSV-1 alone or in mice receiving simultaneous TC + AC infection with WT HSV-1. Thus, infection with an HSV-1 strain that is poorly immunogenic, or induction of specific cell-mediated immune tolerance to HSV-1 antigens was associated with failure to develop a mononuclear cell infiltrate, neovascularization and destruction of corneal tissue following infection.

It was interesting to note the lack of epithelial disease in the TC + AC infected group. Epithelial lesions are thought to reflect the direct cytopathic effect of the virus because they respond well to antiviral drugs, and occur during primary infections prior to the onset of the immune response. It is not clear, therefore, why simultaneous AC infection would protect mice from epithelial lesions. This effect appears not to be due to the mechanics of the AC infection since TC infection with HSV-1 followed by mock infection (injection of medium into the AC) does not alter the course of corneal disease. However, we have observed a more rapid elevation of natural killer activity in the peripheral blood of mice receiving TC + AC infection when compared with those receiving TC infection alone (unpublished observation). The elevated natural killer activity following virus infection is thought to reflect increased levels of circulating interferon. Thus, protection from epithelial lesions following anterior chamber infection may be due to the combined effect of the destruction of infected epithelial cells by natural killer cells and the inhibition of virus replication by interferon.

Based on the results of this and previous studies we propose the following sequence of immunologic events following corneal infection with HSV-1. HSV-1 infection of the cornea results in an early inflammatory response mediated predominantly by PMNs which peaks by day 7. In the absence of a CMI response to HSV-1, the inflammation subsides and normal corneal architecture is retained. In the presence of a CMI response to HSV-1, mononuclear cells infiltrate the cornea resulting in neovascularization and destruction of corneal tissue. In our model AC injection of HSV-1 induces CMI tolerance, thereby halting the progression of stromal disease.
Similarly, infection of the cornea with the poorly immunogenic gC- HSV-1 results in a reduced CMI response and a concomitant reduction in susceptibility to severe stromal disease. We conclude that one approach to preventing the development of corneal stromal disease is through proper manipulation of the CMI response to HSV-1.

Key words: HSV-1, cornea, cell-mediated immunity, anterior chamber, immune tolerance

Acknowledgments
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