Cell-Mediated Immune Tolerance to HSV-1 Antigens Associated With Reduced Susceptibility to HSV-1 Corneal Lesions

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We have investigated the involvement of cell-mediated immune responses to Herpes simplex virus type 1 (HSV-1) in the pathogenesis of HSV-1 induced corneal stromal lesions in mice. Topical corneal (TC) HSV-1 infection induced a vigorous delayed hypersensitivity response, as well as lymphoproliferative and cytotoxic responses in the regional lymph nodes. The cytotoxic response involved HSV-1 specific and genetically restricted cytotoxic T lymphocytes, and activated natural killer cells. Half of the TC HSV-1 infected mice developed corneal stromal inflammation and scarring, the cause of visual morbidity in human herpetic disease. However, injection of HSV-1 into the ocular anterior chamber (AC) prior to, or simultaneously with, TC HSV-1 infection resulted in a profound state of cell-mediated immune tolerance of HSV-1 antigens. The tolerance was characterized by a substantial reduction in delayed hypersensitivity, lymphoproliferative, and cytotoxic responses to HSV-1 and was associated with virtually complete protection from corneal stromal lesions induced by HSV-1. These findings suggest a pathogenetic role for cell-mediated immunity and indicate the feasibility of preventing stromal disease through proper manipulation of the immune response. Invest Ophthalmol Vis Sci 28:1986–1993, 1987

Herpes simplex virus type 1 (HSV-1) corneal lesions are the leading infectious cause of corneal blindness in the United States. Visual morbidity is primarily associated with HSV-induced corneal stromal inflammation. It is generally believed that lesions of the corneal epithelium are directly attributable to the cytopathic effect of the virus. Stromal disease, on the other hand, is thought to be due, at least in part, to an immunopathologic response to HSV antigens within the corneal stroma. This latter belief stems from the observations that (1) although HSV-1 viruses have been demonstrated in stromal keratocytes following incubation of corneal discs from patients with histories of recurrent HSV-1 stromal keratitis, it has been difficult or impossible to isolate infectious HSV-1 directly from stromal lesions; (2) stromal lesions do not respond to drugs that inhibit HSV-1 replication; and (3) in most cases, HSV-1 stromal lesions respond favorably to corticosteroid and immunosuppressive therapy.

Experimental evidence for the involvement of the immune system in the pathogenesis of HSV-1 corneal stromal lesions was provided by Metcalf et al. They demonstrated that congenitally athymic nude mice failed to develop stromal lesions following corneal infection with HSV-1, while their euthymic littermates 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. These findings implicated T-cell responses in the pathogenesis of herpetic stromal keratitis, but did not identify the specific type of T-cell response involved.

We are investigating the involvement of the immune response in the pathogenesis of HSV-1 corneal stromal lesions. We hypothesized that suppression of the cell-mediated immune (CMI) response induced by topical corneal (TC) HSV-1 infection would have a protective effect on the corneal stroma. Selective suppression of specific T-cell-mediated responses could then be used to identify functional populations of cells participating in the immunopathologic response.
We used ocular anterior chamber (AC) infection to induce cellular immune tolerance to HSV-1 antigens. This type of infection has been shown by Whittum et al.6,7 to reduce the delayed hypersensitivity (DH) response to subcutaneous infection through the generation of splenic suppressor cells. The DH-suppressed mice exhibited normal serum anti-HSV antibody titers. In this regard, the AC-induced tolerance appeared similar to the "split tolerance" induced by intravenous injection of HSV-1.8,9 Split tolerance is characterized by suppressed delayed hypersensitivity (DH) but vigorous antibody and cytotoxic T lymphocyte responses to HSV-1. While the tolerance induced by AC and intravenous inoculation of HSV-1 appeared similar, the effect of AC infection on the cytotoxic response to HSV-1 has not been investigated.

This study further characterized the CMI tolerance of HSV-1 antigens induced by AC presentation of HSV-1, and determined the effect this tolerance had on susceptibility of A/J mice to herpetic stromal keratitis. The A/J strain was chosen because it has previously been shown to be highly susceptible to HSV-1 keratitis.10

Materials and Methods

Virus Isolation and Purification

HSV-1 (KOS low passage strain) was grown in VERO or HEp-2 cell lines, and intact virions were purified on a Percoll gradient, as previously described.11 The virus used for in vivo challenge was prepared from HSV-1 infected HEp-2 cells grown in RPMI 1640 plus 10% normal rabbit serum. To prevent a response to cell fragments or serum components, the virus used for in vitro stimulation was prepared from HSV-1 infected VERO cells grown in RPMI 1640 plus 10% calf serum. The virus in each preparation was quantitated as plaque forming units (PFU) on VERO cell monolayers.12

Vesicular stomatitis virus Indiana strain (VSV) was similarly prepared and used as a specificity control in some experiments. The VSV was provided by Dr. Newton Khooibaian (University of Illinois, Chicago, IL). The virus was propagated in L929 cells and purified on percoll gradients. The VSV was titered on monolayers of L929 cells that were overlayed with 1% agarose (Agarose B, Pharmacia, Piscataway, NJ) in RPMI-1640 medium containing 5% fetal calf serum (FCS).

Ultraviolet light inactivated HSV-1 (UV-HSV) or VSV (UV-VSV) was prepared by exposing VERO-grown HSV-1 or VSV (2 × 10^7 PFU/ml) to a UV lamp for 10 min at a distance of 10 cm. The UV-HSV and UV-VSV lacked detectable PFU.

Virus Challenge

Five- to eight-week-old female A/J mice (Jackson Laboratories, Bar Harbor, ME) were acclimatized for 1 week before initiating the experiments. Mice were anesthetized with 2.0 mg of ketamine hydrochloride (Vetalar, Park-Davis, Morris Plains, NJ) and 0.04 mg of acepromazine maleate (Aveco Co., Inc., Fort Dodge, IA) in 0.1 ml of RPMI 1640 injected intra-muscularly in the left hind leg. The anesthetized mice were then inoculated with HSV-1 at one of two sites using a 33 g needle and an automatic Hamilton diluter (model #100004, Hamilton Co., Reno, NV).

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

TC virus challenge was achieved by scarifying the central cornea ten times with a 33 g needle in a criss-cross pattern. Three microliters of HSV-1 or VSV suspension (4 × 10^4 PFU) were applied topically to the scarified cornea and rubbed in with the eyelid.

AC HSV-1 inoculation was accomplished by inserting the 33 g 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 reinserted into the original puncture wound and 3 ml of virus (4 × 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.

Simultaneous topical corneal and anterior chamber (TC+AC) inoculation involved sequential infection with HSV-1 by the TC route followed by the AC route using the procedures described above. Unless otherwise indicated, no more than 15 to 20 min elapsed between TC and AC infections. Control mice were administered AC inoculations of RPMI 1640 culture medium.

Clinical Effects of Virus Challenge

The eyes of infected mice were observed in a masked fashion on alternate days by slit lamp biomicroscopy for the presence of corneal stromal keratitis. Corneal lesion invariably began as epithelial defects 2–4 days after inoculation, which subsequently progressed to stromal disease with frequent perforation and loss of corneal tissue. Since our primary interest in this study was in the development of stromal disease, and to avoid a possible direct effect of fluorescein on the replication of HSV-1 in the epithelium,
treated by the lack of susceptibility to herpetic stromal keratitis of T-cell deficient (nude) mice and the development of lesions in nude mice following adoptive transfer of T-cells from HSV-immune mice. The nude mice did, however, develop disseminated infections and succumb to viral encephalitis. In contrast, similarly infected immunologically normal euthymic mice developed stromal necrosis but not disseminated disease. These studies suggested that T-cell responses to HSV-1 antigens contribute both to the potentially blinding stromal inflammation and to containment of HSV-1 infections.

AC presentation of HSV-1 antigens has produced a distinct pattern of immunity characterized by suppressed DH but a normal antibody response. Our data confirm and extend these findings by showing that HSV-1 injection into the AC simultaneous with or prior to TC infection of the same eye resulted in virtual abrogation of the DH response that is induced by TC infection. AC infection with HSV-1 did not, however, impair the capacity of mice to mount a DH response to other antigens. In addition, our findings demonstrated that the AC-induced tolerance of HSV-1 antigens extends beyond DH to other aspects of the CMI response including lymphoproliferation and cytotoxicity. We are currently investigating the mechanism(s) by which these responses are suppressed.

The RLN of TC + AC infected mice were characteristically smaller and yielded significantly fewer lymphocytes than RLN of mice infected by the TC route alone (data not shown). This may reflect reduced extravasation of lymphocytes from the peripheral blood and/or reduced HSV-1 antigen stimulated proliferation within the RLN. The RLN cells of TC + AC infected mice did exhibit reduced levels of proliferation in both unstimulated and UV-HSV-1 stimulated cultures, when compared with the RLN cells of mice infected by the TC route alone. Horohov et al demonstrated enhanced proliferation of splenocytes from HSV-1 infected mice following depletion of Lyt-2+,I-J+ suppressor cells. It is possible that the activity of such a suppressor cell is enhanced by AC infection, resulting in reduced proliferation both in vivo and in vitro.

Simultaneous TC + AC infection provided a model in which CMI tolerance of HSV-1 antigens was induced in an otherwise immunocompetent mouse. We exploited this model to test the involvement of the immune response in the pathogenesis of corneal lesions. The results of these studies clearly demonstrated an association between the absence of CMI reactivity to HSV-1 antigens and reduced susceptibility to HSV-1-induced stromal lesions. Thus some aspect of the CMI response appeared to contribute to the tissue necrosis associated with corneal stromal disease. Since both DH and cytotoxic tolerance was induced by AC injection of HSV-1, it was not possible to determine the relative contribution of these two responses in the pathogenesis of corneal lesions. However, we have recently found that AC injection of a mutant of KOS HSV-1 that lacks the gene coding for glycoprotein C induces cytotoxic but not DH tolerance (manuscript in preparation). Mice lacking only the cytotoxic response to HSV-1 exhibited similar protection against corneal lesions to those tolerized for both cytotoxicity and DH. This is consistent with the findings of Russell et al that nude mice can be rendered susceptible to corneal stromal lesions by adoptive transfer of T lymphocytes exhibiting cytotoxic activity against HSV-infected targets. Taken together, these studies suggest an immunopathologic role for HSV-1 specific cytotoxic T lymphocytes in the pathogenesis of herpetic stromal keratitis.

In summary, this study suggests that it may be feasible to prevent the potentially blinding corneal stromal inflammation and scarring often associated with herpetic keratitis through proper manipulation of the CMI response.

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

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References

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