Herpes keratitis in the absence of anterograde transport of virus from sensory ganglia to the cornea

Katarina Polcicova*†, Partha Sarathi Biswas‡, Kaustuv Banerjee‡, Todd W. Wisner*, Barry T. Rouse‡, and David C. Johnson*§

*Department of Molecular Microbiology and Immunology, Oregon Health and Science University, Portland, OR 97239; and ‡Comparative and Experimental Medicine, College of Veterinary Medicine, University of Tennessee, Knoxville, TN 37996

Edited by Patricia G. Spear, Northwestern University Feinberg School of Medicine, Chicago, IL, and approved June 20, 2005 (received for review April 19, 2005)

Herpes stromal keratitis is an immunopathologic disease in the corneal stroma leading to scarring, opacity, and blindness, and it is an important problem in common corneal surgeries. Paradoxically, virus antigens are largely focused in the epithelial layer of the cornea and not in the stromal layer, and viral antigens are eliminated before stromal inflammation develops. It is not clear what drives inflammation, whether viral antigens are necessary, or how viral antigens reach the stroma. It has been proposed that herpes simplex virus (HSV) travels from the corneal epithelium to sensory ganglia then returns to the stroma to cause disease. However, there is also evidence of HSV DNA and infectious virus persistent in corneas, and HSV can be transmitted to transplant recipients. To determine whether HSV resident in the cornea could cause herpes stromal keratitis, we constructed an HSV US9$^-$ mutant that had diminished capacity to move in neuronal axons. US9$^-$ HSV replicated and spread normally in the mouse corneal epithelium and to the trigeminal ganglia. However, US9$^-$ HSV was unable to return from ganglia to the cornea and failed to cause periocular skin disease, which requires zosteriform spread from neurons. Nevertheless, US9$^-$ HSV caused keratitis. Therefore, herpes keratitis can occur without anterograde transport from ganglia to the cornea, probably mediated by virus persistent in the cornea.

ocular infection | stroma | neurons | US9 cell-to-cell spread | persistent herpes simplex virus

Herpes simplex virus (HSV) is a highly prevalent human pathogen infecting 60–90% of adults. HSV most often infects mucosal epithelium but also commonly infects the eye and, in some patients, causes herpes stromal keratitis (HSK). Even with anti-HSV drugs available, HSV is still the major infectious cause of blindness in the western world (1). HSK in mice is dependent on cellular immune responses that produce inflammation in the corneal stroma leading to neovascularization, edema, tissue injury, corneal opacity, and eventually blindness (2–4). Damage to the cornea is initiated by T lymphocytes that recognize viral antigens, and replication of HSV is required for disease (2, 4). In the absence of T cells, HSK does not develop, consistent with immunopathology rather than viral cytopathology. There also is evidence for T cells with specificity for corneal autoantigens and bystander effects that may be more important at later stages of the disease (4–6).

HSV infection in the cornea is accompanied by entry of virus into sensory neurons; virus is transported in neuronal axons toward nerve cell bodies, and latency is established in sensory ganglia. It is believed that cycles of HSV reactivation in latently infected neurons, accompanied by anterograde axonal spread to the cornea, lead to recurrent infections and scarring of the cornea. HSV reactivation can be triggered by stress or UV light, but also by corneal surgeries, including the now commonly used laser *in situ* keratomileusis (LASIK), in which HSV reactivation can cause corneal damage in a fraction of patients with a previous history of HSV in the eye (7, 8). Patients with ocular HSV can be treated with steroids and anti-HSV, drugs although this treatment is not always successful, and corneal transplantation may be required. In these patients, HSV infection of the cornea is the major cause of graft rejection (9, 10).

In the murine cornea, HSV predominantly replicates and spreads in the epithelial layer of the cornea (refer to Fig. 1*A*). Viral proteins peak in the epithelium after 2 or 3 days and then dramatically decline so that antigens are not detected after 6 days (2–4). Throughout this infection there are very low or undetectable levels of viral proteins in the stromal layer of the cornea (refer to Fig. 1*A*), yet the most devastating keratitis eventually occurs in the stroma. The corneal stroma and epithelium are separated by a basement membrane that likely impedes virus movement into the stroma. Moreover, HSK begins after most viral antigens are cleared from the epithelium, and disease peaks after 14 days. Thus, there is a perplexing separation between infection and expression of viral antigens in the epithelium and subsequent inflammation in the stroma. This lag has been explained by suggesting that keratitis requires HSV transport to sensory ganglia followed by return to the corneal stroma (2).

There is also substantial evidence that HSV can persist, either as infectious virus or in a latent state, for long periods within the cornea. Persistence of HSV in the eye can be defined as the presence of viral DNA (that may or may not be transcriptionally active) or viral proteins and infectious virus (associated with low-level replication) long after acute infection has subsided. This description differs from the more commonly held notion that HSV is delivered back into the eye after reactivation in sensory neurons. Viral DNA and infectivity are frequently found in human tears and in corneas removed for transplantation (9, 11–13). Moreover, infectious virus can cause damage to corneas during storage or be transmitted to recipients (9, 14, 15), and this virus is unlikely to be derived from reactivating neurons, given the time frame of the surgery. Similarly, in rabbits and mice, persistent HSV DNA has also been detected in the corneas long after acute infections have subsided (16, 17). Therefore, the presence of persistent HSV in the cornea may provide an alternative source of viral antigens that trigger HSK. Rather than taking a roundtrip to the nervous system, HSV resident in the cornea may cause HSK.

Previous efforts to determine the source of HSV that causes keratitis have included the use of virus mutants, e.g., unable to express HSV thymidine kinase (tk) or ribonucleotide reductase, which replicate poorly in the nervous system (18, 19). However, these mutants also displayed reduced replication and spread in the cornea, e.g., an HSV tk mutant was reduced by two orders of magnitude in production of infectious HSV after 36–48 h of

This paper was submitted directly (Track II) to the PNAS office.

Abbreviations: HSK, herpes stromal keratitis; HSV, herpes simplex virus; LASIK, laser *in situ* keratomileusis; pfu, plaque-forming unit; PRV, pseudorabies virus; TG, trigeminal; tk, thymidine kinase.

†Present address: Slovak Academy of Sciences, Bratislava, Slovak Republic.

§To whom correspondence should be addressed. E-mail: johnsoda@ohsu.edu.

www.pnas.org/cgi/doi/10.1073/pnas.0503230102

infection in the eye (18). We similarly found that HSV tk$^-$ mutants spread poorly in the cornea and likely do not reach the stroma well (data not shown). Thus, it was difficult to draw conclusions about the source of HSV that causes keratitis based on studies with these mutants. To investigate this source further, we constructed an HSV mutant that replicated normally in the cornea but was unable to return to the cornea from sensory ganglia. Movement of HSV and the related α-herpesvirus pseudorabies virus (PRV) from the nerve cell bodies of infected neurons to the periphery apparently involves transport by means of microtubule motors (20, 21). There is evidence that nucleocapsids and viral membrane glycoproteins (that make up the virion envelope) move toward axon termini separately, i.e., on different sets of axonal microtubules. Assembly of envelopes onto capsids is thought to occur at axon termini. A PRV membrane protein, US9, functions to promote movement of viral membrane glycoproteins to axon termini, perhaps by tethering glycoprotein-laden vesicles onto microtubule motors (21). In the absence of US9, PRV glycoproteins remain in neuron cell bodies, and no infectious progeny are produced at axon tips (21, 22). HSV expresses a homolog of PRV US9 (23). We reasoned that an HSV US9 mutant might be unable to move in an anterograde fashion from the ganglia to cornea and, thus, we constructed a US9$^-$ HSV and used this mutant to test the idea that roundtrip to the nervous system is required for HSK.

Materials and Methods

Viruses. WT HSV-1 strain F and all virus recombinants were propagated and titered by using Vero cells (24). US9$^-$ HSV was constructed by using a plasmid, pUC-US7/8/9, containing HSV-1 nucleotides 7809–11694 (23) inserted into plasmid pUC-19, replacing US9$^-$ coding sequences (nucleotides 10667–11062) with GFP sequences coupled to the human CMV IE promoter, and recombining this into HSV-1 strain F as described (24). A rescued version of US9$^-$ HSV was derived by transfecting Vero cells with US9$^-$ HSV DNA and plasmid pUCUS7/8/9 and identifying viruses that express US9 and not GFP.

Infection and Spread of Virus in Epithelial Cells and in Mice. ARPE-19 retinal pigmented epithelial cells were infected with low doses of HSV-1, and plaques were stained for HSV antigens as described (24). BALB/c mice were infected on the cornea by corneal scarification as described (25, 26). Animals were killed after 2 days, eyes were removed, and corneas were dissected. Corneas were permeabilized by incubation with 0.2% Triton X-100 for 45 min, blocked with 2% goat serum, and then incubated with anti-HSV antibodies overnight (DAKO). The corneas were washed, incubated with Alexa-594-conjugated goat anti-rabbit IgG, washed, flat mounted on coverslips, and viewed with a confocal microscope. Mice were infected on the snout by first shaving the skin, scarifying as with corneas, and applying 2–4 μl of virus containing 3×10^5 plaque-forming units (pfu) (27); then trigeminal (TG) ganglia and corneas were removed after 4–5 days, and infectious HSV was measured in plaque assays. Mice were infected in the retina by injecting HSV into the vitreous fluid of the eye as described (28). After 5 days, mice were killed and brains were removed, sectioned, and stained with anti-HSV antibodies as described (28).

Keratitis and Periocular Disease in Mice. BALB/c mice were infected with HSV by corneal scarification using 5×10^4 or 5×10^3 pfu, and HSK or periocular skin disease was scored daily between 2 and 15 days by an observer who was unaware of the virus groups and in some cases by using a slit lamp biomicroscope or photographed as described (18, 26).

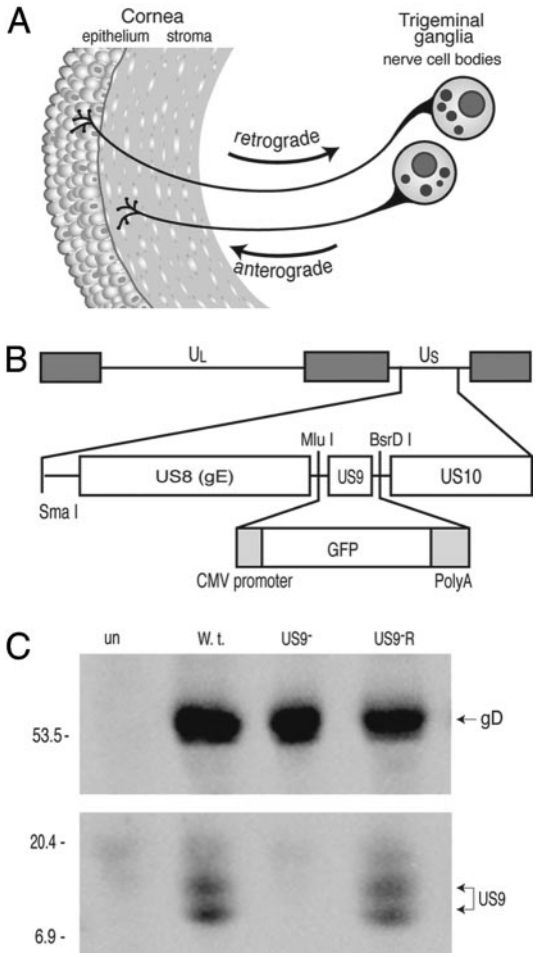

Fig. 1. HSV transport between the cornea and ganglia and construction of an HSV US9$^-$ mutant (US9$^-$ HSV). (*A*) Cartoon depicting HSV infection spread from the cornea epithelium to the trigeminal (TG) ganglia, followed by anterograde spread to the corneal stroma. Sensory neurons possess both retrograde and anterograde modes of transport; however, for simplicity, the neuron projecting to the epithelium is shown only with retrograde transport. (*B*) The HSV-1 genome, including *US8*, *US9*, and *US10* genes, with the *US9* gene replaced with GFP sequences. (*C*) Western blot showing the loss of US9 in US9$^-$ HSV-infected cells. gD is a second HSV membrane protein.

Results and Discussion

An HSV-1 mutant in which the *US9* gene was replaced by a GFP gene was constructed (Fig. 1*B*). Western blot analyses indicated the loss of expression of US9 but not the adjacent gD glycoprotein (Fig. 1*C*). Similarly, gE, encoded by the adjacent *US8* gene, was expressed by US9$^-$ HSV (data not shown). To rule out secondary or separate mutations, a virus was derived from US9$^-$ HSV by rescuing the *US9* gene, denoted *US9$^-$R*, and this recombinant expressed US9 (Fig. 1*C*). US9$^-$ HSV replicated normally in the cultured epithelial cells (data not shown). US9$^-$ HSV plaques produced on human retinal epithelial cells were the same size as those produced by US9$^-$R and WT HSV (Fig. 2*A*). A mutant lacking gE, a glycoprotein important for spread in epithelial cells (24, 25), formed smaller plaques. US9$^-$ HSV formed lesions on the mouse cornea after 2 days that were the same size as those produced by WT and US9$^-$R HSV, whereas gE$^-$ HSV produced small lesions (Fig. 2*B*). In addition, the US9$^-$ mutant produced titers of infectious virus similar or identical to that of WT and US9$^-$R HSV in mouse corneas (Fig. 3*A*). The obvious differences between US9$^-$ HSV and gE mutants in virus spread provided compelling evidence that the

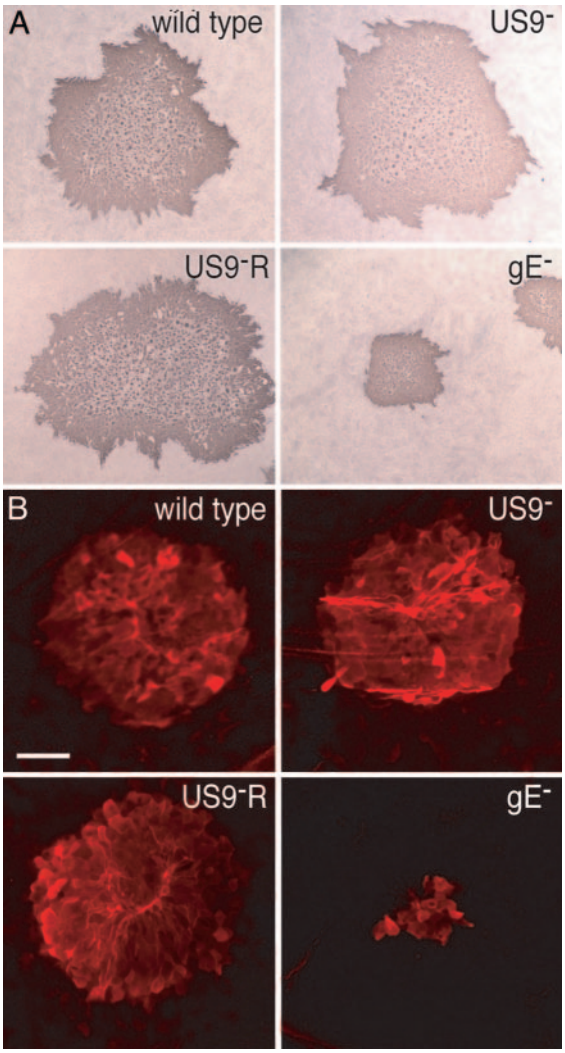

Fig. 2. US9$^-$ HSV spreads normally in epithelial cells and in mouse cornea. (*A*) Cultured ARPE-19 epithelial cells were infected with WT, US9$^-$, US9$^-$R, or gE$^-$ HSV for 48 h, and then the cells were fixed and stained with rabbit anti-HSV antibodies and secondary fluorescent antibodies. (*B*) Mice were infected with HSV by corneal scarification; corneas were removed after 2 days and stained by using rabbit anti-HSV antibodies and secondary fluorescent antibodies. (White bar, 100 μm.)

US9 deletion/substitution did not affect expression of gE or other genes that affect HSV replication or spread. We concluded that US9 is not required for HSV replication and spread in the cornea.

As with transport in the anterograde direction, spread of α-herpesviruses in the retrograde direction, from axon termini to nerve cell bodies, involves axonal microtubule motors that carry capsids to the nucleus, where virus replication occurs (20, 21). When HSV enters sensory neurons, viral glycoproteins are deposited in host membranes and capsids move to the nucleus and initiate infection without the glycoproteins (21). Because US9 is thought to promote axonal movement of glycoproteins, and not capsids, we anticipated that US9$^-$ HSV might move from the cornea to the ganglia normally. To examine this possibility, mice were infected in the cornea, TG ganglia were removed, and virus was quantified. US9$^-$ HSV produced similar quantities of infectious virus in TG ganglia compared with WT and US9$^-$R (Fig. 3*A*). Thus, US9 is not required for retrograde spread to sensory ganglia.

Anterograde spread (from neuronal cell bodies to axon termini) apparently involves the separate transport of nucleocapsids and glycoprotein-laden membrane vesicles, and both are necessary for production of infectious virus (21). PRV US9 promotes traffic of viral glycoproteins toward axon termini, and, thus, we anticipated that US9$^-$ HSV might display defects in anterograde spread. To examine this possibility, we initially used a well characterized model in which virus has direct access to retinal neuron cell bodies and spreads along axons that project to retinorecipient regions of the brain (28). WT HSV was injected into the vitreous body of the eye, and this virus infected and replicated in retinal ganglion neurons and then spread to the superior colliculus, the lateral geniculate nucleus, and the suprachiasmatic nucleus in all animals (Fig. 3*B*). By contrast, 80% of mice infected with US9$^-$ HSV displayed no viral antigens in any region of the brain (Fig. 3*B*), and in 20% of these animals there was limited HSV antigens only in the suprachiasmatic nucleus. Therefore, the US9$^-$ mutant appeared to be severely compromised in its ability to spread in an anterograde direction. One caveat here relates to the fact that HSV must not only move from the retina to the brain but must also spread between neurons to be detected in the brain. Thus, it remained possible that US9 was required for spread between second-order brain neurons.

To further study effects of US9 on HSV spread specifically from the TG ganglia to the cornea, we infected mice with US9$^-$ or US9$^-$R HSV by introducing the viruses onto the snout. After infection of snout epithelial tissues, HSV infects sensory neurons and travels to the TG ganglia, where there is infection of neurons that project to the cornea, leading to infection of the cornea (27). Similar levels of infectious US9$^-$ and US9$^-$R HSV were observed in the TG ganglia of mice (Fig. 3*C Left*), again consistent with the conclusion that US9 is not required for retrograde spread. The majority (71%) of mice infected with these relatively high doses of US9$^-$ HSV displayed no detectable virus in the cornea, and the titers of HSV found in the cornea were extremely low, averaging 3 pfu per cornea (Fig. 3*C*). By contrast, 81% of US9$^-$R HSV-infected mice exhibited virus in the cornea, and titers of virus were substantially higher (666 pfu per cornea). We concluded that US9$^-$ HSV is severely compromised in anterograde spread from the TG ganglia to the cornea so that, in most animals, there was no infectious virus in the cornea. Other results showed that the US9$^-$ mutant failed to transport viral glycoproteins along axons of cultured neurons (K.P, unpublished results), as described for US9$^-$ PRV (21, 22). To our knowledge, US9 is the only HSV gene product described to date that functions exclusively in neurons and not obviously in epithelial cells. As noted above, HSV tk and ribonucleotide reductase mutants are reduced by at least 100-fold in the murine eye (18, 19). Therefore, US9 provides a much more selective tool with which to determine the role of the nervous system in disease.

To ascertain whether US9$^-$ HSV could cause keratitis, mice were infected in the cornea and HSK was followed over the course of 15 days (26). US9$^-$ HSV caused HSK similar or identical to that produced by WT and US9$^-$R HSV; in all animals there was a striking inflammatory response characterized by neovascularization, significant cell influx (histopathology picture not shown), edema, necrosis, and ulceration (Fig. 4). The disease was scored (by an observer who was unaware of the treatment) in groups of 15 mice, as described (26). As shown in Fig. 5*A*, there was no significant difference in the severity of HSK in mice infected with US9$^-$ HSV compared with US9$^-$R- and WT HSV-infected mice. The kinetics of HSK were not different (Fig. 5*B*), and similar comparative results were observed at 10-fold lower virus doses (Fig. 5*C*). US9$^-$ HSV expresses GFP, an immunogenic protein, whereas US9$^-$R HSV does not. It is possible, although we feel unlikely, that GFP expression en-

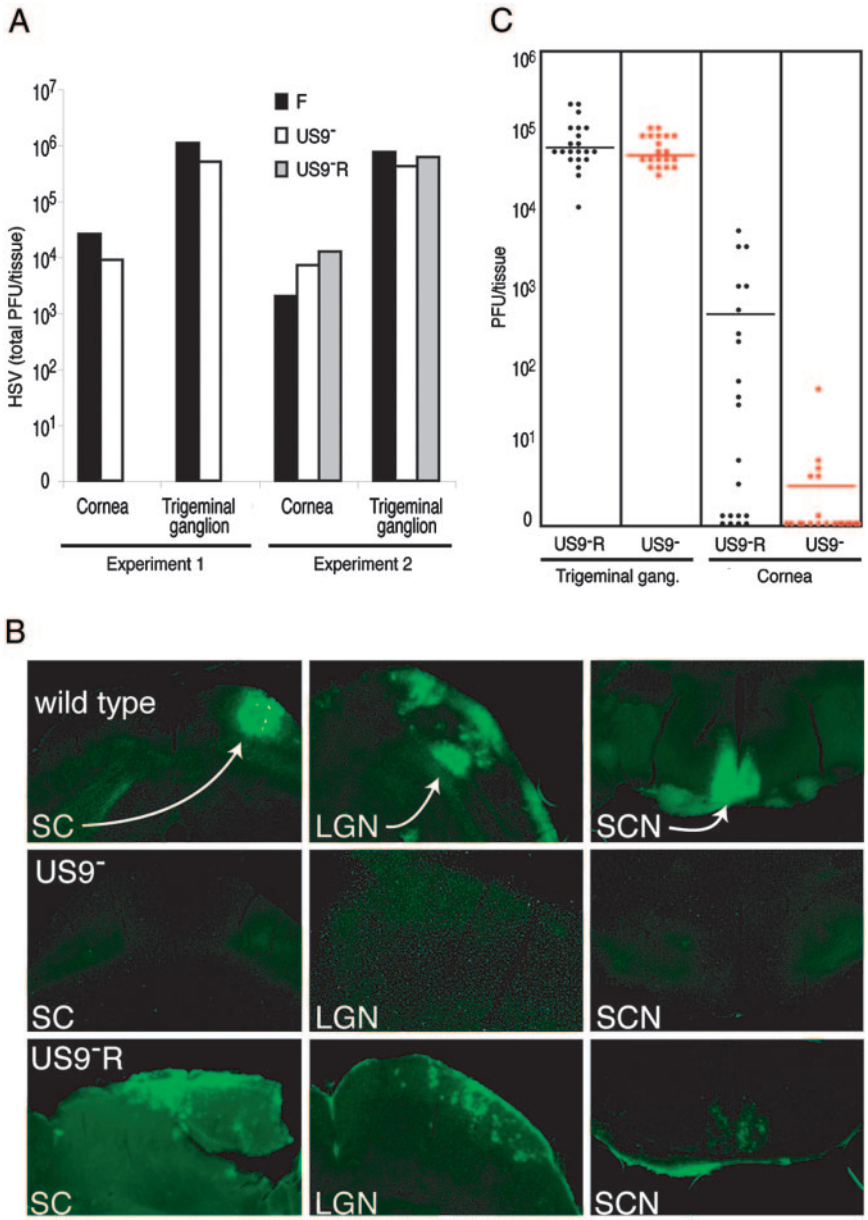

Fig. 3. US9⁻ HSV spreads in neuronal axons in a retrograde but not in an anterograde direction. (*A*) Groups of five mice were infected with HSV (5×10^5 pfu) by corneal scarification. Corneas and TG ganglia were removed at day 4, homogenized, and pooled within each group, and infectious HSV was assayed by plaque titration. (*B*) Spread of HSV from the retina to optic centers of the brain was assessed by injecting each virus (2×10^5 pfu) into the vitreous body of the eye. After 5 days, brain sections were stained for viral antigens as in Fig. 2*B*. SC, superior colliculus; LGN, lateral geniculate nucleus; SCN, suprachiasmatic nucleus. (*C*) Mice were infected with HSV (3×10^5 pfu) by scarification of the snout. TG ganglia and corneas were removed after 5 days, and infectious HSV was measured in plaque assays.

hanced keratitis. We concluded that a roundtrip to sensory ganglia is not necessary for HSK in mice.

Mice infected with HSV in the cornea also become infected in the periocular skin, causing blepharitis and conjunctivitis (29). There is an interval of several days between corneal and periocular skin disease. Studies of HSV tk⁻ mutants have suggested that periocular HSV infection involves zosteriform spread from the cornea to periocular skin via sensory ganglia (18). We compared periocular skin disease in animals infected with US9⁻ HSV with US9⁻R HSV. There was mild to moderate disease with US9⁻R HSV by day 4, and this progressed to severe disease by day 7, when animals began to die of encephalitis (Fig. 5*D*). By contrast, there was no obvious disease in the vast majority of mice infected with US9⁻ HSV and there was mild disease in 1 animal in the group of 15. Therefore, in agreement with previous studies (26), periocular skin infection requires spread from sensory neurons. Moreover, these observations further substantiate our conclusion that US9 is required for return from sensory ganglia to the periphery.

HSK remains an intriguing, complex, and, in some respects, poorly understood example of immunopathology. Anti-HSV T lymphocytes recognize viral antigens or self antigens that mimic HSV and trigger relatively unregulated inflammation in the stroma. In mice, HSV replicates in the corneal epithelium, and viral antigens largely or entirely disappear before inflammation becomes evident, and there are low or undetectable levels of viral antigens in the stroma, which is separated from the epithelium by a basement membrane. Observations of this type,

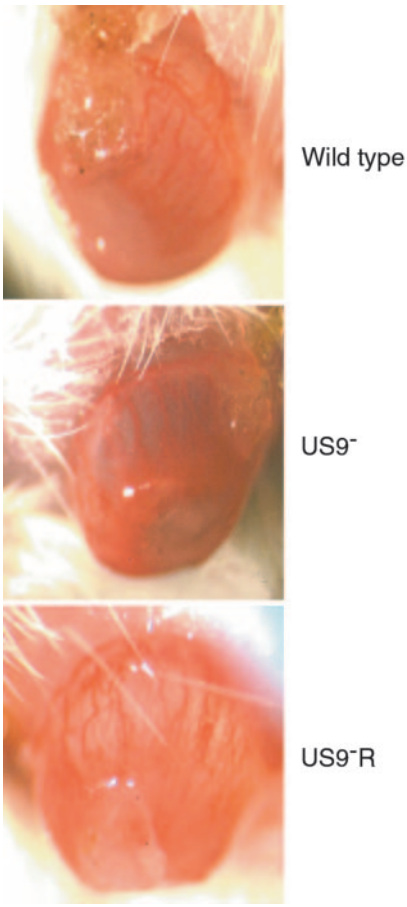

Fig. 4. US9⁻ HSV causes HSK. Mice were infected with 5×10^4 pfu of WT, US9⁻, or US9⁻R HSV-1 by corneal scarification. Photomicrographs were taken of representative corneas after 15 days.

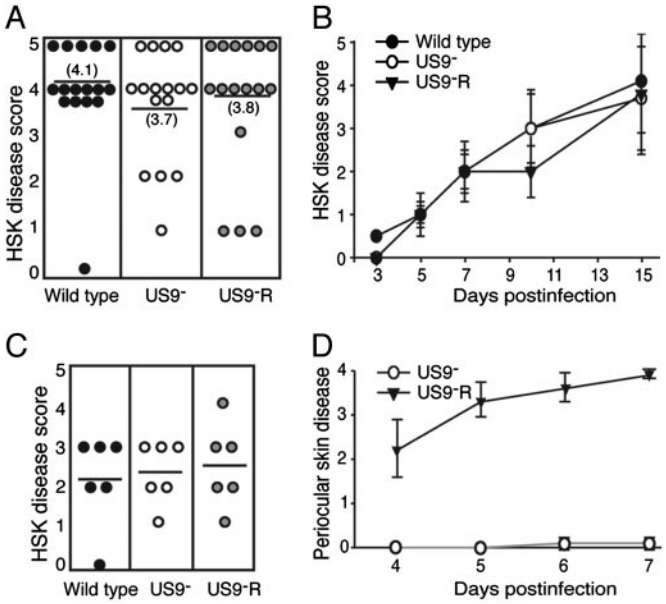

Fig. 5. HSK and periocular skin disease in mice infected with US9⁻ HSV. (*A*) Mice infected with 5×10^4 pfu of WT HSV-1, US9⁻, or US9⁻R by corneal scarification were scored for HSK: 0, normal cornea; 1, mild haze; 2, moderate haze, iris visible; 3, severe haze, iris not visible; 4, severe haze, corneal ulcer; and 5, corneal rupture, after 15 days. (*B*) HSK scores recorded after various times of infection as in *A*, in groups of 15 mice. (*C*) Mice infected with 5×10^3 pfu of each virus and scored as in *A*. (*D*) Groups of 15 mice were infected with 5×10^4 pfu of each virus as in *A* and scored for periocular skin disease: 0, no lesions; 1, minimal eyelid swelling; 2, moderate eyelid swelling accompanied by crusty ocular discharge; 3, severe eyelid swelling and moderate hair loss in periocular skin; and 4, severe swelling with eyes crusted shut, severe periocular hair loss, and skin lesions. The mean disease scores and SDs are shown.

especially the long lag between expression of viral antigens and disease, supported the notion that HSV must travel to sensory ganglia before returning at a later time to the cornea to initiate disease. However, our results demonstrate that HSV can cause HSK in mice without a roundtrip to the nervous system. It is important to clarify that these observations involve infection of mice and do not exclude the real possibility that, in humans with recurrent disease, HSV returns to the cornea from sensory neurons after reactivation to cause HSK. As well, HSV could potentially leave the cornea associated with trafficking immune cells and then return at a later time with these cells. Nevertheless, there are numerous observations of HSV either persistent or latent in human corneas (10–16). Our observations that anterograde spread from ganglia to cornea is not required for disease predict that HSV that remains in the cornea can cause HSK.

The majority of mice infected with higher doses of US9⁻ HSV (3×10^5 pfu) exhibited no detectable infectious HSV in the cornea by sensitive plaque assays. Also consistent with a defect in anterograde spread were observations that virus spread from the retina to the brain was largely abolished. Therefore, in the absence of US9, spread along axons toward the cornea was largely or, in most animals, entirely eliminated. Nevertheless, one could propose that small amounts of HSV antigens were elicited into the stroma derived from HSV-infected neurons, and these were sufficient to activate HSV-specific T cells that cause disease. It is well established that low concentrations of viral antigens can sensitize T cells. When mice were infected with higher doses of virus (3×10^5 or 5×10^4 pfu) there might be sufficient HSV antigens to produce inflammation and HSK. However, when animals were infected with 10- to 60-fold less US9⁻ or WT HSV (5×10^3 pfu), there was identical disease. If low levels of viral antigens were sufficient for disease at high virus doses, at these substantially lower doses, and given the 222-fold lower return of HSV to the cornea, one would anticipate that the levels of viral antigens delivered to the cornea would drop below a threshold required for disease, at least in some animals. Further support for the concept that US9⁻ HSV is sufficiently impaired in retrograde spread came from studies of periocular skin disease, a process that requires spread from the cornea via sensory neurons. The US9⁻ HSV caused little or no periocular skin disease. Together, these results strongly argue against the notion that small amounts of HSV antigens arriving in the stroma from infected neurons can promote HSK. Instead, the data are consistent with the hypothesis that HSV resident in the cornea can initiate this disease. Other studies, perhaps involving deinnervation of the cornea might address this question by a different approach, although such surgical procedures cause inflammation that promotes HSK.

The question remains as to how HSV antigens, if these are necessary, reach the stroma to cause HSK. Although viral antigens are relatively high during initial phases of replication in the epithelium, viral proteins are extremely low in the stroma. These and other observations suggested models in which T cells reactive with cellular autoantigens that mimic viral proteins carry out immunopathology or bystander effects (5, 6). However, the simplest idea is that small quantities of viral antigens reach the stroma from the epithelium. Indeed, we can detect low levels of GFP expressed from the HSV genome in murine stroma (K.P., unpublished data). HSV infection or the act of corneal scarifi-

cation may compromise the underlying basement membrane so that virus antigens and infection spills into the stroma. Alternatively, professional antigen-presenting cells, such as Langerhans cells or monocyte–macrophages, may acquire viral antigens in the epithelium, then move to the stroma and promote inflammation there. The long delay between virus infection and spread in the epithelium and HSK may reflect time required to overcome control mechanisms that dampen host immunity in the stroma.

Our observations that HSV derived after anterograde spread from sensory ganglia is not necessary for the initiation of HSK have important implications in the design of HSV vaccines, which are widely considered necessary to prevent recurrent disease. For example, certain vaccines or delivery methods might produce anti-HSV T lymphocytes that can promote or maintain latency in seropositive individuals by controlling virus reactivation in neurons (30). However, these T cells might also exacerbate corneal disease, especially if virus that persists in the cornea becomes a target for enhanced immunity. The potential of HSV in the cornea to cause disease must also be considered in common corneal surgeries such as keratoplasty and LASIK.

We thank Aurelie Synder for fluorescent microscopy and Jay Nelson for helpful comments on the manuscript. We are indebted to Nancy Sawtell and Tony Simmons for advice on animal models. This research was supported by National Institutes of Health Grants EY11245 and AI055051 (to D.C.J.) and EY05093 (to B.T.R.).

1. Pepose, J. S., Leib, D. A., Stuart, P. M. & Easty, D. L. (1996) in *Ocular Infection and Immunity*, eds., Pepose, J. S., Holland, G. & Wilhelmus, K. (Mosby, St. Louis), pp. 905–932.
2. Streilein, J. W., Dana, M. R. & Ksander, B. R. (1997) *Immunol. Today* **18,** 443–449.
3. Hendricks, R. L. (1999) *Chem. Immunol.* **73,** 120–136.
4. Deshpande, S. P., Zheng, M., Lee, S. & Rouse, B. T. (2002) *Vet. Microbiol.* **86,** 17–26.
5. Zhao, Z. S., Granucci, F., Yeh, L., Schaffer, P. A. & Cantor, H. (1998) *Science* **279,** 1344–1347.
6. Deshpande, S. P., Lee, S., Zheng, M., Song, B., Knipe, D., Kapp, J. A. & Rouse, B. T. (2001) *J. Virol.* **75,** 3077–3088.
7. Davidorf, J. M. (1998) *J. Refract. Surg.* **14,** 667.
8. Dhaliwal, D. K., Romanowski, E. G., Yates, K. A., Hu, D., Goldstein, M. & Gordon, Y. J. (2001) *Am. J. Ophthalmol.* **131,** 506–507.
9. Biswas, S., Suresh, P., Bonshek, R. E., Corbitt, G., Tullo, A. B. & Ridgway, A. E. (2000) *Br. J. Ophthalmol.* **84,** 701–705.
10. Cockerham, G. (2001) *Cornea* **20,** 774–775.
11. Openshaw, H., McNeill, J. I., Lin, X. H., Niland, J. & Cantin, E. M. (1995) *J. Med. Virol.* **46,** 75–80.
12. Neufeld, M. V., Steinemann, T. L., Merin, L. M., Stroop, W. G. & Brown, M. F. (1999) *Cornea* **18,** 489–492.
13. Fukuda, M., Deai, T., Hibino, T., Higaki, S., Hayashi, K. & Shimomura, Y. (2003) *Cornea* **22,** S55–S60.
14. Remeijer, L., Maertzdorf, J., Doornenbal, P., Verjans, G. M. & Osterhaus, A. D. (2001) *Lancet* **357,** 442.
15. Robert, P. Y., Adenis, J. P., Denis, F., Alain, S. & Ranger-Rogez, S. (2003) *J. Med. Virol.* **71,** 69–74.
16. Sabbaga, E. M., Pavan-Langston, D., Bean, K. M. & Dunkel, E. C. (1988) *Exp. Eye Res.* **47,** 545–553.
17. Mitchell, W. J., Gressens, P., Martin, J. R. & DeSanto, R. (1994) *J. Gen. Virol.* **75,** 1201–1210.
18. Summers, B. C., Margolis, T. P. & Leib, D. A. (2001) *J. Virol.* **75,** 5069–5075.
19. Brandt, C. R., Kintner, R. L., Pumfery, A. M., Visalli, R. J. & Grau, D. R. (1991) *J. Gen. Virol.* **72,** 2043–2049.
20. Penfold, M. E., Armati, P. & Cunningham, A. L. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 6529–6533.
21. Tomishima, M. J., Smith, G. A. & Enquist, L. W. (2001) *Traffic* **2,** 429–436.
22. Tomishima, M. J. & Enquist, L. W. (2001) *J. Cell Biol.* **154,** 741–752.
23. McGeoch, D. J., Dolan, A., Donald, S. & Rixon, F. J. (1985) *J. Mol. Biol.* **181,** 1–13.
24. Wisner, T., Brunetti, C., Dingwell, K. & Johnson, D. C. (2000) *J. Virol.* **74,** 2278–2287.
25. Dingwell, K. S., Brunetti, C. R., Hendricks, R. L., Tang, Q., Tang, M., Rainbow, A. J. & Johnson, D. C. (1994) *J. Virol.* **68,** 834–845.
26. Biswas, P. S., Banerjee, K., Kim, B. & Rouse, B. T. (2004) *J. Immunol.* **172,** 3736–3744.
27. Shimeld, C., Dyson, H., Lewkowicz-Moss, S., Hill, T. J., Blyth, W. A. & Easty, D. L. (1987) *Curr. Eye Res.* **6,** 9–12.
28. Dingwell, K. S., Doering, L. C. & Johnson, D. C. (1995) *J. Virol.* **69,** 7087–7098.
29. Maggs, D. J., Chang, E., Nasisse, M. P. & Mitchell, W. J. (1998) *J. Virol.* **72,** 9166–9172.
30. Khanna, K. M., Lepisto, A. J. & Hendricks, R. L. (2004) *Trends Immunol.* **25,** 230–234.