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Immunity in the Female Genital Tract After Intravaginal Vaccination of Mice with an Attenuated Strain of Herpes Simplex Virus Type 2

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Herpes simplex virus type 2 is a common human venereal pathogen which causes lethal neurological illness after intravaginal inoculation into BALB/cJ mice. To investigate whether an attenuated, nonlethal strain of this virus would confer immunity after inoculation of mice, we constructed a strain containing a partial deletion of the thymidine kinase gene, which is necessary for viral replication and spread in sensory ganglia. Unlike its wild-type counterpart, this deletion-containing strain of herpes simplex virus type 2 caused mild clinical disease and was not lethal when studied in an age-dependent murine model of intravaginal infection. Furthermore, after intravaginal infection, the deletion-containing strain could not be isolated from sensory ganglia at a time when wild-type virus was abundant. Of greater significance, intravaginal inoculation with the deletion-containing strain rendered mice completely resistant to rechallenge with a 10-fold 50% lethal dose of wild-type virus. These results suggest that a strain of herpes simplex virus type 2 containing a deletion of the thymidine kinase gene will be useful in studying the cellular basis of mucosal immunity in the genital tract.

Several studies indicate that the generation of immunity in the genital tract can be achieved by vaccination approaches that are different from those inducing systemic immunity. Exposure of the female genital tract to soluble (6), cellular (7, 12, 13, 22, 48, 51), or viral (36) antigens results in an antigen-specific antibody response, particularly secretory immunoglobulin A (IgA), in cervicovaginal secretions which is not necessarily reflected in the circulation. This finding correlates with a marked increase in the number of IgA- and IgG-containing plasma cells found in the cervicovaginal mucosa (12). Similar observations have been made at the mucosae of the intestinal and respiratory tracts and at the salivary, lacrimal, and lactating mammary glands (9, 26, 30, 31). In contrast, parenteral immunization does not usually produce adequate mucosal immunity (9, 26). An experimentally supported explanation for these findings, at least in part, is the existence of a common mucosal immunological system linking the various mucosae through the selective migration of mucosally derived precursor cells that are reactive to environmental antigens (9, 26–28, 50). Thus, an approach to vaccination against genital-tract infection would be the stimulation of mucosal immune responses, perhaps in concert with systemic immunity.

Herpes simplex virus type 2 (HSV-2) is a common cause of sexually transmitted disease for which there is inadequate medical prevention or cure (1). Primary infection of adults and older children with HSV-2 may result in ulcerative lesions of the genital mucosa, and HSV-2 can spread, in the absence of viremia, via neural routes to the spinal cord and meninges, causing encephalitis (2). Recrudescent disease is thought to occur by activation of persistent but latent HSV-2 from infected ganglia (2, 49). Although immunity to or recovery from primary systemic infection by HSV-2 appears to be mediated by humoral and cellular immune mechanisms acting in concert (2, 5, 11, 32, 34), systemic immunization with nonviable, attenuated, or subunit HSV-2 vaccines (10) has not proven to be sufficiently efficacious in generating protective immunity at the genital mucosa. However, HSV-2-specific antibodies are found in cervicovaginal secretions of HSV-2-infected women (29) and mice (34) and, indeed, Morahan et al. (32) commented that mice surviving intravaginal (IVAG) inoculation with HSV-2 were resistant to subsequent IVAG infection but not systemic infection. Thus, stimulation of mucosal immunity might confer resistance to HSV-2 infection in the genital tract.

To investigate this hypothesis, we established a murine model of lethal primary HSV-2 infection in the female genital tract. Since attenuated, replicating antigens elicit maximal mucosal immune responses (9, 26), we constructed a nonlethal attenuated HSV-2 strain deficient in thymidine kinase (TK) activity (TK^-). IVAG inoculation of mice with TK^- HSV-2 induced protective immunity to lethal challenge with wild-type (TK^+) virus. These results indicate that attenuated TK^- HSV-2 will be useful in unraveling the cellular basis of mucosal immunity in the genital tract.

MATERIALS AND METHODS

Cell and virus propagation. Monolayers of Vero cells were grown in Eagle minimal essential medium supplemented with 5% heat-inactivated fetal bovine serum, 1% penicillin-streptomycin (GIBCO, Burlington, Ontario), 0.3% glutamine, 0.075% $NaHCO_3$, and 0.01 M HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) buffer. HSV-2 strain 333, a wild-type TK^+ strain, human host virus adapted for murine infection (41), and the TK^- HSV-2 strain described below were propagated by infecting Vero cells with virus suspended in 5 ml of medium at 37° for 1 h at a multiplicity of 0.1 PFU per cell, and then the cell monolayers were resupplied with fresh medium. When the cytopathogenic effect was extensive, the cells were collected by agitation, washed by centrifugation (200 × *g* for 10 min), and suspended in medium containing 2% fetal bovine serum. Virus was released by the freezing and thawing of cells, followed by sonication. The cell lysate was clarified by centrifugation at 1,500 × *g* for 20 min. Samples of virus stock were stored at −70°C. The virus titer of samples thawed at 0 to 4°C was assayed by plaque formation on Vero cell monolayers (38). A single batch of virus stock was used

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throughout each experiment. We have not noted any appreciable differences in virus activity between virus batches.

Construction of TK$^-$ HSV-2. Strain 333, a wild-type HSV-2 that contained a partial TK$^-$ gene deletion (ΔTK$^-$), was isolated by using a two-step procedure analogous to that used for TK gene deletion in herpes simplex virus type 1 (HSV-1) (43, 44) and is conceptualized in Fig. 1. First, a partial deletion was generated in the TK gene coding region of an HSV-2 DNA fragment which had been cloned in a plasmid vector. Second, the partial-deletion-containing TK gene was transferred into the intact viral genome by recombination in vivo.

The plasmid L3PK1 contains a *Bgl*II-*Xho*I fragment of HSV-2 strain 333 DNA bearing the TK gene cloned between the *Sal*I and *Bam*HI sites of pKC7 (Fig. 1) (S. Bacchetti, McMaster University, personal communication). Treatment of L3PK1 with the restriction endonuclease *Kpn*I (Bethesda Research Laboratories, Gaithersburg, Md.) excised a fragment ca. 180 nucleotide bases long from the TK gene coding region. Recircularization of the plasmid was then accomplished with T4 DNA ligase. After plasmid-mediated transformation of *Escherichia coli* HB101 to ampicillin resistance, plasmids were screened for the desired TK gene deletion by restriction cleavage site mapping. One plasmid bearing a deletion of ca. 180 nucleotide bases was amplified and used as a source of the deleted TK gene DNA for marker rescue, which was conducted as previously described (43, 44). Infectious TK$^+$ HSV-2 strain 333 DNA was transfected into Vero cells along with 100 ng of circular TK-deletion-containing plasmid DNA by using the calcium phosphate coprecipitation method of Graham and Van der Eb (17). The rare TK$^-$ recombinant virions emerging from the transfected cells were positively selected by their ability to form plaques on Vero cell monolayers in the presence of 100 μg of thymine arabinoside (Calbiochem-Behring, La Jolla, Calif.) per ml. Individual TK$^-$ HSV-2-containing plaques were transferred into microwells (1.0 cm^2; Linbro, Hamden, Conn.) of Vero cell monolayers and used to establish working stocks of virus. Stocks were used to infect flask monolayers (25 cm^2) of Vero cells, and viral DNA was selectively extracted from the infected cultures by the procedure of Hirt (20). Samples of viral DNA were cleaved with the restriction endonuclease *Pvu*II and analyzed for the presence of the relevant TK gene deletion by Southern blot hybridization (45) with ^{32}P-labeled L3PK1 DNA as a probe. Nucleic acid hybridization and nick translation were done as previously described (40). An arbitrarily chosen virus isolate bearing the desired TK gene deletion (ΔTK$^-$, designated Δ11a in Fig. 1) was plaque purified twice and expanded into a high titer stock for further study.

Animals. Females of strain BALB/cJ, a strain that is moderately susceptible to lethal infection with HSV-2 (18, 25), were obtained from Health Research Inc., West Seneca, N.Y., and used between 3 and 19 weeks of age.

IVAG inoculation with HSV-2. Mice were inoculated IVAG with 10 μl of HSV-2 suspended in phosphate-buffered saline. No fluid leakage from the vaginal orifice was observed. Mice were individually coded by ear punch and examined daily for vaginal inflammation, neurological illness, and death.

Tissue processing. Animals were sacrificed by cervical dislocation, with care being taken to ensure that expelled urine did not enter the vaginal orifice. A small cotton pledget soaked in medium was used to swab the vaginal canal. A second vaginal swabbing was conducted with a dry pledget. Both pledgets were transferred into 1.0 ml of medium at 0°C and stored at −70°C. Specimens were thawed at 0 to 4°C, the pledgets were agitated in the medium before removal by centrifugation (1,500 × *g* for 20 min), and the supernatants were assayed for virus titer on Vero cell monolayers (38).

Dorsal sensory ganglia were removed at the spine as previously described (49). Explanted ganglia (3 to 4 per animal) were rinsed in medium and cultured on Vero cell monolayers in 24-well macrotiter plates (Costar, no. 3524; Johns Scientific, Toronto, Ontario) or homogenized and sonicated at 0°C in 1.0 ml of phosphate-buffered saline. After thawing from storage at −70°C, homogenates were clarified by centrifugation, and virus titers were assayed by plaque formation on Vero cell monolayers (38).

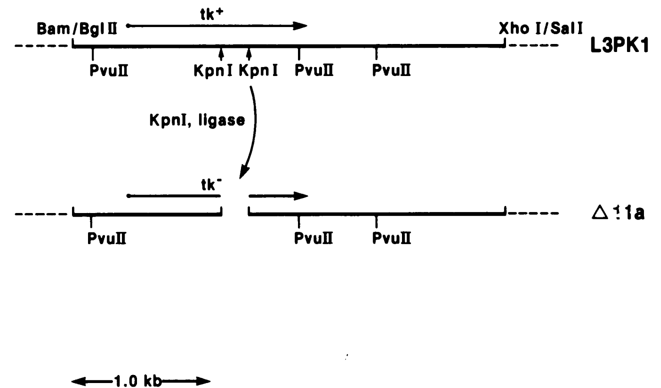

FIG. 1. Structure of the bacterial plasmids L3PK1 and Δ11A. The plasmid L2PK1 contains a *Bgl*II-*Xho*I fragment of HSV-2 strain 333 DNA bearing an intact TK gene cloned between the *Sal*I and *Bam*HI sites of pKC7. The position of the TK coding sequence is indicated, along with *Pvu*II and *Kpn*I restriction endonuclease cleavage sites. A deletion of ca. 180 nucleotide bases from the TK gene of the parental plasmid L3PK1 was accomplished with *Kpn*I. The deletion-containing plasmid, Δ11A, was isolated as described in the text. The cleavage maps and the position of the TK gene are described in reference 40. Southern blot hybridization analysis was used to identify viral isolates containing the partially deleted TK gene. kb, Kilobase.

RESULTS

Isolation of an HSV-2 TK$^-$ deletion mutant. The genome of HSV-2 is too large for easy application of the techniques of direct, site-specific mutagenesis. Consequently, to generate a partially deleted TK gene (ΔTK$^-$) in HSV-2, we employed a two-step procedure previously described for the isolation of comparable HSV-1 mutants (43, 44). Briefly, a partial deletion of ca. 180 bases was introduced into the HSV-2 TK gene sequence present in a bacterial plasmid and transferred into the intact viral genome by homologous recombination in vivo (Fig. 1). The resulting TK$^-$ HSV-2 progeny were isolated by drug selection methods and screened for the presence of the correct partial TK gene deletion by Southern blot hybridization analysis (45). In this method, DNAs from 12 TK$^-$ HSV-2 isolates were cleaved by *Pvu*II, and the resulting fragments were hybridized to a ^{32}P-labeled TK gene region probe.

Figure 2 shows that 9 of these 12 isolates (lanes 1 to 6, 8, 11, and 12) lacked the TK$^+$ 1.5-kilobase *Pvu*II fragment and, instead, displayed a new fragment that comigrated more rapidly with the corresponding *Pvu*II fragment (lane 11a) from the Δ11a plasmid containing the relevant deletion. Therefore, these nine virus isolates have acquired a partial TK gene deletion by in vivo recombination with the plasmid DNA that was present during transfection. The remaining

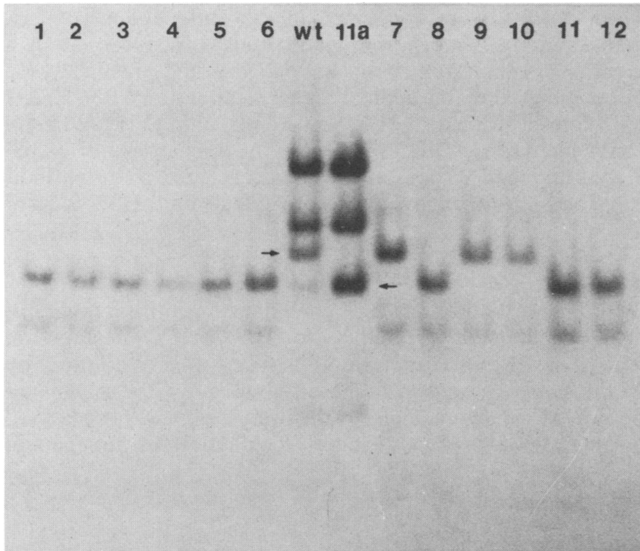

FIG. 2. HSV-2 isolates containing a partial deletion of the TK gene. Samples of viral DNA prepared from 12 TK⁻ virus isolates were cleaved with the restriction endonuclease *Pvu*II, and the resulting fragments were separated by electrophoresis through a 1.4% agarose gel. After transfer to nitrocellulose, the TK-deleted fragments were detected by hybridization to a ^{32}P-labeled L3PK1 DNA probe. Lanes 1 through 12, DNA from viral isolates 1 through 12; wt, L3PK1 marker plasmid DNA; 11a, Δ11a marker containing the relevant TK deletion from plasmid DNA. The positions of the wild-type and deleted versions of the 1.5-kilobase TK *Pvu*II fragments are indicated with arrows.

three TK^- HSV-2 isolates (lanes 7, 9, and 10) retained a normal version of the 1.5-kilobase *Pvu*II fragment and are presumably spontaneously occurring TK^- point mutants. The ΔTK^- HSV-2 isolate analyzed in lane 5 was arbitrarily chosen for use in all subsequent experiments.

Animal model of lethal IVAG infection with TK^+ HSV-2. The clinical progression of disease was followed after a primary IVAG infection of 8-week-old mice with 10^3 PFU of TK^+ HSV-2. A severe vaginal inflammation accompanied by mucus discharge and occasionally local hemorrhage developed 3 to 4 days postinfection, with death occurring within 11 to 13 days. In most mice, ataxia and hind-limb paralysis preceded death by 1 to 2 days. In this age group, the 50% lethal dose (LD_{50}) was found to be from 10^3 to 10^4 PFU of TK^+ HSV-2 per animal. We have repeatedly observed that IVAG inoculation with greater than 10^4 PFU of TK^+ HSV-2 caused 80 to 100% mortality in 8-week-old mice and that greater than 10^5 PFU given via this route was invariably lethal in a shorter period of time. Regardless of IVAG HSV-2 dose, all mice that developed clinical disease after primary infection eventually succumbed.

Age-dependent resistance to TK^+ HSV-2. Age-dependent resistance to IVAG infection with HSV-2 has been reported to occur in outbred mice (4). Thus, resistance to TK^+ HSV-2 challenge after immunization of inbred BALB/cJ mice might be difficult to attribute to vaccination-induced resistance or potentially to nonspecific age effects. The susceptibility of BALB/cJ mice of various ages to primary IVAG infection with TK^+ HSV-2 is shown in Fig. 3. The ability of 10^3 PFU of TK^+ HSV-2 inoculated IVAG to cause death declined exponentially with increasing host age. These results indicated that ca. 100% of neonatal mice would be susceptible to IVAG HSV-2 infection with 10^3 PFU, an observation consistent with neonatal systemic infection by HSV-1 or HSV-2 (46), and that less than 10% of mice are susceptible to TK^+ HSV-2 beyond 14 to 16 weeks of age. An independent study confirmed these findings. Further studies indicated that the LD_{50} increased accordingly in older mice, being ca. 10^5 PFU in 10- to 12-week-old mice (data not shown).

Nonlethal IVAG infection with ΔTK^- HSV-2. Since HSV-1 and HSV-2 possess a virion-encoded TK gene possibly necessary for viral DNA synthesis in quiescent host cells, such as in sensory ganglia (47), ΔTK^- HSV-2 may be incapable of lethal neurological spread. To determine whether ΔTK^- HSV-2 was infective and possibly lethal in vivo, mice were inoculated IVAG at 5 to 6 weeks of age with 7.5×10^2 to 7.5×10^6 PFU of virus. Figure 4 shows the virus dose response for the presentation of mild vaginal inflammation 5 to 6 days postinfection. This mild disease elicited by ΔTK^- HSV-2 did not subsequently progress to the severity seen in TK^+ HSV-2-infected animals. Of greater significance, all mice, even those inoculated with 1,000 times the TK^+ HSV-2 LD_{50} in 8-week-old mice (Fig. 3), completely recovered normal health within 2 weeks after the initiation of mild clinical disease and survived. These results were confirmed by two independent experiments. Other experiments indicated that the age-related susceptibility of BALB/cJ mice to IVAG infection with nonlethal ΔTK^- HSV-2 was similar to their susceptibility to IVAG infection with lethal TK^+ HSV-2 (data not shown). Thus, the mild clinical disease observed in ΔTK^- HSV-2-infected mice was not related to the ability of this virus strain to achieve host infection as a result of animal age.

Detection of HSV-2 in vaginal secretions and sensory ganglia. Death after TK^+ HSV-2 IVAG inoculation in mice is

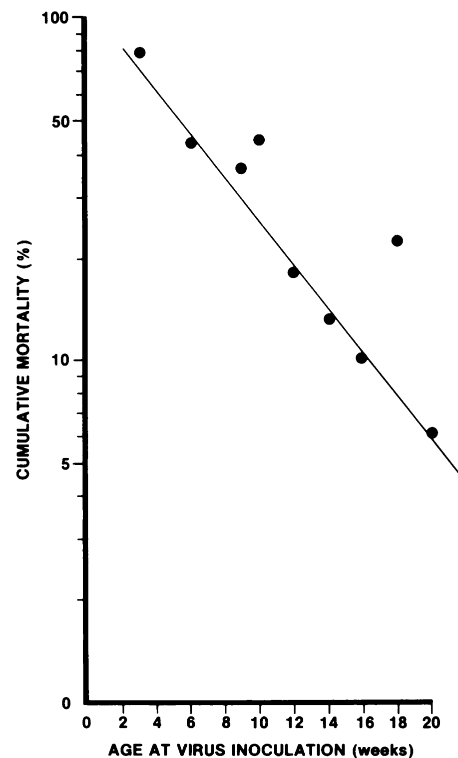

FIG. 3. Influence of age on susceptibility to IVAG infection with HSV-2. Groups of 20 BALB/cJ mice at various ages were inoculated IVAG with 10^3 PFU of wild-type HSV-2 strain 333. The cumulative mortality, expressed as a percentage in each group, was determined 14 days later.

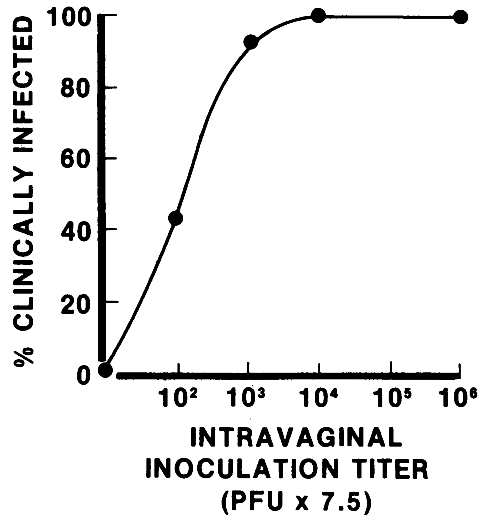

FIG. 4. Susceptibility to IVAG infection with HSV-2 containing a partial deletion of the TK gene (ΔTK⁻). Groups of 10 BALB/cJ mice were inoculated IVAG with ΔTK⁻ HSV-2, and each group was monitored for 14 days to determine the percentage of animals showing clinical disease.

due to a productive neurological infection (1, 2) and, thus, survival after ΔTK⁻ HSV-2 inoculation might be attributed to a lack of virus replication and spread in sensory ganglia (47). Figure 5 shows a parallel decrease in TK⁺ and ΔTK⁻ HSV-2 titers in vaginal secretions after inoculation. Although mice infected with TK⁺ HSV-2 died within 6 days postinfection, ΔTK⁻ HSV-2 was not lethal and was not detectable in vaginal secretions at this time. Infective TK⁺ HSV-2 could be recovered from homogenized sensory ganglia within 2 days postinfection and, here, the virus titer rose dramatically until death ensued at 6 days postinoculation. ΔTK⁻ HSV-2 was not detectable in homogenized spinal ganglia by this method at any time after IVAG inoculation.

Since HSV-2 may be labile to homogenization procedures (49), intact ganglia were cultured from 5-week-old mice (five per group) that had been inoculated IVAG 2 days previously with 2 × 10^6 PFU of either TK⁺ or ΔTK⁻ HSV-2. Within 5 days of culture, viral plaques on Vero cell monolayers were observed in ganglia explant cultures from all animals infected with TK⁺ HSV-2. However, not one ganglion taken from ΔTK⁻ HSV-2-infected mice yielded any viral plaques after 14 days of culture on Vero cell monolayers.

Protection from lethal TK⁺ HSV-2 infection by ΔTK⁻ HSV-2. To determine whether primary IVAG infection with ΔTK⁻ HSV-2 could protect mice from lethal IVAG challenge with TK⁺ HSV-2, groups of 5- to 6-week-old mice were inoculated IVAG with various doses of ΔTK⁻ HSV-2 and challenged IVAG 6 weeks later with 10^6 PFU of TK⁺ HSV-2, i.e., 10-fold the LD_{50} in 11-week-old mice, as mentioned above. Table 1 shows that mice inoculated with ΔTK⁻ HSV-2 were resistant to lethal IVAG challenge with TK⁺ HSV-2. These results were based upon the cumulative mortality in each group determined 14 days after challenge with TK⁺ HSV-2, i.e., a time when maximal mortality was observed in the control group. Indeed, ΔTK⁻ HSV-2-inoculated mice did not show the development of clincal disease when challenged IVAG with TK⁺ HSV-2, and they remained apparently healthy. Furthermore, we did not observe the delayed development of TK⁺ HSV-2-associated disease in animals after 2 weeks of additional observation. These results were confirmed in two independent experiments. In the studies shown in Table 1, as well as in a number of other studies not described in the present work, mice which showed clinical signs of infection after IVAG inoculation with ΔTK⁻ HSV-2 were resistant to lethal IVAG challenge with TK⁺ HSV-2. Thus, the appearance of ΔTK⁻ HSV-2-induced vaginal inflammation seemed to be an indicator of subsequent resistance to lethal TK⁺ HSV-2 challenge.

DISCUSSION

In the present studies, we established a murine model of age-dependent susceptibility to IVAG HSV-2 infection which was used to determine whether protective immunity in the genital mucosa could be elicited by an attenuated ΔTK⁻ HSV-2 strain. The results indicate that ΔTK⁻ HSV-2 is able to achieve a productive infection of the genital

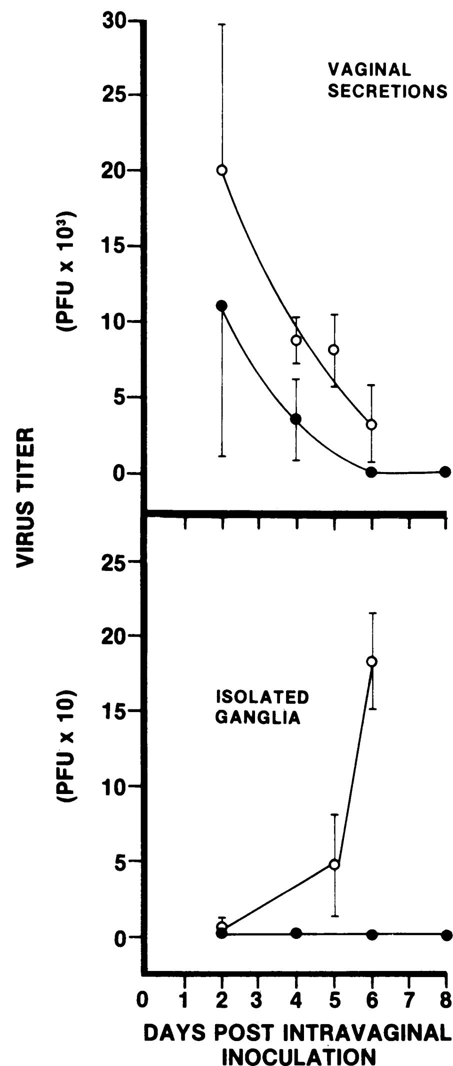

FIG. 5. Detection of HSV-2 in vaginal secretions and isolated, homogenized dorsal sensory ganglia. BALB/cJ mice (10 per group) were inoculated IVAG with 2.5 × 10^6 PFU of wild-type HSV-2 or 7.5 × 10^6 PFU TK⁻ HSV-2. Virus titers in vaginal secretions and homogenized spinal ganglia were determined by plaque formation on Vero cell monolayers. Symbols: ○, Wild-type HSV-2; ●, ΔTK⁻ HSV-2. Data represent means ± standard deviations.

TABLE 1. Protection from lethal TK$^+$ HSV-2 genital infection by IVAG ΔTK$^-$ HSV-2 inoculation[a]

ΔTK$^-$ HSV-2 inoculation dose (PFU)[b]	Proportion of animals after ΔTK$^-$ HSV-2 inoculation		Mortality after IVAG challenge with TK$^+$ HSV-2[c]		
	Healthy	Diseased	Healthy mice	Diseased mice	%
None			9/10		90
10^3	2/10	8/10	1/2	0/8	10
10^4	1/10	9/10	0/1	0/9	0
10^5	0/10	10/10		0/10	0
10^6	0/10	10/10		0/10	0

[a] ΔTK$^-$ HSV-2 is a TK-deficient (TK$^-$) strain of wild-type (TK$^+$) HSV-2. ΔTK$^-$ HSV-2 contains a partial deletion of the TK gene and does not cause lethal infection after IVAG inoculation of mice.

[b] Groups containing 10 BALB/cJ female mice, ages 5 to 7 weeks, were inoculated IVAG with ΔTK$^-$ HSV-2.

[c] ΔTK$^-$ HSV-2-inoculated mice were challenged 6 weeks later with a 10-fold LD_{50} dose of TK$^+$ HSV-2. Ratios represent the proportion of ΔTK$^-$ HSV-2-inoculated or control mice killed by TK$^+$ HSV-2.

mucosa but, unlike primary IVAG infection with wild-type virus, it did not achieve lethal neurological spread. Furthermore, mice were rendered resistant to lethal IVAG challenge with wild-type virus after primary IVAG inoculation with ΔTK$^-$ HSV-2. These findings indicate that ΔTK$^-$ HSV-2 will be useful in unraveling the local immune response in the female genital tract.

IVAG infection with lethal wild-type HSV-2 has been described in both inbred (11, 32) and outbred (34, 49) mice, and susceptibility to this pathogen is probably under genetic control (25). In this study, the local inflammation readily produced by small IVAG inoculations was similar to that reported previously and invariably predicted neurological illness and death. However, a dramatic age-dependent decrease in susceptibility to IVAG HSV-2 was observed (Fig. 3). Although age-related decreases in systemic susceptibility of neonatal mice to HSV-1 and HSV-2 have been correlated with macrophage activity (19, 46), the mechanisms responsible for this age-related resistance in adults are unknown. This observation has been noted in outbred mice (46) and has an important bearing upon the interpretation of HSV-2 host resistance after mucosal immunization. For example, others have reported (32) that the few BALB/cJ mice surviving primary IVAG HSV-2 infection were resistant to IVAG but not intravenous rechallenge at a later time. These earlier studies used mice in broad age ranges and, thus, it is difficult to attribute the observed viral resistance to age effects or to the development of active immunity. Nevertheless, these observations suggest that after mucosal exposure to antigen, when active immunity or age-related nonspecific resistance was operative, it was restricted to the genital mucosa in a manner similar to that seen after exposure of the genital tract to protein antigens (6), various bacteria, (22, 48, 51), spermatozoa (7), and poliovirus (36).

Since maximal protective and durable mucosal immunity is elicited by persistent, replicating antigens (9, 26), an approach to investigating genital immunity might be the use of an attenuated pathogen such as TK$^-$ HSV-2. In rodents, lethal HSV-2 infection spreads, in the absence of viremia, via intraganglial replication (47) and ascending intraaxonal transport from mucosal surfaces (3, 23). Evidently, the presence of intracellular, virally encoded TK is necessary for viral DNA synthesis in nonproliferating ganglia (47) and, thus, TK$^-$ HSV-2 should be incapable of lethal neurological spread. However, since TK$^-$ point mutations in HSV-2 have a relatively high potential for reversion to the wild-type genotype, we reasoned that a sizeable, genetically engineered deletion of the TK gene (Fig. 1 and 2) would still allow virus infection of the vaginal wall but would be unlikely to revert to a lethal wild-type genotype. Our results support this hypothesis. ΔTK$^-$ HSV-2 grew well in tissue culture, remained genetically stable, and infected the vaginal wall in mice, causing mild clinical disease (Fig. 4). However, although ΔTK$^-$ HSV-2 was readily detected in vaginal secretions as reported previously for wild-type virus (32), it could not be isolated from either homogenized or explanted dorsal sensory ganglia at a time when TK$^+$ HSV-2 was readily detected (Fig. 5). The finding that ΔTK$^-$ HSV-2 did not cause lethal neurological illness suggests that it did not achieve ganglial infection or, alternatively, did so but became latent in this site due to a lack of functional TK. Although we are not presently able to choose between these hypotheses, there is no evidence to support the notion that TK activity is required for the infective process to occur at the host cell surface membrane and, thus, the establishment of persistent but latent infection appears most likely. Molecular hybridization studies are currently being conducted to determine whether the ΔTK$^-$ gene can be detected in ganglia taken from ΔTK$^-$ HSV-2-infected animals.

Preexposure of the vaginal mucosa to ΔTK$^-$ HSV-2 rendered mice resistant to lethal IVAG challenge with TK$^+$ HSV-2 (Table 1). Although it is not clear whether such mucosal resistance was specific, the development of mild clinical disease may be an adjuvant as well as an indicator of resistance to rechallenge with TK$^+$ HSV-2. Certainly, antibodies specific for HSV-2 antigens appeared in cervicovaginal secretions taken from both women (29) and mice (34) after genital exposure to HSV-2, and patients without detectable circulating antibody to HSV-2 seroconverted shortly after acquiring a genital infection with the virus (1). Protection from systemic HSV-1 and HSV-2 appears to be mediated by a combination of circulating antibody, macrophages, and T lymphocytes (2, 5, 32), and in vivo studies suggest that several systemic immunological effector mechanisms, such as cytotoxic T cells (24, 42), macrophages (19, 37), natural killer cells (15, 16), antibody (5, 35), and interferon (15, 16), may also be involved in resistance to these serotypes. However, the degree to which any of these mechanisms are expressed at the female genital mucosa and associated lymph nodes remains to be investigated.

Little is known about immunity at the genital mucosa. A number of humoral indices of genital immunity to IVAG antigen have been reported (6, 34, 36), and cellular responses to alloantigens can be expressed in the reproductive organs (14). We previously showed (27, 28) that the gut-associated lymphoid tissue is an enriched source of IgA plasmacyte precursors and, to a lesser extent, IgG plasma cells in the cervix and vagina. Indeed, the mucosally derived cell localization and the humoral immune response in the female genital tract appear to be influenced by sex hormones (12, 21, 28, 52). Whether immunocompetent mucosal precursor cells, including both T and B lymphocytes, are attracted to the genital tract in response to antigen, analogous to their antigen-driven traffic to the intestinal wall (9, 26), is not known. Alternatively, some precursor cells in the genital tract might be indigenous or possibly in transit to other sites. Nevertheless, the observation that the mucosal immune response in the female genital tract resembles that found at other mucosal sites (9, 26) and the restriction of mucosal immunity to the genital tract after local exposure to antigen (6, 7, 12, 13, 22, 32, 36, 48, 51) suggest that host resistance to

mucosal infection by HSV-2 might be best achieved by local immunization acting in concert with systemic immunity.

The results of this work indicate that ΔTK^- HSV-2 may be an appropriate pathogen with which to investigate the genital immune response against HSV-2 and will be helpful in understanding and manipulating the elements of immunity that defend the genital mucosa. Although it might appear that the ΔTK^- HSV-2 is a candidate for incorporation into a specific vaccine, it should be cautioned that HSV-2 genital infection has been correlated with the development of cervical carcinoma (39) and, thus, as an attenuated vaccine, ΔTK^- HSV-2 would presumably offer unacceptable risk. Indeed, it is noteworthy that systemic immunization of mice with inactivated HSV-2 appears to protect against HSV-2-induced cervical neoplasia (8). Furthermore, the murine model of genital infection with HSV-2 is not entirely representative of the adult human disease situation where encephalitis and death rarely occur. Nevertheless, ΔTK^- HSV-2 may be an appropriate tool to identify viral-antigen components which might be most efficacious in eliciting protective immunity at the genital mucosal surface.

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