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Genital *Herpesvirus hominis* Infection in Mice

I. Development of an Experimental Model[1]

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Pregnant female mice, after intravaginal inoculation with *Herpesvirus hominis* (HVH) type 2, developed vaginitis on days 5 to 7 after virus challenge, followed by hunching and hind limb paralysis on days 7 to 9 and death from encephalitis on days 9 to 11. After initial replication in the mucous membranes of the genital tract, virus spread to the spinal cord and ascended to involve the brain. Viremia or replication of *H. hominis* type 2 in the liver or spleen was not detected. Virus was cleared from vaginal secretions by days 8 to 10 after infection. Pregnant mice were more susceptible to the infection than nonpregnant mice. This experimental infection in female mice provides a model for genital herpesvirus infection and for herpesvirus encephalitis in which one can evaluate potentially promising antiviral chemotherapeutic agents.

Infections of the human genital tract with *Herpesvirus hominis* (HVH) type 2 have assumed increasing importance during recent years. The incidence of genital HVH type 2 infection in female patients varies from 0.03% up to 8% depending on the population studied and the method used for diagnosis (25, 30). Although many of these infections are asymptomatic, local pain and irritation can be severe and recurrent disease is not uncommon. In the patient with an underlying immunosuppressive disease, genital herpes infection may be prolonged and disabling. In addition, the incidence of genital herpes appears to be increased during pregnancy (26). If primary disease occurs during the latter stages of pregnancy, there is a significant risk of transmission of the infection to the newborn infant during the birth process and cesarean section has been recommended for women with evidence of active disease at the time of delivery (20). Infection of the neonate with HVH type 2 is frequently associated with disseminated disease and has been reported to result in death in over 70% of cases with neurological sequelae in a significant proportion of the survivors (20). Finally, a number of investigators have demonstrated the significant association, perhaps etiological, of HVH type 2 with carcinoma of the cervix in women (25, 30).

These factors clearly indicate the need for effective antiviral chemotherapy in the treatment of HVH type 2 infection in humans. Although successful treatment of serious HVH disease has been reported with iododeoxyuridine (27), cytosine arabinoside (14), and adenine arabinoside (4), conclusive proof of efficacy based on controlled studies is lacking for all these compounds. Topical therapy for genital herpesvirus infection with neutral red appears to be promising (8, 11); however, additional proof of both safety and efficacy is needed.

After the initial observations of Nahmias et al. (22, 23), we developed an experimental HVH type 2 genital infection in mice as a model of human disease in order to: (i) establish a test system for antiviral compounds which might have potential use in the human disease; (ii) determine the pathogenesis of the infection; and (iii) define the requirements for successful therapy with a given antiviral drug. The results of studies comparing the susceptibility of pregnant and nonpregnant mice to an intravaginal inoculation with HVH type 2 and experiments concerning the pathogenesis of the infection in pregnant mice are presented in this report.

MATERIALS AND METHODS

Animals. Pregnant and nonpregnant adult female Swiss-Webster mice (Simonsen Laboratories, Gilroy, Calif.) were inoculated intravaginally by instillation of 0.05 ml of undiluted virus pool (approximately 10^6 plaque-forming units [PFU]) directly into the vagina by means of a small plastic catheter attached to a syringe.

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Virus, media, and cell cultures. The MS strain of HVH type 2 was obtained from Andre Nahmias, Emory University, Atlanta, Ga. The virus pool used in these studies was prepared in primary rabbit kidney cells and titered 2.0 × 10^7 PFU/ml in fetal lamb kidney cells by methods previously described (16). The media utilized and the preparation of cell cultures have been described previously (16).

RESULTS

Susceptibility of pregnant and nonpregnant mice to intravaginal HVH type 2 infection. Preliminary investigations in our laboratory indicated that intravaginal inoculation of pregnant female mice with HVH type 2 resulted in an initial local vaginitis with erythema, hair loss, and ulceration in the perineal area and mucopurulent discharge (Fig. 1), followed by hunching, hind limb paralysis, and death from encephalitis.

Since there is an increased incidence of genital HVH infections in humans during pregnancy (26, 30), the initial experiments were designed to determine if there was a difference in susceptibility to the infection between pregnant and nonpregnant female mice. A group of 12 pregnant and 12 nonpregnant mice were inoculated intravaginally with 10^6 PFU of HVH type 2 and observed daily for signs of clinical vaginitis and mortality. Only those mice that demonstrated definite erythema with hair loss or ulceration were considered to have clinical vaginitis. Mucopurulent discharge was a late finding, frequently not appearing until a day or two before death. As shown in Fig. 2, the pregnant animals first exhibited signs of clinical vaginitis on days 5 and 6, followed by hunching and paralysis by days 6 to 8 and death on days 8 to 11. The nonpregnant mice developed vaginitis at the same time as the pregnant mice, but death occurred later. All nine of the pregnant mice that developed vaginitis went on to die from the infection, whereas two of the eight nonpregnant mice recovered fully. All 12 of the pregnant mice died from the infection including three that never exhibited signs of vaginitis. These latter three mice developed hunching and/or paralysis before death. In contrast, only 6 of the 12 nonpregnant mice died; all of these 6 had vaginitis. In pooled results from three separate experiments, 30 of 32 (93.8%) pregnant mice died from the infection, whereas only 47 of 95 (49.5%) nonpregnant mice died with the same inoculum of virus ($P < 0.001$, chi-square test). These results indicate that pregnant mice are more susceptible to genital infection with HVH type 2 than nonpregnant mice.

FIG. 1. *Mucopurulent vaginal discharge and perineal hair loss in a pregnant mouse inoculated intravaginally with H. hominis type 2.*

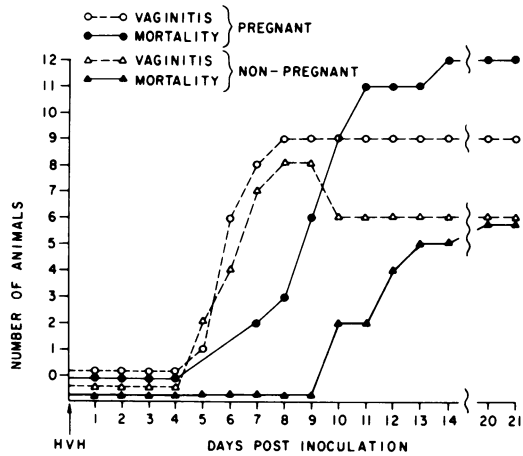

FIG. 2. *Cumulative vaginitis and mortality rates in pregnant and nonpregnant mice after intravaginal inoculation with H. hominis type 2.*

Vaginal swabs were obtained daily from each of the 12 pregnant and 12 nonpregnant animals in the experiment described above and placed in 1 ml of tissue culture media for subsequent virus assay. The daily virus titers in vaginal secretions of individual pregnant mice are illustrated in Fig. 3. The majority of the animals had virus titers of 10^5 to 10^6 PFU/ml in their vaginal secretions by 24 to 48 h after inoculation. High titers of virus were present for several days before the onset of clinical signs of vaginitis and persisted until day 8 to 10. The daily vaginal virus titers in nonpregnant mice are presented in Fig. 4. The results suggested that animals could be grouped into different patterns of viral replication that correlated with the clinical course and outcome from the infection. In the

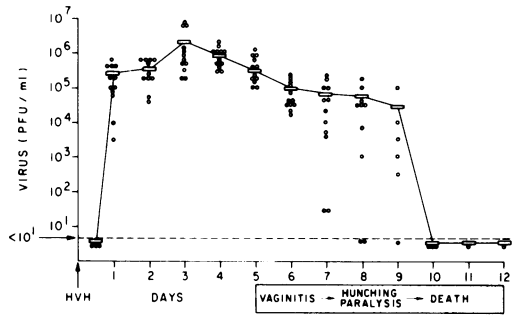

FIG. 3. *Titers of virus in vaginal secretions of pregnant mice after intravaginal inoculation with H. hominis type 2.*

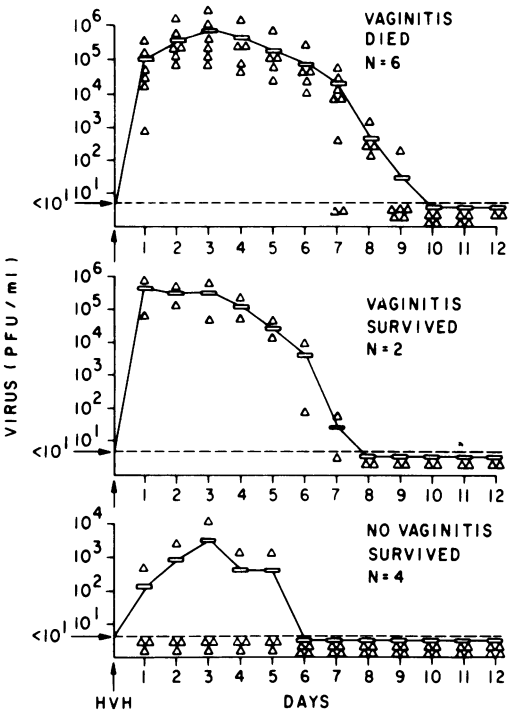

FIG. 4. *Titers of virus in vaginal secretions of nonpregnant mice after intravaginal inoculation with H. hominis type 2. The mice are grouped according to clinical outcome from the infection.*

top graph, the six animals that developed vaginitis and died from the infection all had vaginal virus titers similar to those observed in the pregnant mice. In the center graph, the two animals that developed vaginitis, yet survived the infection, had similar high titers of virus but appeared to clear the virus sooner. Of the four animals that never developed vaginitis and survived the infection (bottom graph), only one demonstrated virus in the vaginal secretions, and these titers were low. The results indicate that after intravaginal inoculation of HVH type 2, pregnant mice regularly develop high titers of virus in vaginal secretions and die from the infection, whereas only half of the inoculated nonpregnant mice exhibit a similar course of infection.

Pathogenesis of the infection in pregnant mice. Studies were then initiated to further define the pathogenesis of the infection. Pregnant mice were inoculated with HVH type 2 as previously described. At daily intervals, vaginal swabs, blood, liver, spleen, spinal cord, cerebellum-brain stem, cerebrum, and olfactory bulb were obtained from three separate animals, prepared as 10% homogenates, and assayed individually for HVH. The mean virus titers pooled from two separate experiments are shown in Fig. 5. The titers of virus in vaginal secretions followed the pattern previously described. Virus was never isolated from any of the blood samples and only rarely was an occasional PFU recovered from liver or spleen. Herpesvirus was first detected in the spinal cord on day 5 with titers ranging from 10^4 to 10^5 PFU/g from day 6 until the death of the animal. Virus did not appear in the cerebellum-brain stem until day 7, with peak titers reaching 10^5 PFU/g, and in the cerebrum until days 8 and 9. Virus was never detected in the olfactory bulb. The results from these experiments indicate that HVH type 2, after initial replication in the mucous membranes of the genital tract, spreads to the spinal cord and then ascends to involve the brain. Viremia, or replication of virus in liver or spleen, was not detected.

DISCUSSION

The results of these studies indicate that, after intravaginal inoculation with HVH type 2, pregnant mice regularly develop a local vaginitis followed by spread of the virus to the spinal cord. The viral infection then ascends the spinal cord to involve the central nervous system

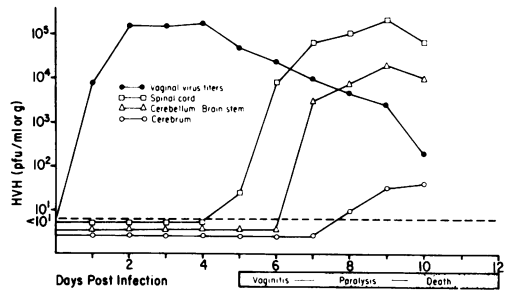

FIG. 5. *Pathogenesis of genital H. hominis type 2 infection in pregnant mice.*

(CNS), resulting in an initial hind limb paralysis followed by encephalitis and death. Neither viremia nor involvement of systemic organs was detectable in this model infection. Nahmias et al. (23, 24) previously reported that female mice inoculated intravaginally by means of a cotton pellet soaked with HVH type 2 developed titers of virus in vaginal secretions for as long as 12 days after inoculation and died of encephalitis within 20 days. Details of quantity of virus recovered from vaginal secretions and the pathogenesis of the infection were not reported. After genital inoculation with HVH type 2, cebus monkeys develop only a localized vaginal infection and recover uneventfully (9, 19, 22). In contrast, intravaginal inoculation of marmosets with HVH type 2 results in extensive involvement of the genitourinary tract, with viral isolation and hemorrhagic necrosis being noted in the vagina and bladder, and the animals die from the infection (10). Results of virologic or pathologic examination of the CNS were not provided in this latter report. Our results confirm the initial observations of Nahmias et al. (23, 24) of genital infection of mice with HVH type 2 and provide more detailed information concerning the kinetics of virus replication in the mucous membranes of the genital tract and the spread of virus to and within the CNS. It is apparent from our results that although mice are able to clear virus from vaginal secretions after genital infection with HVH type 2, once virus reaches the spinal cord it ascends to involve the brain and results in an encephalitis and death. The experimental infection in mice, therefore, appears to provide a model for the evaluation of potential antiviral chemotherapeutic agents in a local infection of the mucous membranes of the genital tract where daily samples of vaginal secretions may be monitored to determine the effect of treatment on viral replication. In addition, it provides a model of a fatal herpesvirus encephalitis which is initiated at surface mucous membranes. The model also provides the opportunity to evaluate both systemic and topical forms of chemotherapy.

Although the transmission of HVH type 2 to the CNS by means of the peripheral nerves was not directly examined as part of the investigations reported here, there is ample evidence in animal experiments from other investigators (13, 18, 32–34, 36, 37) which indicates that the neural route is the major route of transmission to the CNS. In addition, the type 2 strains have been shown to be more neurotropic than type 1 strains in mice and rabbits (28, 29). These results, together with the initial appearance of herpesvirus in the spinal cord and the absence of virus in visceral tissues in the experiments in mice described here, would support the hypothesis that HVH type 2 is transmitted from its initial site of replication in the vaginal mucosa to the spinal cord and the rest of the CNS primarily, if not exclusively, by neural routes.

H. hominis type 2 is a common cause of venereal disease in humans (21); the painful ulcerative lesions may be disabling and recurrent. Although HVH type 2 infections in older children and adults are usually confined to the skin and mucous membranes, spread to involve the meninges and even the spinal cord has been reported (5). Although the genital infection with HVH type 2 in female mice reported here does provide a model for human genital herpetic infection, it should be pointed out that the encephalomyelitis regularly observed in the mice rarely occurs in humans. The vast majority of the human cases of HVH encephalitis are caused by the type 1, rather than the type 2, strain (5, 25, 27). There is strong circumstantial evidence, however, from both human (3) and animal studies (2, 17, 33) that HVH type 1 spreads to the CNS by neural routes and persists as a latent infection in cells of the spinal ganglia. More recently Baringer (1) demonstrated the recovery of HVH type 2 from sacral, but not thoracic or trigeminal, ganglions of unselected human cadavers indicating that the type 2 virus also may spread to the nervous system of humans from a local infection of the mucous membranes, presumably by neural routes.

The enhanced susceptibility of the pregnant mouse as compared with the nonpregnant mouse to genital herpesvirus infection parallels the clinical observations made in humans (26). In both humans (12, 35) and experimental animals (7), pregnant females have been shown to exhibit enhanced susceptibility to several viral infections. In addition, pregnancy has been shown to be associated with alteration of several components of the immune response in humans (6, 15, 31). The model genital herpesvirus infection reported here provides the additional opportunity to examine the possible mechanisms underlying the enhanced susceptibility of the pregnant female to herpesvirus and other viral infections.

ACKNOWLEDGMENTS

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