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Neonatal Herpes Simplex Virus Infection in Guinea Pigs (41459)

TINA C. CHOW AND G. D. HSIUNG[1]

Department of Laboratory Medicine, Yale University School of Medicine, New Haven, Connecticut 06520, and Virology Laboratory, Veterans Administration Medical Center, West Haven, Connecticut, 06516

Abstract. The mode of neonatal herpes simplex virus types 1 and 2 infection in guinea pigs was explored. There was no evidence of transplacental transmission of either virus type from mother to fetus following genital infection of the former. Infectious virus was not recoverable from placenta, cord blood, or amniotic fluid following either genital or intracardiac inoculation of HSV-2 into pregnant guinea pigs. However, a low rate of neonatal transmission of HSV-2 from mother to newborn via an infected birth canal during delivery was demonstrated.

Over the past 10 years, herpes simplex virus (HSV) has been increasingly recognized as a significant venereal disease and contributed to serious neonatal infection (1). The natural history of HSV infection of mother and newborn was summarized in a recent prospective study (2). It is apparent that genital herpes infection has become a major health problem for human neonates. In the latter study, it was noted that 39 of 56 (70%) of the women studied were without either signs or symptoms of genital HSV infection at time of delivery (2). Thus, since the source of neonatal HSV infection is often obscure and its manifestation often unanticipated, it was thought to be of value to identify an experimental model whereby the pathogenesis of neonatal HSV infection could be monitored and defined.

In earlier studies transplacental transmission of HSV-1 and HSV-2 infection from mothers to fetuses was demonstrated in hamsters (3) and rabbits (4), provided viremia occurred. However, neonatal infection was not examined in these early experiments. Unlike the hamster and rabbit, the anatomical structure of the guinea pig placenta resembles the placenta of humans (5). Furthermore, our laboratory, as well as others, have demonstrated that two herpesviruses, i.e., guinea pig cytomegalovirus (GPCMV) and guinea pig herpes-like virus (GPHLV), were readily transmitted transplacentally from infected pregnant animals to their offspring at various stages of gestation (6–11). Since guinea pigs can be readily infected with HSV by the intravaginal route (12, 13), it was of interest to investigate whether transplacental transmission of HSV could be demonstrated in offspring born from genitally infected mothers. Our findings indicate that neonatal HSV infection is acquired via the birth canal and not transplacentally; the results are included in the present report.

Materials and Methods. *Cell culture and virus assay.* Guinea pig embryo (GPE) fibroblast cell cultures were prepared as previously described (13). All cells were grown in Eagle's minimal essential medium (MEM) containing Hanks' balanced salt solution (BSS) and 10% fetal bovine serum (FBS). Secondary cells were seeded in 24 multiwell plates for virus-induced cytopathic effect (CPE). For plaque assay, confluent monolayer cultures were inoculated with serial 10-fold dilutions of virus suspension and were overlayed with 1% methyl cellulose in MEM containing Earle's BSS and 3% FBS. All plate cultures were incubated at 35° in a 5% CO_2 incubator. At 3 days after virus infection, overlay medium was removed and the infected cells were fixed with 5% formalin and stained with 1.3% crystal violet as previously described (14). The plaques were enumerated for

[1] To whom correspondence should be addressed at the VA Medical Center.

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virus infectivity titers. Tube cultures were used for initial virus isolation from infected guinea pig tissues.

Virus strains. HSV-2 strain 186, originally isolated from genital lesions (15), and HSV-1 strain NYU-78, recovered from brain tissue (16), were employed in this study. Both virus strains had been passaged in primary rabbit kidney cultures 5 to 10 times; infectivity titers were $10^{6.5-7.0}$ plaque-forming units (PFU)/ml for HSV-1 and $10^{5.5}$ PFU/ml for HSV-2. The HSV-1 and HSV-2 typing was based upon the result of differential plaque assay, i.e., since chick embryo (CE) cells are susceptible to HSV-2 but not to HSV-1 infection (17), the virus causing plaque formation in both CE and GPE was identified as HSV-2, while virus inducing plaques only in GPE cells was identified as HSV-1. In selected cases, plaque reduction neutralization test was performed using type-specific antisera produced in rabbits.

Animal inoculation. Pregnant and nonpregnant Hartley guinea pigs were inoculated with 0.5 ml of either virus suspension into the vaginal vault which was then plugged with soluble gel foam surgical pads to retain the fluid. All guinea pigs were observed daily for clinical manifestations of herpetic lesions, neurologic symptoms, or death. The gestation period of guinea pigs ranges from 65 to 70 days. In addition, pregnant and nonpregnant guinea pigs were inoculated intracardially with 0.5 ml of HSV-2 containing 5.5 log $TCID_{50}$. These animals were tested for the presence of virus in blood, neural tissues, and nonneural tissues and were observed for evidence of clinical manifestations of disease in mother and/or fetus.

Virus isolation from inoculated guinea pigs: Vaginal swabs. Samples were taken from the vagina of each pregnant guinea pig on the third day postvaginal inoculation to ensure that the genital tract was infected. Within 24 hr of delivery of the newborns, vaginal swabs were assayed again for virus infection.

Guinea pig tissues. Within 24 hr of birth, the eyes and mouths of newborns were swabbed and tested for infectious virus. Neural and nonneural tissues of mothers, newborns, and fetuses aged over 40 days were processed for virus isolation; when the age of the fetus was less than 40 days, the entire embryo was used. Neural tissues studied included posterior brain, lumbosacral spinal cord, thoracic spinal cord, cervical spinal cord, and the lumbosacral dorsal root ganglia. Nonneural tissues studied included cervix, vagina, urinary bladder, liver, spleen, kidney, lung, heart, pancreas, and adrenal glands. A 10% (w/v) suspension of minced tissue in Hanks' BSS was cocultivated with GPE cell monolayers (0.2 ml suspension/culture tube). Inoculated cultures were incubated at 35° and examined for herpesvirus induced CPE for 14 to 21 days.

Results. *Genital HSV infection in pregnant guinea pigs at different stages of gestation.* Clinically, HSV-2 infection was manifested by the appearance of typical herpetic lesions of the genitalia of guinea pigs within 2 to 6 days of vaginal inoculation of the virus. The vulva was often swollen and erythematous. Vesicular lesions appeared first followed by ulcerations and/or crusts. The amount of virus recovered from the vaginal swabs taken 3 days after inoculation and from the neural and nonneural tissues taken 2–12 days postinoculation are shown in Table I. Whereas maximum virus infectivity titers were recovered from vaginal swabs of guinea pigs inoculated with a large dosage, i.e., 5.5 log $TCID_{50}$, no significant difference in virus recovery from neural and nonneural tissues was observed in pregnant guinea pigs inoculated with different dosages of virus during different gestational stages. In addition, eight pregnant guinea pigs inoculated at about 20–30 days of gestation were sacrificed 30–40 days postinoculation; no virus was isolated from the vaginal swabs or any of the neural and nonneural tissues tested in a similar manner. Among the three pregnant guinea pigs inoculated intravaginally with HSV-1, genital lesions appeared similar to those induced by HSV-2, but ulceration did not occur. All three animals shed virus from the genital tract 3 days postinoculation; HSV-1 was isolated from the lumbosacral spinal cord of one animal sacrificed 5 days postinoculation.

TABLE I. GENITAL HERPES IN PREGNANT GUINEA PIGS AT DIFFERENT STAGES OF GESTATION

Virus type	Expt group	At inoculation maternal gestation (days)	Total No. studied	Virus inoculum (log $TCID_{50}$	Vaginal swab (3 days postinoc.)	Average virus infectivity titers[a] (log $TCID_{50}$/0.1 ml)	
						Neural tissue[b]	Nonneural tissue[c]
HSV-2	A	<20	4[d]	4.5	2.6	0.5	<0.5
	B	30–40	2[d]	5.5	3.9	1.9	1.3
	C	50	6	3.7	2.3	1.8	0.7
	D	60	9	4.8	2.6	1.9	0.5
HSV-1	E	<20	3	4.5	1.0	<0.5	0.5

[a] At sacrifice, 2–12 days postinoculation.
[b] Neural tissues included brain, spinal cord, and lumbosacral dorsal root ganglia.
[c] Nonneural tissues included vagina, cervix, uterus, and urinary bladder.
[d] An additional eight pregnant guinea pigs, five in experiment A and three in experiment B were sacrificed 30–42 days after inoculation but no virus was isolated from any of the tissues tested.

A total of 32 fetuses of different gestational ages were obtained from nine pregnant guinea pigs that were sacrificed 3–9 days postinoculation of HSV-2. No virus was isolated from the fetal neural and nonneural tissues, placentas, or amniotic fluid. However, HSV-2 was isolated from the maternal genital tract in five, and from the lumbosacral spinal cord in six of the nine animals studied.

Infection of pregnant and nonpregnant guinea pigs following intracardiac inoculation with HSV-2. In order to determine whether transplacental transmission of HSV-2 can occur following viremia, six pregnant guinea pigs were inoculated intracardially with 5.5 log $TCID_{50}$ of HSV-2 and sacrificed 1–10 days after inoculation. Infectious virus was isolated from the maternal blood taken from the axillary and uterine blood vessels within 2 days after inoculation. However, no virus was isolated from the placenta, cord blood, amniotic fluid, or fetal neural and nonneural tissues from a total of 21 fetuses studied.

In a separate study of 12 nonpregnant guinea pigs, each animal was inoculated by the intracardiac route with HSV-2 and sacrificed daily for up to 10 days. Although none of the inoculated animals showed clinical disease, HSV-2 was isolated from the blood, heart, and lung up to 3 days after inoculation. Figure 1 illustrates the sequential progress of virus distribution from the blood to the neural tissues, including spinal cord and brain. Virus was first detected in the neural tissues on the third day and increased in titers thereafter up to 10 days, the longest time studied. Thus, viremia as a result of HSV infection in guinea pigs, if it occurs, must be brief.

Neonatal HSV-2 infection after maternal genital infection. A total of 12 pregnant guinea pigs divided into four groups were studied (Table II). Eleven were inoculated intravaginally with HSV-2 during the third trimester and gave birth between 7 and 17 days postinoculation. One was inoculated during the first trimester and gave birth 54 days later. All inoculated guinea pigs showed infectious virus in their vaginal swabs 3 days postinoculation. At delivery,

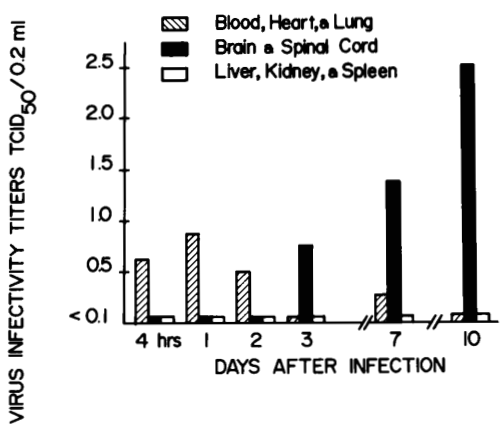

FIG. 1. Distribution of infectious virus in nonpregnant guinea pigs following intracardiac inoculation with herpes simplex virus type 2, 5.5 log $TCID_{50}$ per guinea pig.

TABLE II. NEONATAL TRANSMISSION OF HERPES SIMPLEX VIRUS TYPE 2 IN NEWBORN GUINEA PIGS

Mother					Virus isolation from newborn tissues[a] (No. showed virus/No. studied)		
		At delivery					
Expt group	No. studied	Days postinoc.	Genital lesion	Virus isolation	No. studied	Newborns died at birth	Newborns died 1–8 days after birth
A	6	7–12	+[b]	+	22	0/7	1[c]/15
B	2	8 and 11	–	+	8	ND[d]	0/8
C	2	7 and 12	+	–	8	0/1	0/7
D	2	17 and 54	–	–	6	0/1	0/5

[a] Newborns were sacrificed 1–3 days after birth; tissues for virus isolation were brain lumbosacral, thoracic and cervical spinal cord, liver, kidney, adrenal gland, pancreas, heart and lung.
[b] +, genital lesions or virus isolation from vaginal swabs; –, absence of lesions or no virus isolated.
[c] This baby was born from an infected guinea pig 8 days postinoculation; virus was isolated from the brain and spinal cord of this newborn, which died 8 days after birth.
[d] Not done.

all animals were examined for the presence of herpetic lesions and vaginal swabs, which were taken immediately after they gave birth and were tested for virus infectivity. A total of 44 newborns were examined but infectious virus was not isolated from the eye and mouth swabs taken at birth, nor from the tissues taken at autopsy of stillborn fetuses. However, in samples taken on subsequent days, infectious virus was isolated from the brain and spinal cord of one newborn which was found dead 8 days after birth (Table II, Experiment A). This guinea pig was born to a mother 8 days postinoculation. At time of delivery, severe herpetic lesions were observed in the mother and approximately 0.5 log $TCID_{50}$/0.1 ml virus was present in the vaginal swabs. The baby was separated from the mother within 24 hr of birth, but subsequently became ill, developed neurological symptoms, and died on Day 8. HSV-2 was isolated from the brain and spinal cord but not from nonneural tissues including lung, heart, liver, spleen, pancreas, and adrenal gland of the newborn.

Discussion. This study attempts to define a model for study of the pathogenesis of neonatal herpes simplex virus infection in guinea pigs. Following genital infection of the mother, the incidence of neonatal HSV infection in newborn guinea pigs (3.3%) was considerably lower than that reported in mouse studies (26%). However, in the latter, Amstey and Kobos (18) replaced the virus-soaked pledgets containing 3.5 log $TCID_{50}$/0.025 ml of virus in the mouse vaginal canal daily to ensure contact with the virus by neonates during delivery. In the present study the amount of virus in the vagina of guinea pigs at delivery was minimal. In addition, the presence of fur on newborn guinea pigs at birth may have prevented direct contact and exposure to the virus by the newborn's skin, thus further accounting for the lower incidence of neonatal infection observed in guinea pigs than in mice.

There is no concrete evidence of transplacental HSV transmission in humans. However, transplacental transmission of HSV has been demonstrated in experimental infection of hamsters and rabbits when the virus was present in the blood (3, 4). In previous studies in this laboratory two other herpesviruses, i.e., GPCMV and GPHLV, were successfully transmitted through the placentas of guinea pigs from mothers to offspring (6, 8, 11). Thus it was considered that since the guinea pig placenta resembles the human placenta anatomically and since human CMV and probably VZV are also capable of crossing the human placenta, it would be of particular interest if transplacental transmission of HSV following viremia in guinea pigs could be demonstrated. However, there was no evidence in the present study of transpla-

cental HSV-2 transmission in guinea pigs, even when the virus was introduced into the heart. These data suggest that in guinea pigs as in humans, among the different herpesvirus types, there are differences in the pathogenic process whereby the virus is disseminated within the host. It was noted that although HSV disappeared rapidly from the circulating blood after administration (Fig. 1), an increase in virus titer was observed in the nervous system. The dissemination of HSV from the blood stream to the central nervous system is not well understood (19). In a previous *in vitro* study (14), HSV replication could not be demonstrated in guinea pig leukocytes, although attachment onto and penetration into B and T cells was noted. It is possible that *in vivo* HSV-2 carrying leukocytes settle in the small vessels of the nervous system which then acquire the infection.

Recent reports indicate that 5–10% of human genital herpes simplex virus infections are due to HSV-1. In our experiments, no difference was seen in the initial appearance of lesions caused by HSV-1 or HSV-2 when they were inoculated into the vagina. However, genital lesions were more severe and healing was slower in HSV-2 than in HSV-1-inoculated guinea pigs. It is possible that the pathogenesis of infection of different HSV types may also differ in the guinea pig.

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1. Visintine AM, Nahmias AJ, Josey WE. Genital herpes. Perinatal Care 2:32–41, 1978.
2. Whitley RJ, Nahmias AJ, Visintine AM, Fleming CL, Alford CA. The natural history of herpes simplex virus infection of mother and newborn. Pediatrics 66:489–494, 1980.
3. Kurata K, Kurata T, Aoyama Y. Vertical transmission of type 1 and type 2 herpes simplex virus infection in hamsters. Japan J Exp Med 46:187–195, 1976.
4. Biegeleisen JZ, Jr, Scott LV. Transplacental infection of fetuses of rabbits with herpes simplex virus. Proc Soc Exp Biol Med 97:411–412, 1958.
5. Enders AC. A comparative study of the fine structure of the trophoblast in several hemochorial placentas. Amer J Anat 116:29–67, 1965.
6. Lam KM, Hsiung GD. Herpesvirus infection of guinea pigs. II. Transplacental transmission. Amer J Epidemiol 93:308–313, 1971.
7. Lam KM, Hsiung GD. Further studies on the mode of transmission of herpes-like virus in guinea pigs. Proc Soc Exp Biol Med 138:422–426, 1971.
8. Choi YC, Hsiung GD. Cytomegalovirus infection in guinea pigs. II. Transplacental and horizontal transmission. J Infect Dis 138:197–202, 1978.
9. Kumar ML, Nankervis GA. Experimental congenital infection with cytomegalovirus. A guinea pig model. J Infect Dis 138:650–654, 1978.
10. Johnson KP, Connor WS. Guinea pig cytomegalovirus. Transplacental transmission. Arch Virol 59:263–267, 1979.
11. Griffith BP, Hsiung GD. Cytomegalovirus infection in guinea pigs. IV. Maternal infection at different stages of gestation. J Infect Dis 141:787–793, 1980.
12. Scriba M. Recurrent genital herpes simplex virus (HSV) infection of guinea pigs. Med Microbiol Immunol 162:201–208, 1976.
13. Anderson CA, August MJ, Hsiung GD. Pathogenicity of wild-type and temperature-sensitive mutants of herpes simplex virus type 2 in guinea pigs. Infect Immun 30:159–169, 1980.
14. Chow TC, Hsiung GD. Interaction of herpesvirus with spleen cell subpopulations. Comparison of a neurotropic and a lymphotropic virus. Infect Immun 30:678–685, 1980.
15. Rawls WE, Laurel D, Melnick JL, Glicksman JM, Kaufman RH. A search for virus in smegma, premalignant and early malignant cervical tissues. The isolation of herpesviruses with distinct antigenic properties. Amer J Epidemiol 87:647–655, 1968.
16. Monif G, Burnell P, Hsiung GD. Visceral involvement by herpes simplex virus in eczema herpeticum. Amer J Dis Child 116:324–327, 1968.
17. Nordlund JJ, Anderson C, Hsiung GD, Tenser RB. The use of temperature sensitivity and selective cell culture systems for differentiation of herpes simplex virus types 1 and 2 in a clinical laboratory. Proc Soc Exp Biol Med 155:118–123, 1977.
18. Amstey MS, Kobos K. An experimental model for disseminated herpesvirus infection of the neonate. Amer J Obstet Gynecol 125:40–44, 1976.
19. Johnson RT. The pathogenesis of herpes virus encephalitis. I. Virus pathways to the nervous system of suckling mice demonstrated by fluorescent antibody staining. J Exp Med 119:343–356, 1964.

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