

Persistent Infection with Bovine Herpesvirus Type 1: Rabbit Model

D. L. ROCK* AND D. E. REED

Veterinary Medical Research Institute, Iowa State University, Ames, Iowa 50011

Received 17 July 1981/Accepted 18 September 1981

Persistent infection with bovine herpesvirus type 1 (BHV-1) was established in all rabbits after conjunctival inoculation of virus. Spontaneous reactivations of BHV-1 with and without the appearance of recurrent ocular lesions were observed in persistently infected rabbits. BHV-1 was reactivated predictably and shed from all persistently infected rabbits after the administration of dexamethasone. During all reactivations, BHV-1 isolation was restricted to the inoculated eye.

Bovine herpesvirus type 1 (BHV-1) is responsible for a variety of disease conditions in cattle, including respiratory disease, conjunctivitis, vulvovaginitis, balanoposthitis, meningoencephalitis, and fatal systemic infection (5). Like other members of the herpesvirus group, BHV-1 is capable of establishing persistent infections in its natural host species (1, 4, 15).

Rabbits have been infected experimentally with BHV-1 and suggested as possible laboratory models for studying the pathogenesis of BHV-1 infection (6, 8, 9). This study was conducted to determine whether rabbits could be persistently infected with BHV-1 and to evaluate the rabbit as a laboratory model for studying persistent BHV-1 infection.

Adult white rabbits (2.5 to 5.0 kg) were purchased from a local commercial source and were housed in individual cages in an animal isolation room. The Cooper strain of BHV-1 was supplied by A. Strating, National Veterinary Services Laboratory, Ames, Iowa, and was used at passage level 13. Bovine lung cells were used throughout the experiment and were maintained as previously described (13). Ocular and nasal swabs were collected and immediately immersed in 2.0 ml of Eagle minimum essential medium containing antibiotics and amphotericin B (100 IU of penicillin, 100 µg of kanamycin sulfate, 100 µg of streptomycin sulfate, and 5 µg of amphotericin B per ml); these were vigorously shaken for 15 s before plating 0.5 ml onto bovine lung monolayer cell cultures. Cultures were observed daily for the presence of cytopathic effect and discarded if negative after 7 days. Viral isolates obtained were identified as BHV-1 by direct immunofluorescence or by neutralization with BHV-1 antiserum (8).

Rabbits ($n = 22$) were lightly anesthetized (Metaphane, Pitman-Moore Inc., Washington Crossing, N.J.) and inoculated by instilling $1.5 \times$

10^6 PFU of BHV-1 (in 0.2 ml of minimum essential medium) in the right conjunctival sac. Control animals ($n = 4$) were mock infected with 0.2 ml of minimum essential medium in an identical manner.

Four BHV-1-infected rabbits and one noninfected control rabbit were swabbed (right eye, left eye, and nose) for 125 consecutive days and monitored for spontaneous reactivation and shedding of BHV-1.

At selected dates postinfection (2 to 7 months), groups of rabbits were treated with dexamethasone (Azium, Schering Corp., Bloomfield, N.J.) in an attempt to induce recurrent BHV-1 infection (14). BHV-1-infected and control rabbits were treated with a 4-day regimen consisting of daily intramuscular injections of 4 mg of dexamethasone. In a similar manner a group of rabbits ($n = 4$) received multiple dexamethasone treatments during the course of the experiment. Before dexamethasone treatment, 1 to 2 weeks of daily attempts to isolate BHV-1 from the right eye, left eye, and nose of infected rabbits were unsuccessful.

The acute phase of the infection has been described (8; D. Rock, W. Hagemoser, and D. E. Reed, submitted for publication). Virus was isolated consistently from the right ocular swabs for 9 to 15 days postinfection. Virus was isolated sporadically from a few animals until 24 days postinfection. Virus was not recovered from the noninfected left eye of BHV-1-infected rabbits or from noninfected control animals during this period. Sera from all inoculated rabbits contained BHV-1 neutralizing antibody when examined at 30 days postinfection.

Spontaneous reactivation and shedding of BHV-1 from the right eye were observed in three of four infected rabbits which were continuously monitored. A single episode of recurrent virus shedding was observed in rabbits 415 and

417 at 33 and 86 days postinfection, respectively. During this same period, rabbit 418 experienced three episodes of recurrent virus shedding at 70, 89, and 104 days postinfection. Spontaneous reactivation of BHV-1 was characterized by virus shedding for 1 to 2 days and by the absence of clinically apparent infection. However, an ocular lesion consisting of a small ulcer on the outer right eyelid was observed in rabbit 418 concomitant with virus isolation at 104 days postinfection. The noninfected control animal monitored in a similar manner was virus-free throughout the observation period.

BHV-1 was isolated from the right eye of all 22 of the previously infected rabbits after dexamethasone treatment at 2 to 7 months postinfection. Virus was first reisolated from 40, 55, and 5% of the rabbits at 2, 3, and 4 days after the initiation of treatment, respectively. Virus was shed for 5 to 7 days. BHV-1 also was isolated from nasal swabs for 1 to 3 days. Virus was not isolated from the left eye of any of the treated rabbits. Untreated BHV-1-infected rabbits and dexamethasone-treated noninfected control rabbits remained clinically normal and free of virus throughout the treatment period.

The dexamethasone-induced recurrent infection was characterized by a mild to moderate conjunctivitis and by the presence of small ulcers on the outer right eyelid. These conditions developed on the last day of dexamethasone treatment and persisted for 6 to 7 days. This

recurrent ocular lesion was observed in all rabbits undergoing an initial reactivation with dexamethasone.

Over a period of 15 months, multiple reactivations of BHV-1 in the same rabbit were obtained using dexamethasone (Table 1). Virus was isolated from the right eye, but not the left, in each case. Subsequent reactivations observed in the rabbits were quite similar to the initial reactivations. A decrease in the duration of virus shedding was observed in rabbits 416 and 418 in the later reactivations. Clinically apparent recurrent infection was observed only during the initial reactivation of rabbit 418. An attempt to reactivate rabbit 418 at 15 months postinfection was unsuccessful. Untreated BHV-1-infected rabbits and dexamethasone-treated noninfected rabbits were included as controls for each reactivation attempt. Control rabbits remained clinically normal and free of virus throughout each reactivation period.

The results show that BHV-1 established a persistent infection in all experimentally inoculated rabbits. The rabbit model described in this report is consistent with the following observations made for cattle persistently infected with BHV-1: (i) most, if not all, animals are persistently infected after experimental infection (2, 14); (ii) spontaneous sporadic isolation of virus without evidence of clinical disease is seen in persistently infected animals (1, 4, 15); and (iii) virus can be reactivated predictably with or

TABLE 1. Reactivation of BHV-1 in dexamethasone-treated rabbits^a: multiple reactivations

Rabbit no.	Time (mo) postinfection ^b that reactivation was attempted	Result of reactivation attempt	Day posttreatment initiation that BHV-1 was first reisolated	No. of days of shedding	Recurrent ocular lesions
216	3	+ ^c	2	6	+
	6	+	3	ND ^d	+
	11	+	3	6	+
415	4	+	3	5	+
	7	+	2	5	+
	11	+	3	6	+
	15	+	3	ND	+
416	4	+	3	6	+
	7	+	4	3	+
	11	+	4	3	+
418	4	+	3	4	+
	7	+	4	1	-
	11	+	5	3	-
	15	-			-

^a Rabbits were treated with a 4-day regimen of dexamethasone, consisting of daily intramuscular injections of 4 mg.

^b Before treatment, 1 to 2 weeks of daily attempts to isolate BHV-1 from the right eye, left eye, and nose of infected rabbits were unsuccessful.

^c +, Positive instance; -, negative instance.

^d ND, Not determined.

without the appearance of clinical disease in approximately 100% of the experimentally infected animals treated with dexamethasone (2, 7, 14).

Studies on BHV-1 pathogenesis in cattle have suggested that BHV-1 persists in the trigeminal ganglia after intranasal and intraconjunctival inoculation of virus and is transported centrifugally via the trigeminal nerve to produce recurrent virus shedding and disease (3, 10-12). Lupton et al. (8) isolated BHV-1 from the trigeminal and optic nerves of rabbits during acute conjunctival infection. The restriction of virus isolation during reactivation of persistently infected rabbits to the ipsilateral inoculated eye (right eye) described in this report, along with the isolation of BHV-1 from rabbit trigeminal ganglia during the persistent phase of the infection (Rock et al., submitted for publication), suggests that the pathogenesis of persistent infection in rabbits is similar to that thought to occur in cattle. The similarities discussed above suggest the usefulness of rabbits in the study of persistent BHV-1 infection.

We thank H. W. Lupton for many helpful discussions. We thank J. Wheeler and M. Tymeson for their helpful assistance.

LITERATURE CITED

1. Bitsch, V. 1973. Infectious bovine rhinotracheitis virus infection in bulls, with special reference to preputial infection. *Appl. Microbiol.* 26:337-343.
2. Davies, D. H., and L. E. Carmichael. 1973. Role of cell-mediated immunity in the recovery of cattle from primary and recurrent infections with infectious bovine rhinotracheitis virus. *Infect. Immun.* 8:510-518.
3. Homan, E. J., and B. C. Easterday. 1980. Isolation of bovine herpesvirus-1 from trigeminal ganglia of clinically normal cattle. *Am. J. Vet. Res.* 41:1212-1213.
4. Huck, R. A., P. G. Millar, and D. G. Woods. 1973. Experimental infection of maiden heifers by the vagina with infectious bovine rhinotracheitis/infectious pustular vulvovaginitis virus. *J. Comp. Pathol.* 83:271-279.
5. Kahrs, R. F. 1977. Infectious bovine rhinotracheitis: a review and update. *J. Am. Vet. Med. Assoc.* 171:1055-1064.
6. Kelly, D. F. 1977. Experimental infection of rabbits with the virus of infectious bovine rhinotracheitis. *Br. J. Exp. Pathol.* 58:168-176.
7. Kubin, G. 1969. Intermittent recovery of IPV virus from a naturally-infected bull. *Wien. Tierärztl. Monatsschr.* 56:336-337.
8. Lupton, H. W., H. J. Barnes, and D. E. Reed. 1980. Evaluation of the rabbit as a laboratory model for infectious bovine rhinotracheitis virus infection. *Cornell Vet.* 70:77-95.
9. Lupton, H. W., and D. E. Reed. 1979. Experimental infection of eastern cottontail rabbits (*Sylvilagus floridanus*) with infectious bovine rhinotracheitis virus. *Am. J. Vet. Res.* 40:1329-1331.
10. Narita, M., S. Inui, K. Namba, and Y. Shimizu. 1976. Trigeminal ganglionitis and encephalitis in calves intranasally inoculated with infectious bovine rhinotracheitis virus. *J. Comp. Pathol.* 86:93-100.
11. Narita, M., S. Inui, K. Namba, and Y. Shimizu. 1978. Neural changes in calves after intraconjunctival inoculation with infectious bovine rhinotracheitis virus. *J. Comp. Pathol.* 88:387-394.
12. Narita, M., S. Inui, K. Namba, and Y. Shimizu. 1978. Neural changes in recurrent infection of infectious bovine rhinotracheitis virus in calves treated with dexamethasone. *Am. J. Vet. Res.* 39:1399-1403.
13. Rock, D. L., and D. E. Reed. 1980. The evaluation of an experimental porcine herpesvirus-1 (Aujeszky's disease virus) subunit vaccine in mice. *Vet. Microbiol.* 5:291-299.
14. Sheffy, B. E., and D. H. Davies. 1972. Reactivation of a bovine herpesvirus after corticosteroid treatment. *Proc. Soc. Exp. Biol. Med.* 140:974-976.
15. Snowdon, W. A. 1965. The IBR-IPV: reaction to infection and intermittent recovery of virus from experimentally infected cattle. *Aust. Vet. J.* 41:135-142.