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Pathogenesis of Simian Foamy Virus Infection in Natural and Experimental Hosts

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Inoculation of simian foamy virus type 1 into New Zealand white rabbits resulted in an infection which was very similar to that observed in naturally infected nonhuman primates. Both intraperitoneal and intranasal inoculations were found to be efficient procedures for the establishment of the infection in rabbits. Infection by the nasal route was found to be the best method, whereas no infection could be established by feeding virus in the drinking water. Once infection was established, virus persisted in the tissues and organs for as long as 264 days after inoculation, during which time the animals maintained significant levels of neutralizing antibody. Infectious virus was recovered from spleen, liver, lung, salivary gland, kidney, and, to a lesser extent, the brain. Virus was isolated from the blood only during early infection and never from the urine. A comparison of the distribution of foamy virus in naturally infected monkeys and baboons with experimentally infected rabbits showed that both groups harbored infectious virus in the same internal tissues and organs. Recovery of infectious virus from both groups of animals was accomplished by cultivation and/or co-cultivation of infected cells onto Vero cells.

The use of simian tissues for the preparation of primary cell cultures is generally complicated by the fact that these cultures are often contaminated with one or more endogenous viruses, of which the most prevalent are the foamy viruses (3, 5, 13). Although these viruses were first isolated and described more than 20 years ago (1, 10), very few reports have been made concerning the development of disease as a result of foamy virus infection in the natural hosts. Studies of the pathogenesis of this group of viruses in monkeys are complicated by the fact that primate species are expensive for laboratory study, require elaborate facilities for care and housing, and often are already infected with foamy viruses.

Recently Johnston (8) reported that foamy virus could be recovered from kidney cell cultures of rabbits 1 year after intradermal inoculation. These results suggested that rabbits might serve as a model system for the studies of the pathogenesis of this group of viruses. Systematic studies on infection with foamy virus in experimental animals have not been extensively explored. The present paper describes detailed studies of foamy virus type 1 infection in rabbits. These include virus replication, distribution, and persistence, together with a comparison of results obtained from experimental infections in rabbits with those obtained from naturally infected, captive, nonhuman primates.

MATERIALS AND METHODS

Cell cultures. Primary rabbit kidney cell cultures were prepared in our laboratories from 2- to 3-lb (ca. 907.2 to 1,360.8 g) New Zealand white rabbits, using a modified human kidney (HK) growth medium containing 10% fetal bovine serum (2). When confluent, the cells were maintained in a medium composed of Earle basal salt solution containing 0.5% lactalbumin hydrolysate and 5% newborn calf serum. Vero cultures, a cell line derived from green monkey kidney (15), were kindly supplied by N. Karabatsos, Yale University School of Medicine. These cells were grown in Eagle medium containing Hanks salts and 5% fetal bovine serum. When confluent, the cultures were maintained in Eagle medium containing Earle salts and 2% newborn calf serum.

Viruses. The foamy virus type 1 used in these studies was isolated from a primary rhesus monkey kidney cell culture. Identification was accomplished by the neutralization test using known antisera and comparison with known virus types obtained through the courtesy of Paul Johnston. Department of Microbiology, School of Medicine, University of Louisville, Louisville, Ky. Stock viruses were prepared in primary rabbit kidney cell cultures and stored at −70 C. Each virus suspension was frozen and thawed twice before use.

Animal inoculation. New Zealand white rabbits

weighing approximately 3 lb were inoculated either intraperitoneally (i.p.) with approximately 10^6 mean tissue culture infective doses ($TCID_{50}$) or intranasally (i.n.) with approximately 10^4 $TCID_{50}$ of virus suspension. The latter was accomplished by dropping virus suspension directly into the nostrils of rabbits physically restrained by being wrapped in a towel. Animals were observed daily for any symptoms of disease. Attempts to establish infection by oral administration of virus were carried out by mixing foamy virus suspension with an equal volume of drinking water. Animals were deprived of water overnight before being fed the virus-water mixture, which was usually consumed within a 45-min period.

Source of nonhuman primates. These studies were conducted with the cooperation of the Laboratory for Experimental Medicine and Surgery in Primates, New York University. Tissues and organs for virus assay and serum for antibody study were obtained aseptically as animals and/or tissues became available.

Virus isolation and titrations. At various time intervals after inoculation, rabbits were sacrificed and a 10% suspension of freshly trypsinized cells (vol/vol) was prepared from selected tissues. Vero cell monolayer cultures were inoculated with 0.1 ml of serial 10-fold dilutions of cell suspension, using 5 to 10 tubes per dilution. Cultures were observed one to two times per week for characteristic cytopathic effect and were held for 30 days before they were considered to be virus negative.

Antibody determinations. Serum-neutralizing antibody was measured by mixing twofold dilutions of serum with an equal volume of virus suspension containing approximately 100 $TCID_{50}$ of foamy virus type 1. After incubation at room temperature for 60 min, virus-serum mixtures were inoculated onto Vero cell monolayers, 0.2 ml per tube culture, and then incubated at 35 C. The highest dilution of serum which prevented the appearance of foamy virus cytopathic effect was considered to be the neutralizing antibody titer. All sera were heat inactivated at 56 C for 30 min before testing.

RESULTS

Foamy virus infection in rabbits. Foamy virus type 1 infection was established in rabbits by both i.p. and i.n. inoculation. Although no evidence of clinical disease was ever observed during these studies, infectious virus was readily isolated from the various tissues of these experimentally infected rabbits.

After i.p. inoculation, virus was recovered from one rabbit as early as day 6 after injection and in all additional rabbits tested thereafter (Table 1, top section). Infectious virus with significant titers was obtained from the spleen, liver, kidney, lung, and salivary gland of practically every rabbit tested, but only occasionally from the blood and brain. In these latter samples the virus titers obtained tended to be lower than those of the other tissues. Table 1 summarizes the recovery of virus from various tissues and blood during the first 42 days after inoculation. It was noted that the virus was first isolated from the blood 10 days after infection and was present in low concentrations through day 38. Virus could not be detected on day 42 or in any subsequent blood samples, although virus persisted in the spleen, liver, kidney, lung, and salivary gland for as long as 264 days after i.p. inoculation, the longest period tested. Figure 1 summarizes data obtained from assays of the spleen and kidney during the entire study

TABLE 1. *Distribution of foamy virus type 1 in experimentally infected rabbits*

Inoculation		No. of rabbits studied	Days postinoculation	Avg virus titer log $TCID_{50}/cm^3$ of packed cells						
Method	Dose ($TCID_{50}$)			Spleen	Liver	Kidney	Lung	Salivary gland	Brain	Blood
i.p.	10^6	3	1-3	—[a]	—	—	—	—	—	—
		1	6	2.6	2.8	1.6	—	2.5	—	—
		2	10	2.5	3.4	1.2	3.4	2.8	—	0.9
		1	17	2.7	2.8	3.0	2.3	—	2.6	0.7
		1	38	2.2	2.2	1.6	2.7	—	1.2	0.7
		1	42	2.3	2.0	1.7	1.2	1.6	—	—
i.n.	1.6×10^4	1	3	0.6	—	—	0.6	0.6	—	—
		1	5	—	—	—	—	—	—	—
		2	10	2.8	1.6	2.3	3.0	1.9	0.3[b]	0.4[b]
		1	31	2.6	1.5	1.8	2.5	1.5	—	—
Oral (feeding)	8×10^5	1	18	—	—	—	—	—	—	—
	2×10^7	1	18	—	—	—	—	—	—	—
	1.5×10^7	1	40	—	—	—	—	—	—	—

[a] No virus isolations obtained from inoculation of 10% cell suspensions onto Vero cell monolayers.

[b] Only one of two animals positive.

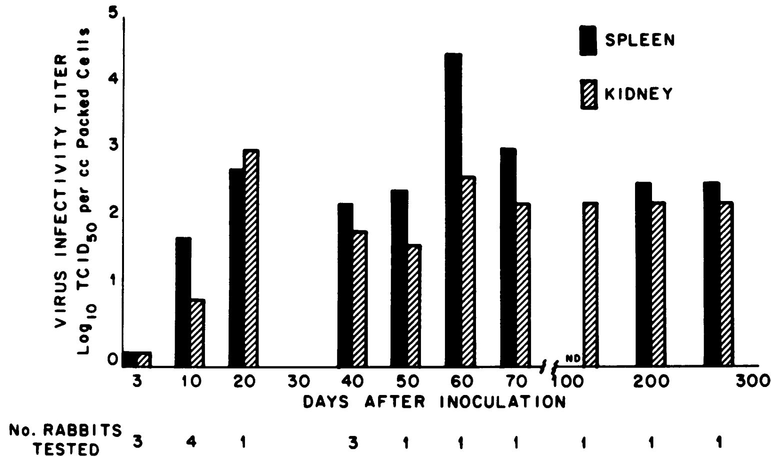

FIG. 1. *Growth and persistence of simian foamy virus type 1 in rabbits. ND, Not done.*

period. These results were typical for values obtained for the other internal organs containing infectious virus.

In the i.n.-inoculated rabbits the titers of infectious virus in the various tissues examined did not differ greatly from the i.p.-infected animals even though the latter group received approximately 100-fold more virus (Table 1, middle section). Again virus was recovered from the blood on day 10 from one of the two i.n.-inoculated rabbits, the other of which also harbored a low concentration of virus in the brain.

Attempts to initiate infection by feeding three rabbits with various concentrations of foamy virus type 1 in the drinking water were not successful. No virus was recovered from any of the tissues or blood samples tested 18 and 40 days after feeding (Table 1, bottom section). Administration of 8×10^6 and 2×10^7 $TCID_{50}$ of virus was accomplished by a single feeding within a 45-min period. The third rabbit received approximately equal amounts of virus suspension for 4 consecutive days, constituting a total of 1.5×10^7 $TCID_{50}$. In a separate control study, no difference in virus titer was noted between a stock virus suspension mixed with an equal volume of sterile water and a similar mixture held at room temperature for 45 min.

Antibody response and virus persistence in experimentally infected rabbits. The antibody response to foamy virus infection in rabbits was found to be the same in both the i.p.- and i.n.-infected animals. A rise in the neutralizing antibody levels was generally noted at about 9 to 10 days after inoculation (Table 2). Virus was isolated from the blood from the second week of the infection at the time antibody levels were increasing until about 40 days

TABLE 2. *Virus isolation and antibody studies of rabbits after i.p. inoculation with foamy virus type 1*

Days after inoculation	Virus isolation[a]			Mean neutralizing antibody titer
	Blood	Urine	Kidney	
0-8	0/6	0/4	1/5	1:2
9-40	5/7	0/4	3/4	1:5
41-80	0/6	0/5	6/6	1:14
81-264	0/3	0/3	3/3	1:40

[a] Number of positive/number of rabbits tested.

after infection, after which no virus could be isolated. The mean neutralizing antibody titer was 1:40 during the later stages of infection and persisted for the remainder of the test period. No attempt was made to detect the presence of virus-antibody complexes in the blood. On the other hand, virus was never isolated from the urine during the entire study period even though infectious virus was repeatedly isolated from the kidneys as well as many other internal organs for as long as 264 days. In two separate experiments the addition of virus suspension to the urine, which was allowed to remain at room temperature for 1 h, had no appreciable effect on the virus titer, suggesting that the absence of virus in the urine of infected rabbits was not due to inactivation.

Comparison of foamy virus infections in rabbits and simian primates. Experimental foamy virus infection in rabbits was compared with the natural infection in nonhuman primates. Virus isolations from the internal tissues and the neutralizing antibody status of rhesus monkeys and baboons which were in captivity for 1 year or longer were compared with data obtained from rabbits which were infected for

more than 40 days (Table 3). Seven of the 10 isolates from rhesus monkeys and three isolates from baboons were identified as foamy virus type 1. Two of the isolates from rhesus monkeys were lost before they could be typed and one isolate has not yet been typed. Whereas virus is commonly isolated from the simian primate kidney tissues, a common source for routine tissue culture preparation, foamy virus isolations were also made from the spleen, liver, salivary gland, and brain of these animals. As observed in rabbits, virus was not isolated from the blood of these long-term captive primates although virus persisted in the various organs. The presence of circulating neutralizing antibody does not appear to be effective in ridding the tissues of infectious virus since isolations were readily made from the antibody-positive animals.

DISCUSSION

Whereas simian foamy viruses are widely encountered as common contaminants in primary cell cultures prepared from simian tissues, very little is known concerning the infection and pathogenesis due to this virus in the natural host. Studies of foamy virus infection in monkeys, the natural hosts, are expensive and difficult since the incidence of natural infection is high and the antibody tests used to select virus-free animals are inconclusive. Screening for antibody-negative animals has been proposed as a method to obtain cell cultures free of this virus. Using the complement fixation test, Stiles (11) was able to select rhesus and grivet monkeys free of foamy virus with 85 to 95% effectiveness. However, using the neutralization test and subjecting primary rhesus monkey kidney cell cultures to long-term incubation, Swack et al. (14) showed that knowledge of the antibody status was of little value as a screening procedure to select virus-free animals. In another study it was found that 28% of rhesus and 19% of African green monkeys were infected with foamy viruses and that virus could be recovered from both neutralizing antibody-positive and -negative animals (13).

Johnston showed that foamy virus types 1 and 2 persisted in the kidneys of rabbits 1 year after intradermal inoculation (8). The present study confirmed and expanded his observation that rabbits are indeed a convenient model for studies of foamy virus infection. Furthermore, experimental infection of foamy virus type 1 in rabbits showed many similarities to the natural infection in monkeys. Despite the fact that foamy virus type 1 was readily recovered from practically all of the tissues and organs of inoculated rabbits, virus isolations from the blood and brain occurred only occasionally. In the natural host O'Brien et al. reported that foamy virus types 1 and 2 were isolated from the brains of rhesus monkeys (9), and more recently a new foamy virus type was isolated from the brain of a spider monkey (4). The results obtained in the present study on experimental infection indicate that the presence of virus in the rabbit brain may be due to the occurrence of a viremia since the virus was not isolated from the brain during the later stages of infection.

Infectivity titers of foamy virus in the tissues of i.n.-infected rabbits were not significantly different from those obtained from i.p.-inoculated animals. Considering the small inoculum used plus the possibility of virus loss during the process of i.n. inoculation, it appears that infection by the respiratory route is a considerably more efficient process than by i.p. injection. In the natural hosts, Johnston isolated foamy virus types 1, 2, 4, and 5 from throat swabs of simian species where the virus was observed to persist for 10 weeks, even though no virus was isolated from any of the internal organs examined except the kidneys (6,

TABLE 3. *Comparison of foamy virus type 1 isolation from naturally infected primates and experimentally infected rabbits*

Animal species[a]	Foamy virus infections	Foamy virus isolations from various tissues						Neutralizing antibody[b]
		Spleen	Liver	Kidney	Salivary gland	Brain	Blood	
Rhesus monkey	10/18[c]	2/2	2/2	10/13	1/2	1/4	0/7	5/5
Baboon	3/16	1/4	ND[d]	2/8	1/4	ND	0/8	5/12
Rabbit	9/9	7/7	6/7	9/9	7/8	2/9	0/7	6/6

[a] Rhesus monkeys and baboons kept in captivity 1 year or longer; rabbits infected more than 40 days.

[b] Titers ≥1:5 considered to be positive.

[c] Number of positive/number of animals tested. Data includes two untyped isolates lost in passage and one isolate as yet untyped.

[d] ND, Not done.

7). Since virus was not isolated from the urine or rectal swabs in the latter studies, it was suggested that foamy viruses are probably respiratory viruses. In the present study i.n. inoculation appeared to be an efficient method for infecting rabbits with foamy virus type 1. It would seem that spreading of the virus in primate colonies probably does occur via the respiratory route. Once an infection has become established it appears to persist for a long time, probably for the life of the animal species infected.

Virus isolation and serological studies of monkeys have shown that rhesus monkeys are heavily infected with foamy virus type 1 (11, 12). In our study, all of the foamy virus isolated either from rhesus or from baboons were identified as type 1. The latter was probably due to the large population of rhesus monkeys in the primate colony, including a large number of recent arrivals.

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