Guinea Pig Herpes-Like Virus Infections

II. Antibody Response and Virus Persistence in Mice and Rabbits

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Mice inoculated with guinea pig herpes-like virus produced minimal, if any, antibody response, and no virus was isolated from the inoculated animals 64 days after administration. The antibody response in rabbits inoculated with the same virus was prompt and reached a high level within 14 to 29 days regardless of the site of inoculation. Long-term persistence of viremia was observed only in intravenously inoculated rabbits; a brief period of viremia was observed in intraperitoneally inoculated rabbits, but no viremia was obtained in rabbits inoculated by the subcutaneous route. Maternal antibody was transferred readily to offspring; however, transference of infectious virus from mother to offspring was demonstrable in only one of 75 fetuses tested.

In the preceding paper we described the antibody response to and virus persistence of a guinea pig herpes-like virus (GPHLV) in its natural host, the guinea pig. Once infected with this virus, either naturally or experimentally, guinea pigs produced viral-specific neutralizing antibody while infectious virus continued to be readily isolated from the blood (5-7). Infected guinea pigs showed no apparent sign of illness, nor were any nuclear inclusions detectable in their tissues.

The host range of the GPHLV infection was narrow. No cytopathic effect (CPE) was observed in infected cultured cells derived from man, monkey, horse, hamster, and mouse even though the infected hamster and mouse cells retained infectious virus for some time (1, 5). Rabbit cells were susceptible to GPHLV infection and produced intranuclear inclusions similar to those observed in guinea pig cells (5). These results indicated that *in vitro* rabbit cells were highly susceptible to the GPHLV, whereas mouse cells were resistant.

The present study was undertaken to investigate the susceptibility of rabbits and mice to GPHLV infection *in vivo*; the parameters studied included virus multiplication, long-term persistence, and antibody response in these two animal species after experimental infection with the GPHLV. The transmission of immunity and transplacental transmission of virus from

infected rabbits to their offspring were also investigated and are included in this report.

MATERIALS AND METHODS

Virus strain. GPHLV, strain Y-407, isolated from a healthy strain 2 guinea pig (5), was used throughout the study. The stock virus was prepared by Hartley guinea pig kidney cell culture. After freezing and thawing three times, the culture fluid had a virus infectivity titer of approximately 10^6 TCID₅₀/ml.

Cell cultures. Hartley guinea pig kidney cell cultures were prepared according to the procedures described previously (5). The trypsinized cells were grown in roller tubes and were used as indicator cells for virus isolation and assay.

Animal sources. Swiss albino mice of different ages and sexes were purchased from Charles River Breeding Laboratories, Inc., Wilmington, Mass.; adult New Zealand white rabbits were obtained from Samolis' Rabbitry, Waterville, Conn.

Animal inoculation and sampling. Mice, 1 to 4 weeks old, were each inoculated with varying amounts of GPHLV suspension by various routes. Infected mice were sacrificed at different time intervals after inoculation. Whole blood samples obtained from several infected mice were pooled and used for virus isolation and the serum was used for antibody studies. Spleen and lung tissues were tested for the presence of virus, and portions of these tissues were fixed in Formalin and stained with hematoxylin and eosin for histological studies.

Rabbits. Each rabbit received 0.5 ml or 5 ml of stock virus intraperitoneally (i.p.) or 5 ml subcutaneously (s.c.). Inoculated rabbits were bled either by cardiac puncture or from ear veins.

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Blood samples were used for virus isolation and serum samples were used for antibody measurement. Tissues including spleen, lung, kidney, and liver were obtained from infected rabbits at the time of sacrifice and were used for virus isolation studies. Portions of each tissue were fixed in Bouin's fixative or in 10% Formalin and processed for histological examination.

When a pregnant rabbit was sacrificed, the whole uterus was removed. Maternal and fetal tissues were tested for virus infectivity in two separate rooms to avoid the possibility of cross-contamination. To obtain the fetuses, the uterus of the rabbit was opened, the embryonic fluids were collected for antibody studies, and the surrounding membranes were removed. The naked fetus was then dipped into an ether bath to inactivate possible surface contamination with GPHLV. Portions of fetal spleen, lung, and thymus were fixed in Formalin for histological examination. Samples of fetal blood were used for viral isolation and antibody studies.

Virus isolation from blood and tissues. Whole blood samples, 0.1 to 0.2 ml, were inoculated directly into guinea pig kidney cell cultures. At 24 to 48 h after inoculation the blood was removed and the maintenance medium was changed. All inoculated cultures were examined for CPE as the criterion for virus growth. The presence of virus in the various tissues was demonstrated by at least one of three methods: (i) a 10 to 20% suspension of minced tissues or (ii) a 10% suspension of trypsinized cells, or both, were inoculated into feeder-layer cultures of guinea pig kidney cells at serial 10-fold dilutions, and (iii) trypsinized cells were allowed to propagate into mono-layer cultures.

Antibody studies. Blood samples obtained before and after inoculation were tested for the presence of virus, and the serum was tested for the appearance of neutralizing antibody to GPHLV. Neutralization tests were performed as described in the preceding paper. The highest dilution of serum that inhibited CPE which appeared in the cultures inoculated with a challenge dose of approximately 100 TCID₅₀ of infectious virus was considered to be the neutralizing antibody titer to GPHLV.

RESULTS

Virus infection and antibody response in mice. Table 1 shows the antibody response in mice inoculated with GPHLV. Although three of the six mice inoculated i.p. with 5.3 log TCID₅₀ of virus at 1 week of age had infectious virus in their spleens when sacrificed 1 to 6 days after infection, no virus was isolated from the blood of the same group of mice 64 days after inoculation. Furthermore, no demonstrable antibody titer was detected in these mice after inoculation with either a large or small dose of virus. Mice inoculated s.c. at 1 week of age also did not

produce any appreciable amount of neutralizing antibody. The six control mice caged next to the infected animals showed no antibody titers. A low but positive antibody response was demonstrated in mice inoculated i.p. at age 2, 3, and 4 weeks or older when the inocula were large. However, when the amount of virus inoculum was small, antibody titers were detected only in mice inoculated at 2 to 3 weeks of age and not in the older group. Intranasal inoculation of 5.3 log TCID₅₀ of virus into 4-week-old mice elicited neutralizing antibody titers ranging from 1:20 to 1:40. At no time was virus isolated from a total of 79 mice when examined 64 days or longer after inoculation.

Eleven infected mice became pregnant and were sacrificed. Virus was isolated from the blood and spleen of seven of them 17 to 30 days after inoculation but not from the 11 pools of embryos tested. The neutralizing antibody titers from the pooled maternal mouse sera ranged from 1:10 to 1:20.

Viral distribution, persistence, and antibody response in rabbits. Rabbits inoculated intraperitoneally with varying doses of GPHLV were observed for 4 months. No evidence of illness attributable to the virus was noted. At different times, members in each group were sacrificed and examined for virus infectivity and antibody titers. Representative results are shown in Table 2. Rabbits no. 3 and no. 4 were inoculated with 5.7 and 6.7 log TCID₅₀ of GPHLV, respectively, and were sacrificed 20 and 27 days

TABLE 1. *Virus infection and antibody response in mice after intraperitoneal inoculation with GPHLV*

Age at time of inoculation (wk)	Inoculum log TCID ₅₀	Days after inoculation	No. virus isolates/no. tested	Neutralizing antibody titer range ^a
≤1	5.3 ^b	1-6	3/6	<5
	5.3 ^c	64	0/7	<5
	3.3	64	0/6	<5
	None	64	0/6	<5
2	5.3	64-109	0/9	5-10
	3.3	64	0/6	10-20
	None	64	0/6	<5
	5.3	64	0/7	10-20
3	3.3	64	0/6	20
	None	64	0/5	<5
	5.3	87	0/9	5
	3.3	87	0/6	<5
4	None	87	0/6	<5

^a Reciprocal of the serum dilutions.

^{b,c} Same group of mice.

TABLE 2. *Virus distribution and antibody response in rabbits inoculated intraperitoneally with GPHLV*

Rabbit no.	Inoculum log TCID ₅₀	Days after inoculation	Virus titers (log TCID ₅₀ /ml of packed cells)				Isolation virus from blood	Neutralizing antibody titers ^a
			Spleen	Liver	Lung	Kidney		
3	5.7	20	3.0	3.0	2.0	— ^b	—	1:80
4	6.7	27	5.0	3.0	2.0	—	—	1:80
7	5.7	142	+ ^c	—	—	—	—	1:160
9	6.7	126	+ ^c	+ ^c	—	+ ^c	—	1:160

^a Reciprocal of the highest serum dilutions.^b Virus was not isolated.^c Virus CPE was observed in monolayer cultures derived from these tissues.

after inoculation. Both showed virus in their visceral tissues. The lung and liver of both animals had about the same amount of virus, but the spleen of rabbit no. 4 which received a larger inoculum had approximately 2.0 log TCID₅₀ more virus than rabbit no. 3. No virus was isolated from the kidneys of either animal. Both rabbits had neutralizing antibody titers of 1:80. Two other rabbits, no. 7 and no. 9, counterparts of no. 3 and no. 4, were sacrificed 4 months after inoculation. The recovery of virus from these two animals required long-term cultivation of cells into monolayer cultures. Virus was isolated from the spleen monolayer cultures of rabbit no. 7 but not from any other tissue cells tested. Rabbit no. 9 showed virus infections in its spleen, liver, and kidney monolayer cultures but not in its lung culture. Both rabbits had neutralizing antibody titers of 1:160, which is twofold higher than the titers of rabbits no. 3 and no. 4. Rabbit no. 9 appeared to have more virus in its tissues than rabbit no. 7. No virus was isolated from the blood of any of the four rabbits at the time of sacrifice.

The virus isolated from the tissues of inoculated rabbits was neutralized by antiserum to GPHLV but not by antiserum to a latent herpesvirus of rabbits originally described by Nesburn (8). There were no inclusions or any other histopathological changes observed in any of the tissue sections examined regardless of presence or absence of infectious virus in the various tissues.

Comparison of viremia and antibody titers in rabbits inoculated by various routes. A total of 6 rabbits, 2 in each group, were each inoculated intravenously (i.v.), i.p., or s.c. with 6.7 log TCID₅₀ of GPHLV. All rabbits were bled at weekly intervals after inoculation. Whole blood was tested for the presence of virus, and the serum was assayed for its neutralizing antibody titer. The results are presented in Fig. 1. Neutralizing antibody was first detected on day 5 in rabbits given i.p. and i.v. injections of virus, whereas in rabbits inoculated by the s.c. route,

the antibody was delayed to day 8 postinoculation. The levels of neutralizing antibody in the i.p.-inoculated animals reached a maximum on days 14 and 26 and remained high thereafter. Virus was recovered from the blood of the i.p.-inoculated animals between the days 5 and 14 but not thereafter. The antibody titers in the intravenously inoculated rabbits reached a maximum on day 15 and remained stable thereafter. Virus was isolated from the blood of these animals for as long as 180 days in spite of the presence of high levels of neutralizing antibody. The antibody level in the s.c.-inoculated rabbits reached the same magnitude as those inoculated by i.p. or i.v. route at about the same time; however, no virus was ever isolated from the blood of the rabbits in this group.

Transmission of infectious virus and immunity from inoculated rabbits to offspring. Rabbits inoculated either i.p. or i.v. with GPHLV for various lengths of time were mated and sacrificed as they exhibited apparent signs of pregnancy. In certain instances, noninfected pregnant rabbits near term were inoculated with virus intravenously. Maternal and fetal blood samples were used for virus isolation and antibody studies. Maternal and fetal tissues, including spleen, liver, lung, kidney, and thymus were trypsinized and used for virus isolation. The results of a total of nine experiments are listed in Table 3. All of the six i.v.-inoculated rabbits had virus in their spleens and placenta. The presence of virus in the spleen could be demonstrated in only one of the three i.p.-inoculated rabbits. Since only tissue suspensions were used for virus isolation in the two negative rabbits, the presence of small amounts of virus may not have been detected by this method. Of a total of 75 offspring tested, only one fetus showed infectious virus in its lungs and kidneys (Table 3). This fetus was obtained from rabbit no. 18 which received 5 ml of virus suspension intravenously only 5 days prior to the test and showed no antibody titer to the virus.

FIG. 1. Virus recovery from and antibody response in rabbits inoculated with GPHLV by IP (intraperitoneal), IV (intravenous), or SC (subcutaneous) routes, two rabbits in each group: A (X) and B (O).

TABLE 3. Transplacental transmission of antibody and virus in rabbits inoculated with GPHLV

Rabbit no.	Route ^a of inoculation	Days after inoculation	Virus isolation			Antibody response						
			Maternal		Offspring (no. pos./no. tested)	Maternal antibody titers ^b	Fetal antibody					
			Blood	Tissue (spleen)			No. positive/no. tested ^c	No. of fetuses showing titers of				
								20	40	80	160	
27	i.v.	4	—	+	0/7	<5	0/7					
18	i.v.	5	+	+	1/9	<5	0/9					
21	i.v.	7	—	+	0/9	5	Not done					
22	i.v.	8	—	+	0/8	5		0/8				
8	i.v.	48	+	+	0/6	80	6/6 ^d	2	3	1		
12	i.v.	180	+	+	0/8	80	8/8		4	4		
11	i.p.	24	—	—	0/6	160	6/6			2	4	
5	i.p.	27	—	+	0/7	160	7/7	1	2	2	2	
6	i.p.	32	—	—	0/7	320	7/7	3	3	1		

^a i.v., intravenous; i.p., intraperitoneal.
^b Reciprocal of the highest serum dilutions.
^c Antibody titer of 1:10 or greater was considered positive.
^d All six fetuses also showed positive antibody titers in their amniotic fluids.

All rabbits that were inoculated for 7 days or longer showed neutralizing antibody titers to the virus (Table 3). The levels of antibody titers were high in rabbits infected for 20 days or more, especially when the virus was administered intraperitoneally. The offspring of rabbits that were inoculated for less than eight days showed no detectable antibody titers to GPHLV in their

serum (rabbits no. 18, 22, and 27 in Table 3), but antibody titers ranging from 1:20 to 1:160 were detected in the offspring of those rabbits that were inoculated for more than 20 days. Amniotic fluid from six fetuses of rabbit no. 8 also contained neutralizing antibody titers ranging from 1:20 to 1:80 similar to those obtained from the fetal sera. No histological change or

intranuclear inclusions were observed in any of the inoculated rabbits or their offspring.

Figure 2 shows an example of the rise and fall of the antibody titers to GPHLV in an infected rabbit during three serial pregnancies. This rabbit showed no antibody titer or virus infectivity in its blood on the day of arrival, day 0, at which time she was given an i.p. injection of 6.7 log TCID₅₀ of GPHLV. She was mated after virus administration and observed closely. Thirty days later she gave birth to her first litter of six. Infectious virus was isolated from the blood of the inoculated mother and a neutralizing antibody titer of 1:80 occurred concurrently. Four of her six offspring also had neutralizing antibody titers of 1:80, and of the remaining two, one had a titer of 1:40 and the other 1:20. No virus was isolated from any of the offspring. A second litter of two rabbits was born 60 days after inoculation. At this time, the maternal antibody titer rose to 1:160 and infectious virus was not isolated from the maternal blood. Both newborn rabbits had low levels of antibody titers, 1:10, but no infectious virus was recovered. When this rabbit was pregnant for the third time, she was given an additional injection of 6.7 log TCID₅₀ of GPHLV intravenously. Two days after the i.v. injection, this rabbit was sacrificed for virus isolation and antibody determination.

No virus was isolated from either the maternal or the fetal tissues. The maternal antibody titer was 1:320. A total of seven fetuses were obtained. Two of the fetal sera had antibody titers of 1:160, two had titers of 1:80, two had titers of 1:40, and one had a titer of 1:20.

DISCUSSION

The differences in response to GPHLV infection in mice and rabbits are well illustrated in the present study. Mice inoculated at 1 week of age showed no antibody response to the GPHLV, although virus was recovered from inoculated mice 1 to 6 days after infection, but not thereafter. Older mice inoculated by the i.p. or intranasal route gave a minimal rise in antibody titer but no virus could be detected. These data confirmed our earlier report that mice are comparatively less susceptible to GPHLV infection than guinea pigs.

In contrast to mice, rabbits are highly susceptible to GPHLV infection. Rabbits inoculated with the guinea pig virus produced antibody promptly and at higher levels. At no time was there any evidence of illness. Infectious virus could be isolated from inoculated rabbits prior to the development of antibody. Once serum antibody appeared, the isolation of virus from the

FIG. 2. Demonstration of transplacental transmission of immunity to offspring in three serial pregnancies. Infectious virus was isolated from the maternal blood at 30 days but not at 60 and 90 days post-inoculation. No virus was isolated from the offspring. Antibody titers were detected in the mother's blood and all of her offspring. (The numbers of offspring with positive antibody are indicated by the numbers in the clean bars at the level of the antibody titers.) Cross bars represent maternal antibody titers and open bars represent antibody titers of fetal blood.

various tissues was achieved with difficulty except in rabbits inoculated intravenously.

The levels of neutralizing antibody to GPHLV in rabbits exceeded those found in guinea pigs. However, viremia was more persistent in guinea pigs than in rabbits when virus was administered by routes other than i.v. (5, 7). In the case of rabbits, only the i.v. injection resulted in persistent viremia; i.p.-inoculated rabbits had a temporary viremia, whereas no viremia was demonstrable in rabbits which received s.c. injections. Recovery of infectious virus from tissues of rabbits inoculated intraperitoneally required long-term cultivation of the cells (Table 2). It was possible that a small amount of virus which persisted in the rabbits was masked by the high levels of antibody. In a separate study (9) it was shown that specific antibody could prevent GPHLV from spreading in organ culture, and infectious virus was recovered from such cultures when the specific antibody was removed.

It was noteworthy that the maternal antibody to GPHLV in rabbits was readily transferable to the offspring. The levels of neutralizing antibody in the offspring were generally lower than the maternal antibody titer. In addition, significantly high titers of neutralizing antibody were found in the amniotic fluids of infected rabbits. According to Brambell the route of transfer of antibodies in rabbits to offspring is probably through the yolk sac of the embryos (2). Although no attempt was made to determine whether the transferred maternal immunity was due to 19S or 7S antibodies, it is felt to be due to the latter.

In contrast to observations with guinea pigs (6), infectious virus appeared to be extremely difficult to demonstrate in the offspring of infected rabbits. Of a total of 75 live offspring examined, with only one exception, none had any recoverable virus from fetal tissues or blood. This fetus was obtained from a rabbit five days after i.v. inoculation and before she developed any detectable levels of maternal antibody (Table 3). Thus, the failure of recovery of infectious virus from the offspring of infected rabbits may be due to: (i) the high levels of neutralizing antibody in both maternal and fetal blood that prevented fetal infection and (ii) the structural barrier of the placenta that prevented the transfer of virus from the maternal blood to fetal tissues. According to Enders (3), rabbits have two layers of

trophoblasts in their placentas, whereas guinea pigs have only one layer.

Again, the GPHLV provides an excellent opportunity for studying the behavior of a persistent virus infection not only in its natural host, the guinea pig, as described in part I of this study (Lam and Hsiung), but also in a foreign host, the rabbit. In contrast to the guinea pigs, antibody response to GPHLV in rabbits was prompt and the titers were high. Recovery of persistent virus in rabbits required long-term cultivation of tissue cells. Transplacental transmission of virus was found only rarely, but transferred immunity was readily demonstrated. Apparently, the high levels of specific antibody have played an important role in the masking of GPHLV infection in rabbits.

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