

Comparison of Guinea Pig Cytomegalovirus and Guinea Pig Herpes-Like Virus: Pathogenesis and Persistence in Experimentally Infected Animals

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The pathogenesis of guinea pig cytomegalovirus (GPCMV) and guinea pig herpes-like virus (GPHLV) in guinea pigs was compared. Animals were inoculated with the two viruses by different routes and sacrificed after varying periods of time. GPCMV was consistently isolated from salivary gland 2 weeks postinoculation and thereafter following intraperitoneal or subcutaneous inoculation. Virus was less frequently found in other tissues including blood, spleen, and kidney. Intranuclear inclusions were seen in tissue sections of salivary gland after inoculation with GPCMV-infected tissue suspension, but were only rarely found after inoculation with tissue culture virus. In GPHLV-infected guinea pigs, consistent latent infection of leukocytes and other tissues was detected by cocultivation techniques. Intranuclear inclusions were not found in the spleen, salivary gland, or other infected tissues after GPHLV infection with either tissue culture virus or infected tissue suspension. Guinea pigs inoculated with GPCMV produced high titers of specific neutralizing antibody to the homologous virus; those inoculated with GPHLV developed long-term viremia accompanied by minimal neutralizing antibody levels to the virus.

It was first demonstrated in 1926 that a filterable agent caused the enlarged cells bearing nuclear inclusions in the salivary glands of guinea pigs (4). The typical Cowdry type A intranuclear inclusions in the salivary gland duct cells provided the basis for determining the presence of an infectious agent in many of the early *in vivo* studies in guinea pigs. Inclusion formation by the guinea pig salivary gland virus, later called the guinea pig cytomegalovirus (GPCMV), in explant cultures was described by Andrewes in 1930 (1), but the virus was not successfully propagated in cell cultures until 1957 by Hartley et al. (6). Several investigators explored the pathogenicity of GPCMV and demonstrated fatal meningitis after intracerebral (*i.c.*) inoculation with infected salivary gland tissue suspension (1, 11, 12) as well as lethal infection after subcutaneous (*s.c.*) or intraperitoneal (*i.p.*) inoculation with a virulent strain of the virus (17). In addition, Kuttner et al. (13) showed that GPCMV was species specific and would not induce inclusions in tissues of mice or hamsters. Since the method for cultivation of GPCMV *in vitro* was not available before 1957, it was difficult to obtain precise and quantitative information on the basis of the early reports.

Another herpesvirus, the guinea pig herpes-like virus (GPHLV), was isolated in our laboratory from kidney cell cultures of strain 2 guinea pigs in 1969 (7). Since then, a number of studies have been performed in which the properties of GPHLV have been investigated (2, 8, 15, 18). Studies of GPHLV in this laboratory demonstrated long-term latent infection (8), viremia due to infection of leukocytes (9), and transplantable transmission of infectious virus (14). Infection with GPCMV, on the other hand, has not been extensively studied since the development of *in vitro* cultivation methods. With the current interest in human cytomegalovirus disease, GPCMV infection in guinea pigs may provide a valuable model for further study of this virus.

In the *in vitro* experiments described in the preceding paper (10), the growth characteristics of the two guinea pig herpesviruses were compared. In the present study an *in vivo* comparison between these viruses was made. Guinea pigs of different ages were inoculated with GPCMV or GPHLV by several routes and sacrificed after varying periods of time. Efforts were made, using virus infectivity titration and light and electron microscope examination, to determine the distribution of virus in various tis-

sues, and thereby to assess the status of viral persistence in guinea pigs infected with either of these two viruses.

MATERIALS AND METHODS

Virus stocks. The prototype GPCMV, strain 21222, was obtained from the American Type Culture Collection. This virus was passaged three times in guinea pig embryo (GPE) cell cultures before inoculation of animals. Some guinea pigs were inoculated with GPCMV-infected salivary gland suspension. Virus titers of the tissue culture fluid ranged from 10^4 to 10^5 mean tissue culture infective doses (TCID₅₀) per 0.1 ml and for the salivary gland tissue suspensions ranged from $10^{2.5}$ to $10^{4.5}$ TCID₅₀/0.1 ml of packed cells. The GPHLV used, strain LK40, was isolated in our laboratory. This virus was passaged four times in guinea pig kidney (GPK) cell cultures, and infectivity titers ranged from 10^5 to 10^6 TCID₅₀/0.1 ml. GPHLV-infected spleen suspensions, with infectivity titers of 10^3 to 10^5 TCID₅₀/0.1 ml of packed cells, were also used for guinea pig inoculation.

Tissue culture. Methods of preparation of primary GPK epithelial cell cultures were similar to those described in the preceding paper (10). In brief, kidney tissues from 1- to 2-month-old Hartley guinea pigs were dispersed in 0.25% trypsin. The dispersed cells were suspended in growth medium, consisting of Hanks balanced salt solution, 0.5% lactalbumin hydrolysate, 10% medium 199, and 10% heat-inactivated calf serum. Cell suspensions were planted into culture tubes or bottles and were incubated at 35 C. When cell sheets were confluent, the growth medium was replaced by maintenance medium containing Earle balanced salt solution, 0.5% lactalbumin hydrolysate, and 2% heat-inactivated calf serum. GPE fibroblast cell cultures were prepared from 20- to 40-day-old embryos. Methods of preparation of GPE cell monolayers were similar to those for the GPK cell cultures. Both primary and passaged fibroblast cell cultures were used.

Source of animals and animal inoculation. Random-bred Hartley guinea pigs, 2 to 3 months of age, were obtained from CAMM Research Institute, Wayne, N.J., and the Yale University School of Medicine, Division of Animal Care. Newborn guinea pigs, 1 to 3 days old, were obtained from guinea pigs in the Animal Resources Facilities at the West Haven Veterans Administration Hospital. Animals were inoculated i.p. or s.c. (anterior neck) either with infected tissue culture fluid or 10% infected tissue suspension, 0.5 to 1.5 ml for adult animals and 0.5 ml for newborns.

Virus isolation. Guinea pigs were sacrificed 3 days to 20 weeks after virus inoculation. All animals were exsanguinated under ether anesthesia by cardiac puncture. Heparinized blood was used for virus isolation and serum was used for antibody studies. The salivary glands, lungs, liver, spleen, and kidneys were removed aseptically and portions of tissues were fixed for histological examination. Urine samples were obtained from some animals. Each tissue was minced with separate instruments and was trypsinized separately. A cell suspension of each tissue was prepared as described above for the

kidney tissue cultures. Serial 10-fold dilutions of each cell suspension were inoculated onto monolayer cultures of GPE for GPCMV and GPK for GPHLV. Virus infectivity titers of heparinized whole blood or of urine were determined by serial 10-fold dilutions which were inoculated into GPE or GPK cells. The cell cultures, three to four tubes per dilution, were examined for the presence of cytopathic effect (CPE), and virus infectivity titers were determined by the 50% end-point method (16).

Neutralizing antibody tests. Neutralizing antibody titers were determined by inhibition of virus-induced CPE in monolayer cultures. Equal volumes of virus suspension, containing approximately 100 TCID₅₀ of virus, and serial twofold dilutions of heat-inactivated serum were mixed and incubated at room temperature for 1 h and then inoculated into GPE or GPK tube cultures. After 4 to 5 days of incubation at 35 C, cultures were examined for virus-induced CPE. The highest serum dilution that inhibited the development of CPE, in comparison to the virus control cultures, was considered to be the titer of the serum.

Histopathology. Portions of salivary gland, lung, liver, spleen, and kidney were fixed in 10% formalin or Bouin solution. Tissues were stained with hematoxylin and eosin or with Giemsa for histological examination. In selected instances, infected tissues were fixed in 3% glutaraldehyde. Thick sections (1 μ m) of the latter were stained with toluidine blue and examined by light microscopy. Tissues showing intranuclear inclusions were further processed for thin section electron microscopy (5).

RESULTS

GPCMV distribution after experimental infection. (i) **s.c. inoculation.** The distribution of GPCMV in various tissues after s.c. inoculation of virus is seen in Fig. 1A. During the initial 1- to 3-week period virus was isolated from the salivary gland in seven of eight guinea pigs, from the blood of one guinea pig, and the kidney of another. Virus with significantly high infectivity titers was consistently recovered from the salivary gland of all 13 guinea pigs tested 4 to 20 weeks postinoculation and from the kidney of one at 9 weeks. GPCMV was not recovered from other tissues including spleen, lung, or liver of any of the 21 animals tested.

(ii) **i.p. inoculation.** Virus was not isolated from salivary gland or blood of the three animals tested 3 to 7 days after i.p. inoculation, although virus was recovered from lung of one and liver of another. The distribution of GPCMV 1 week or thereafter following i.p. inoculation is seen in Fig. 1B. Virus was isolated from salivary gland of five of seven tested at 2 to 3 weeks, four of five at 4 to 9 weeks, and four of four animals at 10 to 20 weeks postinoculation; virus was not isolated from the blood of any of these i.p.-inoculated animals. GPCMV isolations were made from kidney, lung, liver,

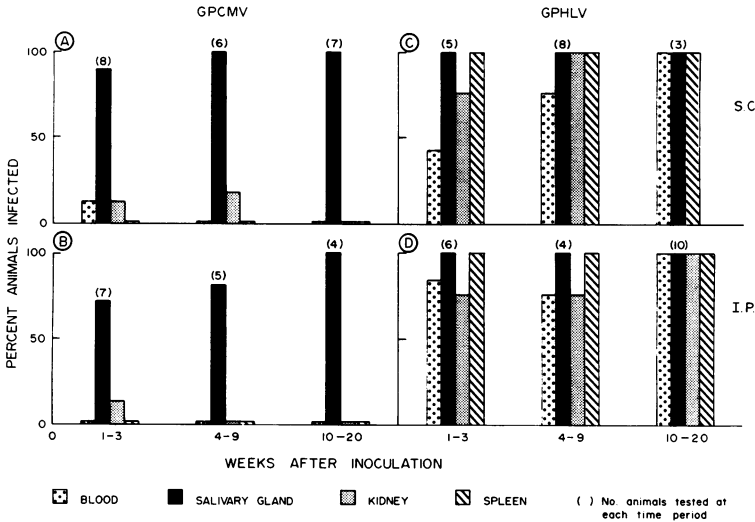


FIG. 1. Distribution of virus in different tissues of guinea pigs inoculated with GPCMV (A and B) and with GPHLV (C and D). (A and C) Animals inoculated s.c.; (B and D) animals inoculated i.p.

and urine 2 to 3 weeks after inoculation, and from liver of one animal 8 weeks after inoculation. Thus, occasional virus isolations were made from several tissues, but not from spleen. Two to three weeks postinoculation, virus was widespread but was most frequently isolated from salivary gland. In the 4- to 20-week period virus was found only in the salivary gland, similar to those animals inoculated s.c.

GPHLV distribution after experimental infection. In contrast to the limited pattern of GPCMV distribution described above, GPHLV was widely distributed in a variety of tissues after s.c. or i.p. inoculation (Fig. 1C and D). Virus was recovered more frequently from blood of i.p.-inoculated animals than from guinea pigs inoculated s.c. during the 1- to 3-week period, but the incidence of virus isolations from blood was similar during the subsequent periods tested. Regardless of route of inoculation, infectious virus was consistently recovered from internal organs including spleen, kidney, and salivary gland of infected animals; the highest virus infectivity titers were obtained uniformly from the spleen. Long-term infection and distribution of GPHLV after different routes of inoculation have been described in several reports from this laboratory (3, 14, 18).

Development of intranuclear inclusions. Tissue sections of salivary gland, spleen, lung, liver, and kidney were carefully searched for the presence of intranuclear inclusions. Single inclusions were found in the salivary gland of only two of ten guinea pigs that had been inoculated with GPCMV tissue culture fluid; none

was found in other tissues. However, intranuclear inclusions were seen frequently in salivary gland duct cells of seven of nine guinea pigs inoculated with GPCMV-infected salivary gland tissue suspension (Table 1). Typical Cowdry type A intranuclear inclusions (Fig. 2A), occasionally accompanied by basophilic cytoplasmic inclusions (Fig. 2B), were seen. On electron microscope examination, intranuclear herpesvirus nucleocapsids and cytoplasmic clusters of enveloped virions were observed in infected salivary gland tissue (Fig. 2C). Inclusions were seen as early as 2 weeks after inoculation with infected salivary gland tissue suspension and up to 11 weeks, the longest time tested. There was no significant relationship between virus infectivity titers in salivary gland ranging from 2.5 to 4.5 log TCID₅₀/0.1 ml of packed cells and the presence or absence of inclusions. Neither was there any significant difference in the GPCMV titers obtained from the salivary gland of animals inoculated with infected tissue suspension and those inoculated with tissue culture fluid (Table 1). Inclusions were not found in other tissues examined. In several salivary gland sections, where inclusion-bearing cells were seen, mononuclear cell infiltrates were also present, sometimes adjacent to the inclusions.

In contrast to animals inoculated with GPCMV, intranuclear inclusions were not found in salivary gland, spleen, or other tissues of guinea pigs inoculated either with tissue culture fluid containing GPHLV or with GPHLV-infected spleen tissue suspension (Table 1). Significant GPHLV titers, however, were obtained

TABLE 1. Comparison of virus titers and inclusion formation in guinea pigs after experimental infection with GPCMV and GPHLV

Virus type	Material inoculated	No. of animals studied	Time post-inoculation (weeks)	Avg infectivity titer (log TCID ₅₀ /0.1 ml of packed cells)				No. of animals showing inclusions	
				Blood	Kidney	Spleen	Salivary gland	Spleen	Salivary gland
GPCMV	Tissue culture fluid	10	3-10	<1.0	<1.0 ^a	<1.0	4.1	0	2
	Salivary gland tissue suspension	9	2-18	<1.0	<1.0	<1.0	3.8	0	7
GPHLV	Tissue culture fluid	20	3-16	1.5	2.5	3.8	2.8	0	0
	Spleen tissue suspension	6	4-11	0.9	2.0	4.0	3.0	0	0

^a Virus was not detected at 1.0 log TCID₅₀/0.1 ml of packed cells or whole blood except in two instances where virus was isolated.

from various tissues of all animals tested with the highest titers in the spleen ranging from 3.0 to 5.5 log TCID₅₀/0.1 ml of packed cells.

Clinical observation and antibody responses. None of the guinea pigs inoculated with GPCMV-infected tissue culture fluid or salivary gland tissue suspension showed symptoms of disease, nor was there any clinical evidence of disease in guinea pigs inoculated with GPHLV.

Animals inoculated s.c. or i.p. with GPCMV showed a good antibody response 5 to 9 weeks postinoculation. Neutralizing antibody titer was minimal 1 to 2 weeks postinoculation but increased to 1:20 to 1:40 after 3 to 4 weeks, and reached a titer of 1:640 by 9 weeks (Table 2). There was no consistent difference in antibody titer in animals inoculated s.c. compared with those inoculated i.p., and there was no correlation between neutralizing antibody titers and infectious virus titers in the salivary gland. Antibody responses to inoculation with GPCMV tissue culture fluid and salivary gland tissue suspension were also similar. In all cases serum obtained from animals inoculated with GPCMV did not neutralize GPHLV at 1:5, the lowest serum dilution tested.

In guinea pigs inoculated with GPHLV, neutralizing antibody to this virus reached levels of only 1:10 to 1:20 up to 20 weeks after virus inoculation (Table 2). Again, there was no correlation between antibody levels and infectious virus titers in the tissues tested. Infectious GPHLV was repeatedly isolated from the blood or leukocytes, but it is not known whether an antigen-antibody complex existed in the animal tissues. There was no evidence of cross-neutral-

ization of GPHLV antisera with GPCMV at a dilution of 1:5, the lowest serum dilution tested.

DISCUSSION

The present study illustrated that the pathogenesis of cytomegalovirus infection in guinea pigs following i.p. or s.c. inoculation was similar. In a separate study, guinea pigs inoculated with GPCMV i.c. showed essentially the same results (unpublished data). Regardless of route, virus was easily recovered from the salivary gland soon after inoculation and persisted for weeks and months. Infectious virus was not commonly found in other tissues, and viremia was brief and detected only occasionally. Connor and Johnson (51st Annu. Meet. Am. Assoc. Neuropathol. p. 50, 1975) reported that GPCMV was isolated from blood 3 and 9 days post-s.c. inoculation but not thereafter. In the present experiment we found that significant neutralizing antibody titers were consistently detectable in guinea pigs 4 weeks after inoculation (Table 2). It is possible that GPCMV viremia was limited by the rapid development of humoral antibody.

A striking difference between the histopathology, i.e., inclusion formation in the duct cells of salivary gland, induced by GPCMV which was passaged in animals, as compared with GPCMV grown in tissue culture, was noted. Typical intranuclear inclusions were frequently seen in guinea pig salivary glands after inoculation with GPCMV-infected salivary gland tissue suspension, whereas they were rarely found in animals after inoculation with infected tissue culture fluid. However, the vi-

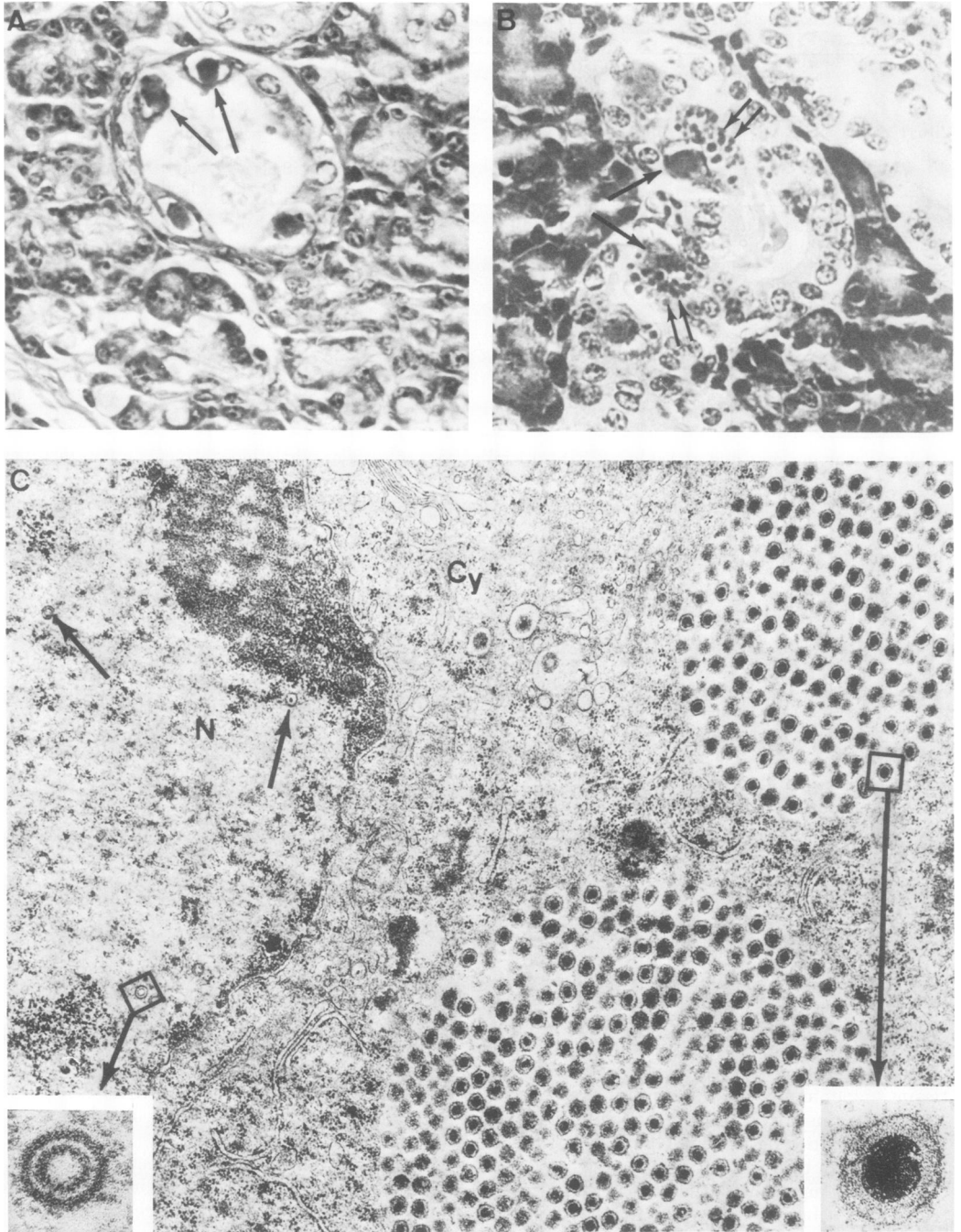


FIG. 2. Guinea pig salivary gland cells infected with salivary gland-passaged GPCMV: (A) and (B) hematoxylin and eosin-stained preparations. $\times 400$. (A) Eosinophilic intranuclear inclusions in duct cells (single arrows). (B) Basophilic cytoplasmic inclusions (double arrows) in cells with eosinophilic intranuclear inclusions (single arrows). (C) Electron micrograph of a salivary gland cell showing intranuclear nucleocapsids (arrows) and clusters of cytoplasmic virions. N, Nucleus; cy, cytoplasm. $\times 16,000$. Inserts: Higher magnification of intranuclear and cytoplasmic virus.

TABLE 2. Antibody response in guinea pigs experimentally inoculated with GPCMV or GPHLV

Virus type	Material inoculated ^a	Time post-inoculation (weeks)	No. of animals studied	Virus isolation (mean virus infectivity titer [log TCID ₅₀ /0.1 ml]) ^b		Neutralizing antibody titer to homologous virus	
				Blood	Salivary gland	Mean	Range
GPCMV	Tissue culture fluid	1-2	7	<1.0	3.4	1:10	<1:5-<1:10
		3-4	5	<1.0	3.5	1:30	1:20-1:40
		5-9	8	<1.0	4.8	1:210	1:160-1:640
		10-20	6	<1.0	3.7	1:280	1:160-1:640
	Salivary gland tissue suspension	1-3	7	<1.0	3.7	<1:10	<1:5-<1:10
		4-9	2	<1.0	4.5	1:80	1:80
		10-20	3	<1.0	3.9	1:345	1:160-1:640
GPHLV	Tissue culture fluid	1-3	11	1.3	3.1 ^c	1:5	<1:5
		4-9	7	1.2	3.7 ^c	1:10	1:5-1:20
		10-20	8	1.7	4.2 ^c	1:10	1:5-1:20
	Spleen tissue suspension	1-3	2	1.0	4.0 ^c	<1:5	<1:5
		4-9	5	1.0	3.5 ^c	1:5	1:5
		10-20	2	1.5	4.3 ^c	1:5	1:5

^a All guinea pigs inoculated i.p. except for those inoculated with GPCMV-infected salivary gland tissue suspension, which was given s.c.

^b Log TCID₅₀/0.1 ml of whole blood or packed salivary gland or spleen cells.

^c Spleen.

rus infectivity titers obtained from the salivary glands of both groups of animals were essentially the same (Table 2). The GPCMV used in the present study was obtained from the American Type Culture Collection and was passaged in tissue culture a number of times before and after we received it. The infrequent intranuclear inclusions seen in guinea pigs inoculated with the tissue culture passaged virus may have been due to altered virus populations with passage and/or storage. Infected tissue suspensions were used in experiments performed during the 1920s and the 1930s (1, 4, 11-13, 18), and the severe disease and intranuclear inclusion formation described in those studies were probably due to the high virulence of the animal-passaged virus strains.

The in vivo pattern of GPHLV distribution differed markedly from that of GPCMV described above. Latent infection of leukocytes and other tissues, as a result of GPHLV infections, has been reported previously (3, 8, 9, 14, 18). Infectious GPHLV was readily detectable in the blood and other tissues, with highest virus titers obtained from the spleen, regardless of route of inoculation (3, 14, 18). Intranuclear inclusions of virions have not been seen after light and electron microscope examination of infected tissues (9), even after serial passage of infected spleen suspension as described in the present study. These data indicate that GPHLV infection resembles in sev-

eral respects the Epstein-Barr herpesvirus infection of humans. GPCMV infection, on the other hand, probably resulted in a brief period of viremia and induced histopathological changes in the salivary glands of short- and long-term-infected animals. Thus, GPCMV infection in guinea pigs more closely resembles cytomegalovirus infection in other species than it does GPHLV infection in guinea pigs. In both cases no clinical disease was observed.

The mechanism of viral persistence has been studied by various investigators using different virus-host systems. However, the terminology used in the literature regarding persistent infections has not been consistent (19). Our data showed that although the two guinea pig herpesviruses induced long-term infections in their natural host, the status of the viral genome differed significantly. In guinea pigs infected with GPCMV, virus-induced inclusions and virus particles were detected in infected tissues, demonstrating persistent infection. In GPHLV-infected guinea pigs, on the other hand, the infection was latent, since virus particles and inclusions were not found in the infected tissues and the actual state of the GPHLV genome has not yet been ascertained.

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