Comparison of Guinea Pig Cytomegalovirus and Guinea Pig Herpes-Like Virus: Growth Characteristics and Antigenic Relationship

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The growth characteristics of guinea pig cytomegalovirus (GPCMV) and guinea pig herpes-like virus (GPHLV) in cell cultures were compared. Guinea pig fibroblast cells were highly susceptible to infection with both viruses, whereas guinea pig kidney cells were sensitive only to GPHLV. No cytopathic effect was observed in the latter cell system after infection with GPCMV, nor was there an increase in virus titer, although the virus persisted in the kidney cells for 2 to 3 weeks postinfection. Electron microscope studies showed nonvirion tubular structures in GPCMV-infected fibroblast cells, but not in GPHLV-infected cells. Large packages of enveloped nuclear virus particles were commonly seen in GPHLV-infected cells, especially kidney epithelial cells, but none were found in the GPCMV-infected fibroblasts. Complete enveloped extracellular virus particles were present in both virus-cell systems. Both viruses showed narrow host spectra and replicated well only in guinea pig cells although GPHLV multiplied to some degree in rabbit cells. No antigenic relationship could be demonstrated between the two viruses using antisera specific for each virus that was produced in rabbits and guinea pigs. Rabbits produced high neutralizing antibody titers to GPHLV, whereas guinea pigs were the animals of choice for GPCMV antiserum production.

Guinea pig cytomegalovirus (GPCMV) was first demonstrated by Cole and Kuttner as a filterable virus responsible for the swollen epithelial cells with nuclear inclusions in the ducts of guinea pig salivary glands (3, 12). However, cultivation of GPCMV in vitro was not accomplished until 1957, when Hartley et al. described the successful propagation of GPCMV in embryonic muscle cells as well as a spontaneously degenerated salivary gland cell culture (7). According to the Herpesvirus Study Group, International Committee on Taxonomy of Viruses, GPCMV is designated as Cavid herpesvirus 1 (18).

In 1969 we reported the isolation of another guinea pig herpesvirus from a spontaneously degenerated kidney cell culture derived from a leukemia-susceptible strain 2 guinea pig, the guinea pig herpes-like virus (GPHLV) (8). Subsequently, a similar virus was isolated from strain 13 guinea pigs and infrequently from Hartley guinea pigs (1, 4, 9, 15). This virus is now designated as Cavid herpesvirus 2 (B. Roizman, personal communication).

During the past several years the pathogenicity and persistence of GPHLV in guinea pigs has been studied extensively (2, 9, 13, 20). However, quantitative analyses of experimental GPCMV infections in guinea pigs are limited. Since latent infections with either herpesvirus are common in guinea pigs, it was of interest to compare the biological properties and the growth characteristics of the two viruses in vitro and in vivo. In the present paper replication in cell culture and affinity for cell type in vitro of these two herpesviruses, as well as their antigenic relationship, are compared in detail. Data regarding virus distribution and histopathology in different guinea pig tissues after short- and long-term infection with these viruses will be reported in the accompanying paper (21).

MATERIALS AND METHODS

Virus strain and assay. The prototype strain of GPCMV, obtained from the American Type Culture Collection (strain 22122), was passaged in guinea pig fibroblast cell cultures three or more times before use. Virus titers ranged from 10⁴ to 10⁶ mean tissue culture infective doses (TCID₅₀) per 0.1 ml in guinea pig fibroblast cells. GPHLV was isolated in our laboratory. Strain LK40 was obtained from a spontaneously degenerated kidney cell culture derived...
from a leukemic strain 2 guinea pig. Virus titers of the latter ranged from 10^4 to 10^6 TCID_{50}/0.1 ml. Dimethyl sulfoxide (Me_{2}SO) was added to a concentration of 10% to all virus stocks before storage at -70 C. GPHLV infectivity titrations were performed in primary guinea pig kidney (GPK) cell cultures. Guineapig embryo fibroblast cultures were used for GPCMV titrations. The highest virus dilution producing cytopathic effect (CPE) was used as the end-point indicator and TCID_{50} was calculated by the 50% end-point method (17). In certain instances, the plaque method in agar overlaid bottle cultures was also used for virus assay.

**Preparation of cell cultures.** Methods for preparation of primary GPK cell cultures were similar to those described previously (2, 9). In brief, kidney tissues obtained from virus-free 1- to 2-month-old Hartley guinea pigs were dispersed in 0.25% trypsin. The dispersed cells were suspended in an enriched growth medium, HK, consisting of Hanks balanced salt solution, 0.5% lactalbumin hydrolysate, 10% medium 199, and 10% heat-inactivated fetal bovine serum. The cell suspensions were planted into culture tubes, Leighton tubes containing cover slips (11 by 22 mm), or 3-ounce (about 90 ml) bottles and were incubated at 35 C. When the cell sheets were confluent the growth medium was replaced by a maintenance medium containing Earle balanced salt solution, 0.5% lactalbumin hydrolysate, and 2% heat-inactivated calf serum. Guinea pig embryo (GPE) fibroblast cultures were prepared from 20- to 40-day-old embryos. The methods for preparation resembled those used for the kidney cell cultures. Both primary and passaged fibroblast cell cultures were used.

**Infection of cell cultures for light and electron microscopy.** Monolayer GPE or GPK cell cultures, prepared in culture tubes, Leighton tubes containing cover slips, or 3-ounce prescription bottles, were infected with either GPCMV or GPHLV at an input multiplicity of approximately 1 TCID_{50}. Virus was adsorbed for 2 h at 35 C. Unadsorbed virus was removed by washing infected cells with Hanks balanced salt solution, followed by replacement of maintenance medium and incubation at 35 C. At selected time intervals, duplicate samples were taken and treated as follows: (i) infected culture tubes were removed from the incubator, Me_{2}SO was added to each tube to a final concentration of 10%, and the cultures were stored at -70 C until further assay; (ii) infected cells on cover slips were fixed in Zenker acetic acid solution and stained with hematoxylin and eosin for light microscopy; (iii) infected cells in 3-ounce prescription bottles were fixed in situ with 2% glutaraldehyde for electron microscopy as previously described (5).

**Production of antiserum.** Adult rabbits and guinea pigs were inoculated with 5 ml of infectious GPCMV or GPHLV, either intraperitoneally (i.p.) or subcutaneously (s.c.). Both single injections and multiple inoculations were used. A sample of antiserum produced in rabbits to GPCMV was kindly supplied by J. N. Middelkamp of Washington University, St. Louis, Mo. Serum samples received from several investigators who have isolated guinea pig herpes-like virus in their laboratories were included in the present study for comparison (1, 4).

**Neutralization tests.** Serum neutralizing antibody titer was determined by inhibition of CPE in tube cultures. Equal volumes of virus suspension containing approximately 100 TCID_{50} of virus and serial dilutions of serum were mixed and incubated at room temperature for 1 h. The mixtures were inoculated into GPE cell culture tubes for GPCMV and GPK cell cultures for GPHLV. After adsorption for 1 h at 35 C, 1 ml of maintenance medium was added to each tube. After an additional 2 to 5 days of incubation at 35 C, all cultures were examined for CPE. The highest serum dilution, which limited CPE to less than that seen in the virus control cultures, was considered to be the titer of the serum.

**RESULTS**

**Growth characteristics of GPCMV and GPHLV in cell cultures.** (i) Cytopathic effect and nuclear inclusion formation. The pattern of CPE induced by GPCMV in GPE and GPK cells was somewhat similar (Fig. 1), although GPK cells infected with GPHLV became more rounded and swollen than did the GPE cells infected with GPCMV. CPE was not observed in GPK cells infected with GPCMV. Foci of CPE induced by GPCMV spread more slowly than did those observed in GPHLV-infected cells. Intranuclear inclusions induced by both viruses in GPE cells were similar (Fig. 2A-C) and resembled those observed in human embryo fibroblast cells infected with human CMV; they formed a homogeneous eosinophilic mass with a clear halo between the inclusion and the nuclear membrane (Fig. 2B and C). In contrast, GPK cells infected with GPHLV showed large swollen cells with somewhat basophilic inclusions in a distinctive pattern (Fig. 2F). Occasionally halo zones were observed in the latter, but these were not as distinct as those seen in the GPHLV-infected GPE cells (Fig. 2C). Intranuclear inclusion-bearing cells were found only occasionally in GPK cell cultures infected with GPCMV (Fig. 2E). It is possible that these inclusion-bearing cells were actually fibroblast cells which were present in the kidney cell population.

(ii) Ultrastructure of virus-infected cells. Certain distinct differences were apparent in GPE cells infected with either GPCMV or GPHLV when examined by electron microscopy. In GPCMV-infected cells, numerous tubular nonvirion structures were seen evenly distributed within the nuclear inclusions (Fig. 3A). These tubular structures were not found in the GPHLV-infected GPE cells. However, in the latter, clusters of enveloped virus particles enclosed in large vacuoles were often seen near...
the nuclear periphery (Fig. 3B), but they were rarely found in GPCMV-infected nuclei. Nucleocapsids with or without electron-dense cores were present throughout the nucleus of cells infected with either virus, and extracellular enveloped virus particles were commonly seen in preparations made from guinea pig cells infected with both viruses.

Comparison of growth rates of GPCMV and GPHLV in cell cultures. (i) GPCMV. GPE monolayers infected with GPCMV showed an initial increase in virus infectivity titer at 24 h after infection and reached a maximum virus yield at 5 days (Fig. 4). Virus-induced CPE progressed steadily from day 3 until the day 7, when the cell sheet was destroyed almost completely. Typical CMV intranuclear inclusions were observed in 15 to 18% of cells 2 to 3 days after infection and progressed to involve over 90% by day 5, after which time extensive cellular destruction precluded an accurate count. In contrast, GPCMV produced minimal, if any, new virus in GPK cells even after 8 days of infection. No evidence of CPE was observed in GPK cells infected with GPCMV for 14 days or longer. However, examination of hematoxylin and eosin-stained preparations of infected GPK cells on cover slips revealed typical intranuclear inclusions in a very low proportion, 0.5%, of cells 6 to 10 days after infection.

(ii) GPHLV. Monolayer cultures infected with GPHLV showed significant virus yield in both cell systems, although virus titers were somewhat higher in the GPE cells than GPK

Fig. 1. CPE induced by GPCMV and GPHLV in GPE and GPK cells, respectively. ×100. (A) Uninfected GPE culture; (B) GPE cells infected with GPCMV, 7 days postinfection; (C) uninfected GPK culture; (D) GPK cells infected with GPHLV, 4 days postinfection.
cells. Increased virus titer first occurred 20 h after infection and reached a maximum 4 to 5 days postinfection (Fig. 4). The degree of virus-induced CPE and the number of cells containing intranuclear inclusions were essentially the same in the two cell systems. In GPHLV-infected GPE bottle cultures, minute plaques were seen under agar overlay 7 to 10 days after inoculation, thus providing a quantitative assay system for GPHLV.

Host range. Guinea pig cells appeared to be the most susceptible host system for the replication of both GPCMV and GPHLV. CPE and nuclear inclusions were induced in primary

Fig. 2. Intranuclear inclusions induced by GPCMV and GPHLV in GPE and GPK cells, respectively. ×400. (A) Uninfected GPE culture; (B) GPE cells infected with GPCMV, 7 days postinfection; note numerous inclusion-bearing cells; (C) GPE cells infected with GPHLV, 4 days postinfection; note many inclusion-bearing cells; (D) uninfected GPK culture; (E) GPK cells infected with GPCMV, 7 days postinfection; note only one inclusion-bearing cell; (F) GPK cells infected with GPHLV, 4 days postinfection; note many inclusion-bearing cells.
FIG. 3. Electron micrographs of GPE cells infected with GPCMV or GPHLV. (A) GPE cell infected with GPCMV showing tubular structures (double arrows); viral capsids with or without dense cores throughout the nuclear inclusions (single arrows). ×13,000. Insert showing tubular structure at higher magnification (×40,000). (B) GPE cell infected with GPHLV showing enveloped virus particles enclosed in nuclear vacuoles; viral capsids with or without dense cores throughout the nuclear inclusion. ×13,000. Insert showing nuclear enveloped virus particles at higher magnification (×40,000).
rabbit kidney cells by GPHLV but not by GPCMV, although the latter was found to persist in rabbit cells for 2 to 3 weeks. Neither virus produced CPE or inclusions when inoculated into human cells (human embryonic kidney cells and human diploid lung-fibroblast cells, WI-38), mouse embryo fibroblast cells, or primary green monkey kidney cells. Infectious virus could not be recovered from the latter cell systems infected with either virus 14 to 21 days postinoculation.

**Antigenic relationship between GPCMV and GPHLV.** The absence of any antigenic relationship between GPCMV and GPHLV is illustrated in Table 1. Cross-neutralization tests in GPE and GPK cell cultures showed no inhibition of CPE induced by either virus when heterologous antisera were used, although significant serum titers were obtained when homologous virus antibody was used in the same test system. It was noted that GPCMV produced significant antibody titers in guinea pigs after either i.p. or s.c. inoculation; GPHLV produced only low levels of neutralizing antibody titers in its natural host. Rabbits showed better antibody response than guinea pigs to GPHLV inoculation but a somewhat lower antibody response than guinea pigs to GPCMV.

**Table 1.** Cross-neutralization tests of GPCMV and GPHLV using antisera produced in rabbits or guinea pigs

<table>
<thead>
<tr>
<th>Immunizing virus</th>
<th>Source of serum*</th>
<th>Method of immunization*</th>
<th>Serum titer (reciprocal dilution) against virus strain tested</th>
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<tr>
<td><strong>GPCMV</strong></td>
<td></td>
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<td></td>
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<tr>
<td>Rabbit (St. Louis) Reference serum</td>
<td>i.p. 4×</td>
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<tr>
<td>Rabbit no. 1 (W.H.) Guinea pig S-19</td>
<td>s.c. 1×</td>
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</tr>
<tr>
<td>Guinea pig F-41</td>
<td>i.p. 1×</td>
<td>320 &lt;5</td>
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<tr>
<td><strong>GPHLV</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabbit (W.H. pool) no. 691318</td>
<td>i.p. + i.v.</td>
<td>&lt;5 160</td>
<td></td>
</tr>
<tr>
<td>Rabbit (Swiss) Guinea pig 74-53</td>
<td>i.p. 6×</td>
<td>&lt;5 80</td>
<td></td>
</tr>
<tr>
<td>Guinea pig AD216</td>
<td>i.p. 1×</td>
<td>&lt;5 20</td>
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* St. Louis serum obtained through the courtesy of J. N. Middelkamp of St. Louis University (14). Rabbit serum no. 691318 was supplied by P. Bhattacharya (1).
* i.v., Intravenous; i.p., intraperitoneal; s.c., subcutaneous.

**Fig. 4.** Growth curves of GPCMV and GPHLV in GPE and GPK cell cultures. (Note: Number of inclusion-bearing cells in GPCMV infected GPK cells was exceedingly low.)
DISCUSSION

Detailed studies of cell cultures infected with GPCMV or GPHLV revealed many distinct differences in the biological properties of these two guinea pig herpesviruses. The affinity of GPCMV for embryo fibroblast cells, but not kidney epithelial cells, in vitro resembles that of human CMV in cell culture (6). In contrast, GPHLV induced CPE faster in kidney epithelial cells than in embryo fibroblast cells although virus yields in both cell systems were comparable. Furthermore, whereas kidney cell cultures were generally resistant to GPCMV infection, the virus could persist in this system for several weeks. Fibroblast cells were necessary for assaying GPCMV.

In the ultrastructural studies of GPCMV-infected GPE cells, Middelkamp et al. (14) reported the presence of tubular structures similar to those observed in the present study. Since these structures were not found in GPE or GPK cells infected with GPHLV, they are therefore thought to be associated specifically with GPCMV infection, but their relationship to the virus has not been determined. It was also noted that in the GPHLV-infected GPE or GPK cells, enveloped virus particles were commonly found in the nucleus. This observation may account for the fact that intracellular virus titers were higher than cell-free virus titers in GPHLV-infected GPK cultures (unpublished data).

In studies by Hartley et al. (7) and Smith (19), the host specificity of CMV isolated from humans, guinea pigs, and mice was shown. The narrow host range of GPCMV was confirmed in the present study. Although mouse CMV was found to be capable of replicating in rabbit kidney cells (10, 11), only limited growth, if any, was found in rabbit kidney cells infected with GPCMV. In contrast, GPHLV replicates well in rabbit cells but not in cells of other animal species. Thus, the host spectrum of GPCMV and GPHLV were similar with the exception of rabbit cells.

The complete lack of antigenic relationship between GPCMV and GPHLV was demonstrated in the present study using antisera obtained from various sources. Since the isolation of GPHLV in 1969 (8), there has been some question among many investigators regarding the characteristics distinguishing these two guinea pig herpesviruses (16). Data presented here, together with those described in the accompanying paper, indicate distinct differences between these two herpesviruses. Infection with GPCMV resembles human CMV infection in human cells whereas GPHLV infection, which is associated in vivo with leukocytes, more closely resembles Epstein-Barr herpesvirus infection of humans (21).

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LITERATURE CITED


