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Dual Infection with Endogenous Herpes- and C-Type Viruses in Cultured Cells Derived from Normal and Leukemic Guinea Pigs[1] (38404)

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Mixed infections with two or three types of virus in cultured cells have been studied by several investigators. Experimental infection of single cells with two different animal viruses have been reported (1–4). Furthermore, replication of murine and feline C-type viruses in a human lymphoblastoid cell line carrying EB herpesvirus (5) and budding of vesicular stomatitis virus in a reptile cell line carrying C-type virus (6) have been noted. However, a naturally occurring dual infection with both C-type virus and herpesvirus in the same cultured cells has not been extensively explored. The present paper describes such a dual infection with guinea pig C-type virus (GPCV) and guinea pig herpes-like virus (GPHLV) in cell cultures derived from normal guinea pigs. The successful activation of the two endogenous viruses in guinea pigs was dependent upon the conditions under which the cultured cells were maintained.

Materials and Methods. Source of guinea pigs. Inbred strain two guinea pigs were originally obtained from the Animal Care Division of the National Institutes of Health. Random-bred Hartley guinea pigs were purchased from CAMM Research Institute, Wayne, NJ. Hybrid guinea pigs were obtained through crosses between strain two and Hartley guinea pigs.

Primary cell cultures. Various tissues including spleen, kidney or liver were removed aseptically from normal healthy guinea pigs. The tissues were minced and dispersed by trypsin, 0.25%. Cell pellets were resuspended in the human kidney (HK) growth medium and the monolayer cultures were prepared by the method previously described (7). Primary cultures were maintained in Basal Medium, Eagle's (BME) supplemented with 5% fetal bovine serum. These cultures were kept for observation as long as the cells were in good condition with weekly medium changes.

Virus isolation and induction. Characteristic herpesvirus induced CPE or intranuclear inclusions were indicative of the presence of the HLV. Detection of HLV virus in the blood or spleen required co-cultivation with guinea pig kidney cell cultures (7). Final identification of the herpesvirus was made by neutralization test using type-specific antiserum against GPHLV. Induction of guinea pig C-type virus was achieved by maintaining the monolayer cultures derived from various tissues in BME medium which contained 5-bromo-2′-deoxyuridine (BrdU), 40 μg/ml, for 3–4 days (8). The cells in the monolayer cultures were fixed *in situ* with 2% glutaraldehyde in 0.05 *M* cacodylate buffer, pH 7.3 for electron microscopic examination.

Electron microscopy. After glutaraldehyde fixation, all samples were postfixed with 1.33% osmium tetroxide in *s*-collidine buffer at pH 7.4 for 1 hr. They were then dehydrated in increasing concentrations of ethanol and embedded in Epon 812. Thin sections were obtained with a LKB Ultrotome, stained with uranyl acetate and lead citrate, and examined with a Philips EM 300 electron microscope.

Results. Manifestation of herpes- and C-type viruses in guinea pig cell cultures with or without BrdU. The expression of herpes- or C-type virus in guinea pig cell cultures was dependent upon the conditions under which the cultures were maintained. Figure 1 illustrates an example of such a dual infection. This culture was prepared from the spleen of AD007, a 2 mo old hybrid guinea pig (see Table I). When the spleen

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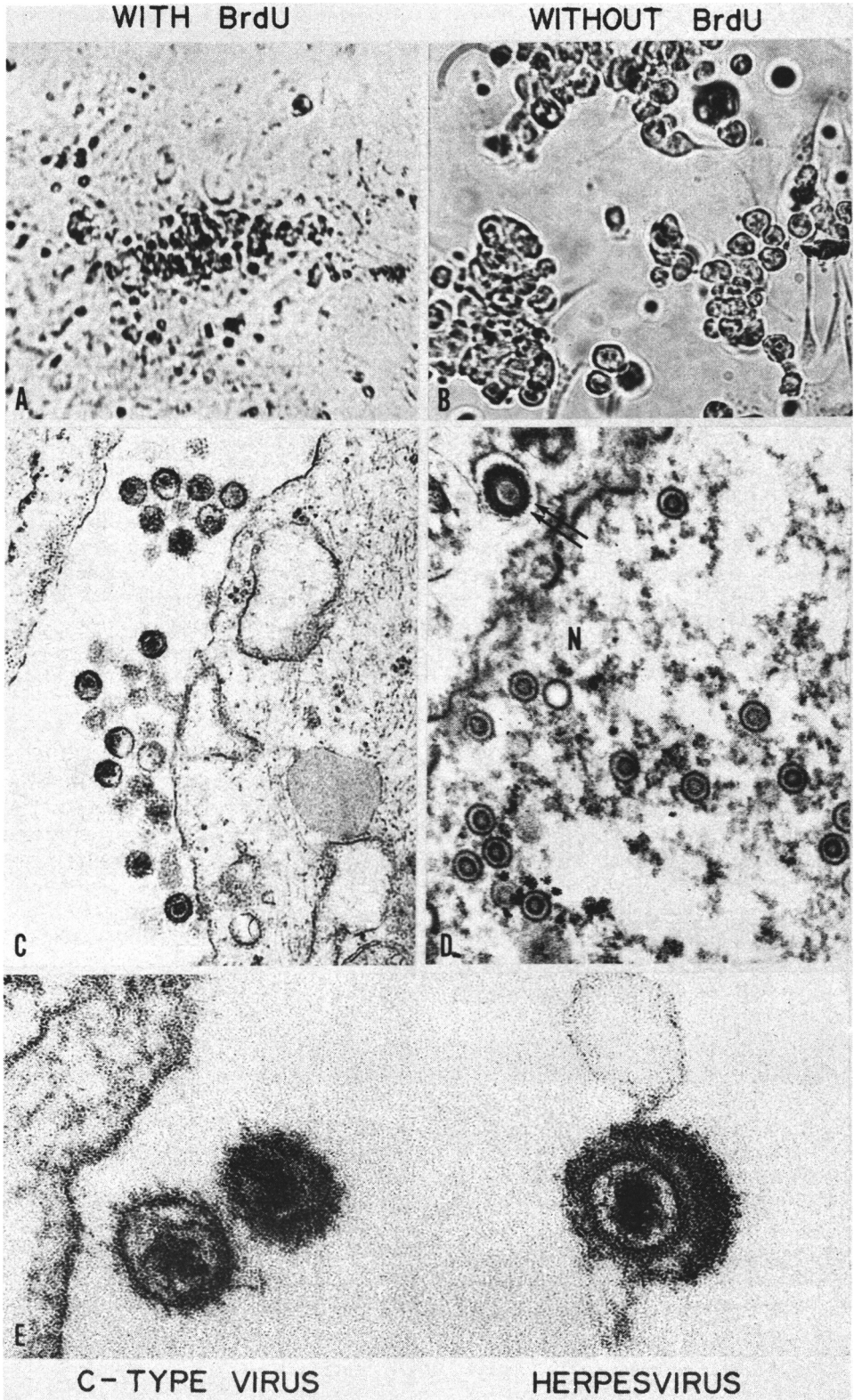
WITH BrdU
WITHOUT BrdU
A
B
C
N
D
E
C-TYPE VIRUS
HERPESVIRUS

monolayer was maintained in a medium containing BrdU, no evidence of virus induced CPE was observed although some granulation was noted in many of the cells (Fig. 1A). However, when the cells were fixed and examined under electron microscopy, numerous C-type virus particles with a diameter of 110 nm were observed (Fig. 1C). On the other hand, when a parallel spleen culture was maintained in a similar medium containing no BrdU, virus induced CPE was observed in the second passage of the culture, 40 days after the original culture was planted (Fig. 1B). Herpesvirus particles were observed when these infected cells were fixed and examined under the electron microscope (Fig. 1D). Numerous herpesvirus nucleocapsids were noted in the nucleus, and mature virus particles with a diameter of 150 nm were observed in the cytoplasm or extracellular space. When BrdU-containing medium was added to a culture that showed early herpesvirus CPE, single cells dually infected with both types of virus were found. In general, the herpesvirus was in the nucleus and the C-type virus in the extracellular space. An example taken from such a mixed infection showing both herpes- and C-type viruses is illustrated in Fig. 1E. In both instances the guinea pig C-type virus and the guinea pig herpesvirus

TABLE I. Prevalence of GPCV and GPHLV in Cultured Cells Derived from Normal Guinea Pigs.

Animal no.	Strain origin[a]	Age (at time of sacrifice)	Virus activation[b] from spleen, liver and/or kidney cells: Cultures without BrdU (GPHLV)	Cultures with BrdU (GPCV)
St. 2AA	Strain 2	26 mo	+ (3.8)	+
St. 2A	Strain 2	20 mo	+	+
St. 2B	Strain 2	12 mo	+	+
C7D	Hartley	12 mo	−	+
HAR74-1	Hartley	4 mo	−	+
ADH 102	Hartley	8 mo	+ (3.5)	+
AD 065	Hybrid F_m	1 day	−	+++
AD 007	Hybrid F_2	2 mo	+[c]	++
AD 055	Hybrid F_m	2 mo	−	++
AD 050	Hybrid F_m	5 mo	+ (1.0)	++
AD 023	Hybrid F_1	6 mo	+ (2.5)	+
AD 002	Hybrid F_2	10 mo	+ (3.3)	+
AD 023	Hybrid F_m	7 mo	+ (< 1.0)	+
AD 023 Embryos	Hybrid F_m	(30 day gestation)	−	+++
AD 133	Hybrid F_m Leukemic[d]	2 mo	+	+++
LA 355	Hybrid F_1 Leukemic	13 mo	+	+++

[a] Strain 2 and Hartleys were used as parents; F_m = back crossed with strain 2 or Hartley strain.

[b] GPHLV by virus induced CPE and intranuclear inclusion; numbers in parenthesis represent virus titer in spleen log $TCID_{50}$/0.1 ml packed cells; +, virus present; −, no virus isolated.

GPCV was identified by electron microscopy; +, a few virus particles; ++, moderated number of virus particles; +++, large aggregates of virus particles.

[c] Isolation of GPHLV from AD007 was obtained in the second passage of the spleen cell culture.

[d] See addendum.

FIG. 1. (A) and (B) Spleen cell monolayer cultures derived from a normal hybrid guinea pig, AD 007. Wet preparations, × 100. (A) Culture maintained in a medium with BrdU for 4 days; some cells showed granulation. (B) Culture maintained in a medium without BrdU; virus-induced CPE appeared in the second cell passage, 40 days after the original culture was planted. (C), (D) and (E) Electron micrographs; C and D, × 38,000; E × 168,000. (C) Two spleen cells shown in A; many extracellular C-type virus particles. (D) A spleen cell shown in B; many herpesvirus nucleocapsids in the nucleus (N), and a virion with outer envelope in the cytoplasm (arrows). (E) A sample taken from a hybrid guinea pig kidney cell culture treated with BrdU after early herpesvirus CPE appeared; both herpes- and C-type virus particles observed.

would have gone unrecognized had not the medium contained BrdU or the incubation time of the monolayer cultures not been lengthened.

Prevalence of herpes and/or C-type virus infections in guinea pigs. Table I lists examples of GPCV and GPHLV infection in cultured cells derived from tissues of normal healthy guinea pigs. GPCV was obtained from the cultured cells prepared from all the animals examined, regardless of age and strain of guinea pig, as long as the cultures were maintained in a medium containing BrdU. The number of cells carrying GPCV was greater in cultures obtained from embryos and young guinea pigs than in adults. Furthermore, large aggregates of C-type virus were found in cultures derived from embryos.

GPHLV was consistently isolated from inbred strain two and hybrid guinea pigs, especially in animals 5 mo or older (Table I). Herpesvirus titers in spleen were high, with a maximum yield of 3.5–4.0 log $TCID_{50}$/0.1 ml of packed cells. Hartley guinea pigs, on the other hand, showed a low incidence of infection although occasionally HLV infection was noted (ADH 102 in Table I). During the past 5 years, a total of 450 Hartley guinea pigs ranging in age from 2 to 6 mo, were examined; only 4% showed HLV infection. Although transplacental transmission of GPHLV was demonstrated in experimentally infected guinea pigs receiving large dosage of virus (9), virus was not isolated from embryos when the naturally infected animals showed low titer of infectious virus as in the case of AD 023 in Table I.

Age distribution of guinea pigs naturally infected with herpesvirus. As shown in Table I, hybrid guinea pigs that reached the age of 5 mo showed herpesvirus infection. A group of ten randomly selected hybrid guinea pigs were bled monthly and serial blood samples from each animal were tested for the presence of HLV. As shown in Table II, none of the ten guinea pigs showed HLV infection when tested at 2–4 mo. HLV started to appear in their blood when they reached 5–6 mo and almost all of the guinea pigs were infected with this virus when they reached 8 mo of age. Only one guinea pig, No. 185, showed no virus in its blood when tested at 9 mo.

Discussion. That the appearance of herpesvirus in guinea pigs is dependent upon the age of the animals confirms our earlier report (9) which indicated a higher incidence of HLV infection in guinea pigs older than 2 mo. Similarly, Cole and Kuttner (10) reported that 84% of full grown guinea pigs showed intranuclear inclusions in their salivary glands whereas only 3 of 43 guinea pigs aged less than 1 mo showed similar infection. Apparently infectious virus titers rise in guinea pigs as their age increases, although horizontal transmission of herpesvirus infection in older guinea pigs cannot be completely eliminated.

Prior to our studies (8), C-type virus was observed only in tissues and leukocytes of leukemic guinea pigs, but not in healthy animals (11–13). Data presented in the present study demonstrate that C-type virus can be induced in cell cultures derived from normal healthy guinea

TABLE II. Age at Which Hybrid Guinea Pigs First Showed Herpesvirus Infection.

G. P. no.	Strain[a] origin	Age of guinea pig at time blood sample tested[b] (month) 2	4	5	6	7	8	9	First showed virus infection (age/month)
185	F_m	–	–	–	–	–	–	–[c]	
191	F_m	–	–	–	– (s)				
194	F_m	–	–	–	–	– (s)			
195	F_1	–	–	–	+				6
189	F_1	–	ND	+					5
190	F_1	–	–	–	–	–	+		8
196	F_1	–	–	–	–	+			7
197	F_m	ND	ND	–	+				6
198	F_m	ND	–	–	–	–	+		8
203	F_1	ND	–	–	–	–	+		8

[a] F_m = hybrid back cross with strain 2 or Hartley strain.
[b] –, no virus isolated; +, GPHLV isolated; (s), sacrificed.
[c] See addendum.

pigs, regardless of age or strain of the animals. Furthermore, more abundant virus particles were observed in cultures derived from embryos than from adult tissues. In a separate study, C-type virus particles were present in gonadal and placental tissues of guinea pigs at various stages of gestation; this would account for the large number of virus particles in the cultured cells derived from them. These findings would support the theory of Huebner and Todaro that C-type viruses probably are present in cells of all mammalian species (14, 15). However, the association of either C-type virus or herpesvirus with neoplastic disease in guinea pigs is unclear.

Guinea pigs have been used exclusively for biomedical research, especially for immunological studies. The presence of both C-type virus and herpesvirus in guinea pigs would have gone unrecognized had not the culture medium contained BrdU or the cultivation or co-cultivation of tissue cells been performed. Recognition of these two endogenous viruses in normal guinea pigs is of particular significance since thousands of guinea pigs are used yearly for various research purposes. Investigators should be aware that their results may have been obtained from guinea pigs or guinea pig cell cultures which were endogenously infected with herpes- and C-type viruses.

Summary. Dual infection with endogenous herpes- and C-type viruses in cultured cells derived from normal guinea pigs as well as in single guinea pig cells, was observed. The guinea pig C-type virus was activated by maintenance of the cultured cells in BrdU containing medium and was detected in all guinea pigs tested, regardless of age, strain or source of animals. Manifestation of the herpesvirus required prolonged cultivation of the cells or co-cultivation with susceptible monolayer cultures. The herpesvirus isolation was age and strain dependent; it occurred most frequently in older inbred animals.

Note added in proof: Guinea pig No. AD185 showed GPHLV infection when tested at 11 mo. Furthermore, both GPHLV and C-type virus were observed in cultured spleen and/or kidney cells derived from leukemic guinea pigs.

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