

In Vitro Transformation of Hamster Embryo Cells by a Guinea Pig Herpes-Like Virus¹ (37723)

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Several investigators have demonstrated the transforming capability of Epstein-Barr (EB) herpesvirus in cultured leukocytes (1-5). Duff and Rapp (6) reported that uv-inactivated herpes simplex virus type 2 induced transformation of hamster embryo cells *in vitro*.

In the study of the viral etiology of guinea pig leukemia, a herpes-like virus was consistently isolated from leukemia-susceptible strain 2 guinea pigs (7). To determine if a relationship existed between the guinea pig herpes-like virus (GPHLV) and guinea pig leukemia, the oncogenic potential of the GPHLV was investigated. We previously reported (8) that the GPHLV was able to persist in leukocytes of guinea pigs *in vitro* and induce cellular transformation characterized by cell clumping similar to EB virus-transformed human leukocytes in cultures. The present paper describes some of our recent findings on the transformation of cultured hamster embryo cells by GPHLV.

Materials and Methods. *Virus.* Guinea pig herpes-like virus strain LK31, LK40, and LK51, originally isolated from strain 2 guinea pigs with leukemia, and strain H-62, H-114, and H-125, isolated from nonleukemic Hartley guinea pigs, were used. Stock viruses were prepared in kidney cell cultures of normal Hartley guinea pigs. Ten percent dimethyl sulfoxide (DMSO) was added to some of

the stock virus suspensions for stabilization of the virus, but virus stocks containing no DMSO were also used. All viruses were stored at -70° .

Cell cultures and media. (a) Hamster embryo (HE) cells: Monolayer cell cultures of HE cells were prepared from 12-13-day-old whole embryos of Syrian golden hamsters and were grown in Eagle's basal medium supplemented with 10% fetal bovine serum. (b) Guinea pig kidney cells: Primary guinea pig kidney cell cultures were prepared as described previously (7). Cells grown in roller tubes were used for virus assay, and cells grown in 16-oz. prescription bottles were used for stock virus preparation.

Transformation experiment. Monolayer hamster embryo cell cultures grown in 8-oz prescription bottles were infected with GPHLV at an input multiplicity ranging from 20 to 100. In certain experiments, uv-inactivated virus preparations were also used. Virus was allowed to adsorb onto the cells at 35° for 2-4 hr. Each infected culture was then subcultured and divided into 4-6 new cultures in Falcon flasks (75 cm²). The infected cells were grown in an Eagle's enriched medium (EEM) containing 10% tryptose phosphate broth, double the amount of Eagle's minimum essential amino acids and vitamins in Hanks' solution, and supplemented with 10% fetal bovine serum. In some experiments, the infected cells showed cellular degeneration and detached from the glass surface during adsorption, apparently as a result of the toxic effect of DMSO. In these instances, subcultures were not made, but fresh EEM was added to the inoculated

¹ This work was supported in part by Research Grant HD05400 from the Institute of Child Health and Human Development, AI-08648 from the Institute of Allergy and Infectious Diseases, National Institutes of Health, and by Veterans Administration Research funds.

cultures immediately after adsorption, followed by subculture 1–2 days later. Uninfected hamster embryo cell cultures were set up as controls in parallel to the virus-infected cultures. Both uninfected and infected hamster cells were subcultured in a similar manner. All cultures were incubated at 35° with medium changes weekly.

Virus assay. To determine virus infectivity titers, infected cultures were frozen and thawed three times to release intranuclear virus. Virus infectivity was assayed in primary guinea pig kidney cell cultures grown in roller tubes using four tubes per dilution. The infectivity titers were expressed as TCID₅₀ per 0.1 milliliter.

Immunofluorescent staining. Cells grown on 11 × 22 mm glass coverslips were washed with phosphate-buffered saline (PBS), fixed with cold acetone for 10 min, and air dried. Anti-guinea pig herpes-like virus serum prepared in a rabbit and conjugated with fluorescein isothiocyanate was added to the acetone-fixed cells at 1:5 dilution and incubated at 35° in a moist petri dish. After 30 min incubation, coverslips were washed with PBS, mounted with buffered glycerin, and examined under a Leitz microscope with a uv light source.

Light and electron microscopy. Cells grown on coverslips were fixed with Zenker acetic acid and stained with hematoxylin and eosin. For electron microscopy, fixation and embedding were the same as previously reported (9). Ultrathin sections were examined under a Philips EM 300 electron microscope.

Results. In vitro cell transformation. Hamster embryo cells infected with GPHLV and subcultured as described above showed complete cell sheets in about 7–10 days (Fig. 1A). No cytopathic effect was observed in these newly growing cells. Three weeks after infection, 1–3 morphologically transformed foci per flask were observed. The number of foci obtained varied from experiment to experiment, and only about 10–50% of the infected cultures developed transformed foci. Although the presence of 10% DMSO in the stock virus used for inoculation produced early cytopathic effect, no noticeable effect on cell transformation was observed. The transformed cells were

characterized by loss of contact inhibition, resulting in multilayer cell growth. Transformed foci were not seen in any of the uninfected HE control cultures. Figure 1B illustrates a transformed focus of an unstained preparation. The foci were treated with trypsin to permit removal of the transformed cells with a capillary pipette and subcultured. The resulting cell lines were maintained in serial passage. The uninfected hamster embryo cells were composed mostly of fibroblasts (Fig. 1C), while the transformed cultures were dominated by epithelial-like cells containing many nucleoli and dense chromatin granules (Fig. 1D, E). Fibroblast-like cells and cells with enlarged nuclei were also observed in the transformed cell colonies (Fig. 1E).

Transforming capacity of GPHLV isolated from different sources. Since GPHLV has been isolated from both leukemic and non-leukemic guinea pigs, attempts have been made to compare the transforming capacity of virus strains from the two different sources. The results are summarized in Table I. It was noted that with virus strains isolated from leukemic guinea pigs, 13 of 18 trials (72%) induced noticeable cell transformation. Both infectious and uv-inactivated virus preparations showed similar effects. When the virus strains isolated from nonleukemic guinea pigs were used, 5 of 11 trials (45%) showed cell transformation. In the uninfected HE control experiments, spontaneous transformation was not observed.

Detection of viral-specific antigen and virus particles in GPHLV-transformed cells. To detect the presence of GPHLV-specific antigen in the transformed hamster cells, the direct immunofluorescent technique was used. Transformed cells were stained with fluorescein-conjugated anti-GPHLV serum which was prepared in a rabbit. Intracytoplasmic fluorescence was observed in approximately 7–20% of the transformed cells in the six cell lines tested. An example is illustrated in Fig. 1F. No viral antigen was present in the uninfected parallel control hamster embryo cells treated with the same conjugated antiserum.

Four of the cell lines at passages 3, 14, and

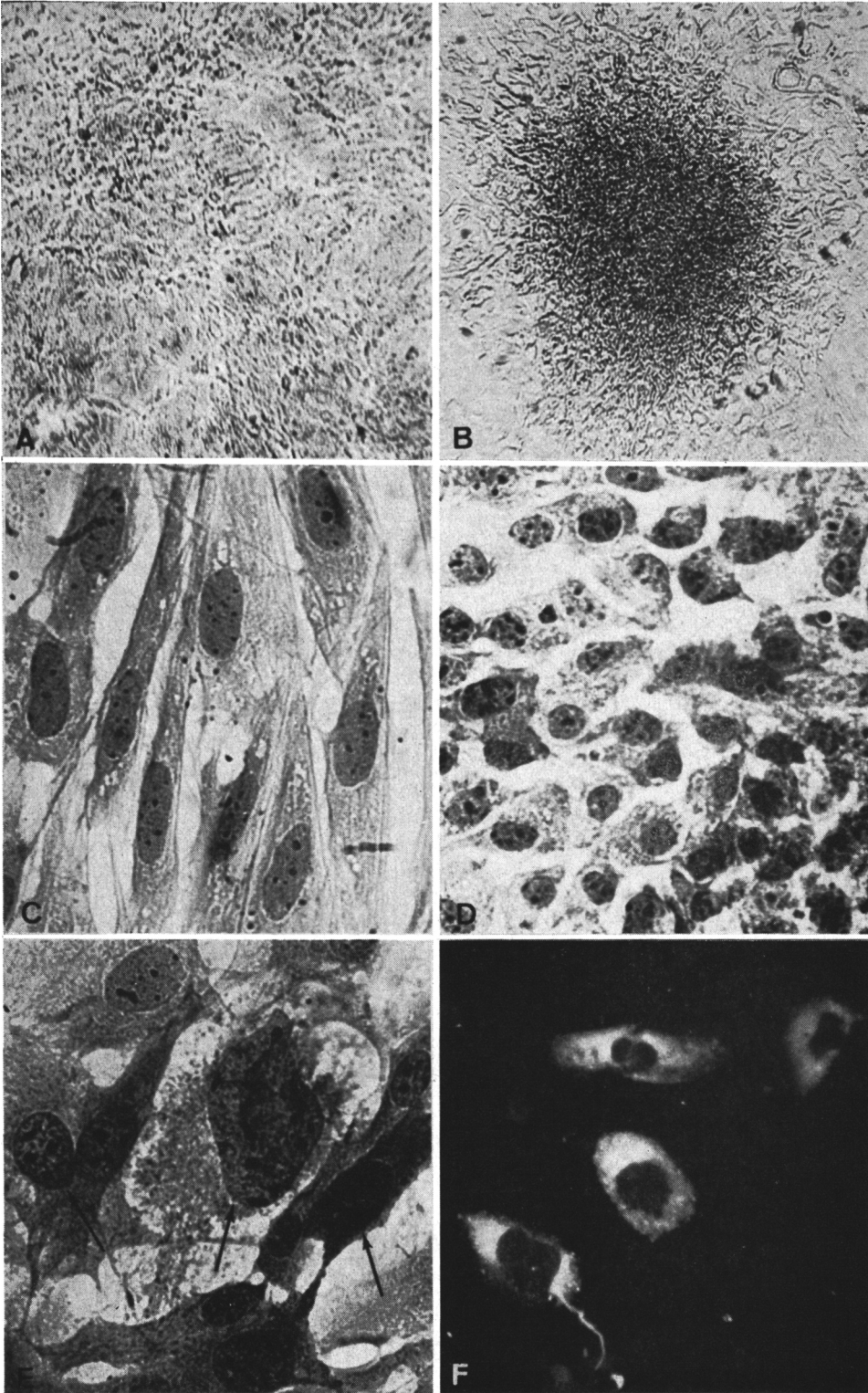


TABLE I. *In Vitro* Transformation of Hamster Embryo Cells by GPHLV Isolated from Leukemic and "Normal" Guinea Pigs.

Virus strain ^a	Virus-infected cultures		Control uninfected cultures	
	No. of experiments performed	No. of experiments showing cellular transformation	No. of experiments performed	No. of experiments showing cellular transformation
LK31	4	3	3	0
LK40	2	2	1	0
LK51	10	7	6	0
LK51 (uv-inactivated)	2	1	ND ^b	ND
Total	18	13	10	0
H-62	4	2	3	0
H-114	4	2	4	0
H-125	3	1	2	0
Total	11	5	9	0

^a LK = leukemic guinea pigs (strain 2); H = "normal" guinea pigs (Hartley strain).

^b ND = not done.

15 were fixed, embedded, and examined under the electron microscope for the presence of virus particles. Neither herpesvirus nor C-type virus particles were found in these cells. Two of the cell lines, LK51-11B-2 and LK51-19-6, were treated with 5-bromo-2'-deoxyuridine, a method previously used for the induction of guinea pig C-type virus (10), but C-type virus was not found in the samples examined.

Recovery of infectious virus from GPHLV-transformed cells. To determine whether or not infectious virus could be recovered from the transformed hamster cells by co-cultivation, several cell lines at various passage levels were co-cultivated with primary guinea

pig kidney cells. As shown in Table II, GPHLV was recovered in 7 of 8 cell lines tested. However, the number of cells yielding infectious virus was exceedingly low. Of the three cell lines tested, 12-47/100,000 cells yielded virus (Table II). Viruses recovered from these cell lines were neutralized by the antiserum produced in a rabbit immunized with the prototype GPHLV.

Discussion. The results obtained in this study demonstrated that GPHLV could induce cell transformation *in vitro*. Our studies are similar to those reported by Duff and Rapp with the uv-inactivated herpes simplex virus type 2 (HSV-2) (6). Unlike HSV-2, GPHLV-induced cell transformation was ob-

FIG. 1A. A monolayer of hamster embryo cells 10 days after GPHLV infection; unstained preparation. $\times 50$.

B. A transformed focus of hamster embryo cells 3 weeks after GPHLV infection; unstained preparation. $\times 50$.

C. Uninfected hamster embryo cells stained with hematoxylin and eosin, $\times 430$.

D. A portion of a GPHLV-transformed focus of hamster cells stained with hematoxylin and eosin, $\times 430$.

E. GPHLV-transformed hamster cells showing an enlarged nucleus and nuclei containing many nucleoli and dense chromatin granules (arrows), $\times 430$.

F. GPHLV-transformed hamster cells stained with fluorescein-conjugated rabbit anti-GPHLV serum showing intracytoplasmic fluorescence, $\times 180$.

TABLE II. Recovery of Infectious Virus from GPHLV-Transformed Hamster Cells by Co-cultivation with Guinea Pig Kidney Cell Cultures.

Transformed cell line	Passage level	Virus recovery by co-cultivation	No. cells yielding virus per 10 ⁶ cells
H-114-20-1 ^a	P5	—	ND ^b
LK31-12-1 ^a	P9	+	ND
LK31-23-3	P7	+	ND
LK31-41-3	P5	+	20
LK40-8B-4	P6	+	ND
LK51-11A-1	P9	+	ND
LK51-11B-2	P11, 21	+	44, 21
LK51-19-6	P14, 21	+	47, 12

^a Designation of the cell lines according to the virus strains originally used for transformation; for details of virus strains, see Table I.

^b ND = not done.

tained by using infectious virus as well as uv-inactivated preparations. There was no evidence of spontaneous cellular transformation in the uninfected hamster embryo cell controls under similar experimental conditions.

Since GPHLV antigen was demonstrated in the transformed hamster cells and infectious virus was recovered by co-cultivation with guinea pig kidney cells, it is apparent that the GPHLV genome must persist in the transformed cells. How the viral genome persists in the transformed hamster cells has not been determined as yet.

It is noteworthy that virus strains isolated from leukemic guinea pigs appear to have a somewhat higher percentage of transforming capacity than those virus strains isolated from nonleukemic guinea pigs. These differences may be due to the particular virus strains. Since C-type virus has been observed in leukemic guinea pigs (10-13), it is possible that GPHLV isolated from leukemic guinea pigs may be contaminated with the C-type virus. However, C-type virus particles were not observed by electron microscopy in the two GPHLV-transformed cell lines examined, even after treatment with 5-bromo-2'-deoxyuridine for the induction of endo-

genous C-type virus. Therefore, the ability of GPHLV to transform hamster cells is most likely due to the herpesvirus infection. Whether the GPHLV-transformed hamster cells are capable of inducing tumor formation in hamsters is currently under investigation.

Summary. Hamster embryo cells infected with GPHLV showed morphological cellular transformation. Virus strains isolated from leukemic guinea pigs showed somewhat higher transforming capacity than the isolates from "normal" Hartley guinea pigs. Virus specific antigen was detected in the cytoplasm of 7-20% of the transformed hamster cells. Infectious virus could be recovered from the transformed cells by co-cultivation with guinea pig kidney cells. Quantitative analysis showed that in these cell lines 12-47/100,000 transformed hamster cells yielded infectious virus.

The authors are grateful to Dr. R. H. Green for his valuable suggestions during the preparation of this manuscript. The excellent technical assistance of JoEllyn Bradley and Judy Wnek is appreciated.

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Received July 10, 1973. P.S.E.B.M., 1973, Vol. 144.