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Persistence and Expression of Herpes Virus in Guinea Pig B and T Spleen Cells (40647)

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A herpes-like virus (GPHLV) was originally isolated from kidney cell cultures derived from leukemic strain 2 guinea pigs (1). Subsequently, the virus was also isolated from leukocytes and other tissues of nonleukemic strain 2, strain 13, and occasionally Hartley guinea pigs without clinical disease (2). Although virus particles or inclusions could not be found in tissues obtained from either naturally or experimentally infected guinea pigs, the virus could be isolated following cultivation or cocultivation of the infected cells. Earlier reports have shown that the virus is widely distributed in leukocytes and various tissues; however, the highest virus titers were always demonstrated in the spleen (2–5). The detection of GPHLV particles in guinea pig leukocytes only after *in vitro* cultivation resembles the reported observations of Epstein–Barr virus (EBV) particles in cultured human leukocytes or tumor cells (6). The main target for EBV has been defined as the human B lymphocytes (7) and it has been shown that normal human lymphocytes, which have a limited life span *in vitro*, will persist in culture if the EBV genome is present in the cells (8, 9). The ability of GPHLV to convert normal lymphocytes into permanently growing cell lines has not been demonstrated, although a limited transformation of circulating guinea pig leukocytes has been described after *in vitro* infection with GPHLV (10). The specific cell type in which GPHLV persists has not been determined; GPHLV particles have been seen in bone marrow macrophages after *in vitro* cultivation, but it was not certain whether they were part of the cell genome or only the result of the macrophage ingesting the virus secondarily (11). Identification of the specific cell type harboring the GPHLV genome is of particular interest since guinea pig leukemia has been classified as a B-cell proliferation (12).

Materials and methods. Virus strain. The LK40 strain of GPHLV was originally isolated from a leukemic guinea pig and was used throughout this study. The virus was serially passaged in guinea pig embryo cell monolayer cultures. Virus infectivity titers of the stock virus varied from 10^6 to 10^7 $TCID_{50}$/ml.

Tissue culture for virus assay. Guinea pig embryo fibroblast (GPE) monolayer cell cultures were prepared from 30- to 40-day-old fetuses of normal Hartley guinea pigs. They were grown in Eagle's basal medium with 10% fetal bovine serum as described previously (13). GPHLV infectivity titers were measured by inoculating serial 10-fold dilutions of infected tissue culture suspensions into GPE monolayer cultures. Virus infectivity titers in separated spleen cells were evaluated by cocultivation of serial 10-fold dilutions of 10^6 cells with GPE monolayer cell cultures.

Animal inoculation. Virus-free Hartley guinea pigs were inoculated intraperitoneally with 5 ml of 10^6 to 10^7 $TCID_{50}$ per milliliter of GPHLV. Animals were sacrificed at different intervals after inoculation and the spleens were removed, minced, and separated into the different subpopulations as described below.

Spleen cell separation. Spleens were removed aseptically from uninfected or infected guinea pigs. The spleens were minced and the cell suspension obtained was washed, resuspended in RPMI 1640 medium with 20% fetal bovine serum, and separated into cell fractions according to the following procedure. The red blood cells were lysed with a solution of ammonium chloride (0.83%). The spleen cells were allowed to adhere to a glass surface for 1 hr at 37° and the glass-adherent

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cell fraction was removed by scraping the glass surface. The cell population which did not adhere to the glass was then separated into fractions enriched in B or T cells on nylon wool columns, as described by Julius *et al.* with some modifications (14). Before application of cells, the columns were washed with Hanks' balanced salt solution (HBSS), equilibrated with RPMI 1640 medium for 1 hr at 37°, and then rinsed with fresh RPMI 1640 medium. About 3 ml of cell suspension containing 10^7 to 10^8 cells/ml were applied to a column. The cells that passed directly through were reconcentrated by centrifugation and approximately 10^7 cells were applied to a second column which was then incubated at 37° for 1 hr. The enriched B-cell fraction adherent to the first column was obtained by vigorous elution of the cells from the nylon wool. The cells that did not adhere to the second column were considered the T-cell-enriched fraction. All enriched cell fractions were pelleted, resuspended in fresh RPMI 1640 medium with 20% fetal calf serum, and the number of viable cells was determined by trypan blue exclusion.

Cell characterization. The T cells were enumerated by rosetting with papain-treated rabbit erythrocytes as described previously (15, 16). B cells were characterized by a direct surface fluorescence assay using rabbit anti-guinea pig immunoglobulin (17). Macrophages were identified by their capacity to adhere to glass and to phagocytize latex particles.

Cultivation of lymphocytes. Aliquots of B- and T-enriched fractions containing 3 to 5 × 10^6 cells/ml either obtained from uninfected or infected animals were kept in culture in RPMI 1640 medium with 20% fetal bovine serum. The cultures were supplemented weekly with fresh media. Lymphocytes infected *in vitro* with virus were kept in the same manner. Viable cells were enumerated daily and identified at selected time intervals for up to 4 weeks; virus infectivity titers were determined in parallel.

Results. Localization of GPHLV in separated spleen cells derived from acutely and latently infected guinea pigs. Enriched fractions obtained from acutely or latently infected guinea pigs were cocultivated with GPE fibroblasts on the day of sacrifice, and GPHLV virus infectivity titers per 10^6 cells were determined. The results obtained during acute GPHLV infection, 1 to 18 days, and latent infection, 25 to 60 days after virus inoculation, are shown in Table I. GPHLV was detected in the macrophage and B-cell fractions as early as 1 day after inoculation whereas the virus was not detected in the T-cell fraction during the first 4 days postinoculation. GPHLV was only detected in the T fraction in animals sacrificed 5 days postinoculation or thereafter. Virus infectivity titers showed an increase of approximately one log in all three cell fractions from day 1 to 18. GPHLV infectivity titers of spleen cell populations obtained from latently infected guinea pigs indicated that GPHLV was isolated from all three fractions. However, the highest virus titers were consistently measured in the B-cell fraction, and the lowest titers were always evident among the T cells.

Expression of GPHLV after in vitro cultivation of B- and T-cell fractions derived from infected guinea pigs. In order to assess GPHLV expression, B and T cells obtained from latently infected guinea pigs were cultured *in vitro* for 20 days and virus infectivity titers and cell count were determined daily. The results are shown in Fig. 1. On Day 0, cell counts were high but virus titers were low since the virus was not as yet expressed. Cell counts in each population decreased slowly thereafter but virus titers increased gradually due to expression of GPHLV in both B- and T-cell fractions. On the basis of four experiments, maximum virus expression was noted in the T-cell fraction after 8 days in culture, whereas 13 days were necessary to obtain

TABLE I. LOCALIZATION OF GPHLV IN SEPARATED SPLEEN CELLS OBTAINED FROM GUINEA PIGS DURING ACUTE AND LATENT INFECTION[a]

Days postinoculation	No. Expts	Average $\log_{10}TCID_{50}/10^6$ cells			
		Whole spleen	[M]	[B]	[T]
1–2	5	0.28	0.23	0.24	0[b]
3–4	3	0.54	0.88	0.31	0
5–8	5	0.97	1.19	1.11	0.36
11–18	4	1.71	1.58	1.62	0.78
25–60	10	1.88	2.21	2.56	1.47

[a] Each animal was inoculated with 10^7 $TCID_{50}$ intraperitoneally. [ ], enriched population; M, macrophages; B, B lymphocytes; T, T lymphocytes.

[b] Virus was not detected.

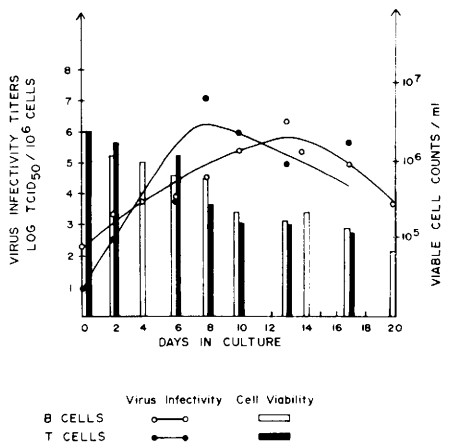

FIG. 1. Expression of GPHLV after *in vitro* cultivation of spleen cell fractions derived from latently infected guinea pigs; average of four experiments. Each animal was inoculated with 10^7 $TCID_{50}$ and sacrificed 25 to 60 days postinoculation.

maximum virus expression for the B cells.

In vitro cultivation of B and T spleen cells with or without GPHLV. In order to determine if spleen cells could support the replication of GPHLV *in vitro*, spleens were obtained from noninfected guinea pigs and were separated into subpopulations. Cell identification in each of the subpopulations on the day of sacrifice showed that about 90% of the cells in the T fraction formed rosettes, approximately 85% of the cells in the B population presented surface fluorescence, and about 90% of the cells in the macrophage fraction phagocytized latex particles. B- and T-cell-enriched fractions were then infected with GPHLV (MOI of 1) for 1 hr at 37°. Unadsorbed virus was removed by three successive washings with HBSS. The infected cells were resuspended in RPMI medium with 20% fetal bovine serum at a concentration of 3 to 5 × 10^6 cells per ml and incubated at 37°. Cell counts and virus infectivity titers were determined daily for 18 days (Fig. 2). GPHLV infectivity titers decreased rapidly in the absence of cells. Although B- and T-cell fractions infected *in vitro* with GPHLV showed no significant increase in virus titer during the 18 days of cultivation, virus infectivity titers decreased at a much slower rate than those without cells. It was also noted that the number of viable cells decreased in both B- and T-cell fractions, although the survival rate of the noninfected cells was better than that of infected cells (Fig. 2). In addition, a diminution in the number of rosette-forming cells in the T fraction and in the number of fluorescent cells in the B fraction was observed after 6 to 12 days in culture. Neither virus titers nor cell viability was increased when similar experiments were conducted with spleen cells that had been kept in culture for 2 or 6 days before virus inoculation.

Discussion. The role of bone marrow-adherent cells in the latency of GPHLV was explored in a previous study by our laboratory (11). Data indicated that GPHLV was present *in vivo* in the macrophage-enriched bone marrow fractions of latently infected guinea pigs. The present study showed that the different spleen subpopulations obtained from latently infected animals also harbored the virus. GPHLV was not only isolated from the macrophage-enriched fraction but also from the B- and T-cell-enriched spleen pop-

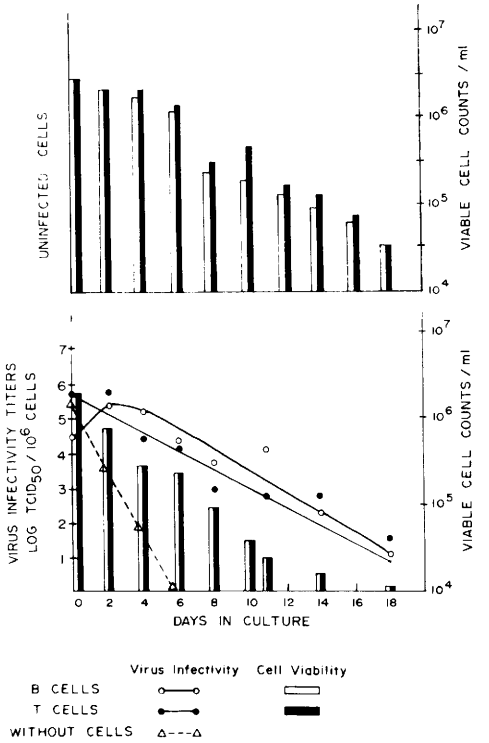

FIG. 2. Cultivation of B and T cells *in vitro* with or without GPHLV infection (average of four experiments). Virus inoculum on Day 0 was 10^6 $TCID_{50}/10^6$ cells.

ulations; however, the highest GPHLV infectivity titers were consistently measured in the B-cell fraction. Results obtained in acutely infected animals demonstrated that GPHLV could be detected in B cells as early as 1 day after inoculation whereas isolation of virus from the T-cell subpopulation was not achieved until 5 days or more after virus inoculation. Furthermore, the lowest infectivity titers were always observed in the T fraction during latent infection. Since each enriched fraction contained an average of 10% of other cells, it is possible that the presence of a contaminating cell harboring GPHLV accounts in part for the presence of the infectious virus detected in each cell fraction. However, despite the technical restrictions imposed as a result of contamination by other cell types, it appeared that in latently infected guinea pigs, GPHLV localized in the B cells preferentially. The presence of GPHLV in T cells could not be ruled out since this fraction had only rarely been found free of virus. Further, whether the virus observed in the spleen macrophages was an integral part of the cell genome or whether it occurred as a result of phagocytosis of an infected cell remains to be determined.

GPHLV expression in organ cultures of thymus and spleen tissues and in adherent bone marrow cells derived from infected guinea pigs has been described (4, 11). The present study showed that GPHLV was also expressed in cultured B and T lymphocytes derived from infected guinea pigs. It appeared that B cells, T cells, or an additional cell fraction might be responsible individually or in association for viral expression *in vitro*. Indeed, the enriched B- and T-cell populations that were infected with GPHLV contained approximately 10% of contaminating cells. In addition, the number of rosette-forming cells in the T fraction and in the number of fluorescent cells in the B-cell fraction decreased after several days in culture. It was not certain whether this was due to the loss of the specific cell receptors or to the preferential growth of non-T, non-B cells during cultivation.

In contrast with what has been observed with bone marrow macrophages (11) neither B nor T spleen cells were able to support GPHLV replication when infected *in vitro*. Serial counts of viable cells showed that the number of B and T cells infected *in vitro* declined at a faster rate than both uninfected controls and cells infected *in vivo*. This was probably due to the lytic effect of GPHLV on guinea pig leukocytes. It is possible that the use of lower MOI and/or mitogen-activated lymphocytes might be more satisfactory for the *in vitro* infection of lymphocytes with GPHLV.

Natural infection of leukocytes has been described for many herpes viruses in both humans and animals. In addition, a number of herpes viruses have been shown to be capable of infecting and replicating in lymphocytes (6, 18–21). Several reports have indicated that some herpes viruses are closely associated with T lymphocytes (20, 21) whereas others, including EBV, have been classified as B lymphotropic (7). We report here that B cells, T cells, and macrophages obtained from the spleen of GPHLV latently and acutely infected guinea pigs harbor GPHLV, although there is some evidence of a GPHLV tropism for B cells. The capacity of GPHLV to persist in lymphocytes and travel to various tissues as well as the possibility of affecting the immunocompetence of such cells may be significant for understanding the pathogenesis of GPHLV as well as other latent herpes virus infections.

Summary. The role of spleen lymphocytes in acute and latent guinea pig herpes-like virus (GPHLV) infection and the *in vitro* susceptibility of the lymphocytes to GPHLV were explored. Macrophage-, B-cell-, and T-cell-enriched populations obtained from infected guinea pigs were examined for infectious GPHLV. During acute infection, virus was first detected in the macrophage and B-cell fractions, whereas, infectious virus was only evident in the T-cell fraction 5 days or more after inoculation. During latent infection, infectivity titers in the B fractions were consistently higher than in the T fractions. In both the B and the T lymphocytes derived from latently infected guinea pigs, virus was expressed only after *in vitro* cultivation or cocultivation with susceptible cells. Lymphocytes infected *in vitro* did not support GPHLV replication, although latent infection of lymphocytes with GPHLV was readily accomplished *in vivo*.

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