Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 4563–4567, July 1984
Microbiology

Transformation of owl monkey T cells *in vitro* with *Herpesvirus saimiri*

(T lymphocytes/interleukin 2)

HARVEY RABIN*†, RALPH F. HOPKINS III*, RONALD C. DESROSIERS‡, JOHN R. ORTALDO§, JULIE Y. DJEU¶, AND RUSSELL H. NEUBAUER*||

*Litton Bionetics, Inc.–Basic Research Program and §Biological Therapeutics Branch, National Cancer Institute–Frederick Cancer Research Facility, Frederick, MD 21701; ‡New England Regional Primate Research Center, Harvard Medical School, Southborough, MA 01772; and ¶Division of Virology, National Center for Drugs and Biologics, Bethesda, MD 20205

Communicated by George Klein, March 14, 1984

ABSTRACT **Owl monkey peripheral blood mononuclear cells were treated with phytohemagglutinin and expanded in interleukin 2 (IL-2)-containing medium. The cells were then exposed to *Herpesvirus saimiri* (HVS strain S295C)-infected owl monkey kidney monolayer cells. Four to 6 weeks later, the lymphocytes showed increased clumping and cell growth and the ability to grow in the absence of IL-2. Control lymphocyte cultures not exposed to HVS eventually died out at ≈6–8 weeks, even in the presence of IL-2. Although infected lymphocytes grew continuously in the absence of IL-2, their growth was enhanced by addition of IL-2 to the cultures. Natural killer cell-like cytotoxicity and γ-interferon release were also enhanced by IL-2. All cultures were positive for HVS antigens, infectious centers, or DNA. The reactivity of monoclonal antibodies to cell surface markers suggested that the resultant cell lines were comprised of activated T cells. The properties of the *in vitro*-transformed cells were similar to those of cells established from HVS-induced owl monkey tumors. Our results suggest that infection of T lymphocytes with HVS results in decreased dependence of T cells upon exogenous IL-2 for growth.**

Herpesvirus saimiri (HVS) is a horizontally spread T-cell tropic herpesvirus of squirrel monkeys. As such, it is a member of a large group of primate lymphotropic herpesviruses, which includes the B-cell tropic Epstein–Barr virus (EBV) of man, the EBV-like viruses of Old World monkeys and apes, and the T-cell tropic viruses of New World monkeys (1). HVS is not pathogenic for squirrel monkeys but is potently oncogenic for several other species of New World monkeys and rabbits, where it produces fatal T-cell lymphomas and leukemias (2–8). Although HVS readily produces T-cell lymphomas in New World primates, only one lymphoid cell line has been established by *in vitro* transformation (9). It is not clear whether HVS-induced lymphomas involve a progression of events including proliferation and subsequent neoplastic changes or if a single virus-induced event results in lymphoma. However, as normal T cells require interleukin 2 (IL-2; T-cell growth factor) for growth, in this report we will use the term transformation to indicate the ability of T lymphocytes to grow continuously in the absence of added IL-2. The ability to transform cells reproducibly with HVS *in vitro* would provide an important tool in the study of the molecular biology of oncogenic transformation by this virus. Additionally, transformation would provide the means for generating autologous target cells for the study of potential cytotoxic T cells, which may control infection *in vivo* in a similar way that HLA-restricted cytotoxic T cells control EBV-positive B cells in humans (10). Moreover, it may be possible to establish immortalized lines of antigen-specific T cells in a fashion analogous to that in which EBV was used to immortalize lines of B cells that produce specific antibody (11, 12).

Previous attempts to transform lymphocytes by cocultivation with HVS producer lymphocytes or by exposure to cell-free HVS have, with the rare exception noted above, been unsuccessful (refs. 3 and 13; unpublished results). Recently, cell lines were established with the aid of IL-2 from owl monkeys bearing HVS-induced tumors (14, 15). These cell lines are not dependent on IL-2 for growth and survival as are normal T cells but respond to it strongly in terms of increased cell growth. Based on this observation and the failure of previous *in vitro* transformation attempts made without IL-2, it was reasonable to assume that HVS transformation *in vitro* might also be aided by IL-2. Therefore, a transformation protocol was established in which cells were first activated by phytohemagglutinin (PHA) to induce IL-2 receptors and then were expanded in IL-2-containing medium prior to exposure to virus. The cells were then exposed to HVS and further cultured in IL-2-containing medium prior to testing them for IL-2-independent growth, the criterion used for transformation. In this paper we report the establishment and characterization of a series of cell lines established *in vitro* by HVS transformation of owl monkey lymphocytes. A preliminary account of these results has been reported (16).

MATERIALS AND METHODS

Transformation Procedure. Peripheral blood mononuclear leukocytes (PBL) from normal adult owl monkeys (*Aotus trivirgatus*) were separated from whole blood by density centrifugation on Ficoll gradients (Histopaque, Sigma). PBL at 1×10^6 cells per ml in RPMI 1640 medium supplemented with 10% heat-inactivated (56°C for 60 min) fetal bovine serum, 2 mM L-glutamine, 50 units of penicillin per ml, and 50 μg of streptomycin per ml (growth medium) were treated for a 5-day period with purified PHA (Burroughs Wellcome, Greenville, NC) at 1 μg/ml. At the end of this time, the cells were transferred to medium consisting of equal volumes of growth medium and conditioned medium from MLA144 cells as a source of IL-2 (17). The final concentration of IL-2 in the medium was ≈300 units/ml, which is in the optimal range for growth of owl monkey T cells. The activated PBL were ex-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: HVS, *Herpesvirus saimiri*; IL-2, interleukin 2; PHA, phytohemagglutinin; IC, infectious centers; MAb, monoclonal antibodies; IFN, interferon; EBV, Epstein–Barr virus; PBL, peripheral blood mononuclear leukocytes; GaLV, gibbon ape leukemia virus; OMK cells, owl monkey kidney cells; TPA, phorbol 12-*O*-tetradecanoate 13-acetate.

†Present address: DuPont Biomedical Products, North Billerica, MA 01862.
||Present address: E. I. duPont de Nemours and Co., Glenolden Laboratory, Glenolden, PA 19036.

panded in the IL-2-containing medium for 2 wk and then cocultivated with HVS (strain S295C)-infected monolayers of owl monkey kidney (OMK) cells grown in 16-mm wells (Costar, Cambridge, MA). A starter culture of OMK cells was generously provided by L. Falk (New England Regional Primate Research Center, Harvard Medical School, Southborough, MA). Cocultivation was in the absence of added IL-2 for 2 days and in the presence of IL-2 for 5 additional days. After cocultivation, the lymphocytes were removed from the infected monolayers and cultured in IL-2-containing medium. As the cells grew, they were transferred from 16-mm wells to flasks (Costar) of 25-cm^2 surface area and then to flasks of 75-cm^2 surface area. Controls consisted of owl monkey PBL from the same bleeding of each individual animal handled in the same way except that exposure to HVS-infected OMK cells was omitted. Cultures were observed at regular intervals for changes in cell morphology and growth pattern. All cultivation was at 37°C in a humidified atmosphere of 5% CO_2 in air.

Infectious Center (IC) Assays. HVS-producing lymphocytes were detected by cocultivation of lymphocytes with Vero cells growing in 16-mm wells. Serial dilutions of lymphocytes from 10^5 to 10^1 cells were used, and each cell concentration was tested in quadruplicate. Viral plaques were scored at 13–14 days after inoculation. Cytopathology was confirmed to be HVS-induced by immunofluorescence examination of representative cultures.

Analysis of Viral DNA. Total cell DNA was isolated by using Sarkosyl lysis and preparative cesium chloride gradient centrifugation as described (18, 19). DNA was cut with restriction endonuclease (Bethesda Research Laboratories), electrophoresed through a 0.8% agarose gel, and transferred to nitrocellulose by the procedure of Southern (20). Transferred DNA on filters was hybridized with ^{32}P-labeled unique HVS DNA, at 67°C in 4× NaCl/Cit, pH 7/0.1% Na-DodSO$_4$ (1× NaCl/Cit = 0.15 M NaCl/0.015 M sodium citrate, pH 7). Details for preparation of ^{32}P-labeled DNA, hybridization, rinsing of the filters, and autoradiography have been described (19, 21).

Immunofluorescence Analysis. Monoclonal antibodies (MAb) to subpopulations of human lymphocytes that have been shown to cross-react with owl monkey lymphocytes were used in these studies (15). Specific MAbs used were OKT11a (a gift of G. Goldstein, Ortho Pharmaceutical, Raritan, NJ) and anti-Leu 5 (Becton Dickinson, Mountainview, CA), which block sheep cell rosetting; anti-Leu 3a, which reacts with the helper T-cell subset; and anti-HLA DR (Becton Dickinson) and B1, which detect determinants on B cells (Coulter). Cells (1–2 × 10^6 cells per assay) were first stained with MAb, then with fluorescein isothiocyanate-labeled goat antiglobulin to murine IgG (Tago, Burlingame, CA) and then washed with propidium iodide (Sigma). Fluorescence from 20,000 viable cells was determined as described (15) by using a FACS IV fluorescence-activated cell sorter (Becton Dickinson FACS Systems, Sunnyvale, CA).

Assays for HVS-Intracellular and Membrane Antigens. Intracellular viral antigens were detected by indirect immunofluorescence of acetone-fixed cells with well-characterized owl monkey sera and fluorescein isothiocyanate-labeled goat anti-human IgG (Tago) essentially as described (22). For detection of HVS-membrane antigen, indirect immunofluorescence staining was performed on viable cells (23). Induction of membrane antigens was accomplished by treatment of the cells with 20 ng of phorbol 12-*O*-tetradecanoate 13-acetate (TPA)/ml and 0.5 mM *n*-butyrate for 3 days as described (24).

Assays for Interferon (IFN). IFN was measured by the inhibition of the cytopathic effect of vesicular stomatis virus in WISH and MDBK cells, as described (25). IFN titers were expressed in international units standardized with National Institutes of Health reference IFN-α, G-0234-901-527.

Assays for Cellular Cytotoxicity. A ^{51}Cr-release assay was performed as described (26). Target cells, MOLT 4 and K562, were labeled with 150 μCi (1 Ci = 37 GBq) of Na-$^{51}CrO_4$ (New England Nuclear) for 30–45 min at 37°C. The cells were then washed and resuspended at the desired concentration in RPMI 1640 medium containing fetal bovine serum. Effector cells were washed, enumerated, resuspended, and mixed at various effector-cell-to-target-cell ratios with target cells at 5 × 10^3 cells per reaction. Incubation was performed for 4 hr at 37°C in 96-well round-bottom microtiter plates. Supernatants were then taken for determination of isotope release. Unlabeled target cells were added to labeled target cells as autologous controls. All experiments were done in triplicate. The percentage of isotope released was determined from the formula:

$$\% \text{ lysis} = \frac{\text{experimental cpm} - \text{autologous cpm}}{\text{total cpm incorporated}} \times 100.$$

RESULTS

Cell Transformation. PBL of five individual owl monkeys were separated, treated with PHA, expanded in IL-2, exposed to HVS, and cultured on IL-2-containing medium as described. After 4–6 wk of incubation, cells in the HVS-exposed cultures showed increased growth and, in four out of five cultures, also showed increased cell clumping compared to the cells in the non-HVS-exposed controls as determined by microscopic examination. At this time, cells of the virus-exposed cultures were tested and found to be able to grow in the absence of added IL-2. Parallel cultures of each cell line, one set maintained in the presence of IL-2 and the other in its absence, were established for each line shortly after determining the ability of the cells to grow in the absence of added IL-2.

Cells of all infected lines grew in the absence of added IL-2 but grew much better in the presence of IL-2. This is illustrated in Fig. 1 for one representative cell line, 190(HVS). Cells, which had been growing without added IL-2, were seeded at 5 × 10^5 cells per ml in flasks of 25-cm^2 surface area in 8 ml of medium that either contained or lacked IL-2. The cells were counted in a hemocytometer and reseeded at 5 × 10^5 cells per ml at 3- to 4-day intervals. The cumulative net cell increase was calculated from the individual cell counts. Fig. 1 shows that while the cells grew in the absence of IL-2, they achieved an approximately 2.5-fold greater increase in the presence of IL-2. In a separate experiment, it was shown that highly purified IL-2 at 300 units/ml (27) would enhance the growth of HVS-transformed cells (data not shown), suggesting that IL-2 was responsible for the increase in cell proliferation. Cells of four of the original lines were growing, with or without added IL-2, at >10 mo after exposure to HVS. The fifth cell line, 15(HVS), was lost to microbial contamination at 3 mo after virus exposure. Four of the non-HVS-exposed cultures gradually died out between 1.5 and 2 mo after initiation, even in the continued presence of IL-2. The fifth line, 224(PHA), grew better than the other control lines but was lost to microbial contamination at 4 mo. This cell line had been tested at 10 wk and was found to be dependent on IL-2 for growth.

The HVS-exposed cells grown either with or without IL-2 were morphologically distinguishable from non-virus-exposed IL-2-dependent cells. Fig. 2 shows a comparison of cells from transformed line 8(HVS) with IL-2-dependent control cells of the same donor. The HVS-exposed cells (Fig. 2 *Left*) appear larger, more heterogeneous in size and shape, and tend to have more cytoplasm than the non-HVS-exposed control cells (Fig. 2 *Right*). Their cytoplasm also appears to be less dense and to be more clumped than that of the control cells.

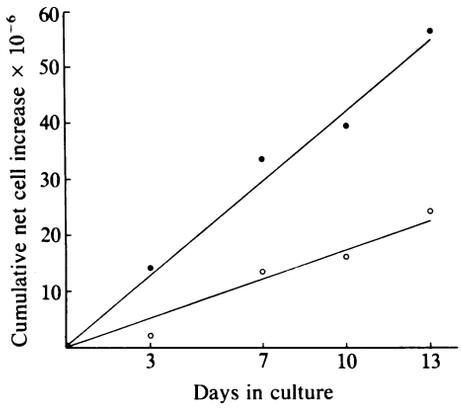

FIG. 1. Growth of HVS-transformed cells, 190(HVS), in the presence (●) and absence (○) of IL-2. Cells were seeded at 5×10^5 cells per ml in 8 ml of medium and were counted and reseeded at the initial concentration and volume at days 3, 7, 10, and 13 (counted only). The cumulative net increase in cell number was calculated from the invidual cell counts.

To determine the reproducibility of the transformation procedure, we obtained peripheral blood samples from two of the owl monkeys used in the initial study, 190 and 265. PBL from these two monkeys were treated in the same fashion as in the initial experiment except that the expanded lymphocytes were exposed to an HVS-infected Vero cell monolayer instead of an infected OMK cell monolayer, and IL-2 was present for the entire cocultivation period. Cells of monkey 190 showed IL-2-independent growth and HVS IC at 5 wk after virus exposure. These cells were growing 4 mo after virus exposure. Uninfected control 190 lymphocytes persisted in culture in the presence of IL-2 for 2.5 mo before dying out. PBL of monkey 265 did not respond normally to PHA and IL-2 treatment, with all control cells dead by 3 wk. Virus-exposed cells persisted in culture for about 8 wk but then also died out.

To examine variables in the transformation procedure, fresh PBL from three owl monkeys were cocultivated directly with HVS-infected OMK cell monolayers for 1 wk in the absence of IL-2. The lymphoid cells were then removed and cultured without IL-2. No transformation was observed in these cultures. Similarly, fresh PBL from four owl monkeys were cocultivated with HVS-infected OMK cell monolayers in the absence of IL-2, removed, and then cultured in the presence of IL-2. No transformation was observed in these cultures.

HVS Antigens and IC. Two and one-half months after initial exposure to HVS, all five virus-exposed lines were tested for virus-associated intracellular antigens by indirect immunofluorescence of acetone-fixed cells. Cells were tested after growth in the absence of IL-2 and after 3 days of growth in the presence of IL-2. Tests with a serum positive for both virus capsid antigen and early antigen showed that four of the five lines were positive for HVS intracellular antigens at levels between <1% and >6% of the cells scored. There was no obvious difference between cells grown on or off IL-2. Most of the antigen appeared to be early antigen, as staining with a virus capsid antigen-positive but early antigen-negative serum failed to stain cells of any of the lines except for 190(HVS), where a few (<1%) of the cells were positive (Table 1).

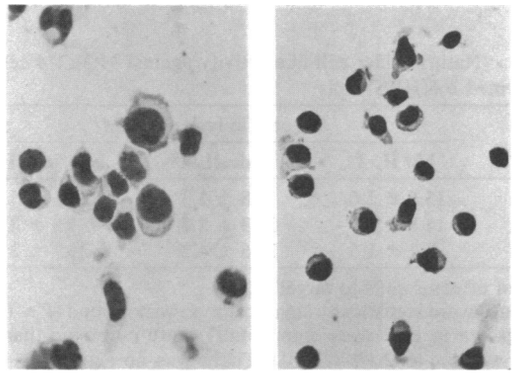

FIG. 2. Photomicrographs (×670) of Giemsa-stained, cytospin preparations of cells of HVS-transformed line 8(HVS) (*Left*) and of non-HVS-exposed control cells from the same donor (*Right*).

At 3 mo after initial exposure to virus, the cells were tested for IC on Vero cell monolayers. The results showed that lines 8(HVS) and 190(HVS) were high in IC, line 224(HVS) had a moderate level, while line 265(HVS) was low (Table 1). Cocultivation was repeated 3 mo later with essentially the same results.

At 5.5 mo after initial exposure to virus, the cell lines were tested for the presence of HVS membrane antigen (Table 1). Three of the four cell lines were positive, while line 265(HVS) was negative. The cell lines were also treated with 20 ng of TPA per ml and 0.5 mM *n*-butyrate for 3 days to determine if membrane antigen levels could be boosted. The results indicated that all lines, except for 265(HVS), showed increased levels of membrane antigen after TPA and butyrate treatment.

Analysis of Viral DNA in Cell Lines. Total cell DNA was prepared from cell lines 224(HVS) and 265(HVS). Southern blot hybridizations were used to determine approximate viral genome copy number and to obtain information on the nature of the viral DNA (Fig. 3). By comparison to known amounts of virion DNA electrophoresed in parallel slots, we estimated that viral DNA represented about 0.06% of total DNA from 265(HVS) cells and about 1.0% of total DNA from 224(HVS) cells after 6 mo in culture. If one assumes the cellular diploid genome to be 6×10^6 kilobase pairs, cell lines 265(HVS) and 224(HVS) contained ≈24 and 400 genome copies per cell, respectively. The restriction endonuclease fragmentation patterns of viral DNA in the 224(HVS) cell line were the same as the parental virion DNA (Fig. 3 and other restriction endonuclease fragmentation patterns not shown). Since restriction endonuclease fragmentation patterns of different HVS strains are distinctive (28), this verified that the 224(HVS) cell line contained the appropriate viral DNA. Also, this indicated that gross rearrangements and/or deletions were not prevalent in this cell line at this time. Circularization of viral DNA would not have been de-

Table 1. HVS intracellular antigens, membrane antigens, and IC of *in vitro*-transformed cell lines

Cell line	Intracellular antigen*		Membrane antigen*		IC¶
	VCA⁺/EA⁺†	VCA⁺/EA⁻‡	Control	TPA + butyrate§	
265(HVS)	Neg	Neg	Neg	Neg	0.5
8(HVS)	6.7	Neg	2.9	24.7	69.7
190(HVS)	2.6	<1	1.4	15	56.3
224(HVS)	1	Neg	1.1	13.8	16.5
15(HVS)	0.8	Neg	ND	ND	ND

ND, not determined; Neg, negative result.
*Percent positive.
†Serum positive for antibodies to both viral capsid antigen (VCA) and early antigens (EA).
‡Serum positive for antibody to VCA but negative for EA.
§Cells treated for 3 days with 20 ng of TPA per ml and 0.5 mM *n*-butyrate.
¶Percent positive.

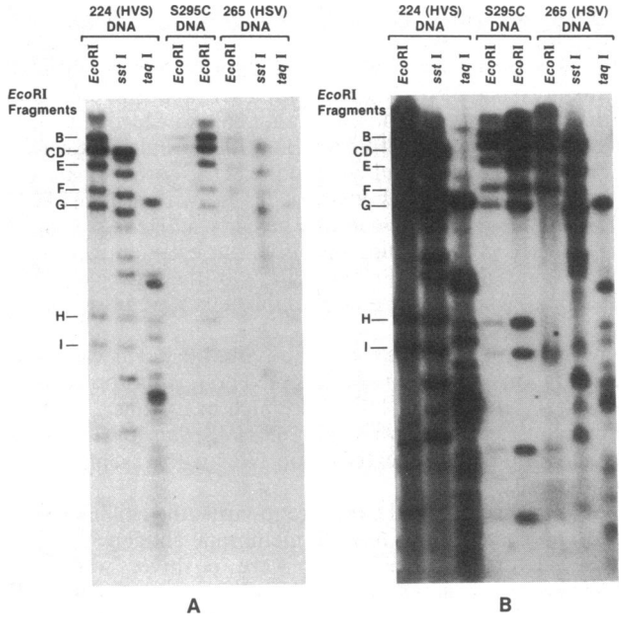

FIG. 3. Southern transfer hybridization of cellular DNA to ^{32}P-labeled HVS DNA. DNA from cell line 224(HVS) (2 μg), DNA from cell line 265(HVS) (5 μg), and total DNA from purified HVS strain S295C virions (2 ng in slot 4 and 10 ng in slot 5) were digested with the indicated restriction endonucleases, electrophoresed through a 0.8% agarose gel, stained with ethidium bromide, photographed, and transferred to nitrocellulose. Transferred DNA was hybridized with ^{32}P-labeled virion L-DNA as described. (*A*) Twenty-four-hour film exposure. (*B*) Ten-day film exposure.

tected by these procedures because of the highly repetitive DNA at the termini of virion DNA. The *Eco*RI G and H fragments were highly underrepresented, however, in the 265(HVS) cell line. This was clearly evident upon long film exposure (Fig. 3*B*). The results indicated that sequences contained within these fragments were deleted in most viral DNA molecules in the 265(HVS) cell line. This region of viral DNA has been found to be deleted in some tumor cell lines (19, 29).

Cell Surface Phenotypes. At 2–2.5 mo after virus exposure, the transformed cell lines were tested with a series of MAb to human lymphocyte surface antigens that cross-react with owl monkey lymphocytes (15). All four lines tested showed the same staining pattern, reacting with OKT11a, anti-Leu 5, and HLA DR. The cells uniformly failed to react with anti-Leu 3a and B1. Thus, the cells would appear to be activated (i.e., HLA DR-positive) T cells, lacking the marker for helper T cells.

Cytotoxic Activity and IFN Release. Cell lines established *in vitro* from HVS-induced marmoset tumors have been shown to have natural killer cell-like activity (30) and to release IFN (31). Additionally, noncontinuous cultures of owl monkey HVS-induced tumors were found to be cytotoxic for xenogeneic, nonlymphoid target cells and to release IFN (32). It was of interest, therefore, to determine if the *in vitro*-transformed cells also possessed these characteristics. Cells from all transformed cell lines had low levels of natural killer cell-like cytotoxicity against MOLT 4 target cells when tested at various times after transformation. Cytotoxicity was consistently enhanced after growth of the cells in IL-2. In a representative experiment (Table 2), 265(HVS) cells showed low but significant levels of activity when grown without IL-2. Cultivation for 5 days in the presence of either crude or purified IL-2 (both at 300 units/ml) enhanced cytotoxicity 2- to 3-fold. Little activity was observed by any of the lines against K562 target cells except after cultivation in IL-2 (data not shown).

Culture fluids from four of the *in vitro*-transformed cell lines were tested for the release of IFN at 4 mo after exposure to virus. Cells grown continually without IL-2 were found to release low levels of IFN—10–125 units/ml. However, when grown in the presence of IL-2-containing medium for 1 wk, the level of IFN increased to between 625 and 1325 units/ml. Further experiments with line 265(HVS) showed that IFN levels increased as early as 6 hr after exposure to IL-2. Purified IL-2 was also found to boost IFN release by 265(HVS) cells (250 to 3125 units/ml after 5 days). The IFN released by the various *in vitro*-transformed cells appeared to be IFN-γ, as it was effective on WISH cells but not on MDBK cells (33).

Cells grown in the absence of added IL-2 were also tested for the production of IL-2. Culture fluids were concentrated on trimethylsilyl-controlled pore glass beads as described (27) and assayed on an IL-2-sensitive marmoset cell line (14) at a final concentration of 25-fold. Culture fluids from MLA144 cells were used as a positive control. No evidence of IL-2 activity was found in the fluids of any of the HVS-transformed cells, although activity in the MLA144 culture fluid was readily demonstrable (data not shown).

Assays for Gibbon Ape Leukemia Virus (GaLV). MLA144 cells were used as the source of IL-2 in this study. These cells are known to be infected with a type C retrovirus, GaLV. It was conceivable that GaLV might have contaminated the IL-2 preparations used and contributed to the cell transformation. Lysates of 10^7 cells of each of the transformed lines (grown without IL-2), of normal PHA-stimulated owl monkey PBL that had been grown for 3 wk in IL-2, and of MLA144 cells were tested in a broadly reactive competition RIA for primate type C retroviruses. This assay used ^{125}I-labeled CPC-1 p28 and a hyperimmune goat antiserum and can detect the major core antigens of all known primate type C retroviruses (L. Arthur, personal communication). Viral antigen was not detected in lysates of any of the transformed cells nor in that of the normal owl monkey PBL. Antigen was readily detected in the MLA144 cell lysate at a level of 4000 ng/ml. Moreover, concentrated culture fluids from all HVS-transformed cell lines were tested on two separate occasions for retrovirus RNA-dependent DNA polymerase activity with negative results. In addition, DNA samples from all of the transformed cell lines were analyzed for the presence of GaLV sequences by Southern blot hybridization. GaLV sequences were not detected in any of these samples under conditions where GaLV sequences in MLA144 cell DNA were strongly evident. We estimate that one genome copy per cell would have been readily detected.

DISCUSSION

The ability to transform cells with HVS on a regular basis would be of importance in the study of the molecular biology

Table 2. Natural killer cell-like activity against MOLT 4 cells of transformed 265(HVS) cells

	% specific lysis ± SEM†		
E:T*	No IL-2	Crude IL-2‡	Purified IL-2‡
100:1	13.6 ± 1.6	34.8 ± 3.3	42.5 ± 5.9
33:1	14.8 ± 1.5	32.3 ± 2.7	34.9 ± 2.8
11:1	9.1 ± 1.5	27.6 ± 4.2	29.7 ± 2.1

*Ratio of effector cells to target cells.
†All values were statistically significant vs. background ($P \leq 0.004$). Cells grown in IL-2 were significantly more cytotoxic than cells grown without IL-2 ($P \leq 0.01$). There was no significant difference in cytotoxicity between cells grown in crude or purified IL-2.
‡Cells were cultivated with 300 units of crude or purified IL-2 per ml for 5 days prior to assay. To prevent IL-2 carry-over into the assay, cells were washed thoroughly before mixing with target cells.

of cell transformation by this virus. At the present time, viral genes contributing to cell transformation have not been identified. Analysis of nonproducer tumor cell lines and of the single marmoset *in vitro*-transformed line indicate that large deletions can occur in the unique viral DNA of these cells, L-DNA, and the growth-transformed state still persists (19, 29). Viral deletion mutants or temperature-sensitive mutants could conceivably be tested for the ability to transform T cells by using assay procedures described in this report.

The transformation protocol described may not be the only or the optimal means of transforming T cells with HVS. However, in contrast to all other procedures that have been tried, it has been very effective in generating continuous lines of HVS-transformed cells. It should now be possible to sort out the steps and components of the procedure that are critical for transformation. Thus, the role of cell activation and use of IL-2 could be carefully evaluated. Because of availability and expense, the IL-2 used in the transformation studies was not purified as was the IL-2 used in selected experiments on cell growth and function. To date, however, IL-2 is the only lymphokine which has been identified conclusively in MLA144 culture fluids. Therefore, while a role for another, as yet unidentified, lymphokine cannot be ruled out, it is likely that the support of T-cell growth and transformation observed in these experiments was due to IL-2.

Preliminary assays on the ability of the transformed cells to form colonies in semi-solid medium in the presence or absence of IL-2 and to transplant to nude mice by the subcutaneous or intracranial routes have been negative, as have similar experiments with HVS-induced-tumor-derived cell lines (unpublished results). Thus, *in vitro* transformation by HVS may be principally associated with continuous cell growth. The *in vitro*-transformed cells have shown several characteristics in common with cells established in culture from experimental HVS-induced tumors. These similarities include deletions in the viral genome of 265(HVS) cells, the presence of natural killer cell-like activity, the release of IFN, and growth without added IL-2.

The results on *in vitro* transformation of owl monkey lymphocytes that we have described are in agreement with preliminary work by others on transformation of marmoset lymphocytes, although it appears that transformation of the marmoset cells required a longer interval after exposure to virus than did the owl monkey lymphocytes (34). It is apparent from studies both of cell lines established from HVS-induced tumors and from cell lines initiated *in vitro* that transformation of T cells by HVS results in a decrease in dependency of the cells on IL-2 for growth. Human T lymphocytes transformed *in vitro* with human T-cell leukemia virus also have shown a decrease in dependency on IL-2 (35, 36). This raises the possibility that there may be additional parallels between T-cell transformation induced by these two distinct types of viruses. If the behavior of HVS-transformed cells *in vitro* reflects the behavior of HVS-induced tumor cells *in vivo*, it would appear that the ability of these cells to replicate in the absence of added IL-2 and their ability to respond to exogenous IL-2 by increased growth and function may be of importance in the pathogenesis of HVS-induced lymphoma.

We thank Mr. Michael Adams for technical support and Dr. Larry Arthur for performing the RIAs for retrovirus core antigen. We also thank Drs. Ronald B. Herberman, Martin Zweig, and Francis W. Ruscetti for valuable discussions of the manuscript. This work was supported in part by the National Cancer Institute under contract NO1-CO-75380 with Litton Bionetics, Inc., and by Grant 31363 awarded by the National Cancer Institute, by a Special Fellowship from the Leukemia Society of America (R.C.D.), and by Grant RR00168 to the New England Primate Research Center from the Division of Research Resources.

1. Rabin, H. (1983) in *Viral and Immunological Diseases in Non-human Primates*, ed. Kalter, S. S. (Liss, New York), pp. 111–133.
2. Falk, L. J., Jr. (1980) in *Oncogenic Herpesviruses*, ed. Rapp, F. (CRC, Boca Raton, FL), pp. 145–173.
3. Fleckenstein, B. (1979) *Biochim. Biophys. Acta* **560**, 301–342.
4. Falk, L. A., Jr. (1980) in *Viral Oncology*, ed. Klein, G. (Raven, New York), pp. 813–831.
5. Deinhardt, F. & Deinhardt, J. (1979) in *The Epstein–Barr Virus*, eds. Epstein, M. A. & Achong, B. G. (Springer, Berlin), pp. 373–415.
6. Rangan, S. R. S. & Gallagher, R. E. (1979) *Adv. Virus Res.* **24**, 1–123.
7. Neubauer, R. H. & Rabin, H. (1979) in *Naturally Occurring Biological Immunosuppressive Factors and Their Relationship to Disease*, ed. Neubauer, R. H. (CRC, Boca Raton, FL), pp. 203–231.
8. Gilden, R. V. & Rabin, H. (1982) *Adv. Virus Res.* **27**, 281–334.
9. Schirm, S., Muller, I., Desrosiers, R. & Fleckenstein, B. (1984) *J. Virol.* **49**, 938–946.
10. Rickinson, A. B., Moss, D. J., Wallace, L. E., Rowe, M., Misko, I. S., Epstein, M. A. & Pope, J. H. (1981) *Cancer Res.* **41**, 4216–4221.
11. Steinitz, M., Klein, G., Koskimies, S. & Makel, O. (1977) *Nature (London)* **269**, 420–422.
12. Rosen, A., Persson, K. & Klein, G. (1983) *J. Immunol.* **130**, 2899–2902.
13. Falk, L., Johnson, D. & Deinhardt, F. (1978) *Int. J. Cancer* **21**, 652–657.
14. Brown, R. L., Griffith, R. L., Neubauer, R. H. & Rabin, H. (1982) *J. Immunol.* **129**, 1849–1853.
15. Neubauer, R. H., Briggs, C. J., Noer, K. B. & Rabin, H. (1983) *J. Immunol.* **130**, 1323–1329.
16. Neubauer, R. H., Hopkins, R. F., III, & Rabin, H. (1983) in *Leukemia Reviews International*, ed. Rich, M. (Dekker, New York), Vol. 1, pp. 109–110.
17. Rabin, H., Hopkins, R. F., III, Ruscetti, F. W., Neubauer, R. H., Brown, R. L. & Kawakami, T. G. (1981) *J. Immunol.* **127**, 1852–1856.
18. Bornkamm, G., Delius, H., Fleckenstein, B., Werner, F.-J. & Mulder, C. (1976) *J. Virol.* **19**, 154–161.
19. Desrosiers, R. C. (1981) *J. Virol.* **39**, 497–509.
20. Southern, E. M. (1975) *J. Mol. Biol.* **98**, 503–517.
21. Desrosiers, R. C. (1982) *J. Virol.* **43**, 427–435.
22. Klein, G., Pearson, G., Rabson, A., Ablashi, D. V., Falk, L., Wolfe, L., Deinhardt, F. & Rabin, H. (1973) *Int. J. Cancer* **12**, 270–289.
23. Pearson, G., Orr, T., Rabin, H., Cicmanic, J., Ablashi, D. & Armstrong, G. (1973) *J. Natl. Cancer Inst.* **51**, 1939–1943.
24. Hopkins, R. F., III, Witmer, T. J., Neubauer, R. H. & Rabin, H. (1982) *J. Infect. Dis.* **146**, 734–740.
25. Djeu, J. Y., Stocks, N., Zoon, K., Stanton, G. J., Timonen, T. & Herberman, R. B. (1982) *J. Exp. Med.* **156**, 1222–1234.
26. Ortaldo, J. R., Bonnard, G. D. & Herberman, R. B. (1977) *J. Immunol.* **119**, 1351–1357.
27. Henderson, L. E., Hewetson, J. F., Hopkins, R. F., III, Sowder, R. C., Neubauer, R. H. & Rabin, H. (1983) *J. Immunol.* **131**, 810–815.
28. Desrosiers, R. C. & Falk, L. A. (1982) *J. Virol.* **43**, 352–356.
29. Kaschka-Dierich, C., Werner, F. J., Bauer, I. & Fleckenstein, B. (1982) *J. Virol.* **44**, 295–310.
30. Johnson, D. R. & Jondal, M. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 6391–6395.
31. Wright, J., Falk, L. & Deinhardt, F. (1974) *J. Natl. Cancer Inst.* **53**, 271–275.
32. Wallen, W. C., Neubauer, R. H. & Rabin, H. (1974) *J. Med. Prim.* **3**, 41–53.
33. Wiranowska-Stewart, M. (1981) *J. Interferon Res.* **1**, 315–321.
34. Falk, L. A., Silva, D., Byington, R. & Schooley, R. (1982) *Proc. 15th Int. Leucocyte Cult. Conf. Immunobiol.* **163**, 227 (abstr.).
35. Miyoshi, I., Kubonishi, I., Yoshimoto, S., Akagi, T., Ohtsuki, Y., Shiraishi, Y., Nagata, K. & Hinuma, Y. (1981) *Nature (London)* **294**, 770–771.
36. Markham, P. D., Salahuddin, S. Z., Kalyanaraman, V. S., Popovic, M., Sarin, P. & Gallo, R. C. (1983) *Int. J. Cancer* **31**, 413–420.