JOURNAL OF VIROLOGY, Oct. 1974, p. 745-750
Vol. 14, No. 4

Printed in U.S.A.

Stimulation of *Herpesvirus saimiri* Expression in the Absence of Evidence for Type C Virus Activation in a Marmoset Lymphoid Cell Line

RUSSELL H. NEUBAUER, WILLIAM C. WALLEN, AND HARVEY RABIN

Department of Virology and Cell Biology, Litton Bionetics, Inc., Kensington, Maryland 20795

Received for publication 6 June 1974

Nucleic acid base analogues were used to examine a *Herpesvirus saimiri* (HVS)-infected marmoset lymphoid cell line (MLC-1) for possible association with type C viruses. Synthetic templates poly(rA)·$d(pT)_{10}$ and poly(dA)·$d(pT)_{10}$ were used to detect RNA-directed DNA polymerase activity in 100-fold concentrated tissue culture fluids. HVS was monitored by immunofluorescence for early, late, and membrane antigens. MLC-1 cells were exposed to 30 μg of 5-bromo-2'-deoxyuridine (BUdR) per ml for 24 h and examined daily. Similar experiments used 5-iodo-2'-deoxyuridine (IUdR) (20 μg/ml) for 30 h or IUdR (20 μg/ml) for 3 days followed by 2% dimethyl sulfoxide for 4 days. Results of these experiments failed to show any type C virus-like polymerase; however, HVS expression was greatly stimulated. BUdR and IUdR enhanced expression of HVS-associated antigens five- to sevenfold, with maximal stimulation being observed 3 to 4 days after removal of the analogue. IUdR-dimethyl sulfoxide treatment was generally less effective. Although more cells showed HVS antigens, the treatments did not increase cell-free infectious virus. The data suggest that HVS-infected lymphoid cells can be stimulated to express virus in a manner similar to that of the Epstein-Barr virus in Burkitt's lymphoma cells. No evidence of type C virus was found in stimulated cultures.

Herpesvirus saimiri (HVS) was the first virus of primate origin shown to be oncogenic in primates (24). HVS can be isolated from squirrel monkeys (*Saimiri sciureus*) which, on the basis of virus isolations and serological studies, appear to be the natural hosts (9, 20, 31). Natural or experimental infection of squirrel monkeys with HVS has not resulted in disease (10, 31), although few such animals have been studied. The inoculation of owl monkeys (*Aotus trivirgatus*) or marmosets (*Saguinus oedipus*, *S. nigricollis*, and *S. fuscicollis*) with HVS has consistently produced malignant lymphoma, and the pathogenesis of this disease has been well characterized with respect to clinical, virological, and humoral immunological parameters (3, 28, 36).

A number of reports indicate possible herpesvirus-type C virus interactions in lymphomas. Frankel and Groupe reported that avian leukosis virus (RAV-2)-infected chicken embryo fibroblast cultures show an increase in type C viral antigens with a concomitant reduction in Marek's disease herpesvirus titer as compared with the appropriate controls (11). Peters et al. reported that in vivo exposure of specific pathogen-free chickens to either Marek's disease herpesvirus or RAV-2 alone did not result in tumors, whereas a co-infection induced tumors in 100% of these animals (29). In additional studies, the coexistence of type C and herpesvirus particles in the feather follicle epithelium (the replication site of enveloped, infectious Marek's disease herpesvirus [6]) of chickens has been reported (17). Burkitt's lymphoma, in which the Epstein-Barr herpesvirus (EBV) has been implicated as being the etiological agent (16), Hodgkin's disease, and other human lymphomas have also shown biochemical evidence for the presence of RNA viruses (21). Along similar lines, both a herpesvirus (19) and a type C virus (13) have been isolated from leukemic strain 2 guinea pigs. Although the oncogenicity of these viruses has not been demonstrated, the inoculation of both of them into day-old guinea pigs resulted in the enlargement of spleen and lymph nodes (18). In HVS studies, the examination of viral inocula as well as tumor and other tissues from owl monkeys and marmosets with lymphomas has not revealed evidence for the presence of type C viruses (8, 26, 27). RNA-dependent DNA polymerase activity has been

reported from a monolayer cell line that was established from a lymphoma tumor mass previously induced by HVS in an owl monkey, in the absence of demonstrable HVS (2, 37). In this report, chemical treatments were used in an effort to increase or activate type C viral expression from HVS tumor cells. A continuous lymphoblastic cell line (MLC-1), derived from an HVS-infected cotton-topped marmoset (*S. oedipus*) (32), was examined after exposure to halogenated pyrimidines by using techniques that have been reported to stimulate type C viral expression in cells of other species (1, 23, 33). Similar treatments have also been reported to enhance the frequency of EBV expression in human lymphoblastoid cell lines (12, 14). Cells and culture fluids were examined for HVS as well as type C viral expression after these treatments.

MATERIALS AND METHODS

Cell culture. A cell line (MLC-1) derived from an HVS-infected cotton-topped marmoset and which has been in continuous culture for over 3 years was generously supplied by Alan S. Rabson, National Cancer Institute, Bethesda, Md. (32). These cells, like other HVS tumor cell lines, produced little or no infectious virus, and only a few cells contained herpesvirus particles or viral-specified antigens (30). The cells contained HVS that could be demonstrated by cocultivation with permissive cells (e.g., Vero) and had surface properties consistent with those of thymus-dependent lymphocytes (35). MLC-1 cells were maintained on RPMI 1640 medium supplemented with 15% heat-inactivated (56 C for 60 min) fetal calf serum, 2 mM L-glutamine, 50 U of penicillin per ml, and 50 μg of streptomycin per ml. Cultures were incubated at 37 C in a humidified atmosphere of 5% CO_2 in air.

Detection of HVS. Indirect immunofluorescence assays for HVS membrane antigen (MA) and for internal early (EA) and late (LA) antigens were performed as previously described by using a fluorescein isothiocyanate-conjugated caprine anti-human gamma globulin reagent (Hyland Laboratories, Los Angeles, Calif.). Previously characterized sera from HVS-infected owl monkeys (28) were used at a 1:20 dilution. Serum with antibodies against LA but not EA had a titer of 160. Serum with antibodies against EA, LA, and MA titered 640 against each antigen and was used for live as well as acetone-fixed cell staining. HVS-associated MA was detected by using viable cells (27). For the detection of internal antigens (20), acetone-fixed (10 min) smears of cells were prepared on glass slides pretreated with a Teflon-epoxy coating (Roboz Surgical Instrument Co., Inc., Washington, D.C.). All acetone-fixed smears were kept at −70 C until used in immunofluorescence assays. A minimum of 500 cells was counted to determine the percentage of antigen-positive cells.

For the detection of infectious HVS, cell-free culture fluid was centrifuged at 65,000 × *g* for 90 min. The viral pellet was gently resuspended overnight at 2 C in 1/20 of the original volume of Hanks balanced salt solution (HBSS). Vero cell monolayers, grown in 16-mm culture wells (Linbro Chemical Co., New Haven, Conn.), were inoculated in quadruplicate with virus. After 1 h of adsorption at room temperature, the cells were overlaid with Eagle minimal essential medium containing 5% heat-inactivated fetal calf serum, 2 mM L-glutamine, and antibiotics. When cytopathic effects were well developed, the cells were fixed with acetic acid-methanol (1:3) and stained with 0.1% crystal violet as described (7). Plaques were counted with the use of a dissecting microscope. The cytopathic effects from sample cultures were confirmed to be HVS induced by indirect immunofluorescent microscopy.

Detection of type C virus. The presence of type C viruses was measured by the reverse transcriptase assay (4). Culture fluids were centrifuged at 65,000 × *g* for 90 min, and the potential virus-containing pellets were resuspended in 1/100 of the original volume of HBSS-virus buffer (1:1) and assayed for viral RNA-dependent DNA polymerase activity. Virus buffer contained 5 mM Tris (pH 7.9), 0.5 M KCl, 0.25% Triton X-100, and 20 mM dithiothreitol. This material was incubated in the presence of 50 mM Tris (pH 7.3), 1 mM $MnCl_2$, dATP, dCTP, and dGTP (80 μM each), tritiated thymidine triphosphate (5 to 6 mM, specific activity 18.5 Ci/mmol; New England Nuclear Corp., Boston, Mass.), and the synthetic template poly(rA)·d(pT)$_{10}$ (20 μg/ml) or poly(dA)·d(pT)$_{10}$ (60 μg/ml) (Calbiochem, San Diego, Calif.) in a final volume of 50 μliters for 20 min. The reaction was stopped by the addition of 50 μg of yeast transfer RNA, as carrier, and cold 10% trichloroacetic acid containing 0.02 M sodium pyrophosphate. Acid-precipitable material was collected on filter pads (Millipore Corp., Bedford, Mass.) followed by extensive washing with 5% trichloroacetic acid and 95% ethanol. The filters were air-dried and counted by using a liquid scintillation counter.

Chemical stimulations. MLC-1 cells were examined after exposure to halogenated pyrimidines by techniques that have been reported to stimulate type C viral expression in other species. Each type of stimulation experiment was repeated at least three times. The nucleic acid base analogues were purchased from Schwarz/Mann, Orangeburg, N.Y. Cells at a concentration of 10^6/ml were exposed to 5-bromo-2'-deoxyuridine (BUdR) at a concentration of 30 μg/ml for 24 h, washed twice with HBSS, and refed with complete medium (1). Cells and tissue culture fluids were then examined daily for evidence of HVS and type C viral expression. Similar experiments were performed by using 5-iodo-2'-deoxyuridine (IUdR) at 20 μg/ml for 30 h (23). In a third group of experiments, cells were exposed to media containing 20 μg of IUdR per ml for 3 days, washed twice, and refed with media containing 2% dimethyl sulfoxide for 4 days (33). Although the above treatments severely suppressed cellular replication, the viable cell population remained at approximately 10^6/ml throughout the experiments.

RESULTS

The exposure of the HVS lymphoid cell line MLC-1 to halogenated pyrimidines resulted in increased levels of HVS expression. In cells exposed to 20 μg of IUdR per ml for 30 h and then monitored for 8 days, or after exposure to BUdR at a concentration of 30 μg/ml for 24 h, immunofluorescent microscopy revealed a marked increase in HVS-associated antigens. HVS internal antigens reached peak levels 4 to 5 days after initial exposure to IUdR or BUdR and then gradually declined (Table 1). Generally, discordant sera that contained antibodies to LA but not to EA stained at slightly lower frequencies than did sera positive for antibodies to both EA and LA, suggesting that the expression of both antigenic groups had been stimulated. Usually internal antigens increased a minimum of five- to sevenfold by days 4 to 5 (unstimulated, 0.1 to 0.6%; stimulated, 2.6 to 12.9%); however, increases of up to 30-fold could be detected after BUdR stimulation. In stimulated as well as unstimulated (day 0) MLC-1 cells, both concordant and discordant sera exhibited the same staining pattern by fluorescent microscopy. This pattern appeared as focal accumulations, which were irregular in size and distributed throughout the nucleus. The difference observed was in the frequency of positive cells. The expression of HVS-associated MA showed a similar increase after treatment with BUdR (Table 1). Membrane antigen was increased four- to sixfold and tended to reach maximal levels at later times after the removal of the nucleic acid base analogue than did the internal antigens. Generally, IUdR induced higher levels of MA than did BUdR. No increased levels of cell-free infectious HVS were detected after these treatments (Table 1). MLC-1 cells were also examined to determine whether BUdR affected the ability to rescue HVS by cocultivation with Vero cells. No change in the frequency of HVS-positive cells was detected. The percentage of cells giving rise to plaques was about 25%, in agreement with original reports (32).

Cell-free tissue culture fluids were concentrated 100-fold by ultra-centrifugation and examined for the presence of an RNA-directed DNA polymerase with a preference for synthetic templates characteristic of type C viruses. Control preparations of viral (simian sarcoma virus, type 1 [SSV-1]) or uninfected Vero cell DNA polymerases were included in these assays. The SSV-1 polymerase synthesized at least 30-fold more trichloroacetic acid-precipitable material with poly(rA)·$d(pT)_{10}$ than with poly(dA)·$d(pT)_{10}$ as a synthetic template. The Vero cellular polymerases synthesized only one-tenth as much, or less, under identical conditions. The concentrated culture fluids from IUdR- or BUdR-stimulated MLC-1 cells exhibited only polymerase activity with a template specificity characteristic of normal cellular enzymes (Table 2). Samples collected at all days showed low levels of activity with poly(rA)·$d(pT)_{10}$ (rA/dA $\leq$ 0.3). An exception to this was noted at day 1 for BUdR (rA/dA = 1.02), where only background activity was detected.

Longer treatment with IUdR (20 μg/ml for 3 days) followed by media containing 2% dimethyl sulfoxide for 4 days has been reported to induce type C-like viral particles from human tumors (33). When MLC-1 cells were examined by this procedure, no type C virus could be activated as detected by reverse transcriptase

TABLE 1. *Stimulation of Herpesvirus saimiri expression in MLC-1 cells by the use of halogenated pyrimidines*

Day[a]	HVS-positive cells (%) after treatment with:						Infectious HVS/ml[g]
	IUdR[b]			BUdR[c]			
	LA[d]	EA-LA[e]	MA[f]	LA	EA-LA	MA	
0	0.2	0.3	0.7	0.3	0.1	0.4	5.0
1	0.7	0.5		1.1	1.7		
2	0.9	1.8	0.6	4.3	6.1	0.9	1.8
3	1.7	3.2		8.5	9.2		
4	3.6	6.5	1.9	10.9	12.7	1.3	3.8
5	2.4	6.7		12.4	11.0		
6	2.0	2.9	6.6	6.7	8.0	1.8	1.0
7	0.8	3.3		5.7	6.4		
8	0.4	3.9	ND[h]	4.2	4.8	1.5	ND

[a] Day after initial exposure to nucleic acid base analogue.

[b] 20 μg/ml for 30 h; daily samples were collected at 24-h intervals after the removal of IUdR. No infectious virus was detected as a result of this treatment.

[c] 30 μg/ml for 24 h; daily samples were collected at 24-h intervals after the removal of BUdR.

[d] Percent of HVS-positive cells as determined by indirect immunofluorescent microscopy using sera positive for antibodies to HVS late internal antigen.

[e] Percent of HVS-positive cells as determined by indirect immunofluorescent microscopy using sera positive for antibodies to HVS early and late antigens.

[f] Percent of HVS-positive cells as determined by indirect immunofluorescent microscopy on viable cells using sera positive for antibodies to HVS membrane antigen.

[g] Titer per milliliter of unconcentrated tissue culture fluids as determined by plaque assay in Vero cells.

[h] ND, Not determined.

TABLE 2. *Attempts to stimulate type C viral expression in MLC-1 cells by the use of halogenated pyrimidines*

Day[a]	IUdR[b]			BUdR[c]		
	Template[d]	Counts/min[e]	rA/dA[f]	Template	Counts/min	rA/dA
0	rA	332	0.26	rA	259	0.08
	dA	1,284		dA	3,213	
1	rA	298	0.08	rA	281	1.02
	dA	3,658		dA	276	
2	rA	596	0.10	rA	477	0.28
	dA	5,692		dA	1,719	
3	rA	487	0.04	rA	281	0.11
	dA	10,834		dA	2,675	
4	rA	651	0.03	rA	220	0.11
	dA	23,222		dA	2,082	
5	rA	1,656	0.12	rA	153	0.31
	dA	13,675		dA	487	
6	rA	981	0.11	ND[g]	ND	ND
	dA	8,758		ND	ND	ND
7	rA	833	0.19	rA	133	0.04
	dA	4,338		dA	3,094	
8	rA	537	0.06	rA	302	0.28
	dA	9,072		dA	1,062	

[a] Day after initial exposure to nucleic acid base analogue.

[b] 20 μg/ml for 30 h; daily samples were collected at 24-h intervals after the removal of IUdR.

[c] 30 μg/ml for 24 h; daily samples were collected at 24-h intervals after the removal of BUdR.

[d] rA, Poly(rA)·d(pT)$_{10}$; dA, Poly(dA)·d(pT)$_{10}$.

[e] After a reaction time of 20 min.

[f] Ratio of activities obtained by using the synthetic templates poly(rA)·d(pT)$_{10}$ and poly(dA)·d(pT)$_{10}$. For control preparations of viral enzyme, rA/dA ≥ 30.0; for cellular enzymes, rA/dA ≤ 0.10.

[g] ND, Not determined.

assays (Table 3). Both HVS internal antigens and MA were stimulated two- to threefold by such treatment. Once again, even though the expression of all three groups of HVS antigens was enhanced, no increase in infectious virus was demonstrated (Table 3).

DISCUSSION

Evidence for herpesvirus-type C virus interactions in several lymphoma systems has been reported (11, 17, 21, 29); however, HVS-induced lymphomas in New World primates have not indicated an interaction of this type. HVS that was purified from possible viral contaminants by velocity sedimentation and isopycnic banding centrifugation has been shown to retain its oncogenicity (22). Additionally, HVS inocula and tumor tissue, as well as other tissues from infected animals, have been examined by electron microscopy, uridine labeling, reverse transcriptase, and group-specific antigen radioimmunoassays, plus the hybridization of cellular RNA with DNA complementary to the SSV-1 genome, and have been found to be free of detectable type C virus (25). In the event that quantities of virus existed below the levels of detection of these assays, a continuous line of HVS tumor cells, MLC-1, was exposed to nucleic acid base analogues in an effort to stimulate the expression of a type C virus. Results of these studies, using techniques known to be effective in stimulating type C viruses of other species, have further shown that there is not a complex viral etiology for HVS-induced lymphoma in susceptible New World primates. This conclusion is also supported by hybridization experiments in which the DNA of normal marmoset tissue was reported to be free of sequences homologous to SSV-1, the gibbon-associated lymphoma virus, or the endogenous primate type C virus M-7 (5; R. Benveniste, C. J. Sherr, M. Lieber, R. Callahan, and G. Todaro, Abstr. Annu. Meet. Amer. Soc. Microbiol., V195, 1974).

In culture, HVS lymphoid cells produced little or no infectious virus, and only a few cells expressed viral antigens or contained herpesvirus particles upon electron microscopic examination (30, 32). In this respect they resembled certain human lymphoid cells derived from Burkitt's lymphoma patients which contain the EBV herpesvirus. The EBV viral genome could be stimulated in these cells by techniques similar to those reported for type C viruses. Arginine deprivation plus short- or long-term exposure to halogenated pyrimidines enhanced viral expression either partially or completely, depending upon the procedures used (12, 14, 15, 34). Short-term exposure of nonproducer human lymphoblastoid cell lines to nucleic acid base analogues generally resulted in incomplete activation of the EBV genome. Antigenic groups

TABLE 3. *IUdR-dimethyl sulfoxide stimulation of MLC-1 cells*[a]

Day	HVS-positive cells (%)			Infectious HVS/ml	Template	Counts/min	rA/dA
	LA	EA-LA	MA				
0	0.7	0.9	1.4	0.5	rA	430	0.07
					dA	6,547	
7	1.5	3.7	4.4	<0.25	rA	525	0.08
					dA	6,639	

[a] IUdR (20 μg/ml) for 3 days followed by media containing 2% dimethyl sulfoxide for 4 days. Other details as in Tables 1 and 2.

were stimulated (predominantly EA and MA), but more extensive treatment was required to induce the production of complete virus particles. By using HVS tumor cell cultures subjected to the conditions described above, we found that all three major viral antigenic groups were stimulated (most clearly LA and MA), whereas the production of infectious HVS was not. Electron microscopic examination of treated cells at the time of maximal antigenic stimulation detected only a few herpesvirus particles, suggesting that there was not a large increase in the production of non-infectious particles. The percentage of cells that contained herpes particles was not different from that originally reported for MLC-1 cells (2 out of 1,000) (32). It may be possible to stimulate infectious HVS from MLC-1 cells by using cultures adapted to high levels of BUdR, as had been reported for EBV cell lines. Current studies are investigating this possibility.

ACKNOWLEDGMENTS

We wish to thank Elizabeth Kingsbury for electron microscope examinations, and Donna West, Sue Goyer, and Douglas Stevens, Jr., for their technical assistance.

This work was supported by Public Health Service contract NO1-CP-43224 with The Virus Cancer Program of the National Cancer Institute.

LITERATURE CITED

1. Aaronson, S. A., G. J. Todaro, and E. M. Scolnick. 1971. Induction of murine C-type viruses from clonal lines of virus-free Balb/3T3 cells. Science **174**:157-159.
2. Ablashi, D. V., W. F. Loeb, G. R. Armstrong, S. S. Yang, M. G. Valerio, and R. H. Adamson. 1972. Oncogenicity of *Herpesvirus saimiri* induced lymphoma and the DNA polymerases of the lymphoma derived cell line and *Herpesvirus saimiri*. Medical Primatology 1972, p. 134-142. Proc. 3rd Conf. Exp. Med. Surg. Primates, Lyon 1972, part III. Karger, Basel.
3. Ablashi, D. V., W. F. Loeb, M. G. Valerio, R. H. Adamson, G. R. Armstrong, D. G. Bennett, and U. Heine. 1971. Malignant lymphoma with lymphocytic leukemia in owl monkeys induced by *Herpesvirus saimiri*. J. Nat. Cancer Inst. **47**:837-855.
4. Baltimore, D. 1970. Viral RNA dependent DNA polymerase. Nature (London) **229**:1209-1211.
5. Benveniste, R. E., R. Heinemann, G. L. Wilson, R. Callahan, and G. J. Todaro. 1974. Detection of baboon type C viral sequences in various primate tissues by molecular hybridization. J. Virol. **14**:56-67.
6. Calneck, B. W., T. Ubertini, and H. K. Adldinger. 1970. Viral antigens, virus particles, and infectivity of tissues from chickens with Marek's disease. J. Nat. Cancer Inst. **45**:341-351.
7. Daniel, M. D., H. Rabin, H. H. Barahona, and L. V. Melendez. 1971. *Herpesvirus saimiri*. III. Plaque formation under multi agar, methyl cellulose and starch overlays. Proc. Soc. Exp. Biol. Med. **136**:1192-1196.
8. Deinhardt, F. 1974. Oncogenic herpesviruses in species other than owl monkeys. A review. J. Med. Primatol. **3**:79-88.
9. Falk, L. A., L. G. Wolfe, and F. Deinhardt. 1972. Isolations of *Herpesvirus saimiri* from blood of squirrel monkeys (*Saimiri sciureus*). J. Nat. Cancer Inst. **48**:1499-1505.
10. Falk, L. A., L. G. Wolfe, and F. Deinhardt. 1973. *Herpesvirus saimiri*; experimental infection of squirrel monkeys (*Saimiri sciureus*). J. Nat. Cancer Inst. **51**:165-170.
11. Frankel, J. W., and V. Groupe. 1971. Interactions between Marek's disease herpesvirus and avian leucosis virus in tissue culture. Nature N. Biol. **234**:125-126.
12. Gerber, P., and S. Lucas. 1972. Epstein-Barr virus-associated antigens activated in human cells by 5-bromodeoxyuridine. Proc. Soc. Exp. Biol. Med. **141**:431-435.
13. Gross, P. A., C. K. Y. Fong, and G. D. Hsiung. 1973. Characterization of guinea pig C-type virus. Proc. Soc. Exp. Biol. Med. **143**:367-370.
14. Hampar, B., J. G. Derge, L. M. Martos, and J. L. Walker. 1972. Synthesis of Epstein-Barr virus after activation of the viral genome in a "virus-negative" human lymphoblastoid cell (Raji) made resistant to 5-bromodeoxyuridine. Proc. Nat. Acad. Sci. U.S.A. **69**:78-82.
15. Henle, W., and G. Henle. 1968. Effect of arginine-deficient media on the herpes-type virus associated with cultured Burkitt tumor cells. J. Virol. **2**:182-191.
16. Henle, W., and G. Henle. 1969. Evidence for a relationship of Epstein-Barr virus to Burkitt's lymphoma and nasopharyngeal carcinoma. Bibl. Haematol. **36**:706-713.
17. Hirumi, H., J. W. Frankel, C. O. Prickett, and K. Maramorosch. 1974. Brief communication: coexistence of particles resembling herpesvirus and type-C virus in feather follicle epithelium of chickens. J. Nat. Cancer Inst. **52**:303-306.
18. Hsiung, G. D., C. K. Y. Fong, and P. A. Gross. 1973. Oncogenic potential of guinea pig herpes- and C-type viruses. Cancer Res. **33**:1436-1442.
19. Hsiung, G. D., L. S. Kaplow, and J. Booss. 1971. Herpesvirus infection of guinea pigs. I. Isolation, characterization and pathogenicity. Amer. J. Epidemiol. **36**:556-573.
20. Klein, G., G. Pearson, A. Rabson, D. V. Ablashi, L. Falk, L. Wolfe, F. Deinhardt, and H. Rabin. 1973. Antibody reactions to *Herpesvirus saimiri* (HVS)-induced early and late antigens (EA and LA) in HVS-infected squirrel, marmoset and owl monkeys. Int. J. Cancer **12**:270-289.
21. Kufe, D., I. T. Magrath, J. L. Ziegler, and S. Spiegelman. 1973. Burkitt's tumors contain particles encapsulating RNA-instructed DNA polymerase and high molecular weight virus-related RNA. Proc. Nat. Acad. Sci. U.S.A. **70**:737-741.
22. Laufs, R., and B. Fleckenstein. 1972. Malignant lymphoma induced by partially purified *Herpesvirus saimiri* and recovery of infectious virus from tumorous lymph nodes. Med. Microbiol. Immunol. **158**:135-146.
23. Lowy, D. R., W. P. Rowe, N. Teich, and J. W. Hartley. 1971. Murine leukemia virus. High-frequency activation *in vitro* by 5-iododeoxyuridine and 5-bromodeoxyuridine. Science **174**:155-156.
24. Melendez, L. V., M. D. Daniel, R. D. Hunt, and F. G. Garcia. 1968. An apparently new herpesvirus from primary kidney cultures of the squirrel monkey (*Saimiri sciureus*). Lab. Anim. Care **18**:374-381.
25. Neubauer, R. H., W. C. Wallen, W. P. Parks, H. Rabin, and J. L. Cicmanec. 1974. Attempts to demonstrate type-C virus in normal and neoplastic tissues of nonhuman primate origin. Lab. Anim. Sci. **24**:235-240.
26. Neubauer, R. H., W. C. Wallen, H. Rabin, G. R. Pearson, and D. V. Ablashi. 1974. Virological investigations of *Herpesvirus saimiri*-infected owl monkeys. J. Med. Primatol. **3**:27-40.
27. Pearson, G., D. Ablashi, T. Orr, H. Rabin, and G.

Armstrong. 1972. Cytoplasmic and membrane immunofluorescence investigations on cells infected with *Herpesvirus saimiri*. J. Nat. Cancer Inst. **49**:1417-1424.

28. Pearson, G. R., T. Orr, H. Rabin, J. Cicmanec, D. Ablashi, and G. Armstrong. 1973. Antibody patterns to *Herpesvirus saimiri* (HVS)-induced antigens in owl monkeys infected with HVS. J. Nat. Cancer Inst. **51**: 1939-1943.
29. Peters, W. P., D. Kufe, J. Schlom, J. W. Frankel, C. O. Prickett, V. Groupe, and S. Speigelman. 1973. Biological and biochemical evidence for interaction between Marek's disease herpesvirus and avian leucosis virus *in vivo*. Proc. Nat. Acad. Sci. U.S.A. **70**:3175-3178.
30. Rabin, H., G. Pearson, H. C. Chopra, T. Orr, D. V. Ablashi, and G. R. Armstrong. 1973. Characteristics of *Herpesvirus saimiri*-induced lymphoma cells in tissue culture. In Vitro **9**:65-72.
31. Rabin. H., G. Pearson, G. Klein, D. Ablashi, W. Wallen, and J. Cicmanec. 1973. *Herpesvirus saimiri* antigens and virus recovery from cultured cells and antibody levels and virus isolations from squirrel monkeys. Amer. J. Phys. Anthropol. **38**:491-496.
32. Rabson, A. S., G. T. O'Conor, D. E. Lorenz, R. L. Kirschstein, F. Y. Legallais, and T. S. Tralka. 1971. Lymphoid cell-culture line derived from lymph node of marmoset infected with *Herpesvirus saimiri*—preliminary report. J. Nat. Cancer Inst. **46**:1099-1109.
33. Stewart, S. E., G. Kasnic, Jr., C. Draycott, and T. Ben. 1972. Activation of viruses in human tumors by 5-iododeoxyuridine and dimethyl sulfoxide. Science **175**:198-199.
34. Sugawara, K., F. Mizuno, and T. Osato. 1973. Induction of Epstein-Barr virus-related membrane antigens by 5-iododeoxyuridine in non-producer human lymphoblastoid cells. Nature N. Biol. **246**:70-71.
35. Wallen, W. C., R. H. Neubauer, and H. Rabin. 1974. *In vitro* immunological characteristics of lymphoid cells derived from owl monkeys infected with *Herpesvirus saimiri*. J. Med. Primatol. **3**:41-53.
36. Wolfe, L. G., L. A. Falk, and F. Deinhardt. 1971. Oncogenicity of *Herpesvirus saimiri* in marmoset monkeys. J. Nat. Cancer Inst. **4**:1145-1162.
37. Yang, S. S., D. Ablashi, G. Armstrong, and R. C. Ting. 1974. RNA-dependent DNA polymerase associated with a simian lymphoid cell line derived from a *Herpesvirus saimiri*-induced lymphoma. Int. J. Cancer **13**:82-90.