Herpesvirus Saimiri: *In Vitro* Sensitivity to Virus-Induced Interferon and to Polyriboinosinic Acid:Polyribocytidylic Acid[1] (35451)

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Virus-induced interferon and the synthetic double-stranded RNA, polyriboinosinic acid:polyribocytidylic acid (Poly I:C) have been shown to inhibit the growth of some members of the herpesviruses group (1–6). Interferon and Poly I:C have also been shown to inhibit the growth of tumors produced by viruses in mice and hamsters, as well as other tumors induced by chemical carcinogens or tumors not known to contain infectious oncogenic viruses (7–9).

Previous studies from this laboratory (10, 11) reported the isolation of a herpesvirus (H. saimiri) from the squirrel monkey (*Saimiri sciureus*) which is capable of inducing a malignant lymphoma of the reticulum cell type in nonhuman primates and in rabbits.

This report deals with the induction of resistance against H. saimiri in owl monkey kidney cultures treated with Newcastle disease virus (NDV) interferon or with Poly I:C. The significance of these findings is discussed.

Materials and Methods. Cells. Primary and continuous cultures of owl monkey kidney (OMK) were grown as monolayers on plastic 250-ml flasks (Falcon) with 15 ml of Eagle's minimum essential medium containing 10% fetal calf serum (MEM-10). Full grown monolayers were maintained in the same medium. For virus titration and virus plaque-inhibition assay of interferon the cells were transferred into 35- and 60-mm plastic dishes and kept at 37° in a 5% CO_2 atmosphere. Penicillin and streptomycin were added to all media employed in this work, at a concentration of 250 units/ml, and 250 μg/ml, respectively. OMK cultures at least 2-months-old were employed in these studies to avoid interference due to the presence of indigenous viruses.

Viruses. H. saimiri strain S-295C was used in these studies. Stocks were prepared and assayed in OMK cells. Vesicular stomatitis virus (VSV), strain New Jersey, was obtained from Dr. R. P. Hanson, The University of Wisconsin, Madison. Stocks were prepared in rabbit kidney primary cultures (RKP) and assayed in RKP and OMK cells. NDV, strain Kansas-Manhattan, was obtained from the same source and stock pools were grown and assayed in the allantoic chamber of 10-day-old embryonated eggs.

Virus titrations and plaque assay. The infectivity assay (ID_{50}) was carried out in triplicate 35-mm dishes. The plaque assay method and the agar overlay medium employed have been described elsewhere (12). The PFU determinations were done in 60-mm dishes and for counting of the plaques, the monolayers were stained with 1% gentian violet in 20% alcohol.

Preparation and assay of interferon. Stock owl monkey interferon (OM-IF) was prepared following a procedure described previously (13). Confluent OMK cultures were infected with NDV at a m.o.i. of approximately 3 PFU/cell and incubated in a 5% CO_2 atmosphere for 18–19 hr. To rid the interferon-containing medium of residual virus, the tissue culture fluids were centrifuged at 100,000*g* and dialyzed against large volumes of 0.03–0.1 *M* HCl (pH 2.0) followed by dialysis in buffered saline solution containing phenol red (pH 7.2). Interferon samples were stored at −20° and assayed by the plaque-inhibition technique with approximately 50–80 PFU of VSV.

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The end point of the titration was expressed as that dilution of interferon in which the plaque number was reduced to 50% compared with the control count. The stocks of owl monkey interferon prepared had a titer of 1:1000 per 3 ml. Other characteristics of this interferon were its acid stability, species specificity, failure to sediment at 10,000*g* for 1.5 hr, and heat stability.

Reagents. Poly I:C was purchased from Microbiological Associates, Bethesda, Maryland, in concentrations of 1 mg/ml. Each lot used was tested for its ability to protect OMK cells against 50 to 80 PFU of VSV. In every test, significative protection (50% plaque reduction) was obtained at concentrations of 0.1 to 0.01 μg/ml after a pretreatment time of 5–6 hr. Diethylaminoethyldextran (DEAE-D) from Pharmacia, Uppsala, Sweden, was prepared as a 1% solution in demineralized water, autoclaved for 10 min at 121° and stored at 4°.

Results. Expt. 1. Sensitivity to NDV-induced interferon. The sensitivity of H. saimiri to the effect of interferon was tested in OMK cultures grown in 35-mm dishes pretreated for 6 hr with 2 ml of a dilution 1:16 of OM-IF in Eagle's basal medium with 0.5% fetal calf serum (BME-0.5). After withdrawing the interferon medium the cultures were challenged with serial tenfold dilutions of H. saimiri, incubated for 1 hr at 37°, and overlaid with 2 ml of MEM-10. A second group of similarly treated cultures were overlaid with 2 ml of a dilution 1:16 of OM-IF and this was kept for the duration of the experiment. Untreated control dishes were also infected. Results presented in Fig. 1 indicate that in the first group of OM-IF-treated cultures (6 hr pretreatment alone) there was a delay of 24–48 hr in the onset of cytopathic effect (CPE). The onset of CPE was even further delayed (6 to 7 days) in the second group of cultures kept in OM-IF after infection. Furthermore, the degree of the CPE was reduced in both groups of interferon-treated cells when compared with the nontreated infected controls. By the 12th day after infection there was no more increase in virus titers in any of the three infected groups of cultures. The end-point titration

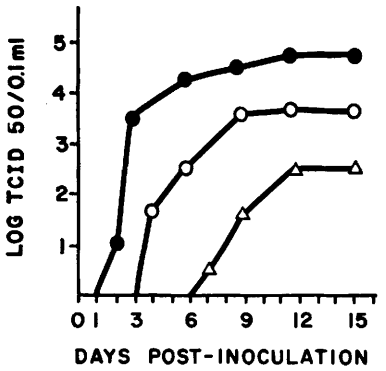

FIG. 1. Cytopathic effect of H. saimiri (log_{10} $TCID_{50}$) in OMK cultures: (○) cells treated with interferon for 6 hr before inoculation; (△) cells treated as the previous group and maintained with interferon after inoculation; (●) untreated control cultures.

indicated differences of approximately 1 and 2 logs in titer in cultures pretreated for 6 hr and in cultures kept in interferon medium for the duration of the experiment, respectively.

Expt. 2. Action of Poly I:C on H. saimiri multiplication. Several concentrations of Poly I:C in BME-0.5 ranging from 20 to 0.001 μg/ml were added to groups of different sets of OMK cultures. These were then incubated for 6 hr at 37° in a CO_2 incubator. Thereafter the monolayers were washed twice with 5 ml of BME-0.5 and infected with serial tenfold dilutions of H. saimiri. After an incubation period of 1 hr, the cultures were overlaid with agar medium and the plaques were counted 10–12 days later.

Pretreatment of the cultures with the polynucleotide at concentrations of 10 and 20 μg/ml gave approximately 1 log protection (81% inhibition) when compared with titrations done in untreated cultures (Table I). However, with 10 μg/ml there were occasional experiments in which protection was not constant. However, Poly I:C at a concentration of 20 μg/ml always gave a constant level of protection.

Expt. 3. Effect of DEAE-D on the protective activity of Poly I:C. Previous reports which indicate that the protective activity of polynucleotides could be enhanced by the presence of polycation DEAE-D (14, 15) led us to study its effect on OMK cultures infected with H. saimiri. These experiments

TABLE I. Inhibition of H. saimiri Multiplication in OMK Cultures by Poly I:C.

Poly I:C (μg/ml)	H. saimiri titer (PFU/0.1 ml)	Inhibition (%)
20	4.5×10^4	81.0
10	4.0×10^4	83.5
1	2.0×10^5	16.6
0.1	2.1×10^5	12.5
0.01	2.3×10^5	4.2
0.001	1.9×10^5	20.0
0.0 (control)	2.4×10^5	—

were conducted in the same way as Expt. 2, with the difference that a group of cultures were pretreated for 6 hr with a medium containing 50 μg/ml of DEAE-D and varying concentrations of Poly I:C. As shown in Table II, the protective activity of Poly I:C at 10 and 20 μg/ml was considerably increased by the addition of the polycation. The size of the plaques was also reduced. This enhancing activity was not observed when DEAE-D was added to polynucleotide concentrations lower than 10 μg/ml. The polycation as the only additive in the medium failed to induce resistance of H. saimiri infection, and did not have toxic effect on the OMK cell cultures.

Expt. 4. Effect of Poly I:C plus DEAE-D added 1 hr after the injection of the cells. To evaluate further the activity of the polycation as an enhancer of the protective effect of Poly I:C, OMK cultures were infected with tenfold dilutions of H. saimiri and, after an incubation period of 1 hr for virus adsorption, the cells were treated for 6 hr with Poly I:C at various concentrations, and with Poly I:C and DEAE-D.

TABLE II. Enhancement of Poly I:C Inhibition of H. saimiri Multiplication in OMK Cultures by DEAE-D.

Poly I:C (μg/ml)	DEAE-D (μg/ml)	H. saimiri titer (PFU/0.1 ml)	Inhibition (%)
20	0	1.2×10^4	94.0
10	0	3.5×10^4	82.5
1	0	1.4×10^5	30.0
20	50	3.0×10^2	99.9
10	50	6.3×10^2	99.7
1	50	1.6×10^5	20.0
0.1	50	4.5×10^5	0.0
0	50	2.4×10^5	0.0
0	0 (control)	2.0×10^5	—

The results presented in Table III show that when the cells were treated with the polynucleotide alone at concentrations of 40, 20, and 10 μg/ml, there was an induction of resistance against H. saimiri similar to the one observed in Expts. 2 and 3 in which the cells were treated with Poly I:C for 6 hr previous to the challenge with the virus. However, the resistance induced in cells preinoculated with the virus and then exposed to similar varying concentrations of the ribonucleotide was considerably enhanced by the inclusion in the inducer medium of 50 μg/ml of DEAE-D. When Poly I:C at concentrations of 40 and 20 μg/ml plus DEAE-D at the concentration previously indicated were used together, no visible plaques were observed with dilution 10^{-1}; however, microscopic areas of CPE were detected with the same dilution and occasionally with dilution 10^{-2}. With Poly I:C at 10 μg/ml plus DEAE-D, the protective activity was not as effective as with higher concentrations, though it was still quite high since almost 3 logs (99.5% inhibition) in virus titer reduction was observed. With this latter concentration of the polynucleotide a reduction in the size of the plaques was also observed.

TABLE III. Suppression of PFU Development in OMK Cultures Preinfected with H. saimiri by Poly I:C and DEAE-D.

Poly I:C (μg/ml)	DEAE-D (μg/ml)	H. saimiri titer (PFU/0.1 ml)	Inhibition (%)
40	0	3.3×10^4	76.5
20	0	3.0×10^4	78.5
10	0	4.9×10^4	65.0
1	0	1.0×10^5	28.5
40	50	0	100.0
20	50	0	100.0
10	50	3.3×10^2	99.5
1	50	1.2×10^5	14.2
0	50	1.5×10^5	0.0
0	0 (control)	1.4×10^5	—

Discussion. Previous studies have shown that interferon and interferon inducers could partially inhibit the replication of some members of the herpesvirus group, as well as to

delay the development of certain virus-induced leukemias and the growth of some tumors in animals (1–9). These studies led us to search for the possibility that H. saimiri, a recently recognized oncogenic herpesvirus (10, 11) could be controlled in its replication and oncogenic activity by interferon inducers.

Most herpesvirus infections tend to develop long-term carrier states which are not necessarily eliminated by the presence of circulating antibodies. Besides, some herpesviruses have been reported to be poor producers of interferon (1, 16, 17) and also to be relatively resistant to the action of interferon (1, 18). Glasgow *et al.* (1) think that the capacity of some of these viruses to circumvent the host interferon response may be one of the factors related to chronic infection by these agents. However, recent reports have indicated that potent interferon inducers can be effectively used for the treatment of herpes simplex keratitis in rabbits (2, 5), as well as to reduce the mortality in mice due to encephalitis produced by intracerebral administration of the same virus (6). Based on these findings, the suggestion has been made that animal infections produced by some herpesviruses may be treated with synthetic polynucleotides (4, 6).

The present results indicate that H. saimiri, like other herpesviruses, seems to be relatively resistant to a short-term treatment of the cells with virus-induced interferon. The interferon used in these experiments only delayed the appearance of H. saimiri-produced CPE and decreased the spread of cell destruction while the same interferon diluted 1:64 produced 50% plaque reduction of VSV. The relative effectiveness with which the cells are protected against H. saimiri, seems to depend upon the length of time that interferon is in contact with the cultures. Monolayers kept in interferon medium after infection were more effectively protected.

As reported for Herpesvirus hominis (3, 5) and for a human cytomegalovirus strain (4), microgram amounts of poly I:C induced effective resistance against a direct challenge with H. saimiri in OMK cells pretreated with the synthetic polynucleotide. The protective activity was even more remarkably enhanced when Poly I:C was added together with DEAE-D into the media previously or 1 hr after the infection of the cells (Expts. 3 and 4).

The observed potentiation of the protective activity of Poly I:C by the addition of DEAE-D, even in previously infected cells, might open new possibilities to study the means to increase the resistance of susceptible nonhuman primates against the malignancy produced by H. saimiri. At this point, however, it is important to consider the possibility that H. saimiri during its replication may be actually blocking the mechanism of interferon production, the immune response or perhaps both of these. These possibilities deserve investigation.

Summary. The sensitivity of H. saimiri to virus-induced interferon and to the synthetic polynucleotide Poly I:C was determined in OMK cell cultures. These studies indicated that H. saimiri is relatively insensitive to the interferon activity in cultures pretreated for 6 hr with NDV-induced interferon. The degree of protection was improved when the cells were kept in interferon medium after the challenge with the virus. A direct pretreatment of the cells with low doses of Poly I:C induced a good protection which was greatly increased by a simultaneous addition of DEAE-D in the medium. The action of the Poly I:C/DEAE-D complex was considerably more effective when the treatment was initiated 1 hr after the inoculation of the virus. These findings suggest that the use of synthetic polynucleotides might prove to be useful for the inhibition of the malignancy produced by H. saimiri in susceptible nonhuman primates.

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