Effect of Poly I:C-induced Interferon on Herpes Virus Hominis Infection in Vitro and in Vivo

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(Z. Naturforsch. **27 b**, 220—222 [1972]; received January 19, 1972)

Dedicated to Prof. WERNER SCHÄFER *on the occasion of his 60th birthday*

In this study the effect of poly I:C-induced interferon on Herpesvirus hominis (HVH) infection *in vitro* and *in vivo* was investigated.

The *in vitro* results revealed a significant effect of interferon on infections of HVH strains of different origin in mouse embryonic cells.

The *in vivo* experiments performed in the HVH-mouse system indicate that the effective induction of interferon in an aminal increases its resistance to subsequent infection with HVH.

This study represents results from an investigation of three related problems. This first problem was to determine whether mouse serum containing poly I:C-induced interferon was an effective inhibitor of herpes virus hominis (HVH) infection in mice. The second problem was prompted by the previous work of HAMILTON et al. [1], who showed that addition of complexed polynucleotides to cell cultures resulted in a reduced number of plaques in a plaque test for HVH. Our aim was to find whether the addition of poly I:C-induced interferon to cell cultures would result in an even greater decrease in the number of plaques than that obtained by HAMILTON et al. [1]. Finally, the sensitivity to interferon-containing sera of HVH strains of different origins was investigated.

Mice of the incest strain STU were used, not only for the production of interferon, but also for the *in vivo* tests, and as the source of embryonic cells for the *in vitro* tests.

Studies on the effect of interferon on HVH-induced encephalitis were reported by CATALANO and BARON [2]. Also, the effect of interferon induced by complexed polynucleotides on herpes virus infections, *in vitro* and *in vivo*, was studied by HAMILTON et al. [1].

Materials and Methods

Virus: The strains of HVH used in this study were: (1) WAL-oral; (2) HOF, ALD-genital; and (3) TUP-corneal. The isolation procedure and analysis of the biological properties of these strains were described previously by MUNK and DONNER [3]. All strains were passaged in HeLa cells. Strain WAL, used in the *in vivo* experiments, was passaged in mouse brain.

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Cell culture: Primary mouse embryonic cell cultures were prepared by trypsinization of embryonic cells from fetal mice, or occasionally, from mice 0 to 24 hours old. Secondary cell cultures were obtained by trypsinizing primary mouse embryonic cell cultures grown in plastic petri dishes (6.0 cm diameter). These were used for the plaque reduction assay. In one experiment, plaque assays were performed with HeLa cells.

All cell cultures were maintained in Hank's solution containing 0.5% lactalbumin, 10% calf serum and antibiotica.

Poly I:C: The poly I:C was kindly supplied by Boehringer GmbH, Tutzing, Germany. The lyophylized preparation contained 10% double-stranded poly I:C and 90% NaCl – PO_4-buffer, pH 7.0. The dosages of poly I:C chosen were in the same range as those used by FIELD et al. [4].

Plaque assay: For plaque assay according to the method of DULBECCO and VOGT [5], confluent cell cultures grown in plastic petri dishes (6.0 cm diameter) were inoculated with 0.5 ml virus suspension. Two hours post infection, 4.0 ml nutritional agar overlay was added. Three days post infection, the second nutritional agar overlay was added; five days post infection, the third nutritional agar overlay containing the vital stain was added. The petri dishes were incubated at 37 °C in 5 percent CO_2. The cultures were then checked for the presence of plaques.

Plaque reduction test: Cells for the plaque reduction test were prepared as described for the plaque assay. However, 0.5 ml mouse serum containing the poly I:C-induced interferon and 1.5 ml nutritional medium was added to the petri dish cultures 16 hours before addition of the virus (LINDEMANN and GIFFORD [6]).

Mouse strain: The incest mouse strain STU (SCHÄFER, Tübingen), a strain with more than 100 incest

generations, was used. For the induction of interferon and for the *in vivo* experiments, only adult mice were used. The mice were fed with pellet standard diet (Altromin)-water.

Statistics: The results of the plaque reduction test were evaluated using tables based on the statistical method of LORENZ [7]. These tables can be used to demonstrate whether the number of plaques is significantly reduced.

To evaluate the results of the *in vivo* tests, the LD_{50} of the treated animals and of the controls were compared using the method of Spearman and Kaerber (LORENZ [8]). This method was simplified by using the same number of animals per each virus dilution and by using constant differences between the infectious doses (symmetric test).

Experiments and Results

Effect of poly I:C-induced interferon in vitro: Interferon was induced by intraperitoneal (i. p.) injection of poly I:C into adult mice. The effect of the interferon-containing sera derived from mice injected with different doses of poly I:C was determined. This was done using the plaque reduction test with the WAL strain of HVH. In our experiments, poly I:C in injections of 100, 200, 400 and 600 μg/mouse was used. HAMILTON et al. [1] previously tested the *in vitro* effect of interferon induced by intraperitoneal injections of 150 μg poly I:C per mouse. FIELD et al. [4] tested a similar effect with interferon induced by 135 – 525 μg poly I:C per mouse. Two and one-half hours after the injection, the mice were bled; 0.5 ml of serum from these mice was added to each petri dish for the plaque reduction test.

The results of this experiment (Table 1) indicate (a) the sensitivity of HVH infection, strain WAL, *in vitro*, to poly I:C-induced interferon and (b) that all of the concentrations of poly I:C tested were sufficient to reduce the number of plaques to a number no more than about 5% of the number in the control culture, which means a significant redution with a probability of error of less than 1 percent.

Herpesvirus Strain WAL Virus dilution	Dosage of Poly I : C used 100 μg	200 μg	400 μg	600 μg	Control
	Number of plaques				
10^{-2}	2	4	1	2	140
10^{-3}	0	0	0	1	11
10^{-4}	0	0	0	0	0

Table 1. Results of plaque reduction test using different amounts of poly I:C for the induction of interferon in mice.

To determine whether strains of HVH of different origins differed in their reaction to poly I:C-induced interferon, the same experiment was performed using oral strain WAL, genital strain ALD, and corneal strain TUP. Poly I:C in doses of 200 μg was used for interferon production.

Virus Dilution	Herpesvirus Strains WAL		ALD		TUP	
	Treated with interferon-containing serum	Control	Treated with interferon-containing serum	Control	Treated with interferon-containing serum	Control
	Number of Plaques					
10^{-1}	120	200	10	200	200	200
10^{-2}	4	130	0	25	200	200
10^{-3}	0	10	0	0	42	200
10^{-4}	0	0	8	0	62	112
10^{-5}	—	—	—	—	0	0

Table 2. Results of plaque reduction test using HVH strains of oral, genital and corneal origin.

The results shown in Table 2 indicate that the application of poly I:C-induced interferon resulted in a significant decrease in the number of plaques for each of the three strains. The decrease was of the same magnitude for strains WAL and ALD, reducing the number of plaques to about five per cent or less than in the control cultures. With strain TUP, however, the plaque number was only reduced by 80% at the 10^{-3} dilution.

Effect of poly I:C-induced interferon on virus production in vitro by HVH strains of different origins: The production of cell-associated HVH was investigated by determining the amount of progeny virus in mouse embryonic cells at 15 hours post infection. Parallel embryonic mouse cell cultures were infected with the same inocula of virus (strain WAL). Half of the cell cultures were treated with mouse serum containing poly I:C-induced interferon, as described above, the other half, with normal mouse serum. The virus yield of both was determined by plaque assay of HeLa cells. Two HVH strains were included in this experiment: WAL (oral) and HOF (genital).

The production of plaques in cells from HVH strains of both genital and non-genital origin was similarly reduced by the interferon-containing mouse serum (Table 3).

Virus Dilution	Herpesvirus Strains WAL		HOF	
	Treated with interferon-containing serum	Control	Treated with interferon-containing serum	Control
	Number of Plaques			
10^{-2}	140	200	70	200
10^{-3}	23	65	12	70
10^{-4}	0	4	0	50
10^{-5}	0	0	0	3
10^{-6}	—	—	0	1

Table 3. Reduction of the yield, 15 hrs. post infection, of cell-associated HVH (strains WAL and HOF) as determined by plaque assay of HeLa cells.

Effect of poly I:C-induced interferon on HVH infection in mice: The effect of poly I:C-induced interferon on HVH infection *in vivo* was investigated with the strain WAL. Two groups of mice were injected with 300 or 600 μg poly I:C, respectively. Two and one-half hours after injection of the drug, both groups and one control group were injected with various dilutions of HVH.

Herpesvirus Strain WAL	Dosage of Poly I:C used 300 μg	600 μg	Control
$-\log LD_{50}$	3,5	3.9	4.7

Table 4. Effect of poly I:C-induced interferon on HVH infection in mice. Five mice per virus dilution were used. Poly I:C was injected i.p. $2^1/_2$ hrs. before infection.

The LD_{50} of the treated mice and of the controls was compared. A significant increase, of the order of 10-fold, in the LD_{50} of HVH-infected mice after induction of interferon by both 300 μg and 600 μg poly I:C was observed (Table 4). This result, which was reproduced repeatedly, indicates that poly I:C induction of interferon leads to significant protection against HVH infection *in vivo* using the method of statistical analysis described above, we conclude that the probability that this conclusion is correct is 95 percent.

Discussion

We investigated the effect of poly I:C-induced interferon on HVH infection *in vitro* and *in vivo*. FIELD et al. [4] described the production of interferon induced by complexed polynucleotides. Interferon was found in the serum of animals injected with the polynucleotide compound, and was identified on the basis of its biological and biochemical properties.

Based on these results, we used the serum of mice injected with various doses of poly I:C as a source of interferon and tested its effectiveness with the plaque reduction test. Our results indicate that interferon induced *in vivo* for use *in vitro* seems to be more effective than are complexed polynucleotides applied directly to cells subsequently infected with HVH as described in the experiments of HAMILTON et al. [1].

Our results revealed both (a) a significant effect of interferon on HVH infections in secondary mouse embryonic cells derived from the same mouse strain, in which the interferon was induced, and (b) in addition, that the effect on infection and virus production of HVH strains of different origins was significant for the three strains tested, especially for the oral and genital strains.

The *in vivo* experiments were performed only with the HVH strain WAL-mouse system. The results revealed a significant increase (approximately 10-fold) in the resistance of interferon-treated mice to infection with HVH as compared with the controls. Although we administered only a single dose of poly I:C, our results were in good correlation with those of HAMILTON et al. [1], who administered the interferon-inducing drug in repeated injections.

The results of these experiments indicate that the effective induction of interferon in an animal increases its resistance to subsequent infection with HVH. Thus, poly I:C induction of interferon offers an effective treatment for HVH infection *in vivo*.

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