Protection Against Herpes Virus and Encephalomyocarditis Virus Encephalitis with a Double-Stranded RNA Inducer of Interferon (34544)

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Herpes simplex virus (HSV) encephalitis is probably the most common cause of fatal, sporadic encephalitis in the United States (1). In light of recent advances in ability to stimulate interferon (see below), it is clearly important to determine the effect of potent interferon inducers on HSV encephalitis.

In a previous report (2), an early, homologous interference effect was demonstrated in HSV encephalitis of mice after a subcutaneous infection with HSV. Since subcutaneous HSV infection also was found to afford protection against encephalomyocarditis virus (EMCV) encephalitis, it was concluded that both the homologous and heterologous interference effects could be due to interferon (Catalano, Moosy, and Sell, unpublished).

A variety of synthetic substances have been shown experimentally to induce interferon. These synthetic inducers include pyran copolymer (3, 4), cyclohexamide (5), and RNA polynucleotides (6, 7). Polyinosinic·polycytidylic ribonucleic acid (In·Cn) has shown to be among the most active of the latter substances and induces interferon both *in vivo* and *in vitro*. Recently, In·Cn has been shown to eradicate established HSV keratoconjunctivitis in the rabbit (8). For these reasons, a study was undertaken to determine whether this material could afford protection in mice with experimental HSV encephalitis.

In addition, interferon-produced protection against encephalitis caused by an interferon-sensitive virus, EMCV (9, 10), was compared to the protection afforded against encephalitis due to the relatively interferon-resistant HSV (11–13).

Materials and Methods. Specific pathogen free male C3H/NIH mice (16–20 g) were used for all experiments. Stocks of a Type I HSV (strain VR_3)[2] were grown in primary rabbit kidney (PRK) cell monolayers in 32-oz glass bottles, and the virus was titered in roller tissue culture tubes containing PRK cells (Microbiological Associates, Bethesda, Maryland). Stocks of EMCV (r+ strain)[3] were grown and titered in primary mouse embryo cell monolayers in 32-oz glass bottles and tissue culture tubes, respectively. Endpoints were determined on Day 5 and were calculated by the Reed-Muench method (14). The stocks of HSV and EMCV contained $10^{7.6}$ and $10^{7.5}$ $TCID_{50}$/1.0 ml, respectively.

Double-stranded polyinosinic·polycytidylic ribonucleic acid duplexes were prepared as described previously (7, 8); the final concentration of this material was 1.0 mg/1.0 ml. The diluent solution served as the control material and consisted of 0.01 *M* phosphate-buffered saline (PBS) at pH 7.2 containing 5×10^{-3} *M* MgCl.

The number of mice used in each group is detailed under figure legends. Mice were treated with either 200 μg (0.2 ml) In·Cn or 0.2 ml PBS given intraperitoneally. Pretreated animals were challenged intracerebrally (ic) 18–20 hr later under light ether anesthesia. For all ic inoculations, volumes of 0.03 ml and 0.02 ml were used for EMCV and HSV, respectively. Subsequently, the mice were

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TABLE I. Amount of Interferon (in units[a]) Assayed in Serum and Brain After a Single Intraperitoneal Injection of 200 μg of In • Cn in C3H Mice.

	Hours after treatment	Serum[b] (units/ml)	Brain[b] (units/g tissue)
Controls	0	<100	<10
	4	<100	<10
	8	<100	<10
	25	<100	<10
In • Cn	2	1000	1000
	4	1000	1000
	8	5000	1000
	25	800	5000

[a] Unit of interferon is the reciprocal of the highest dilution of the sample which inhibits the single-cycle yield of vesicular stomatitis virus by 0.5 $\log_{10}$ in primary mouse embryo cell cultures.

[b] Pool of eight mice.

given 0.1-ml doses of either In•Cn (100 μg) or PBS three times per week for a total of 5 doses. Animals were observed for at least 21 days after the ic injection of virus. Deaths due to ic inoculation trauma or In•Cn toxicity were rare and considered nonspecific and discarded.

A unit of interferon was determined as the reciprocal of the highest dilution of the sample which inhibited the single cycle yield of vesicular stomatitis virus (Indiana strain) by 0.5 $\log_{10}$ in primary mouse embryo cell cultures (15).

Statistical analysis was performed using the chi-square method or Fisher's exact (F.E.) test when appropriate. Protection was defined as control mortality minus experimental mortality, divided by control mortality.

Results. Table I shows the time course of the interferon response in serum and brain after the intraperitoneal administration of a single dose of 200 μg of In•Cn. The maximum interferon response appeared earlier in the serum than in the brain. Since intracerebral virus challenge was at 18–20 hr after the first dose of In•Cn, the challenge occurred at near maximal levels of brain interferon.

Results presented in Figs. 1 and 2 represent the combined data from two experiments. The results of each experiment were sufficiently similar so that data could be pooled to obtain larger populations for statistical analysis. Since very few deaths occurred after Day 10, none of which altered the statistical results, data are presented only for the first 10 days (after ic infection) in each group.

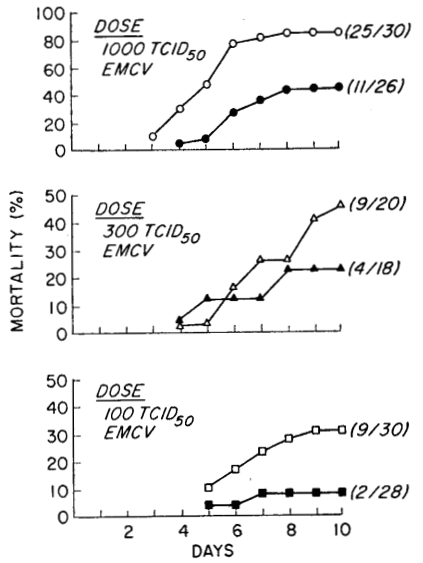

FIG. 1. EMCV. Day of death versus mortality and approximate number of $TCID_{50}$ in ic inoculum. Open symbols indicate PBS-treated controls; closed symbols are animals treated with In•Cn. Note that there are different ordinate scales in order to portray differences at different mortality levels. Numbers in parentheses show number of dead animals at Day 10 over total number of animals used in that group.

EMCV. Figure 1 illustrates that a clear-cut protective effect was achieved by intraperitoneal treatment with In•Cn against ic infection with EMCV. For 1000 $TCID_{50}$ and 100 $TCID_{50}$ of EMCV the p values were .003 ($\chi^2 = 8.50$) and .05 (F.E. = .047) respectively. At 300 $TCID_{50}$, with the number of animals used, results approached significance (F.E. = .189). The calculated percent protection for 1000, 300, and 100 $TCID_{50}$ was 49.1%, 50.7%, and 76.3%, respectively. Thus it is felt that the mortality found for the 300 $TCID_{50}$ group was in accord with the other dosages, even though statistical significance was not found because fewer animals were used in this group

HSV. Results are presented in Fig. 2. Sig-

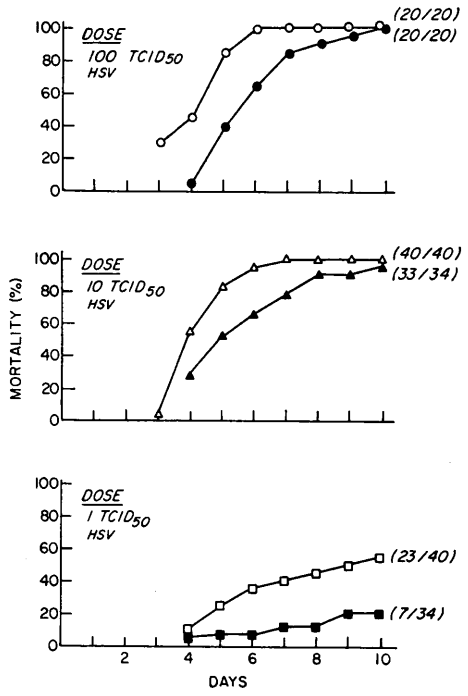

FIG. 2. HSV. Day of death versus mortality and approximate number of $TCID_{50}$ in ic inoculum. Open symbols indicate PBS-treated animals; closed symbols are animals treated with In·Cn. Numbers in parentheses show number of dead animals at Day 10 over total number of animals used in that group.

nificant decrease in mortality ($\chi^2 = 15.27$, $p = .002$) was achieved only against 1 $TCID_{50}$ of HSV, the percent protection being 64.2%. As can be seen, however, there is a full 1-day delay in onset of death and subsequent delay of mortality at 100 and 10 $TCID_{50}$. Furthermore, at the times when PBS controls had reached 100% mortality in the latter two groups, a significant number of In·Cn treated animals were surviving ($p \leq .01$). Although the data are not presented, this was also true for animals treated with In·Cn and challenged with 10,000, 1,000, and 300 $TCID_{50}$ of HSV given ic. However, all of these animals also eventually died.

In an additional experiment, animals were pretreated with only 100 μg of In·Cn, then given 50 μg In·Cn three times a week for a total of five doses. No protection (9 dead out of 20 animals treated *vs* 23 dead out of 40 controls) was attained at 1 $TCID_{50}$. Although statistical significance was not achieved with the number of animals used, the small amount of protection attained as compared with Fig. 1, probably represents an effect of the lower dose of In·Cn.

Figure 3 summarizes the percent protection observed in mice receiving the standard treatment with In·Cn as a function of (1) the number of LD_{50} contained in the ic inoculum and (2) as a function of mortality in controls. Each point represents the sum of two experiments, including the results from Figs. 1 and 2 plus two additional experiments. As can be seen, equal protection was observed for both EMCV and HSV when the lethality of the ic challenge was low. Protection against HSV encephalitis fell off rapidly between 4–8 LD_{50} and disappeared at 10 LD_{50}. Although the protective effect of

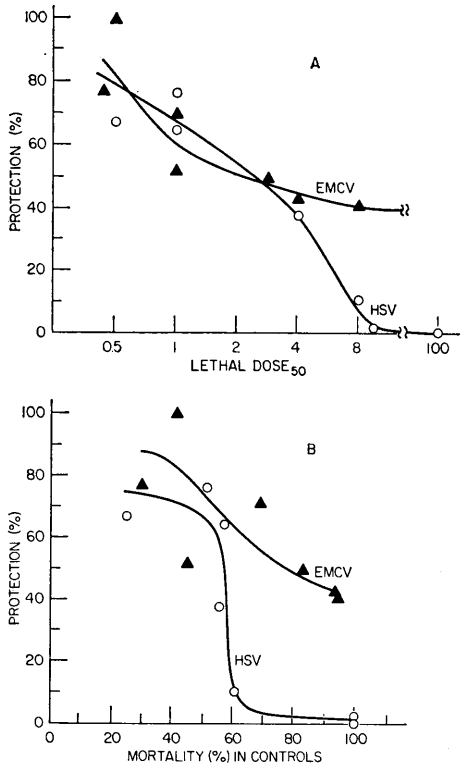

FIG. 3. Protection afforded by In·Cn as (a) function of the LD_{50} of the ic inoculum or (b) a function of the percentage of mortality in controls. One 1 LD_{50} of EMCV represents approximately 300 $TCID_{50}$; 1 LD_{50} of HSV (VR_3) is about 1 $TCID_{50}$ of herpesvirus.

In·Cn against EMCV also fell, 41% protection was still evident at 8 LD_{50}.

Discussion. As has been pointed out in the introduction, previous information has generally regarded HSV as relatively resistant to interferon. The present findings show that HSV, in comparison with the known interferon-sensitive EMCV, appears to be more resistant to interferon induced by In·Cn, but this may be related to the lethal magnitude of the challenge dose as considered below. Our data demonstrate that if a sufficiently small dose of HSV is given, induction of interferon *in vivo* can protect against herpes encephalitis. This confirms the previous work with interferon and ocular infection in animals with HSV (8, 10, 16). It would also appear that the early homologous and heterologous interference effects (2) of a subcutaneous HSV infection in mice, followed by ic challenge with HSV and EMCV, could possibly be due to interferon elicited by the subcutaneous infection. In particular, interferon-mediated interference would account for the early and short-lived nature of the protection. Further studies are needed, however, to establish this interpretation.

Although EMCV has been felt to be very sensitive to interferon, the data illustrate that at about 1 LD_{50} (approximately 300 $TCID_{50}$), 60% protection was achieved with In·Cn treatment, whereas such treatment and challenge ic with 1 LD_{50} (1 $TCID_{50}$) of HSV resulted in 70% protection. The present findings raise the possibility that sufficiently low doses of interferon-sensitive and resistant viruses will manifest the same levels of protection in animals treated with high titered interferon or potent interferon inducers.

Summary. The interferon sensitivities of intracerebrally administered HSV (VR_3 strain) and EMCV (r+ strain) were determined in mice with a potent inducer of circulating interferon, polyinosinic·polycytidylic ribonucleic acid (In·Cn). With the treatment as described, delay in mortality occurred at most challenge doses of HSV and EMCV. Significant delay in mortality and increased survival was obtained with 1000 and 100 $TCID_{50}$ of EMCV, whereas, significant protection was achieved for only 1 $TCID_{50}$ of HSV with mortality decreasing to about 12–20% as compared to 52–58% in the controls. The data show that sufficient interferon can be induced in the mouse with In·Cn to significantly alter intracerebral infection with HSV and EMCV. The protective effect of In·Cn against intracerebral infection of mice with HSV and EMCV was equal only when the challenge dose of each was sufficiently low. The protective effect against HSV was lost with increasing virus doses of virus whereas the protective effect against EMCV decreased only slightly with increasing virus dose.

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