INFECTION AND IMMUNITY, Feb. 1972, p. 199-202

Vol. 5, No. 2
Printed in U.S.A.

Effect of an Interferon Stimulator, Polyinosinic: Polycytidylic Acid, on Experimental Fungus Infections

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Received for publication 8 September 1971

In the present study, the administration of an interferon stimulator, polyinosinic: polycytidylic acid (In:Cn), increased the severity of both experimental *Candida albicans* and *Coccidioides immitis* infections in mice, as evidenced by earlier time of death and higher final mortality. In addition, kidney tissue levels of *C. albicans* at 3 and 5 days after infection were found to be about 40- to 300-fold higher in In:Cn treated mice than in control mice. Experimental *Cryptococcus neoformans* infection, however, was not significantly affected by In:Cn treatment.

Recently it has been shown that interferon or interferon stimulators may exert a protective effect in a number of experimental infections with nonviral agents. Interferon and four different interferon stimulators, including polyinosinic: polycytidylic acid (In:Cn), have been reported to protect mice against *Plasmodium berghei* (4). In addition, In:Cn has been reported to protect mice against infection with a number of different bacteria, although this effect may not always be mediated by interferon (11). Interferon itself has been reported to inhibit the growth of *Toxoplasma gondi* in chick and mouse cell monolayers (9).

The present investigation was designed to study the effect of In:Cn on three experimental fungus infections of mice.

MATERIALS AND METHODS

NIH general-purpose Swiss female mice (18 to 22 g) were used throughout. Double-stranded In:Cn ribonucleic acid duplexes were prepared as described previously (2); the final concentration of this material was 0.5 mg/ml. Interferon assays were determined by exposing mouse L cells to the interferon samples overnight. The L cells were then washed and challenged with GD-7 virus at an input multiplicity of 10:1. The hemagglutinin yield of GD-7 virus was determined after another overnight incubation. The interferon titer was taken as the reciprocal of the highest dilution of serum which reduced the hemagglutinin yield of GD-7 virus by 0.5 $\log_{10}$. Titers were adjusted in accordance with a titer obtained with a laboratory reference interferon which was included in each assay. The international reference mouse serum interferon titered $10^{4.5}$ units/ml.

Strain B-311 of *Candida albicans* was used in the present studies. Inocula were prepared from organisms grown on Sabouraud agar slants for 48 hr at 37 C, and the yeast cell growth was harvested, washed, and suspended in pyrogen-free 0.9% NaCl solution. Organisms were counted directly in a hemocytometer, and the inoculum was adjusted to contain 5×10^6 yeast cells in 0.3 ml. In addition, serial 10-fold dilutions were plated for determination of viable count. Strain 3769 of *Crytococcus neoformans* was also used in the present studies. Inocula were prepared in a manner identical to that described for *C. albicans*. The final inocula also contained 5×10^5 yeast cells in 0.3 ml.

Coccidioides immitis strain 6228 was used and had been cultured at ambient temperature on Sabouraud agar for 15 days before these experiments. Inocula were prepared in the manner already described, except that the final concentration was 100 arthospores per 0.3 ml.

The number of *C. albicans* viable units in the kidneys of infected mice was determined by sacrificing control and In:Cn-treated mice 3 or 5 days after infection. Organs were aseptically removed and homogenized in saline by using sterile glass tissue grinders, and colony counts were determined from pour plate cultures.

In each experiment there were three groups of 20 mice. Group 1 received a single dose of 100 μg of In:Cn in 0.2 ml 1 day before the injection of fungi. Group 2 received 100 μg of In:Cn 1 day before the injection and 1 day after the injection and then every 2 days for a total of seven doses. Group 3 in all experiments was the untreated control group and received 0.2 ml of phosphate-buffered saline (PBS) instead of In:Cn every 2 days, beginning 1 day before fungus injection, for a total of seven doses. Mice of all three groups were injected with the fungus intra-

venously (iv) on day 0. In addition to these three groups, in each experiment 10 mice were injected only with In:Cn every 2 days for seven doses (In:Cn controls). The percentages of survivors were compared by the chi-squared test for proportions (1 degree of freedom).

RESULTS

(i) C. albicans. The effect of In:Cn on *C. albicans* infection is shown in Fig. 1. Mice receiving multiple doses (group 2) of In:Cn began dying earlier and had a significantly higher final mortality than infected mice which had not received In:Cn (group 3). Mice receiving a single dose of In:Cn (group 1) had a higher final mortality than control mice (group 3), but this difference was not statistically significant. In a second experiment, essentially identical results were obtained; when the two experiments were combined, mortality in group 1 was significantly greater than the control group ($P < 0.05$).

(ii) C. immitis. The effect of In:Cn on *C. immitis* infection is shown in Fig. 2. Again mice receiving multiple doses of In:Cn (group 2) died earlier and had a significantly higher final mortality than the untreated controls. Mice receiving a single dose of In:Cn (group 1) also had a higher final mortality than the controls (group 3), but this difference was again not statistically significant. A second experiment yielded similar results. When the two experiments were combined, mice receiving a single dose of In:Cn had a significantly higher final mortality than the untreated controls ($P < 0.05$).

(iii) C. neoformans. The effect of In:Cn on *C. neoformans* infection is shown in Fig. 3. Mice receiving multiple doses of In:Cn died somewhat sooner than control mice, but this difference is not statistically significant. Control mice receiving just *C. neoformans* died more rapidly than the control mice in previous experiments.

Because it had been reported that In:Cn would protect against intracranial (ic) challenge with *C. neoformans* but not against iv challenge with this fungus (8), we studied the effect of In:Cn on ic challenge with this fungus. The results from this experiment are shown in Fig. 4. Mice receiving multiple injections of In:Cn did die somewhat later than control mice not receiving In:Cn, but this difference again was not statistically significant.

Mice receiving In:Cn alone did not sicken or die.

C. albicans tissue levels. The effect of In:Cn on tissue levels of *C. albicans* in the kidneys of treated mice is shown in Table 1. On days 3 and 5 after injection of fungi, In:Cn-treated mice had about 40- to 300-fold higher levels of *C. albicans* in their kidneys.

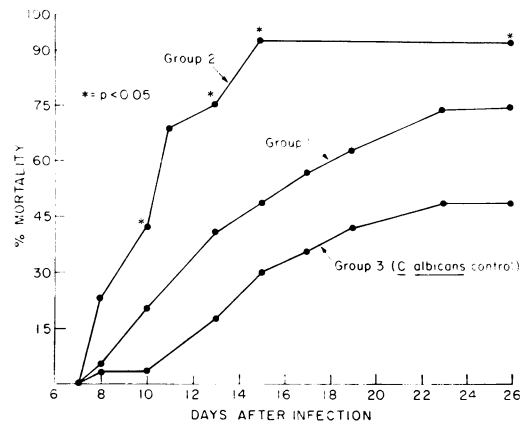

FIG. 1. *Effect of a single (group 1) or multiple (group 2) dose of In:Cn on iv C. albicans infection in mice.*

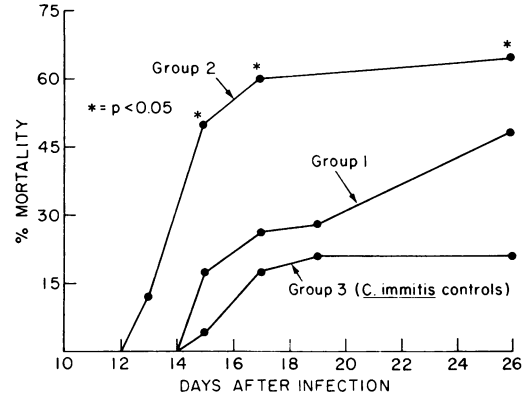

FIG. 2. *Effect of a single (group 1) or multiple (group 2) dose of In:Cn on iv C. immitis infection in mice.*

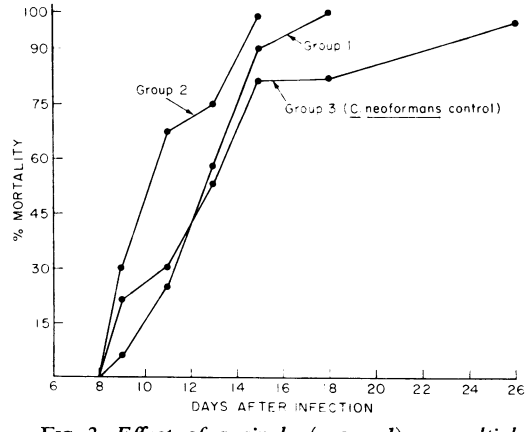

FIG. 3. *Effect of a single (group 1) or multiple (group 2) dose of In:Cn on iv C. neoformans infection in mice.*

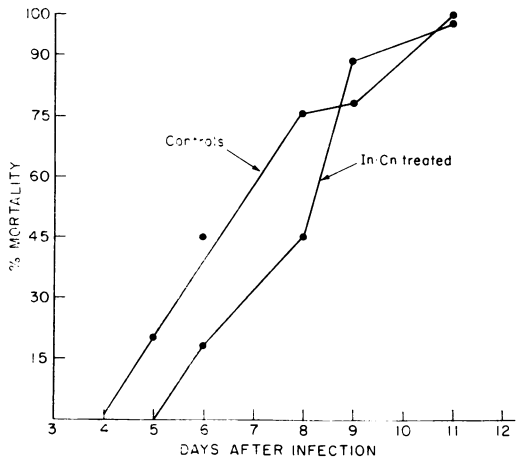

FIG. 4. *Effect of multiple doses of In:Cn on ic C. neoformans infection in mice.*

TABLE 1. *Effect of In:Cn on kidney tissue levels of Candida albicans*

Day	No. of *C. albicans* viable units in both kidneys of individual mice	
	Control mice	In:Cn-treated mice
Day 3	39×10^3	12×10^5
	88×10^3	150×10^5
	290×10^3	14×10^5
Average	139×10^3	59×10^5
Day 5	30×10^3	50×10^5
	40×10^3	89×10^5
	20×10^3	160×10^5
Average	30×10^3	100×10^5

[a] In:Cn-treated mice received 100 μg of In:Cn ip every 2 days for a total of seven doses, beginning the day before iv infection with *C. albicans*.

Interferon titers in normal Swiss female mice. Mice were injected with 100 μg of In:Cn intraperitoneally (ip) every 2 days for a total of seven doses (dose schedule of In:Cn administered to group 2 in each experiment). Six hours after injection of In:Cn, serum samples were obtained from five mice and pooled at each interval. The interferon titer was $10^{4.1}$ units/ml after the first dose of In:Cn and between $10^{2.9}$ and $10^{3.4}$ units/ml after subsequent doses.

DISCUSSION

In the present study, the ip administration of an interferon stimulator, In:Cn, increased the severity of both experimental *C. albicans* and *C. immitis* infections in mice, as evidenced by earlier time of death and higher final mortality. In addition, kidney tissue levels of *C. albicans* at 3 and 5 days after infection were found to be about 40- to 300-fold higher in In:Cn treated mice than in control mice. Experimental *C. neoformans* infection, however, was not significantly affected by In:Cn treatment. It has been reported that In:Cn treatment protected to some extent against ic, but not iv, challenge with *C. neoformans* (8). Details of these experiments have not been published, and it is difficult to compare these results with ours. In our experiments, In:Cn-treated mice challenged ic with *C. neoformans* died slightly later than control mice which did not receive In:Cn, but this difference was not statistically significant. It should be noted that control mice receiving just *C. neoformans* iv or ic died much more rapidly than control mice in the *C. albicans* and *C. immitis* experiments. The possibility remains that some effect of In:Cn on *C. neoformans* infection in mice might have been noted if a smaller challenge dose of *C. neoformans* had been used in these experiments.

In addition to inducing interferon, In:Cn is known to enhance humoral and cellular immune response, to affect the reticuloendothelial system (RES) and, in excessive amounts, to cause drug toxicity (6, 8). In:Cn also appears to have a direct antitumor effect in some systems (6) and may have other effects which have not been delineated. It is clear that large amounts of interferon were stimulated by the dose schedule of In:Cn used in these experiments, but we have no direct evidence that interferon itself enhanced these infections. The direct effect of interferon on fungal infections is currently under investigation. Immunosuppression by a variety of techniques has been shown to potentiate fungal infections in man and in experimental animals (1, 3, 10). It would therefore appear very unlikely that the potentiation of these fungus infections by In:Cn was mediated by its action as an immunological adjuvant.

In:Cn has been reported to have a biphasic effect on the RES, with a period of initial suppression followed by several days of hyperactivity of the RES (8). In our system, we have found no effect of multiple doses of 50 μg of In:Cn on RES activity as measured by the clearance of carbon; this suggests that blockade of the RES is not a likely explanation for the more severe fungal infections. There was no obvious evidence of drug toxicity in any of the mice receiving In:Cn alone; in addition, a single dose of 100 μg of In:Cn, which clearly potentiated *C. albicans* infection, is less than one-thirtieth of the LD_{50} for a single dose of this drug (H. duBuy, *personal communication*).

Presently it is not possible to specify the mechanism or mechanisms by which In:Cn potentiated fungal infections in these experiments. Together

with recent reports of potentiation of *Trypanosoma cruzi* infection in mice by In:Cn (5, 7), these results raise the possibility that this drug may have a similar effect in man. Each of the three fungi studied is a human pathogen, and *C. albicans* infection in particular can be a serious problem in patients with leukemia and other tumors who may also become candidates for In:Cn treatment (1). It should be noted that the degree of potentiation of *C. albicans* and *C. immitis* infections by In:Cn is as great as or greater than we have observed in our laboratory with a variety of immunosuppressive methods, (M. Worthington and H. F. Hasenclever, *unpublished observations*).

ACKNOWLEDGMENTS

We thank W. O. Mitchell for his excellent technical assistance and S. Baron for review of the manuscript.

This investigation was supported by Public Health Service grant 1-F03-A1-42, 860-01 MCBY from the National Institute of Allergy and Infectious Diseases.

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