# Effect of the Interferon Stimulator Polyinosinic-Polycytidylic Acid on Experimental *Trypanosoma cruzi* Infection[1,2] (35686)

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It has recently been shown that interferon or interferon stimulators may exert a protective effect in a number of different protozoan parasitic infections. Four different interferon stimulators have been reported to protect mice against *Plasmodium berghei* (1). In addition, interferon itself has been reported to inhibit the growth of *Toxoplasma gondii* in chick and mouse cell monolayers (2). Interferon stimulators have not been shown to have a consistent effect on the parasite counts in the livers of mice infected with *Leishmania donovani* (3).

The present investigation was designed to study the effect of an interferon stimulator, polyinosinic-polycytidylic acid (In. Cn.), on *Trypanosoma cruzi* infection in mice. This seemed of particular interest since this protozoal infection is of considerable clinical importance and without effective therapy.

*Methods.* Two separate experiments, A and B, were carried out 6 months apart, utilizing 60 and 90, five-week-old, male C3H mice, respectively. Animals were housed five to seven per cage (5 × 8 × 5 in.) and kept at a temperature of approximately 22°. They were fed water and standard Purina Rat Chow *ad libitum*.

*Origin, inoculation, and counts of parasites.* A Colombian strain of *Trypanosoma cruzi* was used (4, 5); it has been maintained in Harvard Swiss mice by monthly passages. After the 129th and 135th passages in Harvard Swiss mice, for experiments A and B, respectively, the parasite was passed once through weanling, male C3H mice prior to the inoculation of C3H mice in the present experiments. All mice were similar in age and were inoculated with 1000 *T. cruzi* in 0.25 ml saline (5). The degree of parasitemia was estimated semiquantitatively by counting motile parasites in 25 highpower fields (HPF) (×430) of blood obtained from the tail twice weekly. Mice were weighed twice a week.

*Polyinosinic-polycytidylic acid (In. Cn.).* The potassium salts of In. Cn. were used for interferon stimulation (P-L Biochemicals, Inc., Milwaukee). In. Cn. was prepared in a concentration of 1 mg/ml by complexing these nucleotides in equimolar concentration in phosphate-buffered saline (PBS), pH 7.6 (0.01 *M* sodium phosphate, 0.15 *M* sodium chloride) (6). Complex formation was confirmed by demonstrating a hypochromic effect (6). The In. Cn. was stored at −20° and diluted in PBS to a final concentration of 50 μg/0.25 ml prior to use.

*Determination of interferon.* Mouse L-cells were exposed to interferon samples overnight. The L-cells were then washed and challenged with GD-7 virus. 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 the titer obtained with a laboratory reference interferon which was titered in each

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[2] Presented, in part, at the 70th Annual Meeting of the American Society for Microbiology on 27 April 1970 in Boston, Mass.

TABLE I. Effect of In. Cn. on Mean Survival and Parasitemia in Mice Infected Intraperitoneally with *T. cruzi* (Expt. A).[a]

| Group | No. animals in group | Intervention | Mean survival (days) | *T. cruzi* blood counts (no./25 HPF) |
|---|---|---|---|---|
| I | 10 | Control | > 100 | |
| II | 10 | In. Cn. control (50 μg ip alternate days for 5 doses) | > 100 | |
| III | 10 | *T. cruzi* ip | 55 ± 11 | 32 ± 7 (10) |
| IV | 15 | *T. cruzi* ip and In. Cn. starting 24 hr prior to inoculation | 30 ± 1[b] | 56 ± 6[c] ( 9) |
| V | 15 | *T. cruzi* ip and In. Cn. starting 24 hr after inoculation | 27 ± 1[b] | 40 ± 7 ( 6) |

[a] All counts 21 days after inoculation and the number of animals in parentheses.
[b] Significantly different from Group III, $p < 0.01$.
[c] Significantly different from Group III, $p < 0.05$.

assay. The international reference mouse serum interferon titered $10^{4.5}$ units/ml.

*Reticuloendothelial (RE) clearance studies.* The effect of In. Cn. on RE clearance was studied in normal 5-week-old C3H male mice. Carbon clearances were determined by a standard method (7). Following injection of India ink into a tail vein, 20 μl of blood was obtained from the retroorbital plexus at 3, 6, 9, and 12 min after injection. The half-time of blood clearance ($T_{½}$) of carbon was calculated as the time required for the carbon concentration to decline to one-half that of the 3-min sample.

*Experimental design.* Two separate experiments were carried out. In Exp. A (Groups I–V; Table I), In. Cn. was administered in dosages of 50 μg ip on alternate days for five doses, beginning 24 hr before inoculation with *T. cruzi* in Group IV and 24 hr after inoculation in Group V. Inoculation of mice with *T. cruzi* was by the intraperitoneal (ip) route only. In Exp. B (Table II), *T. cruzi* were inoculated either ip or subcutaneously (sc), while In. Cn. was administered in 50 μg doses ip on alternate days for 3 weeks.

Experiment A was terminated when all the mice in Groups III, IV, and V were dead. Experiment B, on the other hand, was terminated when mortality exceeded 75% in any group; at that time surviving animals of all the groups were sacrificed for histopathological study. Multiple sections of the heart, lungs, liver, spleen, colon, and skeletal muscle

TABLE II. Effect of In. Cn. on Mortality in Mice Infected with *T. cruzi* Subcutaneously or Intraperitoneally (Expt. B).

| Group | No. animals in group | Intervention | % Mortality[a] |
|---|---|---|---|
| VI | 10 | Control | 0 |
| VII | 10 | In. Cn. control (50 μg ip alternate days for 3 weeks) | |
| VIII | 15 | *T. cruzi* sc | 27 |
| IX | 20 | *T. cruzi* sc + In. Cn. | 80[b] |
| X | 15 | *T. cruzi* ip | 27 |
| XI | 20 | *T. cruzi* ip + In. Cn. | 75[c] |

[a] Percentage mortality on 31st day after inoculation.
[b] Significantly different from Group VIII, $p < 0.05$.
[c] Significantly different from Group X, $p < 0.05$.

were examined. The *T. cruzi* blood counts of different groups were compared by Student's *t* test and the mortality by chi-square distribution.

*Results. Experiment A.* The effect of In. Cn. on mean survival and *T. cruzi* blood counts in mice inoculated with *T. cruzi* intraperitoneally is summarized in Table I, and the cumulative mortality is shown in Fig. 1. The control mice (Group I) and the mice receiving In. Cn. alone (Group II) survived more than 100 days and did not show any significant difference in body weight. The mean survival in Group III (infection alone) was significantly longer compared to the infected mice given In. Cn. (Groups IV and V). The number of *T. cruzi* on the 21st day was significantly greater in the infection + In. Cn. group (IV) than in the infection control group (III). The difference between Groups III and V was not significant.

*Experiment B.* Effect of In. Cn. on mortality and *T. cruzi* blood counts in mice infected with *T. cruzi* intraperitoneally or subcutaneously. The data on mortality are given in Table II, and *T. cruzi* counts in blood are shown in Fig. 2. By 31 days after inoculation, the mortailty was 27% in both groups receiving just *T. cruzi* (Group VIII and X), but 80 and 75% in the two groups receiving In. Cn. in addition to *T. cruzi* (Groups IX and XI, respectively). All the noninjected control mice (Group VI) and the In. Cn. control mice (Group VII) survived; mice in these two control groups also did not differ significantly in body weight.

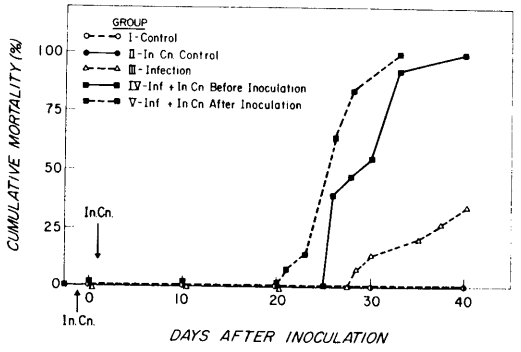


FIG. 1. Effect of In. Cn. on cumulative mortality of mice inoculated with *T. cruzi* ip (Expt. A). Mice receiving In. Cn. received 50 μg of In. Cn. ip on alternate days for five doses, beginning 24 hr before (Group IV) or 24 hr after (Group V) *T. cruzi* inoculation. All the mice in Groups I and II survived. By 40 days all the mice died in Groups IV and V, while only 35% were dead in Group III.

*T. cruzi* appeared in the tail blood on the 13th day after inoculation in Groups VIII–XI (Fig. 2). Twenty-seven days after inoculation, the parasite counts were significantly higher in mice receiving In. Cn. (Groups IX and XI) compared to the controls (Groups VIII and X). Trypanosome counts were 26 ± 4 (SEM) and 76 ± 10/25 HPF in Groups VIII and IX, respectively ($p<0.001$), and 43 ± 6 and 90 ± 9/25 HPF in Groups X and XI, respectively ($p<0.001$).

These results indicate that In. Cn. increased the severity of *T. cruzi* infection, as manifested by increased mortality and higher *T. cruzi* blood counts, even when the *T. cruzi* and In. Cn. were administered by different routes.

*Interferon titers in normal C3H mice.* Mice were injected with 50 μg of In. Cn. ip every other day for five doses (dose schedule of In. Cn. as in Expt. A). Six hours after injection of In. Cn. serum samples were obtained from five mice and pooled at each interval. The interferon titer was $10^{3.9}$ after the first dose of In. Cn. and between $10^{3.0}$ and $10^{3.2}$ after subsequent ones. Such levels of interferon are highly effective against many experimental viral infections (8).

*Effect of In. Cn. on RE clearance in normal C3H mice.* Mice were injected with 50 μg of In. Cn. ip every other day for five doses (the dose schedule of In. Cn. used in Expt. A). Carbon clearances were measured in groups of four mice 3, 5, 7, and 9 days after the last dose of In. Cn., which would correspond to the period just before and just after the onset of parasitemia. The mean $T_{½}$ of the four control mice was 13.2 min, and those of the groups of four mice measured 3, 5, 7, and 9 days after the In. Cn. treatment were 14.3, 12.7, 14.2, and 12.2 min, respectively. There was no significant difference of RE clearance between experimental and control mice.

*Histopathology of treated and untreated mice.* No histopathologic lesions were found

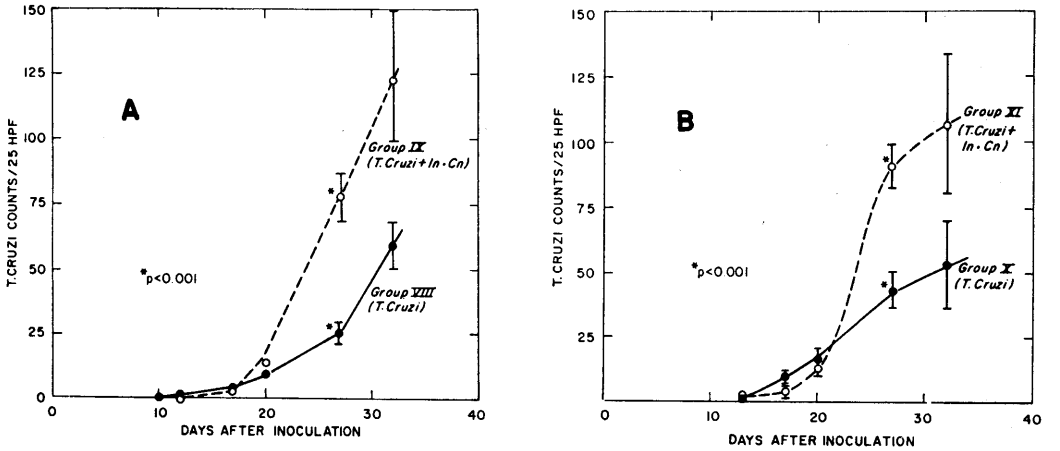


FIG. 2. Effect of In. Cn. on *T. cruzi* blood counts in mice inoculated with *T. cruzi* ip or sc (Expt. B). A. *T. cruzi* counts were significantly higher on the 27th day after inoculation in Group IX (*T. cruzi* sc + In. Cn.) compared to Group VIII (*T. cruzi* sc). B. *T. cruzi* counts were significantly higher on the 27th day after inoculation in Group XI (*T. cruzi* ip + In. Cn.) compared to Group X (*T. cruzi* ip).

in uninfected control animals. All infected animals examined showed evidence of moderate to severe, acute and subacute myocarditis and skeletal myositis. Parasites were seen in every animal. These inflammatory lesions resembled those reported previously from this laboratory (4, 5) in all respects. Chronic passive congestion was seen frequently in sections of lung, liver, and spleen. A comparative analysis was made of sections from four animals each of Groups VIII, IX, X, and XI, which had been sacrificed on the 32nd day after infection. The lesions were described and graded without knowledge of identity of the slide. No differences between groups in the severity or extent of tissue involvement were evident.

*Discussion.* In the present study the intraperitoneal administration of an interferon stimulator, polyinosinic-polycytidylic acid (In. Cn.), increased the mortality from acute experimental *T. cruzi* infection in mice, regardless of whether the parasites were inoculated intraperitoneally or subcutaneously. The enhancement of *T. cruzi* infection was accompanied by significantly greater parasitemia in the In. Cn.-treated mice. A similar enhancement of *T. cruzi* infection in mice by In. Cn. was independently observed by Martinez-Silva *et al.* (9).

In addition to inducing interferon, In. Cn. is known to enhance cell-mediated defense mechanisms, to exert an antitumor effect, and, in excessive amounts, to cause drug toxicity (10). In. Cn. may have other effects, yet to be delineated. We have no direct evidence that it was the induction of interferon by In. Cn. that produced the enhancement of *T. cruzi* infection. In fact, it has been shown that *T. cruzi* infection itself can induce formation of interferon (11).

Although the increased mortality in the In. Cn.-treated mice might have been a reflection of drug toxicity, there was no evidence of such toxicity. Specifically, control mice receiving In. Cn. suffered no mortality or obvious morbidity, and their weights were not significantly different from noninjected control mice. Also, the amount of In. Cn. given in Expt. A over a 10-day period is only about one-twelfth of the $LD_{50}$ for a single dose of this drug in mice (personal communication, H. DuBuy, 1970).

Blockade of the RE system with various agents lowers resistance to *T. cruzi* infection in mice and rats (12, 13). However, the dose schedule of In. Cn. used in Expt. A had no effect on the RE system as measured by the clearance of carbon; therefore, blockade of the RE system is not a likely explanation for

the more severe *T. cruzi* infection.

It is not possible at present to specify the mechanism(s) by which In. Cn. potentiated *T. cruzi* infection in these experiments. However, these results raise the possibility that In. Cn. may have a similar effect in acute *T. cruzi* infection in man.

*Summary.* The effect of an interferon stimulator, polyinosinic-polycytidylic acid (In. Cn.), on the course of experimental *T. cruzi* infection in C3H mice was investigated. In the first experiment In. Cn. was given ip in 5 doses of 50 μg each on alternate days, starting 24 hr before and after *T. cruzi* inoculation. The In. Cn. enhanced the parasitemia and decreased the mean survival by about 50%. In the second experiment In. Cn. was administered intraperitoneally on alternate days for 3 weeks starting 24 hr after infection. *T. cruzi* were inoculated either intraperitoneally or subcutaneously. Again the mice receiving In. Cn. and *T. cruzi* showed increased parasitemia and mortality, irrespective of the route of inoculation of the parasite. The mechanism or mechanisms by which In. Cn. enhanced the severity of *T. cruzi* infection in these experiments remains unknown. The possibility should be considered that In. Cn. might also potentiate *T. cruzi* infection in man.

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