Effectiveness of an Interferon Stimulator in Immunosuppressed Mice (35262)

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Increased severity of viral infections has been reported in immunosuppressed and immunologically deficient persons (1–5). Such individuals would be candidates for treatment with an interferon stimulator, such as polyinosinic·polycytidylic acid (In·Cn). In this paper we describe the effect of three clinically important immunosuppressive agents, cyclophosphamide (Cytoxan), 6-mercaptopurine (6-MP), and azathioprine (Imuran), on the stimulation of interferon by In·Cn. In addition the ability of In·Cn to influence the course of a fatal systemic viral infection in an immunosuppressed host was evaluated.

Materials and Methods. Mice. NIH general purpose Swiss male mice 22 to 26 g were used in all experiments.

Viruses. Neurovaccinia virus was obtained from Dr. W. A. Cassel, Emory University, Atlanta. Vaccinia virus was grown on the chick embryo chorioallantoic membrane; the pool titered $10^{7.0}$ LD_{50}/0.05 ml when inoculated ic into weanling Swiss male mice. Newcastle disease virus (NDV, Herts strain) was grown in the allantoic sac of 11-day-old embryonated eggs. The NDV had a hemagglutinin titer of 2500 units/ml in a standard assay using chicken erythrocytes.

Interferon assays. At various time intervals after injection of either In·Cn or NDV groups of 5 mice were bled by decapitation and their blood pooled. The blood was centrifuged at 1500 rpm for 20 min, and serum was then stored at —20° in a mechanical freezer. The serum interferon titers were determined as the reciprocal of the highest dilution of serum which inhibited the hemagglutinin yield of GD-7 virus during a single growth cycle in mouse L cells by 0.5 $\log_{10}$. Titers were adjusted in accordance with the titer of a laboratory reference interferon which was titered in each assay. The international reference mouse serum interferon titered $10^{4.5}$ units/ml.

In·Cn. Double-stranded polyinosinic·polycytidylic ribonucleic acid was prepared as described previously (6). Final concentration of this material was 0.5 mg/1.0 ml.

Immunosuppressive drugs. Cyclophosphamide (Cytoxan) was obtained from Mead-Johnson and a solution in phosphate buffered saline (PBS) with a final concentration of 20 mg/ml was prepared just prior to use. Azathioprine (Imuran) and 6-mercaptopurine (6-MP) were obtained from Burroughs-Wellcome; suspensions of both drugs in phosphate buffered saline were prepared immediately prior to use. The suspension of Imuran had a concentration of 10 mg/ml, while the suspension of 6-MP had a concentration of 5 mg/ml.

Statistics. In the protection experiments the percentage survivals were compared by the chi-square test for proportions (1 degree of freedom).

Results. 1. Interferon production during immunosuppression. Each of the three immunosuppressive drugs evaluated was used at three different dose levels. Each of these drugs has been reported to be an effective immunosuppressive agent in mice at the lowest of the three dose levels tested (7), and this has been confirmed in our laboratory using sheep RBC as the antigen. Twenty-four hr after the last dose of each drug, mice were inoculated with either 100 μg of In·Cn in 0.2 ml of PBS ip or 500 hemagglutinin units of NDV in 0.2 ml of allantoic fluid iv.

A. Cytoxan. Figure 1 shows that one ip dose of 200 mg/kg of body weight of cytoxan had no effect on interferon stimulation by

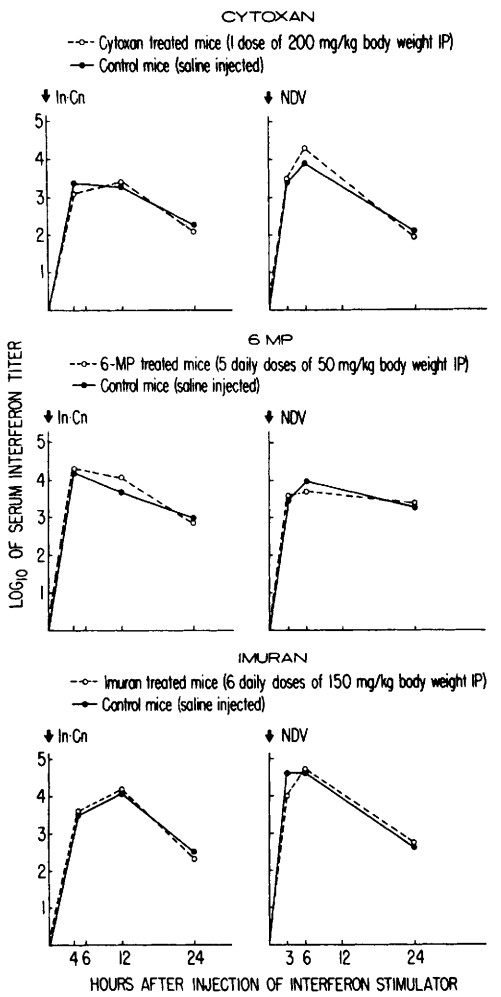

FIG. 1. Effect of 3 immunosuppressive drugs on interferon stimulation by In·Cn and NDV in mice. Twenty-four hr after the last dose of each drug mice were inoculated with either 100 μg of In·Cn ip or 500 hemagglutinin units of NDV iv. The sera from groups of 5 mice were pooled for interferon determinations at each of the time points indicated.

either In·Cn or NDV. A similar lack of effect was noted with one dose of 350 mg/kg of Cytoxan; 500 mg/kg had no effect on interferon stimulation by In·Cn, but did decrease the interferon response to NDV from 4- to 30-fold at all time points in two separate experiments. This latter dose of cytoxan was 100% lethal for mice.

B. 6-MP. Figure 1 shows that five daily doses of 50 mg/kg of 6-MP ip had no effect on interferon stimulation by either In·Cn or NDV. A similar lack of effect on interferon response was noted with 100 and 150 mg/kg/day for 5 days. This latter dose of 6-MP was lethal for the majority of mice.

C. Imuran. Figure 1 shows that 6 daily doses of 150 mg/kg body weight of Imuran ip had no effect on interferon stimulation by In·Cn or NDV. In addition no effect on interferon response was noted when 3 daily doses of 300 mg/kg or a single dose of 900 mg/kg were used.

These results indicate that even very large doses of these 3 immunosuppressive agents have little effect on stimulation of interferon in the mouse by In·Cn and NDV.

2. Protection studies. In all protection studies a large number of mice were inoculated intravenously with 10^6 weanling mouse ic LD_{50} of vaccinia virus in 0.1 ml of Eagles medium containing 2% FBS. Twenty-four hr later they received 125 mg/kg of body weight of Cytoxan ip in 0.15 ml of PBS. In each experiment 40 mice which were infected with vaccinia virus and treated with Cytoxan were kept as controls, while groups of 20 mice were treated with 4 daily doses of 100 μg of In·Cn ip beginning either before or after virus inoculation and Cytoxan treatment. Groups of 20 mice also received only vaccinia virus or only Cytoxan in each experiment.

Table I summarizes the first three protection studies. Mice which received only vaccinia virus iv had about 3% mortality, while 93% of mice which received vaccinia virus plus Cytoxan died. None of the mice which received just Cytoxan died. Pretreatment (beginning on day −1) with In·Cn protected most infected mice receiving Cytoxan and a significant decrease in mortality was noted when treatment with In·Cn was begun as late as 4 or 5 days after injection of virus.

It was considered of interest to determine if In·Cn could be begun after "clinical" evidence of infection appeared and still influence the course of this systemic infection. Mice which received vaccinia virus alone or vaccinia virus plus Cytoxan developed discrete "pock-like" lesions and generalized edema of their tails between the third and seventh day after infection. A large number of infected mice which received Cytoxan were

TABLE I. Effect of In·Cn on Vaccinia Virus Infection in Immunosuppressed Mice.

Expt. no.	Only vaccinia virus	Only Cytoxan	Vaccinia virus and Cytoxan; no In·Cn	Day:	% Mortality relative to day In·Cn therapy started 1[a]	2[a]	3[a]	4[a]	5[a]
1	0[b]	0	97		5[c]	ND[e]	70[c]	90	ND
2	10	0	90		10[c]	45[c]	50[c]	65[d]	ND
3	0	0	92		ND	50[c]	30[c]	50[c]	60[c]
Av	3	0	93		7[c]	47[c]	50[c]	68[c]	60[c]

[a] Mice were injected iv with 10^6 weanling mouse ic LD_{50} of vaccinia virus in 0.1 ml of Eagles medium with 2% FBS; 24 hr later they received 125 mg/kg of body weight of Cytoxan ip. In·Cn treatment was begun either 1 day before or 2 to 5 days after virus infection; 100 μg of In·Cn was administered ip daily for 4 days. All mice were observed for at least 4 weeks.

[b] % Mortality.

[c] $p < 0.01$.

[d] $p < 0.05$.

[e] ND = not done.

examined and those with tail lesions on the third and fourth day after injection of virus were studied for protection by In·Cn. Specifically mice having definite tail lesions on days 3 and 4 were randomly assigned to one of two groups: one group was treated with 4 daily doses of 100 μg of In·Cn ip, while the other group received control injections of PBS. Figure 2 summarizes the two experiments performed in this manner. In the 2 experiments there were a total of 122 mice in the In·Cn-treated groups and 124 mice in the nontreated control groups. There was a significant delay in death of mice receiving In·Cn in both experiments. The only mice which survived in each experiment were in the In·Cn-treated groups, but this difference in final mortality was significant in only one of the two experiments. When the two experiments were combined, the In·Cn-treated mice had a significantly decreased final mortality ($p < 0.01$). Thus when treatment with In·Cn is begun after the development of definite tail lesions, survival is prolonged and mortality may be significantly decreased.

Discussion. In the present study very large but not uniformly lethal doses of three clinically important immunosuppressive agents, Cytoxan, 6-MP, and Imuran, had no effect on interferon stimulation by In·Cn or NDV in mice. A dose of Cytoxan which was 100% lethal for mice (500 mg/kg of body wt) did decrease the interferon response to NDV but not to In·Cn. In addition In·Cn was highly effective in protecting Cytoxan-treated mice from fatal systemic vaccinia virus infection, even when therapy with In·Cn was begun 3 or 4 days after virus injection. Treatment with In·Cn begun after the appearance of definite tail lesions in vaccinia virus-infected, immunosuppressed mice resulted in a highly significant delay in death and in a significant decrease in final mortality.

The effect of a number of immunosuppressive agents on interferon stimulation has previously been reported. In general the results have been quite variable and would appear to depend to a large extent on the specific interferon stimulator selected, as well as the particular immunosuppressive agent used (8–16). Antilymphocyte serum has been reported to decrease the interferon response to In·Cn 3- to 5-fold in mice (14), while a lethal dose of X-irradiation did not significantly decrease the interferon response to In·Cn in mice in another study (8). Evidently, no other immunosuppressive agents have been studied for their effect on interferon stimulation by In·Cn.

Immunosuppressed and immunologically deficient persons have been noted to be particularly susceptible to fatal viral infections (1–5). Progressive systemic vaccinia virus infection has been a particularly severe problem in individuals with depression of both antibody mediated and cellular immunity (1). Cytoxan suppresses both humoral and cellular immunity in the mouse (7, 17, 18),

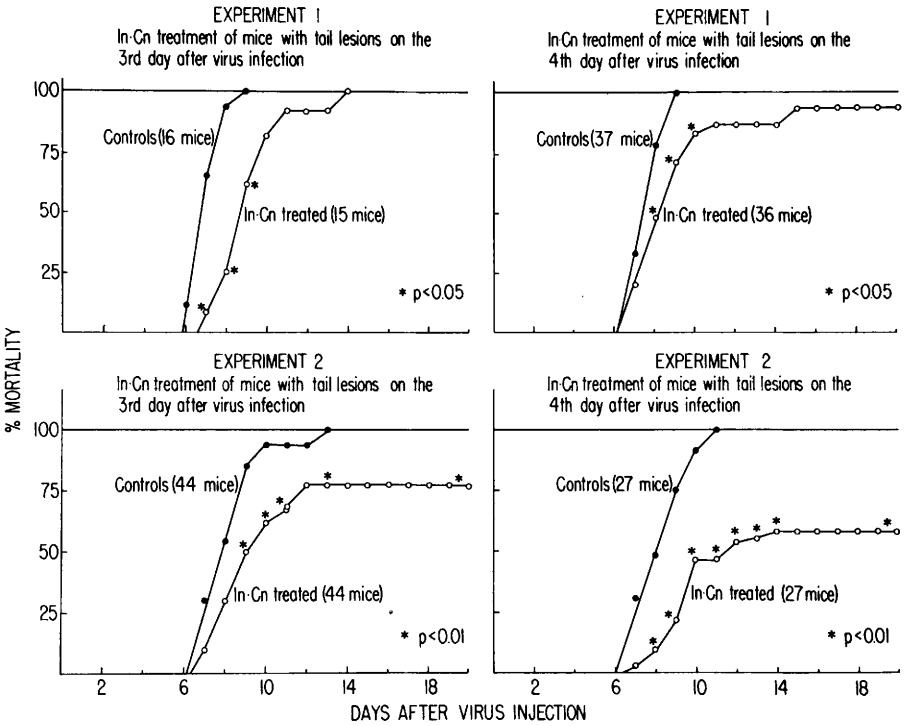

FIG. 2. Effect of In·Cn on vaccinia virus infection in immunosuppressed mice. Mice were injected iv with 10^6 weanling mouse ic LD_{50} of vaccinia virus in 0.1 ml of Eagles medium with 2% FBS; 24 hr later they received 125 mg/kg of body weight of Cytoxan ip. Mice having definite tail lesions 3 or 4 days after virus injection were randomly assigned to one of two groups; one group was treated with 4 daily doses of 100 μg of In·Cn ip, while the other group received control injections of PBS.

and the fatal systemic vaccinia virus infection which Cytoxan-treated mice develop may therefore be a model of the same infection in immunologically deficient patients. It is encouraging that at least one interferon stimulator, In·Cn, can be effective in protecting an immunosuppressed host and that the stimulation of interferon by both NDV and In·Cn is not influenced by doses of immunosuppressants which profoundly suppress immune responses. It has recently been demonstrated that another interferon stimulator, pyran copolymer, could still exert an antiviral effect against Rauscher virus in mice severely immunosuppressed by adult thymectomy and treatment with antilymphocyte serum (16). The present results and previous studies suggest that at least under some conditions a fully functioning immune system is not necessary for a normal interferon response (8, 11, 12, 13, 16). It is particularly encouraging that In·Cn favorably influenced this fatal viral infection even when treatment was begun after virus dissemination, as evidenced by the appearance of tail lesions.

Summary. This study was undertaken to determine whether several clinically important immunosuppressive agents influenced interferon production and the protective effect of an interferon inducer in mice. Large but not uniformly lethal doses of 3 potent and clinically important immunosuppressive agents, Cytoxan, 6-MP, and Imuran had no effect on the stimulation of interferon by In·Cn and Newcastle disease virus in mice. A single dose of 500 mg/kg of body weight of Cytoxan, which is 100% lethal for mice, decreased interferon stimulation by NDV 4- to 30-fold. Treatment with In·Cn was effective in protecting Cytoxan-treated mice from fatal systemic vaccinia virus infection, even when In·Cn was begun 4 or 5 days after virus

injection. Treatment with In·Cn, begun after the dissemination of this virus, as evidenced by development of definite tail lesions, resulted in a highly significant delay in death and in a significant decrease in overall final mortality. In this systemic viral infection an interferon stimulator has been shown to exert a protective effect even when treatment was begun after the appearance of clinical signs of infection. Thus under conditions comparable to clinical usage 3 immunosuppressive agents did not alter interferon production or prevent an interferon inducer from protecting mice against a disseminated vaccinia virus infection.

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