Coxsackievirus Myocarditis: Prophylaxis and Therapy with an Interferon Stimulator[1] (36975)

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It is becoming increasingly evident that viral infections play a significant role in the etiology of heart diseases (1–3). Coxsackie B viruses, which have been implicated as responsible agents in fatal myocarditis in the newborn (4–6) as well as adult myocarditis and pericarditis (3, 7–9), are currently thought to be the commonest cause of virus-induced heart illness in man (3, 9). The susceptibility of weanling mice to myocarditis in a nonlethal, experimental coxsackievirus B-3 infection (10), has provided us with a host–virus model which imitates the coxsackievirus–human relationship.

Numerous studies have demonstrated that interferon (IF) and interferon inducers are highly effective when used prophylactically in a variety of experimental viral infections (11–14). The present investigation was conducted to determine the effect of treatment with an IF inducer polyinosinic·polycytidylic acid (poly I·poly C) on myocarditis produced during an experimental coxsackievirus B-3 infection of mice.

Materials and Methods. Mice. Thirteen-day-old general purpose Swiss mice obtained from the Animal Colony, University of Hawaii, were used in all experiments.

Virus. Coxsackievirus B-3 was obtained from the Department of Health, State of Hawaii, and was passed three times in suckling mice (< 72 hr) by the intraperitoneal route (ip). A stock 20% carcass suspension was prepared after the final passage and yielded a LD_{50} of $10^{-5.3}$/ml in 1-day-old mice inoculated ip.

Histological examination and scoring of lesions. The hearts were removed and examined microscopically. They were then fresh frozen and cut into 8 μm sections. Each 30th section was mounted and stained with haematoxylin and eosin. The severity of microscopic lesions was scored as described by Grodums and Dempster (15) with 1+ = lesions involving $< 25\%$ of the myocardium, 2+ = lesions involving 50% of the myocardium, 3+ = lesions involving 75% of the myocardium, and 4+ = most of the myocardium has undergone pathological change. Susceptibility of each experimental group was graded on a percentile basis using "cumulative lesion score", divided by "maximum possible score" as described by Rytel and Kilbourne (16).

Interferon inducer. A commercial sterile preparation of double-stranded poly I·poly C obtained from Microbiology Associates, Bethesda, MD, was used for interferon induction.

Interferon assay. Serum IF concentrations were determined by the viral plaque reduction method (17) employing vesicular stomatitis virus (VSV) and mouse L cells.

Results and Discussion. Testing host–virus system and determination of virus dose. To establish the degree of myocarditis to be expected, and to determine the appropriate virus challenge dose to be used in interferon studies, groups of 4–6 mice were inoculated ip with 0.5 ml of varying dilutions of the stock virus suspension. After 7 days, the mice were sacrificed and the hearts were examined. The affected hearts with gross damage exhibited yellow-white streaks or patches on the ventricular surface. Microscopic examination revealed focal areas of necrosis and degeneration of the myocardium with a mononuclear cell infiltrate. The results of histological examination of a representative experiment are shown in Table I.

All of the mice challenged with virus dilu-

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TABLE I. Degree of Myocarditis in Mice Inoculated Intraperitoneally with (0.5 ml) Varying Dilutions of Virus.

Dilution of stock virus suspension	Frequency and severity[a] of histological lesions					Score %[b]
	—	1+	2+	3+	4+	
Undiluted	0	0	4	2	0	58
10^{-1}	0	0	1	0	3	87
10^{-2}	0	0	0	1	3	94
10^{-3}	0	0	2	3	0	65
10^{-4}	2	2	0	1	0	25

[a] Severity is graded as follows: 1+ = lesions involving <25% of the myocardium, 2+ = lesions involving 50% of the myocardium, 3+ = lesions involving 75% of the myocardium, 4+ = most of the myocardium involved.

[b] The score percentage was calculated according to the method of Rytel and Kilbourne (16).

tions to 10^{-3} developed myocarditis with the most severe damage occurring when the 10^{-2} dilution was used. However, animals inoculated with the higher concentration of virus (10^{-1} or undiluted) showed less damage. The reason for the decreased severity of damage with the lower dilutions is unknown. It can be speculated that at these dilutions the virus suspension contains a factor(s) which either passively inhibited the viral infection or induced a host response which interfered with the infectious process. This "factor(s)," which could be either IF in the inoculum or IF-induction by the large virus inoculum, is no longer effective at the higher dilutions. Also, it is possible that inoculation of high concentrations of virus may induce early antibody formation which may play a role here (21).

Effect of poly I·C on virus induced myocarditis. To determine the prophylactic and therapeutic effect of poly I·C on coxsackievirus B-3-induced myocarditis, groups of 4–6 mice were given a single 150 μg dose of the interferon inducer ip at intervals from 48 hr prior to, to 96 hr after virus challenge. The challenge dose consisted of 0.5 ml of a 10^{-2} dilution of the stock virus suspension administered by the ip route. Seven days after challenge the mice were sacrificed and the frequency and severity of histological lesions of the hearts were examined.

The results of a representative experiment shown in Fig. 1 indicate that significant protection was provided when the poly I·C was given between 48 hr prior to, and 24 hr after challenge. Protection was almost complete when poly I·C was given 12 hr prior to challenge. In contrast no protection was observed when the inducer was given on the second or fourth day after challenge.

When serum IF concentrations were determined at different times after poly I·C inoculation, maximal production was obtained 12 hr after induction (Table II). This time period coincided with the period when the

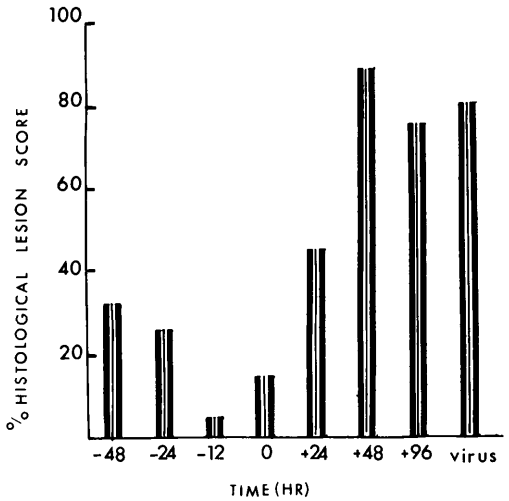

FIG. 1. Comparison of lesion severity in mice inoculated ip with 150 μg of poly I·C at intervals from 48 hr prior to, to 96 hr after challenge with 0.5 ml of 10^{-2} dilution of the stock coxsackievirus B-3 (ip). Histological lesions were graded as described in the text. Score percentage for each experimental group was calculated according to the method of Rytel and Kilbourne (16).

TABLE II. Serum Interferon Response in Mice at Different Times After Poly I·C Inoculation.[a]

Poly I·C Time (hr)	Log_{10} IF/ml
None	<1.0
4	3.6
12	4.3
24	3.6
48	3.3

[a] A single dose of 150 μg of poly I·C/animal was injected intraperitoneally in mice. The animals were sacrificed at different times after inoculation and their serum interferon levels were determined by the 50% VSV plaque reduction technique (17).

animals exhibited maximal protection to challenge with coxsackievirus. The protection decreased with increasing intervals between induction and challenge.

Previous studies have shown that poly I·C induces high levels of serum IF in mice within a few hours of inoculation (13, 18). Numerous studies have also demonstrated that IF provides resistance to a variety of experimental viral infections (12–14). The inhibition of myocarditis by poly I·C in the present study can therefore also be attributed to be due to the induction of IF. The fact that the inducer no longer provides protection when administered 48 hr after challenge indicated that by this time the infectious process may have either progressed to the point that IF was ineffective in preventing damage, or the protective effect of the single induction IF may have waned. Rytel and Kilbourne (16) have shown that in experimental infection of mice with coxsackievirus B-3, the severity of cardiac lesions begins to increase rapidly after 24 hr postchallenge attaining almost maximum severity by 72 hr. Furthermore, the present results show that the protective effect induced by poly I·C decreases as a function of time when it is given 24 hr or more before the challenge. This is in agreement with the reported kinetics of IF induction and duration (16, 19, 20).

Since a single dose of poly I·C in the present experiment provided a significant degree of therapeutic effect, it would be interesting to examine the effect of a multiple dose schedule on coxsackievirus-induced myocarditis. Daily treatments of Semiliki Forest virus-infected mice with 100 μg of poly I·C (ip) have been reported to be most effective in reducing mortality compared to other dose schedules tested (22).

Summary. A single dose of the synthetic double-stranded polynucleotide poly I·C inoculated intraperitoneally into mice 12 to 48 hr before challenge with coxsackievirus B-3 resulted in almost complete protection from virus-induced myocarditis. Protection was related to the presence of high titers of circulating IF in the serum. Significant protection was also obtained even when poly I·C was given 24 hr after challenge with coxsackievirus.

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