Relationship Between Dose Size and Dose Interval of Polyinosinic·Polycytidylic Acid and Interferon Hyporesponsiveness in Mice (35047)

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Hyporesponsiveness to interferon induction may be defined as decreased interferon production after repeated administration of interferon inducers including polyinosinic·polycytidylic acid (poly I·poly C) (1–7). In the intact animal hyporesponsiveness is an important factor which influences the approach taken for long-term production of interferon. The present study was undertaken to help determine whether the hyporesponsive state in the mouse could be altered or overcome by varying the dosage schedule of the synthetic double-stranded RNA inducer of interferon, poly I·poly C.

Materials and Methods. Poly I and poly C were purchased from P-L Biochemicals and were annealed in equimolar concentrations in 0.15 molar NaCl to give a final concentration of 1 mg/ml. Annealing was evidenced by a 40% hypochromic shift and by an increase in activity as an inducer of the interferon system in tissue culture (8).

Adult female Swiss mice (NIH strain), weighing 20–25 g were injected with 25, 50, or 200 μg poly I·poly C per mouse, corresponding to 1.25–1.0, 2.5–2.0, or 10–8 mg/kg, respectively.

Serum samples for interferon assay were obtained at appropriate intervals by the orbital bleeding technique. Samples from five mice were pooled at each time point and the serum was stored at a 1:10 dilution in Eagle's medium containing 2% fetal bovine serum at −20°. The interferon titer was determined as the reciprocal of the highest dilution of serum which inhibited the hemaglutinin yield of GD-7 virus in mouse L cells by 0.5 $\log_{10}$ (9). Titers were adjusted in accordance with the titer obtained with a laboratory reference interferon which was titered in each assay. The international reference mouse serum titered $10^{4.5}$ units per ml. Determination of interferon titers by this method produces values with a 95% confidence interval for the estimated titer of ± .35 $\log_{10}$ units.

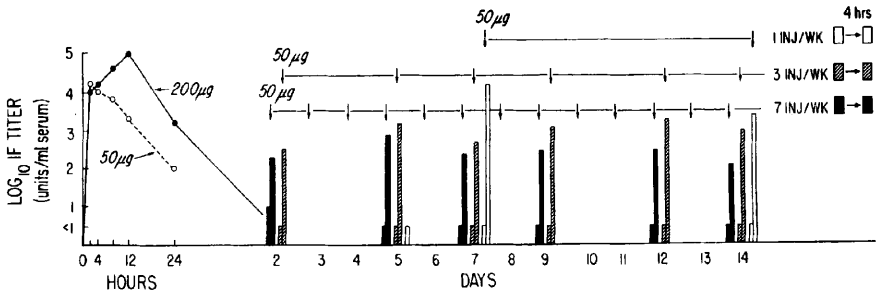

FIG. 1. Serum interferon response of mice during a 14-day period of poly I·poly C injection. Two groups of mice were injected at 0 hr with either 50 or 200 μg poly I·poly C (ip). At day 2 the group which received the initial 200 μg dose was divided into three groups and injected with 50 μg poly I·poly C (ip) at the times indicated by the vertical arrows. On experiment days 2, 5, 7, 9, 12, and 14 pooled sera were obtained from each group for interferon assay just prior to and 4 hr after the injection given on that day.

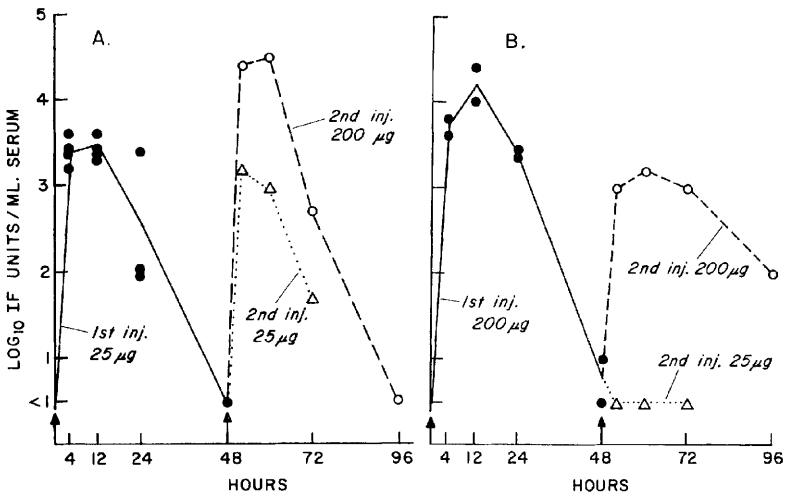

FIG. 2. Effect of the amount of poly I·poly C in the first ip dose on the interferon response to a second dose given 2 days later. All mice were injected at 0 and 48 hr with the amount indicated. A. First injection: 25 μg poly I·poly C; second injection: 25 or 200 μg poly I·poly C. B. First injection: 200 μg poly I·poly C; second injection: 25 or 200 μg poly I·poly C. Arrows indicate the times of injection.

Results. Serum interferon after repeated injections of poly I·poly C. Groups of mice were initially stimulated with 50 μg or 200 μg of poly I·poly C given either intravenously (iv) or intraperitoneally (ip). The groups which received 200 μg were divided into subgroups which were restimulated with 50 μg once weekly, three times weekly or seven times weekly beginning on day 2. The findings with ip dosage or iv dosage were indistinguishable and so only the results for ip dosage are presented in Fig. 1. It may be seen that hyporeactivity after the initial dose of 200 μg of poly I·poly C was most marked with daily doses, less marked with doses given three times a week, and not evident with doses given once weekly.

Effect of amount of poly I·poly C in the first ip dose on the interferon response to a second dose given 2 days later. Initially groups of mice were injected ip with either 25 or 200 μg of poly I·poly C. Each group was divided in half and after 2 days each subgroup was injected ip with either 25 or 200 μg of poly I·poly C. Serum interferon titers were determined at the time points shown in Fig. 2. It may be seen in Fig. 2A that an initial dose of 25 μg of poly I·poly C did not depress the interferon response to a subsequent dose given 2 days later (either 25 or 200 μg). In contrast, an initial dose of 200 μg clearly depressed the 2-day response to both 25 and 200 μg of poly I·poly C (Fig. 2B).

Time of onset of and time of recovery from hyporesponsiveness. In order to further define the time of onset of and recovery from hyporesponsiveness, the onset and duration of the hyporesponsive period under high- and low-dosage conditions was studied. An initial dose of 25 μg poly I·poly C was administered to one large group of mice and 200 μg to another large group. Subgroups were restimulated with the same dose at various time intervals. The magnitude of the interferon response after each restimulation with poly I·poly C indicated the onset of and the recovery time from hyporesponsiveness. Figure 3A defines the hyporesponsive period after an initial injection of 25 μg of poly I·poly C. It may be seen that the second dose given at 24 hr elicited a poor interferon response, the second dose given at 36 hr still elicited a hyporeactive interferon response in one of two experiments, but the second dose at 48 hr resulted in a normal response. This indicates that the hyporeactive period after an initial dose of 25 μg of poly I·poly C begins

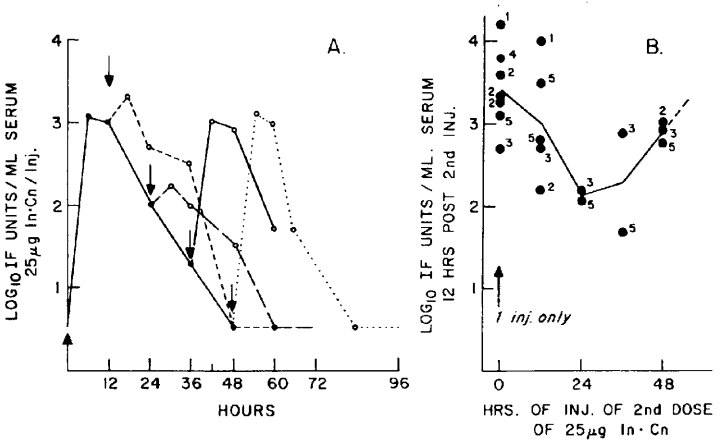

FIG. 3. Onset and duration of hyporesponsiveness after 25 μg poly I·poly C given ip to mice. A. Groups of mice were injected ip with 25 μg poly I·poly C at 0 hr (upward arrow). One group (●) received no additional injections. The other groups received a second injection of 25 μg at either 12, 24, 36, or 48 hr. Downward arrows indicate the times at which the second injections were given. Serum interferon production resulting from the second doses is plotted. B. Summary of all experiments performed to determine the kinetics of hyporesponsiveness to 25 μg poly I·poly C. The serum interferon response determined 12 hr after the second dose of poly I·poly C is plotted against the time of injection of the second dose. Points obtained from the same experiment are designated by a common superscript number. In some experiments multiple determinations were made at a given time.

some time after 12 hr and is gone by hour 48. Figure 3B is a composite of all experiments with this dose schedule, which plots the serum interferon response 12 hr after the second dose of poly I·poly C, injected at the time indicated. The figure clearly indicates the kinetics of development of hyporesponsiveness. This figure also shows the variation in the level of the interferon response observed in different groups of mice, when data obtained over a period of 18 months are compared.

In similar experiments employing 50 μg as the initial stimulus (either as a single injected dose or in a divided dose of 25 μg, 12 hr apart) followed by a second dose of 25 μg at various times. Results essentially the same as those using a single 25-μg initial dose were obtained.

Figure 4A defines the hyporesponsive period after an initial dose of 200 μg of poly I·poly C. As may be seen the hyporesponsive period begins some time after 24 hr and is fully established at 48 hr. Recovery from hyporeactivity had begun by 60 hr after the initial dose. Figure 4B is a composite of all experiments with this dose schedule, derived in a manner similar to Fig. 3B. From Figs. 3 and 4 we can see that the hyporeactive period after an initial dose of 25 μg of poly I·poly C is terminated after 2 days, and the response after a dose of 200 μg of poly I·poly C after 3 days. These results explain the absence of refractoriness, found when 25 μg were used as an initial dose, followed, after 2 days, by a second injection.

Effect of a small amount of poly I·poly C on the interferon response to a second high dose given before, during, and after the hyporesponsive period. The above experiments did not answer the question whether a small initial dose, followed by a larger second dose would affect the response to this second large dose. Accordingly, an initial injection of 25 μg poly I·poly C/mouse was given, followed by a second dose of 200 μg administered 12, 21, 24, 27, 36, and 48 hr after the first, and the interferon titer determined 12 hr after the second injection. The results of three experiments are presented in Table I.

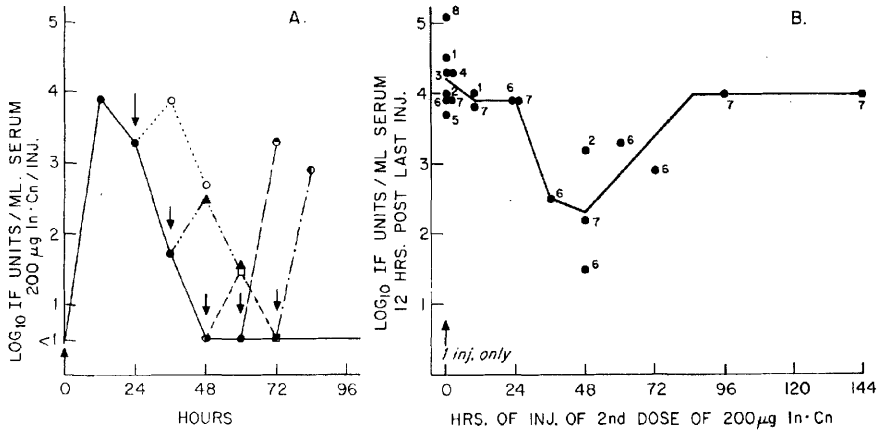

FIG. 4. Onset and duration of hyporesponsiveness to 200 μg poly I·poly C in mice. Groups of mice were injected ip with poly I·poly C at 0 hr (upward arrow). One group (●) received no additional injections. The other groups received a second injection of 200 μg at either 24, 36, 48, 60, or 72 hr (downward arrow). B. Summary of all experiments performed to determine the kinetics of hyporesponsiveness to 200 μg poly I·poly C. Data derived and superscript numbers as in Fig. 3B.

It can be seen that the interferon production resulting from the second injection of 200 μg poly I·poly C during the refractory period of the first injection of 25 μg poly I·poly C is reduced by 0.3–0.8 log.

Discussion. The present findings indicate that the dose of poly I·poly C and the dosage schedule exert a strong influence on the hyporeactive period. A small initial dose of 25 μg gives rise to a hyporeactive period of relatively early onset and relatively short duration (12–24 hr). A large initial dose of 200 μg gives rise to a hyporeactive period of later onset (36 hr) and of longer duration (24–48 hr). These findings have several important implications.

The observation that an initial dose of 200 μg of poly I·poly C was followed by a decreased second interferon response at 48 hr and that an initial dose of 25 μg was not followed by a hyporeactive response at 48 hr may be explained by different hyporesponsive periods after primary doses of different concentrations. Other implications of the present finding are (1) the potential for prolonging the initial interferon response by employing multiple doses of poly I·poly C before hyporeactivity begins; (2) the possibility of employing doses of poly I·poly C of varying concentration to achieve a significant interferon responsiveness over a more prolonged period; and (3) alternating treatment with

TABLE I. Comparison of Interferon Production After Injection of 200 μg Poly I·Poly C Preceded or Not Preceded by an Injection of 25 μg. Interferon Production Was Determined 12 hr After the Last Injection.

	Interferon titer as $\log_{10}$ units/ml serum							
	One injection		Two injections					
			Interval between injections (hr)					
Exp.	25 μg	200 μg	12	21	24	27	36	48
1	3.3	4.0	4.4	—	3.7	—	4.1	4.0
2	3.0	4.2	4.0	3.2	3.6	3.2	4.0	—
3	4.0	4.5	4.6	4.2	4.2	3.7	3.7	—

poly I·poly C and interferon or other interferon inducers to overcome hyporesponsiveness.

The present findings tend to argue against interferon or the antiviral state as direct causes of hyporesponsiveness because hyporesponsiveness frequently did not occur until long after interferon was produced and long after resistance is known to develop under these conditions of interferon production. For example, after a dose of 200 μg of poly I·poly C (Fig. 4) large amounts of interferon were produced by 4 hr and the antiviral state is well developed 6 hr later (10). If hyporeactivity were due to interferon or the antiviral state it would occur by hour 10. However, hyporeactivity did not begin until after 24 hr. This interpretation is valid only if, *in vivo*, the same cell types respond to both injections of poly I·poly C. Evidence that the same cell types do respond to two injections, is given in Table I. The second large dose, given during the refractory period of the first small dose produced only 15–50% of the normal interferon response to this dose. Therefore, those cells that produced the bulk of the interferon after the second dose were also affected by the first, small, dose.

The interferon response in mice after a single injection shows an initial rise, a period of leveling off, and a final declining phase. The declining phase indicates decreased interferon production coupled with rapid removal of interferon from the circulation (11). The declining interferon production may be due to elimination of inducer, with resulting cessation of interferon production, or to hyporeactivity to further interferon production due to feedback inhibition by inducer, interferon, or the antiviral state. The present findings favor the interpretation that elimination of inducer is responsible for the declining production because replacement of inducer by a second dose before the onset of hyporesponsiveness is followed by a second interferon response. This interpretation is in agreement with the finding that poly I·poly C is very rapidly eliminated from the blood of the rabbit (8).

The quantitative relationship between the level of circulating interferon in the mouse and the degree of resistance to virus infection has not been adequately determined. Evaluation of the effect of variation in circulating interferon levels and the *in vivo* resistance to virus infection deserves further investigation.

Summary. The degree and duration of hyporeactivity were determined by the interferon response to subsequent doses of poly I·poly C, given at various time intervals after an initial dose. When giving two doses, a small initial dose (25 μg of poly I·poly C) gives rise to a hyporeactive period of early onset (after 12 hr) and short duration (12–24 hr). A large initial dose (200 μg poly I·poly C) gives rise to a hyporeactive period of later onset (after 24 hr) and of longer duration (24–48 hr). The implications of these findings regarding the maintenance of high interferon levels for extended periods are discussed.

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