Kinetics of Serum Interferon Response in Mice after Single and Multiple Injections of PolyI·PolyC[1] (35272)

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Synthetic ribonucleic acids (RNA) such as polyinosinic·polycytilylic acid (polyI·polyC, In·Cn) stimulate the production of interferon in mice and other animals (1). The mouse is an important model system for studying the role of interferon in the control of viral infections. Understanding of this model system requires knowledge of the interferon response to RNA stimulators. The present study was done to help determine the dose–response kinetics of interferon production following single and multiple injections of the synthetic RNA, polyI·polyC.

Materials and Methods. Mice. NIH Swiss mice weighing 16 to 25 g were used in all experiments.

RNA. The synthetic homopolymers polyI and polyC were obtained from P-L Biochemicals, Inc. as lyophilized powders. They were solubilized and hybridized to form the double-stranded polyI·polyC as described elsewhere (2).

Interferon induction. Solutions of polyI·polyC were injected intraperitoneally (ip) in mice at the appropriate concentration as indicated in the Results section. Whenever possible the stock solution of polyI·polyC (1000 μg/ml) was diluted so that an inoculum volume of 0.2 ml/mouse could be used. Serum for interferon assay was obtained at appropriate intervals after inducer injection by the orbital bleeding technique as described previously (2).

Interferon assay. Serum interferon concentration was determined in a mouse L-cell GD-7 viral hemagglutinin (HA) yield reduction assay as previously described (2). In this assay the NIH international mouse reference interferon titers $10^{4.5}$ units/ml. Determination of interferon titers by this method produces values with a 95% confidence interval of $\pm$ 0.35 $\log_{10}$ units.

Results. Response to a single injection of varying amounts of polyI·polyC. Figure 1 shows the results of two representative experiments on the interferon response in groups of mice following a single ip injection of increasing amounts of polyI·polyC. Each point represents the titer of pooled sera obtained from five mice, at several times after injection of the indicated amount of polyI·polyC. In general, an increased dose of inducer was followed by both a higher peak titer and a longer duration of serum interferon production, even at the highest and most toxic concentration of polyI·polyC. This pattern of serum interferon production was reproducible in all experiments but the absolute amount of interferon produced had approximately a 10-fold overall variation in different experiments. The extent of the variation in interferon response observed over an 18-month period is illustrated in Fig. 2 where all of the results obtained using 10, 25, 50, or 200 μg of polyI·polyC/mouse have been plotted. The solid line is the average value for any time period and compares well in shape with any individual experiment. The extreme variation at some time points probably reflects variations in response of the different groups of mice and is not a reflection of the interferon assay, since all values have been corrected to a standard reference interferon which was titered in each assay. Table I shows the variation in the interferon response of individual mice 12 hr after a single injection of 200 μg of polyI·polyC. The variation of individual

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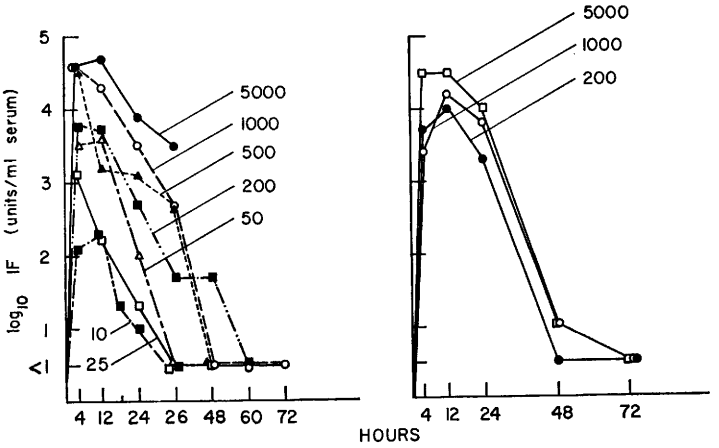

FIG. 1. Serum interferon (IF) response of mice to varying amounts of polyI·poly C: A single ip injection of the indicated concentration/mouse (μg) was administered at 0 hr. Results of two experiments are shown.

titers was too small to account for variations between groups of mice.

Response to multiple injections of polyI·polyC. In order to determine if the initial response would be influenced by multiple injections administered during the first 48 hr, groups of mice were injected with 200 μg of polyI·polyC every 6, 12, or 24 hr. Their serum interferon response was compared to a group of mice which received only a single dose of 200 μg. The results are shown in Fig. 3. Multiple doses extend the duration of peak

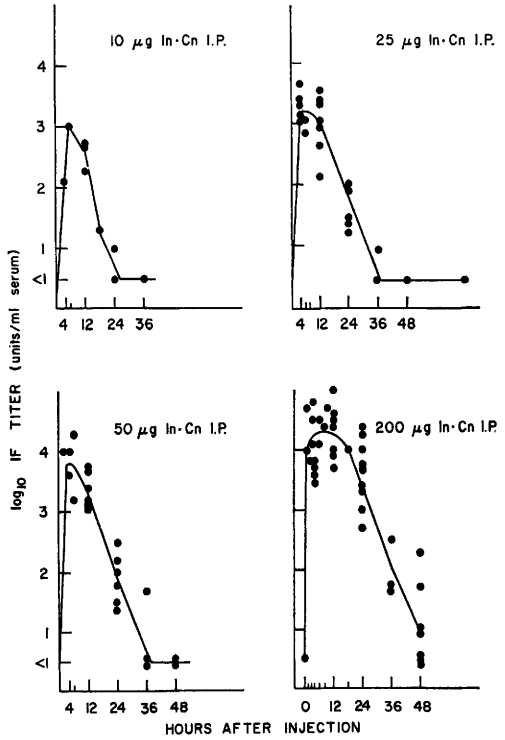

FIG. 2. Variation in serum interferon response in different groups of mice: (—) the average of all observations during an 18-month period.

TABLE I. Interferon (IF) Response in Individual Mice 12 hr After Injection of 200 μg of PolyI·PolyC, ip.

Mouse	$\log_{10}$ IF units/ml of serum
1	5.0
2	4.6
3	4.3
4	4.0
5	3.8

levels of interferon by about 12 hr. Two additional daily doses (total dose of 600 μg) were just as effective as four or eight additional doses (total dose of 1000 or 1800 μg) given over the same 2-day period of time. Also, no increase in the peak titer was observed. As discussed in another paper (2), the decreased interferon levels observed after 2 days of repeated doses of polyI·polyC is due to a hyporesponsive state which begins to develop about 36 hr after an initial dose of 200 μg of polyI·polyC.

The effect of multiple doses given over a period of 6 to 10 days was also studied. In

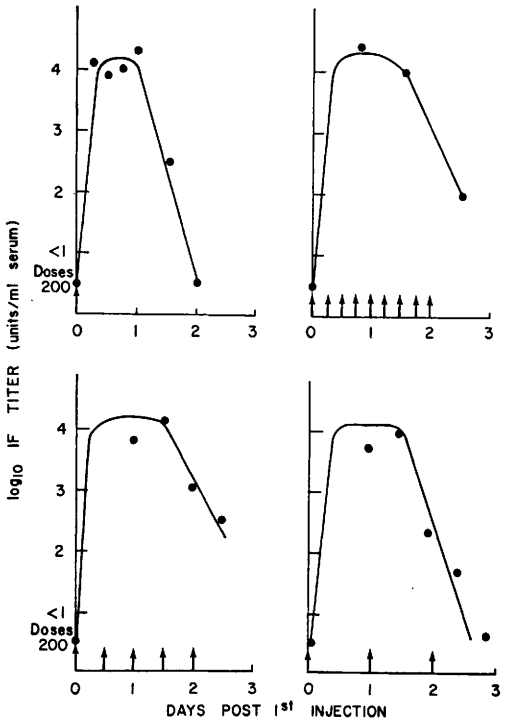

FIG. 3. Serum response of mice to a single or to multiple injections of polyI·polyC. Mice were injected with 200 μg/mouse, ip at times indicated by vertical arrows. Serum was obtained and interferon titers determined at times indicated by (●).

one experiment groups of mice were injected with either 200 or 25 μg of polyI·polyC every 12 hr. Prior to each injection, pooled serum was obtained for serum interferon determinations. Figure 4 shows the results of this experiment. Both groups had an initial response of about 10,000 units of interferon/ml of serum. However, after the first 24 hr, a level of about 1000 units/ml, or 10-fold less than the initial response, was observed. The values for the 200-μg group were consistently greater than those of the 25-μg group.

In another experiment, the effect of variable dose schedules were studied (Table II). A group of mice was given increasing doses (10 to 100 μg) every 12 hr for the first 3 days. This was done to see if the hyporesponsive state could be overcome by starting with small amounts of polyI·polyC. Following this initial 3-day dose schedule, the mice in this group were given seven daily injections of polyI·polyC in increasing amounts (100 to 200 μg). The response of this group of mice was compared to that of a group receiving large initial doses. There was no significant difference in the observed amount of serum interferon produced except for the low response of the high dose group at 4 and 5 days. A similar experiment did not reproduce this difference at 4 and 5 days, indicating that the hyporesponsive state was not overcome by starting treatment with small amounts of polyI·polyC.

Discussion. The results of these studies help define some of the factors governing the production of circulating interferon by mice following polyI·polyC injection. The level of serum interferon observed is proportional to

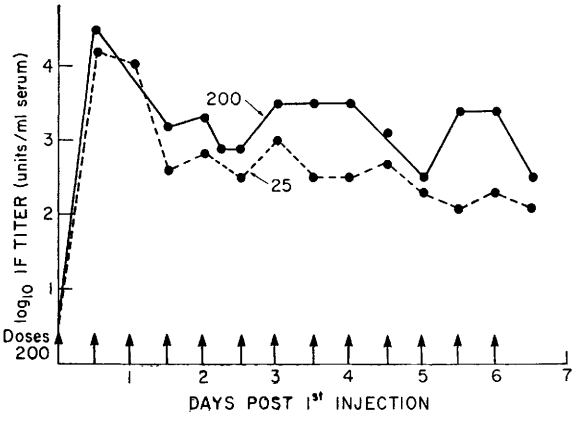

FIG. 4. Serum interferon response in mice following multiple injections, ip of polyI·polyC. Mice were injected every 12 hr (vertical arrows) with either 200 μg (● ——) or 25 μg (● - - -) per mouse.

TABLE II. Serum Interferon (IF) Response of Groups of Mice to Varying Doses of PolyI • PolyC Injected Intraperitoneally for 9 Days.

Day	Group I		Group II	
	μg of In·Cn	$\log_{10}$ IF/ml	μg of In·Cn	$\log_{10}$ IF/ml
0	10	<1.0	200	<1.0
0.5	10	2.7	—[a]	—
1	25	3.4	100	3.3
1.5	25	—	—	—
2	50	2.6	50	2.3
2.5	50	—	50	—
3	100	2.5	100	2.3
4	100	1.5	100	<1.0
5	150	2.3	150	<1.0
6	150	1.4	150	1.7
7	200	2.5	200	2.4
8	200	2.8	200	1.7
9	200	1.7	200	1.9
10	—	1.7	—	1.7

[a] Not done.

the concentration of a single dose of RNA injected, up to 5000 μg. It could not be determined whether doses above 5000 μg would continue to induce increasing amounts of interferon because such doses were toxic resulting in 90 to 100% lethality. It is therefore possible that the observed maximum response of $10^{5.0}$ units of interferon/ml of serum is not the maximum amount which the mouse can produce.

A repeated dose(s) of polyI·polyC given during the 24-hr period after the first dose extends the duration of maximum interferon production from 24 to 36 hr. Further peak production is prevented at 36 hr by the development of the hyporesponsive period (2). Although it must be tested directly, it is possible that the additional 12 hr of maximum interferon production could be important for the control of those viral infections in which a few critical days of virus multiplication determine the outcome of the infection.

Concentrations of polyI·polyC, which are below the toxic level for mice (200 μg/mouse), induce less serum interferon than a good nontoxic viral inducer (2). NDV ($10^{7.9}$ plaque-forming units given intravenously), for example, will induce $10^{4.0}$ to $10^{5.0}$ units of interferon/ml of serum as determined by the assay methods used in the present work (4). Many other viruses, however, stimulate considerably less interferon than does polyI·polyC (5).

Continued doses of polyI·polyC, given twice a day, are capable of maintaining the production of significant levels (ca. 1000 units/ml) of serum interferon for at least 10 days and probably longer (2). These repeated doses elicit only about 1/10 the peak interferon response which is stimulated by the first dose. Similar hyporeactivity to polyI·polyC has been observed in the rabbit (6).

Comparison of the antiviral effects of interferon with polyI·polyC has raised the possibility that exogenous interferon may be more effective than the endogenous interferon stimulated by polyI·polyC (7, 8). The amounts of polyI·polyC used in these early studies were relatively small. The present findings demonstrate that small doses of polyI·polyC elicit only a fraction of the amount of interferon which the mouse is capable of producing. It would therefore be desirable to extend the comparison using the broad dose range of polyI·polyC employed here.

The amount of resistance to viral challenge does not necessarily parallel interferon titers. In fact, interferon-producing cells may exhibit a degree of resistance to virus infection out of proportion to the amount of interferon produced (9). For this reason it is not possible to predict the relative degree of viral resistance of mice by measuring the amount of interferon produced. For example, it would be important to determine the viral resistance of mice after primary stimulation and after restimulation during the hyporesponsive period.

Similar patterns of serum interferon response have been observed following intravenous injection of polyI·polyC (Finter, personal communication). In these studies significant protection against virus challenge was obtained 5 to 9 days following a single injection of polyI·polyC (7.4 mg/kg or about 200 μg/mouse).

Summary. In mice, a single intraperitoneal injection of the synthetic double-stranded RNA, polyI·polyC, produces a serum inter-

feron response with peak titer and total duration of production proportional to the amount of inducer injected. Repeated injections within the first 48 hr after an initial induction only increase the total duration of production without increasing the peak titer. The ability to obtain high, sustained levels of interferon is hampered by hyporesponsiveness to restimulation. However, adjustment of the dosage schedule can result in sustaining moderate levels of serum interferon production for a period of 7 or more days.

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