

Hyporeactivity to Interferon Production by Double-Stranded RNA Associated with Hyporeactivity to Antiviral Protection and Hyporeactivity to Toxicity (36772)

E. DE CLERCQ¹

Rega Institute for Medical Research, University of Leuven, Leuven, Belgium

Repeated injections of the synthetic double-stranded RNA polyriboinosinic acid-polyribocytidylic acid [poly (I)·poly (C)] in mice lead to a decreased interferon production (1, 2). Hyporeactivity to interferon production has also been noted following repeated administration of a natural double-stranded RNA extracted from *Penicillium mycophages* (3). As interferon production by double-stranded RNA seems to be linked to its protection against virus infections (4) and its *in vivo* toxicity (5), studies were undertaken to determine whether hyporeactivity to interferon production was associated with hyporeactivity to antiviral protection and hyporeactivity to toxicity. These studies were performed in mice with both synthetic [poly (I)·poly (C)] and natural (mycophage) double-stranded (ds) RNAs.

Materials and Methods. Animals. Young (20 day old) female NMRI mice, weighing 10–12 g, were used in all experiments. They were housed in an air-conditioned room at 25°.

Poly (I)·poly (C) was prepared as described previously (6). The individual homopolynucleotides were purchased from P-L Biochemicals, Inc., Milwaukee, WI. Poly (I)·poly (C) was stored frozen in aliquots of 1 mg/ml at –20°.

Double-stranded mycophage RNA (BRL-5907) was generously provided by Dr. D. N. Planterose, Beecham Research Laboratories, Betchworth, England. The mycophage RNA was stored frozen in aliquots of 5 mg/ml at –20°. Immediately before use samples of poly (I)·poly (C) and mycophage RNA were thawed and diluted in phosphate buffered

saline to the appropriate concentration.

Interferon production. Serum interferon titers were measured with a plaque reduction technique in mouse L 929 cell monolayers using bovine vesicular stomatitis virus (VSV, Indiana strain) as the challenge virus.

Antiviral protection. Antiviral protection was evaluated with the intranasal VSV assay. Mice were inoculated intranasally with 10⁵ PFU (plaque forming units, measured in L 929 cells) of VSV, according to previously described procedures (4, 7). Most animals died between Days 5 and 9 after virus challenge. Mice surviving 15 days after virus challenge without visible signs of illness were counted as survivors.

Toxicity. Toxicity measurements were based on lethality of poly (I)·poly (C) and mycophage ds-RNA in mice treated with lead acetate [Pb(OOC-CH₃)₂]. Lead acetate has been used previously to sensitize mice to the lethal effects of poly (I)·poly (C) (8). Deaths due to poly (I)·poly (C) or mycophage ds-RNA in lead acetate-treated mice all occurred within 48 hr after their injection.

Statistical significance of the mortality data obtained in the antiviral protection and toxicity experiments was assessed by the X² test with Yates' correction; X² was computed for the final numbers of mice that died or survived.

Results. With both poly (I)·poly (C) and mycophage ds-RNA two different treatment schedules were used to induce an hyporeactive state: (a) a first injection of the compound, followed by a second dose 4 days later, and (b) three repeated injections of the compound, on day 0, 1, and 2, followed by a fourth dose on day 4. All injections were given intravenously. The dosage levels of

¹ "Aangesteld Navorsers" of the Belgian "Nationaal Fonds voor Wetenschappelijk Onderzoek."

poly (I)·poly (C) and mycophage ds-RNA used in the interferon production and antiviral protection experiments (10 $\mu\text{g}/\text{mouse}$) were far below the toxic doses [LD₅₀: 250 $\mu\text{g}/\text{mouse}$ for both poly (I)·poly (C) and mycophage ds-RNA].

Prior injection of poly (I)·poly (C) on Day 0 did not cause a reduction in the interferon response to a second stimulus of poly (I)·poly (C) on Day 4, did not affect the protective effect of this second dose against intranasal VSV challenge, and failed to reduce the mortality of a second dose of poly (I)·poly (C) in lead acetate-treated mice (Table I: treatment regimen No. 2 compared to No. 5).

Prior injection of mycophage ds-RNA, however, brought about an appreciable reduction in the interferon response to a second stimulus of the phage RNA 4 days later (Table II: treatment regimen No. 2 compared to No. 5); the protective activity against VSV infection and the mortality in lead acetate-treated mice were diminished in parallel.

Although a single injection of poly (I)·poly (C) on Day 0 did not induce hyporesponsiveness to a second dose on Day 4, three repeated doses of poly (I)·poly (C) administered on Days 0, 1 and 2 led to a marked reduction of the interferon inducing capacity of a fourth dose of poly (I)·poly (C) on Day 4 (Table I: treatment regimen No. 4 compared to No. 5); antiviral protection and toxicity were lost in parallel.

Three repeated injections of mycophage ds-RNA exerted an even greater depressing effect on the interferon response of a fourth dose of mycophage ds-RNA on Day 4 (Table II: treatment regimen No. 4, compared to No. 5); the mortality was markedly diminished, but antiviral protection was not, apparently because the treatment regimen used to induce the hyporeactive state (No. 3, Table II) was still effective against VSV inoculated 2 days after the last of the three injections.

Discussion. Circulating interferon induced by poly (I)·poly (C) in mice has been quantitatively correlated to the degree of resistance against VSV inoculated intranasally shortly after intravenous injection of the pol-

ynucleotide (4): exogenous interferon administered in amounts which closely mimicked the levels of interferon induced endogenously by poly (I)·poly (C) gave essentially the same protection as poly (I)·poly (C) itself.

Recent reports, however, tend to de-emphasize the role of circulating interferon levels in the protective effects of double-stranded RNA and other interferon inducers against virus infection. Thus, Sharpe, Birch and Planterose (3) found that during the hyporeactive state of interferon production animals were well protected by mycophage ds-RNA against encephalomyocarditis virus challenge. Merigan *et al.* (9) and Pindak *et al.* (10) found that the amounts of circulating interferon induced with a variety of interferon inducers including poly (I)·poly (C), endotoxin, statolon and pyran copolymer were not proportional to the degree of protection afforded by these compounds against Mengo virus or MM virus infection in mice. Furthermore, Campbell and Buera (11) noted differences in the protective effects of different sources of interferon against Mengo virus infection in mice, poly (I)·poly (C)-induced interferon being as effective as poly (I)·poly (C) itself, but significantly more effective than Mengo virus-induced interferon. It should be pointed out, however, that in most experiments, mentioned in these studies (3, 9, 10), both the interferon inducer and the virus were given by the intraperitoneal route. Local, noninterferon mediated effects may have been operating in these conditions: e.g., direct inactivation of the virus within the peritoneal cavity, increased phagocytosis of the virus by activated peritoneal macrophages.

The role of interferon in the resistance induced by double-stranded RNA to virus infections has been reevaluated in the present study. Hyporeactivity to interferon production was generated by two different treatment schedules: (a) two injections of the double-stranded RNA, 4 days apart, and (b) repeated injections of the double-stranded RNA on Days 0, 1, 2 and 4. With both treatment schedules, hyporeactivity to interferon production closely paralleled hyporeactivity to antiviral protection and hyporeactivity to toxicity. Mice given two injections of myco-

TABLE II. Hyporeactivity to Interferon Production, Antiviral Protection and Toxicity Following Repeated Injections of Mycophage ds-RNA.

No.	Treatment regimen	Circulating interferon production ^e (units/4 ml serum)	Protection against intranasal VSV challenge ^b		Toxicity ^e	
			Percentage survival	Statistical significance p^c	Percentage mortality	Statistical significance p^d
	Mycophage ds-RNA injected/ on day ...					
1	0, —, —, —, —	3200	15	NS	—	—
2	0, —, —, —, 4	800	45	<.10	<.001	<.20
3	0, 1, 2, —, —	280	70	<.002	NS	—
4	0, 1, 2, —, 4	40	65	<.005	NS	.025
5	—, —, —, —, 4	4200	80	<.001	—	—
6	Control	<30	15	—	—	—

^a Measured 4 hr after last injection of mycophage ds-RNA. Ten mice per group.

^b 10⁵ PFU/mouse; administered on day 4 for all treatment regimens [4 hr after injection of mycophage ds-RNA for treatment regimens Nos. 2, 4 and 5]. Twenty mice per group.

^c Compared to the control group. NS = nonsignificant.

^d As compared to treatment regimen No. 5.

^e Only recorded with treatment regimens Nos. 2, 4 and 5. Mycophage ds-RNA was injected at 30 µg/mouse on day 4, simultaneously with an intravenous injection of Pb (OOC-CH₃)₂ (1 mg/mouse). Twenty mice per group.

^f Intravenously; 10 µg/mouse, unless stated otherwise (see *e*).

phage ds-RNA, on Days 0 and 4 respectively, showed a marked hyporeactivity to all three parameters; in contrast, mice given two injections of poly (I)·poly (C), 4 days apart, failed to develop hyporeactivity. Three repeated doses of poly (I)·poly (C) (10 μ g/mouse) were required to install hyporesponsiveness with poly (I)·poly (C). Again hyporeactivity to interferon production occurred in parallel to hyporeactivity to antiviral protection and toxicity. Repeated doses of mycophage ds-RNA on Days 0, 1 and 2 caused a marked hyporesponsiveness to interferon production and toxicity of a fourth dose on Day 4; hyporeactivity to antiviral protection could not be fully assessed in these conditions, because the treatment regimen used to induce hyporeactivity was still effective against VSV inoculated 2 days after the last injection of the RNA (Table II: treatment regimen No. 3).

Both similarities and differences were observed in the patterns of interferon production, antiviral protection and toxicity of poly (I)·poly (C) and mycophage ds-RNA. Single doses of poly (I)·poly (C) and mycophage ds-RNA did not differ significantly in antiviral protection or interferon production (both measured 4 hr after injection of the RNA), or in toxicity. Mycophage ds-RNA might have been slightly superior in induction of interferon and resistance to virus infection. However, striking differences were noted following repeated injections of the nucleic acid. Hyporeactivity to interferon production was more pronounced with mycophage ds-RNA than with poly (I)·poly (C) (compare treatment regimens No. 4 of Tables I and II; compare also treatment regimens No. 2). In addition, the protective effect of mycophage ds-RNA against VSV challenge persisted for a longer time than the protective effect of poly (I)·poly (C) (compare treatment regimens No. 3 of Tables I and II).

The longer duration of the protective effect of the mycophage ds-RNA compared to poly (I)·poly (C) may reflect quantitative differences in the influence of these compounds on host defense mechanisms, *e.g.*, a more prolonged interferon production. A more pro-

longed interferon response may also account for the significantly greater duration of antiviral protection of the intact mycophage as compared to its free ds-RNA, noted in other studies (12). It may be concluded, therefore, that the persistence of the protective effect of mycophage ds-RNA, observed with treatment regimens Nos. 3 and 4 (Table II), is related to the amounts of interferon produced in response to the injections of ds-RNA administered on days 0, 1 and 2, and not to the amounts of interferon produced in response to the fourth dose given on Day 4.

Summary. Mice given repeated injections of either synthetic [poly (I)·poly (C)] or natural (mycophage) double-stranded (ds) RNA developed a marked hyporeactivity in their interferon response to these agents. Two different treatment schedules were used to induce hyporeactivity: (a) repeated injections of poly (I)·poly (C) or mycophage ds-RNA on Days 0, 1, 2 and 4 and (b) two injections of either ds-RNA on days 0 and 4. During the hyporesponsive period mice were significantly less protected against intranasal vesicular stomatitis virus (VSV) challenge, but markedly more tolerant to the lethal effects of the double-stranded RNA. However, repeated injections of the mycophage ds-RNA on Days 0, 1, 2 and 4 brought about a significant decrease of interferon production and toxicity, but no loss of protection against VSV challenge. In all other systems, hyporeactivity to interferon production closely paralleled hyporeactivity to antiviral protection and hyporeactivity to toxicity. The findings presented herein tend to reinforce the interpretation that, in well-defined conditions, these three parameters of the biological activity of double-stranded RNA are interrelated.

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