Interferon Production by ø6 Pseudomonas Phaseolicola Phage and Its Double-Stranded RNA (37578)

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Double-stranded RNA (DS-RNA) has been found to be an effective inducer of interferon in animals and in tissue culture (1). A primary source of DS-RNA is synthetic double-stranded poly rI:rC (2) and the DS-RNA of mycophages of Penicillia and other filamentous fungi (3–5).

A new source of DS-RNA was recently revealed by the discovery of Vidaver, Koski and Van Etten (6) of the first DS-RNA containing bacteriophage. This page, called ø6, is produced by the host *Pseudomonas phaseolicola*.

We have tested ø6 DS-RNA for its interferon-inducing capacity and find it to be an excellent inducer. The phage can be grown to relatively high titers of 2–3 $\times$ 10^{11} PFU/ml in fermenters and is, therefore, a good source of DS-RNA.

Materials and Methods. Pseudomonas phaseolicola *and ø6 bacteriophage.* The host strain of *Pseudomonas phaseolicola* and phage ø6 were kindly made available to us by Dr. A. Vidaver of the University of Nebraska.

Production and purification of ø6. Phage ø6 was grown essentially according to the methods of Vidaver *et al.* (6) in a semisynthetic medium and titered according to classical methods (7). Pilot scale production was accomplished in 100-liter fermenters. ø6 was isolated and purified by means of centrifugation according to methods described previously (8). The lysates were first clarified on the Sharples centrifuge and then sedimented on the wall of a high-speed sedimentation rotor. The resuspended sediment was further purified and concentrated by a combined rate zonal-isopycnic banding in a sucrose gradient in the B-XXIX rotor. A 350-fold purification based on PFU/μg protein was obtained. The fraction with the highest phage concentration contained 6 $\times$ 10^{13} PFU/ml.

Preparation of ø6 DS-RNA. Concentrated purified ø6 was diluted to 1–2 $\times$ 10^{11} PFU/ml in a medium composed of 10^{-3} *M* Tris buffer, pH 7.0, 10^{-3} *M* EDTA, and 0.05 *M* NaCl and extracted at 25° by means of 88% phenol equilibrated with the same medium. Since lipid is associated with ø6, sodium dodecyl sulfate (0.3 mg/ml) was employed in the extraction.

Poly rI:rC. The synthetic double-stranded polyribonucleotide was obtained from P & L Laboratories (Lot 047231) in the form of the lyophilized sodium salt.

Treatment of the ø6 phage with organic solvents and pancreatic lipase. An equal volume of chloroform or ether was added to the ø6 at room temperature and the mixture was shaken intermittently for 10 min. After centrifugation, the water phase was removed and freed of residual solvent by aspiration with nitrogen. Lipase (Nutritional Biochemicals) treatment was accomplished by incubating the ø6 phage with 0.5 mg of enzyme per ml.

Interferon production and assay. ø6 Phage, ø6 DS-RNA, or poly rI:rC were injected intraperitoneally into 15-g mice; 3–6 mice were employed per dose. At appropriate times, 16 hr after injection of the inducer (unless otherwise specified) the mice were bled and the serum separated. Interferon titers were determined by the mouse L cell–vesicular stomatitis virus tissue culture system. Titers were read from the decrease in the number of plaques relative to untreated control

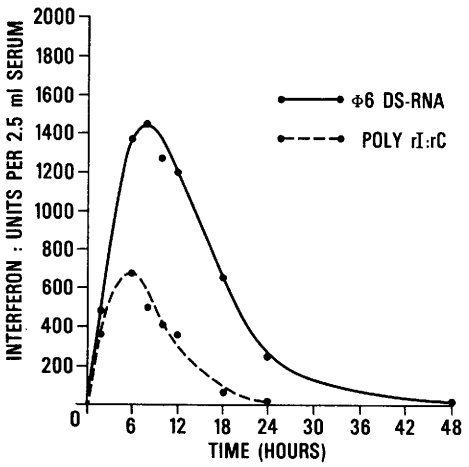

FIG. 1. Appearance and decay of interferon induced by Φ6 DS-RNA and poly rI:rC.

cells; a unit of interferon being the reciprocal of the dilution producing a 50% decrease in plaque count.

Results. Appearance and decay of ø6 DS-RNA and poly rI:rC induced interferon with time. ø6 DS-RNA and poly rI:rC were administered intraperitoneally into mice at 1 mg/kg. The mice were bled at appropriate times beginning at 2 hr after treatment. The results are given in Fig. 1. A maximum interferon level was attained at 6 hr with poly rI:rC and at 8 hr with ø6 DS-RNA. The response with ø6 DS-RNA was approximately twice that seen with poly rI:rC and the decay of interferon activity with time was essentially equivalent in each instance.

Dose response of ø6 DS-RNA and poly rI:rC. Mice were injected with varying quantities of ø6 DS-RNA and poly rI:rC ranging from 1 to 100 μg per mouse. The mice receiving poly rI:rC were bled 6 hr later and those receiving ø6 DS-RNA were bled at 8 hr. Saturation levels of interferon were reached at 40 μg and the total response of ø6 DS-RNA was 1.6 times that of poly rI:rC (Fig. 2). In our experience ø6 DS-RNA has proved to be somewhat more potent as an inducer, generally giving a response 1.5–2.5 times that of poly rI:rC.

Our studies with statolon, an interferon inducer derived from *Penicillium stoloniferum,* showed that protective activity could be attained lasting for 30 days against MM virus when the inducer was administered intraperitoneally, and up to 14 days against influenza virus when statolon was administered intranasally (9, 10). The protection obtained with mycophage particles of *Penicillia* appears to be of longer duration than that seen with the extracted DS-RNA (11, 12). The longer duration of activity of the particle can be ascribed to protection of the DS-RNA against nuclease activity (13) by the phage coat along with concomitant slow release of DS-RNA. We were, therefore, interested in determining the interferon-inducing capacity of the ø6 phage particle. With mycophage of *Penicillium stoloniferum* we were able to obtain significant circulating interferon in mice injected with approximately 3–5 $\times$ 10^{10} particles per mouse. Even T_4 coliphage, a DNA containing phage, has been found to elicit a response of interferon production at 1 $\times$ 10^{10} particles/mouse (14). In the case of ø6, however, the response was less than expected. No response was seen with ten times as many particles (10^{11} particles/mouse) administered either intraperitoneally or intravenously. When the quantity of particles was increased to 8.5 $\times$ 10^{11} per mouse, only 355 units of interferon were produced (Table I).

Vidaver, Koski, and Van Etten have found ø6 phage to contain a significant quantity of lipid, about 25% (6). The lipid apparently

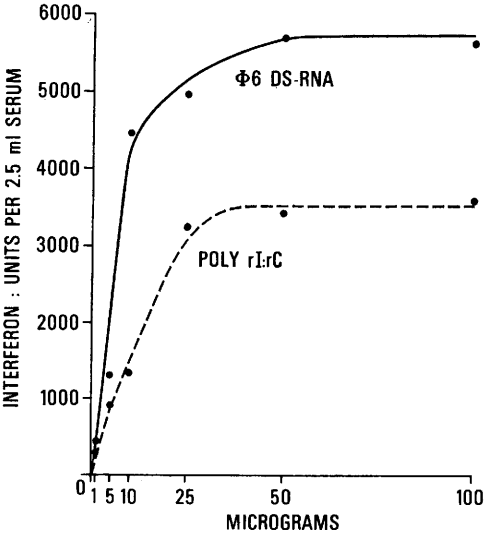

FIG. 2. Interferon production with various doses of Φ6 DS-RNA and poly rI:rC.

TABLE I. Interferon Inducing Activity of Φ6 Phage.

Treatment	Route	PFU/mouse $\times 10^{11}$	Interferon units/2.5 ml
None	ip	1.0	<30
None	ip	8.5	355
None	iv	1.0	<30
$CHCl_3$	iv	1.0	525

is arranged as an envelope around the phage particle and its removal by means of organic solvents, sodium deoxycholate, or pancreatic lipase results in loss of infectivity. It seemed likely that the lipid envelope could also be responsible for the decreased interferon response with ø6 phage relative to that seen with other phage particles. When the ø6 was extracted with chloroform, a stimulation of interferon production was seen in mice injected intravenously. While the untreated phage at 1×10^{11} phage particles per mouse showed no response, the same quantity of phage treated with chloroform produced 525 units (Table I).

In another experiment ø6 phage was treated with ether and pancreatic lipase as well as with chloroform. There were no signs of an insoluble material separating out as a result of these treatments indicating that minimal, if any, denaturation with concomitant release of DS-RNA had occurred. In each instance an increase in interferon production was seen with the treated phage over that of the untreated suspension (Table II). Ether apparently is more efficient at extracting the lipid, for it has consistently given the best results.

Discussion. ø6 Bacteriophage of *Pseudomonas phaseolicola* affords a new source of DS-RNA. ø6 is readily grown to high titer and in relatively large quantities. Its DS-RNA has been found to be an effective inducer of interferon producing a response somewhat greater than that seen with poly rI:rC.

Though the ø6 DS-RNA is a good inducer of interferon, the ø6 phage itself has proved not to be as effective an inducer as expected relative to the degree of response seen with other DS-RNA containing virus particles. The lipid envelope surrounding the ø6 appears to prevent the full exertion of the interferon-inducing capacity of the phage. Removal of lipid by means of ether or chloroform or digestion with pancreatic lipase potentiates the inducing capacity in mice, as much as sixfold when ether is used.

The lipid envelope very likely decreases the uptake of the ø6 phage particles by cells. In the animal, interferon is elicited by cells of the reticuloendothelial system (RES). DeMaeyer, DeMaeyer-Guignard and Julien (15) have obtained evidence that lymphocytes are the primary source of interferon induced by myxoviruses. Stuart and Davidson (16) and Diluzio and Blickens (17) have shown that a variety of fatty acid esters such as methyl palmitate and cholesterol oleate administered to mice depress the phagocytic function of the reticuloendothelial system. In like manner, the lipid envelope probably decreases the uptake of ø6 by RES cells resulting in a depression of interferon production. Removal of the lipid envelope by solvent or enzymatic treatment should expose more attachment sites. Exposure of such sites on ø6 permits increased uptake of the phage by RES cells resulting in greater production of interferon.

Summary. *Pseudomonas phaseolicola* bacteriophage ø6 containing DS-RNA is capable of inducing interferon with a lower potency than with mycophage obtained from *Penicillium stoloniferum.* The smaller response with ø6 appears to be due to the lipid envelope surrounding the phage, since removal of lipid potentiates ø6's interferon-inducing capacity. ø6 DS-RNA induces high levels of interferon in mice and its interferon-inducing activity is somewhat greater than that seen with poly rI:rC.

TABLE II. Effect of Lipid Removal on Interferon Inducing Activity of Φ6 Phage.[a]

Treatment	Interferon units/2.5 ml
None	134
Ether	801
$CHCl_3$	370
Lipase	320

[a] Φ6 Phage, 2×10^{11} PFU/mouse, administered ip.

We gratefully acknowledge the expert assistance of Dr. W. S. Boniece, Mr. E. L. Hayes, Mr. J. Redmond,, Mr. M. Wilson, and Ms. Patricia Wood.

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Received May 17, 1973. P.S.E.B.M., 1973, Vol. 144.