Dose Responsiveness and Variation Among Inbred Strains of Mice in Production of Interferon After Treatment with Poly (I)·Poly (C) [Poly-D-Lysine] Complexes

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Poly-D-lysine forms a stoichiometric complex with poly(I)·poly(C) which has a higher Tm (83°C in 0.15 M NaCl) than the uncomplexed double-stranded polyribo- nucleotide (63°C). The complex was superior to poly(I)·poly(C) alone as an interferon inducer in vivo. Significant serum interferon titers were attained in Swiss mice during a 24-hr period after intraperitoneal injection of 10 to 300 μg of poly(I)·poly(C) [1.0 poly-D-lysine] complex, at concentrations of 100 to 1,000 μg/ml. The serum interferon responses (average and maximum titers) of a series of inbred strains of mice to a single intraperitoneal injection of 100 μg of complex decreased in the order: Swiss > DBA/2 > C3H > BALB/c > CF-1 > AKR, C57BL/6, NZB > SJL > NZW and varied by a factor of approximately 100 from the most to the least responsive.

Poly(I)·poly(C), the double-stranded synthetic ribonucleic acid (RNA) generated by mixing equimolar quantities of polyribocytidylic and polyriboinosinic acids, was first described by Davies and Rich in 1958 (5) and reported by Hilleman et al. in 1967 (10) to give rise to a high titer of interferon in the serum of animals which had received on the order of 10 to 100 μg of this material intravenously. Subsequent studies have amply confirmed that poly(I)·poly(C) is an effective antiviral agent both in vitro (11, 18) and in vivo (4, 8, 9, 14, 16, 17) and is the most effective synthetic interferon inducer generally available. For this or any other polymer to be biologically effective, however, it is necessary that its rate of uptake by cells exceed the rate at which it is degraded into biologically inactive fragments. In the case of polyanionic nucleic acids, this competition is weighted against the polymers, not only by the ubiquity and efficiency of ribonucleases but by electrostatic repulsion of the negatively charged cellular membranes which they must penetrate.

Complex formation between nucleic acids and oligofunctional basic substances of low molecular weight, such as spermine and streptomycin, or polyfunctional bases of high molecular weight, such as protamine, is known to inhibit ribonuclease activity; furthermore, the transport of nucleic acids from solution into mammalian cells is facilitated when the nucleic acid is in the form of a complex with protamine (3). Efforts to improve interferon-inducing efficiency (interferogenicity) by the addition of streptomycin, spermine, and related compounds of low molecular weight to preparations of poly(I)·poly(C) were encouraging on the basis of in vitro studies (12), but only the high-molecular-weight polycation diethylaminoethyl (DEAE) dextran had been found to be significantly effective in vivo (7) until we reported the marked efficiency of poly(I)·poly(C) [poly-D-lysine] complexes as interferon inducers in mice (19). The pronounced hepatotoxic effects of repeated DEAE dextran administration were not observed in mice which received intraperitoneal injections of poly(I)·poly(C) [poly-D-lysine], and the very high titers of interferon attained in their sera suggested that complex formation between poly(I)·poly(C) and polymers of cationic amino acids might significantly enhance its effectiveness in the prophylaxis and therapy of viral infections.

We report here a series of studies on the complexes formed between polylysines of different conformations and molecular weights with several poly(I)·poly(C) preparations, the effects of dosage and concentration of such complexes
on the magnitude and duration of serum interferon titers achieved by highly responsive Swiss mice in response to single intraperitoneal injections, and some observations on the variation in interferon production by different inbred strains of mice given a single large dose (100 μg) of poly(I)·poly(C) [poly-D-lysine] complex.

**MATERIALS AND METHODS**

Young adult mice (1 to 3 months old) of strains DBA/2, C3H, CF-1, C57Bl/6, AKR, Balb/c, and SJL, Calif. were obtained from Microbiological Associates, Inc., Bethesda, Md. Swiss, NZB, and NZW mice were obtained from the Animal Production Section, Laboratory Aids Branch, National Institutes of Health. They were housed in plastic cages with free access to a commercial pelleted diet and tap water. Blood for interferon assays was obtained from the jugular vein or heart under deep ether anesthesia. Blood from at least three animals was pooled for each time point, and the pooled, undiluted sera were stored frozen at −20 °C until titrated.

Polyriboinosinic acid [poly(I)] and polyrribcytidylic acid [poly(C)] were purchased as lyophilized potassium salts from Schwarz BioResearch Inc., Orangeburg, N.J., and from Calbiochem Inc., Los Angeles, Calif. Each polymer was dissolved individually in phosphate-buffered physiological saline (PBS; 0.15 M NaCl, 0.006 M Na2HPO4, pH 7.0; reference 10) at concentrations of 2 mg/ml (5 mM polyribonucleotide phosphorus). Three different poly(I)·poly(C) preparations, designated stocks J, K, and L, were obtained by mixing equal volumes of the following pairs of solutions: Schwarz poly(I) and poly(C), both lot 6701 (stock J); Schwarz poly(I) and poly(C), both lot 6801 (stock K); and the Calbiochem products (stock L). Each mixture was warmed to 65 °C, cooled slowly to room temperature, and stored frozen in portions at −20 °C. Each homopolymer solution was characterized quantitatively and qualitatively by its ultraviolet absorption spectrum before mixing, and poly(I)·poly(C) formation was confirmed by spectroscopic measurement of hypochromism at 250 nm (5, 10). All stock poly(I)·poly(C) solutions were annealed a second time before use, at whatever final concentration was required for mixing with polylsine solutions, to insure dissolution of aggregates formed during the freezing process.

Lysine homopolymers of three different sizes, each with a different configuration, were purchased as the hydrobromide salts from Nutritional Biochemicals Corp., Cleveland, Ohio. These included poly-L-lysine HBr (molecular weight, ~3,000), poly-DL-lysine HBr (molecular weight, ~15,000), and poly-D-lysine HBr (molecular weight, ~160,000). Each was dissolved in PBS at a concentration of 1.09 mg/ml (5 mM ε-amino nitrogen) and stored frozen at −20 °C. Poly(I)·poly(C) [polylsine] complexes were prepared by combining equal volumes of poly(I)·poly(C) and polylsine stock solutions, previously diluted to twice their intended final concentration. We have used the following notation for describing preparations of polynucleotides complexed with polycations. (i) The polynucleotide is designated by the rules of the IUPAC-IUB Commission on Biochemical Nomenclature (Biochemistry 20:4022–4027, 1970) and is preceded by its quantity: 100 μg poly(I)·poly(C). (ii) The polycation follows, in square brackets together with such information as is necessary for its characterization, and is preceded by the molar ratio of positively charged groups in the polycation to polynucleotide phosphorus (i.e., ratios less than unity indicate an excess of polynucleotide phosphorus, ratios greater than unity indicate an excess of polycation): 50 μg of poly(A)·poly(U) [10 streptomycin], 100 μg of poly(I)·poly(C) [0.5 DEAE dextran, molecular weight 2 × 106]. (iii) To define a concentration, the preceding expression is followed by a diagonal, then the volume in which the quantity of polynucleotide mentioned is contained, and finally by an abbreviation for the solvent: 100 μg of poly(I)·poly(C) [3.0 poly-D-lysine, molecular weight 160,000/0.1 ml PBS or 100 μg of the double-stranded complex of poly(I) and poly(C) dissolved in 0.1 ml of phosphate-buffered saline containing an amount of polynucleotide having a molecular weight of 160,000, such that the molar ratio of polynucleotide ε-amino groups to polynucleotide phosphorus is 3:1]. Diluted poly(I)·poly(C) stock solutions were heated to 70 °C, allowed to cool slowly to 40 to 50 °C, and swirled vigorously by hand or by a Vortex-type mixer while the polylsine solution (also at 40 to 50 °C) was added dropwise. In the event of precipitate formation, the suspension of poly(I)·poly(C) [polylsine] complex was heated to ~75 °C, just below its melting temperature (Tm = 81 to 83 °C), and stirred vigorously until the solution was complete or no further dissolution appeared likely. Residual precipitate was uniformly dispersed or removed by centrifugation, and the final concentration of poly(I)·poly(C) in the complex solution was determined by ultraviolet spectroscopy. Although thermal dissociation of poly(I)·poly(C) [1.0 polylysine] complexes is reversible in dilute solution (~40 μg/ml), concentrated preparations (~1 mg/ml) maintained at temperatures above the Tm for more than a few minutes frequently form insoluble deposits on the glass walls of the container. Accordingly, temperatures above the polynucleotide Tm should be avoided when preparing these mixtures. Solutions of poly(I)·poly(C) [polylsine] complexes were stored at 4 °C and warmed to 56 °C before use to disperse any gel or precipitate formed during storage.

Melting curves, ultraviolet absorption spectra, and interferon titrations (a cytopathic end-point method using tube cultures of cloned L cells [CCL-1] and vesicular stomatitis virus, Indiana strain) were carried out as previously described (19). A standard mouse interferon sample of 30,000 culture-protective units (CPU) per ml, obtained from Samuel Baron of the National Institute for Allergy and Infectious Diseases, titered 10,000 CPU/ml in our system.

**RESULTS**

Complex formation between poly-D-lysine (molecular weight 160,000) and poly(I)·poly(C).

The addition of poly-D-lysine solution, 5 mM in ε-amino nitrogen, to an equimolar solution of
poly(I)·poly(C) stock L (2 mg/ml or 5 mM polynucleotide phosphorus), or to much more dilute solutions of very large polyanions such as highly polymerized deoxyribonucleic acid resulted in the immediate formation of an intractable, fibrous precipitate, which afforded prima facie evidence of complex formation. The tendency toward precipitation was less pronounced when more dilute solutions of 500 μM concentration were combined, although it became increasingly severe as the molar ratio of ε-amino nitrogen to polynucleotide phosphorus approached unity. At equivalence, precipitation was pronounced in mixtures of 500 μM solutions, but it was significantly less extensive when stock K was used in place of stock L and was negligible with stock J. This progressive decrease in precipitability indicated a progressive decrease in average molecular weight of the double-stranded RNA in these stock solutions in the order L > K > J, a conclusion expected from the manufacturers' molecular weight estimates for the constituent homopolymers. (These ranged from 10⁶ to 10⁸ in the same order, but we have not attempted to confirm these data.) These precipitates redissolved or dispersed uniformly, however, during vigorous stirring at 60 C. With stock J, even at stoichiometric equivalence at a final polynucleotide phosphorus concentration of 2.5 mM, faintly opalescent solutions were obtained by heating and stirring the partially precipitated mixture of equal volumes of 5 mM stock solutions. In these preparations, the ultraviolet absorption spectrum of the poly(I)·poly(C) component was unchanged qualitatively and quantitatively reflected the fraction of polynucleotide which remained in solution. This fraction varied from 0.80 to 0.95 and was larger in more dilute preparations.

That complex formation between polylsine and poly(I)·poly(C) actually occurs quantitatively under conditions where no precipitate is formed is clear from the biphasic nature of poly(I)·poly(C) melting curves in the presence of less than equimolar amounts of polylsine (Fig. 1). The midpoint of the thermal transition (Tm) of poly(I)·poly(C) in 0.15 M NaCl in the absence of polycations is 63 C and that of poly(I)·poly(C) [1.0 poly-D-lysine] complexes is nearly 20 C higher (19). Biphasic melting curves of appropriate stoichiometry were obtained from poly(I)·poly(C) solutions containing poly-D-lysine at polycation to RNA molar ratios of 0.1 and 0.3 (Fig. 1). Since the addition of an equimolar amount of poly-D-lysine completely converts all poly(I)·poly(C) in solutions of 20 μg/ml or greater concentration to the high-melting complex and since solutions of other than equimolar quantities of poly(I)·poly(C) and poly-D-lysine simply contain a mixture of complex and excess polymer, we have used only equimolar mixtures of poly-D-lysine and poly(I)·poly(C) in our studies of interferon induction.

Influence of genetic background on murine interferogenic response to poly(I)·poly(C) and its poly-D-lysine complexes. The serum interferon titers achieved by mice of one random-bred line (Swiss) and eight inbred strains after a single intraperitoneal injection of 100 μg of poly(I)·poly(C) stock J [1.0 poly-D-lysine] complex in 1 ml of PBS prepared as previously described (19) are summarized in Table 1. The various breeds are listed in descending order of responsiveness, judged from the magnitude and duration of the interferon titers observed. It is readily apparent that there is a marked difference in responsiveness among these different genotypes of the single species Mus musculus. The peak interferon titers
achieved vary by two powers of ten, from > 12,800 CPU/ml in Swiss and DBA/2 to 100 CPU/ml in strain NZW.

Nearly all animals tested showed a similar pattern of response in that a measurable titer appeared in the serum within 4 hr, rose to a maximum between 8 and 18 hr, and had fallen to a level distinctly below the maximum 24 hr after injection. This pattern is apparent both in highly responsive strains such as Swiss, in which the peak titer reached 12,800 CPU/ml between 8 and 18 hr after injection but had fallen to 800 CPU/ml by 24 hr, and in the minimally responsive strain SJL, in which the only measurable titers (400 CPU/ml) were obtained at 12 and 18 hr. The only exception to this pattern occurred in the response of strain NZW, in which detectable levels of interferon were observed 4 and 8 hr after injection but no later. The experiment was, therefore, repeated in this strain and in the NZB strain as well, with poly(I)-poly(C) itself and with the poly(I)-poly(C) [1.0 poly-D-lysine] complex, with the results presented in Table 2. In both New Zealand strains, the interferogenic response to the complex was much greater than the response to an equivalent amount of poly(I)-poly(C). Moreover, strain NZB was highly responsive to the complex and achieved a high interferon titer which persisted between 12 and 24 hr after injection. Strain NZW achieved a higher peak titer in this second experiment, but again it occurred 6 hr after injection and decayed rapidly over the next 12 hr, the period of time during which other strains exhibited a maximum titer. Furthermore, the NZW 6-hr titer was comparable to that achieved by other strains, including NZB. The data suggest that the interferogenic response to poly(I)-poly(C) [1.0 poly-D-lysine] begins normally in strain NZW but is swiftly abrogated by some process which serves to clear either circulating interferon or the poly-nucleotide inducer from these animals' systems with unusual speed. The nature of this interference is not known.

Dose-effect relationships. To determine the range of effective doses over which single injections of poly(I)-poly(C) [1.0 poly-D-lysine] complexes elicit interferon production in mice of the highly responsive Swiss line, volumes of 0.1 to 3.0 ml of buffered saline solutions containing 100 μg of poly(I)-poly(C) [1.0 poly-D-lysine] per 1.0 ml were injected intraperitoneally, and interferon titers in the serum were determined 6, 12, 18, 24, and 48 hr after injection.

### Table 1. Serum interferon titers in mice after a single intraperitoneal injection of 100 μg of poly(I)-poly(C) stock J [1.0 poly-D-lysine] complex in 1.0 ml of phosphate-buffered saline

| Strain | Serum interferon titer (CPU/ml) at various times after injection |
|--------|---------------------------------------------------------------|
|        | 4 hr | 8 hr | 12 hr | 18 hr | 24 hr | Avg 4-24 hr |
| Swiss  | 800  | 12,800 | >12,800 | 12,800 | 800  | 8,000 |
| DBA/2  | 200  | 6,400  | 3,200   | 12,800 | 1,600 | 4,840 |
| C3H    | 800  | 800    | 6,400   | 1,000  | 200  | 1,660 |
| BALB/c | 160  | 2,560  | 1,280   | 2,560  | 320  | 1,370 |
| CF-1   | 0    | 1,600  | 1,600   | 800    | 800  | 960   |
| AKR    | 0    | 800    | 800     | 400    | 200  | 480   |
| C57BL/6| 200  | 800    | 400     | 800    | 200  | 480   |
| SJL    | 0    | 0      | 400     | 400    | 0    | 160   |
| NZW    | 100  | 100    | 0       | 0      | 0    | 40    |

* Serum from at least three mice was pooled for each time point. The lowest titer determined in these studies was 100 CPU/ml; titers lower than this are recorded as zero.

### Table 2. Serum interferon titers in New Zealand black (NZB/BIN) and white (NZW/BIN) mice after a single intraperitoneal injection of 100 μg of poly(I)-poly(C) stock J or its equimolar poly-D-lysine complex

| Treatment (1.0 ml in PBS ip) | Strain  | Serum interferon titer at various times after injection |
|-----------------------------|---------|--------------------------------------------------------|
|                             | Strain  | 6 hr | 12 hr | 18 hr | 24 hr |
| 100 μg of poly(I)-poly(C)   | NZB     | 200  | 50   | 0     | 50    |
|                             | NZW     | 50   | 0    | 0     | 0     |
| 100 μg of poly(I)-poly(C)   | NZB     | 800  | 1,600| 1,600 | 1,600 |
| [1.0 poly-D-lysine]         | NZW     | 800  | 200  | 200   | 0     |
| Swiss                       |         | 3,200| 6,400| 1,600 | 200   |
| (positive control)          | NZB     | 0    | 0    | 50    |       |
| Poly-D-lysine, equivalent to | NZW     | 0    | 0    | 50    |       |
| 100 μg of poly(I)-poly(C)   |         |      |      |       |       |

* Serum from at least three mice was pooled for each time point. Titers lower than 50 CPU/ml were not determined and are recorded as zero.

* PBS, phosphate-buffered saline; ip, intraperitoneally.
Poly(I)·poly(C) stocks J, K, and L all formed sufficiently soluble complexes with poly-D-lysine (molecular weight, 160,000) at this concentration to permit their use in this experiment (Table 3). Peak interferon titers and the average titers during the first 24 hr after injection varied directly with the volume of solution injected for each poly(I)·poly(C) [1.0 poly-D-lysine] preparation studied at the concentration of 100 μg of poly(I)·poly(C) per ml, over a range of volumes which include the practical limits for intraperitoneal injections in mice. At each dose level above 10 μg of poly(I)·poly(C), the peak titers produced by all three preparations were either identical or varied by no more than one dilution. The mean titers between 6 and 24 hr after injection tended to be highest in the animals which received complex prepared from poly(I)·poly(C) of stock K, which contains poly(I) and poly(C) of intermediate molecular weight. The absence of a progressive increase in interferon titers with increasing molecular weight of the inducer is one respect in which the poly(I)·poly(C) [1.0 poly-D-lysine] complexes differ in biological activity from poly(I)·poly(C) preparations, among which such a trend is characteristic [compare the titers obtained from uncomplexed poly(I)·poly(C) of stocks J, K, and L; Table 3]. The low solubility of polylysine complexes precluded the study of more highly concentrated solutions of poly(I)·poly(C) stocks K and L. More concentrated solutions of stock J [1.0 poly-D-lysine] complexes were successfully prepared, however, and these induced higher mean interferon titers in the serum during the 24 hr immediately after injection. From the data in Table 3, it is apparent that the mean serum interferon titer generated by 100 μg of poly(I)·poly(C) stock J [1.0 poly-D-lysine] increased irregularly from 1,150 to 1,425 to 2,200 CPU/ml as the concentration increased from 100 to 333 to 1,000 μg/ml, although the peak titer achieved was invariant over this range of concentrations.

The solubility properties of poly(I)·poly(C) [polylysine] complexes can be improved by using a polylysine of much lower molecular weight than the poly-D-lysine used here, but in our experience such complexes are inferior inducers of interferon and generate lower mean and peak serum titers than complexes of high-molecular-weight polylysine with the same poly(I)·poly(C) preparations. The interferon titers achieved with poly(I)·poly(C) stock L [1.0 poly-L-lysine; molecular weight, 3,000] were identical to those obtained with an equivalent dose of this high-

### Table 3. Serum interferon titers in female Swiss mice given a single intraperitoneal injection of poly(I)·poly(C) [1.0 polylysine] complexes

| Determination | Poly(I)·poly(C) [1.0 poly-D-lysine, mol wt 160,000] | Serum interferon titer at various times after injection | Poly(I)·poly(C) [1.0 poly-L-lysine, mol wt 3,000] | Poly(I)·poly(C) alone |
|---------------|-------------------------------------------------|---------------------------------------------------|------------------|
| Dose (μg) | Volume injected (ml) | 6 hr | 12 hr | 18 hr | 24 hr | 48 hr | Avg over 24 hr | Dose (μg) | Volume injected (ml) | 6 hr | 12 hr | 18 hr | 24 hr | 48 hr | Avg over 24 hr | Dose (μg) | Volume injected (ml) | 6 hr | 12 hr | 18 hr | 24 hr | 48 hr | Avg over 24 hr |
| J 10 | 0.1 | 100 | 100 | 200 | 200 | 0 | 150 | J 100 | 1.0 | 800 | 800 | 1,600 | 1,600 | 0 | 1,425 | J 100 | 1.0 | 800 | 800 | 1,600 | 1,600 | 0 | 1,425 |
| J 30 | 0.3 | 200 | 400 | 1,600 | 1,600 | 0 | 650 | J 300 | 3.0 | 400 | 400 | >6,400 | >6,400 | 0 | 2,600 | K 10 | 0.1 | 400 | 400 | 800 | 800 | 0 | 600 |
| J 100 | 1.0 | 800 | 200 | 3,200 | 3,200 | 0 | 1,150 | J 100 | 0.1 | 800 | 1,600 | 3,200 | 3,200 | 0 | 2,200 | K 30 | 0.3 | 400 | 800 | 1,600 | 800 | 0 | 900 |
| J 300 | 3.0 | 400 | 400 | >6,400 | >6,400 | 0 | 2,600 | J 300 | 3.0 | 6,400 | 1,600 | >6,400 | >6,400 | 25 | 5,200 | L 10 | 0.1 | 0 | 50 | 800 | 100 | 0 | 237 | L 100 | 1.0 | 400 | 800 | 3,200 | 1,600 | 0 | 1,500 |
| J 100 | 1.0 | 800 | 400 | 800 | 200 | 0 | 550 | L 30 | 0.3 | 100 | 400 | 800 | 200 | 0 | 375 | L 300 | 3.0 | 3,200 | 3,200 | 6,400 | 3,200 | 25 | 4,000 |
| J 300 | 1.0 | 1,600 | 800 | 400 | 200 | 0 | 750 | L 100 | 1.0 | 1,600 | 800 | 400 | 200 | 0 | 750 | L 300 | 3.0 | 3,200 | 3,200 | 6,400 | 3,200 | 25 | 4,000 |
| J 100 | 1.0 | 100 | 200 | 50 | 87 | L 100 | 1.0 | 1,600 | 800 | 800 | 50 | 813 | K 100 | 0.4 | 800 | 1,600 | 1,600 | 400 | 1,100 | L 100 | 0.4 | 1,600 | 1,600 | 1,600 | 800 | 1,400 |

* Serum from at least three mice was pooled for each interferon titration.
molecular-weight poly(I)·poly(C) preparation alone (Table 3), although in the case of stock J some enhancement was observed. Experiments with poly-DL-lysine of intermediate molecular weight (15,000) showed no significant improvement over low-molecular-weight poly-L-lysine.

**DISCUSSION**

Interferon titers in Swiss mice were higher and persisted for longer periods of time when a single dose of poly(I)·poly(C) was administered in the form of its poly-D-lysine complex. This enhancement was most pronounced when the double-stranded polynucleotide was composed of homopolymers of relatively low molecular weight. The greatest utility of poly-D-lysine complex formation thus appears to consist in improving the interferon-inducing activity of poly(I)·poly(C) preparations which, because of the low molecular weights of their constituent homopolymers, are relatively inactive in vivo when given alone. The greater solubility of equimolar poly-D-lysine complexes with poly(I)·poly(C) of relatively low molecular weight reinforces this conclusion. In the experiments reported here, only the complexes prepared from poly(I)·poly(C) stock J (lowest molecular weight) were completely soluble at the concentrations used. Preparations of poly(I)·poly(C) stock K complexes contained fine precipitate, and those of stock L consisted of an exasperating mixture of fibrous precipitate and stiff gel. Although such preparations are clearly capable of eliciting interferon production, they are difficult to handle and are likely to be dangerous or ineffective when given by any other than the intraperitoneal route.

Mice of different genetic lines differed markedly, by as much as two powers of ten, in their interferogenic response to a single intraperitoneal injection of 100 μg of poly(I)·poly(C) [1.0 poly-D-lysine]. Interferon titers in highly responsive Swiss mice rose progressively over the dose range 10 to 300 μg; no evidence of saturation of the ability of these animals to respond was observed over this dose range with any of the three preparations tested. The variability in peak titers attained and the different shapes of the interferon induction curves characteristic of different strains of mice allow the inference that a broad spectrum of intrinsic responsiveness to poly(I)·poly(C) [poly-D-lysine] complexes probably exists in other animals which are found in large populations of genetically unrelated individuals, such as the human and many domestic animal species. Such variability must be considered in planning experiments on antiviral prophylaxis and therapy.

Poly-D-lysine (molecular weight, 160,000) was clearly more effective in enhancing interferon induction by poly(I)·poly(C) than was poly-L-lysine (molecular weight, 3,000). The importance of conformation in the polyamino acid component of these complexes remains to be determined, however, since in the preparations available for these studies the variables of molecular weight and of conformation about the α carbon atoms of the lysine residues were not independently variable. The stability of complexes formed between poly(I)·poly(C) and various polylysine preparations of the same molecular weight may depend significantly on whether each successive amino acid residue has the same conformation or whether conformation varies in random fashion from one residue to the next.

Poly(I)·poly(C) is capable not only of inducing high titers of serum interferon when given in vivo but in stimulating both humoral and cellular antibody formation (21, 23) and in protecting animals against rapidly progressive bacterial infections, apparently by nonspecific endotoxin-like stimulation of the reticuloendothelial system (22). Whether these modes of host resistance to infectious agents are enhanced more strongly by poly(I)·poly(C) [poly-D-lysine] than by poly(I)·poly(C) alone is yet to be determined. The effect of poly-D-lysine complex formation on the toxic effects (1, 2, 13, 15, 20) of poly(I)·poly(C) also remains to be evaluated, but preliminary observations in mice indicate that the acute toxic effects of large doses of the polynucleotide are at least not enhanced by poly-D-lysine. The prospects for use of these complexes for nonspecific antiviral therapy therefore appear encouraging.

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**LITERATURE CITED**

1. Abachar, M., and W. R. Stinebring. 1969. Endotoxin-like properties of poly I·poly C, an interferon stimulator. Nature (London) 223:715–717.
2. Adamson, R. H., and S. Fabro. 1969. Embryotoxic effects ofpoly I·poly C. Nature (London) 223:718.
3. Aron, H., and K. E. Kearns. 1963. Influence of bacterial ribonucleic acid on animal cells in culture. II. Proteine enhancement of RNA uptake. Exp. Cell Res. 32:14–25.
4. Catalano, L. W., Jr., and S. Baron. 1970. Protection against herpes virus and encephalomyocarditis virus encephalitis with a double-stranded RNA inducer of interferon. Proc. Soc. Exp. Biol. Med. 133:684–687.
5. Davies, D. R., and A. Rich. 1958. The formation of a helical complex between polyinosinic acid and polycytidylic acid. J. Amer. Chem. Soc. 80:1003–1004.
6. Dianzani, F., W. Cantagalli, S. Ganoni, and G. Rita. 1968. Effect of DEAE-dextran on production of interferon induced by synthetic double-stranded RNA in L cell cultures. Proc. Soc. Exp. Biol. Med. 128:708–710.
7. Dianzani, F., G. Rita, P. Cantagalli, and S. Ganoni. 1969.
Effect of DEAE-dextran on interferon production and protective effect in mice treated with the double-stranded polynucleotide complex polyinosinic-polycytidylic acid. J. Immunol. 102:24-27.

8. DuBuy, H. G., M. L. Johnson, C. E. Buckler, and S. Baron. 1970. Relationship between dose size and dose interval of polyninosinic-polycytidylic acid and interferon hypersensitivity in mice. Proc. Soc. Exp. Biol. Med. 135:340-344.

9. Fenge, P., and B. Postic. 1970. Protection of rabbits against experimental rabies by poly I:poly C. Nature (London) 226:171-172.

10. Field, A. K., A. A. Tytell, G. P. Lampson, and M. R. Hilleman. 1967. Inducers of interferon and host resistance, II. Multistranded synthetic polynucleotide complexes. Proc. Nat. Acad. Sci. U.S.A. 58:1004-1010.

11. Field, A. K., A. A. Tytell, G. P. Lampson, and M. R. Hilleman. 1968. V. In vitro studies. Proc. Nat. Acad. Sci. U.S.A. 61:340-346.

12. Lampson, G. P., A. A. Tytell, A. K. Field, M. M. Nemes, and M. R. Hilleman. 1969. Influence of polyanamines on induction of interferon and resistance to viruses by synthetic polynucleotides. Proc. Soc. Exp. Biol. Med. 132:212-218.

13. Lindsay, H. L., P. W. Trown, J. Brandt, and M. Forbes. 1969. Pyrogenicity of poly I:poly C in rabbits. Nature (London) 223:717-718.

14. Nemes, M. M., A. A. Tytell, G. P. Lampson, A. K. Field, and M. R. Hilleman. 1969. Inducers of interferon and host resistance. VI. Antiviral efficacy of poly I:C in animal models. Proc. Soc. Exp. Biol. Med. 132:776-783.

15. Ostler, H. B., J. O. Oh, C. R. Dawson, and W. L. Burt. 1970. Toxicity of poly I-poly C for rabbit eyes. Nature (London) 228:362-364.

16. Park, J. H., and S. Baron. 1968. Herpetic keratoconjunctivitis: therapy with synthetic double-stranded RNA. Science 162:811-813.

17. Pearson, J. W., W. Turner, P. S. Ebert, and M. A. Chirigos. 1969. Effectiveness of therapy with a multistranded synthetic polynucleotide complex against a murine sarcoma virus. Appl. Microbiol. 18:474-478.

18. Rhim, J. S., C. Greenawalt, and R. J. Huebner. 1969. Synthetic double-stranded RNA: inhibitory effect on murine leukemia and sarcoma viruses in cell cultures. Nature (London) 222:1166-1168.

19. Rice, J. M., W. Turner, M. A. Chirigos, and N. R. Rice. 1970. Enhancement by poly-D-lysine of poly I:C-induced interferon production in mice. Appl. Microbiol. 19:867-869.

20. Serota, F. T., and R. Baserga. 1970. Polyinosinic acid: polycytidylic acid: inhibition of DNA synthesis stimulated by isoproterenol. Science 167:1379-1380.

21. Turner, W., S. P. Chan, and M. A. Chirigos. 1970. Stimulation of humoral and cellular antibody formation in mice by Poly L, C. Proc. Soc. Exp. Biol. Med. 133:334-338.

22. Weinstein, M. J., J. A. Waitz, and P. E. Carne. 1970. Induction of resistance to bacterial infections of mice with poly I-poly C Nature (London) 226:170-171.

23. Woodhour, A. F., A. Friedman, A. A. Tytell, and M. R. Hilleman. 1969. Hyperpotentiation by synthetic double-stranded RNA of antibody responses to influenza virus vaccine in adjuvant 65. Proc. Soc. Exp. Biol. Med. 131:809-817.