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Preparation and biological properties of a highly active poly(G) · poly(C) inducer of interferon

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Summary

Experimental conditions necessary for the full expression of interferon-inducing activity by a complex of polyguanylic acid and polycytidylic acid included: (i) a sufficiently high molecular size of each homopolymer; (ii) annealing conditions which insured complete denaturation of polyguanylic acid self-structure; and (iii) the specific biological assay employed to assay interferon-inducing potency. The complex of polyguanylic acid and polycytidylic acid possessed several properties that suggested it may be an atypical polynucleotide interferon inducer. For instance, it was inactive in primary rabbit kidney cell cultures, usually exquisitely sensitive to polynucleotide interferon inducers, unless it was incubated on the cell cultures for prolonged times or in the presence of DEAE-dextran. Polyguanylic acid · polycytidylic acid could induce interferon in rabbits and mice but gave a more protracted response than did poly(I) · poly(C). Finally, poly(G) · poly(C) was, without any modification, resistant to degradation by serum nucleases.

interferon induction; poly(G) · poly(C); poly(I) · poly(C)

Introduction

A plethora of natural and synthetic chemicals are able to induce interferon production in vitro or in vivo [1]. Among the most potent inducers, the double-stranded RNAs (dsRNAs), structural modifications of the heterocyclic bases, the ribose moieties, or the phosphate groups have, in some instances, given rise to potent interferon inducers [1–3]. The dsRNA poly(G) · poly(C) may be considered as a poly(I) · poly(C) analog in which the position 2 hydrogen of the hypoxanthine ring has been replaced by an amino function. The interferon-inducing capability of poly(G) · poly(C) has been a subject of considerable controversy in the literature; for instance, Colby and Cham-

berlin [4], Aksenov et al. [5], Timkovsky et al. [6], Vilner et al. [7] and Novokhatsky et al. [8] reported that poly(G) · poly(C) was an active interferon inducer, but Field et al. [9], Matsuda et al. [10], De Clercq et al. [11] and De Clercq and Torrence [12] could not ascribe any interferon-inducing ability to their preparations of poly(G) · poly(C). After a number of attempts, we finally have succeeded in obtaining an interferon response with poly(G) · poly(C). This demonstration of interferon-inducing activity was dependent upon a number of variables associated with the preparation of the complex, on the one hand, and the methodology of its assay, on the other hand.

Materials and Methods

Nucleic acids

Poly(G) purchased from Sigma Chemical Co. (St. Louis, Mo.) had an $s_{20,w}$ of 13.6 S whereas poly(G) purchased from P-L Biochemicals had an $s_{20,w}$ of 9.0 S. Poly(C) was obtained from P-L Biochemicals in batches that had $s_{20,w}$ units of 3.08 S, 8.2 S or 13.2 S. The poly(I) · poly(C) used as a reference for the interferon induction assays also was from P-L Biochemicals. A sample of poly(G) · poly(C) that was the same as that described by the Russian workers [5–8] was obtained from S.L. Bresler, N.S. Tikhomirova-Sidorova and A.L. Timkovsky of the Leningrad Nuclear Physics Institute, Institute of High Molecular Weight Compounds, U.S.S.R. Academy of Sciences, Leningrad. The Leningrad poly(G) · poly(C) sample was prepared in April 1981 by direct annealing of poly(G) ($M_r \sim 3 \times 10^5$) and poly(C) ($M_r \sim 5 \times 10^5$), both at 10^{-2} M, in 0.005 M sodium phosphates, pH 7.5, supplemented with 0.1 M NaCl, for 10 h at room temperature, followed by 10 min at 90°C. The sample was then filtered through a membrane with pore diameter of 0.2 μm and lyophilized. For preparation of solutions of poly(G) an extinction coefficient of 9.8×10^{-3} [13] was used whereas for poly(C) an extinction coefficient of 6.3×10^{-3} [14] was employed.

Preparation of poly(G) · poly(C)

Typically, poly(G) ($s_{20,w}$ = 13.6 S, 475 μl, 21 μmol/ml, 10 μmol) was mixed at room temperature with poly(C) ($s_{20,w}$ = 13.2 S, 1.49 ml of 6.7 μmol/ml, 10 μmol), and the resulting mixture was diluted to 5.0 ml after the addition of sufficient urea (2.4 g) to give a final urea molarity of 8 M and a final polynucleotide phosphate molarity of 4×10^{-3} M. The mixture then was placed in a boiling water bath and heated (2–3 min) until the temperature of the solution reached > 95°. After one additional minute at > 95°, potassium chloride (4 M) and Hepes (pH 7.5, 1 M) were added to give a final concentration of 0.01 M KCl, 0.01 M Hepes. The solution then was removed from the water bath and allowed to cool slowly (≈ 30 min) to room temperature. The entire mixture was transferred to a dialysis bag and dialyzed at 4° against three 2-l changes of water over a period of 48–72 h. After completion of the dialysis, the concentration of the poly(G) · poly(C) solution was determined spectrophotometrically using as extinction coefficient $\varepsilon_p = 7.7 \times 10^{-3}$.

Determination of interferon-inducing activity

Interferon production was measured in primary rabbit kidney (PRK) cells, murine L-929 cells, diploid human embryonic skin-muscle (E_6SM) fibroblasts, aneuploid MG-63 human fibroblasts, trisomic-21 (RL) human fibroblasts, diploid human VGS fibroblasts, and in mice and rabbits according to previous published methodology [15]. The routine procedure for measuring interferon production in PRK cells was as follows: confluent PRK cells were incubated for 1 h with the polynucleotide in serum-free Eagle's minimal essential medium (MEM) and subsequently treated ('superinduced') with cycloheximide (2 μg/ml) for 3 h and actinomycin D (3 μg/ml) for 30 min. Following this superinduction period, the cells were washed extensively with MEM and further incubated for 20 h with MEM containing 3% heated fetal calf serum. The cell culture fluid then was harvested for interferon titration. Alternatively, PRK cells could be exposed for 24 h to the polynucleotide before the superinduction period was initiated (see also Results). When the cells (either PRK, human fibroblasts or mouse L-929) were exposed to the polynucleotides in the presence of DEAE–dextran (100 μg/ml), the incubation period with the nucleic acid could be shortened to 1 h and was followed immediately by the interferon production period (20 h); in this situation, the cells were not superinduced with the cycloheximide and actinomycin D regimen.

Rabbit IFN titers were determined by inhibition of the cytopathic effect (CPE) of vesicular stomatitis virus (VSV) in PRK cells. Mouse IFN-titers were determined by reduction of VSV plaque formation in murine L-929 cells, and human IFN titrations were carried out in human fibroblast (strain VSG) cultures by using a VSV CPE inhibition technique similar to that used for rabbit IFN titrations. The human, rabbit and mouse IFN titers are expressed in terms of the U.S. National Institutes of Health (NIH) human fibroblast reference interferon G023-902-527, rabbit reference interferon G019-902-958 and mouse reference interferon G002-904-511 standards, respectively.

Ultraviolet spectra

Ultraviolet spectra were determined in a Hewlett Packard 8450A UV/VIS spectrophotometer, using as buffer 0.1 M KCl, 0.01 M Hepes, pH 7.5.

Results

Method of complex formation

The induction of IFN by poly(G) · poly(C) was dramatically dependent on the manner in which the component homopolymers were annealled. Table 1 presents data that demonstrate that the poly(G) · poly(C) complex, if any, formed at room temperature in 8 M urea was devoid of interferon-inducing ability whereas that annealled in 8 M urea at a temperature in excess of 90° possessed considerably increased interferon-inducing ability. The annealing method used in the latter case was a modification of the methodology developed by Englander et al. [13].

TABLE 1

Interferon production by poly(G)·poly(C): dependence on the method of duplex formation

Procedure	IFN titer of resulting complex (log_{10} units/ml)[b]
(1) Poly(G)[a] and poly(C)[a] mixed in 8 M urea, heated to 95°C for 2 min; 4 M KCl and 1 M Hepes (pH 7.5) added to give a final concentration of 0.01 M each; solution cooled to ambient temperature over 30 min period, then dialyzed at 4°C against 3 changes of H_2O	3.0
(2) Identical procedure to 1, but polynucleotides not heated to 95°C	<1.3

[a] Poly(G) and poly(C) in both procedures had $s_{20,w}$ values of 13.6 S and 13.2 S, respectively.

[b] Determined in PRK cells exposed to the complex for 1 h in the presence of DEAE-dextran (100 μg/ml); cells were *not* superinduced. The concentration of poly(G)·poly(C) applied to the cells was 10^{-4} M.

Molecular size of the component homopolymers

Table 2 presents the results of experiments that demonstrate that only poly(G) · poly(C) prepared from the highest available molecular weight homopolymers had maximum interferon-inducing activity. Poly(G) · poly(C) prepared from poly(G) of high molecular weight (13.6 S) and of poly(C) medium molecular weight (8.2 S) had intermediate interferon-inducing ability, 1.5 log_{10} units less than that obtained for poly(G) · poly(C) constituted from both high molecular weight homopolymers. If, on the other hand, the poly(G) · poly(C) was constituted from poly(G) of intermediate

TABLE 2

Interferon inducing activity of poly(G)·poly(C)[a]: dependence on the molecular size of the component homopolymers

Sedimentation constants ($s_{20,w}$)[a,b]		IFN titer[c,d] (log_{10} units/ml)
Poly(G)	Poly(C)	
13.6	13.2	3.5
13.6	8.2	2.0
13.6	3.08	<1.3
9.0	13.2	<1.3

[a] Complexes of poly(G)·poly(C) were prepared from the various molecular sizes of poly(G) and poly(C) according to the procedure outlined in Materials and methods (procedure 1 of Table 1).

[b] Expressed in Svedberg units.

[c] Determined in PRK cells superinduced with cycloheximide and actinomycin D. The cells were exposed to the polymers for 24 h before initiation of the superinduction procedure. The polynucleotides were evaluated at a final concentration of 10^{-4} M.

[d] Under the same conditions, poly(I)·poly(C) induced 2.0 log_{10} IFN units/ml when applied to the cells as 10^{-4} M for 24 h.

molecular weight (9.0 S) and poly(C) of high molecular weight (13.2 S), it was devoid of detectable interferon-inducing activity. Finally, poly(G) · poly(C) prepared by annealling poly(G) of high molecular weight (13.6 S) and poly(C) of low molecular weight (3.08 S) also gave a complex without any detectable interferon-inducing activity.

Dependence of interferon-inducing ability upon the conditions of the biological assay

When poly(G) · poly(C) was prepared from high molecular weight polymers and annealled by the 8 M urea/heating procedure and then incubated on PRK cells for 1 h before superinduction by cycloheximide and actinomycin D (the routine procedure for interferon induction by polynucleotide interferon inducers), then it was completely devoid of interferon-inducing activity (Table 3). On the other hand, if the cells were exposed to the complex for 24 h before the superinduction protocol was initiated, then poly(G) · poly(C) stimulated interferon production as efficiently as did poly(I) · poly(C) tested under the same assay conditions (Table 3). Even in the absence of the superinduction procedure, measurable (1.8 $\log_{10}$ units/ml) amounts of IFN were produced (data not illustrated).

High titers of IFN could be obtained if the cells were exposed to poly(G) · poly(C) in the presence of DEAE-dextran or $CaCl_2$. In these cases, no superinduction procedure was required and the polynucleotides needed to be incubated on the cells for only 1 h as opposed to 24 h (Table 3). The positively charged polyamino acids, poly(L-lysine) and poly(L-ornithine), could not substitute for the $CaCl_2$ or DEAE-dextran treatment (data not shown). Priming the cells with rabbit interferon did not significantly alter the yield of interferon in response to poly(G) · poly(C); nor did it alter the requirement for exposure of the cells to the poly(G) · poly(C) complex for 24 h (data not illustrated). If, after the 24 h exposure period, the cell culture medium was harvested and incubated on fresh PRK cells for 1 h, no interferon was produced; in addition, if poly(G) ·

TABLE 3

Interferon induction by poly(G)·poly(C): dependence upon assay conditions

Procedure	IFN titer[a] ($\log_{10}$ units/ml)	
	poly(G)·poly(C)[b]	poly(I)·poly(C)[b]
(1) Cells exposed to the complex for 1 h, then superinduced	<0.8	4.7
(2) Cells exposed to the complex for 24 h, then superinduced	2.5	2.0
(3) Cells exposed to the complex for 1 h in the presence of DEAE-dextran (100 μg/ml)	3.0	3.5
(4) Cells exposed to the complex for 1 h in the presence of $CaCl_2$ (5 mM)	3.5	4.5

[a] Determined in PRK cells, superinduced with cycloheximide and actinomycin D (procedures 1 and 2), or not superinduced (procedures 3 and 4).

[b] Final concentration of complex, 10^{-5} M.

poly(C) was incubated in MEM (without cells) at 37° for 24 h and then incubated on PRK cells for 1 h, no interferon production was detectable (data not shown).

As shown in Table 4, the properties of the antiviral principle induced by poly(G) · poly(C) in PRK cells corresponded to those expected for rabbit IFN. It was destroyed by trypsin and had no activity on human cells, but was stable toward treatment with pancreatic RNase A, EDTA, pH 2 treatment and was not neutralized by antiserum against human IFN-β.

The interferon-inducing activity of the poly(G) · poly(C) complex was not limited to rabbit cells. Table 4 shows the results of experiments in murine L-929 cells and compares the interferon-inducing ability of the poly(G) · poly(C) prepared in this current study to that prepared by S.L. Bresler, N.S. Tikhomirova-Sidorova and A.L. Timkovsky and to poly(I) · poly(C) purchased from P-L Biochemicals. It is clear that both preparations of poly(G) · poly(C) could induce interferon, albeit less effectively than poly(I) · poly(C). Significantly, the only induction protocol which led to interferon production in L-929 cells was that which involved the use of DEAE–dextran. Other procedures (24 h incubation on the cells, superinduction) did not give rise to interferon production as they had in the case of PRK cells.

The poly(G) · poly(C) prepared in this study also was able to induce interferon in human cells, specifically MG-63 fibroblasts. In this case, the poly(G) · poly(C) prepared in the present study was equally effective as the poly(G) · poly(C) sample from Leningrad and poly(I) · poly(C) from P-L Biochemicals: the interferon titer obtained after exposure of the cells to the complex (10^{-4} M) for 24 h, followed by superinduction, amounted to 10^2 units/ml. That this antiviral principle was truly human IFN-β was established by its neutralization by anti(Hu-IFN-β) serum (Table 6). In other human cell cultures that were examined, including E_6SM, T-21(RL) and VGS human fibroblasts, regardless of the induction protocol used, no interferon

TABLE 4

Characterization of antiviral principle induced by a mixture of poly(G)·poly(C)[a] and DEAE–dextran[a]

Treatment	Interferon titer ($\log_{10}$ units/ml)	
Antiviral principle induced by:	poly(G)·poly(C)	poly(I)·poly(C)
None	3.0	3.2
Incubate 1 h 37°C	3.2	3.2
Incubate 1 h 37°C with trypsin (0.25 mg/ml)	<1.0	1.2
Incubate 1 h 37°C with 10^{-3} M EDTA	3.2	3.5
Incubate 1 h 37°C with RNase A (0.1 mg/ml) in 10^{-3} M EDTA	3.2	3.5
Incubate 1 h at 50°C	2.3	2.4
Incubate 24 h at pH 2.0	3.0	3.4
Exposure to anti-human IFN-β at 10^5 U/ml	3.2	3.7
Activity upon titration in human cells	<0.8	<0.8

[a] Antiviral principle was induced by treatment of PRK cells with poly(G)·poly(C) (10^{-5} M) and DEAE–dextran (100 μg/ml).

TABLE 5

Interferon-inducing ability of poly(G) · poly(C) samples in murine L-929 cells[a]

Complex	Concentration (M)	Interferon titer (log_{10} units/ml)	
		exp. 1	exp. 2
Poly(G)·poly(C)[b] (this study)	10^{-4}	1.8	1.5
Poly(G)·poly(C)[c] (Leningrad)	10^{-4}	1.9	1.0
Poly(I)·poly(C) (P-L Biochemicals)	10^{-4}	2.5	3.1
	10^{-5}	3.5	3.3

[a] Polynucleotides were mixed with DEAE-dextran (100 μg/ml) and incubated on the cells for 1 h.
[b] Prepared according to the procedure outlined in Materials and methods.
[c] As obtained from the Institute of High Molecular Weight Compounds in Leningrad (see also Materials and methods).

response was obtained with poly(G) · poly(C) sample, even though poly(I) · poly(C) could induce interferon in these systems (data not shown).

In vivo induction of interferon

The interferon-inducing ability of poly(G) · poly(C) was not confined to in vitro situations. From Fig. 1, it is readily apparent that the poly(G) · poly(C) prepared in

TABLE 6

Interferon-inducing activity by poly(G)·poly(C) samples in human cells (MG-63) and its neutralization by anti-(Hu-IFN-β) serum[a]

Complex	Concentration (M)	Interferon titer[b] (log_{10} units/ml)	Neutralization titer[c] (log_{10} units/ml)
Poly(G)·poly(C)[d]	10^{-4}	1.2	5.0
(this study)	10^{-5}	1.2	5.0
Poly(G)·poly(C)[e]	10^{-4}	1.2	5.0
(Leningrad)	10^{-5}	1.2	5.0
Poly(I)·poly(C)	10^{-4}	1.2	5.0
(P-L Biochemicals)	10^{-5}	1.2	5.0

[a] Determined in T-21 (RL) human fibroblasts.
[b] Actual interferon titer as used in the neutralization assay.
[c] Mixtures of the interferon samples, diluted so as to contain about 10 IFN units/ml, with serial dilutions of goat anti-(Hu-IFN-β) serum were incubated at 37°C for 1 h, after which the residual antiviral activity was determined in T-21 (RL) cells challenged with VSV. The neutralization titers correspond to the highest dilution of antiserum which neutralized the protective effect of IFN by 50%, multiplied by the interferon titer of the sample assayed. Authentic Hu-IFN-β showed a neutralization titer under these conditions of 5.1 log_{10} units/ml.
[d] Prepared according to Materials and methods.
[e] As obtained from the Institute of High Molecular Weight Compounds in Leningrad (see also Materials and methods).

this study showed in vivo activity in both mice and rabbits. In mice (Fig. 1A), the poly(G) · poly(C) prepared for this study was as effective as an interferon inducer as was poly(I) · poly(C). The present poly(G) · poly(C) preparation induced serum interferon levels that were 10 times greater than those induced by the Leningrad poly(G) · poly(C) [5–8]. In rabbits (Fig. 1B), the poly(G) · poly(C) prepared for the present study produced lower peak interferon titers than did poly(I) · poly(C), whereas, in turn, the Leningrad poly(G) · poly(C) elicited a much lower interferon response than the current poly(G) · poly(C) preparation. The interferon titers produced in response to poly(G) · poly(C) did not diminish over the first 24 h following injection, whereas the interferon titers in response to poly(I) · poly(C) peaked at 3–6 h and declined rapidly thereafter. The Leningrad poly(G) · poly(C) preparation at 10^{-4} M induced low levels of detectable interferon (~ 10 units/ml) in rabbits at 6 h after injection (Fig. 1B).

Comparison of poly(G) · poly(C) prepared in Leningrad to that prepared in the present study

Our poly(G) · poly(C) preparation differed from the Leningrad sample both in physical properties as well as biological properties. The ultraviolet spectra of these

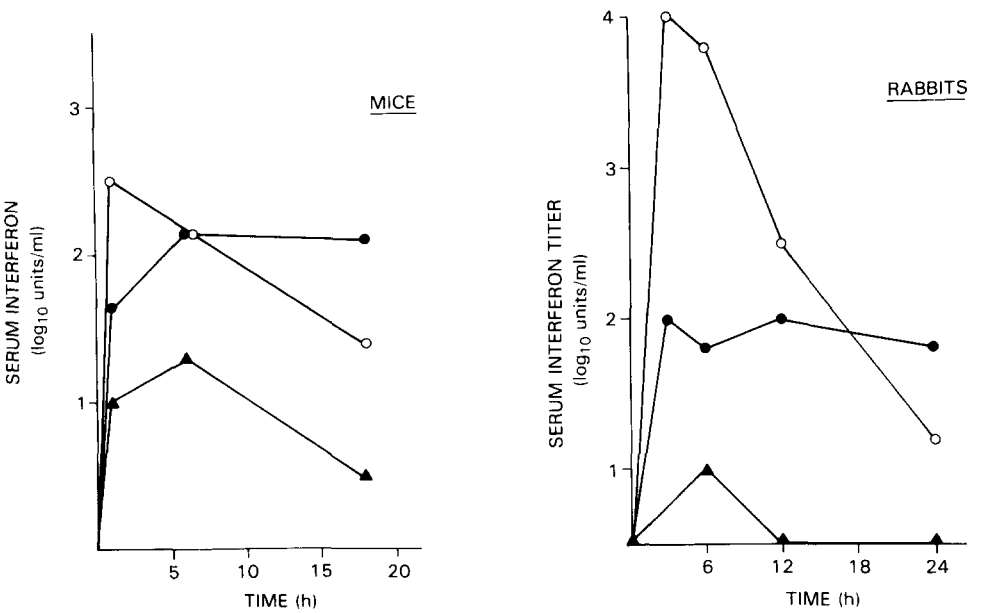

Fig. 1. Interferon-inducing activity of poly(G) · poly(C) (this study), poly(G) · poly(C) (Leningrad) and poly(I) · poly(C) in mice and rabbits. (A) Female NMRI mice (weighing 20–25 g) were injected intravenously with poly(G) · poly(C) or poly(I) · poly(C) at 10^{-5} M in 0.2 ml Dulbecco's PBS (3 mice per group). (B) Albino-white rabbits (weighing ~ 1 kg) were injected intravenously with poly(G) · poly(C) or poly(I) · poly(C) at 10^{-4} M in 1 ml Dulbecco's phosphate buffered saline (PBS). Symbols: ●, poly(G) · poly(C) this study; ▲, poly(G) · poly(C) Leningrad; ○, poly(I) · poly(C).

two preparations showed that the 260 nm/280 nm absorbance ratio was 2.22 for the Leningrad poly(G) · poly(C) while the corresponding ratio for the material prepared in this study was 1.75. The 260 nm/290 nm absorbance ratio also varied considerably: that for the Leningrad sample was 4.02 whereas the ratio for the sample prepared herein was 2.80. An additional difference in biological activity was that while the poly(G) · poly(C) prepared in this study was active in PRK cells, the Leningrad sample was not, regardless of the induction procedure followed (data not shown). Neither nucleic acid complex showed any evidence of strand separation (as monitored by ultraviolet hyperchromicity) even when the temperature was increased to > 95°C using 8 M urea, 0.001 M EDTA, pH 7.5 as buffer.

Ribonuclease resistance

The poly(G) · poly(C) prepared herein was evaluated for its sensitivity toward degradation by pancreatic RNase A and human serum. For comparison purposes the degradation of poly(I) · poly(C) also was followed. The nucleic acid was incubated at a final concentration of 10^{-5} M with either ribonuclease A or human serum for 1 h at 37°C. DEAE-dextran then was added at a final concentration of 100 μg/ml and the reaction mixtures were incubated with the PRK cells for 1 h. The cells were then further processed for interferon induction. Poly(I) · poly(C) was rapidly degraded by pancreatic ribonuclease A and also by human plasma (serum) as has been documented previously [16–18]. However, in contrast to poly(I) · poly(C), poly(G) · poly(C) was completely resistant to degradation either by ribonuclease A or human serum ((Fig. 2). These observations were in accord with the findings of Vilner et al. [19] that monkey serum cannot inactivate poly(G) · poly(C), even after a 3 h incubation period.

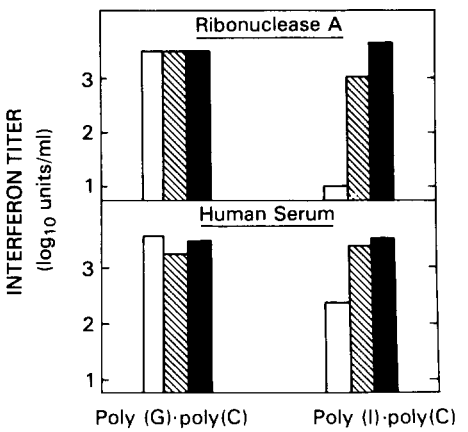

Fig. 2. Sensitivity of poly(G) · poly(C) to degradation by pancreatic ribonuclease A and human serum, as monitored by residual interferon induction in primary kidney cells. Poly(G) · poly(C) and poly(I) · poly(C) were incubated at a final concentration of 10^{-5} M with either ribonuclease A or human serum for 1 h at 37°C. DEAE-dextran was then added at a final concentration of 100 μg/ml and the reaction mixtures were incubated with the cells for 1 h. The cells were then further monitored for interferon production. Concentrations of ribonuclease A: □, 100 μg/ml; □, 10 μg/ml; ■, control. Concentrations of human serum: □, 50% (v/v); □, 10% (v/v); ■, control.

Discussion

The results presented herein aid in a final resolution of the uncertainty regarding the interferon-inducing capacity of poly(G) · poly(C). Thus, significant interferon-inducing activity of poly(G) · poly(C) depends upon a sufficiently high molecular size of the constituent homopolymers, the method by which the two complementary strands are annealled, and the manner in which the resulting complex is assayed for interferon-inducing ability. As to the latter criterion, our studies have established that, for the interferon-inducing activity of poly(G) · poly(C) to be expressed, it must be incubated on the cells for 24 h, a much longer exposure time than is required for other active inducers such as poly(I) · poly(C). Alternatively, either DEAE-dextran or calcium chloride, when administered together with the poly(G) · poly(C), can eliminate the necessity of the 24-h exposure. These results suggest, but by no means prove, that poly(G) · poly(C) is poorly (or slowly) taken up by cells. Changes in poly(G) · poly(C) structure do not appear to account for the peculiar behaviour of poly(G) · poly(C), since the medium from cells exposed to poly(G) · poly(C) for 24 h did not stimulate the production of interferon when exposed to fresh cells for 1 h.

Previous work has revealed the need for sufficiently high molecular weight of the component poly(G) and poly(C) homopolymers [6,8]. This earlier work also suggested a requirement for denaturing the poly(G) before complex formation with poly(C) [8,20], but these studies were carried out in aqueous solution without the use of denaturing agents. While under these conditions, some breakdown of the extremely stable secondary structure of poly(G) may occur, it would be far from complete according to the work of Englander et al. [13]. Heating the poly(G) to a temperature $>$ 85°C in 8 M urea was found to be necessary to induce complete collapse of the poly(G) self-structure. Thus, attempted denaturation of the poly(G) in aqueous buffer alone may lead only to partial denaturation of the poly(G); limited annealling with poly(C) could take place to give a mixture of poly(G) · poly(C) helix interrupted with poly(G) self-structure. In another context, it seems possible that the finding of Colby and Chamberlin [4] that poly(G) · poly(C) could induce resistance to sindbis virus infection may have been due to their method of preparation of poly(G) · poly(C). Their use of RNA polymerase to transcribe poly(G) from a poly(C) template would have obviated the difficulty of poly(G) self-structure.

That the antiviral principle induced by poly(G) · poly(C) in cell cultures was truly interferon has been established by a variety of criteria. That induced in rabbit cells was destroyed by trypsin, was stable at pH 2 and was inactive on heterologous cells. The interferon induced by poly(G) · poly(C) in human MG-63 cells was ascertained to be Hu-IFN-β, since it was neutralized by antiserum to Hu-IFN-β to the same extent as was authentic Hu-IFN-β.

The interferon-inducing ability of poly(G) · poly(C) was not confined to cell cultures since it was also induced interferon in rabbits and mice. However, the kinetics of the interferon response to poly(G) · poly(C) was different from that of poly(I) · poly(C). While poly(I) · poly(C) gave higher peak titers of interferon than did poly(G) · poly(C), the interferon response to poly(G) · poly(C) tended to last for a longer time. Even at 18–24 h post-injection no decrease in interferon titer was observed.

This protracted interferon response generated by poly(G) · poly(C) in vivo may be

related to a longer persistence of the polynucleotide in biological fluids. Poly(I) · poly(C) is rapidly degraded by human plasma nuclease(s) [16–18], and these nuclease(s) resemble pancreatic ribonuclease A in substrate specificity since they specifically hydrolyze the poly(C) strand of the poly(I) · poly(C) duplex [18]. In contrast with poly(I) · poly(C), poly(G) · poly(C) was completely resistant to degradation by ribonuclease A and human serum.

According to the results of this present study, poly(G) · poly(C) is endowed with several properties that suggest it may be an atypical interferon inducer. First, it is active on PRK cells, usually exquisitely sensitive to polynucleotide interferon inducers, but only after prolonged exposure or in the presence of DEAE-dextran or calcium chloride. Secondly, poly(G) · poly(C) also induces interferon in murine L-929 cells and human MG-63 cells, albeit less efficiently than poly(I) · poly(C) under similar conditions. In human fibroblast lines such as VGS or E_6SM, which respond well to poly(I) · poly(C), poly(G) · poly(C) does not induce detectable interferon. Thirdly, although poly(G) · poly(C) induces interferon in mice and rabbits, the kinetics of induction differ significantly from the kinetics of induction by poly(I) · poly(C) in that poly(G) · poly(C) gives a more protracted response. This latter property may relate to the resistance of poly(G) · poly(C) to degradation by serum nucleases. Finally, according to Smorodintsev [21], poly(G) · poly(C) is considerably less toxic to mice and rabbits than is poly(I) · poly(C), and, unlike poly(I) · poly(C) [22] does not cause an increased death rate when injected into mice shortly after infection with Newcastle disease virus or vesicular stomatitis virus [23].

The rather unusual behavior of poly(G) · poly(C) as an interferon inducer suggests that it may be valuable to characterize further this nucleic acid as to its protective activity against virus infection in vivo, its effect on tumor growth, hyporeactivity following repeated administration, its acute and chronic toxicity, and its activity in higher animal species such as monkeys.

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