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Studies on the Mechanism of the Priming Effect of Interferon on Interferon Production by Cell Cultures Exposed to Poly(rI)·Poly(rC)

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Interferon induction by poly(rI)·poly(rC) in primary rabbit kidney and mouse L-929 cell cultures was markedly increased if the cells were previously treated with homologous interferon. This priming effect has been established with different times of exposure of the cells to poly(rI)·poly(rC), and was most pronounced for short pulses of contact of the polynucleotide with the cells (10 s, 1 min). Treatment of the cells with pancreatic ribonuclease immediately after their exposure to poly(rI)·poly(rC) brought about a relatively greater reduction of the interferon response in interferon-primed cells than it did in unprimed cell cultures. Priming of the cells with interferon did not increase cell-binding of poly(rI)·poly(rC), whether this cell-binding was measured quantitatively (by radioactivity, upon exposure of the cells to radiolabeled polymer) or qualitatively (by antiviral activity, by assaying the cell extract for virus plaque reduction). Similarly, interferon priming did not alter the sensitivity of cell-associated poly(rI)·poly(rC) to extraneous ribonuclease treatment. Finally, priming with interferon did not decrease the rate of degradation of cell-bound poly(rI)·poly(rC) by cellular nucleases nor did it increase the anti-nuclease potency of the cells. The exact mechanism by which previous exposure of the cells to interferon enhances subsequent interferon production, induced by either synthetic polynucleotides or viruses, has not yet been resolved.

In cells exposed to (viable or inactivated) viruses, both increased and decreased interferon production have been obtained if the cells were pretreated with interferon. Whether pretreatment of the cell cultures with interferon resulted in a decreased or increased interferon production (respectively 'blocking' and 'priming'), depended very much on the concentration of interferon used, the length of interferon treatment, and the multiplicity of virus infection. In general, low to moderate doses of interferon enhanced subsequent interferon production (15, 16, 18, 20, 22-26, 31, 34), whereas high doses of interferon reduced the production of interferon (2, 5, 15, 17, 24, 26, 32, 35, 36).

If poly(rI)·poly(rC) was substituted for virus as the interferon inducing agent, pretreatment of the cells with interferon led generally to a net increase of the interferon response to poly(rI)·poly(rC) (2, 25, 28, 29, 30, 33), although, occasionally, depression of this interferon response has been reported (17, 32, 36). The priming effect of interferon on subsequent interferon induction by poly(rI)·poly(rC) has been established in different cell cultures (mouse L cells, rabbit kidney cells, and human diploid cells); the priming effect was generally greater if suboptimal doses of poly(rI)·poly(rC) were used for eliciting the interferon response. Recently, interferon priming has been proven a successful method for scaling up the production of human interferon in either human diploid cell cultures (3, 19) or leukocyte suspensions (18, 34).

The mechanism of the priming phenomenon is not at all understood. Therefore, attempts have been made to unravel the mechanism by which interferon-treated cells respond to poly(rI)·poly(rC) with an heightened interferon production. In designing the experiments reported in the present paper, we were led by the following questions: (i) Does priming with interferon increase subsequent interferon production by poly(rI)·poly(rC), even with very short periods of contact of the polymer with the cells? (ii) How does pancreatic ribonuclease affect the priming effect of interferon? (iii) Does poly(rI)·

poly(rC) bind more efficiently to interferon-primed than to unprimed cells, and (iv) is poly(rI)·poly(rC) bound to interferon-primed cells more or less susceptible to pancreatic ribonuclease than poly(rI)·poly(rC) bound to normal cells? Finally, (v) does priming of the cells with interferon alter the nuclease activity in these cells, or the rate of degradation of cell-bound poly(rI)·poly(rC), or both?

MATERIALS AND METHODS

Materials (poly[rI], poly[rC], ^{3}H-labeled poly[rC], pancreatic ribonuclease, cell cultures [PRK: primary rabbit kidney and mouse L-929 cells], cell culture medium [MEM: Eagle minimal essential medium], viruses [VSV: vesicular stomatitis virus]), and methods (production, titration, and characterization of interferon, preparation of cell homogenates and determination of cell-associated acid-insoluble radioactivity) have been fully documented in previous papers (7, 8, 9). Further details are presented in the legends to the figures.

RESULTS

Interferon production in interferon-primed and unprimed PRK and L-929 cell cultures exposed to poly(rI)·poly(rC) for different times. In previous investigations concerning the priming effect of interferon on subsequent interferon production by poly(rI)·poly(rC), the polymer was left in contact with the cells for a constant time period, generally 1 h (25, 29, 31). Since induction of interferon and antiviral resistance can be achieved with markedly shorter periods of contact of the cells with the polymer (1, 11, 27), we decided to explore the priming effect of interferon in cells exposed to poly(rI)·poly(rC) for different periods of time. Preliminary experiments had indicated that, in accordance with earlier findings (31), an optimal priming effect was obtained both in PRK and in L-929 cells, if the cells were incubated overnight (16 h) with 100 U of interferon per ml. This priming procedure was, therefore, used throughout all experiments.

Interferon production was measured in the supernatant fluid of PRK cells and L-929 cells which had been primed with homologous interferon or control medium, and then exposed to poly(rI)·poly(rC) for either 10 s, 1, 3, 10, 30, or 60 min. Previous treatment of the cells with interferon resulted in a marked increase of the interferon response to poly(rI)·poly(rC), no matter how long poly(rI)·poly(rC) was left in contact with the cells (Fig. 1). Unprimed L-cells did not produce any interferon. Yet, primed L cells produced nearly maximal amounts of interferon upon an extremely short pulse of poly(rI)·poly(rC): e.g., 10 s. Primed PRK cells produced invariably more interferon than unprimed PRK cells; this priming effect was the more apparent, the shorter time poly(rI)·poly(rC) was put in contact with the cells.

Influence of pancreatic ribonuclease on interferon induction by poly(rI)·poly(rC) in interferon-primed and unprimed PRK and L-929 cell cultures. It has been well established (1, 11) that ribonuclease treatment after exposure of the cells to poly(rI)·poly(rC) or other double-stranded polyribonucleotides reduces the subsequent induction of interferon and resistance to virus infection. The extent of this reduction depends on the time the cells are exposed to the polynucleotide before addition of the enzyme. To determine whether interferon priming may alter the sensitivity of poly(rI)·poly(rC)-induced interferon response to ribonuclease treatment, primed and unprimed PRK and L-929 cells were treated with pancreatic ribonuclease after their initial exposure to poly(rI)·poly(rC) (Fig. 2). This additional treatment with pancreatic ribonuclease significantly reduced the amounts of interferon subsequently

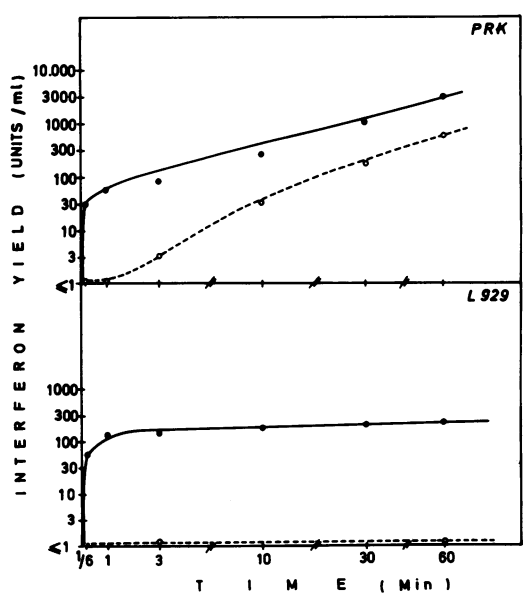

FIG. 1. *Poly(rI)·poly(rC)-induced interferon responses in unprimed and interferon-primed cell cultures. PRK and L-929 cell cultures were exposed to homologous interferon (100 U/ml) (●) or control medium (MEM plus 3% calf serum) (○) (2 ml/petri dish) for 16 h, washed (3 times) with MEM, and then incubated with poly(rI)·poly(rC) (10 μg/ml in MEM; 1 ml/petri dish) at 37 C for different times ranging from 1 to 60 min. The cells were washed again with MEM and further incubated with MEM plus 3% calf serum (4 ml/petri dish). Interferon production was measured in the supernatant fluid 24 h later.*

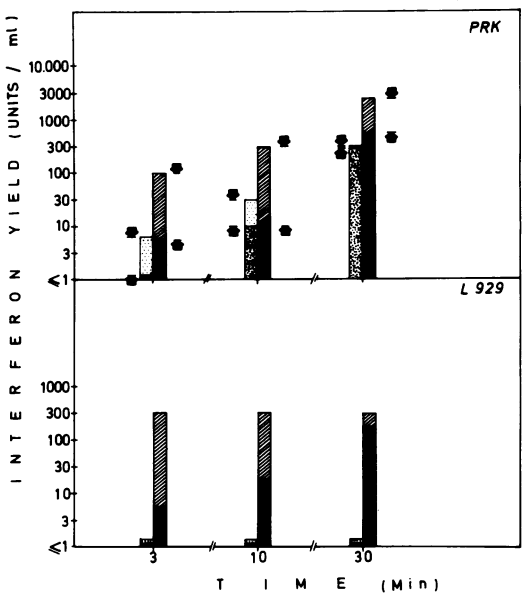

FIG. 2. *Effect of pancreatic ribonuclease on interferon induction by poly(rI)·poly(rC) in unprimed and interferon-primed cell cultures. PRK and L-929 cell cultures were exposed to homologous interferon (100 U/ml) or control medium (MEM plus 3% calf serum) (2 ml/petri dish) for 16 h, washed (3 times) with MEM, and then incubated with poly(rI)·poly(rC) (10 μg/ml in MEM; 1 ml/petri dish) at 37 C for either 3, 10, or 30 min. The cells were washed again with MEM and then exposed for 30 min to either MEM plus 10^{-3} M EDTA or MEM plus 10^{-3} M EDTA plus pancreatic ribonuclease (10 μg/ml) (2 ml/petri dish). Thereafter, the cells were washed once more and incubated with MEM plus 3% calf serum (4 ml/petri dish). Interferon was measured in the supernatant fluid 24 h later. Symbols: ⊡, unprimed, EDTA treatment; ▤, unprimed, EDTA plus RNase treatment; ▨, interferon-primed, EDTA treatment; ■, interferon-primed, EDTA plus RNase treatment.*

produced. The reduction was inversely proportional to the time the cells were left in contact with poly(rI)·poly(rC) before addition of ribonuclease. Moreover, the interferon response induced in interferon-primed cells showed a remarkably greater sensitivity to pancreatic ribonuclease treatment than did the interferon response induced in unprimed cells. With primed (PRK) cells exposed to poly(rI)·poly(rC) for 10 min, ribonuclease treatment brought about a 30-fold drop in interferon production as compared to a threefold reduction in unprimed (PRK) cells. Similar effects were seen if poly(rI)·poly(rC) was incubated on the PRK cells for 3 or 30 min. Relative sensitivities of interferon induction to ribonuclease treatment in primed and unprimed L cells could not be established because of the lack of interferon production in the latter.

It should be pointed out that the effects of ribonuclease treatment depicted in Fig. 2 were obtained by exposing the cells, immediately after their incubation with poly(rI)·poly(rC), to 100 μg of pancreatic ribonuclease per ml of MEM (plus 10^{-3} M ethylenediaminetetraacetic acid [EDTA]) for 30 min at 37 C. Additional incubation of the cells for 30 min at 37 C with MEM (plus 10^{-3} M EDTA) or MEM without EDTA did not affect the subsequent interferon production as compared to cells which were immediately incubated with maintenance medium (MEM plus 3% calf serum) after their initial exposure to poly(rI)·poly(rC).

Kinetics of cell-binding of poly(rI)·poly(rC) in interferon-primed and unprimed PRK and L-929 cell cultures exposed to poly(rI)·poly(rC) for different times. To investigate whether the priming effect of interferon may have been mediated by an increased uptake of the polynucleotide by the cells, the rate of cell binding of poly(rI)·poly(rC) was measured in both interferon-primed and unprimed PRK and L-929 cell cultures. Binding of the polymer to the cells was monitored in the following ways: (i) analysis of acid-insoluble radioactivity in homogenates of cells exposed to ^{3}H-labeled poly(rI)·poly(rC) and (ii) analysis of antiviral activity in homogenates of cells exposed to unlabeled poly(rI)·poly(rC). This antiviral activity was assayed by measuring vesicular stomatitis virus plaque reduction in PRK cells with serial dilutions of the cell homogenates. A reference standard of poly(rI)·poly(rC) was included in the assay, so that a direct estimation could be made of the amounts of poly(rI)·poly(rC) that were taken up by the cells at a given time after their exposure to the polymer.

As demonstrated in both PRK and L-929 cell cultures (Fig. 3), ^{3}H-labeled poly(rI)·poly(rC) attached equally well to interferon-primed and unprimed cells. It should be stressed that the conditions used for measuring cell-associated radioactivity (concentration of the polynucleotide and times of exposure of the polynucleotide to the cells) were identical to those employed for measuring the kinetics of interferon production (Fig. 1). In additional experiments cell-associated radioactivity was measured in interferon-primed and unprimed L-929 cell cultures exposed to suboptimal concentrations of ^{3}H-labeled poly(rI)·poly(rC) (1, 0.5, 0.1 μg per ml per petri dish). Again, no significant differences were observed in the amounts of acid-insoluble radioactivity recovered from primed and unprimed cells (data not shown).

Likewise, if binding of poly(rI)·poly(rC) to the cells was monitored by antiviral activity instead of radioactivity, interferon-primed and

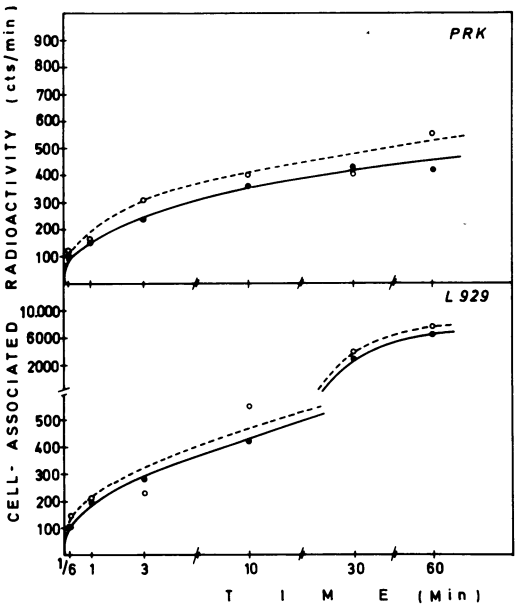

FIG. 3. *Kinetics of binding of ^{3}H-labeled poly(rI)·poly(rC) to unprimed and interferon-primed cell cultures as measured by cell-associated radioactivity. PRK and L-929 cell cultures were exposed to homologous interferon (100 U/ml) (●) or control medium (MEM plus 3% calf serum) (○) (2 ml/petri dish) for 16 h, washed (3 times) with MEM, and then incubated with poly(rI)·poly(rC) (mixture of 5 μg of unlabeled and 5 μg of ^{3}H-labeled poly[rI]·poly[rC] per ml: approximately 110,000 counts/min in 1 ml of MEM per petri dish) at 37 C for different times ranging from 1 to 60 min. At the indicated times, the cells were washed (3 times) with MEM and acid-insoluble radioactivity was determined in the cell homogenates.*

unprimed cell cultures did not show marked differences in the amounts of cell-associated polymer (data not shown). Thus, all attempts designed to reveal any increased binding of poly(rI)·poly(rC) to interferon-primed cells invariably failed to do so, clearly indicating that the priming effect of interferon is not mediated by an increased cellular uptake of the interferon inducer.

Influence of pancreatic ribonuclease on cell-associated poly(rI)·poly(rC) in interferon-primed and unprimed PRK and L-929 cell cultures. Differences in accessibility of cell-associated poly(rI)·poly(rC) to pancreatic ribonuclease (or persistence of the polynucleotide at the outer cell membrane, in as far as sensitivity of cell-associated poly[rI]·poly[rC] to exogenous nuclease treatment can be considered as a parameter of persistence of the polymer at the cell surface) have recently been connected with differences in antiviral activity of poly(rI)·poly(rC) in various cell cultures (9).

To investigate whether differences in accessibility of cell-bound poly(rI)·poly(rC) to pancreatic ribonuclease might also account for the priming effect of interferon, interferon-primed and unprimed PRK and L-929 cell cultures were exposed to ^{3}H-labeled poly(rI)·poly(rC) (for 1, 3, 10, 30, or 60 min) and immediately thereafter were treated with pancreatic ribonuclease or control medium (MEM plus 10^{-3} M EDTA) for 30 min. Cell-associated poly(rI)·poly(rC) was determined by measuring acid-insoluble radioactivity in the cell homogenates. The conditions used for measuring the sensitivity of cell-bound poly(rI)·poly(rC) to pancreatic ribonuclease (concentration and time of exposure of either poly[rI]·poly[rC] or ribonuclease) were exactly the same as those employed for measuring the influence of pancreatic ribonuclease on interferon production (Fig. 2). It has been established previously (9) that pancreatic ribonuclease applied exogenously to PRK or L-929 cell cultures (at 40 μg/ml in MEM plus 10^{-3} M EDTA) for 30 min at 37 C does not degrade ^{3}H-uridine-labeled host cell ribonucleic acid (RNA). Thus, any decrease in cell-associated poly(rI)·poly(rC) upon pancreatic ribonuclease treatment should be ascribed to a release or degradation, or both, of that portion of the polynucleotide that is superficially associated with the cell.

As shown in both PRK and L-929 cell cultures (Fig. 4), pancreatic ribonuclease did not remove a substantially greater part of cell-associated poly(rI)·poly(rC) from interferon-treated than from untreated cells. Similar results were obtained if the amounts of cell-associated poly(rI)·poly(rC) were determined by antiviral activity instead of radioactivity (data not shown). These results suggest that the increased interferon response to poly(rI)·poly(rC) in interferon-primed cells cannot be related to a longer persistence of the polynucleotide at the surface of these primed cells.

Nuclease sensitivity of cell-bound poly(rI)·poly(rC) in interferon-primed and unprimed L-929 cells. A plausible explanation for the priming effect of interferon on the subsequent interferon response to poly(rI)·poly(rC) might have been protection of the polynucleotide from premature degradation by cellular nucleases. Such degradation has been invoked previously (2) to explain the reduced interferon response (tolerance, hyporeactivity) upon repeated administration of poly(rI)·poly(rC) to cell cultures. To explore the possibility that priming of the cells with interferon might alter the degradation of cell-associated poly(rI)·poly(rC) by cellular enzymes, interferon-primed and unprimed L-929 cell cultures were exposed

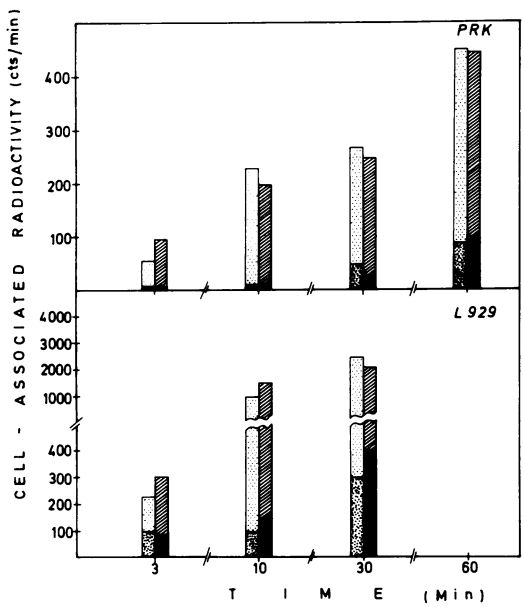

FIG. 4. *Effect of pancreatic ribonuclease on cell-associated radioactivity in unprimed and interferon-primed cell cultures exposed to ^{3}H-labeled poly(rI)·poly(rC). PRK and L-929 cell cultures were exposed to homologous interferon (100 U/ml) or control medium (MEM plus 3% calf serum) (2 ml/petri dish) for 16 h, washed (3 times) with MEM, and then incubated with poly(rI)·poly(rC) (mixture of 5 μg of unlabeled and 5 μg of ^{3}H-labeled poly[rI]·poly[rC] per ml: approximately 110,000 counts/min in 1 ml MEM per petri dish) at 37 C for either 3, 10, 30, or 60 min. The cells were washed again with MEM and then exposed for 30 min to either MEM plus 10^{-3} M EDTA or MEM plus 10^{-3} M EDTA plus pancreatic ribonuclease (100 μg/ml) (2 ml/petri dish). Thereafter, the cells were washed again (3 times) with MEM and acid-insoluble radioactivity was determined in the cell homogenates. Symbols: ▨, unprimed, EDTA treatment; ▨ unprimed, EDTA plus RNase treatment; ▩, interferon-primed, EDTA treatment; ■, interferon-primed, EDTA plus RNase treatment.*

to ^{3}H-labeled polymer for a given period of time and then were further incubated with polymer-free medium (Fig. 5).

A small part of the cell-bound radioactivity was lost during the additional incubation period with control medium. Such loss may be expected if acid-insoluble radioactivity measured in cells which have been exposed to radiolabeled poly(rI)·poly(rC) truly reflects uptake of the polymer by the cell and not incorporation of degraded polymer material into host cell RNA (9). In the experiments presented in Fig. 5 interferon-primed cells showed a slightly higher binding of ^{3}H-labeled poly(rI)·poly(rC) than unprimed cells. However, the decrease in cell-associated radioactivity noted after an additional incubation with control medium did not differ significantly from interferon-primed cells to unprimed cells, suggesting that priming of the cells with interferon did not alter the rate of degradation of cell-bound poly(rI)·poly(rC).

To investigate the possibility that interferon-treated cells may have a heightened anti-nuclease activity, cell extracts (as well as intact cells) prepared from interferon-primed and unprimed L-929 cell cultures were added to a reaction mixture containing ^{3}H-labeled poly(rI)·poly(rC) (0.1 μg/ml) and pancreatic ribonuclease (10 μg/ml). The reaction mixtures were incubated at 37 C and samples were withdrawn at different times for analysis of acid-insoluble radioactivity.

The data presented in Fig. 6 show that (i) in the absence of cells (or cell extracts) ^{3}H-labeled poly(rI)·poly(rC) was rapidly degraded by pancreatic ribonuclease, (ii) addition of cells (or cell extracts) tempered the rate of degradation of poly(rI)·poly(rC) by pancreatic ribonuclease, (iii) cells (or cell extracts) prepared from primed and unprimed cells did not significantly differ in inhibiting the degradation of poly(rI)·poly(rC), and (iv) the cells (or cell extracts) themselves did not cause a breakdown of ^{3}H-

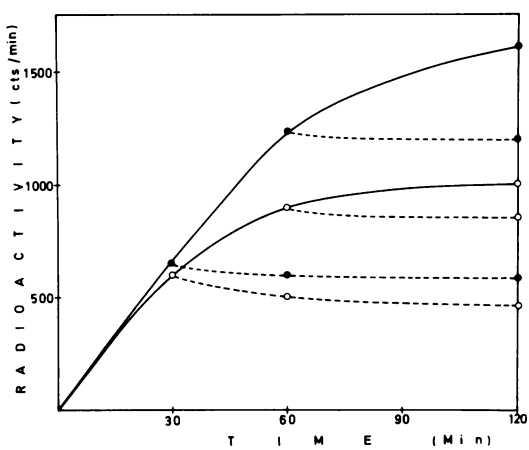

FIG. 5. *Fate of cell-associated ^{3}H-labeled poly(rI)·poly(rC) in unprimed and interferon-primed L-929 cell cultures. L-929 cell cultures were exposed to mouse interferon (100 U/ml) (●) or control medium (MEM plus 3% calf serum) (○) (2 ml/petri dish) for 16 h, washed (3 times) with MEM, and then incubated with ^{3}H-labeled poly(rI)·poly(rC) (22,000 counts/min or 1 μg/ml in MEM; 1 ml/petri dish) at 37 C for either 30 min, 1 of 2 h. All cell cultures were then washed (3 times) with MEM; one part of the cell cultures was immediately analyzed for acid-insoluble radioactivity (——). Another part of the cell cultures was further incubated at 37 C with MEM (4 ml/petri dish) for either 30, 60, or 90 min, and then analyzed for acid-insoluble radioactivity (- - -).*

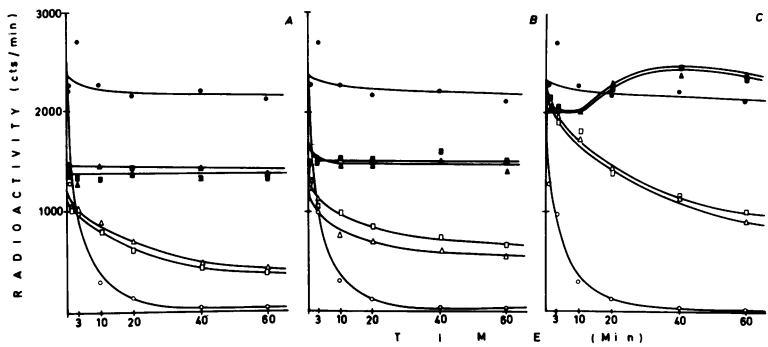

FIG. 6. *Influence of intact cells (A) and cell homogenates, prepared by ultrasonication for 10s (B) or 2 min (C), from unprimed and interferon-primed L929 cell cultures, on the rate of degradation of ^{3}H-labeled poly(rI)·poly(rC) by pancreatic ribonuclease. ^{3}H-labeled poly(rI)·poly(rC) (0.1 μg/ml) was incubated at 37 C for varying periods of either of the following: (●), control medium (MEM plus 10^{-3} M EDTA); (▲), unprimed cell preparation, (■), interferon-primed cell preparation, (○), pancreatic ribonuclease (10 μg/ml) in control medium; (△), pancreatic ribonuclease (μg/ml) plus unprimed cell preparation; (□), pancreatic ribonuclease (10 μg/ml) plus interferon-primed cell preparation. Total volume of the reaction mixture: 10 ml. Cell preparations were used at a ratio of 8 × 10^6 cells (2 confluent petri dish cell cultures) per reaction mixture. At the indicated times 1-ml samples of the reaction mixtures were withdrawn for analysis of acid-insoluble radioactivity.*

labeled poly(rI)·poly(rC) (as measured by trichloroacetic acid-precipitable radioactivity). Thus, interferon-treated cells did not show an increased anti-nuclease activity as compared to normal cells, suggesting that their increased sensitivity to interferon induction by polynucleotides is not due to protection of the polynucleotide against nucleases.

DISCUSSION

Kleinschmidt (21) recently suggested that interferon priming is solely a cell surface and cell membrane phenomenon. This conclusion was essentially based on the observations of Stewart et al. (31) who clearly demonstrated that, unlike the production of interferon itself and the development of antiviral activity induced by interferon, the development of priming did not depend on protein synthesis. If interferon priming is a cell surface (and cell membrane) phenomenon, it should most likely act by increasing the uptake of the interferon inducer (21). In that sense, priming with interferon would be very similar to the potentiation of the interferon response observed after addition of diethylaminoethyl-dextran (10, 13).

Yet, interferon-primed cells did not show such increased uptake of poly(rI)·poly(rC), although poly(rI)·poly(rC) induced significantly higher interferon levels in primed than in unprimed cells (Fig. 1 and 3). Cell-binding of poly(rI)·poly(rC) was studied by measuring cell-associated acid-insoluble radioactivity, upon exposure of the cells to ^{3}H-labeled poly(rI)·poly(rC) as well as by measuring cell-associated antiviral activity, upon exposure of the cells to unlabeled polymer. The first assay system may be interpreted as a quantitative binding of poly(rI)·poly(rC) molecules, irrespective of their antiviral activity. The second system may be described as qualitative binding, since only those poly(rI)·poly(rC) molecules are measured in this assay which are active in virus inhibition. Neither quantitative nor qualitative binding of poly(rI)·poly(rC) appeared to be increased in interferon-primed cells as compared to unprimed cells.

That the efficiency of poly(rI)·poly(rC) as an interferon inducer does not depend on its rate of uptake by the cells has been established in several studies. Thus, De Clercq et al. (12) did not find a significant correlation between the antiviral activity and rate of cell-binding with eight different polyribo- or polydeoxyribonucleotides of widely differing degrees of antiviral activity. Poly(rI)·poly(rC) exposed to a series of eight cell cultures which differed markedly in sensitivity to the antiviral activity of the polynucleotide bound equally well to the most and least sensitive of these cell cultures (9). Colby and Chamberlin (6), Black et al. (4), and Field et al. (14) also failed to demonstrate a preferentially increased cellular uptake of poly(rI)·poly(rC) as compared to other, noninterferon inducing polynucleotides (poly[rI]·poly[dC] [6], poly[rI]·poly[2'-ClC] [4], poly[rI] and poly[rC] [14]) in either chicken embryo cells, human skin fibroblast, or rabbit kidney cell cultures.

May pretreatment of the cells with interferon promote persistence of poly(rI)·poly(rC) at the cell surface, thus preventing the polymer from entering into the cell? Previous studies have revealed (i) a positive correlation between the antiviral activity of poly(rI)·poly(rC) in different cell cultures and its persistence at the outer cell membrane of these cultures (9), and (ii) a positive correlation between the antiviral activity and persistence at the cell surface with polyr(A-U), poly(rI)·poly(rC) and many other polyribo- and polydeoxyribonucleotides upon heating of the individual polymers at 37 C in MEM (11, 12). However, the increased interferon titers obtained with poly(rI)·poly(rC) in interferon-primed cells could not be correlated with a longer persistence of the polymer at the cell surface: poly(rI)·poly(rC) bound to interferon-primed cells proved equally well susceptible to extraneous ribonuclease treatment as poly(rI)·poly(rC) bound to unprimed cells (Fig. 4).

That the priming effect of interferon would be due to a protection of poly(rI)·poly(rC) from premature degradation by cellular nucleases has also been ruled out: there were no differences in the fate of cell-associated poly(rI)·poly(rC) in interferon-primed and unprimed cells (Fig. 5) and cell extracts prepared from interferon-treated cells did not preferentially inhibit degradation of poly(rI)·poly(rC) by pancreatic ribonuclease as compared to cell extracts prepared from normal cells (Fig. 6).

That ultra-brief exposure times of poly(rI)·poly(rC) to interferon-treated cells lead to a nearly full expression of the antiviral activity in these cells (Fig. 1), is reminiscent of the very short exposure times required for initiation of antiviral activity with thermally activated polynucleotides (poly r[A-U] [11]). It is tempting to speculate, therefore, that both interferon priming and thermal activation reside in similar or at least complementary mechanisms. Priming of the cells with interferon may unmask (L-929 cells) or adjust (PRK cells) cellular receptor sites, so that these become able to recognize particular structural features in the polynucleotide. With thermal activation, the configuration of the polynucleotide itself would be altered to fit in with the cellular receptor sites. Thus, both interferon-priming and thermal activation may achieve their effects by a specific adaptation of the cellular receptor sites to the conformation of the polynucleotide.

It has been recently observed that interferon priming not only increases the responsiveness of cells to induction, but also shortens the time required for induction of interferon (15, 22, 26, 29, 31, 32, 34). In this regard it will be particularly interesting to determine whether normal (unprimed) cells produce interferon more quickly in response to thermally activated polynucleotides than they do to nonactivated complexes.

The results presented in Fig. 2 indicate that adaptation of the cellular receptor sites in interferon-primed cells is rather sensitive to pancreatic ribonuclease, for pancreatic ribonuclease treatment of the cell cultures immediately after their exposure to poly(rI)·poly(rC) brought about a relatively greater reduction of interferon production in interferon-primed than in unprimed cells. Yet, pancreatic ribonuclease did not remove significantly greater amounts of cell-bound poly(rI)·poly(rC) from primed than from unprimed cells (Fig. 4). It would appear, therefore, that the relatively greater ribonuclease sensitivity of the interferon response in primed cells as compared to unprimed cells is not caused by an increase in the release or degradation of cell-bound poly(rI)·poly(rC), or both. It may well be ascribed to other mechanisms, e.g., a weakened association of poly(rI)·poly(rC) with the cellular receptor site.

It remains to be determined whether interferon responses induced by polynucleotides result from the same induction process as interferon responses induced by viruses; at least in some cases, this does not appear to be the situation (32). It is, therefore, not presently tempting to speculate on a common mechanism of priming for viral and nonviral inducers. In view of the apparently diverse effects of interferons on cells (30), it is possible that different mechanisms of priming are involved for different types of inducers.

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