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The Epidermal Growth Factor- and Interferon-Independent Effects of Double-Stranded RNA in A431 Cells

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SUMMARY

The human epidermoid carcinoma cell line A431, containing an amplification in the epidermal growth factor (EGF) receptor gene, was examined for its sensitivity to the growth inhibitory effects of synthetic double-stranded RNAs (dsRNAs). Poly(I)·poly(C), poly(A)·poly(U) and $rI_n \cdot r(C_{13},U)_n$ at 5 to 100 μg/ml produced 20 to 60% growth inhibition, whereas poly(ICLC) produced 40 to 80% growth inhibition at 0.05 to 25 μg/ml. Poly(I)·poly(C) did not cause the secretion of interferon (IFN) into the medium, and addition of polyclonal antibodies to IFN-α and IFN-β did not block the growth inhibition produced by poly(I)·poly(C). Clone 29, which proliferates in response to EGF, and clone 29R, which is sensitive to the growth inhibitory effects of EGF, showed sensitivities to the antiproliferative effects of poly(I)·poly(C) similar to those of the parent cell line. Incubation of cell membrane extracts with poly(I)·poly(C) or treatment of cells with the dsRNA did not affect EGF receptor tyrosine kinase activity. On the other hand, poly(I)·poly(C) produced a dose-dependent induction of (2′,5′)oligo(A) synthetase activity and degradation of 45S preribosomal RNA and 28S and 18S rRNA. These results indicate that the growth inhibitory properties of poly(I)·poly(C) in A431 cells are independent of the action of IFN but are associated with degradation of rRNA, an effect that may be related to the (2′,5′)oligo(A)-RNase L pathway.

The synthetic dsRNA poly(I)·poly(C) is a potent antiviral and antitumor agent (1–4), as well as an effective inducer of IFN in several cell systems (5, 6). In these instances, dsRNA is believed to serve as an activator of two IFN-induced enzymes, dsRNA-dependent protein kinase and (2′,5′)oligo(A) synthetase (7, 8). The former enzyme is believed to function via phosphorylation of eukaryotic initiation factor 2 to inhibit translation whereas the latter enzyme effects a similar result by activation of a latent endonuclease (RNase L) to degrade rRNA and mRNA.

Poly(I)·poly(C) can also produce biological effects in the absence of exogenous interferon. Poly(I)·poly(C) exerted an antiproliferative effect in lung and bladder carcinoma, HeLa, and fibrosarcoma cell lines without cotreatment with IFN (9–11). In quiescent fibroblast 3T3 cells, poly(I)·poly(C) produced a mitogenic response that resulted in the increased expression of the proto-oncogenes c-*myc* and c-*fos*, in a manner similar to that of platelet-derived growth factor (12). More recently, it was demonstrated that the proliferative effect of poly(I)·poly(C) in human fibroblasts was *amplified* by anti-IFN-β antibodies (13). Thus, there is some evidence to suggest that dsRNA may produce biological effects independent of and even antagonistic to that of IFN.

Thus far, there have not been any investigations that have sought to determine whether the antiproliferative action of poly(I)·poly(C) as a single agent is related to the IFN-inducible (2′,5′)oligo(A)-RNase L pathway. In the present study, we have investigated this possibility in the human epidermoid carcinoma cell line A431, which we discovered to be highly sensitive to the growth inhibitory properties of dsRNA. This cell line also contains an amplification of the EGF receptor gene (14–16) and thus lent itself to determining whether the antiproliferative properties of dsRNA are associated with its ability to act as a growth factor by direct interaction with the EGF receptor tyrosine kinase.

Experimental Procedures

Materials. [*methyl*-^{14}C]Thymidine (53 mCi/mmol) and [α-^{32}P]ATP (600 Ci/mmol) were purchased from New England Nuclear (Boston, MA). ^{125}I-EGF (228 μCi/μg) and [γ^{32}P]ATP (589 Ci/mmol) were obtained from ICN Radiochemicals (Irvine, CA). Poly(I)·poly(C)-agarose, poly(A)·poly(U), and poly(I)·poly(C) were purchased from Pharmacia-P. L. Biochemicals (Milwaukee, WI). Poly(ICLC) was kindly supplied by Dr. Hilton Levy, National Cancer Institute (Frederick, MD). The mismatched analogue of dsRNA was synthesized according to the

ABBREVIATIONS: dsRNA, double-stranded RNA; IFN, interferon; EGF, epidermal growth factor; IFN-α, leukocyte IFN; IFN-β, fibroblast IFN; IFN-γ, immune IFN; poly(I)·poly(C), polyriboinosinic·polyribocytidylic acid; poly(A)·poly(U), polyriboadenylic·polyribouridylic acid; poly(ICLC), polyriboinosinic·polyribocytidylic acid stabilized with poly-L-lysine in carboxymethylcellulose; $rI_n \cdot r(C_{13},U)_n$, mismatched dsRNA, in which the C:U base ratio is 13:1; (2′,5′)oligo(A), $pppA(2'pA)_n$; TCA, trichloroacetic acid; IC_{40}, concentration producing a 40% reduction in cell count; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; PMSF, phenylmethyl sulfonyl fluoride; EGTA, [ethylene bis (oxyethylene nitrilo)]tetraacetic acid.

method described previously (17). Polyclonal antibodies to IFN-α and IFN-β were obtained from the Research Resources Branch, National Institute of Allergy and Infectious Diseases (Bethesda, MD).

Cell culture. Human epidermoid carcinoma cell line A431 was purchased from American Type Culture Collection (Rockville, MD). Clones 29 and 29R of A431 cells (18–20) were kindly provided by Dr. Gordon N. Gill, University of California (San Diego, CA). Cells were grown under an atmosphere of air and 5% CO_2 in Dulbecco's minimum essential medium supplemented with 10% heat-inactivated fetal calf serum and gentamicin, 50 μg/ml. Clone 29 was maintained in medium supplemented with 10 nM EGF but was grown in the absence of EGF for 1 week before use in the experiments described. Cell inocula were 2×10^5/10 ml in 25-cm^2 flasks and 2×10^6/100 ml in 175-cm^2 flasks. Cell growth was monitored by determining the cell number with a Model ZM Coulter counter.

DNA synthesis. Cells in 25-cm^2 flasks were incubated for 1 hr with 1 μCi of [*methyl*-^{14}C]thymidine. Cold TCA-precipitable radioactivity was collected on glass fiber filter discs and radioactivity was measured by liquid scintillation counting.

IFN assays. The presence of IFN in the medium was bioassayed, using MDBK cells, by Dr. Katherine Zoon, Office of Biologics, Food and Drug Administration, as described previously (21).

(2′,5′)Oligo(A) synthetase. Logarithmically growing cells in 175-cm^2 flasks were treated with poly(I)·poly(C) for varying time intervals. Extracts were prepared after lysing the cells with 20 mM HEPES (pH 7.4)/5 mM $MgCl_2$/120 mM KCl/1 mM dithiothreitol/10% (v/v) glycerol/0.5% Nonidet P-40. Cell extracts containing 20 μg of protein/10 μl were assayed for (2′,5′)oligo(A) synthetase activity as described previously (21). Briefly, cell extracts are adsorbed to poly(I)·poly(C)-agarose and incubated with [α-^{32}P]ATP and Mg^{2+}. After incubation for 1 hr at 30°, unincorporated ATP is hydrolyzed with alkaline phosphatase and labeled (2′,5′)oligo(A) is adsorbed to and eluted from an alumina column. One unit of (2′,5′)oligo(A) synthetase is that amount that synthesizes 1 nmol of (2′,5′)oligo(A)$_n$ per hr at 30°C.

(2′,5′)Oligo(A) levels. Intracellular (2′,5′)oligo(A) levels were measured in log phase cells in 175-cm^2 flasks after 1 day of treatment with 25 or 100 μg/ml of poly(I)·poly(C). Cells were harvested by trypsinization, washed with cold PBS, and extracted with 100 μl of 5% TCA. Extracts were neutralized by shaking with 2 volumes of 0.5 M trioctylamine in trifluorotrichloroethane. (2′,5′)Oligo(A) levels in neutralized extracts were measured by the radiobinding assay as described previously (22).

dsRNA-dependent protein kinase. Exponentially growing cells were treated for 1 day with poly(I)·poly(C) and detergent-solubilized extracts were prepared as described above. Poly(I)·poly(C)-dependent protein phosphorylation was assayed after adsorption of cell extracts containing 20 μg of protein to 20 μl of poly(I)·poly(C)-agarose, which was washed extensively with 20 mM HEPES (pH 7.5)/10 mM $MgCl_2$/120 mM KCl/1 mM dithiothreitol/10% glycerol as described previously (23). The bound proteins were incubated with 10 μM ATP containing 10 μCi of [γ-^{32}P]ATP in a 50-μl reaction mixture at 30°C for 1 hr. The reaction was terminated by adding 50 μl of 2× electrophoresis sample buffer [0.063 M Tris·HCl (pH 6.8)/2% SDS/10% glycerol/0.05 M dithiothreitol/0.001% (w/v) bromophenol blue]. The reaction mixture was heated at 95° for 10 min and centrifuged in an Eppendorf centrifuge, and the supernatant was separated electrophoretically in 10% polyacrylamide slab gels as described by Laemmli (24).

Cell membranes. Cell membranes were prepared by the procedure of Kris *et al.* (25). In brief, cells were washed twice with cold phosphate-buffered saline and the cell pellet was suspended in a buffer containing 20 mM HEPES (pH 7.5), 1.5 mM $MgCl_2$, 1 mM EGTA; and 1 mM PMSF and homogenized in a glass homogenizer. After centrifugation in an Eppendorf centrifuge for 5 min at 4°, the supernatant was applied to a 35% (w/v) sucrose solution and centrifuged for 30 min at 15,000 rpm at 4° in a Sorvall centrifuge. The membrane fraction at the interface was suspended in 10 mM HEPES (pH 7.5) and recentrifuged for 30 min at 15,000 rpm at 4°. The membrane pellet was suspended in 50 mM Tris·HCl (pH 7.5)/1 mM PMSF/2 mM EGTA/10 mM dithiothreitol/5 μg/ml aprotinin/200 μg/ml leupeptin/1% (v/v) Triton X-100, sonicated for 5 sec at 4°, and centrifuged for 6 min at 15,000 × *g* in an Eppendorf centrifuge. Protein concentrations in Triton X-100 extracts were determined using the Bio-Rad protein reagent with bovine serum albumin as a standard.

***In vitro* phosphorylation.** The reaction mixture contained Triton X-100 extract of membranes containing 20 μg of protein, 20 mM HEPES (pH 7.5), 1 mM $MnCl_2$, 15 μM [γ-^{32}P]ATP (10 μCi/assay), 7.5 μg/ml bovine serum albumin, and, when indicated, 100 ng/ml EGF and 100 μg/ml poly(I)·poly(C) in a final volume of 60 μl. The tubes were placed at room temperature without and with EGF or poly(I)·(poly(C). Tubes were then chilled on ice for 10 min, the reaction was initiated by the addition of labeled ATP, and the incubation at 4° was continued for 5 min as described previously (26). The reaction was terminated by addition of 15 μl of 6× electrophoresis sample buffer and the tubes were heated for 2 min in boiling water. Aliquots (50 μl) of each sample were applied to 7.5% polyacrylamide gels and the proteins were separated electrophoretically at 40 V for 18 hr at room temperature. The gels were then dried and autoradiographed using Kodak X-Omat XK-1 film.

RNA characterization. Logarithmically growing cells in 175-cm^2 flasks were prelabeled with 2.5 μCi of [^{14}C]uridine and then treated for 1 day with 100 μg/ml poly(I)·poly(C). Cells were pulse-labeled for 2 hr with [^{3}H]adenosine (1 μCi/ml) and 1 μM 2′-deoxycoformycin to inhibit adenosine deaminase. RNA was extracted and separated as described previously (27). Each gel represents 0.2 A260 units of RNA.

EGF receptor level. The binding of ^{125}I-EGF to cell membrane receptors was performed with 10^6 cells in 25-cm^2 flasks. The monolayer was washed twice with Dulbecco's minimum essential medium containing 10% fetal calf serum and incubated with varying concentrations of radiolabeled EGF in the presence and absence of 1 μg/ml unlabeled EGF. After 1 hr of incubation at 37°, cells were washed 3 times at 4° with the incubation medium and subsequently washed twice with the medium lacking fetal calf serum. Cells were solubilized in 3 ml of 2% sodium dodecyl sulfate and cell-bound radioactivity was measured in a γ counter. Nonspecific binding was determined as the difference in binding of ^{125}I-EGF in the presence and absence of unlabeled EGF and was less than 10% of the total binding.

Results

Cell growth and DNA synthesis. Exponentially growing cells were exposed to varying concentrations of either poly(I)·poly(C), poly(A)·poly(U), poly(ICLC), or $rI_n \cdot r(C_{13},U)_n$ and cell growth was determined 3 days after treatment. Poly(I)·poly(C), poly(A)·poly(U), or $rI_n \cdot r(C_{13},U)_n$ at 5 to 100 μg/ml produced a 20 to 60% reduction in cell growth and 0.05 to 25 μg/ml of poly(ICLC) produced a 40 to 80% inhibition of cell growth (Fig. 1A). The concentration of dsRNA producing a 40% reduction in cell growth after 3 days of treatment was 20 μg/ml poly(I)·poly(C), 100 μg/ml poly(A)·poly(U), 0.2 μg/ml poly(ICLC), and 40 μg/ml $rI_n \cdot r(C_{13},U)_n$. The reduction of DNA synthesis after 3 days of treatment with dsRNA paralleled growth inhibition (Fig. 1B). Three-day treatment with 5 μg/ml of either poly(I)·poly(C), poly(A)·poly(U), $rI_n \cdot r(C_{13},U)_n$, or 0.5 μg/ml poly (ICLC) reduced DNA synthesis by 50%.

To ascertain whether growth inhibition by poly(I)·poly(C) was related to production of IFN, cells were incubated with an excess (400 neutralizing units) of anti-IFN-α and anti-IFN-β during the three day exposure to 100 μg/ml poly(I)·poly(C) (Table 1). This treatment did not alter the cytostatic effect produced by poly(I)·poly(C). Growth inhibition was also determined by the direct addition of IFN-β to the medium (Table 1). In these instances, IFN-β produced no growth inhibition

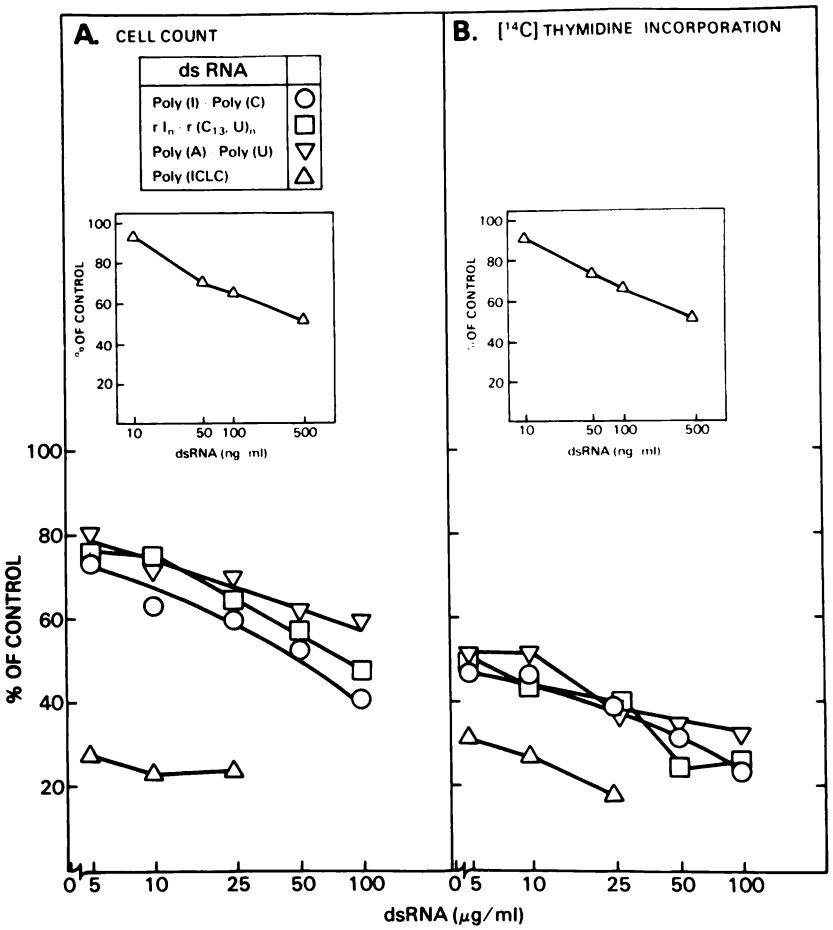

Fig. 1. Effects of different dsRNAs on cell growth and DNA synthesis. A431 cells in log phase were treated for 3 days with the indicated concentrations of either poly(I)·poly(C), $rI_n \cdot (C_{13},U)_n$, poly(A)·poly(U), or poly(ICLC). Cells were pulse-labeled with [^{14}C]thymidine for 1 hr and cell number (A) and TCA-insoluble radioactivity (B) was measured. Each value is the mean of three experiments, for which the standard error did not exceed 5%.

TABLE 1

Effect of IFN-β, anti-IFN-α, and anti-IFN-β on cell proliferation in A431 cells treated with poly(I)·poly(C)

Each value is the mean of duplicate experiments.

Treatment	Cell count
	% of control
Control	100
Poly(I)·poly(C) (100 μg/ml)	27
Poly(I)·poly(C) (100 μg/ml) + preimmune serum	21
Poly(I)·Poly(C) (100 μg/ml) + anti-IFN-α, (400 NU/ml) + anti-IFN-β, (400 NU/ml)*	28
IFN-β (10 units/ml)	88
IFN-β (50 units/ml)	71
IFN-β (100 units/ml)	71
IFN-β (500 units/ml)	51

* NU, neutralizing units.

below 10 units/ml and only marginal growth inhibition at concentrations above 10 units/ml. We also directly measured IFN levels by bioassays after treatment with 100 μg/ml poly(I)·poly(C) for 8, 15, and 24 hr. In each instance, 5 units or less of IFN per ml was produced, which is the background level of the bioassay and is not considered significant (results not shown).

To examine the possibility that poly(I)·poly(C) affected the growth of A431 cells by direct interaction with the EGF receptor, the anticellular effects of poly(I)·poly(C) were investigated in two variants of A431 cells. Clone 29 contains approximately 50% of the gene copy number and EGF receptor level of the parent cell line and proliferates in response to EGF (Fig. 2). The revertant cell line 29R contains a 1.5-fold higher gene copy number and EGF receptor concentration compared with the parent cell line and, like A431 cells, is inhibited by EGF (Fig. 2) (18–20). Both cell variants showed sensitivities to poly(I)·poly(C) similar to that exhibited by the parental A431 cells (Fig. 2).

EGF receptor tyrosine protein kinase activity. The growth responses of the parental and clonal variants of A431 cells correlate with the number of EGF binding sites and the stimulation of EGF receptor tyrosine kinase activity by EGF (18–20). To investigate whether alterations in this activity were responsible for the growth inhibition observed in A431 cells by poly(I)·poly(C), autophosphorylation of the EGF receptor was measured in the absence and presence of EGF or poly(I)·poly(C), using detergent-solubilized cell membrane preparations. Although direct addition of 100 ng/ml EGF stimulated receptor kinase activity, addition of 100 μg/ml of poly(I)·poly(C) alone or together with EGF did not result in any further change in autophosphorylation (Fig. 3). Treatment of cells with poly(I)·poly(C) also did not affect the autophosphorylation of the EGF receptor *in vitro* (results not shown).

Binding of EGF to poly(I)·poly(C)-treated cells. The binding of ^{125}I-EGF to control cells and those pretreated for 1 day with 2.5 μg/ml poly(I)·poly(C) revealed no significant differences (results not shown).

(2′,5′)Oligo(A) synthetase and (2′,5′)oligo(A) levels. In order to assess the role of the IFN-induced, dsRNA-dependent (2′,5′)oligo(A)-RNase L pathway (7), (2′,5′)oligo(A) synthetase activity was measured in cells exposed to varying con-

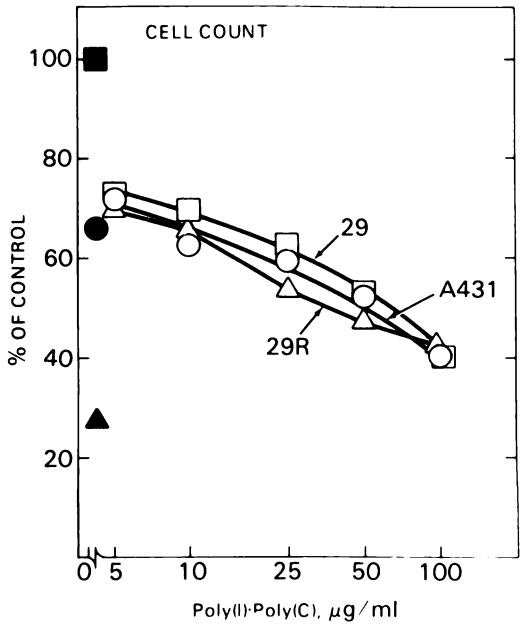

Fig. 2. Sensitivity of variants of A431 cells to poly(I)·poly(C). The cell variants, clone 29 (□, ■) and clone 29R (△, ▲), and parental A431 cells (○, ●) were treated with varying concentrations of poly(I)·poly(C) (open symbols) or 5 ng/ml, EGF (closed symbols) and the cell count was determined after 3 days of exposure to the dsRNAs.

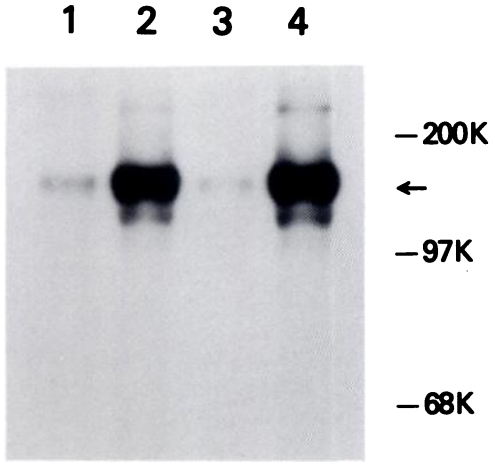

Fig. 3. *In vitro* phosphorylation in membrane extracts from A431 cells in the presence of EGF and poly(I)·poly(C). Detergent-solubilized extracts from A431 cell membranes were phosphorylated in the absence (*lane 1*) or presence of 100 ng/ml of EGF (*lane 2*), 100 μg/ml poly(I)·poly(C) (*lane 3*), or a combination of EGF and poly(I)·poly(C) (*lane 4*) for 5 min at 4°. The reaction products were separated by polyacrylamide gel electrophoresis as described under Experimental Procedures. An autoradiograph of the dried gel is shown.

centrations of poly(I)·poly(C) and after varying exposure intervals (Fig. 4). Treatment of cells with 10 to 100 μg/ml poly(I)·poly(C) resulted in a 2- to 6-fold induction of synthetase activity (Fig. 4A), which reached a maximum 15 to 24 hr after treatment (Fig. 4B). However, no measurable amount of intracellular (2′,5′)oligo(A) could be detected in cell extracts by a radioligand binding assay after 1 day of treatment with 100 μg/ml poly(I)·poly(C) (results not shown). The sensitivity of the radiobinding assay was such that it enabled us to detect levels of (2′,5′)oligo(A) as low as 2 fmol/10^6 cells in cell extracts "spiked" with authentic (2′,5′)oligo(A).

rRNA degradation. To determine whether RNase L was activated in cells after poly(I)·poly(C) treatment, the integrity of prelabeled and newly synthesized rRNA was examined 1 day after treatment (Fig. 5). Fifty to 70% degradation of prelabeled 45S ribosomal precursor RNA and 28S and 18S rRNA was observed in poly(I)·poly(C)-treated cells. Two-hour pulse-labeling of these cells with [^{3}H]adenosine revealed an 80 to 90% reduction in newly synthesized rRNA. Cell viability in control or treated cells, as determined by exclusion of trypan blue, was greater than 90% under these conditions.

dsRNA-dependent protein kinase activity. dsRNA-dependent protein kinase was not detectable in either control or poly(I)·poly(C)-treated A431 cells (results not shown).

Discussion

The human epidermoid carcinoma cell line A431 is unique in comparison with most other cell lines in that it is highly sensitive to growth inhibition by dsRNA. Although all of the dsRNAs were effective in inhibiting cell growth, the optimal concentration for the individual dsRNA's varied to a significant extent. A 100-fold higher concentration of poly(I)·poly(C) (IC_{40} = 20 μg/ml) in comparison with poly(ICLC) (IC_{40} = 0.2 μg/ml) was necessary to produce an equivalent anticellular effect. The greater potency of poly(ICLC) can be attributed to its increased resistance to hydrolysis by ribonucleases (28). Conversely, a 2-fold higher concentration of the mismatched dsRNA $rI_n \cdot r(C_{13},U)_n$ was required, and this difference in concentration can be attributed to its increased sensitivity to ribonuclease A (29). The lesser antiproliferative effect of $rI_n \cdot r(C_{13},U)_n$ on A431 cells in comparison with poly(I)·poly(C) is in agreement with previous reports of its effects on other human tumor cell lines (11) and its reduced effectiveness in potentiating the antiproliferative activity of IFN-γ (17). Similarly, a greater concentration of poly(A)·poly(U) than poly(I)·poly(C) was essential to generate equivalent growth inhibition in A431 cells. Poly(A)·poly(U) was shown previously to be a poor IFN inducer and produced less cytotoxicity than poly(I)·poly(C) in mouse L929 cells pretreated with IFN-β (30). In contrast, poly(A)·poly(U) was superior to poly(I)·poly(C) in potentiating the cytotoxic effect of IFN-γ in human colon carcinoma cells (17).

Because dsRNAs are effective IFN inducers in some cell types, it is possible that the growth inhibitory effects of the dsRNAs were manifestations of the endogenous secretion of IFN. Using a sensitive bioassay, only 5 units or less of IFN per ml of medium were present after treatment with poly(I)·poly(C). These concentrations were not found to be growth inhibitory. Moreover, the addition of anti-IFN-α and anti-IFN-β concomitantly with poly(I)·poly(C) did not affect dsRNA-mediated growth inhibition, a result noted by others investigating the cytotoxicity produced by poly(I)·poly(C) in human fibrosarcoma and epidermal carcinoma cells (10, 11). Thus, it is unlikely from the available evidence that IFN is mediating the cytotoxic effects of poly(I)·poly(C), unless it is by an autocrine mechanism, in which an antibody might not be accessible to the lymphokine, such as found in a recent study in which anti-granulocyte/macrophage colony-stimulating factor could not block autocrine stimulation of a cell line secreting this factor (31). Because our cell line does not constitutively produce IFN, the latter possibility is unlikely.

To ascertain whether the growth inhibitory effect of poly(I)·poly(C) was mediated by its interaction with the EGF receptor, the growth responses of variants of A431 cells that either

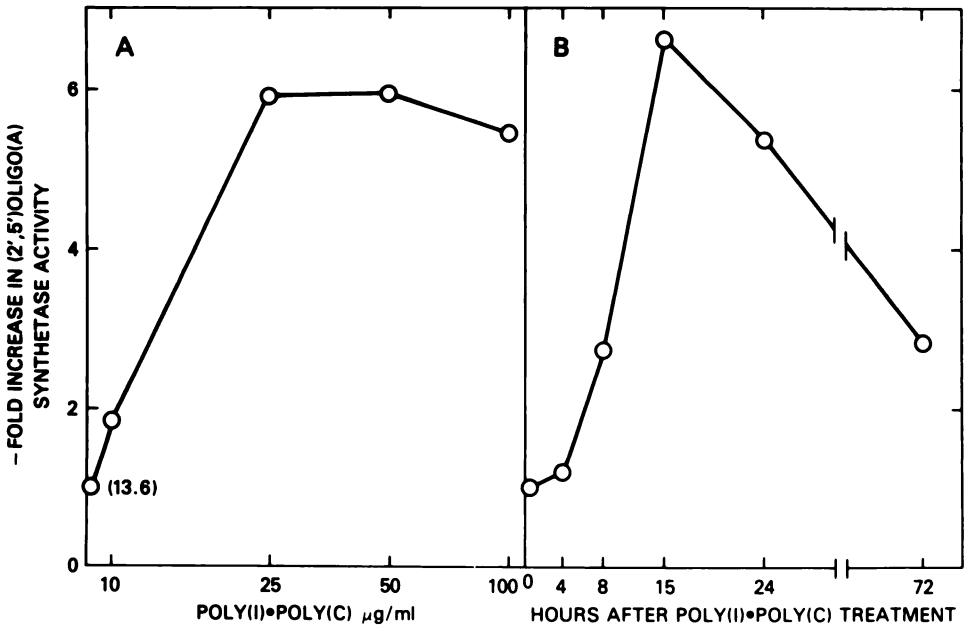

Fig. 4. (2′,5′)Oligo(A) synthetase induction after poly(I)·poly(C) treatment. A431 cells were treated with varying concentrations of poly(I)·poly(C) for 1 day (A) or 100 μg/ml poly(I)·poly(C) (B) for varying time intervals. Extracts of control and poly(I)·poly(C)-treated cells were assayed for (2′,5′)oligo(A) synthetase activity as described in Experimental Procedures. The results are expressed as the fold increase in (2′,5′)oligo(A) synthetase activity in treated cells in comparison with control cells. *Numbers in parenthesis* represent the basal enzyme activity (units/mg) in untreated cells. Each value represents the mean of duplicate assays from two experiments.

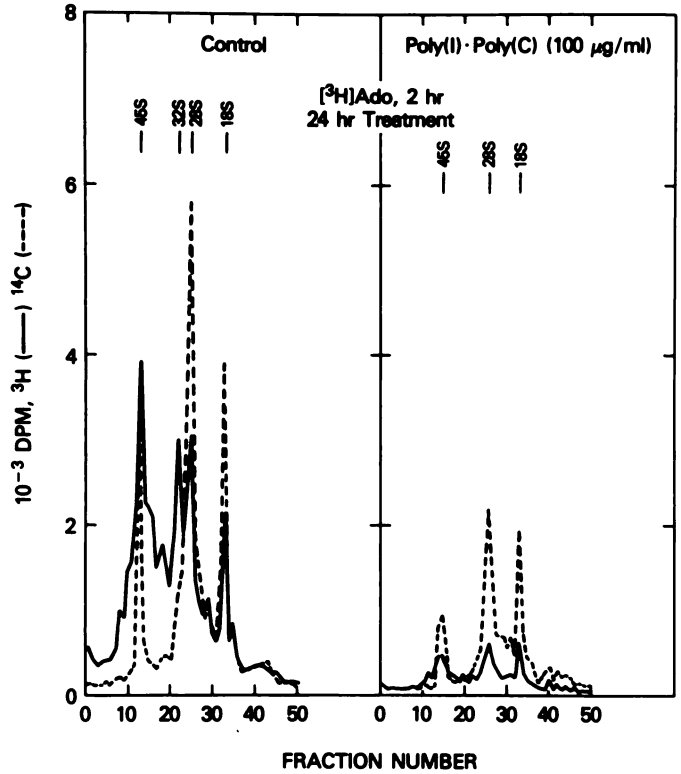

Fig. 5. rRNA degradation in A431 cells treated with poly(I)·poly(C). Cells were prelabeled with [^{14}C]uridine and subsequently exposed to 100 μg/ml poly(I)·poly(C) for 1 day. Cells were pulse-labeled with [^{3}H]adenosine for the last 2 hr of treatment. The viability of control and treated cells was greater than 90% as assessed by the exclusion of trypan blue. RNA was extracted and separated as described under Experimental Procedures.

proliferate or are inhibited by EGF were assessed in the presence of poly(I)·poly(C). The response of the 29 and 29R subclones to EGF is a direct function of the degree of gene amplification in each cell line (18–20), with a lower level of ligand-activated EGF receptors causing growth stimulation (clone 29) and a higher level causing growth inhibition (clone 29R). In each instance, the variants exhibited a sensitivity similar to that of the parental cell line. The level of autophosphorylation of the EGF receptor as well as the receptor level were also unaltered by poly(I)·poly(C). These data preclude a direct interaction of poly(I)·poly(C) with the EGF receptor as a possible mechanism of growth inhibition.

The role of the dsRNA-dependent (2′,5′)oligo(A)-RNase L pathway has been implicated in the antiviral and antiproliferative effects of IFN (7, 8) but not of dsRNA *per se*. Our results demonstrate that (2′,5′)oligo(A) synthetase activity is significantly elevated in A431 cells after poly(I)·poly(C) treatment. Although, an increase in tissue and serum (2′,5′)oligo(A) synthetase activity in mice after treatment *in vivo* with dsRNA has been reported previously (31, 32), elevation of (2′,5′)oligo(A) synthetase after *in vitro* exposure of tumor cells to dsRNA alone has not been reported previously. Despite elevated (2′,5′)oligo(A) synthetase, intracellular (2′,5′)oligo(A) was undetectable after treatment with poly(I)·poly(C), but significant degradation of both prelabeled and pulse-labeled rRNA was observed in dsRNA-treated cells, a characteristic that typifies the involvement of IFNs and dsRNA-activated RNase L (34–39). Nilsen *et al.* (40) have previously demonstrated a significant increase in (2′,5′)oligo(A) levels and mRNA degradation in HeLa cells exposed to the combination of IFN-β and poly(I)·poly(C), but not in cells exposed to either agent alone. The lack of detection of intracellular (2′,5′)oligo(A) levels in our case is probably a result of the high (2′,5′)oligo(A) phosphodiesterase activity present in most cell lines (7) and the limited induction of (2′,5′)oligo(A) synthetase by dsRNA in comparison with IFN (21). On the other hand, localized concentrations of (2′,5′)oligo(A) or compartmentalization of intracellular (2′,5′)oligo(A) might still account for the activation of latent RNase L to foster the endonuleolytic cleavage of rRNA.

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