Cellular and Enzymatic Activities of a Synthetic Heteropolymer Double-stranded RNA of Defined Size*

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We have synthesized a novel heteropolymer double-stranded RNA (dsRNA) molecule of defined length and strandedness (dsRNA309) and evaluated its ability to induce cytokine gene expression, activate dsRNA-dependent enzymes, and inhibit both tumor cell growth and virus replication. Unlike the conventionally studied synthetic homopolymer dsRNAs, polyinosinic acid:polycytidylic acid (poly(I-C)) and its mismatched analogue polyinosinic:polycytidylic acid, poly(I-C12-U), dsRNA309 possessed restricted biological activity. dsRNA309 was unable to inhibit tumor cell growth or efficiently induce cytokine (i.e. interferon-2 and interleukin-1α) gene expression. However, dsRNA309 was able to inhibit virus replication and activate dsRNA-dependent intracellular enzymes, 2′-5′-oligoadenylate synthetase (2′-5′A synthetase) and the dsRNA-activated inhibitor kinase in *in vitro* assay systems. Overall, dsRNA309 provided a means for examining the mechanisms governing the dsRNA-regulated antiviral and antiproliferative responses, and studies with dsRNA309 demonstrated that the ability of a synthetic dsRNA to activate dsRNA-dependent intracellular enzymes does not necessarily predict the same gene inducing capacity.

Poly(I-C) is a synthetic homopolymer dsRNA which has been used extensively as a model for understanding the mechanism by which cells respond to virus infection. It is believed that the viral dsRNA genome or dsRNA replicate intermediates are the molecular species responsible for the antiviral cascade which is triggered upon virus infection (1). Marcus and Sekellick (2) demonstrated that one intracellular dsRNA molecule was sufficient for interferon (IFN) induction and proposed that dsRNA was capable of interacting with and activating a cellular receptor responsible for amplification of a dsRNA-induced signal. Subsequently, poly(I-C) has been shown to possess a variety of activities including the ability to activate intracellular enzymes, induce gene expression, and inhibit both virus replication and tumor cell growth (reviewed in Ref. 3).

Two IFN-induced, dsRNA-activated intracellular enzymes, 2′-5′A synthetase and DAI kinase, have been implicated as key intracellular enzymes involved in the poly(I-C)-induced, antiviral, and antiproliferative responses (reviewed in Ref. 4). Upon activation by dsRNA, 2′-5′A synthetase converts ATP into a series of oligonucleotides containing an unusual 2′-5′ phosphodiester linkage (5, 6). These compounds are capable of activating a latent endonuclease, RNase L, which inhibits protein synthesis by degrading RNA (7, 8). Another IFN-induced intracellular enzyme, DAI, is activated by poly(I-C) and autophosphorylated upon dsRNA activation (9). Once autophosphorylated, DAI phosphorylates the α subunit of eukaryotic protein synthesis initiation factor eIF-2 which prevents further polypeptide chain initiation and results in inhibition of protein synthesis (10).

A number of genes involved in both growth regulation and the antiviral response are induced upon treatment of cells with dsRNA or virus (11-14). The best characterized dsRNA-inducible genes are the type I IFNs (IFN-α and IFN-β) through which many of the dsRNA-induced antiviral and antiproliferative properties are mediated (15). Even though cis-acting sequences within the IFN promoters, required for induction by dsRNA, have been extensively characterized (reviewed in Refs. 16 and 17), the cellular receptors and signal transduction pathways governing the induction of IFN remains poorly understood. The protein kinase inhibitor 2-aminopurine has been shown to inhibit the induction of a number of genes (including IFN-β) by poly(I-C) or virus infection (18, 19). Since 2-aminopurine inhibits DAI activity (20), researchers have speculated that this kinase may be involved in gene induction by dsRNA. However, it still remains to be seen whether DAI is actually involved in mediating gene induction by dsRNA or virus infection.

The biological activity of poly(I-C) and its analogues has been shown to depend primarily upon the length of double-stranded regions within these molecules and not on particular nucleic acid base sequence (21). Studies have also demonstrated that the structural characteristics of synthetic homopolymer dsRNA required for enzyme activation are similar to that required for IFN induction (22, 23). However, one problem with using homopolymer dsRNA to define structural requirements for dsRNA-induced activities is that due to its limited complexity, these molecules do not remain entirely double-stranded in solution. Strand slippage and intermolecular complex formation can alter the molecular size and percentage of double helicity of these molecules. In order to circumvent this problem, we have synthesized complementary random sequence heteropolymer dsRNA of defined length and strandedness. Synthetic heteropolymer dsRNA has been

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‡‡ The abbreviations used are: poly(I-C), polyinosinic-polycytidylic acid; poly(I-C12-U) polyinosinic-polycytidylic acid, uridylic acid; ds, double-stranded; IFN, interferon; IL, interleukin; 2′-5′A synthetase, 2′-5′ oligoadenylate synthetase, DAI, dsRNA-activated inhibitor (dsRNA-dependent protein kinase), CHX, cycloheximide; PBS, phosphate-buffered saline; SDS, sodium dodecyl sulfate; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.
used previously to determine affinity of DAI for dsRNA (24) but the biological activities of these molecules has not been investigated. We report that heteropolymer dsRNA309 unexpectedly possessed restricted biological activity as compared to homopolymer dsRNA. Similar to poly(I-C) and poly(I-C$_3$U), dsRNA309 was a potent activator of dsRNA-dependent enzymes in vitro and possessed antiviral activity. However, dsRNA309 was unable to induce cytokine (i.e. IFN-β and IL-1α) gene expression or inhibit the growth of a dsRNA-sensitive bladder carcinoma cell line, A1698.

**Materials and Methods**

**Reagents**—Ribonuclease A (RNase A), cycloheximide (CHX), Sephadex G-50, and proteinase K were purchased from Sigma; poly(I-C) from Pharmacia LKB Biotechnology Inc.; poly(I-C$_3$U) was obtained from HEM Pharmaceuticals (Rockville, MD); calf alkaline phosphatase, pGEM1, and RNA polymerases T7 and Sp6 were purchased from Promega; deoxyribonuclease 1 (DNase I) and restriction endonucleases NheI and MuAl from Boehringer Mannheim; and radiolabeled compounds from Amersham.

**Synthesis of Heteropolymer dsRNA**—Template (dsdsRNA309) for in vitro transcription was generated by subcloning 4X174 bacteriophage sequences into the polylinker of pGEM1. Template for plus RNA (T7 reaction) was prepared by a NheI restriction and minus strand RNA (Sp6 reaction) by a MuAl restriction. In vitro transcription consisted of 100 μl containing, at final concentrations, 40 mM Tris-HCl, pH 7.5, 1.5 mM MgCl$_2$, 2 mM spermidine, 2 mM ATP, 2 mM CTP, 2 mM GTP, 50 μg/ml RNase-free T7 RNA polymerase, linearized DNA template, and 200 units of T7/Sp6 RNA polymerase. Reaction was incubated at 37 °C for 4 h, and the quantity of plus RNA (T7 reaction) and minus RNA (Sp6 reaction) strand was determined from the percentage incorporation of [α-32P]UTP. DNA template was then removed by incubating completed transcription reactions with 200 units of DNase I for 30 min at 37 °C. Equal quantities of plus and minus strand RNA were then annealed at 65 °C for 15 min in 0.14 M sodium phosphate. After annealing, dsRNA309s were nonRNased aliquot of annealed product was loaded onto a Sephadex G-50 column, eluted with PBS, and stored at −70 °C. dsRNA309 was unable to induce cytokine (i.e. IFN-β and IL-1α) gene expression or inhibit the growth of a dsRNA-sensitive bladder carcinoma cell line, A1698.

**Cell Line**—A1698 cells derived from a human bladder carcinoma were obtained from S. Aronson (National Cancer Institute). These cells were cultured in RPMI 1640 media containing 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin at 37 °C, 5% CO$_2$. These cells were previously described as A2182, a human lung adenocarcinoma cell line which was sensitive to the antiproliferative effects of poly(I-C) and poly(I-C$_3$U). However, karyotype analysis (W. Peterson, Children’s Hospital of Michigan) indicated that cells maintained for previously published studies were the A1698 cell line.

**Antiviral Assays**—Antiviral assays with A1698 cells were performed as described (26) with the following modifications. Five × 10$^5$ exponentially growing cells in 200 μl were seeded into flat bottom 96-well microtiter plates. A1698 cells were grown 2 days post-confluence with frequent media changes (once per day). Media were removed from post-confluent A1698 cells, replaced with media containing synthetic dsRNA, and incubated overnight. Cells were then challenged with a 10–tissue culture infectivity dose (TCID$_{50}$) of polio-1 virus in 20 μl of media. After a 15-min incubation, 150 μl of media was added, and cells were incubated for 3–5 days. Media were removed each day, subjected to a low speed centrifugation (500 × 10 min), and used to reseed virus-challenged cells (1:1 mixture of fresh and virus-containing media) until a cytopathic effect was observed in non-treated cells. Once a cytopathic effect was observed (4–5 days), media were removed, cells were washed three times with PBS, and 100 μl of 0.014% Finters neutral red in growth media were added to each well. After a 1-h incubation at 37 °C, media were removed, cells were again washed three times with PBS, and 100 μl of acidified ethanol (50% ethanol, 1% acetic acid) was added to each well. The amount of absorbed neutral gel was then quantitated colorimetrically by measuring optical density at 490 nm (MR700 microplate reader, Dynatech Laboratories).

**Antiproliferative Assays**—Antiproliferative assays were performed as described (25) with the following modifications. One × 10$^5$ exponentially growing cells were seeded into 35-mm dishes and allowed to attach overnight. After an overnight incubation, cells in day 0 dishes were detached with 1 ml of trypsin, diluted with an equal volume of 0.2% trypan blue, and the number of viable cells per dish was determined with a hemacytometer. Also at day 0, media were removed from cells containing A1698 cells and replaced with complete media containing synthetic dsRNA and proper controls. After a 4-h treatment, media were removed, and cells were provided with fresh media and allowed to incubate for 72 h. After 72 h, media were removed, cells were washed three times with PBS, and the number of cells was determined as described for day 0 points. Values represent different cultures containing A1698 cells.

**Measurement of Gene Induction**—Induction of A1698 cells with synthetic dsRNA and subsequent lyase RNase protection was performed as described (27). Cultures were preincubated with complete medium containing 50 μg/ml CHX at 37 °C for 1 h, then in complete medium containing 50 μg/ml CHX + synthetic dsRNA at 37 °C for an additional 4 h. Cells were removed with trypsin, collected by low speed centrifugation, and dissolved in complete media containing synthetic dsRNA and proper controls. After a 4-h treatment, media were removed, and cells were provided with fresh media and allowed to incubate for 72 h. After 72 h, media were removed, cells were washed three times with PBS, and the number of cells was determined as described for day 0 points. Values represent different cultures containing A1698 cells.

**Expression of Gene Induction**—Induction of A1698 cells with synthetic dsRNA and subsequent lyase RNase protection was performed as described (27). Cultures were preincubated with complete medium containing 50 μg/ml CHX at 37 °C for 1 h, then in complete medium containing 50 μg/ml CHX + synthetic dsRNA at 37 °C for an additional 4 h. Cells were removed with trypsin, collected by low speed centrifugation, and dissolved in complete media containing synthetic dsRNA and proper controls. After a 4-h treatment, media were removed, and cells were provided with fresh media and allowed to incubate for 72 h. After 72 h, media were removed, cells were washed three times with PBS, and the number of cells was determined as described for day 0 points. Values represent different cultures containing A1698 cells.

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RESULTS

dsRNA309 Synthesis—Fig. 1A depicts the vector (pdsRNA309) used for the synthesis of dsRNA309. Even though there are no known heteropolymer dsRNA sequences required for dsRNA-mediated activities in mammalian cells, we chose segmented, noneukaryotic bacteriophage sequences as template for heteropolymer dsRNA synthesis. \( \Phi X_{174} \) bacteriophage sequences were subcloned into the polylinker of pGEM1, and templates for \( \textit{in vitro} \) transcription were generated by NheI (T7 reaction) and MvaI (Sp6 reaction) restriction endonuclease digestions. Component strands were synthesized and subsequently annealed. dsRNA309 was generated by subjecting the annealed complementary strands to RNase A treatment, and the integrity of dsRNA309 was verified by gel electrophoresis. Fig. 1B demonstrates that component strands (\( T7 = 551; \text{Sp6} = 555 \)), and dsRNA309 were of expected size. Fig. 1B also shows the size distribution of homopolymer poly(I-C) and its mismatched analogue poly(I-C12,U). Since both poly(I-C) and poly(I-C12,U) are comprised of varying length component strands, these molecules contain both single- and double-stranded domains. The possibility therefore exists that both single- and double-stranded domains may be required for some or all of homopolymer dsRNA-induced activity. In order to control for heteropolymer dsRNA-induced activities that may require both domains, non-RNased dsRNA309 (dsRNA309ss, Fig. 1B) was also evaluated for cellular and enzymatic activities.

Antiviral and Antiproliferative Activity of dsRNA309—In order to determine whether dsRNA309 possessed antiviral activity, we first examined the ability of this molecule to inhibit polio-1 virus replication in a previously identified (25) dsRNA-sensitive bladder carcinoma cell line, A1698. Table I shows that dsRNA309 was able to inhibit polio-1 virus replication in this cell line. Since dsRNA309ss possessed the same antiviral activity as dsRNA309 in A1698 cells and the single-stranded control, ssRNAT7, had no antiviral activity, it was unlikely that contaminating RNase or products from \( \textit{in vitro} \) transcription contributed to the observed antiviral activity of dsRNA309. In contrast to the antiviral activity of dsRNA309, this synthetic heteropolymer dsRNA was unable to inhibit the growth of A1698 cells. Table II shows that high concentrations of dsRNA309 (10 \( \mu \)g/ml) had relatively little effect on the growth of A1698 cells, while low concentrations of poly(I-C) (0.1 \( \mu \)g/ml) inhibited A1698 cell growth. The inability of heteropolymer dsRNA to inhibit A1698 cell growth was not due to the absence of single-stranded regions since both dsRNA309 and dsRNA309ss lacked antiproliferative activity. Poly(I-C12,U) showed antiproliferative activity, and the concentration of poly(I-C12,U) (2 \( \mu \)g/ml) required to inhibit the growth of the dsRNA-sensitive cell line by 50% was consistent with previously reported observations (25).

Gene-inducing Activity of dsRNA309—The type I IFNs have been implicated as key mediators in the antiproliferative and antiviral activities of synthetic dsRNA (15). Previous experi-

<p>| TABLE I |
| Antiviral activity of dsRNA309 |
| Two-day post-confluent A1698 cells in 96-well microtiter plates were pretreated with synthetic dsRNA and, after a 1-day pretreatment, were challenged with polio-1 virus as described under &quot;Materials and Methods.&quot; Percentage viable cells remaining in dsRNA pretreated A1698 cells after exposure to polio-1 virus was determined by neutral red uptake and calculated by the following formula. |
| ( % \text{ viable cells} = \frac{\text{[dsRNA pretreatment OD] - no pretreatment OD}}{\text{[no virus OD] - no pretreatment OD}} \times 100 ) |</p>
<table>
<thead>
<tr>
<th>dsRNA</th>
<th>Concentration</th>
<th>Viable cells</th>
<th>±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly(I-C)</td>
<td>2.0</td>
<td>80.4</td>
<td>±4.5</td>
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<tr>
<td>Poly(I-C)</td>
<td>0.1</td>
<td>21.1</td>
<td>±3.2</td>
</tr>
<tr>
<td>Poly(I-C12,U)</td>
<td>2.0</td>
<td>73.8</td>
<td>±5.5</td>
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<tr>
<td>dsRNA309</td>
<td>2.0</td>
<td>45.0</td>
<td>±4.1</td>
</tr>
<tr>
<td>dsRNA309ss</td>
<td>2.0</td>
<td>50.0</td>
<td>±5.2</td>
</tr>
<tr>
<td>ssRNAT7</td>
<td>2.0</td>
<td>18.8</td>
<td>±2.5</td>
</tr>
</tbody>
</table>

<p>| TABLE II |
| Antiproliferative activity of dsRNA309 |
| Exponentially growing A1698 cells in 35-mm dishes were treated with synthetic dsRNA for 4 h, and the number of cells was then determined by hemacytometer counting of trypsinized cells 72 h after treatment as described under &quot;Materials and Methods.&quot; Percentage control growth was determined by the following formula. |
| ( % \text{ control growth} = \frac{[\text{treated cells (72 h)} - \text{control cells (0 h)}]}{[\text{control cells (72 h)} - \text{control cells (0 h)}]} \times 100 ) |</p>
<table>
<thead>
<tr>
<th>dsRNA</th>
<th>Concentration</th>
<th>Control growth</th>
<th>±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly(I-C)</td>
<td>2.0</td>
<td>19.6</td>
<td>±2.6</td>
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<tr>
<td>Poly(I-C)</td>
<td>0.1</td>
<td>32.5</td>
<td>±4.8</td>
</tr>
<tr>
<td>Poly(I-C12,U)</td>
<td>2.0</td>
<td>43.9</td>
<td>±7.4</td>
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<tr>
<td>dsRNA309</td>
<td>10.0</td>
<td>84.1</td>
<td>±6.4</td>
</tr>
<tr>
<td>dsRNA309ss</td>
<td>2.0</td>
<td>91.4</td>
<td>±11.5</td>
</tr>
<tr>
<td>ssRNAT7</td>
<td>2.0</td>
<td>86.4</td>
<td>±8.3</td>
</tr>
</tbody>
</table>

Fig. 1. A, generation of pdsRNA309. Bacteriophage \( \Phi X_{174} \) sequences were subcloned into the polylinker of pGEM1, and the corresponding pdsRNA309 consists of the following sequences (starting at the Sp6 transcriptional start site) which comprise the double-stranded domain of dsRNA309, +1 to +31, polylinker sequences, +32 to +106, bacteriophage \( \Phi X_{174} \) sequences from HincII (5024) to HaeIII (4980) sites; +106 to +116, polylinker sequences, +117 to +277, bacteriophage \( \Phi X_{174} \) sequences spanning the HaeIII (2352-2513) sites; +278 to 309, polylinker sequences. NheI and MvaI denote restriction endonucleases used for template preparation. B, electrophoresis of dsRNA309. Synthetic heteropolymers dsRNA309, dsRNA309ss, and component strands were synthesized as described under "Materials and Methods" and subjected to electrophoresis on a 2% agarose gel. Poly(I-C) and poly(I-C12,U) were subjected to electrophoreses on 1% agarose gel. Lane 1, 500 ng of component T7 strand; lane 2, 500 ng of component Sp6 strand; lane 3, 250 ng of dsRNA309ss; lane 4, 250 ng of dsRNA309; lane 5, 2.5 \( \mu \)g of poly(I-C12,U); and lane 6, 2.5 \( \mu \)g of poly(I-C).
ments have shown that the antiproliferative activity of dsRNA was mediated by IFN-β in A1698 cells since neutralizing antibodies to IFN-β relieved growth inhibition induced by poly(I-C) or poly(I-C12,U) (25). In order to better understand requirements for dsRNA-mediated antiproliferative and antiviral responses in A1698 cells, we next examined the ability of dsRNA309 to induce IFN-β gene expression in A1698 cells. Fig. 2A shows direct induction (in the presence of protein synthesis inhibitor, CHX) of IFN-β RNA by synthetic dsRNA. Undetectable levels of IFN-β RNA were observed upon treatment of A1698 cells with either dsRNA309 or dsRNA309ss at concentrations (0.05 μg/ml) which induced a marked increase in IFN-β RNA with poly(I-C) and poly(I-C12,U) treatment. Accumulation of IFN-β RNA was seen at higher concentrations (0.5 μg/ml) of dsRNA309 and dsRNA309ss. The inability of dsRNA309 to efficiently induce IFN-β gene expression was not specific for this cytokine since dsRNA309 was also unable to induce IL-1α. Fig. 2B shows that poly(I-C) and poly(I-C12,U) were both capable of inducing IL-1α RNA accumulation, while dsRNA309 or dsRNA309ss was unable to induce an increase in IL-1α RNA levels over treatment with CHX alone.

Activation of dsRNA-dependent Intracellular Enzymes—Since DAI has been implicated in poly(I-C)-mediated gene induction (17, 18), and both intracellular dsRNA-dependent enzymes have been speculated to contribute to the dsRNA-mediated antiproliferative and antiviral responses (3), we also wanted to address the question of whether DAI or 2'-5' A synthetase displayed differential affinities for dsRNA309. Activation of DAI by dsRNA results in the autophosphorylation of the 68-kDa protein and has been shown to be dependent on dsRNA concentration (24). High concentrations of dsRNA inhibit autophosphorylation of DAI, while low concentrations result in DAI activation and autophosphorylation. Fig. 3 shows the activation and inhibition of purified DAI autophosphorylation by synthetic heteropolymer and homopolymer dsRNA. High concentrations of dsRNA309 (1000 ng/ml) and to a lesser extent dsRNA309ss inhibited the autophosphorylation of DAI, while lower concentrations (100 to 10 ng/ml) of dsRNA309 and dsRNA309ss activated DAI autophosphorylation. Autophosphorylation of DAI was observed with all concentrations of poly(I-C) and poly(I-C12,U). Activation was also observed with high concentrations (1000 to 100 ng/ml) of ssRNAT7, while the lowest concentration (10 ng/ml) of ssRNAT7 was unable to activate DAI. Activation of highly purified DAI by dsRNA contaminants generated during in vitro transcription reactions has been documented previously (29). It is also possible that activation of DAI by ssRNAT7 was a result of secondary structure within ssRNAT7. Nonetheless, failure to activate DAI at 10 ng/ml by ssRNAT7 suggests that DAI activation at the same concentration by the dsRNA309 preparation was from dsRNA309 and not from component single-stranded RNA. Fig. 4 shows that dsRNA309 was also a potent activator of 2'-5'A synthetase. Poly(I-C), poly(I-C12,U), dsRNA309ss, and dsRNA309 at equivalent concentrations all catalyzed the conversion of ATP into oligoadenylate dimers (A2) and trimers (A3) in a dose-dependent manner. Activation of 2'-5'A synthetase was dependent on heteropolymer dsRNA and not on single-stranded heteropolymer RNA, since the component strand of dsRNA309, ssRNAT7, was inactive. The biological activity of dsRNA309 was not due to the component Sp6 strand since ssRNAsp6 was also unable to inhibit virus replication or activate dsRNA-dependent enzymes (data not shown).

**DISCUSSION**

Synthetic homopolymer dsRNA has previously been shown to interact with intracellular proteins, induce a wide range of genes and inhibit both tumor cell growth and virus replication (reviewed in Ref. 3). This paper describes for the first time a synthetic dsRNA which possesses restricted biological activ-
poly(1-C) did not inhibit the IFN-β inducing ability of poly(1-C) with synthetic dsRNA in the presence of nonlabeled ATP and chromatography plate. The plate was developed in 1 M acetic acid, these same cells. However, dsRNA309 was able to inhibit cellular extracts from IFN-treated HeLa cells were incubated at 37 °C to relieve A1698 growth inhibition by poly(1-C) and poly(1-C, U) (25). Therefore, the inability of dsRNA309 to induce DAI and was unable to induce IFN-β RNA synthesis in A1698 cells. The inability of dsRNA309 to growth of a dsRNA-sensitive human bladder carcinoma cell line (A1698) or efficiently induce IFN-β RNA synthesis in these same cells. However, dsRNA309 was able to inhibit polio-1 virus replication in A1698 cells and activate both dsRNA-dependent intracellular enzymes, DAI and 2′-5′A synthetase.

It appears that dsRNA309 was a poor ligand for a cellular receptor responsible for the induction of genes involved in the dsRNA-mediated antiproliferative response. Neutralizing antibodies to IFN-β have previously been shown to partially relieve A1698 growth inhibition by poly(I-C) and poly(I-C, U) (25). Therefore, the inability of dsRNA309 to induce IFN-β may explain the lack of antiproliferative activity of dsRNA309 against A1698 cells. The inability of dsRNA309 to induce cytokine gene expression was most likely a result of its inability to bind and not activate a specific cellular receptor, since treatment of A1698 cells with both dsRNA309 and poly(I-C) did not inhibit the IFN-β-inducing ability of poly(I-C) (data not shown). It is much more difficult to speculate how dsRNA309 induced an antiviral state in A1698 cells. A simple explanation is that the amount of IFN-β required for the inhibition of viral replication was substantially lower than the amount required to inhibit tumor cell growth, and therefore the low levels of dsRNA309-induced IFN-β may be sufficient for observed antiviral activity. Alternatively, dsRNA309 may enter the cell and directly activate dsRNA-dependent intracellular enzymes constitutively expressed in A1698 cells.

Previous work has shown that the structural characteristics of synthetic homopolymer dsRNA required for enzyme activation were similar to those required for IFN induction (22, 23). The present work shows that there are different structural requirements for heteropolymer dsRNA-mediated gene induction and enzyme activation. dsRNA309 was able to activate both DAI and 2′-5′A synthetase but was a poor inducer of IFN-β and was unable to induce IL-1α gene expression. It has been proposed that dsRNA-mediated gene induction occurs through a common signal transduction pathway since 2′-aminopurine inhibits the induction of a wide range of genes (including IL-1α and IFN-β in A1698 cells, data not shown) by dsRNA or virus infection (18, 19). Also, since DAI activity is inhibited by this compound (20), it has been speculated that DAI activation is involved in this second messenger pathway. Presuming DAI is equally accessible in intact cells to poly(I-C) and dsRNA309, experiments presented here argue against this mechanism of dsRNA-mediated gene induction, since dsRNA309 was shown to be an activator of DAI in vitro but was unable to induce IFN-β in intact cells. However, it is possible that dsRNA309 was not internalized and for this reason was unable to activate DAI. Both a cell surface receptor, required for dsRNA recognition and internalization, and an intracellular receptor (i.e. DAI) may be required for dsRNA-induced gene expression. Nonetheless, the present experiments show that competency of a dsRNA to activate DAI and 2′-5′A synthetase does not automatically render the dsRNA competent for gene induction.

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