Effects of Fibroblast and Recombinant Leukocyte Interferons and Double-Stranded RNA on ppp(2'-5')Aₙ Synthesis and Cell Proliferation in Human Colon Carcinoma Cells in Vitro

Mrunal S. Chapekar and Robert I. Glazer¹

ABSTRACT

The antiproliferative effect of human fibroblast interferon (IFN-β), human recombinant leukocyte interferon (IFN-αA), and polyinosinic-polycytidylic acid [poly(I)-poly(C)] was investigated in human colon carcinoma cell line HT-29. Exposure of HT-29 cells for 1 to 3 days with 100 to 2000 units of either interferon preparation resulted in a 30 to 50% inhibition of growth after 3 days. Similar treatment of cells with 100 μg per ml poly(I)-poly(C) resulted in progressive inhibition of growth by 50 to 60% in 2 to 3 days. The inhibitory effects of combination of either IFN-β or IFN-αA with poly(I)-poly(C) were additive with up to 80% inhibition of cell growth after 3 days of exposure. None of the treatment regimens was markedly cytotoxic, and only 25 to 30% reduction in colony formation was noted under optimal treatment conditions following 2 to 3 days of drug exposure. Measurements of DNA, RNA, and protein synthesis following interferon treatment indicated a dose-dependent reduction in all three parameters, particularly when interferon was administered with poly(I)-poly(C). The associated changes in (2',5')oligoadenylate [(2',5')oligo(A)] pathway produced by IFN-β and IFL-αA were measured under growth-inhibitory conditions. A concentration-dependent induction of (2',5')oligo(A) synthetase was produced by IFN-β or IFL-αA with a concomitant decrease in (2',5')oligo(A) phosphodiesterase. Poly(I)-poly(C) alone induced (2',5')oligo(A) synthetase activity but had no effect on the associated activity of phosphodiesterase. The combination of either IFN-β or IFL-αA and poly(I)-poly(C) further reduced (2',5')oligo(A) phosphodiesterase activity. There was no general dose-response correlation between the induction of (2',5')oligo(A) synthetase and the cytostatic activity of interferon treatment alone or in combination with double-stranded RNA.

INTRODUCTION

IFN² is a family of proteins formed in vertebrate cells in response to viral infection (14–16). Although the cellular effects of IFN may be best described as pleiotropic (14), recent, much attention has been focused on the stimulatory effect of IFN on the dsRNA-dependent activities, a protein kinase which phosphorylates and inactivates eIF-2α (the α subunit of eukaryotic initiation factor 2), and (2',5')oligo(A) synthetase which catalyzes the formation of (2',5')oligo(A) from ATP (2). (2',5')Oligo(A) is a potent inhibitor of protein synthesis and acts by activation of an endoribonuclease (RNase L) (5, 27, 28), present in both normal and IFN-treated cells which results in degradation of RNA. The action of RNase L is also indirectly affected by the ubiquitous enzyme, (2',5')oligo(A) PDE, which hydrolyzes (2',5')oligo(A) to its constituent nucleotides (12, 20). The inhibitory action of (2',5')oligo(A) on protein synthesis has also been deduced by its direct introduction into cells after membrane permeabilization (26) or by the calcium phosphate coprecipitation technique (3, 10). Dephosphorylated (2',5')oligo(A), when added to mitogen-treated mouse spleenocytes, causes antimitotic effects similar to those produced by IFN (13). All of these observations suggest a major role of (2',5')oligo(A) in the antiproliferative effects of IFN.

Thus far, the majority of studies examining the antiproliferative effects of IFN in human tumor cells have not focused on whether the induction of the (2',5')oligo(A) pathway is directly reflected in the cytotoxic or cytostatic activity of IFN (1, 6, 7, 11, 19). Two recent investigations have shown no correlation between the extent of induction of (2',5')oligo(A) synthetase and the antiproliferative effects of IFN in human embryonic fibroblast cell lines (24, 25). However, there is no available information regarding this point in human adenocarcinomas.

In the present study, we investigated the (2',5')oligo(A) pathway and growth-related parameters in human colon carcinoma cell line HT-29 in response to IFN-β and IFN-αA, both individually and in combination with the dsRNA, poly(I)-poly(C).

MATERIALS AND METHODS

[α-32P]ATP (10 to 50 Ci/mmol), [methyl-14C]dThd (53 mCi/mmol), L-[35S]methionine (800 Ci/mmol), and [5-3H]uridine (25 Ci/mmol) were purchased from New England Nuclear (Boston, Mass.). Poly(I)-poly(C) agarose and poly(I)-poly(C) were obtained from P-L Biochemicals (Milwaukee, Wis.), and human IFN-αA (3 x 10⁸ IU/mg protein; Lot C116131-062) was kindly provided by Dr. Sidney Pestka, Roche Institute of Molecular Biology (21).

Cell Culture. HT-29 cells originally derived from a human colon carcinoma (8) were obtained from Dr. L. Erickson, Laboratory of Molecular Pharmacology, National Cancer Institute. The cells were maintained as a monolayer under an atmosphere of 5% CO₂-air in Roswell Park Memorial Institute Tissue Culture Medium 1640 supplemented with 10% heat-inactivated fetal calf serum and gentamicin, 50 μg/ml.

Cell Extracts. Logarithmically growing cells were exposed to IFN and/or poly(I)-poly(C) for 20 hr at 37°C. Cells were washed twice with cold phosphate-buffered saline (6.3 mM Na₂HPO₄, 0.8 mM KH₂PO₄, 0.154 mM NaCl, pH 7.4) and lysed at 0°C with 100 μl of 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer (pH 7.4), 5 mM MgCl₂, 120 mM KCl, 1 mM dithiothreitol, and 10% glyceral (Buffer B) containing 0.5% Nonidet P-40. Cyttoplasmic extracts obtained after centrifugation for 0.5 min at 12,000 x g in an Eppendorf centrifuge were kept frozen at −80°C.

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²The abbreviations used are: IFN, interferon; dsRNA, double-stranded RNA; (2',5')oligo(A), ppp(2'-5')Aₙ, PDE, phosphodiesterase; IFN-β, fibroblast interferon; IFN-αA, recombinant leukocyte interferon species A; poly(I)-poly(C), polyinosinic acid:polycytidylic acid; dThd, thymidine.

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JUNE 1983 2683

2683
RESULTS

Cell Growth and Lethality. Exponentially growing HT-29 cells were treated with varying concentrations of either IFN-β or IFN-αA and 100 μg poly(I)-poly(C) per ml for 1 to 3 days, and cell growth and cell viability via a soft agar clonogenic assay were determined (Chart 1). Growth inhibition by either IFN-β or IFN-αA was produced only at concentrations exceeding 400 IU/ml for 2- and 3-day exposure intervals, and to a comparable extent by poly(I)-poly(C) over the same treatment periods (Chart 1, A and B). The combination of either IFN-β or IFN-αA and poly(I)-poly(C) produced a synergistic growth-inhibitory effect at 100 IU of IFN per ml for 3 days of drug exposure, but the other regimens of the 2 agents generally gave an additive effect (Chart 1, A and B). Examination of the lethal effects of the IFNs by colony formation revealed that neither preparation nor poly(I)-poly(C) were markedly lethal (Chart 1, B and D). The combination of either IFN-β or IFN-αA and poly(I)-poly(C) produced a 25 to 35% reduction in colony formation.

DNA and RNA Synthesis. Cells were pulse labeled with [3H]thymidine, [3H]uridine and [35S]methionine as measures of DNA, RNA, and protein synthesis, respectively (Chart 2). Reduction of DNA, RNA, and protein synthesis following treatment with either IFN-β, IFN-αA, or poly(I)-poly(C) generally paralleled growth inhibition and was not very pronounced. Significantly greater inhibition of macromolecular synthesis occurred at 400 to 2000 IU/ml for either IFN in combination with poly(I)-poly(C) versus either agent alone over a 3-day exposure interval (Chart 2, A, B, D, E).
The present study has examined the effects of IFN-β, IFN-αA, and poly(I)-poly(C) on DNA, RNA, and protein synthesis. Log-phase cells were treated as described in Chart 1 and were pulse labeled during the last hr of treatment with 0.5 μCi of [3H]thymidine (A and D), 5 μCi [3H]uridine (B and E), and 10 μCi [35S]methionine (C and F). The amount of trichloroacetic acid-insoluble radioactivity was measured and normalized for cell number. Each value represents the mean of 3 to 4 experiments where the standard error did not exceed 5%. IFN-β, poly(I)•poly(C).

**DISCUSSION**

The present study has examined the effects of IFN-β, IFN-αA,
and poly(l)-poly(C), used singly and in combination, on cell prolifera-
tion and the oligoadenylate biosynthetic pathway in a human colo-
cancer cell line. HT-29 cells were previously reported by Ito and Buffet (11) to be moderately sensitive to the growth-
inhibitory properties of IFN-β upon prolonged exposure, whereas
40% inhibition of cell growth occurred at 5000 IU/ml after 4 days.
Under the latter conditions, a 15 and 65% reduction in colony
formation occurred at 500 and 5000 IU of IFN-β per ml, respec-
tively. Our results of the growth-inhibitory and cytocidal activi-
ty of IFN-β, as well as IFN-αA, are in close agreement with their
results obtained by Ito and Buffet (11) to be moderately sensitive to the growth-
inhibition period and may be a prerequisite for the antiproliferative
action of IFN. The additive effects of IFN and poly(l)-poly(C) on
inhibition of cell growth concomitant with reduced (2',5')oligo(A)
PDE activity and enhanced inhibition of RNA and protein synthesis
suggest that the oligoadenylate pathway may contribute to
inhibition of cell growth by IFN. In addition, despite the fact that
(2',5')oligo(A) synthetase activity is already maximally activated
either alone or in combination with poly(l)-poly(C). In addition,
the duration of this effect was sustained over the growth-inhibi-
tory period and may be a prerequisite for the antiproliferative
action of IFN. The additive effects of IFN and poly(l)-poly(C) on
inhibition of cell growth concomitant with reduced (2',5')oligo(A)
PDE activity and enhanced inhibition of RNA and protein synthesis
suggest that the oligoadenylate pathway may contribute to
inhibition of cell growth by IFN. In addition, despite the fact that
(2',5')oligo(A) synthetase activity is already maximally activated
in poly(l)-poly(C) upon assay in vitro, it is probable that poly(l)-
poly(C) optimally activates the synthetase in situ. Thus, the
combination of IFN and poly(l)-poly(C) would be expected to
create a milieu resulting in an increased net synthesis of
(2',5')oligo(A) as reported by Nilsen et al. (18). Under these
conditions, RNase L would be expected to be activated and
accelerate the degradation of RNA to produce a reduction in
translation. Indeed, reduced RNA and protein synthesis was
observed more consistently with IFN and poly(l)-poly(C).

In conclusion, the combination of IFN-β or IFN-αA with poly(l)-
poly(C) appears to be more effective in inhibiting cell growth than
either drug alone. The available evidence, thus far, suggests that
this effect may be mediated via the oligoadenylate pathway.

ACKNOWLEDGMENTS

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National Cancer Institute, for performing the IFN assays and Beth Singer and Laura
Alpert for preparing this paper.

Table 1

(2',5')Oligo(A) synthetase and (2',5')oligo(A) PDE activities following treatment of HT-29 cells with IFN-β and poly(l)-poly(C)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>1-day treatment</th>
<th>3-day treatment</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Synthetase</td>
<td>PDE</td>
</tr>
<tr>
<td></td>
<td>(units/mg)</td>
<td>(units/mg)</td>
</tr>
<tr>
<td>Control</td>
<td>14 ± 2*</td>
<td>6 ± 0.3</td>
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<tr>
<td>poly(l)-poly(C), 100 μg/ml</td>
<td>55 ± 6</td>
<td>7 ± 0.8</td>
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<tr>
<td>IFN-β</td>
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<tr>
<td>10 IU/ml</td>
<td>16 ± 3</td>
<td>7 ± 0.9</td>
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<tr>
<td>100 IU/ml</td>
<td>119 ± 13</td>
<td>5 ± 0.7</td>
</tr>
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<td>1000 IU/ml</td>
<td>251 ± 4</td>
<td>4 ± 0.5</td>
</tr>
<tr>
<td>IFN-β, 10 IU/ml + poly(l)-poly(C), 100 μg/ml</td>
<td>52 ± 4</td>
<td>7 ± 0.9</td>
</tr>
<tr>
<td>IFN-β, 100 IU/ml + poly(l)-poly(C), 100 μg/ml</td>
<td>111 ± 4</td>
<td>3 ± 0.5</td>
</tr>
<tr>
<td>IFN-β, 1000 IU/ml + poly(l)-poly(C), 100 μg/ml</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

* Mean ± S.D. of duplicate assays from 2 experiments.

Table 2

(2',5')Oligo(A) synthetase and (2',5')oligo(A) PDE activities following treatment of HT-29 cells with IFN-αA and poly(l)-poly(C)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>1-day treatment</th>
<th>3-day treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Synthetase</td>
<td>PDE</td>
</tr>
<tr>
<td></td>
<td>(units/mg)</td>
<td>(units/mg)</td>
</tr>
<tr>
<td>Control</td>
<td>8 ± 0.5*</td>
<td>9 ± 0.3</td>
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<tr>
<td>poly(l)-poly(C), 100 μg/ml</td>
<td>16 ± 0.8</td>
<td>8 ± 1</td>
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<tr>
<td>IFN-αA</td>
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<td>100 IU/ml</td>
<td>67 ± 0.5</td>
<td>5 ± 0.5</td>
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<tr>
<td>400 IU/ml</td>
<td>97 ± 8</td>
<td>5 ± 0.3</td>
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<tr>
<td>1000 IU/ml</td>
<td>121 ± 8</td>
<td>5 ± 0.7</td>
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<tr>
<td>2000 IU/ml</td>
<td>137 ± 8</td>
<td>5 ± 0.3</td>
</tr>
<tr>
<td>IFN-αA, 100 IU/ml + poly(l)-poly(C), 100 μg/ml</td>
<td>111 ± 4</td>
<td>4 ± 0.4</td>
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<tr>
<td>IFN-αA, 400 IU/ml + poly(l)-poly(C), 100 μg/ml</td>
<td>131 ± 10</td>
<td>3 ± 0.2</td>
</tr>
</tbody>
</table>

* Mean ± S.D. of duplicate assays from 3 experiments.
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