Opposite Effects of Recombinant Interferon-αA and Deoxycoformycin on Adenosine Deaminase Activity in the Daudi B Lymphoblastoid Cell Line

By Connie R. Faltynek

Interferon-α and the adenosine deaminase (ADA) inhibitor deoxycoformycin (dCF) have each been shown to be efficacious in the treatment of some lymphoid malignancies and to have potent antiproliferative activities in vitro. This study examined whether dCF and recombinant interferon-αA (rIFN-αA) were additive, synergistic, or antagonistic in their effects on the cultured B lymphoblastoid cell line Daudi. Treatment of Daudi cells for three to four days with doses of rIFN-αA that were growth inhibitory was unexpectedly found to increase the level of ADA activity per cell two- to threefold and therefore to prevent the inhibition of ADA by limiting concentrations of dCF. However, the opposite effects of dCF and rIFN-αA on ADA activity did not lead to antagonistic effects on growth inhibition. The higher concentrations of dCF (with deoxyadenosine) necessary for appreciable growth inhibition could inhibit the increased ADA activity in rIFN-αA–treated cells, thus resulting in additive antiproliferative effects.

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MATERIALS AND METHODS

Cells and IFN. Daudi cells were grown in Dulbecco’s modified Eagle’s medium (DMEM) with 10% heat-inactivated horse serum as previously described.21 Horse serum has negligible ADA activity compared with human or fetal calf serum22 and therefore can be used without complication in studies on cellular ADA. IFN-αA was prepared by recombinant DNA technology and purified with monoclonal antibodies by Hoffmann-LaRoche, Nutley, NJ, to a specific antiviral activity of 2.4 × 10⁵ U/mg protein. This IFN was titrated by using an assay for the reduction of the cytopathic effect of vesicular stomatitis virus on WISH cells, and activity was expressed as units of IFN by comparison with the international standard Ga23-902-530 for IFN-α.

Growth curves. Daudi cells were grown to high density (≥1.2 × 10⁶ cells/mL), centrifuged, resuspended in fresh DMEM with 10% horse serum at 5 × 10⁴ cells/mL, and treated with rIFN-αA, dAdo (Sigma Chemical Co, St Louis), or dCF (obtained from the Natural Products Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD). When the cells were treated with a combination of agents, dCF was added first, the cells allowed to incubate for 30 minutes at 37°C, followed by the addition of dAdo and/or rIFN-αA. Cells were cultured at 37°C in 95% air, 5% CO₂. An aliquot of cells was removed daily and counted with a hemacytometer. Cells were examined for trypan blue exclusion; only viable cells were counted.

For some experiments, the growth of treated cells was expressed as a percentage of the growth of untreated cells as follows: (experimental cell density – initial density) × 100/(control cell density – initial density). For combinations of drugs, the actual cell density was used.

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density was compared with the density expected for additive antiproliferative effects, which was calculated by the multiplicative model, as previously described.24,25

**ADA assays.** By using a previously published method,26 cell extracts were prepared from untreated Daudi cells and from cells treated as described for the growth curves. The protein concentration in each extract was measured with the standard Bio-Rad protein assay (Bio-Rad Laboratories, Richmond, CA) by comparison with a bovine serum albumin standard containing an equivalent amount of homogenization solution.

To measure ADA activity, cell extract (1 μg protein diluted to 10 μL with homogenization solution) was incubated for 20 minutes at 35°C with 0.1 mmol/L [8-14C] adenosine (20 μCi/μg; New England Nuclear, Boston) in 50 mmol/L sodium phosphate, pH 7.4, in a total volume of 50 μL as previously described.26 The reactions were stopped by the addition of perchloric acid, and then neutralized, precipitated proteins were removed, and the supernatants were spotted on Kodak unsubstituted cellulose thin layers on mylar sheets (Eastman Kodak Company, Rochester, NY) by assay (Bio-Rad Laboratories, Richmond, CA) by the DNA polymerase method.28,29 Calculations of the assayed duplicate assays was less than 10%.

Extracts stored for up to 2 weeks at 4°C were stable with the reaction was linear with extract prepared from 0.5 to 5 x 10^6 cells. Similar results were obtained from fresh and frozen untreated Daudi cells and from cells proliferative effects, which was calculated by the multiplicative model, as previously described.24,25

**dATP levels.** Nucleotides were extracted as described and assayed by the DNA polymerase method.28,29 Calculations of the deoxynucleoside triphosphate levels in the extracts were corrected for isotope dilution caused by the deoxyribonucleoside triphosphates in the extracts.29

## RESULTS

**Effects of IFN, dCF, and dAdo on Daudi cell proliferation.** Daudi cells were very sensitive to the antiproliferative activity of rIFN-αA as shown in Fig 1A and as previously reported with both natural and recombinant IFN-α.14,21,22 The effect of ADA inhibition on Daudi cell growth was examined by treating the cells with dCF alone and in combination with dAdo, a substrate for ADA. Relative high concentrations of either dAdo (10^{-5} mol/L) or dCF (10^{-4} mol/L) alone caused only slight growth inhibition (Fig 1B). However, when the cells were treated with both dAdo and dCF at these concentrations, there was complete growth inhibition after three days (Fig 1B). As shown in Fig 1B, there was a dose-response relationship between the concentration of dCF used in combination with 10^{-5} mol/L dAdo and the extent of growth inhibition. In some experiments, similar results were obtained when 10^{-5} mol/L adenosine was used instead of 10^{-5} mol/L dAdo, although in other experiments 10^{-5} mol/L adenosine alone inhibited growth of the cells and was more potent than 10^{-3} mol/L dAdo when combined with dCF (data not shown).

Because either rIFN-αA or dCF (with dAdo) had potent growth-inhibitory effects on Daudi cells, we examined whether the effects would be additive, synergistic, or antagonistic when the drugs were combined. In initial experiments, Daudi cells were treated with 10^{-5} mol/L dCF with or without 10^{-5} mol/L dAdo in combination with varying concentrations of rIFN-αA. As shown in Table 1, 10^{-7} mol/L dCF and 10^{-5} mol/L dAdo were synergistic in their effects on cell growth, whereas the effects of rIFN-αA were additive with dCF or with the combination of dCF and dAdo.

**Effect of rIFN-αA on ADA.** To determine whether not only dCF but also rIFN-αA affected adenosine metabolism, we measured ADA activity in untreated and rIFN-αA-treated Daudi cells. As shown in Fig 2A, it was found that rIFN-αA-treated cells had increased levels of ADA activity. The magnitude of the increase was dependent on the rIFN-αA concentration and the length of treatment. After four days' treatment with 50 U/mL rIFN-αA, the ADA activity was nearly threefold higher than that in untreated cells (Fig 2A). Longer treatments with rIFN-αA did not further increase ADA activity since similar results were obtained after four, five, or seven days' incubation with 50 U/mL rIFN-αA (data not shown). Treatment with 1,000 U/mL rIFN-αA only slightly increased the level of ADA above that obtained with 50 U/mL (data not shown). The concentrations of rIFN-αA that increased ADA activity (Fig 2A) were
expressed per microgram of protein to normalize for differences in cell size, the specific ADA activity was also greater in rIFN-αA–treated cells (Fig 2B).

It was possible that the increased ADA activity in IFN-treated cells was a consequence of different levels of enzyme activity in proliferating and nonproliferating cells. To examine this possibility, ADA activity was measured in cells that were density arrested at high cell concentration and in cells incubated with 0.2% horse serum in DMEM, a condition that maintains cell viability but does not enable cell proliferation. As shown in Table 2, when cells were growth inhibited by either serum deprivation or density arrest, there was no significant change in ADA activity, which indicated that the increased activity in rIFN-αA–treated cells was not a consequence of growth inhibition.

The effect of dCF treatment on ADA activity in Daudi cells was also examined. As shown in Table 2, ADA activity was nearly completely inhibited in cells that had been treated for four days with 10⁻⁶ mol/L dCF. Similar levels of inhibition were observed after one, two, or three days (data not shown).

**Opposing effects of dCF and rIFN-αA.** Because rIFN-αA treatment of Daudi cells increased ADA activity whereas dCF inhibited the enzyme, the effects of combined treatments with rIFN-αA and dCF on ADA activity were studied. As shown in Table 2, treatment of the cells with 10⁻⁹ mol/L dCF alone partially inhibited ADA activity. However, when the cells were treated with the combination of rIFN-αA and 10⁻⁴ mol/L dCF in the absence or presence of dAdo, the ADA activity was greater than or equal to that in untreated cells. In contrast, when cells were treated with a higher dose (10⁻⁸ mol/L) of dCF that caused nearly complete ADA inhibition, rIFN-αA treatment had only a small effect on ADA activity (Table 2). Results obtained after

<table>
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<th>Experiment</th>
<th>Control</th>
<th>IFN</th>
<th>0.2% serum</th>
<th>High density</th>
<th>10⁻⁶ mol/L dCF</th>
<th>10⁻⁴ mol/L dCF + IFN</th>
<th>10⁻⁵ mol/L dCF</th>
<th>10⁻⁴ mol/L dCF + IFN</th>
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<tr>
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<td>7.4</td>
<td>15.9</td>
<td>6.0</td>
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Daudi cells were treated for four days with 50 U/mL rIFN-αA and/or the indicated concentrations of dCF and dAdo. Extracts were prepared and assayed for ADA activity. The enzyme activity was calculated from the effect of dAdo combined with dCF, multiplied by the effect of IFN.
three or five days' treatment (data not shown) were similar to those shown in Table 2 for four-day treatments.

To further study the opposing effects of dCF and rIFN-αA on ADA activity, cells were treated with varying doses of rIFN-αA in combination with 10^{-9} mol/L dCF. As shown in Fig 3, there was a dose-response relationship between rIFN-αA concentration and the increase in ADA activity. By increasing the level of ADA activity, treatment with ≥10 U/mL rIFN-αA opposed the inhibition by 10^{-9} mol/L dCF such that the ADA activity was similar to that present in untreated cells.

We examined whether treatment of the cells with concentrations of rIFN-αA and dCF that had opposing effects on ADA activity resulted in antagonism in growth inhibition. At 10^{-9} mol/L, dCF partially inhibited ADA activity (Table 2), but even when combined with 10^{-5} mol/L dAdo, 10^{-5} mol/L dCF did not affect cell growth or antagonize the growth inhibitory effect of rIFN-αA (Table 3). When the cells were treated with 10^{-4} mol/L dCF plus 10^{-5} mol/L dAdo, there was slight growth inhibition, which was at least additive with that caused by rIFN-αA (Table 3).

Effect on dCF and dAdo on dATP levels. To study the mechanism for the growth-inhibitory effects of dCF with dAdo on Daudi cells, dATP levels were measured. As shown in Table 4, Daudi cells treated with the combination of dCF and dAdo had elevated levels of dATP, which correlated with growth inhibition.

**DISCUSSION**

The purpose of this study was to examine whether dCF (combined with dAdo) and rIFN-αA, which each have antiproliferative activities, were additive, synergistic, or antagonistic in their effects on the Daudi lymphoblastoid cell line. Because IFN treatment has been shown to increase the biochemical effects of the two agents might intersect.

In this study, it was found that rIFN-αA–treated Daudi cells had higher levels of ADA activity and that IFN treatment prevented the inhibition of ADA by limiting concentrations of dCF. However, in spite of the opposite effect of dCF and rIFN-αA on ADA activity, no antagonistic effects were observed on growth inhibition.

Several factors may account for the observed lack of antagonism between dCF (combined with dAdo) and rIFN-αA in antiproliferative action in spite of opposing effects on ADA activity. First, dCF rapidly inhibited ADA activity, but the maximal increase of ADA activity by rIFN-αA required three to four days. Therefore, during combined treatments with dCF and rIFN-αA, ADA inhibition by dCF occurred first. The metabolic changes that resulted from ADA inhibition by dCF might not be readily reversed even when ADA levels subsequently increased as a consequence of rIFN-αA treatment.

Second, treatment with rIFN-αA only prevented the inhibition of ADA activity by low dCF concentrations that caused partial ADA inhibition. These low concentrations of dCF, even when combined with dAdo, were not growth inhibitory so that the growth of cells in the presence of rIFN-αA and low concentrations of dCF (combined with dAdo) was similar to the growth with rIFN-αA alone. Higher concentrations of dCF, which were growth inhibitory when combined with dAdo, effectively inhibited ADA activity even in rIFN-αA–treated cells, and at least additive antiproliferative effects were observed.

In the presence of ADA inhibitors, B cell lines in general are less sensitive to the toxicity of dAdo than T cell lines. However, the Daudi B lymphoblastoid cell line used in the present study was growth inhibited by concentrations of dAdo and dCF similar to those previously demonstrated to be growth inhibitory to T cell lines and phytohemagglutinin-stimulated lymphocytes. Evidence has been presented that the contrast in dAdo sensitivity between T and B cell lines is due to a difference in the capacity of the cells to accumulate deoxyadenosine nucleotides, possibly due to a difference in 5'-nucleotidase activity. Daudi cells have been reported to have low levels of 5'-nucleotidase, similar to those in T cell lines, rather than the much higher levels

![Fig 3. Antagonism between the effects of dCF and rIFN-αA on ADA activity. Daudi cells were treated for four days with no IFN or the indicated concentrations of rIFN-αA in the absence (●) or presence (○) of 10^{-9} mol/L dCF. Cell extracts were prepared and assayed for ADA activity. Results are expressed as the percent substrate converted per 1 × 10^{6} cells.](image-url)
cells are at least as sensitive or more sensitive to adenosine part of the toxicity of adenosine may be due to a nucleotide-activity in rIFN-αA-treated Daudi cells is not presently known. It has been previously reported that in many cell types, including Daudi, IFN-α increases the level of several adenosine-containing compounds, namely, S-adenosylmethionine, S-adenosylhomocysteine, and methylthioadenosine. The increased ADA activity in IFN-treated Daudi cells may be indicative of overall changes in adenosine metabolism. However, because concentrations of dCF that prevented the rIFN-αA--induced increase in ADA activity did not antagonize the antiproliferative activity of rIFN-αA, the elevated ADA activity in rIFN-αA--treated Daudi cells may not be required for the antiproliferative action of rIFN-αA.

The increased ADA activity in rIFN-αA--treated Daudi cells did not appear to be a general effect of rIFN-αA because rIFN-αA up to 1,000 U/mL had no significant effect on ADA activity in four lymphoma--derived cell lines (HT, RL, SR, DB), the T lymphoblastoid MOLT-4 cell line, or the promyelocytic cell line HL-60 (data not shown). However, these cell lines are less sensitive than Daudi cells to the antiproliferative action of IFN-α (data not shown).

Nevertheless, even in the Daudi cell line, in spite of opposite effects of rIFN-αA and dCF on ADA activity, no antagonistic antiproliferative effects were observed because the higher concentrations of dCF (combined with dAdo) required for significant growth inhibition could overcome the increased ADA activity in rIFN-αA--treated cells. These results indicate that even though rIFN-αA and dCF have opposite effects on ADA activity in some cell lines, the cellular changes caused by rIFN-αA are not sufficient to abrogate the in vitro antiproliferative effect of dCF combined with dAdo.

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