2',3'-Dideoxyadenosine Is Selectively Toxic for TdT-Positive Cells

By Zachary Spigelman, Robert Duff, G. Peter Beardsley, Samuel Broder, David Cooney, Nathaniel R. Landau, Hiroaki Mitsuya, Buddy Ullman, and Ronald McCaffrey

The 2',3'-dideoxynucleosides (ddNs) are currently undergoing clinical evaluation as antiretroviral agents in HIV-infected individuals. When phosphorylated, the ddNs (ddNTPs) function as chain-terminating substrate analogues with reverse transcriptase, thereby inhibiting HIV replication. These nucleoside analogues can also inhibit, by chain-terminating additions, the primitive lymphoid DNA polymerase, terminal deoxynucleotidyl transferase (TdT). To determine the effect of possible intracellular chain-terminating additions of ddNMPs by TdT, we exposed a series of TdT-positive and TdT-negative cell lines to 2',3'-dideoxyadenosine (ddA), a representative ddN. At ddA concentrations 25-fold higher than required for inhibition of HIV replication, progressive dose-related cytotoxicity was observed in the TdT-positive cell lines. This was accentuated by the adenosine deaminase inhibitor Cofor-...
Table 1. Comparative Efficiency of Various ddNTPs as Competitive Substrate Analogues for TdT

<table>
<thead>
<tr>
<th>Substrate Analogue</th>
<th>Concentration of ddNTP Producing 50% Inhibition of [3H]-dGMP Incorporation (mM/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ddATP</td>
<td>9.0</td>
</tr>
<tr>
<td>ddGTP</td>
<td>18.0</td>
</tr>
<tr>
<td>ddTTP</td>
<td>20.0</td>
</tr>
<tr>
<td>ddCTP</td>
<td>24.8</td>
</tr>
<tr>
<td>ddTTP</td>
<td>47.2</td>
</tr>
<tr>
<td>AZTTP</td>
<td>133.0</td>
</tr>
</tbody>
</table>

Assayed using oligo (dT)50 and [3H]-dGTP as described in the Materials and Methods section. DNA polymerase-α was not inhibited by any ddNTP at concentrations up to 500 μM/L.

[activated DNA; 5' drought-GTCCGTCTCTGCTC-3'(15 MER synthesized on an automated DNA synthesizer using standard phosphoramidite chemistry and 32P end-labeled by a standard Maxam-Gilbert reaction), YEP-FG-2 plasmid DNA, oligo (dC)18, oligo (dT)18, and various [3H] substrates (dTTP, dATP, dTTP) in place of of [3H]-dGTP. Reactions using the 15-MER initiator contained in (0.1 mL): 0.05 mmol/L Tris-HCl (pH 8.3), 4 mmol/L dithiothreitol, 4 mmol/L MgCl2, 10 μg poly (dAT), 0.05 mmol/L [3H]-dATP, 0.05 mmol/L dTTP, and 0.05 U enzyme. For inhibition studies, ddNTPs were dissolved in deionized distilled water and added to the reaction mixtures at time zero to give the final concentrations shown in Fig. 1. Degree of inhibition was calculated from simultaneous control reactions. Mode of inhibition and inhibition constants were determined using Lineweaver-Burk and Dixon plots. Reactions were stopped with 0.5 mL cold (4°C) 0.1 mol/L sodium pyrophosphate (NaPP) containing 1 mg/mL yeast RNA and precipitated with 0.5 mL cold 25% trichloroacetic acid (TCA). Precipitates were centrifuged at 13,000 g in a microfuge for 1 minute, resublimed in 0.2 mL 0.3 N NaOH and then reprecipitated with NaPP and TCA. Following a second 0.3 N NaOH solubilization, 10 mL ScintiVerse II (Fisher Scientific, Fairlawn, NJ) were added to each sample for counting in a Beckman (Wakfield, MA) LS 7300 liquid scintillation counter.

Endonuclease Assays

Endonuclease activity was evaluated by the method of Deibel by demonstrating the conversion of superhelical DNA (form I) to nicked circular (form II) and linear (form III) DNA on 0.7% agarose gels. pUC 9 plasmid DNA was incubated with and without 100 ng homogenously purified TdT, as previously described, for 30 minutes in a reaction mixture containing 1 μg plasmid DNA, 5 mmol/L MgCl2, and 50 mmol/L Tris HCl, pH 8.0, at 37°C in a final volume of 20 μL. Reactions were stopped at 4°C by the addition of 7 μL 1% sodium dodecyl sulfate (SDS), 0.03 mmol/L EDTA, and 15% glycerol. Reaction products were transferred to a horizontal 0.7%

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PD-31 Cell Lines

DOL-HM1 is a retroviral vector containing a cDNA encoding an enzymatically active TdT protein and a neosporin selective marker gene. DOL-HM1A is a similar retroviral vector containing a TdT cDNA with several point mutations yielding enzymatically inactive TdT protein and a neosporin selective marker gene. PD-31 is a TdT-negative, Abelson murine leukemia virus transformed pre-B-lymphoid cell line. Aliquots of PD-31 cells were infected with either DOL-HM1 or DOL-HM1A virus by culturing 1 x 106 cells for 2 days on a monolayer of ψ 2-viral producer cells. Infected cells were selected by subsequent culturing in G418 (2 mg/mL) or mycophenolic acid (0.5 μg/mL). Expression of TdT enzymatic activity in the parental PD-31 cells and the DOL-HM1 and DOL-HM1A infected cell lines was determined biochemically, as previously described. All PD-31 lines were grown in RPMI 1640 supplemented with 10% FCS.

CEM Mutant Lines

CEM Ara-C is a mutant human T-lymphoblastoid cell line derived from the parent line CEM-CCRF. It is deficient in deoxyuridine kinase and shows impaired phosphorylation of deoxyadenosine (30% of the parental CEM line). CEM TUB-4C is a CEM mutant which is deficient in nucleoside transport across cell membranes. Parent and mutant CEM lines were grown in RPMI-1640 with 15%
ddA IS TOXIC FOR TdT-POSITIVE CELLS

FCS. All parent and mutant CEM cell lines are TdT-positive by biochemical assay.21

Clinical Samples

Leukemic blast cells from bone marrow or peripheral blood were obtained from 12 patients with acute leukemia (6 with acute lymphoblastic leukemia (ALL), 3 with acute myeloblastic leukemia (AML), 2 with chronic myelogenous leukemia (CML), and 1 with acute myelogenous leukemia complicating multiple myeloma). Diagnoses were based on standard clinical and laboratory criteria.21 In 10 of the 12 patients, blast cells constituted >75% of the mononuclear cells; in the eleventh and twelfth patients, 32% and 42% of the cells were blasts. Leukemic cells were further enriched on Ficoll-Hypaque,22 washed in phosphate-buffered saline (PBS) without divalent cations, resuspended in RPMI 1640 with 20% FCS, and further processed as described for cultured cell lines. TdT status of each sample was defined by biochemical assay.22

Growth Inhibition Studies

Cell lines in exponential growth phase and freshly collected leukemic blast cells were exposed to ddA and CF at concentrations defined in Figs 3 through 5 and Table 2 for the exposure periods indicated. Cells seeded at 3 x 10^6 cells/mL in T25 flasks containing 4 mL culture media were counted in a hemocytometer; viability was determined using the trypan blue dye exclusion method. Degree of growth inhibition was calculated from untreated controls.

RESULTS

Effect of ddNTPs on TdT and DNA Polymerase-α

Table 1 summarizes the comparative efficiency of various ddNTPs and AZTTP as competitive substrate analogues for TdT. TdT was assayed under the most optimal conditions for TdT-catalyzed end additions [oligo (dA)50 initiator and dGTP substrate].22 Using the ddNTP and AZTTP concentration producing 50% inhibition of [3H]-dGTP incorporation in the standard TdT assay, a rank order of competitiveness could be established showing ddATP > ddGTP > ddITP > ddCTP > ddTTP > AZTTP. The K_m for ddATP, as defined by Lineweaver-Burk plots, was 1.8 μmol/L. The K_m for the authentic substrate dGTP was 5.0 μmol/L. The mode of inhibition by ddATP was competitive with respect to substrate and noncompetitive with respect to initiator. The competitive rank order noted above was neither initiator nor substrate dependent. ddATP continued to be the most effective competitive inhibitor with various initiators [activated DNA, oligo (dC)18, oligo (dT)18] used in place of oligo (dA)50 or various substrates [dATP, dCTP, dTTP] used in place of dGTP.

The comparative recognition of ddATP as a competitive substrate analogue by TdT is presented in Fig 1. DNA polymerase-α was not affected by any concentration of ddATP up to 500 μmol/L.

ddATP inhibited TdT catalysis by chain termination. As shown in Fig 2, when ddATP was used as substrate only one ddAMP residue was added by TdT. Using the authentic substrate dATP, multiple nucleotide additions were made to the radiolabeled oligomer.

Growth Inhibition Studies

Cell lines. We exposed a series of TdT-positive and TdT-negative cells to ddA,24 the nonphosphorylated precursor of the most efficient competitive substrate analogue, ddATP (Table 1). ddA can enter cells (whereas ddATP cannot) and can be successively phosphorylated to ddATP.
As summarized in Table 2, at 250 μmol/L ddA, growth inhibition (30% to 80%) was noted in all TdT-positive lines, whereas among the TdT-negative lines there was very little effect (0% to 15% growth inhibition). Cytotoxicity increased progressively in a dose-related manner; at 500 μmol/L ddA, the cytotoxicity in the TdT-positive lines showed 92% to 99% growth inhibition. However, progressive, dose-related cytotoxicity was also noted in the TdT-negative cells; at 500 μmol/L ddA, four of the TdT-negative lines tested also showed considerable (26% to 44%) growth inhibition. We attributed this to purine cytotoxicity, which also occurs with adenosine and deoxyadenosine in TdT-negative cell lines.26-33

In the next series of experiments, we attempted to reduce the requirement for high ddA levels by preincubating cells for 30 minutes with the adenosine deaminase (ADA) inhibitor CF.34 Previous studies showed ddA to be rapidly deaminated to ddl by ADA.35 We reasoned that by inhibiting this

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**Table 2. Effect of 48-Hour Continuous Exposure to ddA Alone or ddA Plus CF. on Various TdT-Positive and TdT-Negative Cell Lines in Culture**

<table>
<thead>
<tr>
<th>Cell Lines</th>
<th>TdT Status</th>
<th>Origin</th>
<th>Stage of Differentiation</th>
<th>ddA 250 μmol/L</th>
<th>ddA 500 μmol/L</th>
<th>ddA/CF 250 μmol/L/30 μmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphoid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NALM-6</td>
<td>Positive</td>
<td>ALL</td>
<td>Pre-B cell</td>
<td>30</td>
<td>95</td>
<td>95</td>
</tr>
<tr>
<td>MOLT-4</td>
<td>Positive</td>
<td>ALL</td>
<td>Early T cell</td>
<td>60</td>
<td>92</td>
<td>85</td>
</tr>
<tr>
<td>CEM</td>
<td>Positive</td>
<td>ALL</td>
<td>Early T cell</td>
<td>20</td>
<td>ND</td>
<td>85</td>
</tr>
<tr>
<td>298-26</td>
<td>Positive</td>
<td>Mouse liver</td>
<td>Pre-B cell</td>
<td>80</td>
<td>99</td>
<td>95</td>
</tr>
<tr>
<td>B-244</td>
<td>Positive</td>
<td>Mouse liver</td>
<td>Pre-B cell</td>
<td>40</td>
<td>100</td>
<td>95</td>
</tr>
<tr>
<td>B452</td>
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<td>ALL</td>
<td>Early T cell</td>
<td>80</td>
<td>ND</td>
<td>80</td>
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<tr>
<td>HPB-ALL</td>
<td>Positive</td>
<td>ALL</td>
<td>Early T cell</td>
<td>40</td>
<td>99</td>
<td>95</td>
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<td>H9</td>
<td>Negative</td>
<td>ATL</td>
<td>Mature T cell</td>
<td>15</td>
<td>40</td>
<td>20</td>
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<tr>
<td>ML3</td>
<td>Negative</td>
<td>ANNL</td>
<td>Mixed lineage (lymphoid-myeloid)</td>
<td>0</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td>8392</td>
<td>Negative</td>
<td>ALL</td>
<td>B cell</td>
<td>0</td>
<td>0</td>
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<td>Nonlymphoid</td>
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<td>HeLa</td>
<td>Negative</td>
<td>Cervical CA</td>
<td>Unknown</td>
<td>0</td>
<td>26</td>
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<tr>
<td>3T3</td>
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<td>0</td>
<td>44</td>
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<td>K562</td>
<td>Negative</td>
<td>CML</td>
<td>Pre-erythrocyte blast</td>
<td>0</td>
<td>ND</td>
<td>0</td>
</tr>
</tbody>
</table>

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**Fig 4.** Effect of ddA and CF on TdT-positive and TdT-negative PD-31 cell lines. PD-31 is a TdT-negative pre-B-cell line (<0.001 TdT U/10⁸ cells) PD-31-DOL-HM1 is a daughter line of PD-31 that was rendered TdT-positive (5 TdT U, 10⁸ cells) by infection with the retroviral vector DOL, which contains a TdT-cDNA. PD-31-DOL-HM1 is a daughter line of PD-31 that was infected with a DOL retroviral vector containing a TdT cDNA with several point mutations produced in an enzymatically inactive TdT protein. Following infection, the DOL-HM1A cells remain TdT-negative by enzymatic assay (<0.001 TdT U/10⁸ cells). Cells were continuously exposed to 250 μmol/L ddA and 30 μmol/L CF for 48 hours. Results are expressed as a percentage of control growth for each line. Each determination represents the mean of triplicate flasks. Percent of control growth was determined by biochemical assay.

**Fig 5.** Effect of ddA and CF on fresh leukemic blast cells. Cells were continuously exposed to 250 μmol/L ddA and 30 μmol/L CF for 72 hours. Results are expressed as a percentage of control viability for each sample. Each determination represents the mean of triplicate flasks. TdT status of each sample was determined by biochemical assay, as previously described.35 For the six samples (CW-RN) labeled TdT(−), from patients with AML (3 cases), blastic CML (2 cases), and ANLL following multiple myeloma (1 case), enzyme activity was <0.001 TdT U/10⁸ cells. For the six ALL samples (JC-LF) labeled TdT(+), from six children with ALL, enzyme activity ranged from 0.3 to 2.5 TdT U/10⁸ cells.
reaction we would increase the available intracellular ddA pool for anabolic conversion to ddATP. As summarized in Table 2, the TdT-positive lines became exquisitely sensitive to 250 μmol/L ddA after preincubation with 30 μmol/L CF. In all TdT-positive lines tested (seven cell lines) a 30-minute preincubation with 30 μmol/L CF, followed by 250 μmol/L ddA, resulted in major (80% to 95%) growth inhibition at 48 hours. In these seven TdT-positive cell lines, TdT activity spanned the range seen in normal thymocytes (0.8 to 2.5 TdT U/10^8 cells) and ALL cells (0.3 to 5 TdT U/10^8 cells). Among the TdT-negative cell lines, only two (HeLa and H9 cells) were growth affected (20% growth inhibition) at 48 hours. TdT-negative status was defined as <0.001 TdT U/10^8 cells.

ddA/CF effects on CEM mutant cell lines. To help define the toxic species mediating ddA/CF lymphocytotoxicity, mutant derivatives of the TdT-positive cell line CEM were studied. CEM TUB-4C is a cell line deficient in the protein that facilitates diffusion of all natural nucleosides and several nucleoside derivatives across the cytoplasmic cell membrane. CEM Ara-C is a cell line deficient in deoxycytidine kinase, one of the enzymes believed to be responsible for intracellular ddA phosphorylation. TdT activity in all CEM lines ranged between 0.3 and 0.5 TdT U/10^8 cells. As shown in Fig 3, the same dose of ddA/CF at which the parent CEM cells were growth inhibited 85% produced only 40% inhibition of CEM TUB-4C (nucleoside transport deficient) cells, whereas CEM Ara-C (deoxycytidine kinase deficient) cells were almost completely unaffected. These data strongly suggest that transport and phosphorylation of ddA are deficient in these cell lines and that this in turn is reflected in resistance to cytotoxicity.

ddA/CF effects in PD-31 cells. Figure 4 shows the effects of 250 μmol/L ddA and 30 μmol/L CF on the TdT-negative (<0.001 TdT U/10^8 cells) murine pre-B-cell line PD-31 and its transfected daughter lines PD-31-DOL-HM1 and PD-31-DOL-HM1A. As shown, PD-31 was growth inhibited 30% by 250 μmol/L ddA and 30 μmol/L CF. Likewise, the DOL-HM1A-infected line that remained TdT negative (<0.001 TdT U/10^8 cells) was also growth inhibited to this degree (28% cell death). In contrast, the PD-31 cells, rendered artificially TdT-positive (5 TdT U/10^8 cells) by infection with a DOL vector containing TdT cDNA showed a significant increase in sensitivity to ddA and CF (90% growth inhibition at 48 hours), suggesting that TdT per se plays a central role in ddA/CF toxicity.

Leukemic blast cells. As shown in Fig 5, fresh leukemia cells were continuously exposed ex vivo to 250 μmol/L ddA and 30 μmol/L CF for 72 hours and evaluated for viability as defined by trypan blue staining. Blast cells from six patients with TdT-negative (<0.001 TdT U/10^8 cells) nonlymphoblastic acute leukemias were resistant to the effects of ddA/CF. In contrast, leukemia blasts from six patients with TdT-positive ALL (enzyme activity ranged from 0.3 to 1.8 TdT U/10^8 cells) demonstrated ddA/CF cytotoxicity (70% to 85% cell death). In two of these TdT-positive cases, exposure to 30 μmol/L CF alone had no effect on cell viability.

Endonuclease activity. As shown in Fig 6, when pUC-9 plasmid DNA was incubated with homogeneously purified TdT as defined by polyacrylamide gel electrophoresis, (PAGE) under conditions that promote endonuclease activity, a significant amount of DNA was cleaved and converted from supercoiled (form I) to nicked circular (form II) and linear (form III) DNA. These endonucleolytic sites provide 3'OH groups to which TdT can add mononucleotides, as shown in Fig 7, where the authentic [3H]-nucleotide (dGTP) substrate is used. When 50 μmol/L ddATP is added to the reaction mixture at various times, the rate of further [3H]-dTTP incorporation is dramatically reduced. Thus, these data suggest that, in vivo, TdT may be able to cleave genomic DNA, forming 3'OH ends onto which it can subsequently incorporate chain-terminating ddAMP residues.
The function of TdT has been an enigma since its discovery in 1961 as an end-addition, template-independent DNA polymerase in calf thymus extract. Its strict limitation in normal animals to primitive lymphoid cells suggests a role for it in generating the functional properties of T and B cells. The experiments of Alt and Baltimore, strongly support a role for TdT in insertion of nongermline nucleotides (N segments) at VDJ joining sites during heavy-chain immunoglobulin gene rearrangements. A similar role for TdT in N-segment addition in T-cell receptor gene rearrangements may also exist.

In the series of experiments we report, our objectives were to extend our in vitro ddNMP/TdT chain-terminating observations to intact TdT-positive cells. In the in vitro situation, as shown in Fig 1, the addition of micromolar concentrations of ddATP to a standard TdT polymerization reaction system dramatically inhibits incorporation of the authentic nucleoside substrate. As shown in Fig 2 and as previously reported, the mechanism of inhibition of polymerization was the addition of ddAMP, producing chain termination: the absence of a 3'-OH group on the incorporated ddAMP residues makes further 5' → 3' additions impossible. Because DNA polymerase-α, the major replicative polymerase of mammalian cells, does not recognize ddNTPs, we exposed a series of TdT-positive and TdT-negative cell lines to ddA, the nonphosphorylated precursor form of ddATP, to determine the effect of possible intracellular chain-terminating additions by TdT. In these cellular studies, the nonphosphorylated deoxyxynucleoside was used to allow transport across cytoplasmic cell membranes. Anabolic conversion of ddA to ddATP then provides a substrate recognized by TdT. We have shown this anabolic conversion to occur in three representative TdT-positive cell lines (MOLT-4; NALM-6; CEM) and in one TdT-negative cell line (ML-3) studied to date, using [3H]-ddA, and high-performance liquid chromatography (HPLC) analysis (ref 24 and unpublished observations). To augment further the intracellular pool of ddA available for conversion to ddATP, we also added CF to inhibit the deamination of ddA to ddA.

Our data show that ddA alone, and especially the ddA/CF combination, produce significant cytotoxicity in all TdT-positive cell lines tested. In contrast, the six TdT-negative lines were largely unaffected by the ddA/CF combination. Likewise, when we exposed fresh, TdT-positive leukemic blast cells from six children with acute lymphoblastic leukemia ex vivo to ddA/CF, significant cytotoxicity was evident in all six leukemic samples (70% to 85% cell death at 72 hours). In contrast, six of six blast cell samples from patients with TdT-negative acute nonlymphoblastic leukemia were not affected under identical conditions of ddA/CF exposure. To establish TdT further as a central determinant of ddA/CF cytotoxicity, we exposed a normally TdT-negative murine cell line, PD-31, after it had been rendered artificially TdT-positive by a DOL retroviral vector containing TdT cDNA, to ddA/CF. As shown in Fig 4, TdT expression in PD-31 cells is associated with an increase in sensitivity to ddA/CF (90% cell death at 48 hours). In contrast, the PD-31 cells infected with the DOL vector containing a cDNA for an enzymatically inactive TdT showed no increase in ddA/CF sensitivity, suggesting that the key element in increased sensitivity is TdT expression and not some unknown effect of the DOL infected state.

These data, in the aggregate, suggest that TdT per se plays a central role in the ddA/CF cytotoxicity we observed and is not simply a marker for cells that are ddA/CF sensitive on some other basis. For example, lymphoid cells in general and T cells in particular are known to be sensitive to killing by adenosine and deoxyadenosine. The mechanisms responsible for this "purely cytotoxicity" are poorly understood. Although ddA cytotoxicity theoretically may be a variant of the purine cytotoxicity produced by adenosine and deoxyadenosine, it should be emphasized that adenosine and deoxyadenosine can produce purine cytotoxicity in TdT-negative, mature lymphoid cells. As we show, ddA cytotoxicity is confined to TdT-positive lymphoid cells, making it unlikely that it is mechanistically related to conventional purine cytotoxicity. We likewise believe that DNA polymerase-β or DNA polymerase-γ, both of which are ubiquitously present in mammalian cells and also recognize ddNTPs as substrate analogues of ddATP, may have a role to play in the selective cytotoxicity we observe in TdT-positive cells.

The lower level of TdT activity (TdT U/10⁶ cells) associated with ddA/CF sensitivity remains to be defined. Across the range of activities in the cells we studied (0.3 to 5 TdT U/10⁶ cells) comparable ddA/CF sensitivity was observed (Table 2 and Fig 5).

Although our data suggest that TdT per se is a central determinant of the cytotoxicity we observe, we have not yet definitively established that cell death results from ddAMP chain-terminating additions by TdT; studies are in progress to measure any such additions directly. We have also yet to show that the anabolic conversion of ddA to ddATP occurs equivalently in all the TdT-positive and TdT-negative cell lines we have studied. We have so far shown that the TdT-negative, ddA/CF-insensitive, ML-3 line can generate ddATP from ddA as efficiently as the TdT-positive, ddA/CF-sensitive MOLT-4, NALM-5, and CEM lines. Studies are in progress to determine ddATP levels in our other cell lines; the ML-3 data do not suggest, however, that failure to generate ddATP will be a characteristic of ddA/CF insensitive cells, except for the CEM-AraC mutant line, in which we expect significantly impaired ddATP generation. In the PD-31 cell lines, direct demonstration of equivalent anabolic ddA metabolism in the parental and infected lines will help strengthen our contention that increased sensitivity to ddA/CF in these cells is not owing to altered ddA metabolism following retroviral infection but instead is owing to TdT expression following retroviral expression.

We have assembled our data as they now stand to construct a model to explain the mechanism of cell killing by ddA/CF in TdT-positive cells. Central to this model is the sequential functioning of TdT as an endonuclease and polymerase, as shown in vitro in Figs 6 and 7. TdT, functioning as an endonuclease, would generate 3'-OH ends to which TdT, now functioning as a polymerase, would make ddAMP end-additions from the available pool of ddATP. Multiple
chain-terminating additions of this sort would ultimately lead to DNA fragmentation and cell death. 39

If this model is correct, it may be possible to identify sites of analogue insertion using radiolabeled analogues. The identification of these sites would help determine if the only function of TdT is N-segment insertion and further define the mechanism of ddA/CF toxicity.

The ddNs are currently undergoing study as potential therapeutic agents in HIV-infected individuals. As an anti-HIV agent, ddA is active at concentrations 25-fold lower than those we used. ddA, at 10 μmol/L, when used in cell culture systems, under conditions of substantial viral excess, effectively suppresses HIV replication. ddA at this concentration (10 μmol/L) has no cytotoxic effects. 1,3

Whether the concentrations of ddA required for cytotoxic damage to TdT-positive leukemia cells ex vivo would exceed the range of what would be clinically tolerable is not presently known. Thus, the immediate clinical relevance of the data reported here is not apparent. Nevertheless, we believe that it is appropriate to speculate that the selective elimination of TdT-positive neoplastic cells might be clinically achievable using a ddA/CF regimen. We conclude that further study of this combination in disease model systems, as a potentially useful treatment regimen for TdT-positive malignant disease, is warranted.

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