Determinants of Deoxyadenosine Toxicity in Hybrids between Human T- and B-Lymphoblasts as a Model for the Development of Drug Resistance in T-Cell Acute Lymphoblastic Leukemia

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ABSTRACT

Cultured human T-lymphoblastoid cell lines are more sensitive than B-cell lines to 2'-deoxyadenosine in the presence of 2'-deoxycoformycin, a potent inhibitor of adenosine deaminase. This difference is related to the greater efficiency with which T-lymphoblasts accumulate cytotoxic levels of dATP derived from the adenosine deaminase substrate 2'-deoxyadenosine (dAdo). Previous work has shown that differences in dATP accumulation by cultured T- and B-lymphoblastoid cell lines cannot be explained by large differences in the levels of dAdo-phosphorylating or dAdo nucleotide (dAXP)-degrading activities in cytoplasmic extracts of these cells, although it has been proposed that intact B-cell lines may catabolize intracellular dAXP more rapidly than do T-cell lines. To further examine the determinants of dAdo sensitivity in T- and B-lymphoblasts, we have studied dAdo and dAXP metabolism in the human T- and B-cell lines CEM and WI-L2 and in hybrids generated by fusion of these cell lines. The hybrid nature of the fusion products was established by nutritional studies and by analyses of cellular surface antigens, DNA content, and enzymatic activities. We found that WI-L2 × CEM hybrids and another T × B hybrid derived from fusion of the SB human B-cell line with CEM were 30- to 40-fold less sensitive to dAdo and about 10-fold less sensitive to the dAdo analogue 9-β-o-arabinofuranosyladenine than was CEM, or about as resistant as were their B-cell parental lines. Our studies confirm that CEM avidly accumulates dAXP from dAdo but does not catabolize intracellular dAXP. In contrast, WI-L2, SB, and WI-L2 × CEM and SB × CEM hybrids rapidly degraded intracellular dAXP, which limited their ability to undergo dAXP pool expansion. Expression of dAXP catabolic activity in T × B hybrids behaved as a dominant mechanism, conferring resistance to dAdo- and dAdo-related nucleosides to T × B hybrids. It has been postulated that cell fusion may play a role in the progression of tumors and contribute to diversity among the cells that compose clonal tumors. We have speculated that fusion of a malignant T-lymphoblast with an activated B-cell might be a mechanism for the evolution of drug resistance in acute T-cell leukemia.

INTRODUCTION

Genetic deficiency of ADA³ (EC 3.5.4.4) results in profound lymphopenia, with relative sparing of other organ systems (23, 44). This selectivity has provided a rationale for the treatment of lymphoid cancers with a potent ADA inhibitor, dCF (42, 49–51). Several specific effects of the ADA substrates Ado and dAdo are known, including altered metabolism of cAMP (60), inhibition of pyrimidine nucleotide synthesis (25), S-adenosylmethionine-dependent transmethylation (28, 30, 32, 43, 45), and ribonucleotide reductase (40, 41, 47, 58), and activation of ATP catabolism (5, 54, 62). Together or individually, these effects could contribute to the immune defect in genetic ADA deficiency in various ways, by interfering with lymphocyte differentiation, function, or viability. However, in determining the effectiveness of ADA inhibition as a treatment for lymphocytic leukemia, the most important factor is selective cytotoxicity, which is due primarily to the expansion of the intracellular pool of dATP derived from dAdo (8, 12, 13, 17).

Of the various types of ALL, T-ALL has been most responsive to treatment with dCF (26, 42, 51, 53, 56). Both in vivo during dCF treatment and in vitro when incubated with dCF and dAdo, T-lymphoblasts accumulate large amounts of dATP and are killed (26, 56). In contrast, cultured B-lymphoblastoid cell lines accumulate much less dATP from dAdo than do T-cell lines, and they are more resistant to dAdo toxicity (7, 9, 48, 52). Mutations that abolish the ability of T-cell lines to trap dAdo as intracellular dATP, such as loss of ability to transport or phosphorylate dAdo, markedly diminish the sensitivity of T-cell lines to dAdo in vitro (29, 59). Likewise, we have observed the emergence of relative resistance to dCF therapy in a patient with T-ALL, which was associated with loss of the ability of the patient’s leukemia cells to accumulate dATP in vivo (31).

Attempts have been made to determine the basis for the 10- to 100-fold greater rate of dATP accumulation that occurs in T-lymphoblasts compared with B-lymphoblasts when they are incubated with dAdo in the presence of ADA inhibitors. Different rates of dATP accumulation could result from different levels of enzymes that phosphorylate dAdo or degrade dAdo nucleotides (dAXP). The levels of total dAdo-phosphorylating activity and of the two specific enzymes capable of catalyzing this reaction, dCyd kinase and Ado kinase, differ by no more than 2- to 4-fold in cytoplasmic extracts of a number of T- and B-cell lines (9, 29). More rapid dAXP catabolism by B-cell lines than by T-cell lines has been proposed (7, 10, 61). However, the enzyme(s) specifically involved in dAXP catabolism has not been identified and, as with dAdo phosphorylation, only 2- to 4-fold differences in total cytoplasmic nucleotidase activity have been found in extracts of T- and B-cell lines (10).
The finding of similar levels of nucleoside kinase and nucleotidase activities in extracts of T- and B-lymphoblastoid cells, despite large differences in their rates of dATP accumulation, suggests that there are significant differences in the regulation of dAdo phosphorylation or dAXP catabolism in intact T- and B-lymphoblastoid cells. In earlier studies, we examined the effects of mutational loss of dCyd or Ado kinases on dAdo-mediated dATP pool expansion in the human T- and B-cell lines CEM and WI-L2 (29, 30, 59). We found that dAXP accumulation was primarily dependent on dCyd kinase in the T- (29) and Ado kinase in the B- (30, 59) cell line. Moreover, both kinases appeared to operate far more actively in dAdo phosphorylation in the intact T-cell line than in the B-cell line. We postulated that rapid dAXP degradation might account for the apparent inactivity of dAdo-phosphorylating enzymes in WI-L2 (29).

In order to obtain more information about the nature of the mechanisms that regulate dAdo nucleotide accumulation in intact T- and B-cell lines, we have used the somatic cell genetic technique of generating cell hybrids by fusing WI-L2 and CEM. We have compared the hybrid and parental cell lines with respect to their sensitivity to dAdo and other nucleosides and their abilities to accumulate and degrade intracellular dAXP. In addition to shedding light on the regulation of dAdo metabolism, our studies suggest a possible mechanism by which resistance to chemotherapeutic nucleoside analogues may develop in lymphoid cancers.

MATERIALS AND METHODS

Materials. Hypoxanthine, aminopterin, OUA, 6-thioguanine, Nonidet P-40, azaserine, ara-C, ara-G, 8-azaguanine, and dAdo were purchased from Sigma Chemical Co. (St. Louis, MO). [5-3H]Adenosine and [2-14C]dAdo were obtained from Moravek Biochemicals (Brea, CA). [5-3H]Cyd and [8-14C]AMP were from Amersham/Searle Corp. (Arlington Heights, IL), and [8-3H]Cyd, [2-14C]dCyd, and [8-14C]hypoxanthine were from New England Nuclear (Boston, MA). Polyethyleneimine-cellulose thin-layer plates were from Merck. PEG (Carbowax PEG 1000) was purchased from Fisher. Acridine orange was purchased from Polysciences, Inc. (Warrington, PA). dCF (Pentostatin) was a gift from Warner Lambert-Parke Davis (Detroit, MI). Tiazofurin was provided by the National Cancer Institute. Other chemicals and materials used were of the highest available quality and were purchased as described in the references cited.

Cell Lines and Culture Methods. The HGPRT-containing (HGPRT"), OUA-sensitive (OUA") human splenic B-lymphoblastoid cell line WI-L2 (46) has been used in this laboratory for several years. Cell line CEM.AG1.OU1.5 is an HGPRT-deficient (HGPRT"), OUA-resistant (OUA") clone of the human malignant T-lymphoblastoid cell line CCRF-CEM (19). In the text and charts, CEM.AG1.OU1.5 is referred to simply as CEM or "the T-cell parent." The T x B hybrid cell line S8.CEM.1 (34), derived by fusing the human B-cell line S8 (1) with a CEM.AG1.OU1.5 clone, was obtained from D. Howell and P. Cresswell, Department of Immunology, Duke University Medical Center. Cells were routinely cultured in RPMI 1640 (Grand Island Biological Co., Grand Island, NY) supplemented with 10% horse serum, nonessential amino acids, and 1 mM pyruvate under an atmosphere of 5% CO2 in air at 37 °C. Cloning was performed by limiting dilution either in growth medium or in semisolid medium containing 0.22% agarose (Sea-Kem; Marine Colloids, Inc., Rockville, ME), using as feeder cells (in a separate, lower 0.22% agarose layer) mouse macrophages obtained by sterile 0.9% NaCl solution irrigation of the peritoneal cavity or human fetal foreskin fibroblasts, which were kindly provided by Dr. Sheldon Pinell. All experiments and cell fusions were performed with cultures in the logarithmic phase of growth (4 to 8 x 106 cells/ml). Cultures were deemed free of Mycoplasma contamination by the inability of conditioned medium in which the cell line had been grown to cause the conversion of dAdo to adenosine (in the presence of 5 μM dCF to inhibit ADA) or thymidine to thymine by a cytochemical stain procedure (11).

OHA selective medium consisted of RPMI 1640 + 10% horse serum + 1 mM pyruvate + nonessential amino acids + 50 mM OUA + 100 μM hypoxanthine + 5 μM asazeren. These drug concentrations were selected to completely inhibit growth of the appropriate parent but slow growth of the other parent by no more than 25%. Growth in OHA medium selects for cells that are OUA-resistant and possess HGPRT activity, properties expected of the desired hybrids.

Cell Fusion. Fusions were performed by a method originally developed for murine myeloma-splenocyte fusions (22) with minor modifications. Briefly, 2 x 10⁶ CEM and 2 x 10⁶ WI-L2 were mixed in a 15-ml conical centrifuge tube and pelleted; 0.5 ml of a 50% solution of PEG in RPMI 1640 medium without serum was added dropwise over 1 min, followed by dropwise addition of 11 ml of RPMI 1640 without serum over the next 6 min. The cells were pelleted, resuspended in 20 ml of OHA selective medium, and distributed in 150-μl aliquots in 96-well tissue culture plates (Costar) at a final density of 2 x 10⁴ cells/well. In every fusion, controls were performed by omitting PEG and by carrying out the procedure with each parent alone.

Cell Surface Antigenic Phenotype. HLA-A.B.C. and DR typing were performed by the Duke Medical Center Serologic Laboratories by complement mediated microcytotoxicity (2). Chromosome Analysis. Logarithmically growing cells were exposed to Colcemid (0.1 μg/ml) for 2 h. Cells were then harvested, and slides were prepared for analysis by Giemsa staining according to established protocols (63). At least 50 mitoses were examined by photomicroscopy for determination of chromosome number.

DNA content per cell was determined by flow microcytfluorometry after staining with acridine orange (3, 14). Ten ml of logarithmically growing cells in growth medium were pelleted and resuspended in phosphate-buffered saline containing 0.5% Nonidet P-40 + 20% fetal calf serum. Ten min later, the cells were stained by addition of 1 ml of a solution containing 0.002% acridine orange, 0.1 mM NaCl, and 0.01 mM citrate-phosphate buffer at pH 3.8. After 15 min at room temperature, the fluorescence intensities of the individual cells were measured in a flow cytometer (Cytofluorograf 50H; Ortho Diagnostic Instruments, Bio/Physics Systems, Westwood, MA). Detailed description of the instrument is presented elsewhere (6, 18). The results were analyzed using a multichannel analyzer and plotted as frequency distribution histograms of DNA content.

Enzyme Assays. Extracts of 2 to 5 x 10⁶ cells were prepared for assay by methods that involved disrupting cells by freezing and thawing, followed by centrifugation and chromatography of supernatants over 0.7- 20-cm Sephadex G-25 columns (30). The assay conditions have been described previously for measurement of AdoHcyase (28), ADA (57), HGPRT (33), total dAdo-phosphorylating activity (30), dCyd kinase (29), ecto-5'-nucleotidase (61), PNP (21), Ado kinase (29), and dCyd deaminase (18).

Growth Inhibition by Nucleosides. The effects of the ADA substrates dAdo and ara-A (in the presence of 5 μM dCF) and of ara-C and tiazofurin on growth of the parent and hybrid cell lines were examined after 72 to 96 h of exposure. Results were compared to controls exposed to dCF alone or to no drug using the formula

\[
\text{Final cell count - initial cell count)} \text{in presence of drug} - \text{Final cell count - initial cell count)] \text{in absence of drug}
\]

Results were expressed as EC50 (concentration of drug required to inhibit growth by 50%).

Measurement of dAXP Accumulation. Cultures at a cell density of 5 x 10⁶ cells/ml in growth medium were incubated at 37°C with 5 μM dCF for 1 h, followed by addition of various concentrations of dAdo (range, 5 to 500 μM). After 3 or 4 more h of incubation, duplicate aliquots were removed for harvesting of cells and extraction with HClO4 as described (30). Total adenine deoxyribonucleotides (dAXP = dATP + dADP + dAMP) in neutralized HClO4 extracts of cells were measured by high-pressure liquid chromatography after dephosphorylation of these nucleo-
tides to dAdo by a modification (29) of a procedure described previously (30). dAdo was quantitated by high-pressure liquid chromatography on a Partisil PX5 10/25 SCX column eluted with 50 mM NaH2PO4, pH 2.6, at a flow rate of 1.5 ml/min.

Measurement of dAXP Degradation. Duplicate cultures of CEM, WI-L2, SB, the WI-L2 x CEM hybrid (TBj1.1E6), and the SB x CEM hybrid, each at a cell density of 5 x 10^5 cells/ml in growth medium, were incubated with 5 μM dCF for 1 h at 37°C. Then, WI-L2, SB, and the hybrids were incubated for an additional 3 h with 500 μM dAdo and CEM for 2 h with 20 μM dAdo, conditions chosen to cause approximately equal dATP accumulation in the B-cell lines and CEM (see “Results”). The cultures were chilled on ice for 5 to 10 min and centrifuged at 4°C, and the medium was decanted. The cell pellets were washed once with 10 ml of ice-cold medium containing 5 μM dCF without dAdo. Centrifugation was repeated, the wash medium was aspirated, and the cells were resuspended at the original cell density in 37°C growth medium that contained 5 μM dCF without dAdo. Duplicate aliquots of cultures were then harvested for measurement of intracellular dAXP as described (29, 30), immediately (0 time) and after further incubation at 37°C for 30, 60, 90, 180, and 300 min.

RESULTS

General Characterization of Hybrids

A fusion of CEM.AG1.OU1.5 with WI-L2 yielded growth in 3 wells from two 96-well tissue culture plates. Cells from these wells were cloned in OHA medium in 0.22% agarose layers. Six hybrid clones were recovered and placed in long-term culture in OHA for 2 months and then in regular growth medium. No growth was observed in control experiments in which either parental line was fused with itself or when PEG was omitted from the fusion protocol. As an additional control, we also developed a T- x T-cell hybrid by crossing a CEM.AG1.OU1.5 clone with the HGPRT+, OUA® CEM line from which it was derived. The behavior of this T- x T-cell hybrid cell line is discussed in various sections below. We also conducted some studies with one other T- x B-cell hybrid clone, SB.CEM.1, which was derived from the fusion of a clone of CEM.AG1.OU1.5 with the B-cell line SB.

The hybrid nature of the cloned CEM x WI-L2 fusion products was established on the basis of their growth in various selective conditions, their karyotype and DNA content, the expression of surface antigens, and by measurement of cell-associated enzyme activities. The 6 cloned products of CEM x WI-L2 fusions were found to have very similar characteristics, which have remained stable after almost 2 years in culture. Representative data from one isolate (TBj1.1E6) are presented in more detail; data from the other hybrid clones are summarized.

Growth in Selective Media. The hybrids all grew in selective media that failed to support the growth of one or both parents. Sensitivity of the hybrids to thioguanine and to azaserine in the absence of hypoxanthine established that they contained HGPRT activity, OUA® CEM line from which it was derived. The behavior of this T- x T-cell hybrid cell line is discussed in various sections below. We also conducted some studies with one other T- x B-cell hybrid clone, SB.CEM.1, which was derived from the fusion of a clone of CEM.AG1.OU1.5 with the B-cell line SB.

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Growth in Selective Media. The hybrids all grew in selective media that failed to support the growth of one or both parents. Sensitivity of the hybrids to thioguanine and to azaserine in the absence of hypoxanthine established that they contained HGPRT activity and were not simply azaserine-resistant mutants of the OUA-resistant T-cell parent. The T- x T-cell hybrid control exhibited identical growth properties in the media shown in Table 1 as the T- x B-cell hybrids, but it could be easily distinguished from them on the basis of HLA markers and deoxyribonucleoside metabolism, as discussed below.

DNA Content. Based on cytofluorimetric analysis of nuclear DNA content, WI-L2 and CEM cells contained similar amounts of DNA, while the DNA content of the hybrids was clearly greater than that of either parent (Chart 1). These relationships were confirmed by chromosome counts. The modal chromosome number for the CEM x WI-L2 hybrids was 122 (range, 117 to 130), compared with 92 (range, 86 to 96) for CEM and 89 (range, 70 to 93) for WI-L2.

Surface Antigens. Expression of human class I and class II major histocompatibility antigens was examined in the hybrid and parental cell lines (Table 2). With the exception of CW2 (a class I antigen expressed only on the B-cell parent), the HLA A, B, and C antigens and DR antigens of both parents were expressed by the hybrids. In addition, the hybrids expressed one new antigen, expressed on neither parent, at each HLA locus. The phenomenon of novel class II (DR) antigen expression in T- x B-cell hybrid lymphoblastoid cell lines has been observed previously in studies of the regulation of HLA phenotype in T- and B-cells (34, 35). It was concluded that the new DR marker was encoded by the genome of the T-cell parent but was not expressed in the undifferentiated or unactivated state. Our findings suggest that hybrid formation may also result in expression of class I as well as class II loci, but it is unclear whether these loci were derived from the T- or the B-cell parent.

Enzyme Activities. Cytoplasmic enzyme activities were measured in Sephadex G-25-treated cell extracts for CEM, WI-L2,
systems were >10-fold more resistant to ara-A-mediated growth inhibition than was CEM and were slightly more resistant than was either B-cell parent.

Hypothetically, resistance to nucleosides might be a general property of T- x B-lymphoblast hybrids. To assess this possibility, we compared the sensitivities of the hybrid and parental lines to the nucleoside analogues tiazofurin and ara-C. Tiazofurin is converted to an analog of NAD, which inhibits inosinic acid dehydrogenase (36). Our unpublished studies indicate that tiazofurin, unlike dAdo and ara-A, is activated by neither dCyd nor Ado kinase. In contrast to dAdo and ara-A, tiazofurin is about 10-fold more toxic to WI-L2 than to CEM, with ECso values for the B- and T-cell lines of 6 and 60 μM, respectively. The hybrid was as sensitive to tiazofurin as was WI-L2 (Chart 2C). Like dAdo and ara-A, ara-C is phosphorylated by dCyd kinase, but ara-C is only about 2-fold more toxic to CEM than WI-L2 (Chart 2D). The WI-L2 x CEM hybrid TBJ.1E6 was about as sensitive to ara-C as was WI-L2. These studies show no evidence of a general resistance of the hybrids to nucleosides, so that it is valid to address the resistance of the hybrids to dAdo specifically in terms of the regulation of dAdo and dAdo nucleotide metabolism by the parental T- and B-cell lines.

There is evidence that dCyd kinase in WI-L2 might be less efficient than the enzyme in CEM in activating dAdo. Thus, dAdo-induced dAXP pool expansion or toxicity in WI-L2 (30, 59) compared with CEM (9). In light of these observations, we examined the effect of dCyd on dAdo toxicity to WI-L2 x CEM hybrids. dCyd (20 μM) increased the ECso values for dAdo of CEM by 4- to 6-fold but had almost no effect on dAdo toxicity to WI-L2 or the CEM x WI-L2 hybrid. It is possible that the ineffectiveness of dCyd in diminishing dAdo toxicity to WI-L2 and the WI-L2 x CEM hybrid is related to the 5- to 7-fold higher dCyd deaminase activity in these lines as compared to CEM (Table 3).

The greater dependence of dAXP accumulation on dCyd kinase in CEM compared with WI-L2 does not appear to be the result of different properties of the dCyd kinases present in these cell lines.

### Table 2

<table>
<thead>
<tr>
<th>HLA phenotype of parental and hybrid cell lines</th>
</tr>
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<tbody>
<tr>
<td>HLA antigen</td>
</tr>
<tr>
<td>A1</td>
</tr>
<tr>
<td>A2</td>
</tr>
<tr>
<td>A w19</td>
</tr>
<tr>
<td>B8</td>
</tr>
<tr>
<td>B17</td>
</tr>
<tr>
<td>Bw4</td>
</tr>
<tr>
<td>Bw6</td>
</tr>
<tr>
<td>Cw2</td>
</tr>
<tr>
<td>Cw3</td>
</tr>
<tr>
<td>DR3</td>
</tr>
<tr>
<td>DR7</td>
</tr>
<tr>
<td>DRw6</td>
</tr>
</tbody>
</table>

### Table 3

Enzyme specific activities in parental and hybrid cell lines

Activities were measured in Sephadex G-25-treated cell lysates as described in ‘Materials and Methods’ using the indicated concentrations of radioactive nucleoside substrate.

<table>
<thead>
<tr>
<th>Concentration of labeled substrate (μM)</th>
<th>T-cell</th>
<th>B-cell</th>
<th>Hybrid</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADA</td>
<td>175</td>
<td>8175</td>
<td>1260</td>
</tr>
<tr>
<td>Ado kinase</td>
<td>100</td>
<td>20.0</td>
<td>11.0</td>
</tr>
<tr>
<td>dCyd kinase</td>
<td>50</td>
<td>9.0</td>
<td>2.0</td>
</tr>
<tr>
<td>dAdo kinase</td>
<td>100</td>
<td>19.0</td>
<td>7.0</td>
</tr>
<tr>
<td>AdoHyase</td>
<td>150</td>
<td>320</td>
<td>320</td>
</tr>
<tr>
<td>PNP</td>
<td>200</td>
<td>2978</td>
<td>2987</td>
</tr>
<tr>
<td>dCyd deaminase</td>
<td>200</td>
<td>0.13</td>
<td>0.97</td>
</tr>
</tbody>
</table>

Accumulation of Adenine Deoxyribonucleotides. dAXP accumulation was measured in ADA-inhibited cells after 4 h of
incubation in growth medium containing 5, 25, or 125 μM dAdo (Table 5). At lower concentrations of dAdo, dAXP accumulation by the B-cell lines was barely detectable and, at 125 μM, they accumulated roughly 10% of that by CEM. The WI-L2 × CEM hybrid accumulated only slightly more dAXP than did WI-L2. The SB × CEM.1 hybrid accumulated about 20% as much dAXP as CEM at 25 μM dAdo and nearly as much as CEM at 125 μM. At very high concentrations of dAdo (500 μM or greater), the accumulation of dAXP by the WI-L2 × CEM hybrid also approached that by CEM (not shown).

Catabolism of Intracellular dAXP. In order to study the rate of catabolism of intracellular dAXP, it is necessary to first expand this pool by incubating cells with dAdo, after which the cells are transferred to dAdo-free medium and the rate of fall in dAXP content is followed. It is desirable but difficult to achieve equal levels of intracellular dAXP in the cells under study, given the marked difference in rates of dAXP accumulation between T-

Table 4
Comparison of sensitivity to dAdo and ara-A in T-cell, B-cell, and hybrid cell lines
All experiments contained 5 μM dCF.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Growth inhibition [EC50 (μM)]</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>By dAdo</td>
</tr>
<tr>
<td>CEM (T)</td>
<td>0.74 ± 0.66a (7)</td>
</tr>
<tr>
<td>WI-L2 (B)</td>
<td>48.5 ± 9.47 (4)</td>
</tr>
<tr>
<td>SB (B)</td>
<td>14.0 ± 4.36 (3)</td>
</tr>
<tr>
<td>CEM × WI-L2 (T × B)</td>
<td>27.7 ± 5.35 (7)</td>
</tr>
<tr>
<td>CEM × SB (T × B)</td>
<td>21.8 ± 5.89 (5)</td>
</tr>
<tr>
<td>CEM × CEM (T × T)</td>
<td>0.52 ± 0.39 (5)</td>
</tr>
</tbody>
</table>

*aMean ± SD.
*bNumbers in parentheses, number of experiments.
*cExperiment performed once.

Chart 3. Effect of substrate concentration on the rate of phosphorylation of deoxyadenosine by dCyd kinase from a CEM/WI-L2 hybrid. Extracts of 5 × 10^7 cells of TBJ.1E9 were prepared, centrifuged, and chromatographed on Ultrogel AcA 34 as described previously (29). Abscissa, reciprocal of the concentration of [3H]dAdo concentration. y-axis, reciprocal of the reaction velocity. Inset, plot of the concentration of [3H]dAdo (x-axis) versus velocity (y-axis, arbitrary units).
dAdo TOXICITY IN LYMPHOBLAST HYBRIDS

The objective of this study was to determine whether hybrids formed by fusing a dAdo-sensitive T-cell line with a dAdo-resistant B-cell line would retain the sensitivity of one or the other parent or simply express intermediate sensitivity. We hoped to evaluate our results in terms of the mechanisms that may underlie the different sensitivities of the parental cell lines to dAdo and related nucleosides. Since T-cell lines are also more sensitive than are B-cell lines to the cytotoxic effects of thymidine, we thought that use of the standard hypoxanthine-aminopterin-thymidine medium to select for hybrids [which had been used in isolating the SB × CEM hybrid (34)] might result in preferential isolation of thymidine-resistant clones that might be cross-resistant to dAdo. We therefore selected for WI-L2 × CEM hybrids in medium containing azaserine, which inhibits purine synthesis but not thymidylate synthesis, and hypoxanthine, which is a nontoxic source of purines to cells that possess HPRT activity. A similar type of selection has been developed independently for the isolation of hybrids involving peripheral blood T-lymphocytes (20).

The 6 WI-L2 × CEM hybrid clones we isolated were all much more resistant to dAdo toxicity than was their T-cell parent and were nearly as dAdo-resistant as was their B-cell parent. Likewise, the SB.CEM.1 hybrid was as resistant to dAdo as was its B-cell parent, SB. “B-ness” was also dominant in both the WI-L2 × CEM and SB × CEM systems with respect to the toxicity of ara-A, which is phosphorylated by the same kinases that act on dAdo and which may achieve toxicity in part by mechanisms similar to those of dAdo. These results contrast with the finding that tiazofurin, a nucleoside that differs from dAdo and ara-A in its mechanism of activation and toxicity, was about 10-fold more toxic to WI-L2 and a WI-L2 × CEM hybrid than to CEM. Thus, resistance to nucleoside toxicity is not an inevitable attribute of T- × B-hybrids.

The limited ability of dAdo to cause dATP pool expansion has been proposed as the sole explanation for the relative resistance of WI-L2 and other B-cell lines to dAdo (30). However, there may also be differences between B- and T-cell lines in the intracellular pools of dATP, as suggested by the finding that dAdo arrested CEM cells in the G1 phase of the cell cycle but blocked WI-L2 cells in S phase (37). Thus, our present results, which show that B-like resistance is dominant in T- × B-hybrids, could indicate either that the dATP target in B-cells is less sensitive to dATP than that of T-cells or that a dominant-acting mechanism in the B-cell lines is responsible for limiting dAdo-mediated dATP pool expansion. Our studies shed no direct light on the identity of the dATP target, but the fact that the SB.CEM.1 hybrid accumulated substantially more dATP than did its B-cell parent, SB, but was as resistant to dAdo as was SB, is consistent with the possibility that SB contains a dATP-insensitive target.

Our studies with the WI-L2/CEM and SB/CEM systems provide direct evidence in favor of the hypothesis (7, 61) that more rapid catabolism of dAXP is an important factor in explaining the difference in ability of dAdo to cause dATP pool expansion in T- and B-cell lines. It is clear from our results that a dATP-degrading activity, present in WI-L2 and SB but not CEM cells, is expressed in WI-L2 × CEM and SB × CEM hybrids. At the low concentrations of dAdo that are cytotoxic to CEM cells, the rate of operation of this nucleotidase activity appears sufficient to prevent dAXP accumulation and toxicity in WI-L2 and SB and in WI-L2 × CEM and SB × CEM hybrids. The identity of this nucleotidase
dase has not been definitively established, although it seems likely that it is a cytoplasmic rather than a cell surface-associated activity. If expression of this activity in T- X B-lymphoblastoid hybrid cells is a general phenomenon and, if this expression confers a dAdo-resistant phenotype to hybrids, then it may be possible to map the chromosomal location of the gene for the nucleotidase by constructing hybrids between a dAdo-sensitive murine T-cell line and a dAdo-resistant human B-cell line such as WI-L2. Moreover, expression of this activity should be dominantly selectable in T-cell lines, which could provide a strategy for isolation of its gene using DNA transfer methodology.

It has been proposed that cell fusion involving malignant and nonmalignant cells or between phenotypically variant tumor cells may contribute to the progression of tumors or to the evolution of phenotypic heterogeneity common in tumors (4, 15, 27, 38, 39). Evidence of such fusion has been obtained experimentally in studies of transplantable lymphoid tumors (24), and Sinkovics (55) has purported that hybrid formation involving antibody-producing plasma cells may be a natural process in lymphoproliferative diseases. In general, discussions of the role of hybrid-generated diversity in tumors have focused on the development of more invasive or metastatic characteristics, properties that influence the progression of the tumor prior to therapeutic intervention. Our studies suggest that fusion between a malignant, dAdo-sensitive T-cell with an activated B-cell might result in the acquisition of characteristics that would confer a selective advantage during treatment with dCF or ara-A. In this regard, it is interesting to consider clinical and biochemical observations that we reported recently of a patient with T-cell acute lymphoblastic leukemia who underwent a series of treatments with dCF (31).

After initially responding to dCF infusion, this patient’s leukemia became relatively resistant to dCF treatment, which still caused marked elevation in plasma dAdo. Resistance was associated with a striking decline in accumulation of dAXP in circulating leukemia cells in vivo which could not be accounted for by any alteration in ability of dCF or dAdo to enter the malignant cells or in the activities of dCyd or Ado kinases. Thus, the malignant cells, which still bore T-cell surface markers, had acquired the “B-like” characteristic of inability to respond to increased levels of exogenous dAdo by dAXP accumulation, despite adequate levels of dAdo-phosphorylating activity.

We were not able to conduct studies of dAXP degradation with leukemic cells from this patient, but it is unreasonable to consider the possibility that diminished dAXP accumulation may have resulted from an enhanced rate of dAXP catabolism, given the present evidence that degradation is a dominant factor that determines the ability of dAdo to cause dAXP pool expansion. Acquisition of increased capacity to degrade dAXP could have arisen by several mechanisms, such as gene amplification or a change in regulation of gene expression. However, in light of our present studies and proposals regarding the possible role of cell fusion in tumor progression, we think it intriguing to speculate that resistance to dCF treatment in this patient might have been the result of the fusion of a leukemia T-lymphoblast with an activated B-cell. The postulated type of fusion might also play a role in the evolution of resistance to other chemotherapeutic nucleoside analogues such as ara-A and ara-C, which are converted to cytotoxic nucleotides. It may be fruitful to monitor for changes in karyotype and cell surface antigens, as well as rates of analogue nucleotide accumulation and degradation, in leukemia cells during the course of treatment with these agents as a means of evaluating the occurrence and consequences of hybrid formation in vivo.

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dAdo TOXICITY IN LYMPHOBLAST HYBRIDS


Determinants of Deoxyadenosine Toxicity in Hybrids between Human T- and B-Lymphoblasts as a Model for the Development of Drug Resistance in T-Cell Acute Lymphoblastic Leukemia

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