Conversion of a stem cell leukemia from a T-lymphoid to a myeloid phenotype induced by the adenosine deaminase inhibitor 2'-deoxycoformycin

(hematopoietic differentiation/S-adenosylhomocysteine/methylation inhibition/purine nucleoside toxicity/deoxycytidine deaminase)

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ABSTRACT Selective failure of lymphoid development occurs in genetic deficiency of adenosine deaminase (ADA). We examined the in vivo effects of a potent inhibitor of ADA, 2'-deoxycoformycin, which was used to treat a patient with refractory acute leukemia. Unexpectedly, within 7 days of starting treatment, the leukemic phenotype underwent complete conversion from T lymphoblastic to promyelocytic, with kinetics that suggested a precursor–product relationship between the two cell populations. Pretreatment T lymphoblasts and posttreatment promyelocytes had the same abnormal karyotype. Upon culture in vitro, the former transformed spontaneously over several weeks into mature myeloid cells. We conclude that the leukemia arose from a multipotent stem cell capable of both lymphoid and myeloid differentiation. Effects of ADA inhibition on leukemia cells during treatment included expansion of the deoxyadenosine nucleotide pool and accumulation of S-adenosylhomocysteine, a potent inhibitor of S-adenosylmethionine-dependent methylation. The influence of these changes on the leukemic phenotype is discussed in terms of (i) selective cytotoxicity to T lymphoblasts, which accumulated deoxyadenosine nucleotides more efficiently than did the patient’s promyelocytes during in vitro incubation with deoxycoformycin plus deoxyadenosine, and (ii) induction of an altered program of differentiation.

In this report we describe a closely observed instance in which the phenotype of an acute leukemia changed abruptly from T lymphoid to myeloid in vivo. The phenotypic conversion was remarkable because it entailed not simply further maturation within a cell lineage but an apparent redirection in the program of hematopoietic differentiation. Lymphoid to myeloid conversion was related temporally to treatment with 2'-deoxycoformycin (dCF), a potent inhibitor of adenosine deaminase (ADA). Use of dCF to treat lymphoid malignancies is based on the fact that genetic deficiency of ADA causes selective absence of lymphoid tissues (1). Lymphopenia is thought to result from effects of the ADA substrates adenosine (Ado) and 2'-deoxyadenosine (dAdo), among which are the intracellular pools of the toxic nucleotide dATP, and of S-adenosylhomocysteine (AdoHcy), a potent inhibitor of S-adenosylmethionine (AdoMet)-dependent transmethylation reactions (2). We observed accumulation of these compounds in the leukemia cells of our patient during treatment with dCF, and we have considered their possible influence on the leukemic phenotype.

MATERIALS AND METHODS

Materials. [8,14C]Adenosine (59 mCi/mmol; 1 Ci = 37 GBq) and [5'-3H]deoxycytidine (26 Ci/mmol) were purchased from Amersham; Colcemid and phytohemagglutinin M, from Gibco; and lymphocyte separation medium (LSM); sodium diatrizoate/Ficol, density 1.077–1.080 g/ml, from Litton Bionetics. dCF for clinical use was provided by the Investigational Drug Branch of the National Cancer Institute, and dCF for in vitro studies, by Parke-Davis/Warner-Lambert Pharmaceuticals (Ann Arbor, MI). Reversed-phase C18 Bondapak and Partisil 10 SCX cation-exchange HPLC columns were purchased from Waters Associates and Whatman. Calf intestine ADA, type I, was purchased from Sigma. The nonspecific ADA from Aspergillus oryzae was prepared as described (3).

Methods. We have described our procedures for monitoring the biochemical effects of treatment with dCF, including processing of samples of urine and blood; isolation of circulating leukemia cells by density gradient centrifugation in lymphocyte separation medium; preparation of extracts of cells, plasma, and urine for measuring concentrations of metabolites and activities of enzymes; and conditions for HPLC quantitation of extracellular Ado and dAdo, and of their total intracellular acid-soluble nucleotides (4). The activities of ADA (5), AdoHcy hydrolase (AdoHcyase; EC 3.3.1.1) (6), and 2'-deoxycytidine (dCyd) deaminase (7) in Sephadex-G-25-treated cell lysates (8) were measured as described.

RESULTS

Clinical Course Prior to dCF Treatment. Illness began 2 months prior to treatment with dCF, with lymph node and thymus enlargement, rapidly rising leukocyte count, and central nervous system involvement in a 16-year-old male. Abnormal cells in blood, bone marrow, and a lymph node had lymphoid morphologic and histochemical characteristics, and >90% reacted with monoclonal antibodies to T-cell surface antigens (Table 1, Fig. 1). High leukemia cell ADA activity (9 μmol/hr per mg of protein) was consistent with T-cell acute lymphoblastic leukemia (ALL) (16). An additional

Abbreviations: ADA, adenosine deaminase; dCF, 2'-deoxycoformycin; AdoHcy, S-adenosylhomocysteine; AdoMet, S-adenosylmethionine; Ado, adenosine; dAdo, 2'-deoxyadenosine; dCyd, 2'-deoxycytidine; ALL, acute lymphoblastic leukemia; AdoHcyase, AdoHcy hydrolase.

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Table 1. Leukemia cell reactivity with monoclonal antibodies

<table>
<thead>
<tr>
<th>Monoclonal antibody</th>
<th>% cells positive in peripheral blood</th>
<th>Reactivity of frozen tissue sections</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5/28</td>
<td>7/6</td>
</tr>
<tr>
<td>T cell</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3A1</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>35.1</td>
<td>100</td>
<td>24</td>
</tr>
<tr>
<td>Leu 4</td>
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<td>8</td>
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<tr>
<td>Leu 3A</td>
<td>22</td>
<td>5</td>
</tr>
<tr>
<td>NA1/34</td>
<td>21</td>
<td>1</td>
</tr>
<tr>
<td>Leu 2A</td>
<td>15</td>
<td>ND</td>
</tr>
<tr>
<td>Myeloid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DU-HL60-1</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>DU-HL60-3</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>DU-HL60-4</td>
<td>9</td>
<td>12</td>
</tr>
<tr>
<td>CM02</td>
<td>8</td>
<td>6</td>
</tr>
</tbody>
</table>

Dates of testing: 5/28 = diagnosis; 7/6 = 17 days before treatment with dCF; 7/30 = day 7 after start of dCF. ND, not determined.

Characterization of leukemia cells in peripheral blood. Mononuclear cells were prepared from heparinized blood or leukapheresis packs by density gradient centrifugation. Reactivity of these cells with a panel of monoclonal antibodies was characterized by indirect immunofluorescence and flow cytometry(9). T-cell markers: Antibody 3A1 reacts with 85% of peripheral blood T cells and all thymocytes but does not bind to B cells, granulocytes, or erythrocytes(10). T-cell antigens defined by other monoclonal antibodies are NA1/34, the T6 antigen; Leu 3A, the T4 antigen; Leu 2A, the T8 antigen; Leu 4, the T3 antigen; and 35.1, the erythrocyte rosette receptor. Myeloid markers: CM02 defines a myeloid antigen that is found on monocytes and blasts of acute myelomonocytic leukemia, but not on blasts of acute myeloid leukemia or ALL(11). Monoclonal antibodies DU-HL60-1, DU-HL60-3, and DU-HL60-4 were generated to the HL60 promyelocytic cell line and react with all granulocytes and peripheral blood monocytes but not with normal B or T cells(12). Other markers: 100% of cells on all three days reacted with 3F10, an anti-HLA antibody. On 5/28 and 7/30, 22–23% of cells, and on 7/6, 74% of cells reacted with antibody SE9, which defines the transferrin receptor(13).

Reactivity of frozen tissue sections with monoclonal antibodies. Tissue was obtained by lymph node biopsy at time of diagnosis (5/28) and at autopsy (7/30) from lymph node, thymus, and spleen. Four-micrometer frozen sections were assayed for reactivity with monoclonal antibodies by indirect immunofluorescence(14). Reactivity: + = 1–95% of cells positive; 40%+ = 40–95% of cells positive; 20%+ = 20–95% of cells positive; - = <5% of cells negative.

*Monoclonal antibody A2B5, which defines neuroendocrine thymic epithelium(15), was used to confirm that tissue obtained from the mediastinum was thymus.

T-cell characteristic (17, 18) of pretreatment leukemia cells was the ability to accumulate dATP when incubated in vitro with dCF plus dAdo (Table 2).

The patient had transient responses to systemic treatment with multiple drugs, including vincristine, prednisone, cyclophosphamide, daunomycin, hydroxyurea, 6-mercaptopyrimidine, "high-dose" cytosine arabinoside, and VM-26, as well as intrathelial methotrexate, cytosine arabinoside, and hydrocortisone. Resumption of leukemia cell proliferation 5 days after the last of these regimens led to the decision (with consent) to treat with dCF.

Phenotypic Conversion with Retention of Karyotype. The schedule of dCF administration and response of blood leukocyte count are summarized in Fig. 2A and B. After increasing from 20,000 to 140,000 per μl during the first 2 days of treatment, the leukocyte count became stable. It was lowered by leukapheresis on day 4, when dCF infusion ended, and remained in the 60,000–80,000 range. On day 7 the leukocyte count rose from 84,000 to 195,000. Progressive hypoxemia and circulatory collapse developed, and the patient died.

Table 2. Accumulation of dAdo nucleotides from dAdo in vitro by leukemia cells with inhibited ADA

<table>
<thead>
<tr>
<th>dAdo, μM</th>
<th>Before treatment</th>
<th>After conversion</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>7/7</td>
<td>7/23 (day 1)</td>
</tr>
<tr>
<td>0</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>45</td>
<td>55</td>
</tr>
<tr>
<td>50</td>
<td>200</td>
<td>144</td>
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</tbody>
</table>

Circulating leukemia cells were isolated by density gradient centrifugation and resuspended in RPMI 1640 medium containing 10% horse serum and 5μM dCF at 1.3–1.5 x 10^6 cells per ml. Then dAdo was added to give the indicated final concentration. After incubation at 37°C for 2 hr, cells were harvested and washed, and the concentration of total acid-soluble intracellular dAdo nucleotides was measured as described (4).

*The higher base line content of dAdo nucleotides on day 7 results from the in vivo accumulation of these compounds during treatment.

On days 1–3 at least 90% of circulating leukocytes had the appearance of lymphoblasts on standard Wright-stained preparations, but over the next 4 days these were quantitatively replaced by larger cells, with more abundant cytoplasm and granules, most of which appeared to be promyelocytes (Figs. 1 and 2B). By day 7 virtually all circulating leukocytes were morphologically myeloid: about 40% promyelocytes and the remainder a mixture of more mature forms, including segmented neutrophils. Compared with pretreatment data, on day 7 there was a marked decrease in the percentage of leukemia cells that reacted with the T-cell monoclonal antibody 3A1 and a striking increase in the percentage that reacted with monoclonal antibodies to myeloid cells (Table 1). Autopsy revealed generalized clumping of myeloid cells in blood vessels (leukostasis), which was felt to have contributed to death. The thymus was massively enlarged, weighing 400 g, and, along with lungs, spleen, lymph nodes, and bone marrow, was infiltrated uniformly with tumor cells that had histochemical and electron microscopic characteristics of promyelocytes (not shown). The same change in the surface antigen immunoreactivity of leukemia cells, from T lymphoid to myeloid, was demonstrable in sections from lymph nodes, spleen, and thymus obtained at autopsy (Table 1).

The activity of dCyd deaminase has been found to be high in mature granulocytes and in some myeloid leukemias (19). In contrast, we found very little dCyd deaminase activity in the lymphoblasts of a patient with T-cell ALL (4), or in the CEM human T-lymphoblastoid cell line (data not shown). Consistent with these latter results, dCyd deaminase was low (0.5–1.2 nmol/hr per 10^6 cells) in leukemia cells with a T-cell morphologic and immunologic phenotype that were obtained at diagnosis and on the day treatment with dCF began. The activity increased almost 2-fold in the first 24 hr of treatment with dCF and then doubled every 10–12 hr for the next 4 days, reaching 1,300 nmol/hr per 10^6 cells (Fig. 2C). By extrapolation, the onset of the rise in dCyd deaminase activity, and possibly the onset of phenotypic conversion, can be set at about 18 hr after the start of dCF treatment. A second biochemical correlate of the change from T-lymphoid to myeloid phenotype was loss of the capacity of leukemia cells obtained on the day of death to accumulate dATP when incubated in vitro with dCF plus dAdo (Table 2).

Despite the conversion in phenotype, the same abnormal chromosomes were found in the patient’s T lymphoblasts, obtained at diagnosis, and in his promyelocytic cells, obtained on the day of death (Fig. 3). In each population virtually all evaluable spontaneous mitoses possessed a translocation involving chromosomes 1 and 14, and two partial deletions of chromosome 1, one at the short arm and one at the

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long arm: 46,XY,−14,1p33−d1(14)(p33;q11),+1q11−. The Philadelphia chromosome, present in 90% of patients with chronic myelogenous leukemia (22), was not observed. The karyotype of phytohemagglutinin-stimulated peripheral blood mononuclear cells obtained at diagnosis, which were presumably nonmalignant lymphocytes, was normal (results not shown).

We have examined serially the morphologic and surface antigenic characteristics of the patient's T-lymphoblastoid cells, isolated at diagnosis, during culture in vitro. Although space does not permit presentation of these results in detail, it is pertinent to note that we observed a gradual spontaneous conversion of these cells into myeloid cells at various stages of maturation, including macrophages and segmented neutrophils (Fig. 1 F and G).

**Biochemical Consequences of ADA Inhibition.** Leukemia cell ADA activity was inhibited by >98%. Effective inhibition of total body ADA was indicated by a rise in plasma dAdo from <0.1 μM to 18.9 μM and by daily renal excretion of up to 2.4 mmol of dAdo (data not shown). dAdo nucleotides in leukemia cells rose dramatically from <2 to 1,050 nmol/10⁹ cells by day 5, declining to 70 nmol/10⁹ cells by day 7 (Fig. 4A). Of interest, free (unphosphorylated) dAdo also appeared in circulating leukemia cells (Fig. 4A), reaching a peak on day 6. The time course of appearance and decline of cellular dAdo is consistent with the possibility that it arose from the catabolism of the expanded cellular dAdo nucleotide pool via dephosphorylation of dAMP. AdoHcy derived from transmethylation reactions is catalyzed in eukaryotic cells by hydrolysis to Ado + l-homocysteine, catalyzed by AdoHcyase (23). Because the equilibrium for this reversible reaction highly favors AdoHcy, Ado causes the accumulation of AdoHcy, with inhibition of DNA and RNA methylation, in ADA-inhibited lymphoid cells in vitro (24, 25). AdoHcyase is also a high-affinity binding protein for Ado and its analogs (8). Binding of dAdo irreversibly inactivates the enzyme (6), an effect responsible for the >98% deficiency of AdoHcyase activity in the erythrocytes of children with genetic ADA deficiency (26).

AdoHcyase activity in leukemia cells decreased by 90% by the end of treatment (data not shown). The intracellular concentration of AdoHcy rose from a pretreatment level of <0.2 to 6 nmol/10⁹ cells (Fig. 4B). The AdoMet content also increased from 7.5 to 30 nmol/10⁹ cells, presumably as the result of inhibition by AdoHcy of AdoMet-dependent transmethylation reactions. The further increases in concentra-
tions of both AdoMet and AdoHcy between days 5 and 7 (Fig. 4B) reflect the larger size of the circulating leukemia cells present on those days (Fig. 1). The ratio of AdoMet to AdoHcy, which is an index of the capacity to transmethylate (2, 4), was 39:1 in leukemia cells prior to treatment; it fell to 8:1 by day 2 and was 4:5:1 thereafter (Fig. 4B).

**DISCUSSION**

We believe that the leukemia in this patient arose via the malignant transformation of a stem cell with the potential to differentiate along either a lymphoid or myeloid pathway. This conclusion is consistent with the capacity of the patient's T lymphoblasts to transform slowly in vitro into macrophages and mature granulocytes (Fig. 1F and G). The possibility of developing two independent acute leukemias, one lymphoid and one myeloid, within a period of 2 months is remote. Moreover, the presence of the same abnormal karyotype in both leukemic populations strongly favors their common clonal origin. The existence of a common progenitor of lymphoid and myeloid cells has been suggested by the occasional evolution of chronic myelogenous leukemia into a lymphoblastoid phase (27), by reports of leukemias with mixed lymphoid and myeloid characteristics (28, 29), and by elegant studies of hematopoietic ontogeny in mice (30–32).

We are aware of one other abrupt conversion from T-lymphoblastoid to promyelocytic leukemia after treatment with dCF (33) and of the reported conversion from non-T-cell ALL to myelomonocytic leukemia during treatment with vincristine, L-asparaginase, prednisone, and intrathecal methotrexate (34). Postconversion cytogenetic analysis was not performed in the former case; the karyotype was normal in the latter. Nevertheless, in each case the rapidity of the transition to the second phenotype also led to the suggestion that these leukemias arose from stem cells capable of both lymphoid and myeloid differentiation. In each case, phenotypic conversion was attributed solely to a differential sensitivity of lymphoid and myeloid cells to treatment, since a decline of >90% in total leukemia cell count preceded the appearance of the second, myeloid, cell type. In the present case, such a decline did not occur. For this and other reasons discussed below, we wish to consider not only the role of selective toxicity but also the possibility that treatment may have had a direct effect on the differentiation of the leukemia cells.

The sensitivity of leukemia T cells to dAdo toxicity is dependent primarily on their ability to accumulate dATP (2, 4, 17, 18, 35). Thus, a selective cytotoxic effect is most readily explained in terms of the different capacities of the patient's T-lymphoblastic and promyelocytic leukemia cells to accumulate dATP (Table 2). Although a subpopulation of dAdo-resistant myeloid cells could have undergone selective proliferation during dCF treatment, this alone would have resulted in a mixed population of cell types. The complete conversion that was observed would also have required a lethal effect on T lymphoblasts. However, two kinds of observations argue against such an effect: (i) Sandberg et al. found that untreated ALL patients excreted about 5 times as much uric acid in the urine as did normal controls (36). The mean for 14 patients was 30.2 mg of uric acid per kg per 24 hr (range 11.9–74.8), with a good correlation between amount excreted and increasing leukocyte count. During cytolytic therapy, excretion rose to over 100 mg/kg per 24 hr. Total oxygen use (hypoxanthine + xanthine + uric acid) excretion by our patient rose from 11.7 mg of uric acid equivalents per kg per 24 hr on day 1 of dCF treatment to a peak of 29.2 on day 4, during which his leukocyte count increased 7-fold.
Institutes of Health Training AM 00434 and CA 00030. Jaclyn Biegel helped study poietic stem cells into lymphocytes. Present observations, we propose:

1. It is possible that overall DNA due to T-lymphoblast RNA of tissues exclusively with AdoHcy.
2. In vivo as a result of a precursor-product relationship of a precursor-product relationship.
3. The cytogenetic availability of tissues exclusively with myeloid leukemia cells after their first appearance in the circulation. Finally (v), the direct differentiation hypothesis predicts that even though postconversion myeloid cells had little capacity for dATP expansion in vitro, dATP should have accumulated in these cells in vivo as a result of their evolution from ADA-inhibited T lymphoblasts. It may have been catalepsis of this expanded dATP pool that produced the free dAdo found in circulating, predominantly myeloid, leukemia cells after the end of dCIF infusion (Fig. 4A).

In view of the postulated role of DNA hypomethylation in controlling differentiation (37–40), it is appropriate to consider the possibility that phenotypic conversion was initiated by an inhibition of DNA methylation by AdoHcy. However, although a fall in the AdoMet-to-AdoHcy ratio to 4–5:1 is sufficient to cause inhibition of methylation of pyrimidine residues in T-lymphoblast RNA during dCIF treatment (4), in vitro studies of ADA-inhibited lymphoid cells indicate that a fall in this ratio to <3:1 is necessary to appreciably inhibit overall DNA methylation (2). In fact, we found that 3.6% of dCyd residues were methylated in enzynatic digests (41) of DNA from the patient’s leukemia cells obtained just prior to dCIF treatment, and also on day 4, when treatment ended. It is possible that methylation of regulatory sequences near genes that control differentiation may be particularly sensitive to inhibition by AdoHcy. On the other hand, an effect on differentiation could result from inhibition of some other type of methylation (RNA, protein) that may influence gene expression, from some as-yet-recognized effect of dATP pool expansion, or from an effect of Ado on cyclic AMP levels.

The selective absence of lymphocytes in genetic ADA deficiency has been attributed solely to the cytotoxicity of Ado and dAdo to mature lymphocytes (2). On the basis of our present observations, we propose that lymphopenia may also be partly the result of metabolic effects of ADA deficiency that disrupt or redirect the differentiation of hematopoietic stem cells into lymphocytes. The leukemia cells derived from the present patient may serve as a useful model for in vitro studies relating to this hypothesis and for the study of factors that regulate human hematopoietic stem cell growth and differentiation.

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