Adenosine Deaminase and Ecto-5'-Nucleotidase Activities in Various Leukemias With Special Reference to Blast Crisis: Significance of Ecto-5'-Nucleotidase in Lymphoid Blast Crisis of Chronic Myeloid Leukemia

By Mitsuhiko Koya, Tadashi Kanoh, Hiroyoshi Sawada, Haruto Uchino, and Kunihiro Ueda

Adenosine deaminase (ADA) and ecto-5'-nucleotidase (5'-N) activities were examined in peripheral leukocytes from patients with leukemias, including nine patients with chronic myeloid leukemia (CML) in blast crisis. Four of nine cases of CML in blast crisis were myeloid and the remaining lymphoid morphologically. The diagnosis of CML in lymphoid blast crisis was further contributed by the measurement of terminal deoxynucleotidyl transferase (TdT) activity. In all four cases of lymphoid blast crisis and one of myeloid blast crisis, leukemia cells had high 5'-N activity, while there was a little or no detectable activity in those from four cases of myeloid blast crisis and all of CML.

BLAST TRANSFORMATION commonly occurs in the terminal phase of chronic myeloid leukemia (CML). It usually presents with myeloid blast crisis, but sometimes transforms into acute leukemia resembling acute lymphocytic leukemia (ALL). Leukemia cells in lymphoid blast crisis are morphologically indistinguishable from ALL cells. These cell populations show elevated levels of terminal deoxynucleotidyl transferase (TdT) activity, and clearly express the immunologic characteristics of non-T, non-B ALL cells. TdT, therefore, has been considered to be a biochemical marker for CML in lymphoid blast crisis. No enzyme other than TdT, however, has as yet been known to classify CML in blast crisis.

Since the recognition of the association of purine enzyme abnormalities with specific immune dysfunction, purine metabolism has recently been the subject of renewed interest. Adenosine deaminase (EC 3.5.4.4: ADA) and ecto-5'-nucleotidase (EC 3.1.5.5: 5'-N) in the purine salvage pathway are examples of such enzymes that have been extensively investigated. In addition to the abundance in lymphoid tissues, the levels of these enzymes might reflect the lineage of specific lymphocyte populations. Recent series of studies have indicated that the levels of these enzymes correlate with the conventional immunologic markers. Thus, the evaluation of ADA and 5'-N seems to be of diagnostic help in the characterization of leukemic lymphocytes of unknown origin.

In the present study we assayed ADA and 5'-N activities in leukemia cells from patients with ALL, acute myeloid leukemia (AML), and CML in chronic or acute phase. The first aim of this study is to investigate the possible value of 5'-N as a diagnostic tool for CML in lymphoid blast crisis, since this enzyme activity has not yet been described in blast crisis of CML. The second aim is to analyze the possible relationship between ALL and CML in lymphoid blast crisis in terms of enzyme activities. Our results indicate that 5'-N may be useful as a new biochemical marker for lymphoid blast crisis of CML and provide additional evidence to support the idea that some CML and some ALL originate in a common target cell.

MATERIALS AND METHODS

Patients

Peripheral leukemia cells were studied in a total of 26 patients with various leukemias. Blood was obtained from the patients at presentation or in relapse. None of the patients with CML received cytotoxic drugs at the time of study. The patients studied were as follows: three patients with non-T, non-B ALL, eight with AML, six with CML in chronic phase, and nine with CML in blast crisis. The diagnosis was made at the referring hospitals and based on clinical findings and on conventional morphological, cytochemical, and immunologic studies. The diagnosis of CML in lymphoid blast crisis was further confirmed by the activity of TdT, which was measured according to the method of McCaffrey et al. Controls were provided by blood from healthy volunteers and from patients with no detectable hematologic abnormalities.

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0006-4971/81/5806-0007$0.00/0

Blood, Vol. 58, No. 6 (December), 1981

1107
Preparation of Mononuclear Cells

Peripheral blood was drawn into a heparinized syringe. Erythrocytes were separated from leukocytes by dextran (6%) sedimentation. The leukocyte fraction was then layered onto Ficoll-Hypaque, followed by centrifugation and separation of mononuclear cells from polymorphonuclear leukocytes (PMNs).11 Purified cells were washed three times with buffered saline, and finally suspended in the same solution at a density of 5 \times 10^6 cells/ml.

Enzyme Assays

All enzyme activities were assayed by radioactive tracer methods and in duplicate.

5'-N activity was assayed with intact cells dispersed at a density of 5 \times 10^6 cells/ml. The reaction mixture contained 100 mM Tris-HCl at pH 8.0, 5 mM MgCl2, and 200 mM (8-\textsuperscript{14}C)-5'-AMP (specific activity 10 mCi/mole, Amersham/Searle) in a final volume of 100 \mu{l}. After 30 min at 37\textdegree C, the reaction was terminated by the addition of ice-cold 10% trichloroacetic acid. The supernatant solution obtained by centrifugation was neutralized with NaOH and put on a column of Dowex 1 (formate form, x2, 200-400 mesh)18 Radioactive adenosine was eluted from the column with 0.05 M formic acid. Radioactivity in an aliquot of the eluate was determined in a Triton-toluene scintillator. One unit of enzyme activity is equal to 1 nmole of adenosine produced per hour. Specific activity is expressed as U/10^6 cells.

ADA activity was assayed with freeze-thawed cells originally dispersed at a density of 5 \times 10^6 cells/ml.19 The reaction mixture contained 50 mM potassium phosphate at pH 7.4, and 100 mM (8-\textsuperscript{14}C)-adenosine (specific activity 20 mCi/mole, Amersham/Searle) in a final volume of 50 \mu{l}. After 20 min at 37\textdegree C, the reaction was stopped by the addition of ice-cold ethanol. Products and substrate were separated by Whatman DE-81 ion-exchange paper chromatography in 1.0 mM ammonium formate. One unit of enzyme activity is equal to 1 nmole of inosine produced per minute. Specific activity is expressed as U/10^6 cells.

Check of Specificity of 5'-N Assay

Whether and how much nonspecific alkaline phosphatase contributed to the 5'-N activity assayed as above was estimated by the effects of L-tetramisole and \beta-glycerophosphate. In contrast, \alpha,\beta-methylene adenosine diphosphoric acid, a specific inhibitor of 5'-N, inhibited the 5'-N activity by 96%.

The 5'-N activities in normal lymphocytes, normal considered to be myeloid and the remaining four to be lymphoid. Leukemia cells from the latter four cases exhibited a high TdT activity, while those from one case of the former (case 19) showed a low but significant TdT activity.

The 5'-N activity in leukemia cells was not affected by inhibitors of nonspecific alkaline phosphatase, such as L-tetramisole and \beta-glycerophosphate. In contrast, \alpha,\beta-methylene adenosine diphosphoric acid, a specific inhibitor of 5'-N, inhibited the 5'-N activity by 96%.

Table 1. Hematologic Findings of Patients With Leukemias

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>WBC (1/cu mm)</th>
<th>Blast (%)</th>
<th>Peroxidase</th>
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<td>Acute myeloid leukemia</td>
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<td></td>
<td></td>
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<td>17</td>
<td>F</td>
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<tr>
<td>3</td>
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<td>M</td>
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<td>80</td>
<td>(+)</td>
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<tr>
<td>4</td>
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<td>89</td>
<td>(+)</td>
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<tr>
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<td>8</td>
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<td>*Case 6 was classified as type M2 according to the FAB system.</td>
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Table 2. Hematologic Findings at Blast Crisis of Chronic Myeloid Leukemias

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>WBC (1/cu mm)</th>
<th>Blast (%)</th>
<th>Peroxidase</th>
<th>TdT†</th>
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<td>5,600</td>
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<tr>
<td>22</td>
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<td>96,000</td>
<td>56</td>
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<td></td>
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<tr>
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<td>F</td>
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<td>26</td>
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<td>F</td>
<td>43,100</td>
<td>42</td>
<td>(-)</td>
<td>2.6</td>
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*ND, not determined.
†TdT denotes terminal deoxynucleotidyl transferase. One unit (U) was defined as the enzyme activity that catalyzed the incorporation of 1 nmole of labeled dNTP into acid-insoluble material per hour. Specific activity is expressed as U/10^6 cells.
‡Case 21 and case 12 in Table 1 are of the same patient.
PMNs, and various leukemia cells are summarized in Fig. 1. A very low or negligible activity was detected in normal PMNs, leukemia cells from seven patients with peroxidase-positive AML, and all CML patients in chronic phase. In striking contrast, high 5'-N activities were noted in normal lymphocytes and ALL cells. The mean values for these two kinds of cells were 14.7 ± 6.9 and 27.2 ± 4.5 U/10⁶ cells, respectively. Thus, the level of 5'-N activity in ALL cells was significantly higher than that in normal lymphocytes (p < 0.05). It was also noted that the peroxidase-negative leukemia cells obtained from one case of AML (case 2) had a much higher 5'-N activity than those in peroxidase-positive counterparts.

Of the nine cases of CML in acute phase, all four patients in lymphoid blast crisis and one in myeloid blast crisis (case 19) had leukemia cells with a markedly increased 5'-N activity, while others had leukemia cells with very low or no detectable activity. The mean activities for these two groups of patients were 24.2 ± 10.6 and 1.7 ± 0.8 U/10⁶ cells, respectively. The difference was statistically significant (p < 0.01). It was of interest that the leukemia cells in one case of CML in myeloid blast crisis (case 19) had low TdT, high 5'-N, and no detectable peroxidase activities.

This case was considered to be biochemically analogous with that case of AML (case 2) with high 5'-N and no detectable peroxidase activities.

The ADA activity in leukocytes is shown in Fig. 2. This enzyme activity was found to be higher in leukemia cells from 8 patients with AML and all 3 with ALL. The mean values for the patients with AML and ALL were 281.7 ± 88.4 and 338.7 ± 130.6 U/10⁶ cells, respectively. These values were significantly higher than those of normal lymphocytes (97.8 ± 30.3) (p < 0.01), but the activities observed were so widely distributed that no clear distinction was made between AML and ALL (p > 0.05). The AML cells with partial maturation (case 6) had a similar ADA activity to those of CML cells.

Leukemia cells from all patients with CML in chronic phase had ADA activities much lower than AML cells, but similar to normal PMNs. In acute phase, however, ADA activity was significantly increased in 7 of 9 cases as compared to those in chronic phase. These 7 patients included all 5 patients with CML in myeloid blast crisis and 2 of 4 with CML in lymphoid blast crisis. The mean activity of ADA in these 7 patients was 198.3 ± 32.5 U/10⁶ cells, which...
differed significantly from the value for typical AML, 281.7 ± 88.4 U/10^6 cells. In 2 cases of CML in lymphoid blast crisis, ADA activity remained low in spite of blast transformation.

**DISCUSSION**

It has been well known that CML sometimes progresses into acute leukemia resembling ALL. This notion has been suggested by the similar morphological appearance of the cells and the unexpected responses to therapy for ALL, and was recently confirmed by detection of TdT and cell surface markers. Janossy et al. reported an immunologic phenotype of lymphoid blast crisis cells and its identity with that of ALL cells. Now it seems to be likely that some CML and some ALL cells originate in a common target cell and that CML is a clonal disease of a pluripotent stem cell.

TdT has been found abundant in ALL cells and used as a diagnostic biochemical marker of lymphoid blast crisis of CML. However, no enzyme other than TdT has ever been known to be of diagnostic significance in lymphoid blast crisis of CML. Our observation that all cases of CML in lymphoid blast crisis were associated with increased levels of 5'-N activity seems to qualify 5'-N as a new biochemical marker for CML in lymphoid blast crisis. 5'-N activity has never been investigated in CML in blast crisis, and this is the first report on the diagnostic and biologic significance of 5'-N in lymphoid blast crisis of CML.

5'-N is an ectoenzyme located on the plasma membrane. The function of this enzyme in vivo is uncertain, but cells such as lymphoblasts that do not synthesize purines de novo actively transport nucleosides, which are breakdown products of 5'-nucleotides by 5'-N. This enzyme activity was found to be higher in B cells than in T cells and to correlate with cell maturity. 5'-N activity has been reported to be very low in mature neutrophils, CML cells, T-ALL cells, and most chronic lymphocytic leukemia (CLL) cells, whereas it is high in non-T, non-B ALL cells and normal peripheral mononuclear cells. These and our data clearly indicate that high levels of 5'-N are detected in non-T, non-B ALL cells and a minority of CLL cells among various leukemia cells.

ADA is also an enzyme involved in the purine salvage pathway. Its absence is usually associated with severe combined immunodeficiency. This enzyme is more abundant in thymus and T cells than in B cells. Many investigators have reported the activity of this enzyme in various leukemia cells. High levels of ADA were found in non-T, non-B ALL cells, T-ALL cells, and AML cells, and low levels in CLL cells. We confirmed high levels of ADA in non-T, non-B ALL cells and AML cells. Smyth et al. postulated that the measurement of ADA might be useful for diagnostic differentiation of undifferentiated leukemias. Our results and those of Meier et al. have shown a similar distribution of ADA activity in AML and ALL cells, and thus no clear distinction is possible between these two on the basis of ADA activity alone.

Smyth et al. noted the elevation of ADA activity during blast transformation of CML. We confirmed their observation in seven of nine patients with CML in blast crisis. Our observation did show a range of ADA activity in these blast crisis cells similar to those in AML and ALL cells. This elevation of ADA activity can be explained by the increase in the percentage of myeloblasts. However, the elevation of 5'-N activity in lymphoid blast crisis of CML does not appear to be explicable with the change in the number of myeloblasts, because the latter cells have low 5'-N activity. This elevation might be reasonably explained by the appearance of different cell populations from preexisting ones. In two of four patients with CML in lymphoid blast crisis, high 5'-N and ADA activities were detected in addition to the presence of TdT. It seems plausible that the enzymatic phenotype of non-T, non-B ALL cells is high levels of ADA, 5'-N, and TdT activities. Thus, lymphoblast cells in two of four patients with CML in lymphoid blast crisis appear to be non-T, non-B ALL cells as judged by enzyme activities. This seems to provide further support to the view that some lymphoblasts in lymphoid blast crisis of CML are of null cell origin.

In one AML and one CML case in myeloid blast crisis, high 5'-N and ADA activities were detected in blast cells with myeloid morphology but negative peroxidase staining. It is uncertain whether these myeloblasts with negative peroxidase staining and high ADA and 5'-N activities are biochemically closer to lymphoid cells. Further studies will be needed to answer this question.

**ACKNOWLEDGMENT**

We are grateful to Dr. S. Nakamura, Department of Geriatrics, Kyoto University, for his technical advice and also thank many physicians for studying their patients.

**REFERENCES**

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Adenosine deaminase and ecto-5'-nucleotidase activities in various leukemias with special reference to blast crisis: significance of ecto-5'-nucleotidase in lymphoid blast crisis of chronic myeloid leukemia

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