

Adenosine Deaminase and Terminal Deoxynucleotidyl Transferase: Biochemical Markers in the Management of Chronic Myelogenous Leukemia¹

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ABSTRACT

Serial determinations of adenosine deaminase (ADA) activity in 69 patients with chronic myelogenous leukemia provided a biochemical marker of disease activity. Eighty-nine % of patients in the accelerated phase had an elevation of ADA activity. This elevation was not a direct reflection of an increased absolute blast count. Furthermore, five of seven patients studied serially from the stable phase into the accelerated phase had an increase in ADA activity before the absolute blast count increased. This is the first investigation which clearly demonstrates the potential value of measuring serial ADA activities in a large number of patients with chronic myelogenous leukemia.

INTRODUCTION

CML³ is a clonal neoplasm of the bone marrow pluripotent stem cell (10). The mean survival for patients with this disease treated with standard chemotherapy is 41 months (11). Despite all current therapeutic maneuvers, approximately 70% of these patients will expire after entering an accelerated phase which resembles acute leukemia. A majority of the patients develop an aggressive myeloblastic transformation, which is refractory to standard chemotherapy and is associated with a mean survival of 2.5 months.

About 30% of the patients entering the accelerated phase of CML demonstrate immature cells characteristic of lymphoblasts (3, 10). This subset can be identified by detection of TdT activity in either the peripheral blood or bone marrow. The presence of this enzyme, which is a specific biochemical marker of lymphoblasts, is associated with a 50 to 60% response rate to chemotherapeutic agents which are normally utilized in the treatment of lymphoblastic leukemia (1, 13). Although the best medical regimen for induction and maintenance of remission in this subgroup of patients is not yet known, the median survival of the responders is reported to be 6.5 months (11).

ADA (adenosine aminohydrolase, EC 3.5.4.4, an important enzyme in purine nucleoside catabolism, is elevated in the peripheral blood of patients with acute leukemia (14, 18). Smyth and Harrap (18) investigated ADA activity in a small group of patients with CML and demonstrated that ADA activity

is elevated in the peripheral blood mononuclear cells during the accelerated phase of the disease. We have reported previously (8) that ADA activity is elevated in the peripheral blood buffy coat of patients with accelerated disease and suggested that serial determinations of this enzyme might provide a new biochemical marker of disease acceleration in CML.

We now report the results of an extensive investigation of both ADA and TdT activities in 69 patients with CML conducted over the past 3.5 years. Our objective was to determine if ADA activity could be utilized as a biochemical marker of disease activity and to determine whether TdT-positive patients differed from TdT-negative patients with regard to ADA levels.

MATERIALS AND METHODS

Chemicals. Radioactive nucleosides and nucleotides were obtained from both New England Nuclear (Boston, Mass.) and Amersham Corp. (Arlington Heights, Ill.). Calf intestinal ADA was obtained from Sigma Chemical Co. (St. Louis, Mo.).

Enzyme Assay. Sequential determinations of the ADA activity of the peripheral blood buffy coat preparations were obtained by utilizing an assay that measured the conversion of [8-¹⁴C]adenosine to inosine (9). The buffy coat preparation was obtained from heparinized peripheral blood separated by a dextran sedimentation technique. The cells were washed twice in Seligmann's balanced salt solution with 1% EDTA, resuspended in 5 mM Tris-HCl (pH 7.4)-0.25 M sucrose, and then enumerated. After sonication, the homogenate was centrifuged at 50,000 × g at 4° for 30 min. The resulting supernatant was utilized as the source of the enzyme in each assay, which was run in triplicate.

The enzyme assay was carried out in a total volume of 70 μl containing 5 mM Tris-HCl (pH 7.4) and 5 mM [8-¹⁴C]adenosine at 37°. After 10 min, the assay was terminated by adding 20 μl of 4 M formic acid and placing the sample in an ice bath. A 5-μl aliquot was placed on a cellulose acetate thin-layer chromatography plate (Analabs, Inc., New Haven, Conn.) and developed in water for 45 min. One unit of ADA activity is defined as 1 × 10⁻² μmol inosine formed per hr, and specific activity is expressed as units of activity per 10⁶ cells.

Two types of TdT assays were utilized. The quantitative assay of human TdT activity has been described previously (5, 6). The components of the assay in a total volume of 125 μl were: 0.2 M potassium cacodylate (pH 7.5); 1 mM 2-mercaptoethanol; 0.01 mM p(dA)₅₀; and 1 mM [³H]dGTP (100 cpm/pmol) with 8 mM MgCl₂. The reactions were incubated at 35° for varying time periods and terminated by application of 25-μl aliquots onto GF/C glass fiber papers as described (2). One unit of enzyme activity is defined as 1 nmol of dGTP incorporated per hr, and specific activity is expressed as units of activity per mg of protein. The TdT immunofluorescent assay (3) was performed on peripheral blood or bone marrow aspirates utilizing a commercial rabbit anti-calf TdT (Bethesda Research Laboratories) or rabbit anti-human TdT (4).

Methods of Clinical Study. Sixty-nine individuals with documented CML were identified in the patient population of the Ohio State University Hospital and followed for variable periods of time over the duration

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³ The abbreviations used are: CML, chronic myelogenous leukemia; TdT, terminal deoxynucleotidyl transferase; ADA, adenosine deaminase.

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of the 3.5-year study. The diagnosis of CML was established utilizing the standard criteria including cytogenetic analysis of the bone marrow, leukocyte alkaline phosphatase, review of the bone marrow aspirate, and peripheral blood smear. ADA activity was determined on the peripheral blood buffy coat preparation of the individual stable patients during their routine visits to the outpatient clinic. A simultaneous absolute blast count was determined after reviewing the peripheral blood smear on the same blood specimen utilized to perform the ADA assay. A total of 39 patients were examined during the stable phase of their disease. A thorough review of the clinical presentation and course was completed on each of these patients. Serial determinations were obtained on most of these patients. Seven of the 39 patients were followed from the stable phase into the accelerated phase of their disease.

Clinical recognition of the exact onset of the accelerated phase of the disease is often difficult (10). Although the identification of onset of the accelerated phase is not precise because the rate of onset is often variable, certain criteria were established for the purpose of this study. Demonstration of greater than 20% blasts in the peripheral blood or greater than 25% blasts in the bone marrow aspirate was considered diagnostic of an accelerated phase. A total of 38 patients was studied during the accelerated phase of this disease. As indicated previously, 7 of these patients were followed from the stable phase of the disease into the accelerated phase. The other 31 patients were initially examined by us after a diagnosis of acceleration had already been confirmed.

A total of 30 healthy volunteers were studied to determine the normal ADA activity in the peripheral blood buffy coat. As infection is often a frequent complication of either the underlying leukemia or its treatment, 11 patients without cancer with either documented bacterial or viral infections within the intensive care unit were examined to determine what effect infection might have on the peripheral blood buffy coat ADA.

TdT assays were performed on 33 of the 38 patients. Multiple quantitative TdT assays were performed on 29 of the 33 patients, and immunofluorescence assays were performed on the other 4.

Statistical Analysis. Data were primarily analyzed using the Wilcoxon signed-rank test (15). The coefficient of correlation between the number of immature cells in the peripheral blood and the ADA activity was also determined (15).

RESULTS

Age, chromosome analysis, and ADA values of the patients are presented in Table 1. The mean ADA activity of the initial enzyme determination of each of the 39 patients with stable CML was not statistically different from either the normal control group or those patients with infection. Ninety serial ADA determinations in 11 stable patients were remarkably consistent (Table 2). S.D.s in the individual patients were small.

The ADA determinations in the peripheral blood buffy coat of the 38 patients in the accelerated phase of CML were statistically higher than those of both the normal controls and patients with stable CML ($p < 0.01$) (Table 1). Five of the 7 patients who were serially studied during a time of transition from the stable phase into the accelerated phase of the disease had an increase in their enzyme activity before clinically apparent acceleration of disease. An increase in the ADA activity in the other 2 patients occurred simultaneously with the recognition of their clinical deterioration.

The data in Chart 1 demonstrate the initial enzyme determinations on each of the patients in the 4 populations studied. Patients in the stable phase of the disease are indistinguishable from patients in the normal control group and from patients with infection. Twenty-eight of 38 (74%) of patients in the

Table 1
Characteristics of patient populations

Diagnosis	No. of patients	Age (yr)	% of patients Philadelphia chromosome positive	ADA units ^a
Stable CML	39	47.7 ± 17.0 ^b	86	7.1 ± 2.2
CML accelerated phase	38	51.0 ± 15.0	83	18.8 ± 10.1
Normal volunteers	30	28.4 ± 6.6		6.6 ± 2.1
Nonmalignant infection ^c	11	57.2 ± 22.2		7.8 ± 2.0

^a One unit of ADA activity is defined as 1×10^{-2} μ mol inosine formed per hr, and specific activity is expressed as units of activity per 10^6 cells.

^b Mean ± S.D.

^c Patients with documented viral or bacterial infection but no underlying cancer.

Table 2
Serial ADA determinations in individual patients with stable CML

Patient	(n) ^a	ADA units ^b
C. D.	7	6.0 ± 2.9 ^c
C. E.	9	9.3 ± 2.0
R. F.	5	7.1 ± 3.6
W. K.	6	9.7 ± 1.5
S. L.	10	9.0 ± 1.7
R. M.	8	4.6 ± 0.8
L. M.	12	8.5 ± 2.5
R. R.	4	6.2 ± 1.1
Y. R.	12	7.3 ± 1.9
G. S.	12	6.5 ± 0.7
C. W.	5	6.8 ± 1.2

^a Number of serial determinations made on an individual patient.

^b Ninety serial ADA determinations were done on 11 stable patients during monthly follow-up visits in the outpatient clinic. ADA activity is expressed as units of enzyme activity. One unit of ADA activity is defined as 1×10^{-2} μ mol inosine formed per hr, and specific activity is expressed as units of activity per 10^6 cells. S.D.s in the individual patients were small.

^c Mean ± S.D.

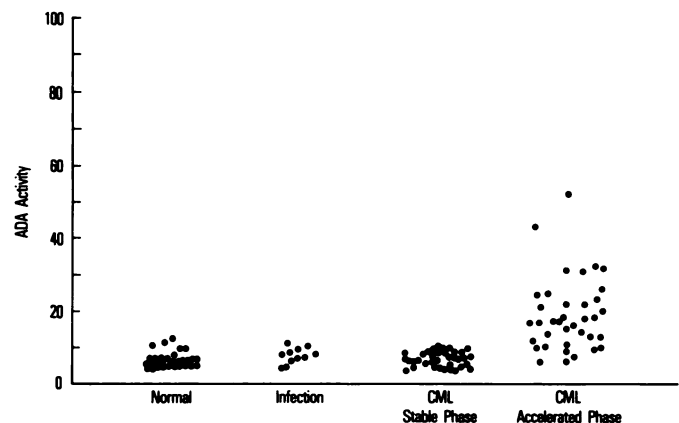


Chart 1. Initial ADA activity in buffy coat preparations of individual patients. Points, single determination of ADA activity. One unit of ADA activity is defined as 1×10^{-2} μ mol inosine formed per hr, and specific activity is expressed as units of activity per 10^6 cells.

accelerated phase had an ADA value which is greater than 2 S.D.s above the mean of the stable population. Six of the 10 patients whose initial ADA values were indistinguishable from patients with stable disease had an increase of the ADA to high levels shortly after diagnosis of the accelerated phase. Therefore, 89% of patients with an established acceleration of the disease ultimately had an elevated ADA value in their buffy coat. The group of patients who failed to demonstrate an elevation of peripheral blood ADA prior to death was small and

Table 3
ADA activity and peripheral blood characteristics of patients with CML

Clinical state	No. of patients	WBC ^a	Absolute blast count ^b	ADA units ^c
Stable untreated	17	169,276 ± 187,021 ^d	2,812 ± 4,398	7.0 ± 2.1
Stable treated	22	50,650 ± 43,444	298 ± 775	7.7 ± 2.4
Accelerated phase	38	81,061 ± 87,571	31,171 ± 61,202	18.8 ± 10.1

^a Peripheral WBC count corresponding to the simultaneously measured peripheral blood buffy coat ADA.

^b Observed in the peripheral blood of the samples utilized to determine the ADA activity.

^c ADA activity expressed as units of enzyme activity. One unit of ADA activity is defined as 1×10^{-2} μmol inosine formed per hr, and specific activity is expressed as units of activity per 10^6 cells. This mean is calculated by utilizing the first ADA determination obtained on each patient from the 3 clinical categories of CML.

^d Mean ± S.D.

had no distinguishing clinical characteristics.

The level of ADA activity in the peripheral blood was not a simple reflection of the absolute number of cells present, since enzyme activity is reported per 10^6 cells. Table 3 demonstrates that patients with markedly elevated WBC counts in the untreated state had ADA values that are indistinguishable from those patients who had been receiving chemotherapy for a variable period of time. Although patients with the accelerated phase of CML had by definition an increase in circulating immature cells, the correlation between the absolute blast count and ADA values was very poor ($r = 0.12$). Likewise, the correlation between absolute immature cell count (blasts and promyelocytes) and ADA was poor ($r = 0.18$).

Chart 2A demonstrates the serial ADA determinations of an individual patient followed over a 3-year period. In September 1981, ADA activity in the peripheral blood buffy coat of this patient was greater than 2 S.D.s above the mean of the stable population. No circulating blasts were observed at that time. The patient was asymptomatic and was clinically stable. Serial determinations of the enzyme activity demonstrated a progressive increase in the ADA values, which were eventually followed by an increasing number of circulating blasts. By the middle of December 1981, the patient was clinically in the accelerated phase of his disease.

Serial ADA determinations are depicted in Chart 2B for an additional patient whose CML entered the accelerated phase. Beginning in May 1980, the spleen progressively increased in size. A slight increase in the number of circulating blasts occurred. The patient's medication was then changed from busulfan to hydroxyurea. Clinically, the patient continued well until May 1981, at which time a bone marrow biopsy confirmed the onset of the accelerated phase of her disease. Over a 4-month period before the clinically evident development of the accelerated phase of her disease, 8 serial ADA values were greater than 2 S.D.s above the mean of the stable population. This is in contrast to previous single determinations which were elevated but always returned to the normal range. A similar pattern was observed in 3 additional patients whose serial ADA values increased before the acceleration of the CML was clinically appreciated.

TdT activity was determined on 33 of 39 patients in the accelerated phase of the disease (Table 4). The median duration of stable disease before the patients eventually entered the accelerated phase was 20 months. The median survival of all patients who entered the accelerated phase of the disease was 4.3 months. The median survival of the 15 patients who were TdT positive was significantly longer than the survival of those patients who were TdT negative ($p < 0.01$).

While 35% of all patients in the accelerated phase demon-

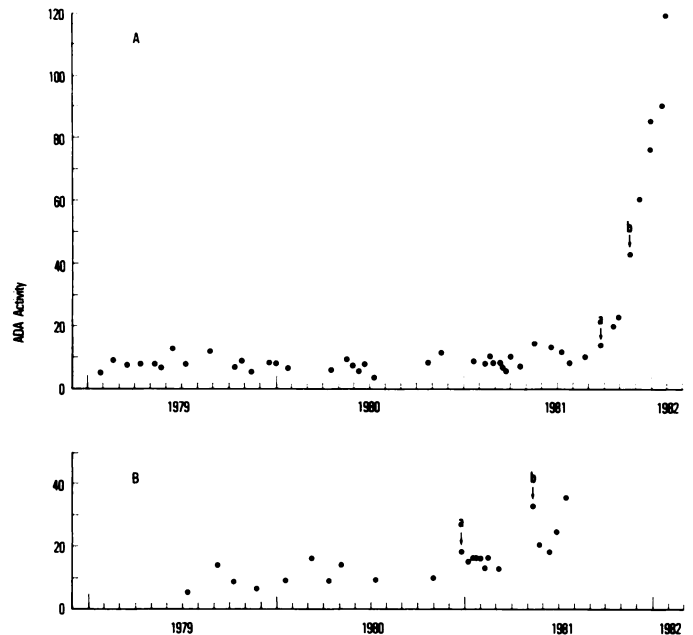


Chart 2. A, serial buffy coat ADA activities on an individual patient followed for an extended period of time. One unit of ADA activity is defined as 1×10^{-2} μmol inosine formed per hr, and specific activity is expressed as units of activity per 10^6 cells. This point (a) represents an ADA greater than 2 S.D.s above the mean of the stable population which is followed by a progressive increase in ADA activity in subsequent samples. The clinical diagnosis of blastic transformation (b) was confirmed in December 1981. B, serial buffy coat ADA activities on an individual patient followed for an extended period of time. One unit of ADA activity is defined as 1×10^{-2} μmol inosine formed per hr, and specific activity is expressed as units of activity per 10^6 cells. This point (a) represents an ADA greater than 2 S.D.s above the mean of the stable population which is followed by serial ADA values that never return to the stable range. The clinical diagnosis of blastic transformation (b) was confirmed in May 1981.

Table 4
Duration of survival after onset of the accelerated phase of CML

Clinical category	No. of patients	Median survival (mos.) ^a
All patients	38	4.3
TdT negative ^b	18	3.0
TdT positive ^b	15	8.0

^a Median duration of survival expressed in months after the accelerated phase of CML was confirmed utilizing criteria described in "Materials and Methods."

^b TdT activity was determined at the time of onset of the accelerated phase utilizing techniques described in "Materials and Methods." Note that 5 patients did not have TdT determinations done.

strated positive TdT activity in either the peripheral blood or bone marrow, 7 of the 12 patients with CML (58%) presenting in the accelerated phase were TdT positive. Correlation between the level of TdT activity and the simultaneously measured ADA activity in the entire group of patients in the accelerated

phase of CML was poor ($r = 0.2$). Three patients who initially had positive TdT activity detected in the peripheral blood at the onset of the accelerated phase had prolonged survival (8, 14, and 15 months, respectively), but they were documented to have negative TdT activity at the time when the disease finally became unresponsive to therapy.

DISCUSSION

CML has a variable clinical course. The mean duration of survival of all patients in this study after diagnosis was 37.7 ± 44.4 months. This wide range in survival is consistent with previous observations (11). The median duration of survival was 18.2 months, emphasizing the actual poor prognosis of this disease. Chemotherapy readily produces a hematological improvement in these patients but appears ineffective in significantly delaying the onset of the accelerated phase (10, 11).

Treatment of the accelerated phase of the disease has improved with the identification of a subgroup of patients who show evidence of lymphoblastic transformation identified by TdT positivity and lymphoblastic morphology (1, 2, 4-6, 8, 9, 13-16, 18). This group of patients responds well to vincristine and prednisone (13).

The majority of patients entering the accelerated phase, however, have a short survival (10, 11). Previous attempts at improving survival including splenic irradiation or splenectomy have not provided significant benefit. Bone marrow transplantation during the chronic phase is being explored in an effort to improve the long-term survival (7). Since survival with CML is so variable, a biochemical parameter that would predict onset of the accelerated phase may identify a subset of patients who have a predictably poor prognosis and who might benefit from an attempt at innovative or aggressive therapy.

In 1975, Smyth and Harrap suggested that serial measurements of ADA activity might predict the onset of the accelerated phase of CML. A recent study by Koya *et al.* (12) demonstrated in a small number of patients that ADA activity was elevated in patients in the blast crisis compared to patients with stable disease. The data that we now present on a much larger number of patients in the accelerated phase of disease confirm our earlier observations and support the contention that serial determinations may indeed predict the onset of the terminal phase. While we have demonstrated that an elevation of this enzyme is not a mere reflection of the absolute blast count, the increase may indicate the emergence of a subpopulation of immature cells characteristic of this terminal phase.

The number of patients in our study that were positive for TdT activity is consistent with that reported previously (10, 11). Our data confirm the observation that patients in the accelerated phase with detectable TdT activity have prolonged survival compared to those patients who are TdT negative. Loss of detectable TdT activity in patients with persistence of the accelerated phase may indicate the emergence of cell lines resistant to chemotherapy (17). Although serial determinations of TdT activity in a group of patients with stable disease would not detect the onset of clinical deterioration in approximately 70% of the patients, serial determination of this enzyme in patients who have entered the accelerated phase may be important in terms of management.

In conclusion, serial determination of ADA activity in the peripheral blood buffy coat of patients with CML may provide

a biochemical marker of disease activity. Once the accelerated phase of the disease has developed, a determination of TdT activity assists in the selection of a therapeutic regimen. Serial determinations of TdT in conjunction with ADA activity might allow the prediction of the emergence of a resistant leukemic cell line. Careful biochemical monitoring of both of these enzymes may improve the management of patients with this disease.

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