Myeloblastic and Lymphoblastic Markers in Acute Undifferentiated Leukemia and Chronic Myelogenous Leukemia in Blast Crisis

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ABSTRACT

Blast cells were obtained from 17 patients with acute undifferentiated leukemia and 13 patients with chronic myelogenous leukemia in blast crisis. The blasts were tested with anti-i serum in cytotoxicity tests and with antisera to myeloblastic leukemia-associated antigens in immunofluorescence tests. The terminal deoxynucleotidyl transferase (TDT) content of the blasts was also measured. Lymphoblasts react strongly with anti-i, do not react with anti-myeloblast serum, and have high levels of TDT; myeloblasts react weakly with anti-i, do react with anti-myeloblast serum, and have very low levels of TDT. Of the 17 patients with acute undifferentiated leukemia, there were six with blasts which reacted like lymphoblasts, six with blasts which reacted like myeloblasts, and five with blasts bearing different combinations of these lymphoblastic and myeloblastic markers. Eight of the 11 patients with lymphoblastic or mixed lymphoblastic-myeloblastic markers, but only one of the six with myeloblastic markers, achieved complete or partial remission in response to therapy. Thus, in acute undifferentiated leukemia, classification of blasts by these markers may be of prognostic value.

Of the 13 patients with chronic myelogenous leukemia in blast crisis, the markers were concordant (for myeloblasts) in only two cases. Three of the 13 patients had TDT-positive blasts, but the reactions of these cells with anti-i and with anti-myeloblast serum differed from those seen with lymphoblasts from patients with acute lymphoblastic leukemia. Although the cell involved in "lymphoid" blast crisis of chronic myelogenous leukemia is similar in many respects to that involved in acute lymphoblastic leukemia, these cells are not identical.

INTRODUCTION

Conventional morphological criteria (5) usually enable one to classify acute leukemia as myeloblastic or lymphoblastic. In difficult cases, special cytochemical stains may be helpful but there remains a group, comprising up to 10% of patients with acute leukemia (10), in which morphology and special stains are inadequate to classify the leukemia.

Several markers have been identified which distinguish myeloblastic from lymphoblastic leukemia (2, 7, 23-25), and some of these have been used in an attempt to classify, as myeloblastic or lymphoblastic, AUL (8, 13, 21, 25) and CML in blast crisis (12, 15, 17, 19, 20, 22). With each marker, blasts from patients with AUL or CML in blast crisis react either like myeloblasts or like lymphoblasts, but there is limited information comparing the classification of these blasts by different markers (19, 20).

Several studies suggest that classification of blasts in patients with AUL or CML in blast crisis may be of value in selecting optimal therapy and predicting prognosis (3, 20, 22). However, the accuracy of the various markers in classifying blasts as myeloblastic or lymphoblastic has been established by studies of morphologically typical AML and ALL. Since there are no absolute criteria by which to assess the accuracy of classification as myeloblastic or lymphoblastic of blasts from patients with AUL or CML in blast crisis, it is important to determine whether their classification by different markers agrees. The possibility that such concordance may not always occur, at least in patients with CML in blast crisis, is suggested by the observation that some of these patients may have, at the same time, some cells reacting like myeloblasts and others reacting like lymphoblasts (19).

The present study was done to compare the classification by tests for 3 different markers (i antigen, myeloblastic leukemia-associated antigen, TDT) of blasts from patients with AUL and from patients with CML in blast crisis.

MATERIALS AND METHODS

AUL. Patients with acute leukemia were considered to have AUL when their attending hematologists were unable to classify the leukemia as AML or ALL using conventional morphological criteria (5). All patients were adults except Patients 6 and 17 who were 10 and 12 years old, respectively. The lack of diagnostic morphological features was confirmed by examination of blood and bone marrow films from these patients by a hematological pathologist who was not otherwise involved in this study. Cytochemical stains had been done on specimens from some of these patients, but the results of these studies were not used to include or exclude any patient.

CML in Blast Crisis. Patients with CML were considered to...
be in blast crisis when 30% or more of the leukocytes in their peripheral blood were blasts (9). All patients were adults and were included for study regardless of whether the CML was Philadelphia chromosome positive or negative.

Cells. Blasts from patients with AUL were collected for study before the institution of any chemotherapy. Blasts from patients with CML in blast crisis were also collected before starting chemotherapy for the crisis, but most of these patients had received myleran during the chronic phase of their disease. Blasts from all patients were obtained from the peripheral blood.

Normal lymphocytes were obtained from the blood of healthy adults.

Defibrinated blood was applied to a Hypaque-Ficoll gradient, and the interface layer was harvested (6). With one exception (Patient 17, 54% blasts), the final leukemic cell populations studied contained at least 80% blasts and most contained >95% blasts.

Cytotoxicity Tests with Anti-i. Anti-i Den. was kindly provided by Dr. D. H. Cowan (Sunnybrook Medical Centre). This serum distinguishes lymphoblasts from myeloblasts in that it is cytotoxic to lymphoblasts as to normal lymphocytes (50% kill in dilutions up to approximately 1:1280 to 1:2560) but much less cytotoxic to myeloblasts (50% kill in dilutions up to approximately 1:10 to 1:20) (25). In cytotoxicity tests, anti-i Den. and rabbit complement were added to cells, and the mixtures were incubated at 4° for 30 min and then at 25° for another 30 min (26). Cytotoxicity was assayed by trypan blue exclusion.

Fluorescence Tests with Anti-Myeloblast Serum. Anti-mye-loblast sera were obtained from patients with AML in remission receiving immunotherapy with Bacillus Calmette-Guérin and irradiated allogeneic leukemic cells (1). Cells were tested by incubation with antisera at 4° for 30 min. Suspensions were washed in medium containing 0.01% sodium azide and were incubated at 4° for another 30 min with fluorescein-conjugated goat anti-human IgG. Cells were counted as positive when ringed with bright surface dots using the Ploem vertical illuminator (1).

Sera used in this study showed a mean reactivity with known myeloblasts of 59 ± 16% (S.D.) of cells per sample, whereas reactivity with known lymphoblasts was 4 ± 2%. Based on these previous tests, a minimum of 30% of cells fluorescing is required to identify blasts as myeloblasts.

TDT Assays. The technique for assay of TDT has been described previously (11). Nucleated cell suspensions at a density of 1 to 2 x 10⁹ cells/ml in 0.25 M phosphate buffer, pH 7.5, containing 1 mM mercaptoethanol were sonicated 4 times in 15-sec bursts, with cooling. The sonicate was centrifuged at 100,000 x g for 60 min, and the resulting supernatant was assayed for TDT. Assay mixtures contained, at final concentration, 0.2 M potassium cacodylate buffer (pH 7.5), 1 mM [³H]dGTP (specific activity, 120 cpm/pmol), 0.02 mM oligo(d(pA)₅o), 8 mM MgCl₂, 1 mM mercaptoethanol, and 50 mM phosphate. One unit of TDT equals 1 nmol of nucleotide polymerized per hr at 35°. Specific activities are expressed as units/10⁶ cells.

Using this assay, blasts from patients with ALL contain >20 units/10⁶ cells, whereas blasts from patients with AML contain <9 units/10⁶ cells (15).

RESULTS

AUL. Blasts from 17 patients with AUL were studied. Blasts from 12 patients were tested with anti-i and anti-myeloblast serum and were assayed for TDT; blasts from 3 patients were tested with anti-i and anti-myeloblast serum but were not assayed for TDT; blasts from the other 2 patients were tested with anti-i and assayed for TDT but not tested with anti-myeloblast serum.

The results of these studies and clinical information about the patients are summarized in Table 1. Of the 12 patients in whom all 3 markers were studied, the blasts reacted as mye-

Table 1

| i antigen, myeloblastic leukemia-associated antigen, and TDT level of blasts from patients with AUL |
|---|---|---|---|---|---|
| Patient | Lymphoblast markers | Myeloblast markers | 'Mixed' markers | Treatment and response | Survival (mos.) |
| | Cytoxicity by anti-i² | Fluorescence with anti-myeloblast serum³ | TDT level (units/10⁶ cells) | V, P → NR, Adria, ara-C → NR² | 6 |
| 1 | 2,560 | 7 | 85.6 | V, P → NR, Adria, ara-C → NR² | 6 |
| 2 | 2,560 | 13 | 3.7 | V, P → NR, Adria, ara-C → CR | 9 |
| 3 | 2,560 | <5 | 256 | DNR, ara-C → CR | In CR at 4 mos. |
| 4 | 2,560 | ND | 4.7 | V, P → CR | In CR at 24 mos. |
| 5 | 5,120 | 18 | ND | DNR, ara-C → PR | 15 |
| 6 | 640 | <5 | ND | V, P, asparaginase → CR | In CR at 24 mos. |
| 7 | 20 | 92 | 0 | V, P → NR, DNR, ara-C → PR | 5 |
| 8 | 10 | 72 | 0 | DNR, ara-C → NR | 0.5 |
| 9 | <10 | 60 | 0 | V, P → NR, Adria, ara-C → NR | 9 |
| 10 | <10 | 60 | 0 | V, P → NR | 1 |
| 11 | <10 | 80 | ND | V, P → NR, Adria, ara-C → NR | 5 |
| 12 | 10 | ND | 0 | DNR, ara-C → NR | 3 |
| 13 | 2,560 | 75 | 22.4 | V, P → CR | 15 |
| 14 | 10,240 | 70 | 8.9 | V, Adria, ara-C, Cyclo → NR | 9 |
| 15 | 20 | 2 | 9.5 | DNR, ara-C → NR | 2 |
| 16 | 10 | 17 | 0 | Adria, ara-C → PR | 14 |
| 17 | 20 | <5 | 1.9 | V, ara-C, Cyclo → CR | In CR at 6 mos. |

¹ Reciprocal of highest dilution of anti-i killing 50% of the cells.
² Percentage of brightly fluorescing cells observed after sequential incubation with anti-myeloblast serum and fluorescein-labeled goat anti-human IgG.
× V, vincristine; P, prednisone; NR, no remission; Adria, Adriamycin; ara-C, 1β-D-arabinofuranosylcytosine; Cyclo, cyclophosphamide.

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loblasts in all tests in 4 cases and as lymphoblasts in all tests in 2 cases. One other patient (Patient 2), classified as lymphoblastic in tests with anti-i and anti-myeloblast serum, had 3.7 units TDT per $10^6$ blasts, a higher level than is found in blasts from most patients with AML but less than what is found in patients with typical ALL. In the other 5 cases (Patients 13 to 17), the classification by one of the markers was different from that by the other 2 markers.

Of the 5 patients tested for only 2 of the 3 markers, there was definite concordance in 4 (lymphoblastic markers in 2, myeloblastic markers in 2); in the other patient (Patient 4), a lymphoblastic reaction in tests with anti-i was associated with a borderline TDT level of 4.7 units/$10^6$ cells.

Including the 2 patients (Patients 2 and 4) with borderline TDT levels, there were therefore 6 patients with AUL which was classified as lymphoblastic in tests for these markers. Four achieved a complete remission and one achieved a partial remission in response to chemotherapy. The mean survival of these patients with lymphoblastic markers is 13+ months (Patients 3, 4, and 6 are still alive and in remission at 4, 24, and 24 months, respectively). None of the 6 patients whose leukemia was myeloblastic by these markers achieved a complete remission, but one did achieve a partial remission in response to therapy. Mean survival of patients in this group was 4 months.

Two of the 5 patients with both myeloblastic and lymphoblastic markers achieved a complete remission, and one achieved a partial remission. Mean survival of patients in this group is 9+ months (Patient 17 is still alive and in remission at 6 months).

**CML in Blast Crisis.** Blasts were studied from 12 patients with CML in blast crisis and from another patient who had had polycythemia rubra vera progressing to myelofibrosis and, eventually, to blast cell leukemia. The Ph$^1$ chromosome was found in 7 of the 12 patients who had cytogenetic studies done.

Blasts from 9 patients were tested with anti-i and anti-myeloblast serum and were assayed for TDT, blasts from 3 patients were tested with anti-i and anti-myeloblast serum but TDT assays were not done; blasts from the other patient were tested with anti-i and assayed for TDT but were not tested with anti-myeloblast serum. The results of these studies and clinical information about the patients are shown in Table 2. The markers were concordant (for myeloblasts) in only 2 of these 13 cases of blast crisis. Moreover, of these 2 cases, in one (Patient 18), only 2 of the 3 markers (i antigen and myeloblast antigen) were studied; in the other (Patient 19), the reaction with anti-myeloblast serum was very weak.

Three patients (Patients 19, 28, and 29) did not receive chemotherapy for their blast crisis. Of the remaining 10 patients, 4 (Patients 22, 24, 25, and 27) responded to chemotherapy for their blast crisis. Of the remaining 10 patients, 4 (Patients 22, 24, 25, and 27) responded to chemotherapy and survived for a mean of 9 months from the diagnosis of crisis, and 6 did not respond, surviving a mean of just less than 4 months from the diagnosis of crisis.

**DISCUSSION**

The relationship of AUL to morphologically typical AML and ALL is unclear. Unlike blasts from patients with the common variety of ALL, blasts from patients with AUL do not react with anti-ALL serum, nor do they possess T-lymphocyte markers which would suggest a relationship to the less common T-cell variety of ALL (14). However, TDT is detected in the blasts of some patients with AUL (13, 14), and in these TDT-positive cases there is therefore a definite relationship to the common non-T, non-B, and the less common T-cell type of ALL, both of which would suggest a relationship to the less common T-cell variety of ALL (14).

### Table 2

**i antigen, myeloblastic leukemia-associated antigen, and TDT level of blasts from patients with CML in blast crisis**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Cyto-toxicity by anti-i</th>
<th>Fluorescence with anti-myeloblast serum</th>
<th>TDT level (units/$10^6$ cells)</th>
<th>Ph$^1$ chromosome</th>
<th>Treatment and response</th>
<th>Survival (mos.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myeloblast markers</td>
<td>18</td>
<td>10</td>
<td>76</td>
<td>ND$^2$</td>
<td>Neg. V, P → NR, DNR, ara-C</td>
<td>3</td>
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<tr>
<td>19</td>
<td>40</td>
<td>0</td>
<td>Pos.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixed markers</td>
<td>20</td>
<td>20</td>
<td>52</td>
<td>58.3</td>
<td>Pos. V, P, DNR → NR</td>
<td>5</td>
</tr>
<tr>
<td>21</td>
<td>2500</td>
<td>10</td>
<td>0</td>
<td>Neg. V, Adria, Cyclo → NR</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>10</td>
<td>2</td>
<td>97.1</td>
<td>Neg. V, P, DNR, Adria, 6TG, ara-C, Cyclo, Mtx → R</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>10</td>
<td>&lt;5</td>
<td>0</td>
<td>Pos. V, P → NR</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>20</td>
<td>&lt;5</td>
<td>0</td>
<td>Pos. V, P, DNR → R</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>&lt;10</td>
<td>&lt;5</td>
<td>74.4</td>
<td>Pos. V, P, DNR → R</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>&lt;10</td>
<td>&lt;5</td>
<td>0.89</td>
<td>Pos. DNR, Adria, 6TG → NR</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>&lt;10</td>
<td>&lt;5</td>
<td>0</td>
<td>Pos. 6TG, ara-C → R</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>2500</td>
<td>60</td>
<td>ND</td>
<td>Neg. No therapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>20</td>
<td>2</td>
<td>ND</td>
<td>Neg. No therapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>&lt;10</td>
<td>ND</td>
<td>0</td>
<td>ND</td>
<td>V, P, Adria, Cyclo → NR</td>
<td>2</td>
</tr>
</tbody>
</table>

$^2$ Reciprocal of highest dilution of anti-i killing 50% of the cells.

$^3$ Percentage of brightly fluorescing cells observed after sequential incubation with anti-myeloblast serum and fluorescein-labeled goat anti-human IgG.

$^4$ ND, not done; Neg., negative; V, vincristine; P, prednisone; NR, no response (blast crisis persisted); DNR, daunorubicin; ara-C, 1-β-D-arabinofuranosylcytosine; Adria, Adriaycin; 6-MP, 6-mercaptopurine; Pos., positive; Cyclo, cyclophosphamide; 6TG, 6-thioguanine; Mtx, methotrexate; R, response.

$^5$ Blast crisis following polycythemia rubra vera and myelofibrosis.
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which are TDT positive (11, 13, 14). If AUL arises in a pluripotential hematopoietic stem cell, it might be possible to demonstrate myeloid markers in at least some cases of AUL (for example, in those which are TDT negative).

In the present study, blasts from patients with AUL were tested for 3 markers which distinguish typical myeloblasts from typical lymphoblasts: the i antigen, detected in much smaller amounts on myeloblasts than on lymphoblasts in cytotoxicity tests with anti-i (25); myeloblastic leukemia-associated antigens, detected on myeloblasts, but not on lymphoblasts in fluorescence tests using serum from patients with AML in remission who have received immunotherapy with Bacillus Calmette-Guerin and irradiated allogeneic leukemic cells (1); and TDT, found in high concentrations in lymphoblasts but not detectable, or detectable in very low concentration, in myeloblasts (15).

Those AUL blasts which contained TDT, had abundant surface i antigen, and lacked the myeloblast antigen were considered to possess lymphoblast markers; those AUL blasts which lacked TDT, had very weak i antigen, and had readily detectable myeloblast antigen were considered to possess myeloblast markers. Blasts with these markers in any other combination were considered to possess “mixed” markers. Within this last group, blast populations which lack an expected marker (e.g., weak i antigen despite detectable TDT) should be distinguished from blast populations with an unexpected additional marker (e.g., myeloblast antigen as well as i antigen and TDT). The lack of a marker may be due to inadequate maturation. However, maturation-dependent changes cannot explain the presence of markers of different lineages. In such a case, either 2 populations (one lymphoid, one myeloid) of leukemic cells must be present or there is a single population with both lymphoid and myeloid markers. The former explanation seems more likely since there is other evidence (in patients with CML in blast crisis) that more than one clone of cells may be involved (12, 19).

In tests of blasts from 17 patients with AUL, the markers detected were those of myeloblasts in 6 cases, lymphoblasts in 6 cases (including 2 classified as lymphoblastic in tests with anti-i and anti-myeloblast serum, in which the TDT levels were only marginally elevated), and “mixed” in the other 5 cases.

Of the 5 cases of AUL discordant for the myeloblastic and lymphoblastic markers tested, 2 (Patients 13 and 14) expressed both the i and myeloblast antigens as well as being TDT positive. In the other 3 cases, the discordant markers appear to reflect lack of maturation rather than mixed myeloid-lymphoid differentiation.

The finding that some patients with AUL have lymphoblastic markers confirms the observations of Hoffbrand et al. (14) and Gordon et al. (13). The findings that patients with AUL may also have myeloblastic markers, either alone or in combination with lymphoblastic markers, and that still other patients with AUL may lack both the myeloblastic and lymphoblastic markers studied support the hypothesis that AUL arises in a pluripotential hematopoietic stem cell capable of expressing a variety of lymphoid and myeloid markers.

Boggs suggested that, in some patients with CML who have entered blast crisis, the crisis may be lymphoblastic rather than myeloblastic (4). This hypothesis was based on morphological considerations but, more recently, the antigen detected by anti-ALL serum (3, 17, 18, 20) and TDT (12, 15, 22) have been found in blasts of some patients with CML in blast crisis. Interestingly, these markers may be found in patients whose leukemia is morphologically myeloblastic as well as in those whose leukemia is morphologically lymphoblastic (20, 22).

In tests for a variety of markers, including those associated with T- and B-cells as well as those characteristic of the common non-T, non-B form of ALL, Janossy et al. (16, 17, 20) found no difference in the reactions of blasts from patients with CML in “lymphoid” blast crisis and those of blasts from patients with common non-T, non-B ALL, leading them to suggest that these diseases may arise from the same pluripotential stem cells.

In the present study, blasts from patients with CML in blast crisis were tested with anti-i and anti-myeloblast serum, and their TDT level was assayed. Blasts from patients with ALL react strongly with anti-i (25) and do not react with anti-myeloblast serum (1). By contrast, none of the 3 cases of TDT-positive blast crisis in the present study reacted strongly with anti-i and one (Patient 20) reacted with anti-myeloblast serum. The reaction with anti-myeloblast serum could have been due to the presence in this patient of a TDT-negative myeloblast population, but this hypothesis does not account for the failure of the TDT-positive population in this patient and in the other patients to react with anti-i.

Thus, in CML in blast crisis, TDT-positive blasts differ from those in the ALL patients that we have tested previously. Although blasts from these ALL patients were not tested for T-cell markers or with anti-ALL serum, it is very likely (because of the high frequency among patients with ALL, of the non-T, non-B form) that at least some of the patients in these previous studies (1, 25) had non-T, non-B ALL. TDT-positive blasts in patients who have CML in blast crisis are therefore probably different from the blasts in the common non-T, non-B form of ALL, the difference most likely being one of maturation. The previous findings of Janossy et al. (16, 17, 20) of a similarity in the markers of these 2 types of blasts appear to have been a function of the markers that they tested rather than an indication of true identity.

The lack of reaction, in many of these patients, of anti-myeloblast serum with blasts which were TDT negative and weakly reacting with anti-i suggests that the cell involved in “myeloid” blast crisis is also less “mature” than the cell involved in typical AML.

In the present study, the variety of drug combinations used for patients with AUL and for those with CML in blast crisis makes it difficult to draw conclusions about the value of tests for i antigen, myeloblast antigen, and TDT in predicting the response to therapy. However, among the patients with AUL, the remission rate was higher and mean survival was longer in those with lymphoblastic or “mixed” markers than in those with myeloblastic markers, and a study using a standard treatment protocol is warranted.

There was no consistent difference in the markers of the 4 patients with CML in blast crisis who responded to treatment and the 6 who did not. The finding that 2 of the 4 responders and only 1 of the 6 nonresponders were TDT positive suggests, as reported by others (20, 22), that had larger numbers of patients been studied a significantly better response rate might have been observed in those patients who were TDT positive than in those who were TDT negative. Studies of additional patients are required to determine whether there is prognostic

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value in classifying CML in blast crisis by tests with anti-i and anti-myeloblast serum as well as by assays for TDT.

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