T Cell Lineage Involvement in Lymphoid Blast Crisis of Chronic Myeloid Leukemia

By M. Allouche, A. Bourinbair, V. Georgoulia, R. Consolini, A. Salvatore, H. Auclair, and C. Jasmin

Cytogenetic and immunologic analysis of cells obtained from two patients with chronic myeloid leukemia (CML) during blast crisis reveals markers suggestive of an immature lymphoid phenotype. Peripheral blood mononuclear cells from both patients generated spontaneous lymphoblastoid colonies in methylcellulose, a phenomenon observed in T cell acute lymphoblastic leukemias and T cell non-Hodgkin’s lymphomas but not in any other type of leukemia.1-3 Colonies derived from one patient were composed predominantly of OKT3+ cells (89%), whereas those from the second patient displayed 42% OKT3+ and OKT6+ cells. In the second patient’s colonies, each of five mitoses contained the Philadelphia chromosome (Ph’), and two of five displayed the same additional karyotypic abnormalities as the blast crisis cells. Cells obtained from the two patients during remission still gave rise to spontaneous T cell colonies (>95% OKT3+) and Ph’ was detected in 33% and 60% of the metaphases, respectively. However, when colony growth was induced by an interleukin 2-containing conditioned medium, <5% of mitoses were Ph’-positive. These data suggest that: (1) the T cell lineage might be involved in CML; (2) a subset of T cells may remain unaffected by the leukemic process, as demonstrated by the virtual absence of Ph’ in induced T cell colonies; and (3) the spontaneous colony assay seems to select for the growth of malignant T cells.

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MATERIALS AND METHODS

Patients. Patient 1, a 44-year-old woman, first presented with leukothrombopenia, 20% to 30% blasts in peripheral blood and 80% undifferentiated blasts in the bone marrow. She had been diagnosed as having ALL and had been treated with cortisone for ten days before admission to our institution in 1980. At this time, her WBC count was 19 x 10⁹ per liter, including 15% blasts, 21% granulocytes, 6% myelocytes, 6% metamyelocytes, 41% lymphocytes, and 11% monocytes. Complete remission was induced by Adriamycin, vincristine, asparaginase, and prednisone, and was maintained for three years with methotrexate, purinethol, and vindesine. During this clinical and hematologic remission, late myeloid elements were consistently observed in the peripheral blood, evoking the diagnosis of CML. In 1983, she relapsed with 40% blasts in the bone marrow; blast cells were undifferentiated and had the same morphological and cytogenetic characteristics (see Results) as in the first acute phase. Karyotypic analysis revealed Ph’ and additional abnormalities (Table 3 and Fig 2) in 96% and 70% of the mitoses, respectively, thus confirming that the disease was a CML in blast crisis. Remission of blast crisis was again induced with the same protocol as in the first acute phase and was maintained for four months, after which the patient relapsed and died. Our study was performed during the 1983 relapse and the subsequent remission.

Patient 2, a 35-year-old man, presented in Aug 1981 with a typical clinical profile of Ph’-positive CML, which was treated with hydroxyurea and busulfan. In 1982, during stable chronic phase, he underwent splenectomy. In Feb 1983, he entered an accelerated phase, as indicated by 5% hyperdiploid and 85% Ph’+ mitoses. Overt blast crisis occurred in July 1983, with 89 x 10⁹ WBCs per liter and 98% lymphoblasts in the peripheral blood. Return to chronic phase CML was induced using the same combination chemotherapy as in patient 1 and was maintained for six months. In March 1984, the patient relapsed and died during the phase of aplasia following reinduction treatment. Our study was performed during the first blast crisis (July 1983) and the subsequent remission. Informed consent for these studies was obtained from the patients under treatment.

Cell separation. PBMCs or bone marrow mononuclear cells (BMMCs) were obtained by density gradient (Ficoll-Paque, Pharmacia, Uppsala, Sweden; density, 1.077 g/L) centrifugation. In some cases, they were further separated by E rosetting overnight at 4°C with 2-aminoethylisothiouronium hydrobromide (AET)-treated sheep erythrocytes. E rosette-forming cells (E+) and nonrosetting cells (E−) were then collected.

From Unité d’Oncogénèse Appliquée (INSERM U268), Hôpital Paul Brousse, 94800 Villejuif, France. Submitted Aug 27, 1984; accepted May 20, 1985. Supported by a grant from the Association de la Recherche sur le Cancer (ARC), France.

Address reprint requests to M. Allouche, MD, Unité d’Oncogénèse Appliquée (INSERM U268), Hôpital Paul Brousse, 94800 Villejuif, France. © 1985 by Grune & Stratton, Inc.
setting cells (E') were recovered from the pellet and the interface of a second density gradient centrifugation, respectively. Sheep erythrocytes in the E' fraction were lysed hypotonically.

**Monoclonal antibodies.** The following monoclonal antibodies were used: OKT3 (peripheral T cells), OKT4 (helper T cells), OKT6 (thymocyte), OKT8 (cytotoxic/suppressor T cells) and OKT10 (thymocytes and actively proliferating cells) were obtained from Ortho (Raritan, NJ); T11 (Coultronics, Margency, France) recognized the E rosette receptor; RFBI (a gift from Dr G. Janossy, London) detects thymic blasts and corticoidresponsive (i.e., immature T cells) and immature hematopoietic precursors but does not react with pre-B or B cells; Leu 9 (Becton Dickinson, Buc, France) and WT1 (a gift from Dr W. Tax, Nijmegen, The Netherlands) are markers of immature and mature leukemic and normal T cells; J5 recognizes CALLA; B1 and B2 (Coultronics) detect B and pre-B; and a fluorescein-conjugated goat anti-human immunoglobulin antibody (Cappel, Cochranville, Pa) was used in direct immunofluorescence. Myeloid lineage markers included MO2 (Coultronics) and OKM1 (Ortho). HLA-DR antigens were detected using monoclonal antibodies I1 (anti-1a-like; Coultronics), BM50, Terminal deoxynucleotidyl transferase (TdT) activity was detected with an immunoenzymoassay.13

**Cytologic and cytochemical studies.** Cytocentrifuge slides and smears of peripheral blood and bone marrow cells were prepared and stained with May-Grunwald-Giemsa for morphological characterization of the blasts. The following cytochemical markers were also analyzed: specific and nonspecific esterase, periodic acid-Schiff (PAS), myeloperoxidase, and acid phosphatase. Terminal deoxynucleotidyl transferase (TdT) activity was detected with an immunoenzymoassay.13

**In vitro differentiation assay.** PBMCs from patient 2 were cultured in a-minimum essential medium (MEM) GIBCO, Grand Island, NY) with 10% heat-inactivated fetal bovine serum (FBS) GIBCO), 2 mmol/L L-glutamine and antibiotics in the presence of various concentrations of phorbol myristic acetate (PMA) Sigma, St Louis) ranging from 0.01 to 10 ng/mL. Control cultures contained no PMA. After a 48-hour incubation at 37 °C, with 5% CO2 in air, cells were harvested, washed three times with α-MEM, and stained with OKT3 in indirect immunofluorescence.

**B cell colony assay.** B cell colonies were obtained using the technique described by Izaguirre et al.14 Briefly, 2 x 10^7-depleted cells (E') from patient's peripheral blood were seeded in 0.8% methylcellulose in α-MEM with 20% FBS, 2 mmol/L glutamine, antibiotics, 20% (vol/vol) PHA-TCM (two-day culture supernatant from PHA-stimulated normal T cells), and 3 x 10^6 pooled normal irradiated (2,500 rad from a 137Cs source) E' lymphocytes from two

<p>| Table 1. Surface Antigen Phenotype of Lymphoid Blast Crisis Cells in Two CML Patients |
|-----------------------------------------------|-------------------------------|</p>
<table>
<thead>
<tr>
<th>Cell Lineage</th>
<th>Surface Marker</th>
<th>Positive Cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mature thymocyte</td>
<td>E</td>
<td>18</td>
</tr>
<tr>
<td>T3</td>
<td>21</td>
<td>9</td>
</tr>
<tr>
<td>T4</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>T8</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>T11</td>
<td>NT</td>
<td>6</td>
</tr>
<tr>
<td>Early and common thymocyte</td>
<td>T6</td>
<td>0</td>
</tr>
<tr>
<td>T10</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>RFBI</td>
<td>NT</td>
<td>86</td>
</tr>
<tr>
<td>Leu 9</td>
<td>NT</td>
<td>67*</td>
</tr>
<tr>
<td>WT1</td>
<td>NT</td>
<td>60*</td>
</tr>
<tr>
<td>B and pre-B</td>
<td>B1</td>
<td>NT</td>
</tr>
<tr>
<td>lymphocyte</td>
<td>B2</td>
<td>NT</td>
</tr>
<tr>
<td>Myeloid lineage</td>
<td>J5</td>
<td>62</td>
</tr>
<tr>
<td>MO2</td>
<td>NT</td>
<td>0</td>
</tr>
<tr>
<td>OKM1</td>
<td>15</td>
<td>3</td>
</tr>
<tr>
<td>Non-lineage specific</td>
<td>HLADR</td>
<td>0</td>
</tr>
</tbody>
</table>

Bone marrow (40% blasts) and peripheral blood (98% blasts) monocloned cells were examined for patients 1 and 2, respectively. Abbreviation: NT, not tested.

*Studied on cryopreserved cells.

†Mean percentage of positive cells obtained with five different anti-HLA-DR monoclonal antibodies (see Materials and Methods).

donors. Cultures were incubated for five to seven days at 37 °C in a humidified atmosphere under hypoxic conditions (5% O2, 5% CO2 in air). Aggregates of more than 20 cells were scored as colonies under an inverted microscope.

**Cytogenetic studies.** Cytogenetic studies were performed both on patients' blasts and on colony cells. Patient's peripheral blood or bone marrow blast cells were cultured for 48 hours in α-MEM containing 10% FBS and antibiotics, with or without 1% PHA-M (Difco). T or B cell colonies were picked individually at day 5 of culture, pooled, and washed once with phosphate-buffered saline (PBS). Cells were incubated with colcemid (Gibco) (0.1 μg/mL) for 60 to 90 minutes at 37 °C, washed, and lysed hypotonically with 0.075 mol/L KCl (30 minutes at 37 °C) and fixed twice in methanol-acetic acid (3:1 vol/vol) at −20 °C. Air-dried slide preparations

Fig 1. Induction of T3 expression on blast cells from patient 2 with PMA.
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BMMCs or PBMCs were cultured for 48 hours. tPBMCs were stimulated with 1% PHA-M for 48 hours.

Table 2. Cell Surface Phenotype of Spontaneous and PHA-LCM-Induced T Cell Colonies in Two CML Patients at Various Clinical Phases

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Clinical Phase</th>
<th>No. of Spontaneous Colonies/10^6</th>
<th>Positive Cells (%)</th>
<th>No. of Induced Colonies/10^6</th>
<th>Positive Cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Blast crisis</td>
<td>573</td>
<td>NT 42 NT 22</td>
<td>386</td>
<td>9 37 NT 27</td>
</tr>
<tr>
<td></td>
<td>Remission</td>
<td>768</td>
<td>NT 60 NT 2</td>
<td>398</td>
<td>21 61 38 NT 43</td>
</tr>
<tr>
<td>2</td>
<td>Blast crisis</td>
<td>13</td>
<td>NT 90 70 NT</td>
<td>308</td>
<td>NT 90 97 84 NT</td>
</tr>
<tr>
<td></td>
<td>Remission</td>
<td>536</td>
<td>82 54 20 NT</td>
<td>350</td>
<td>62 NT NT NT</td>
</tr>
</tbody>
</table>

Values are expressed as the mean numbers of colonies from triplicate cultures. SD was always <12%.

were made, stained with Giemsa, and examined microscopically for well-spread metaphases. R and/or G banding analysis were also performed.

RESULTS

Morphological, cytochemical, and immunologic characterization of patients' blast cells. Blasts of patient 1 were undifferentiated, whereas those of patient 2 were lymphoblasts. In both cases, cells were PAS- and myeloperoxidase-negative, but displayed a positive TdT and acid phosphatase (in spots over the Golgi) reactivity. Specific and nonspecific esterases, only tested in blasts from patient 1, were weakly positive.

The surface antigen phenotype of blast crisis cells is given in Table 1. Blasts from patient 1 were undifferentiated, expressing only CALLA (J5), but not HLA-DR or T or B cell markers. In patient 2, blast cells expressed predominantly RFB1, Leu 9, WT1, CALLA, and HLA-DR, suggesting an immature T cell phenotype.

In addition, blast cells from patient 2, although originally T3, could be induced to differentiate into T3+ cells in the presence of a phorbol ester, PMA, in a dose-dependent fashion (Fig 1).

Spontaneous and induced T cell colony growth from patients' PBMCs. Spontaneous lymphoblastoid colonies were generated from PBMCs obtained from the two patients during blast crisis and remission (Table 2). T3+ cells in pooled colonies always represented >85% of the cells except for colonies obtained from patient 1 during blast crisis, in which 42% cells were T3+, 42% T4+, 43% T6+, and 33% T10+. More than 30% of the colony cells were RFB1+ in all cases, and HLA-DR antigens were observed on 69% of blast crisis colony cells of patient 1 and on 33% of remission spontaneous colony cells of patient 2 (Table 2). J5 was expressed on colonies derived from patient 2 colonies during blast crisis (70%) but not on colonies obtained from both patients during remission (20%).

T cell colonies could be induced by an IL 2-containing PHA-conditioned medium (PHA-LCM) during blast crisis and remission in both patients (Table 2). The surface phenotype of these colony cells was similar to that of spontaneous colonies (Table 2), as observed in T-ALL.12

B cell colony growth. PBMCs obtained from patient 1 during remission generated 357 colonies per 10^5 seeded cells in the B cell colony assay. Pooled colony cells contained 64% surface Ig, 58% HLA-DR, 60% B1, 8% E, and no J5-positive cells.

Table 3. Karyotypic Studies of Blast Cells and Spontaneous T Cell Colonies in Two CML Patients at Various Clinical Phases

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Clinical Phase</th>
<th>No. of Metaphases Studied</th>
<th>Karyotype</th>
<th>No. of Metaphases Studied</th>
<th>Karyotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Blast crisis</td>
<td>67</td>
<td>50, XX, +3, +5, -6, iso7, +8, +15, +18, t(9; 22)</td>
<td>5</td>
<td>47, XX, +3, +5, -13, t(9; 22)</td>
</tr>
<tr>
<td></td>
<td>Remission</td>
<td>24</td>
<td>49, XX, +3, +5, +15, t(9; 22)</td>
<td>3</td>
<td>46, XX, t(9; 22)</td>
</tr>
<tr>
<td>2</td>
<td>Blast crisis</td>
<td>19</td>
<td>47, XX, t(9; 22), 2Ph⁰</td>
<td>10</td>
<td>46, XX, Ph⁰</td>
</tr>
<tr>
<td></td>
<td>Remission</td>
<td>11</td>
<td>46, XX, t(9; 22), 2Ph⁰</td>
<td>8</td>
<td>46, XX</td>
</tr>
</tbody>
</table>

*BMMCs or PBMCs were cultured for 48 hours.
†PBMCs were stimulated with 1% PHA-M for 48 hours.
Fig 2. Abnormal mitoses from blast crisis cells in two CML patients: (A) Patient 1. 49, XX, +3, +5, −6, +8, −13, +15, t(9;22), 2Ph1 (R banding): (B) patient 2. 47, XY, +17q−, 2Ph1 (G banding). Ph1 or t(9;22) are indicated by arrows.
**Cytogenetic analysis of blasts and colony cells.** The karyotype of spontaneous T cell colonies was compared to that of bone marrow or peripheral blood blasts, using the RHG (patient 1) and GTG (patient 2) banding techniques (Table 3 and Fig 2). During blast crisis of patient 1, all five mitoses obtained from spontaneous colonies were Ph'. Moreover, two of these mitoses displayed hyperploidy, as also observed in fresh malignant cells (Table 3 and Fig 2). In remission, 60% of mitoses found in spontaneous T cell colonies (12 of 20, Table 3) were still bearing the Ph' chromosome, whereas two of 20 mitoses (10%) were hyperploidic. Similarly, two of six mitoses detected in spontaneous T cell colonies during remission of patient 2 were Ph'. Unfortunately, no mitoses could be detected in the few colonies derived from blast crisis PBMCs of patient 2 (Table 3). The karyotype of PHA-stimulated PBMCs during complete remission was normal for patient 2, whereas in patient 1, five of 24 Ph' mitoses were detected, of which two of 24 were hyperploidic (Table 3).

Table 4 gives the percentages of Ph' mitoses of spontaneous and induced T and B cell colonies during remission of the two CML patients. The percentage of Ph' mitoses was significantly higher in spontaneous than in induced T cell (patient 2, P < .001, Table 4), and T or B cell (patient 1, .05 < P < .10, Table 4) colonies.

**Discussion**

Very few cases of T cell involvement in CML have been reported, since usually no Ph' mitoses after PHA stimulation of lymphoid blasts with T cell markers are observed. Of interest was the case reported by Griffin et al. of a patient with T lymphoid blast crisis of CML. This suggested that the CML stem cell may, although rarely, differentiate into T lymphocytes, as previously demonstrated for all other hematopoietic lineages, using cytogenetic and G6PD isoenzyme markers, and recently, immunoglobulin gene rearrangements for the B lineage.

In this report we present two cases of CML blast crisis in which the cytologic and cytochemical (Tdt', myeloperoxidase-negative, and PAS' characteristics of blasts suggest a lymphoid origin. The spotlike acid phosphatase positivity over the Golgi apparatus in blasts of both patients was suggestive of T lymphoid cells. The presence of CALLA on cells of both patients might be suggestive of blasts of a pre-B cell origin; however, this marker has also been found in some T lymphomas as well as in normal immature T cells. Since no specific T or B cell markers were found in a significant percentage of bone marrow cells from patient 1, we considered these cells to be immature lymphoid cells.

In patient 2, several arguments concur to indicate a T cell origin of the blasts: (1) RFB1, Leu 9, and WT1 monoclonal antibodies recognize determinants on immature and mature T cells; (2) leukemic blasts could be induced to differentiate in vitro in the presence of PHA into T3' cells in a dose-dependent manner (Fig 1); (3) in vitro stimulation of the blast crisis PBMCs by PHA for 48 hours induced a significant secretion of IL 2 (data not shown).

HLA-DR antigens are frequently expressed on cALL cells, ie, of a pre-B phenotype, but usually not in T-ALL. Note that this marker was not present on our first CML patient's blasts, but that in patient 2, as in the T cell blast crisis described by Griffin et al, it could be detected.

We have previously reported that spontaneous T cell colonies could be generated from PBMCs of patients with acute T cell malignancies (T-ALL and T-NHL) but not with cALL, acute myeloid leukemia, chronic phase CML, solid tumors, nor from normal donors.

Spontaneous colony cells often displayed an immature T cell phenotype, resembling that of the initial leukemic blasts. In addition, spontaneous T cell colonies were also obtained from PBMCs of these patients with T cell malignancies during complete remission (manuscript in preparation). We report here the formation of spontaneous colonies from PBMCs obtained from two CML patients during both blast crisis and remission (Table 2). Colony cells were lymphoblastoid, bearing markers of the T cell lineage. In all cases, >85% T3' spontaneous colony cells were observed, except during the first patient's blast crisis, with 42% cells bearing T3, T4, and/or T6. Thus, we cannot exclude, in this last case, that a fraction of the spontaneous colonies might be either immature T cells or of a non-T cell origin. Note that induced T cell colonies, obtained from PBMCs of both patients in the presence of PHA-LCM (Table 2), also displayed a T cell phenotype, similar to that of spontaneous colonies, as observed in T cell malignancy patients.

Karyotypic analysis of spontaneous colonies during remission revealed 60% (patient 1) and 33% (patient 2) of Ph' mitoses in colonies containing 94% and 87% T3' cells, respectively (Tables 2 and 3). Moreover, in patient 1, 22% of mitoses (five of 24) obtained after PHA stimulation of PBMCs during remission were Ph' and 8% (two of 24) also displayed hyperploidy, as seen in leukemic blasts (Table 3). In addition, during blast crisis of this patient, all five mitoses detected in the spontaneous colonies (containing at least 42% T cells) were bearing Ph', and two of five had additional abnormalities (Table 3). Taken together, these data provide evidence for the existence of Ph' T cells in both patients.
Conversely, <5% of Ph<sup>+</sup> mitoses were observed in induced T cell colonies derived from PBMCs of both patients during clinical remission (Table 4). Similar findings were reported by Sonoda and Abe<sup>21</sup> in two chronic phase CML cases. Thus, there appear to be at least two subsets of T colony-forming cells (T-CFC): the first, detected in the spontaneous colony assay, is Ph<sup>+</sup> and seems to derive from the CML clone; the second, induced by mitogen (this report) or antigen<sup>21</sup> stimulation in the presence of exogenous IL 2, displays a normal karyotype. A similar observation of a dual T cell origin was reported in a patient with acute myeloid leukemia originating in a multipotent stem cell.<sup>22</sup> Moreover, in T-ALL and T-NHL, we have demonstrated that the pool of T-CFC is heterogenous: spontaneous T-CFC, in contrast to induced T-CFC, are highly clonogenic cells, in cell cycle, with self-renewal capacity and relatively high drug and radiation sensitivity.<sup>2</sup>

The B cell colony assay has been used to grow clongenic cells from normal subjects and from patients with B cell malignancies, such as cALL.<sup>14,23</sup> Consolini et al (submitted for publication) demonstrated that the B cell colonies from cALL patients in complete remission displayed an abnormal phenotype (a high J5 and low surface immunoglobulin expression) and karyotypic markers similar to those of the initial leukemic blasts; conversely, B cell colonies from T-ALL patients in complete remission had a normal (E-S Ig<sup>B</sup> I<sup>B</sup> I<sup>J5</sup>) phenotype. This assay should thus allow us to detect B cell involvement in the CML. However, B cell colonies obtained from PBMCs of patient 1 in remission displayed a normal phenotype (no J5· cells) and only 4% of the mitoses were Ph<sup>+</sup>. Note that 8% of pooled colony cells were E<sup>+</sup>, which could be due to limited spontaneous growth. Thus, it seems likely that the B cell lineage was not involved in the CML of patient 1.

Fauser and Löhr<sup>24</sup> have demonstrated a common pluripotent stem cell (CFU-GEMM) for all hematopoietic lineages including lymphocytes. More recently, the same authors, studying the lymphohematopoietic progenitors of a patient with chronic phase CML, observed Ph<sup>+</sup> mitoses in T cell colonies subcloned from multilineage colonies,<sup>25</sup> indicating that T cells can derive from the CML stem cell. It is not known, however, whether both T and B cells can be found in the same multilineage colony and be simultaneously affected by the CML. In the case of our CML patient 1, it seems that the T—but not the B—cells were involved in the CML. Thus, the question remains whether there is a common precursor for the B and T lymphoid lineages. One possibility is that each CFU-GEMM has the potential to differentiate into only one of the two lymphoid branches. This would explain why, either in normal or pathologic conditions, no common stem cell origin has, to date, been demonstrated for T and B cells.

In summary, our study provides evidence for T cell involvement in CML. In the two cases described here, there appears to be at least two subsets of T cell progenitors, one deriving from the CML stem cell, and the other(s) remaining unaffected. The spontaneous colony assay seems to favor the growth of malignant T cells and might be useful in surveying residual leukemic cells during remission.

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