Differentiation Patterns in the Blastic Phase of Chronic Myeloid Leukemia


CHRONIC MYELOID LEUKEMIA (CML) is a myeloproliferative disorder associated in 90% of cases with a characteristic karyotypic marker, the Philadelphia chromosome (Ph'). After a variable period of time, the majority of patients enter a terminal phase that may resemble an acute blastic leukemia (blast crisis). About one-third of blast phase leukemias have a lymphoblastic morphology, expressing a phenotype corresponding to an immature lymphoid cell and were felt to have “lymphoid” blast crisis. None of these lymphoid leukemias were TdT+ or responded to vincristine (V) and prednisone (P). Eleven patients expressed a phenotype similar to acute lymphoblastic leukemia cells and probably reflect maturation to an early B lymphocyte. All of these “lymphoid” leukemias were TdT+, and 67% of evaluable patients had a complete response to V and P. One leukemia had the phenotype of an erythroleukemia, one patient’s cells expressed the phenotype of a megakaryoblastic leukemia, and one leukemia had populations of both myeloid and lymphoid blasts. Six leukemias did not express surface markers characteristic of any lineage and were termed “undifferentiated.” This group was heterogeneous with respect to TdT expression, but no patient had a complete response to V and P. Determination of surface antigen phenotype in CML blast crisis thus provides clinically useful information for the structuring of treatment protocols.

MATERIALS AND METHODS

Patients Studied

Peripheral blood or bone marrow samples were obtained from 30 patients in the blast phase of CML. Twenty-eight patients had a recognized antecedent chronic phase of CML and met standard diagnostic criteria including expression of the Ph' chromosome. Two patients (patients 5 and 7) presented with a Ph'-positive acute leukemia without a diagnosed preceding chronic phase. However, both patients reverted to a Ph'-positive chronic phase CML following treatment. Twenty-six patients were adults (>18 yr old) at the time of diagnosis of blast crisis (mean age 43, range 20–82), and 4 were pediatric patients (mean age 14, range 13–15). All patients with a recognized chronic phase had received either busulfan, hydroxyurea, or both for control of symptoms. Many patients evolved from chronic phase CML through an “accelerated” phase with progressive symptoms, thrombocytopenia, anemia and splenomegaly, then developing overt blastic leukemia. The diagnosis of blast crisis was made with the finding of ≥40% blasts in peripheral blood or bone marrow. All patients received some form of chemotherapy in the blastic phase in an attempt to induce remission. Twenty-two patients received evaluable trials of vincristine and...
prednisone. A complete response was defined as elimination of blasts with a return to a chronic phase CML in blood and bone marrow for a period of ≥1 mo. A partial response was defined as a substantial decrease (≥75%) in circulating blasts without complete clearing of blasts or return to a chronic phase morphology. Patients who received chemotherapy in addition to vincristine and prednisone were not considered evaluable for complete response. Nonresponders were considered evaluable if they received an adequate trial of vincristine and prednisone, regardless of whether or not they simultaneously received other drugs.

The original bone marrow and peripheral blood slides were available for review on 21 of the patients. The slides were coded and independently classified by two experienced hematologists as myeloid, lymphoid, erythroid, megakaryoblast, undifferentiated, or mixed blast crisis.

**Sample Preparation and Immunofluorescence Assays**

Heparinized peripheral blood or bone marrow samples were sent to the Tumor Immunology Laboratory at Sidney Farber Cancer Institute. Erythrocytes and mature myeloid cells were removed by density gradient sedimentation in Ficoll-Hypaque (1.077 g/cm cu). Interface cells were washed twice in minimal essential media (GIBCO, Grand Island, N.Y.) containing 2.5% pooled human AB serum (AB-MEM), and suspended at 10 x 10⁶ cells/ml in AB-MEM for surface antigen determination. Cytocentrifuge preparations of blast crisis cells were stained with Wright's stain for enumeration of the percent blasts in the analyzed sample. All samples had >40% blasts.

TdT was determined by the method of Bollum on cytocentrifuge preparations. Reagents for the TdT assay were from Bethesda Research Laboratory. The characteristic granular nuclear fluorescence of TdT was detected using a Zeiss fluorescence microscope. The TdT assay was quantitatively interpreted as indicated in Table 3 (below). Fifteen patients also had an enzymatic assay performed elsewhere. There was one discordant result (patient 15) where the immunofluorescence assay was negative, while the biochemical assay was reported as positive.

Surface antigens identified by monoclonal antibodies were detected using an immunofluorescence assay as previously described. Fluorescent antibody-coated cells were detected on a fluorescence activated cell sorter (FACS-1, Becton Dickinson, Mountain View, Calif.) or a cytofluorograph (FC200/4800A, Ortho Instruments, Westwood, Mass). Background fluorescence was determined by staining cells not considered to be blasts. Negative samples generally showed moderately bright fluorescence of all or most erythrocytes and megakaryocytes.

The monoclonal antibody panel was selected to identify differentiation antigens of myeloid cells (MY4, MY7, MY8, Mol), erythroid cells (glycophorin-A (LICR/LON/R10)), B cells (CALLA (J5), B1), and platelets (Plt-1). The distribution of these antigens on normal peripheral blood, normal bone marrow, and leukemic cells is shown in Table 1. Methods of antibody production have been previously described. LICR/LON/R10 was generously provided by Dr. Paul Edwards, Surrey, England. All antibodies were used as diluted ascites. Reactivity with normal peripheral blood cells and bone marrow was determined as previously described. Reactivity with megakaryocytes was determined on methanol-fixed bone marrow smears using indirect immunofluorescence. Reactivity with myeloid colony-forming cells (CFU-C) and erythroid burst-forming cells (BFU-E) was determined by complement-mediated cytotoxicity testing or fluorescence activated cell sorting, as described. Where previously reported, the reference is given in Table 1.

**RESULTS**

In order to study surface antigen differentiation patterns of blast crisis cells, we selected a panel of monoclonal antibodies reactive with lineage-restricted antigens of multiple cell types. The reactivity of this antibody panel with normal peripheral blood cells, megakaryocytes, myeloid and erythroid colony-forming cells (CFU-C and BFU-E), CML, AML, and ALL leukemic cells is shown in Table 1.

**Surface Antigen Phenotyping of CML Blast Crisis Cells**

The surface antigen phenotype of 30 patients with CML blast crisis is shown in Table 2. The percent of blasts in the sample is shown for each patient. The

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**Table 1. Reactivity of Monoclonal Antibody Panel With Normal and Leukemic Cells**

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<td>CALLA (J5)</td>
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<td>B1</td>
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<td>Plt-1</td>
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<td>Glycophorin (LICR/LON/R10)</td>
<td>++</td>
<td>NT</td>
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*Erythrocytes have been separated from leukocytes by Ficoll-Hypaque. † -1 Nonreactive (not fluorescent over background in an indirect immunofluorescence assay); (+) - weakly reactive; (+) - moderately reactive; (++) - strongly reactive.

† -1- 1% of leukemias positive; (+) - 1%–50% positive; (++) - >50% positive.

**Table 2. Reactivity of Monoclonal Antibody Panel With Normal and Leukemic Cells**

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introduction at very weak reactivity (+) is uncertain, and a positive reaction is considered to be "++" or "+++". Weak reactivity of myeloid antibodies (anti-MY7, MY8, or Mo) was noted in several patients, and in some cases, was felt to represent reactivity with residual chronic phase myeloid cells when examined by fluorescence microscopy (patients 3 and 26). Two patients whose dominant population of blasts expressed myeloid markers (patients 16 and 21) also had a minority population that expressed Plt-1. Both of these patients had small numbers (<10%) of cells morphologically consistent with micromegakaryoblasts. However, the possibility that a minor population of cells expressed both markers simultaneously was not excluded by the techniques used in this study.

Eighty-three percent of patients' blasts were Ia+ CALLA+ and negative for myeloid, erythroid, or megakaryocyte markers. Five of these Ia+, CALLA+ patients also expressed Bl antigen, and Bl antigen expression was restricted to the CALLA+ phenotype. The frequency of Bl expression in these patients was similar to Bl expression in ALL patients.

Ten patients expressed phenotypes characteristic of early myeloid cells and commonly observed in AML (patients 12–21). These patients expressed MY7 antigen on all or most of their blasts. Nine of these were Ia+, 4 were Mo+, 1 was MY8+, and 1 was MY4+. Lymphoid, megakaryocyte, and erythroid markers were either negative or detected on very small numbers of cells. One patient (no. 13) had a small number of glycophorin-positive cells felt to be residual bone marrow erythroid cells.

One patient expressed glycophorin as the only identified marker on the blasts and was shown to have an erythroleukemia phenotype. This patient was Ia−. One patient expressed Plt-1 as the only marker, and represents a megakaryoblastic phenotype. One patient
(no. 24) had significant populations of both MY7+ and CALLA+ cells, and may have had “mixed” blast crisis. The MY7+ cells of this patient were slightly larger than the CALLA+ cells, but expression of both antigens on the same cells was not excluded. The remaining 6 patients (25–30) did not express markers characteristic of any lineage and are termed “undifferentiated.” Five of these were Ia+. One (no. 28) had a small population of CALLA+ cells. T-cell markers were not detected on the blasts of any of the 30 patients.

The mean age of the lymphoid blast crisis patients (31 yr, range 13–52) was younger than the myeloid blast crisis patients (mean 51 yr, range 15–82). The mean age of the undifferentiated patients was 36 (range 14–62). Similarly, the TdT+ patients were younger than the TdT− patients (mean 29 yr and 46 yr, respectively).

The relationship of surface antigen phenotype to TdT expression, response to vincristine and prednisone chemotherapy, and survival is shown in Table 3. Fourteen of the 29 tested patients were TdT+, including 11 of the 11 tested CALLA+ patients (including patient 24 with mixed blast crisis). None of the 10 myeloid blast crisis patients were TdT+. The patient with megakaryoblastic crisis (no. 23) was TdT+ by immunofluorescence assay and by enzymatic assay in a separate laboratory. Two of 6 undifferentiated blast crisis patients were TdT+.

Responses to vincristine and prednisone were evaluable in 22 patients, and complete responses occurred in 7. Two additional patients had a decrease in blasts without a complete response. No patient with a non-lymphoid phenotype had a complete response to vincristine and prednisone. Six of 9 evaluable CALLA+ patients had a complete response (67%). An additional lymphoid patient (no. 7) had a complete response, but received doxorubicin in addition to V and P. Seven of 12 evaluable TdT+ patients (58%) responded, all of the responders also being CALLA+. Two patients who were TdT+, CALLA− (undifferentiated phenotype) failed to respond to V and P. Four B1+, CALLA+ patients were evaluable, 2 responded. Survival was poor in all groups. Three of 5 patients surviving for more than 1 yr were lymphoid. Twenty-seven percent of lymphoid patients survived for 3 mo or less, as did 60% of myeloid patients, and 67% of undifferentiated blast crisis patients.

The relationship of surface markers to morphology was evaluated by reviewing the original bone marrow and peripheral blood slides on 21 patients. The slides were coded and classified by two reviewers into myeloid, lymphoid, erythroid, megakaryoblast, or mixed blast crisis. Both reviewers agreed with the immunologic classification in 52%. One of the two reviewers agreed in an additional 28%. In the other 20% (4 cases), both reviewers disagreed with the immunologic diagnosis. In 3 of these 4 cases, the reviewers disagreed with each other, and in one case, agreed with each other. Agreement was highest with lymphoid patients (both reviewers agreed in 5/6) and lowest with erythroid (0/1) and undifferentiated (0/4). Overall, both reviewers agreed with each other on 11/21 cases.

DISCUSSION

Chronic myeloid leukemia provides compelling evidence for the existence of a pluripotent hematopoietic stem cell in humans. Although most of the clinical
manifestations of the disease involve the myeloid series, hematopoietic precursors in this disorder acquire a karyotypic marker, the Philadelphia chromosome, which can be detected in myeloid cells, erythroid cells, and megakaryocytes. There is also evidence that B lymphocytes may be Ph$^+$ in some patients. In addition, studies with isoenzymes of the X-linked enzyme glucose-6-phosphate dehydrogenase further indicate that CML originates from a single cell. In many cases the disease progresses to a blastic phase where differentiation ceases and one or more populations of leukemic blast cells replace the chronic phase cells. Although additional cytogenetic abnormalities are common, the new clone of blast crisis cells almost always retains the Ph$^+$, indicating that it was derived from the original CML stem cell. The morphology of blast phase cells has been recognized as heterogeneous, with the majority having morphological patterns resembling AML. Lymphoblast morphology has been noted in about one-third of patients, and other correlations with the cells of ALL have been described, including expression of TdT and cytoplasmic immunoglobulin chains consistent with involvement of the B-cell lineage. The relationship of nonlymphoid blast crisis leukemias to AML cells is less clearly defined. The “myeloid” blast crisis cells are morphologically undifferentiated, rarely have Auer rods, and are usually negative for cytochemical stains such as myeloperoxidase and nonspecific esterase, which are commonly positive in AML.

It has recently become possible to define discrete stages of differentiation of normal T lymphocytes, B lymphocytes, and myeloid cells using monoclonal antibodies to demonstrate unique patterns of cell surface antigens. Furthermore, by determining the surface antigen phenotype, it has been possible to relate leukemic cells to a stage of normal cell differentiation. In most cases, leukemic cell surface antigen phenotypes have been found to reproduce with considerable accuracy the phenotype of a normal cell counterpart. For example, T-cell leukemias and lymphomas have been shown to recapitulate T-cell ontogeny. Likewise, a cell with the phenotype of the common acute lymphoblastic leukemia cell (Ia+, CALLA+, T10+, and TdT+) has recently been detected both in normal bone marrow and fetal liver. These observations again support the notion that the observed state of “differentiation” of the leukemic cell often represents an orderly process and may provide useful information regarding differentiation of normal hematopoietic cells. The CML blast crisis leukemias are of particular interest in this regard, as the malignant transformation may involve a pluripotent stem cell, with the leukemic cell sometimes showing partial differentiation along any of several pathways.

The surface antigen phenotype of the dominant population of blast crisis cells of 30 patients was determined. Eleven patients’ cells expressed a phenotype identical to the common ALL phenotype (Ia+, CALLA+, T10+, and TdT+). Five of these lymphoid phenotypes (45%) were B1+, similar to the percent of ALLs that express B1 and confirming the concept that lymphoid blast crisis cells are phenomenically early B lymphocytes. All of the CALLA+ patients were TdT+, and 67% of evaluable patients responded to ALL-type chemotherapy.

Ten patients had a recognizable myeloid phenotype, expressing one or more myeloid antigens. All of these patients were negative for lymphoid markers, all were TdT−, and all evaluable patients failed to respond to ALL-type therapy. The level of differentiation of these cells is of interest, and this study demonstrates that the
surface antigens of myeloid blast crisis cells are generally characteristic of a less mature cell than most AML cells. The myeloid blast crisis cells were usually Ia+, MY7+, but only one patient’s cells expressed MY4 and MY8, antigens acquired later than MY7 in myeloid differentiation and frequently expressed by AML blasts. Most of the myeloid blast crisis leukemic cells would correspond approximately to the phenotype of the normal CFU-C cell (Table 1).

The erythroblast leukemia was Ia- and glycophorin+, suggesting maturation to a level somewhat more mature than CFU-E cells (most are Ia-, and glycophorin expression begins at about the level of the CFU-E). Likewise, the megakaryoblast leukemia had the phenotype of a megakaryocyte (Table 1). Interestingly, this patient’s cells were TdT+ as well, but lacked CALLA. Erythroblast and megakaryoblast phenotypes are rare, however, and exact correlations with normal cells will require study of additional patients.

Six patients’ cells did not express markers unique to any lineage. This “undifferentiated” group is probably heterogeneous, as Ia antigen and TdT were expressed by some, but not all, of the leukemias. It may be possible in the future to correlate these “undifferentiated” cells with a normal counterpart cell when markers to identify earlier stages of differentiation of myeloid or lymphoid cells are identified.

The results presented in this study have several implications for the understanding of the blastic phase of CML. In most cases, blast crisis cells show orderly, but limited, differentiation (Fig. 1). This is of considerable clinical importance because one phenotype (Ia+, CALLA+, TdT+) has a substantial likelihood of a complete response to vincristine and prednisone. Unfortunately, responses are often short, and relapse occurs.

Most of the patients studied here had a clearly identifiable dominant population of cells that expressed differentiation markers of only one lineage. It has been suggested that many blast crisis leukemias may contain small numbers of cells with another morphology or a marker of a second lineage. Although this study was not designed to evaluate the incidence of small populations of blasts expressing markers of a second lineage, mixed blast crisis (myeloid-lymphoid) was noted in one patient, and several additional patients did have small populations of cells with a second marker such as Plt-1. In some cases, the second marker clearly resulted from a small number of residual chronic phase cells, whereas in others, a second small population of blasts, such as micromegakaryoblasts, was probably present. These observations might suggest that the malignant event triggering blast crisis occurs in the pluripotent stem cell and that abortive differentiation along one, or sometimes several, lineages may occur simultaneously or sequentially. Residual nontransformed stem cells may be rare, except in some patients with lymphoblastic phenotype, accounting for the poor response to chemotherapy and poor survival of most patients.

In summary, we have examined surface antigen differentiation patterns in 30 patients with CML blast crisis and demonstrated phenotypes characteristic of cells of the myeloid, B lymphoid, erythroid, and megakaryocyte lineages. lymphoblast crisis leukemias express a phenotype identical to most non-T-ALL patients and correspond to an early B cell. Most myeloid blast crisis leukemias are similar to “undifferentiated” AML and correspond approximately to the normal CFU-C. Occasional patients’ cells express megakaryocyte or erythroid phenotypes, and about 20% express no lineage-specific markers. This analysis of surface antigens has allowed further definition of those patients likely to have a favorable response to chemotherapy and demonstrates that the surface antigen phenotype in CML blast crisis in many cases accurately reflects the phenotype of a corresponding immature normal cell.

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