T-Cell Surface Antigens in a Patient With Blast Crisis of Chronic Myeloid Leukemia

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There is little evidence to suggest that T lymphocytes are involved in the leukemic process in chronic myeloid leukemia (CML). A case of CML in blast phase is described in which T-cell surface antigens were detected by immunofluorescence on the patient's blasts using monoclonal antibodies. In order to determine that the T-cell blasts

**Chronic Myeloid Leukemia (CML)** is a well-defined myeloproliferative disorder associated in greater than 90% of cases with a characteristic karyotypic marker, the Philadelphia chromosome (Ph'). Studies with isoenzymes of the X-linked enzyme, glucose-6-phosphate dehydrogenase, in heterozygous patients have shown that CML results from proliferation of a single clone of cells. Although most of the clinical manifestations of CML result from excessive production and accumulation of myeloid cells, the Ph' chromosome can also be detected in megakaryocytes, erythroid cells, and B lymphocytes, thus suggesting that CML arises in a pluripotent stem cell. Efforts to identify the Ph' chromosome in T lymphocytes have largely been unsuccessful, and this has been taken as evidence that myeloid cells share a common precursor with some B cells, but not with T cells.

After a variable period of time, the chronic phase disease undergoes transformation to an acute blastic leukemia (blast crisis). During this phase, additional chromosome changes (e.g., trisomy 19 or 21, a second Ph', or isochromosome 17) are superimposed on the Ph' chromosome in 80% of cases. The blast crisis cells often show evidence of partial differentiation along one or more hematopoietic pathways. The blasts of a majority of patients are thought to resemble acute myeloblastic leukemia cells, although they are usually peroxidase negative and rarely have Auer rods. Myeloid cell surface markers have recently been demonstrated in some of these patients. About one-third of patients' blasts morphologically resemble the lymphoblasts of acute lymphoblastic leukemia (ALL). Less commonly, the dominant phenotype is an erythroblast, a megakaryoblast, or an undifferentiated blast with no detectable lineage-associated markers. T-cell markers in CML blast crisis have rarely been reported, consistent with the hypothesis that the malignant clone of cells in CML does not involve T-cell precursors.

We present a case of CML blast crisis in which surface marker studies revealed T-cell antigens. This event is sufficiently uncommon to suggest the possibility of the simultaneous occurrence of two distinct diseases (CML and T-cell leukemia). In this patient, however, the Ph' chromosome could be conclusively demonstrated in blasts with T-cell surface markers.

**CASE REPORT**

A 60-yr-old man was found to have an elevated WBC and platelet count on routine examination in 1979. A chromosome study on bone marrow cells revealed the Philadelphia chromosome, and the patient was treated with busulfan and hydroxyurea to control his leukocytosis and thrombocytosis. He remained asymptomatic until January 1982, when he noted sudden onset of malaise and left upper abdominal pain. He was found to have splenomegaly, a WBC of 25.0 x 10^9/liter with 30% blasts. A bone marrow aspirate revealed 50% undifferentiated blasts, which were specific esterase, nonspecific esterase, and periodic acid-Schiff (PAS) negative. An enzymatic assay for terminal deoxynucleotidyl transferase showed no detectable activity. The patient was treated with cytosine arabinoside 3 g/sq m by i.v. bolus every 12 hr for 4 doses. After a period of aplasia lasting 3 wk, the WBC recovered to 32.9 x 10^9/liter with 80% polys, 6% basophils, and no blasts. The platelet count returned to 879 x 10^9/liter. A bone marrow on day 20 showed a return to stable phase CML with less than 1% blasts.

**MATERIALS AND METHODS**

**Sample Preparation and Immunofluorescence Assays**

Bone marrow was collected into preservative-free heparin, and erythrocytes removed by density gradient sedimentation in Ficoll-Hypaque (1.077 g/cu mm). Interface cells were washed twice in minimal essential medium (GIBCO, Grand Island, N.Y.) containing 2.5% pooled human AB serum (MEM-AB). Surface antigen expression was determined as previously described. Briefly, 10^6
cells were incubated in appropriate dilutions in MEM-AB of monoclonal antibody for 30 min at 4°C. The cells were washed twice and further incubated with fluoresceinated goat anti-mouse Ig (Tago, Burlingame, Calif.) for 30 min at 4°C. Following two wash steps, fluorescent cells were detected on a fluorescence activated cell sorter (EPICS V, Coulter Electronics, Hialeah, Fla.). Background fluorescence was determined by staining cells with a control IgG or IgM monoclonal antibody. Light scatter windows were selected to exclude erythrocytes. Ten-thousand cells were evaluated per sample, and the percent of cells more fluorescent than control was recorded. Cytocentrifugation preparations were stained with Wright-Giemsa stain to determine the percent of blasts in the analyzed sample.

Terminal deoxynucleotidyl transaminase (TdT) was determined by immunofluorescence assay (Bethesda Research Laboratories, Rockville, Md.) as described by Bolli.17

Monoclonal Antibodies

The antibody panel was selected to identify differentiation antigens of T cells, B cells, and myeloid cells. Ia-like antigen (Ia) was detected by anti-Ia, which reacts with a nonpolymorphic region of the human HLA-DR molecule.18 The common ALL antigen (CALLA) was detected by antibody J-5.19 Bi is an antigen expressed by B cells and B-cell malignancies, including about 50% of ALLs and 40% of lymphoid blast crisis of CML.20,21 T1, T3, T4, T5, and T8 are antigens of mature thymocytes and circulating T cells.22-24 T1 and T3 are "pan-T" cell antigens, while expression of T4 defines the inducer cell population and expression of T5 and T8 defines the cytotoxic/suppressor cell population. T6 is a marker for the intermediate, or common, thymocyte.23,24 Anti-T11 detects the structure associated with sheep erythrocyte rosetting.25 Mol is expressed by monocytes, granulocytes, and some non-T, non-B lymphocytes (including some natural killer cells).26 MY7 is expressed by monocytes and granulocytes in the peripheral blood, and by a small number of bone marrow myeloid cells, primarily immature forms.23 Like Mol, it is expressed on some nonlymphoid CML blast crisis leukemias and most acute myeloblastic leukemias.

One heteroantiserum was used in this study. A99 is a rabbit anti-T-cell antiserum that is reactive with T cells and a majority of T-cell malignancies.27

Fluorescence Activated Cell Sorting

Cryopreserved cells (viability >95% by trypan blue exclusion) were separated stably into T3+ and T3− populations28 on two occasions after staining as described above, utilizing the Coulter Epics V cell sorter.

Post-sort viability was 75% after the first sort, and >95% after the second sort. Purity of the FACs-separated T3+ cell subset was >98% in each experiment.

Chromosome Analysis

Approximately 10⁶ T3+ cells from each sort were incubated overnight in RPMI 1640 medium with 15% heat inactivated fetal bovine serum, antibiotics, and L-glutamine. At the end of the incubation period, colcemid (GIBCO) at a final concentration of 0.01 µg/ml was added and incubation continued for an additional 10 min. The cells were centrifuged for 7 min at 800 rpm, and the cell pellet resuspended in 75 mM KCl hypotonic solution. After 10 min at room temperature, the cells were pelleted and fixed in freshly prepared 3:1 (v/v) methanol-acetic acid. Standard air-dried slides were prepared and stained with quinacrine mustard as previously described.29 Q-banded metaphases were photographed using a Leitz Ortholux microscope for analysis.

RESULTS

Surface Antigen Phenotype

The surface antigen phenotype of this patient’s blast cells is shown in Table 1. The blasts were moderately Ia+ and brightly fluorescent with anti-T1, T3, and A99. T11 was detected on most cells, but fluorescence was dim. CALLA, B1, T4, T5, T6, and T8 were not detected. In addition to T-cell markers, two myeloid markers were present. MY7 was weakly positive, while Mol was found on a majority of blasts. Two additional myeloid markers, MY4 and MY8, were negative. Despite the expression of T11, only 5% of cells formed rosettes with sheep erythrocytes. The possibility that the T-cell antibodies bound nonspecifically by Fc binding was unlikely because the assays were performed in the presence of human serum, and isotype-identical control antibodies showed negligible binding. Further, bone marrow cells were obtained from this patient after chemotherapy-induced remission and tested for reactivity with the same panel of antibodies. The remission bone marrow cells showed the pattern of reactivity of normal bone marrow. No increase in T-cell markers was detected.

Analysis of Philadelphia Chromosome in T3+ Blasts

In order to determine if the cells bearing T-cell markers in the peripheral blood were part of the CML population, T3+ cells were isolated by fluorescence activated cell sorting (Fig. 1), and chromosome analysis was then performed on the T3+ cells. A total of 25 metaphases were analyzed. All metaphases showed a Ph1 chromosome [(9q+; 22q-) (Fig. 2). In addition, 13 of 25 metaphases showed a trisomy for chromosome 19.

DISCUSSION

The identification of a "lymphoid" variant of CML blast crisis has been of considerable clinical importance because this subgroup of patients has a high response rate to ALL-type chemotherapy (vincristine and prednisone).31 The lymphoid blast crisis cells morphologically resemble ALL lymphoblasts, and like ALL cells, express TdT30 and CALLA14,19 Cytoplasmic µ chains have been detected, suggesting that these cells are related to early B cells.31,32 Also, the Ph1

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<th>Percent Positive Cells†</th>
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*Percent blasts in the analyzed sample.
† Determined by indirect immunofluorescence assay.
chromosome has been detected in circulating B cells in the chronic phase of the disease,6 8 confirming that B cells share a common stem cell with myeloid cells in CML. In contrast, there is little evidence to show that chronic phase T cells are Ph1+.3,4,6,10 This suggests either that T-cell precursors are derived from a separate stem cell than B cells, or that most phytohemagglutinin-stimulatable circulating T cells were generated prior to the development of CML, and that the new CML stem cell has a limited ability to differentiate into T cells. T-cell markers in the blast phase would be of particular importance, therefore, as an indication of the differentiating potential of the CML stem cell. However, T-cell markers in blast crisis have rarely been reported. No cases were identified in more than 275 cases studied by Greaves or in more than 35 cases that we have investigated using an extensive panel of monoclonal antibodies, (J. Griffin, unpublished observations). Reactivity of blast crisis cells with various heteroantisera against T cells has been reported,14,34 but these antisera are rarely monospecific, and in one case,34 reactivity was also seen against acute myelomonoblastic leukemia cells. The cells of one patient with lymphoid blast crisis were reported to form E rosettes, but no further immunologic evaluation was done.35 Three cases of Ph1+ acute leukemia (without stable phase CML) with possible T-cell characteristics have been reported,36-38 but the relationship of that disorder to CML is unclear. Janossy et al.11 reported a blast crisis patient whose blasts were Ia−, CALLA−, TdT+, weakly positive with an antithymocyte heteroantiserum, but failed to form E rosettes.

In the case presented here, a patient with typical Ph1+ stable phase CML of 2-yr duration entered a blast transformation of undifferentiated morphology. Marker analysis showed that the blasts were Ia+, CALLA−, T1+, T3+ and A99+. Further, two myeloid markers were detected, Mol and MY7, while TdT was negative. In order to confirm that the cells expressing T-cell markers were indeed the blast crisis cells,
T3+ cells were isolated by cell sorting, and chromosome analysis performed on the sorted cells. All of the metaphases examined in the T3+ fraction contained the Ph1 chromosome.

The surface antigen phenotype of this patient is unusual in several respects. Previous studies with T-cell acute leukemias have shown that these cells usually express phenotypes that correlate with normal thymocytes at early stages of development.24 The patient’s cells expressed T1 and T3 antigens, which are acquired late in T-cell ontogeny, suggesting that this leukemia was derived from a mature population. This was further suggested by the absence of the thymocyte-specific antigen T6.24 The additional lack of T4 and T8 antigens may correlate with its derivation from a T3+, T4−, T8− subset of T cells.25 Also of note is the apparent coexpression of two myeloid antigens, Mol and MY7, with the T-cell markers, and the expression of Ia antigen, which is rarely detected on T-ALL. Of interest, there has been a recent report of an OKT3+, OKM1+ chronic leukemia that displayed natural killer activity.26 The significance of this unusual phenotype is uncertain. It is possible that these leukemias may have a normal counterpart cell in the non-T, non-B lymphocyte cell fraction of the peripheral blood that contains natural killer cells. It is also possible that these phenotypes may represent anomalous expression of T-cell or myeloid surface markers. In either event, the case presented here is of biologic interest, as it demonstrates that T-cell markers, although rare, may be expressed in the blast phase of CML.

REFERENCES

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