Membrane Marker and Cell Separation Studies in Ph1-positive Leukemia


From 32 patients with Ph1-positive leukemia whose cells reacted with a specific antiserum made against non-T, non-B acute lymphoid leukemia (ALL) cells, five typical patients were studied in detail. Three of these had had Ph1-positive chronic myeloid leukemia (CML) and later developed acute blast crisis with lymphoid involvement. The other two patients presented with acute leukemia with a mixture of myeloid and lymphoid blasts. In all five cases lymphoid blasts expressed the same phenotype as Ph1-negative non-T, non-B ALL: they reacted with anti-ALL and anti-p28,33 (anti-la) sera but lacked differentiation markers of thymocytes, B lymphocytes, and myeloid cells. In these cell populations high levels of terminal transferase enzyme activity were detected. Blast cells reacting with anti-ALL serum (ALL+) were separated from unreactive cells (ALL-) on a fluorescence-activated cell sorter. ALL+ cells had the morphology of ALL blasts, while ALL- cells appeared to be myeloid. These studies also suggest but do not prove that both ALL+ (lymphoid) and myeloid populations carried the Ph1 chromosome. The implication of these findings is that at least some Ph1-positive leukemias may arise from a prelymphoid, premyeloid progenitor and during blast crisis a variable proportion of blasts may retain their essentially stem cell-like characteristics. Since patients with lymphoid involvement sometimes respond to therapy designed for ALL, the early diagnosis is clinically important. Assays for ALL antigen and for terminal transferase have an important role in the diagnosis of lymphoid blast crisis, particularly if the lymphoblasts are present in the company of myeloid cells.

Leukemias with the Ph1 chromosome are stem cell disorders1 with a variable hematologic and clinical course. The typical form of the disease is chronic myeloid leukemia (CML), which frequently transforms into an acute phase with abundant immature myeloid elements (myeloid blast crisis). Only a few Ph1-positive leukemias present without a preceding chronic phase. Some of these are myeloblastic,2,3 but others clearly express the characteristics of acute lymphoblastic leukemia4 14 (Ph1-positive ALL). The relationship between these different forms is unknown. The view that typical CML derives from an early myeloid stem cell11,15 and that Ph1-positive ALL may derive from another, possibly prelymphoid, stem cell4 is in accord with the clinical features of most cases observed.

There are, however, observations suggesting that at least in some patients the...
Ph\textsuperscript{1} rearrangement might take place in a pluripotential stem cell progenitor of both myeloid and lymphoid stem cells.\textsuperscript{15,16} It has been known for some time that CML can transform into acute leukemias resembling lymphoid (or mixed lymphoid-myeloid) malignancies.\textsuperscript{16,18} These are referred to as lymphoid (or mixed) blast crises. A variable proportion of blast cells has been shown to have the morphologic,\textsuperscript{16,18} cytochemical,\textsuperscript{18,19} and ultrastructural\textsuperscript{20} appearance of lymphoblasts. Besides, these cell populations express the growth characteristics in vitro of Ph\textsuperscript{1}-negative ALL\textsuperscript{21} and show elevated levels of terminal deoxynucleotidyl transferase\textsuperscript{22,24} (reviewed in Ref. 25).

The first aim of this study is a detailed analysis of three patients in this particular group using membrane and enzyme markers as well as cytochemistry, routine morphology, and cytogenetics. The lymphoid blasts in Ph\textsuperscript{1}-positive leukemia react with an anti-ALL serum made against Ph\textsuperscript{1}-negative non-T, non-B ALL\textsuperscript{26} that allows the separation of these cells by the fluorescence-activated cell sorter (FACS).\textsuperscript{27}

Other suggestions for the pluripotential stem cell origin of some leukemias derive from the observation that some patients, having originally presented with Ph\textsuperscript{1}-positive ALL, develop CML.\textsuperscript{25,28} These lymphoid blasts have been shown to react with anti-ALL serum and have been found to express the phenotype of non-T, non-B ALL.\textsuperscript{25,28}

The second aim of this paper is to demonstrate that in some cases of these Ph\textsuperscript{1}-positive acute leukemias mixtures of lymphoid and myeloid blasts can be seen. Two such patients are presented. Thus the positive identification of lymphoid blasts by membrane and enzyme markers may guide rational treatment in the different forms of Ph\textsuperscript{1}-positive disease.

**MATERIALS AND METHODS**

**Hematology and Cytogenetics**

May-Grünewald-Giemsa (MGG), Sudan Black, (SB) and periodic acid-Schiff (PAS) stains were performed by standard methods. Cytogenetic analysis was carried out by a modification of the direct method of Tjio and Whang.\textsuperscript{31} Preparations were stained by Giemsa. Banding was performed in Case 1 by Dr. K. E. Buckton of the MRC Population and Cytogenetic Unit, Edinburgh and in Cases 2 and 4 by V. Pickthall of the Royal Marsden Hospital.\textsuperscript{32} Metaphases were examined for the presence of the Ph\textsuperscript{1} chromosome, and selected cells were karyotyped.

**Marker Analysis**

Immunofluorescence tests (IF) were used to detect membrane antigens. With the exception of anti-human immunoglobulin fluorescein isothiocyanate (FITC), the sera were used in indirect IF tests.\textsuperscript{35,28} \(10^6\) cells were incubated at 4°C for 25 min with rabbit antisera diluted 1:10 in 50 µl saline, washed three times, resuspended (in 50 µl), and incubated at 4°C for 25 min with goat anti-rabbit immunoglobulin FITC (1:10). After washing, the cells were studied in suspension preparations by a standard Model 18 Zeiss phase-contrast microscope equipped with IV/F epicondenser, filters for FITC, and 63x phase oil objective. Stained cell suspensions were also analyzed and separated using the FACS (FACS-I, Becton Dickinson).\textsuperscript{27} The settings used were as follows: photomultiplier tube 700 V, laser 200 mW, fluorescence gain 8:1, scatter gain 2:1. Spot diagrams were made and cells separated as described previously.\textsuperscript{33}

Anti-ALL serum was made by “coating” non-T, non-B ALL cells with rabbit anti-human lymphocyte serum and injecting them into rabbits.\textsuperscript{20} The serum was absorbed with AB red cells (three times), tonsil cells (five times), human bone marrow cells (four times), acute myeloid leukemia (AML) cells (once), and thymocytes (once). This serum specifically reacted with non-T, non-B ALL and some acute lymphoid transformations in Ph\textsuperscript{1}-positive leukemias.\textsuperscript{25,28,28}
An antiserum reacting with human T cell/thymocyte antigen (anti-HuTLA) was made by injecting $2 \times 10^8$ monkey thymocytes into rabbits.\(^34\) After absorption with red cells (three times), CLL cells (three times), B lymphoid cell line (twice), normal bone marrow (once), and AML cells (twice), the serum reacted strongly with thymocytes and ALL of thymocyte phenotype (Thy-ALL). It also reacted with T cells but not with B cells, CLL, AML, CML, and non-T, non-B ALL cells.\(^25\),\(^34\)

Heterologous anti-p28.33 serum (these antigens consist of two chains of approximate molecular weights of 28,000 and 33,000 daltons) was made in rabbits against la-like or B cell-associated antigens.\(^35\) The anti-p28.33 serum reacted strongly with B cells, non-T, non-B ALL cells, and Ph\(^1\)-positive lymphoid blast crisis cells and weakly with some AML cells and myeloblasts in Ph\(^1\)-positive myeloid blast crisis.\(^25\),\(^33\),\(^36\)

Anti-myeloid serum was made against AMML cells and absorbed with tonsil cells (seven times) and thymocytes (twice). It reacted with human bone marrow myeloid cells and AML and CML cells but not with T cells, B cells, or ALL blasts.\(^39\)

Membrane immunoglobulin (SmIg) was detected with F(ab’\(^2\)) fragments of goat anti-human Ig-FITC. The reagent was specific for B lymphocytes and B cell-derived leukemias (CLL, B lymphomas).

Normal rabbit serum (NRS) was absorbed with human tonsil cells and used as a negative control.

All antisera used were ultracentrifuged (90,000 g, 60 min, 4°C), sterile filtered, and kept at 4°C until use.

Rosetting with neuraminidase-treated sheep red cells (E rosetting) was a marker for thymocytes and T lymphocytes and Thy-ALL blast cells.\(^28\),\(^36\)

Terminal deoxynucleotidyl transferase (TdT) enzyme was measured by the method of Hutton and Coleman:\(^23\),\(^24\) $5-10 \times 10^6$ leukocytes were used in each test. This marker for cortical (cortisone sensitive) thymocytes and prethymocytes was absent from medullary (cortisone resistant) thymocytes and peripheral T and B lymphoid cells.\(^41\) The enzyme was also present in lymphoid leukemias (including non-T, non-B and Thy-ALL)\(^22\),\(^25\) and in some acute undifferentiated leukemias\(^42\) but not in AML or in B cell-derived malignancies.\(^22\),\(^24\)

CASE REPORTS

Hematologic findings in five cases are summarized in Table 1.

Case I

C.T., a female age 13 yr, presented with a 2-mo history of lassitude, weakness, and feverishness (October 1975). Examination showed splenomegaly but no lymphadenopathy. The white blood cell count (WBC) was $512 \times 10^9$/liter, predominantly granulocytes (Fig. 1A). The bone marrow was hypercellular with $96\%$ granulocytes and precursors. Cytogenetic studies indicated the presence of the Ph\(^1\) chromosome. The patient was controlled with busulfan and mercaptopurine and subsequently with 6-wk courses of melphalan, hydroxyurea, dibromomannitol, and cyclophosphamide.

Fourteen months after diagnosis the WBC started to rise ($46 \times 10^9$/liter) but still showed predominantly myeloid elements, with $86\%$, granulocytes. Three weeks later, however (12/28/76), the WBC had risen to $110 \times 10^9$/liter, of which $90\%$, were lymphoid blasts (Fig. 1B). This cell population was shown to contain the Ph\(^1\) chromosome. She was treated with vincristine and prednisolone and the blasts disappeared within a week. Pancytopenia developed, which precluded any cytotoxic therapy. On 1/17/77 the bone marrow showed no blast cell infiltration but aplasia, and by 3/30/77 there was partial recovery of erythropoiesis and granulopoiesis, although megakaryocytes were still severely depressed. Then (April 1977) her WBC started to rise, with predominantly myeloid cells (WBC $66 \times 10^9$/liter). One week later (4/13/77) $30\%$, blast cells appeared (WBC $250 \times 10^9$/liter), which were lymphoid. She was again treated with prednisolone and vincristine, and the blast cells disappeared from the blood. On this occasion, despite continued thrombocytopenia, maintenance treatment was instituted with daily mercaptopurine and weekly methotrexate. This therapy has controlled the level of WBC to date, and there is currently no evidence of either myelocytes or blast cells in the blood. However, after a period of intermittent headache and vomiting, her cerebrospinal fluid (CSF) was found to contain 1600 blast cells/cu mm (7/12/77). Her meningeal leukemia was successfully treated with intrathecal methotrexate and craniospinal irradiation.
Table 1. Hematologic Findings At Presentation and in Blast Crisis

<table>
<thead>
<tr>
<th>Case</th>
<th>Date</th>
<th>Source of Sample</th>
<th>Patient Status</th>
<th>Site*</th>
<th>Differential White Cell Count (%)</th>
<th>WBC (x 10^9/liter)</th>
<th>Platelets (x 10^9/liter)</th>
<th>Hb (g/dl)</th>
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<tr>
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<td>Lymphoid Cells</td>
<td>Blasts</td>
<td>Promyelocytes</td>
<td>Myelocytes</td>
<td>Metamyelocytes</td>
<td>Neutrophils</td>
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<td>1</td>
<td>10/9/75</td>
<td>Presentation</td>
<td>PB</td>
<td>3</td>
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<td>6</td>
<td>30</td>
<td>11</td>
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<td></td>
<td></td>
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<td>0</td>
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<td>2</td>
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<td>29</td>
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<td>6</td>
<td>2</td>
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<td>4/1/74</td>
<td>Presentation</td>
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<td>7</td>
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<td>98</td>
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</table>

*PB, peripheral blood; BM, bone marrow.
†For morphology and cytochemistry of blast cells see text and Table 2.
‡Occasional megakaryocytes.
§Occasionally seen.
Case 2

J.B., a male age 37 yr. presented with high count CML (WBC 385 x 10⁹/liter, April 1974). The marrow showed increased cellularity and prominent granulopoiesis. The hemoglobinization in normoblasts was depressed and megakaryocytes were abnormally small. Cytogenetic studies showed the presence of the Ph¹ chromosome. The patient was controlled with busulfan for 17 mo, after which WBC and platelet counts started to rise (platelets 1470 x 10⁹/ml, 12/19/75), and within 3 wk large proportions of blasts had appeared in the peripheral blood (47 x 10⁹/liter blasts) and the marrow showed many blasts with grossly abnormal but still very active granulopoiesis (1/10/76). The proportion of blasts in the marrow increased by 2/3/76 to 95%.

On combination chemotherapy with vincristine, prednisolone, and L-asparaginase the WBC fell and blast cells disappeared from the blood (3/15/76); the bone marrow, however, showed predominantly blast cells with lymphoid morphology, although a few cells had the appearance of myeloblasts. The lymphoid cells lacked azurophil granules, were SB and peroxidase negative, and PAS positivity was seen in 15% blasts. The number of blast cells increased, and they soon replaced the residual differentiating erythroid and granulopoietic elements (5/3/76). At this stage the bone marrow was analyzed by markers (9/1/76; see Results). The peripheral blast cell counts rose terminally to 244 x 10⁹/liter (96% blast, 4% lymphocytes (9/20/76).

Case 3

J.P., a 33-yr-old black male, presented with a 1-mo history of headache and weakness, and examination showed hepatosplenomegaly but no lymphadenopathy (May 1974). The WBC count
was 115 x 10^9/liter (predominantly granulocytes), and the bone marrow was typical of CML. He was treated with busulfan and remained in clinical remission for 14 mo, when hepatosplenomegaly and lymphadenopathy developed. At this stage, the bone marrow showed 93% blasts, most of which appeared undifferentiated with fine nuclear chromatin and two or three nucleoli, had no azurophilic granules and were negative with peroxidase, SB, and acid phosphatase. Only a few (5%) had fine PAS-positive granules (9/25/75; see Results for marker analysis). All 25 metaphases studied showed the 46.XY,Ph+ karyotype. The peripheral blood count revealed, in addition to 34%, blasts, large numbers of granulocytes and their precursors. Elevated levels of B12-binding protein (3700 pg/ml) and serum lysozyme (36 μg/ml) also reflected an increased granulocyte mass at the onset of blast crisis (normal 800–1650 pg/ml and 4–10 μg/ml, respectively).

The patient was treated with three courses of TRAMPOL (thioguanine, rubinomycin, cytosinarabinoside, methotrexate, prednisolone, cyclophosphamide, vincristine, asparaginase) and the blast cells disappeared from the peripheral blood films. The patient died of gram-negative sepsis after returning to Africa (12/26/75).

Case 4

P.S., a female age 52 yr, presented with a 3-mo history of malaise and symptoms of anemia, and had generalized lymphadenopathy without hepatosplenomegaly. In the bone marrow 94% blast cells were seen, appearing to be mostly myeloid (3/20/76; Fig. 2). The majority of them were SB

![Fig. 2. Photomicrograph of bone marrow cells from case 4 at presentation. Myeloblasts are conspicuous, but some blast cells are unidentifiable and have lymphoid or micromyeloblast appearance (arrows). Proportion of ALL blasts in this particular sample higher than proportion of lymphoblasts and micromyeloblasts, implying that some myeloblasts also probably were ALL. These blasts did not react with an antimyeloid serum. PAS positivity and SB negativity were in accord with the membrane marker results, and the clinical course of the patient showed lymphoid involvement (see Fig. 3 and Table 3). x 1500.](http://www.bloodjournal.org)
MEMBRANE MARKERS: Ph<sup>1</sup>-POSITIVE LEUKEMIA

negative; about 40<sup>%</sup>, showed strong granular PAS positivity. The cytogenetic studies showed the presence of Ph<sup>1</sup> chromosome in each cell studied and a diagnosis of Ph<sup>1</sup>-positive leukemia was made. The patient received combination chemotherapy including vincristine and prednisolone, following which the peripheral blasts disappeared rapidly and lymphadenopathy resolved. Four weeks later bone marrow examination showed 5<sup>%</sup>, blasts and a normal karyotype (8/16/76), but 1 wk later in a count of 20 metaphases, 2 cells were again Ph<sup>1</sup> positive (8/24/76; Table 4). Shortly after completing two further courses of therapy, she relapsed with 51<sup>%</sup>, blast cells in the marrow; 15<sup>%</sup>, were myeloid in appearance and 36<sup>%</sup>, were lymphoid (10/19/76; see Table 2 for marker analysis). Cytogenetic analysis showed the Ph<sup>1</sup> chromosome in each cell studied. Further treatment resulted in only a temporary fall in the blast cell count, and a marrow count 6 wk later showed 50<sup>%</sup>, blasts and differentiating myeloid and erythroid elements (11/30/76). This sample was analyzed by FACS (Table 3, Fig. 3). During the final 4 mo the proportion of blasts in her bone marrow continued to increase despite various chemotherapy regimes, and terminally the WBC was 356 x 10<sup>9</sup>/liter (96<sup>%</sup>, blasts, 3/28/77).

Case 5

W.H., a female age 52 yr, presented with bruising and anemia and had generalized lymphadenopathy and hepatosplenomegaly. In the bone marrow 98<sup>%</sup>, blasts were seen, two-thirds of which were morphologically myeloblasts. The remainder were smaller lymphoid blasts with less prominent nuclei and practically no cytoplasm. The SB was negative and the PAS showed granular positivity in about 40<sup>%</sup>, of the blast cells of both morphologic types (8/25/76; see Results for marker analysis). Cytogenetic studies showed the presence of either single or double Ph<sup>1</sup> chromosomes in every cell examined. She was treated with vincristine and prednisolone. The peripheral blast cells disappeared rapidly, and marrow examination 3 wk later showed 4.5<sup>%</sup>, blasts and the loss of Ph<sup>1</sup> chromosomes with a normal karyotype (9/14/76; Table 4). On maintenance therapy, her peripheral blood count remained normal, and marrow examination showed a normal karyotype and a slight increase (6<sup>%</sup>, 14<sup>%</sup>,) in blast cells, two-thirds of which were myeloid in appearance (9/28/76 - 1/25/77). Six months later she developed meningeal leukemia with the blast cells in the CSF having lymphoid morphology (2/14/77). This episode was treated successfully by intrathecal chemotherapy, and as of 9 mo after the initiation of therapy she continued in clinical remission.

RESULTS

Membrane and Enzyme Marker Analysis (Table 2)

Samples taken during blast crisis were analyzed. In all patients large proportions of cells reacted with an anti-ALL serum (ALL<sup>+</sup>: 48<sup>%</sup>, 90<sup>%</sup>,) and with anti-p28,33 serum (p28,33<sup>+</sup>: 50<sup>%</sup>, 97<sup>%</sup>,). The levels of TdT were elevated and were more than ten times higher than the highest values observed in AML and myeloid blast crisis. The diagnosis of lymphoid blast crisis was made in accord with the morphologic and cytochemical data (see qualifying note in legend for Fig. 2).

The majority of leukemic blast cells did not exhibit differentiation markers that characterize thymocytes and T lymphocytes (i.e., E rosetting and anti-HuTLA serum) or B lymphocytes (SmIg), and they did not react with anti-myeloid serum.

The bone marrow samples showed some (<15<sup>%</sup>,) larger cells with granular cytoplasm that reacted with antimonyeloid serum. A few (20<sup>%</sup>,) E rosette positive, anti-HuTLA<sup>+</sup> positive cells (presumably normal T lymphocytes) were present in the blood sample from patient 5. It was also likely that the marrow from patient 5 contained a subset of E rosette negative leukemic blasts of intermediate size that reacted weakly with anti-HuTLA.
<table>
<thead>
<tr>
<th>Case 1*</th>
<th>Case 2†</th>
<th>Case 3*</th>
<th>Case 4†</th>
<th>Case 5*</th>
<th>Non T, Non B</th>
<th>AML and Myeloid Blast Crisis†</th>
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<tr>
<td>First Blast Crisis (12/29/76)</td>
<td>Second Blast Crisis (4/13/77)</td>
<td>Case 2† (2/3/76)</td>
<td>Case 3* (9/25/75)</td>
<td>Case 4† (10/19/76)</td>
<td>Case 5* (8/25/76)</td>
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<td>Anti-ALL (ALL§)</td>
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<td>20% (1–2)</td>
<td>48% (1–3)</td>
<td>50% (2)</td>
<td>56% (3)</td>
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<td>35% (2–4)</td>
<td>97% (2–4)</td>
<td>95% (2–4)</td>
<td>60% (2–4)</td>
<td>80% (2–4)</td>
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<td>9%</td>
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<td>20%</td>
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<td>Anti-HuTLE (HuTLE)</td>
<td>2%</td>
<td>10% (2)§</td>
<td>19% (1)§</td>
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<td>&lt;1%</td>
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<td>118</td>
<td>175</td>
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<td>tidyl transferase (TdT) (U/10⁶ cells)</td>
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NT, not tested.
* Blood sample.
† Bone marrow sample.
§ See Refs. 25, 33, and 43.

† Intensity of fluorescent staining is shown in parentheses by an arbitrary scale based on FACS observations: 1, dim cells; 4, very bright cells.
§ See Refs. 23 and 24. Normal range of bone marrow in our hands, 0–2 U/10⁶ cells. 24
Morphologic Analysis of ALL⁺ and ALL⁻ leukemic cells (Fig. 3, Table 3)

Cells that stained with anti-ALL serum were analyzed on the FACS; spot diagrams, indicating the interrelationship of fluorescence and cell size, are shown in Fig. 3. Many relatively small and intermediate-sized blast cells were ALL⁺. Larger cells and some of intermediate size were ALL⁻. The distinction between the ALL⁺ and ALL⁻ fractions was particularly clear in samples taken from patients 1 and 4, while there was a continuous spectrum in samples from patients 2 and 5.

The ALL⁺ and ALL⁻ cells were separated physically on the FACS and smears were made (Fig. 3). Most of the ALL⁺ cells had lymphoblastic morphology resembling typical ALL, although some small lymphocytes were present. In smears of the ALL⁻ fractions myeloblasts were conspicuous. Most of these were positive with anti-p28,33 serum. Smears of the ALL⁻ cells from the bone marrows of patients 1 and 4 contained only a few myeloblasts but many differentiating myeloid cells and some erythroid precursors. The latter cell types were negative with anti-p28,33 serum.

Cytogenetic Analyses (Table 4)

In patient 1 the dominant karyotype was 46,XX,Ph¹. The Ph¹ chromosome was seen in all but one analyzable metaphases during the chronic phase. During blast crisis only peripheral blood was studied, and the Ph¹ chromosome was seen in 11 of the 13 metaphases studied.

In patient 2 the dominant karyotype was 46,XY,9q+,22q-. The Ph¹ chromosome was observed in all analyzable metaphases during the chronic phase, blast crisis, and relapse. During relapse ALL⁺ and ALL⁻ fractions were separated by the FACS, and mitoses were more frequent in the ALL⁺ than in the ALL⁻ populations (Table 3).

In patient 4 at presentation and in relapse the Ph¹ chromosome was seen in all of the bone marrow cells examined. Virtually all metaphases showed one additional marker chromosome, which in some cells was of G group size and in others of C group size. During the relapse ALL⁺ and ALL⁻ cells were separated on FACS (Table 3). Mitoses were seen in both fractions but were nevertheless not analyzable. During later stages of the disease in the peripheral blood 96% of cells were ALL⁺, and the Ph¹ chromosome was found in all cells examined. The dominant karyotype was 47,XX,9q+,22q-,mar+, the extra marker being metacentric and of C group size (Lawler SD, Pickthall V: personal communication).

Patient Monitoring (Table 4)

In some Ph¹-positive acute leukemias two independent leukemia-associated markers were available (the Ph¹ chromosome and anti-ALL reactivity). The two markers gave parallel observations. Following treatment in case 4, 0.6% ALL⁺ cells persisted. A week later 1% ALL⁺ cells were accompanied by the reappearing Ph¹ chromosome, and the patient soon fully relapsed. In contrast, repeated marrow samples from patient 5 showed no evidence of the Ph¹-positive ALL⁺ clone. Thus in this patient the few blast cells observed in the bone marrow were considered probably to be normal regenerating elements.
Table 3. Morphology of ALL+ and ALL− Cells Separated on the Fluorescence Activated Cell Sorter

<table>
<thead>
<tr>
<th>Case</th>
<th>Separated</th>
<th>Unseparated</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (4/13/77)</td>
<td>13 2 12</td>
<td>20* 20</td>
</tr>
<tr>
<td>2 (9/1/76)</td>
<td>21 90 4</td>
<td>17 50</td>
</tr>
<tr>
<td>4 (11/30/76)</td>
<td>54 2 65</td>
<td>2 1</td>
</tr>
<tr>
<td>5 (8/25/76)</td>
<td>2 0 4</td>
<td>0 1</td>
</tr>
</tbody>
</table>

Lymphocytes
- Unseparated: 13, 21, 54, 2
- Separated: 20*, 4

Lymphoblasts
- Unseparated: 12, 17, 50, 2
- Separated: 20*, 4

Unidentifiable blasts
- Unseparated: 5, 4, 5, 35
- Separated: 16, 33

Myeloblasts
- Unseparated: 5, 4, 35, 2
- Separated: 16, 33

Myeloid cells
- Unseparated: 5, 1, 17, 17
- Separated: 6, 52

Erythroid precursors
- Unseparated: 2, 0, 4, 19
- Separated: 3, 26

Mitotic index
- Case 1: NT
- Case 2: NT
- Case 4: NT
- Case 5: 0.3

TdT activity
- Case 1: 41.5
- Case 2: 114
- Case 4: 118
- Case 5: NT

DISCUSSION

The anti-ALL serum used in this study has been made against Ph1-negative non-T, non-B ALL.26 This reagent was unreactive with AML, myeloid blast crisis, most cases of ALL of thymocyte phenotype (Thy-ALL), and with normal lymphoid and bone marrow cells.4 The specificity of the anti-ALL serum was confirmed here (Fig. 3). Cells were taken during the acute phase of Ph1-positive leukemias, incubated with the antiserum, and separated on the FACS according to their staining intensity. Positively stained bright cells were predominantly lymphoblasts. Nonstaining or dimly stained cells included a few lymphoblasts and were mostly myeloblasts and differentiating myeloid elements. In some leukemic cell populations there was a continuous transition between ALL+ lymphoid and ALL− myeloid forms, with some overlap of unidentifiable blast cells.

Bone marrow cells from cases 2 and 4 and blood cells from cases 1 and 5 were labeled with anti-ALL serum and separated into ALL+ and ALL− fractions as shown in Fig. 3. Smears were prepared and stained with MGG. Lymphoid blasts were ≤10 μm, had a high nuclear/cytoplasm ratio, and only one or no nucleolus could be seen (Figs. 3B and 3E). Myeloblasts were >11 μm and had more extensive cytoplasm and two or three nucleoli (Figs. 2 and 3C). In cases 1 and 2 blasts were essentially negative with PAS and Sudan black staining (although patient 2 had had some PAS-positive cells at an earlier stage of the disease). In cases 4 and 5 PAS positivity was seen in lymphoblasts and also in other blasts with myeloid appearance (see Case Reports). NT, not tested.

*Some of these lymphocytes are almost certainly leukemic and are p28,33 E. These cells are blood T lymphocytes (E rosette ) that accumulate in the ALL− fraction.
†At the time of investigation all analyzable mitoses in the patients' bone marrow were Ph1 positive (see Table 4).

Fig. 3. Separation of cells using anti-ALL serum. Cells from case 2 (A-C) and case 4 (D-F) labeled with anti-ALL serum in indirect immunofluorescence test and processed on the fluorescence activated cell sorter. A and D: two-dimensional spot diagrams of cell size (x axis) and intensity of fluorescence (y axis). Cells were sorted into ALL+ and ALL− fractions and MGG smears were made: B and E, ALL+ cells; C and F, ALL− cells. Some cells with intermediate staining were discarded (shaded area). Cells from case 5 gave findings similar to those from case 2 (A-C). ×1160.
<table>
<thead>
<tr>
<th>Case</th>
<th>Date</th>
<th>Stage</th>
<th>Source of Cells</th>
<th>Blasts (%)</th>
<th>ALL⁺ Cells (%)</th>
<th>No. of Metaphases Studied</th>
<th>No. of Ph⁻¹-positive Cells</th>
<th>Karyotype of Selected Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>12/19/75</td>
<td>Chronic phase</td>
<td>BM</td>
<td>1</td>
<td>NT</td>
<td>7</td>
<td>All cells</td>
<td>46,XY,Ph⁻¹ (two octaploid Ph⁻¹ pos)</td>
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<tr>
<td>2</td>
<td>2/3/76</td>
<td>Blast crisis</td>
<td>BM</td>
<td>78₁</td>
<td>45</td>
<td>8</td>
<td>All cells</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>5/3/76</td>
<td>Relapse</td>
<td>BM</td>
<td>80₁</td>
<td>38</td>
<td>9</td>
<td>All cells</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>7/2/76</td>
<td>Relapse</td>
<td>BM</td>
<td>66₁</td>
<td>35</td>
<td>8</td>
<td>All cells</td>
<td>46,XY,9q+,22q⁻ (Ph⁻¹)</td>
</tr>
<tr>
<td>9</td>
<td>9/1/76</td>
<td>Relapse</td>
<td>BM</td>
<td>94₁</td>
<td>48₁</td>
<td>NT</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>9/6/76</td>
<td>Relapse</td>
<td>BM</td>
<td>90₁</td>
<td>50</td>
<td>NT</td>
<td>—</td>
<td></td>
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<td>4</td>
<td>3/20/76</td>
<td>Presentation, in acute phase</td>
<td>BM</td>
<td>84</td>
<td>52</td>
<td>20</td>
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<td>46,XX,Ph⁻¹ (one cell)</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>47,XX,C +,Ph⁻¹ (three cells)</td>
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<td>47,XX,G +,Ph⁻¹ (three cells)</td>
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<tr>
<td></td>
<td>8/16/76</td>
<td>Remission</td>
<td>BM</td>
<td>5</td>
<td>0.5</td>
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<td>None</td>
<td>46,XX</td>
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<td>8</td>
<td>8/24/76</td>
<td>Remission</td>
<td>BM</td>
<td>3</td>
<td>1</td>
<td>20</td>
<td>2 (10%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8/19/76</td>
<td>Relapse</td>
<td>BM</td>
<td>51</td>
<td>56</td>
<td>15</td>
<td>All cells</td>
<td>47,XX,C +,Ph⁻¹ (one cell)</td>
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<tr>
<td>10</td>
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<td>BM</td>
<td>57</td>
<td>56₁</td>
<td>11</td>
<td>All cells</td>
<td>47,XX,C +,9q+,22q⁻ (Ph⁻¹)</td>
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<tr>
<td>11</td>
<td>1/18/77</td>
<td>Relapse</td>
<td>Blood</td>
<td>95</td>
<td>95</td>
<td>9</td>
<td>All cells</td>
<td>47,XX,C +,9q+,22q⁻ (Ph⁻¹) (one cell)</td>
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<tr>
<td>5</td>
<td>8/25/76</td>
<td>Presentation, in acute phase</td>
<td>BM</td>
<td>93</td>
<td>60₁</td>
<td>16</td>
<td>All cells</td>
<td>46,XX,Ph⁻¹ (one cell)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5 (1 Ph⁻¹)</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>11 (2 Ph⁻¹)</td>
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<td>9</td>
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<td>BM</td>
<td>4.5</td>
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<td>9/28/76</td>
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<td>BM</td>
<td>6</td>
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<td>None</td>
<td>46,XX,C +,G +,2 Ph⁻¹ (two cells)</td>
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<tr>
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<td>BM</td>
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<td>None</td>
<td>46,XX (three cells)</td>
</tr>
<tr>
<td></td>
<td>11/23/76</td>
<td>Remission</td>
<td>BM</td>
<td>6</td>
<td>&lt;0.5</td>
<td>12</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>1/25/77</td>
<td>Remission</td>
<td>BM</td>
<td>14</td>
<td>&lt;1</td>
<td>NT</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>2/14/77</td>
<td>CSF relapse</td>
<td>CSF</td>
<td>80</td>
<td>70</td>
<td>NT</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>3</td>
<td>3/25/77</td>
<td>Remission</td>
<td>BM</td>
<td>3</td>
<td>&lt;0.5</td>
<td>18</td>
<td>None</td>
<td></td>
</tr>
</tbody>
</table>

NT, not tested; BM, bone marrow; CSF, cerebrospinal fluid.
*See Tables 1 and 2 for further results.
¹Most of the other cells were smaller lymphoid cells that were ALL+ and p28,33+. The proportion of mature myeloid cells was <0.6%.
²These samples were also analyzed by the FACS (see Table 3).
with anti-ALL serum (Fig. 2). It is interesting to note, however, that these myeloblasts were SB negative, but some of them were PAS positive. The clinical follow-up confirmed the diagnosis of lymphoid involvement (Fig. 3). Previous crossabsorption studies have shown that the membrane antigens detected by anti-ALL serum on Ph' negative ALL and Ph' positive leukemias are fully shared.44

Cell separation in one experiment indicated that ALL + lymphoblasts contained particularly high levels of TdT enzyme (Table 3). (The study did not exclude, however, the possibility that in these patients some blast cells with myeloid morphology also expressed some “residual” TdT activity.) High levels of TdT in lymphoblasts is in line with the good correlation between these two independent markers in >90% of Ph' positive leukemias.24

Furthermore, ALL + lymphoblasts strongly expressed the p28,33 (la-like) antigen,* but lacked differentiation markers that characterize B lymphocytes (i.e., surface immunoglobulin), thymocytes and T lymphocytes (E rosette and human T lymphocyte antigen), and myeloid cells (e.g., myeloid antigen, Sudan black, and peroxidase positivity). The same phenotype (ALL +, TdT +, p28,33 +, SmIg -, E -, HuTLA -, My -) has been observed in most cases of Ph' negative non-T, non-B ALL25'45 (see below).

In these studies lymphoid leukemic blasts were positively identified in patients who initially presented with CML or had acute leukemic blasts with myeloid morphology. The question therefore arises as to whether the myeloid and lymphoid leukemic cells belong to the same clone or represent independent leukemias.

Conventional membrane marker analysis is incompatible with karyotyping. Furthermore, cells transferred between different institutes and then stained by indirect IF and sorted on FACS fail to yield chromosome spreads of sufficiently high quality. However, there is indirect evidence that strongly suggests that in the patients studied both myeloid and lymphoid leukemic cells are Ph' positive. First, patients in overt lymphoid blast crisis with >85%, ALL + lymphoblasts (patient 1 on 12/22/76 and patient 4 on 1/18/77) retained the Ph' chromosome in all but two metaphases observed. This finding is in keeping with observations made in other patients (Table 5).47,48 These findings do not exclude, however, the possibility that in rare cases a Ph' positive myeloid disorder and Ph' negative lymphoid leukemia can arise independently.49 Second, three cell suspensions were studied that in fresh samples contained 100%, Ph' positive karyotypes. Immediately after FACS separation considerable mitotic activity was seen in the ALL + fraction (Table 3), suggesting that ALL + cells contributed to the Ph' positive metaphases observed.

The simplest explanation is that ALL + lymphoid and myeloid leukemic populations derive from the same Ph' positive clone and represent different

*Unlike anti-ALL serum and TdT activity, anti-p28,33 serum does not distinguish between lymphoid and myeloid blast crises.33 ALL blasts and many immature myeloblasts (including AML and normal myeloblasts) are p28,33 +, although the myeloblasts react weakly.33,36,37 Differentiating myeloid elements are p28,33 negative.33,38 This reagent is important to help in the diagnosis of acute leukemia and in excluding the possibility that leukemic blasts have a thymocyte phenotype.
Table 5. Spectrum of Ph\(^1\)-positive ALL \(^*\) Lymphoid Leukemias

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Patients</th>
<th>Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16</td>
<td>Typical CML evolving into lymphoid blast crisis. Similar to cases 1–3, although six patients had &lt;15% ALL (^*) blasts. Of the nine cases tested, with one exception, all retained the Ph(^1) chromosome during lymphoid crisis.</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>Acute or subacute leukemia with mixtures of myeloid, lymphoid, and unidentified blast cells. Similar to cases 4 and 5.</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>ALL with minimal or no myeloid abnormalities.</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>ALL with minimal or no myeloid abnormalities, but subsequently developing CML.</td>
</tr>
</tbody>
</table>

Analysis of 32 patients (from various U.K. hospitals) studied at the ICRF. Three patients with lymphoid blast crisis who were ALL \(^-\) and many more with myeloid blast crisis were not included. The overall proportion of patients with ALL \(^*\) lymphoid crisis amongst Ph\(^1\)-positive patients is probably <20%. The exact proportion is not known, since cases with lymphoid and undifferentiated morphology were preferentially referred to us.

stages of clonal evolution. We have already expressed our view that the so-called lymphoid leukemic blasts of non-T, non-B phenotype may derive from rare normal cells of similar phenotype and retain these essentially stem cell-like characteristics. [The corollary of this view is that ALL antigen (detected by anti-ALL serum), p28,33 antigen, and TdT enzyme are markers for pluripotential stem cell(s) and/or their close relatives.25,28,33,43,45] Thus both “lymphoid” and myeloid blasts may be derivatives of a pluripotential (prelymphoid, premeloid) stem cell, the “lymphoid” blasts having been fixed or arrested at an early stem cell developmental stage (or perhaps at a prelymphoid stem cell stage). The alternative possibility, that some patients have an inherent susceptibility for repeated Ph\(^1\) translocations in different stem cells, is less likely because in some leukemic populations the transition between the lymphoid and myeloid elements has been continuous (Fig. 3), and because chromosomal evidence32,49 as well as studies with glucose-6-phosphate dehydrogenase heterozygotes1 indicate a unicellular origin of CML.

During the last 2 yr, 32 cases of Ph\(^1\)-positive leukemias were found to react with anti-ALL serum in the ICRF laboratory (Table 5). Sixteen patients had CML that evolved into acute blast crisis with lymphoid involvement. This group included eight patients with frank lymphoid or myeloid-lymphoid blast crisis, such as patients 1–3. Six of these patients had only a few (<15%) ALL \(^*\) blasts. In these cases the diagnosis by hematologic criteria or by a gross enzyme assay, such as TdT test, was frequently equivocal. Nevertheless, some of these patients responded well to vincristine and prednisolone therapy (see below).

Of the other sixteen patients who presented with acute or subacute leukemia, five had a mixture of myeloid and lymphoid blast cells similar to patients 4 and 5. Eleven patients presented with Ph\(^1\)-positive ALL, three of whom later developed CML. Thus one of the striking findings was the extraordinary heterogeneity of CML and Ph\(^1\)-positive acute leukemia with a considerable proportion of transitional forms between myeloid and so-called lymphoid leukemias.

Finally, there is clinical importance in recognizing lymphoid involvement in Ph\(^1\)-positive leukemia. CML blast crisis in general is notoriously unresponsive...
MEMBRANE MARKERS: Ph'-POSITIVE LEUKEMIA

875
to chemotherapy, but the lymphoid and mixed lymphoid-myeloid types may respond dramatically to ALL therapy. Case I was rather exceptional in the sense that both lymphoid blasts and myeloid elements disappeared with vincristine and prednisolone treatment and severe aplasia developed. It is also interesting that in Ph'-positive acute leukemias full remission can be achieved with ALL therapy with the disappearance of the Ph1 chromosome, even if some of the blast cells at presentation show myeloblast cytomorphology (case 5). Favorable responses with prolongation of survival have been noted in lymphoid blast crisis by others. Nevertheless, the response in many cases has been only transient (cases 2 and 4), and in general the prognosis of Ph1-positive lymphoid blast crisis and Ph1-positive ALL is worse than the prognosis of Ph1-negative lymphoid leukemia of non-T, non-B type.10,11

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REFERENCES

15. Boggs DR: The pathogenesis and clinical


Membrane marker and cell separation studies in Ph1-positive leukemia

G Janossy, RK Woodruff, A Paxton, MF Greaves, D Capellaro, B Kirk, EM Innes, OB Eden, C Lewis, D Catovsky and AV Hoffbrand