Ph[1]-positive Childhood Leukemias: Spectrum of Lymphoid-Myeloid Expressions

By Edwin N. Forman, Teresita Padre-Mendoza, Peter S. Smith, Barbara E. Barker, and Patricia Farnes

Two children presented with Ph[1]-positive leukemia, confirmed by Giemsa banding as 22q−. One child showed an initial presentation characteristic of acute lymphoblastic leukemia, followed by development of chronic myelocytic leukemia 2 yr later. A second child presented in blast crisis. Both patients showed blast cells possessing both lymphoid and myeloid characteristics, as demonstrated by histochemical, biochemical, or surface receptor properties of each cell series. The evidence provided supports the assumption of mixed lymphoid-myeloid properties of blast cells in chronic myelocytic leukemia in children. Detailed study of the leukemic cells may aid in the understanding of complex stem cell relationships and suggest more effective therapeutic approaches.

STEM CELL IDENTIFICATION in certain leukemic patients, and especially in patients with chronic myelocytic leukemia (CML) in blast crisis, has recently been the subject of renewed interest. Previously, it had been assumed that the blasts in blast crisis of CML were of myeloid derivation. In 1974, however, Boggs[1] pointed out that leukemic stem cells in certain cases of CML might possess pluripotent (i.e., lymphoid) differentiation capacity. This circumstance was suspected to be the case in some patients in whom evidence suggesting lymphoid cell identity was derived from morphologic impressions and from unexpected responses to "lymphoid leukemia" therapy. Since then, a variety of clinical and laboratory results have lent support to this concept in substantial minorities of CML blast crisis patients.

Some recent experiences with Ph[1]-positive leukemias in our pediatric patients have led us to reevaluate the diagnosis in two cases. In these patients, the new considerations of pluripotent stem cell properties and application of newly developed cytogenetic techniques were found to be relevant to the clinical diagnosis and management. Our observations suggested that, in some cases of CML, blast cells may possess lymphoid properties or combined lymphoid-myeloid characteristics.

MATERIALS AND METHODS

Chromosome preparations were made from direct bone marrow, from direct peripheral blood if blast cells were present, from 2- and 3-day cultures in vitro of peripheral blood with and without phytohemagglutinin (PHA) cultivated by a modified method of Moorhead et al.[2] and from skin fibroblasts. The G-banding technique (GTG) was a modification of Seabright's method.[3] For Q banding, the procedure followed was that of Caspersson et al.[4] Nonspecific esterase,[5] naphthol AS-D chloroacetate esterase,[5] peroxidase,[6] Sudan black B,[6] and periodic acid Schiff reaction[7]

From the Departments of Pediatrics, Pathology, and Oncology, Rhode Island Hospital, and Division of Biomedical Sciences, Brown University, Providence, R.I.

Submitted June 14, 1976; accepted December 13, 1976.

Address for reprint requests: Edwin N. Forman, Director, Division of Pediatric Hematology-Oncology, Rhode Island Hospital, 593 Eddy St., Providence, R.I. 02902.

were demonstrated in leukemic blast cells from bone marrow and/or peripheral blood. Sheep erythrocyte rosetting of blast cells was demonstrated by a modification of the technique of Steel et al.[8] Assays of terminal deoxynucleotidyl transferase activity (TdT) of bone marrow and blood blasts were kindly performed by Dr. Ronald McCaffrey of the Massachusetts Institute of Technology. The range of activity was 0.8–2.1 TdT units/10^8 cells in thymocytes and <0.004 units in normal circulating leukocytes.[9]

CASE REPORTS

Patient 1. C.F., a 4-yr-old white female, was admitted to the Rhode Island Hospital in January 1970. The clinical course and pertinent laboratory findings are summarized in Fig. 1. Physical findings on admission included pallor and right knee tenderness; x-rays revealed lytic bone lesions. The peripheral blood showed blasts with lymphoid features, including deep nuclear clefts and cytoplasmic blebs (Fig. 2). A bone marrow biopsy revealed blastic replacement, and karyotype of the direct bone marrow prior to treatment showed a clone with a G chromosome long arm deletion. Subsequent Giemsa banding established the presence of 22q– with translocation to chromosome 9 (Fig. 3). Initially, on the basis of blast morphology and clinical presentation, a diagnosis of ALL had been made, and treatment begun on ALGB protocol 6801. Rapid improvement occurred with initial L-asparaginase, and complete remission was achieved with vincristine, prednisone, and daunorubicin. Remission continued for 2 yr on methotrexate maintenance and vincristine-prednisone reinduction therapy (without daunorubicin). After this period, a "transitional" phase began, with adenopathy, splenomegaly, intermittent granulocytic leukocytosis, marked thrombocytosis, and progressive myeloid hyperplasia. The fetal hemoglobin at this time was 11%. Leukocyte alkaline phosphatase activity tested on four occasions showed scores of 50, 20, 22, and 2. After March 1973, frank CML was obvious with increasing splenomegaly, rising leukocyte and platelet counts, and ultimately, increasing blasts in the peripheral blood (Fig. 4). Histochemistry of these blast cells showed peroxidase activity and naphthol AS-D chloroacetate in a majority of blast cells. Some cells were positive for nonspecific esterase; glycogen was not demonstrable. The patient responded to busulfan, and was maintained on busulfan, Ara-C, and CCNU until blastic relapse 9 mo later. During the blast phase, karyotype of the leukemic cells showed presence of a single Ph^1 and aneuploidy. Serum vitamin B_{12} level, which was 696 pg/ml during a period of remission, was 1689 pg/ml during this aggressive stage. Blast cell terminal transferase was markedly increased (1.8 units/10^8 cells). Blast cells showed no sheep erythrocyte rosetting and absence of immunoglobulin receptor sites. Brief responses were obtained during the ultimate blastic phase with vincristine and prednisone, but the patient succumbed 5 yr after the original diagnosis.

Patient 2. The course and laboratory data of A.L., a 9-yr-old white male, are summarized in

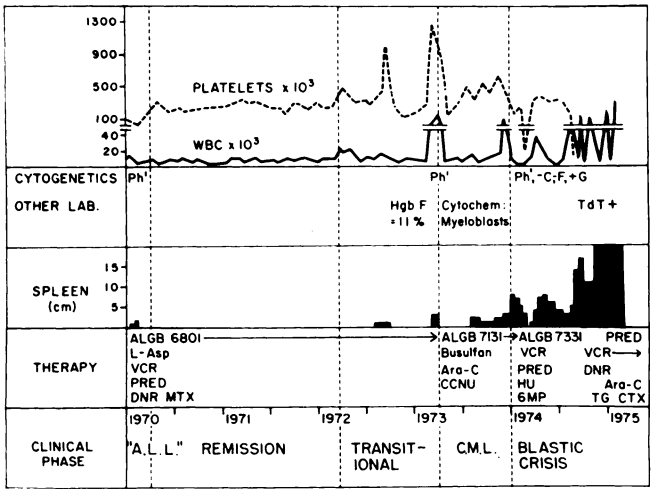

Fig. 1. Clinical course of case 1 (C.F.).

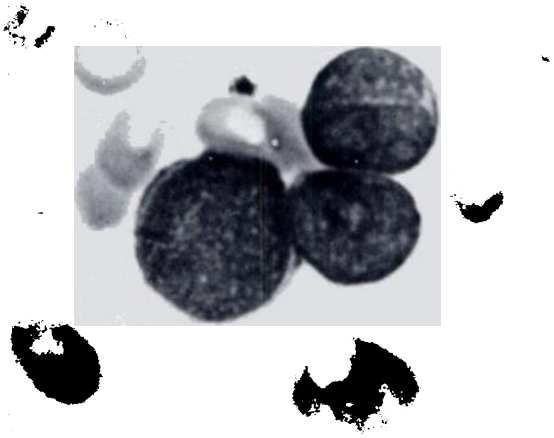

Fig. 2. Peripheral blood from case 1 on presentation. × 1500.

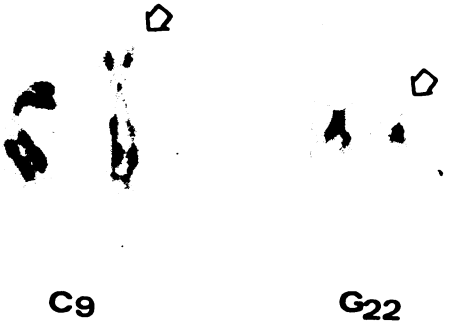

Fig. 3. GTG-banded karyotype of case 1. Arrows point to 22q− and to chromosome 9 with the translocation.

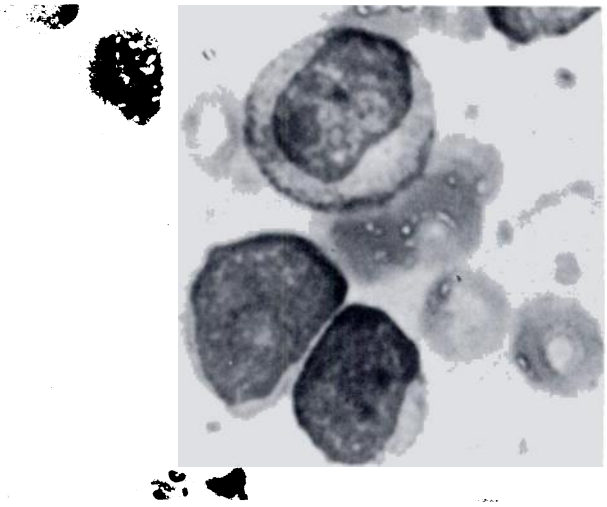

Fig. 4. Peripheral blood of case 1 during CML phase. Most blasts showed more cytoplasm and less definitive nuclear chromatin organization than those of initial presentation. × 1500.

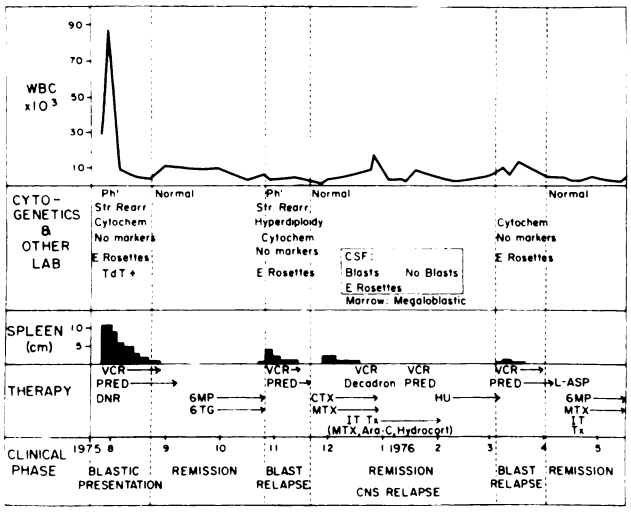

Fig. 5. Clinical course of case 2 (A.L.).

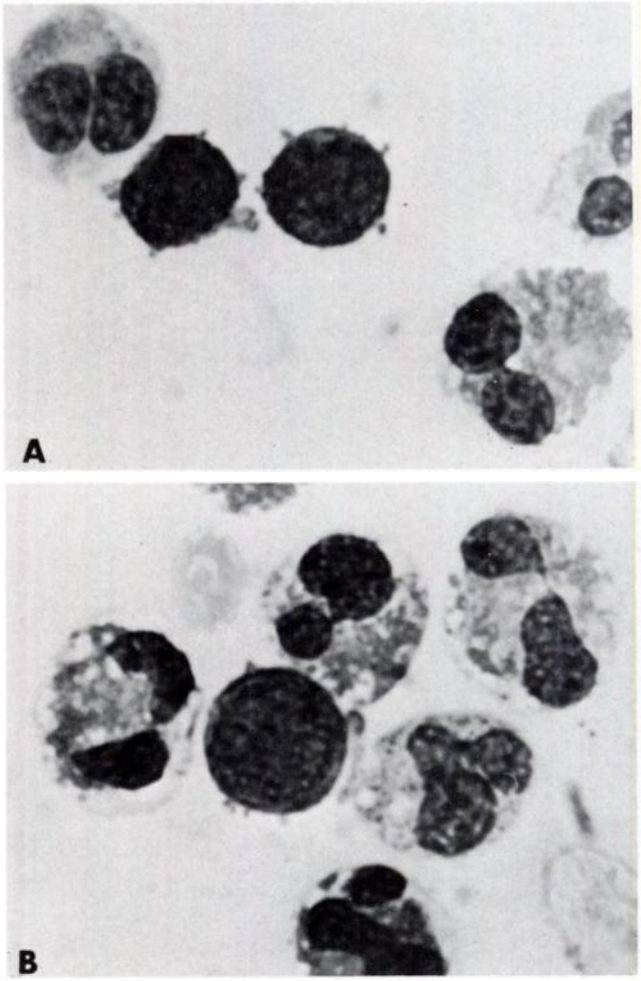

Fig. 6. Peripheral blood from case 2 on presentation. × 1500. (A) Blasts were small with finely divided nuclear chromatin and cytoplasmic blebs. (B) Eosinophils showed sparsity of cytoplasmic granules and variable granule size.

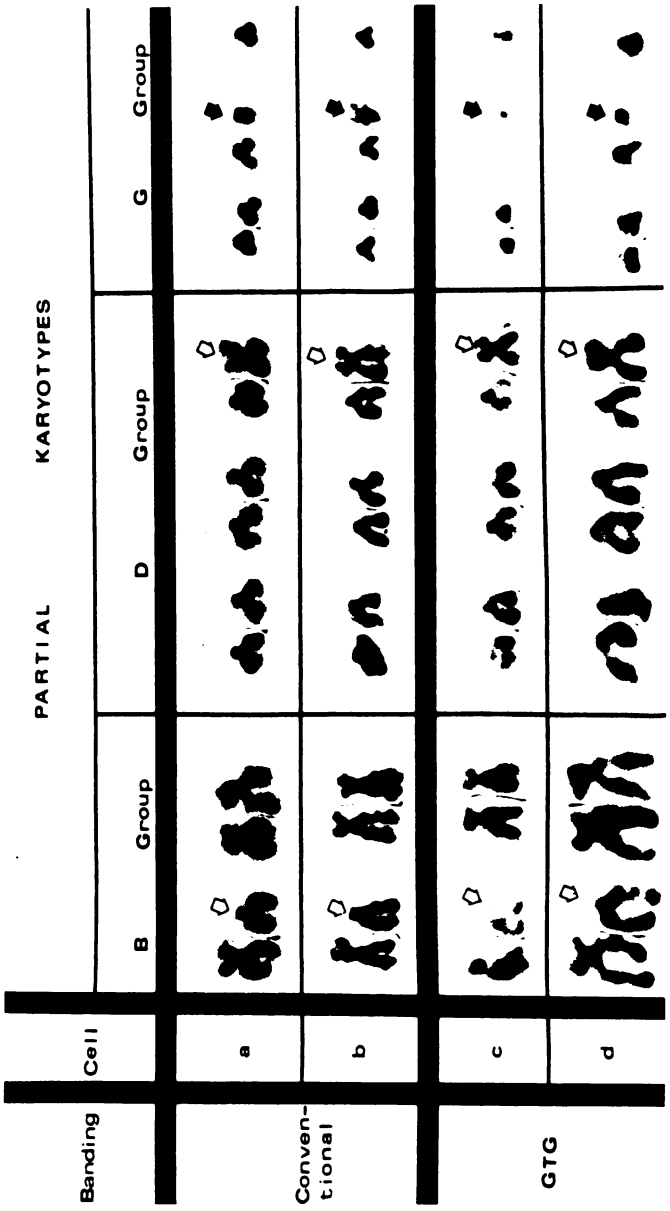

Fig. 7. Partial karyotypes of case 2. Solid arrows point to the Ph[1] chromosomes. Hollow arrows point to rearranged chromosomes, t (B,D).

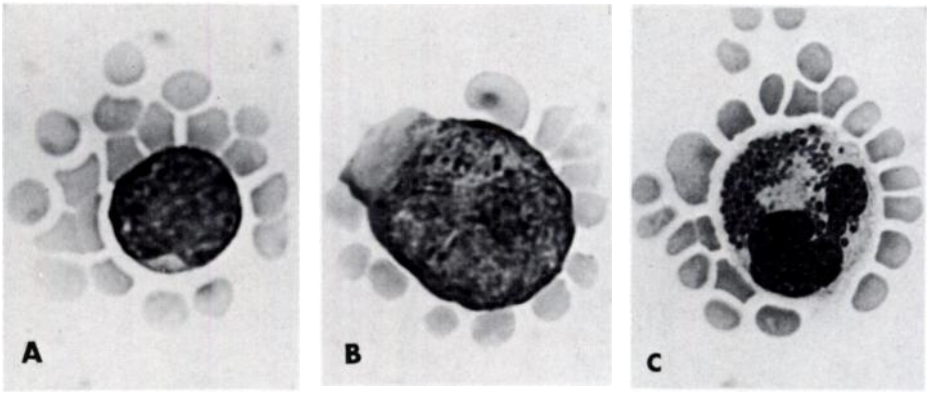

Fig. 8. Bone marrow cells of case 2 at presentation showing E-rosetting. × 1500. (A) Typical blast cell. (B) Large granulated early promyelocyte. (C) Abnormal eosinophil.

Fig. 5. He was admitted in July 1975, with a brief history of fever, puffy eyelids, generalized pruritus, and pallor. Physical findings included generalized adenopathy and marked hepatosplenomegaly. There was anemia, thrombocytopenia, leukocytosis (90,000/cu mm) with left shift, 24% eosinophilia with bizarre forms, and 4% blasts (Fig. 6). The bone marrow showed sheets of blasts which were negative for all histochemical myeloid markers and showed no glycogen. Bone marrow direct preparation revealed a Ph^1 with additional structural rearrangements involving a B and D group chromosome. GTG banding confirmed the presence of 22q−, but the site of translocation could not be demonstrated (Fig. 7). QFQ banding analysis demonstrated a normal Y chromosome. Blast cell terminal transferase was markedly elevated (1.7 units/10^8 cells). Small numbers of blasts, as well as abnormal promyelocytes and eosinophil precursors, formed rosettes with sheep erythrocytes (Fig. 8). Leukocyte alkaline phosphatase score was 35. A complete remission was obtained with vincristine, prednisone, and daunorubicin. The marrow karyotype was normal, with absence of the Ph^1 and of structural rearrangements. Two bone marrow relapses and a CNS relapse followed. During the first relapse, serum vitamin B_{12} was 3180 pg/ml. During relapses, sheep erythrocyte rosetting of blast cells from bone marrow and spinal fluid, and of cells with characteristics of early granulocytic precursors was demonstrated on three occasions. The first bone marrow relapse was accompanied by reappearance of the Ph^1 and the B and D group rearrangements, and both relapses responded to vincristine-prednisone reinductions. Currently, he is in bone marrow and CNS remission.

DISCUSSION

These cases show characteristic clinical and laboratory features of CML, in addition to the Ph^1. Two forms of CML have been described in children.[10] In the juvenile type, there is normal or decreased leukocyte alkaline phosphatase,[11] increased fetal hemoglobin,[12] and absence of the Ph^1, as well as a variety of red cell metabolic and immunologic characteristics interpretable as consistent with fetal red cells.[13] The adult type is characterized by increased vitamin B_{12} levels and transcobalamin I, reduced leukocyte alkaline phosphatase activity, usually normal fetal hemoglobin, and presence of the Ph^1. The clinical and laboratory features of our two cases of CML are most consistent with this adult type. In patient 1, an elevated fetal hemoglobin was present; however, elevated hemoglobin F has been reported in up to one-third of adult patients with CML.[14] The unique features of our patients are the presence of evidence for lymphoid properties of their blastic components.

As suggested in Fig. 9, the clinical evaluation of these patients provides a basis for considering some cases of CML, CML blast crisis, and ALL as part of a spectrum where interconversion recognizable at the clinical level may occur and in which interconversions may be reversible. Recently, evidence has accumulated which supports the notion of "lymphoid-myeloid" multipotentiality of certain leukemic cells, and suggests further that transient expressions of lymphoid or myeloid type may occur in the cells at different times, or at the same time. Evidence has been of two types: (1) coexistence of different (i.e., lymphoid or myeloid) leukemias in the same patient, and (2) recognition of subgroups of blastic crisis patients, in whom morphology and evidence of higher orders supports lymphoid identity of the emerging clone.

Anecdotal reports of ALL and CML concurrently present in the same individual have appeared in the literature.[15-18] In some instances,[15] the interpretation was based on morphological and clinical impressions. In other cases, chromosomal evidence supported identity of the CML.[16-18] In one instance, CLL and CML occurred together in the same patient.[17] Our patient (case 1) was the first reported in which Giemsa banding conclusively demonstrated the Ph[1] chromosome during a lymphoid presentation preceding onset of typical CML.

Morphology, therapeutic response, biochemical properties of the blast cells, in vitro colony-forming properties, histochemical characteristics, surface properties, and cytogenetics have provided evidence to support dual cell type participation in some instances of blast crisis. In Fig. 9, properties that can support lymphoid or myeloid identity of blast cells are listed. Current evidence suggests that a significant minority of CML blast crises ($\simeq$20%-30%) present proliferation of subclones with lymphoid, rather than myeloid, properties, or combined lymphoid and myeloid characteristics.

In 1973, Marmont and DeMasio reported that some patients with blastic crisis of CML showed predominantly "agranular" blasts;[19] these patients were distinguished by their sensitivity to vincristine-prednisone treatment, and resembled lymphoid leukemias in this respect. Morphologic properties of blast cells are often useful in determining leukemic cell types. However, subjectivity of interpretation and cytologic ambiguity of some blasts severely limits morphologic impression as a sole criterion for valid cell identification. A stronger piece of evidence supporting lymphoid identity of blast crisis cells is the presence of elevated levels of terminal deoxynucleotidyl transferase (TdT). This DNA-polymerizing enzyme was initially felt to be specific for the thymus. However, similar high levels have been found in the blasts of many cases of acute lymphoblastic leukemia and some blast crises of CML. Insignificant

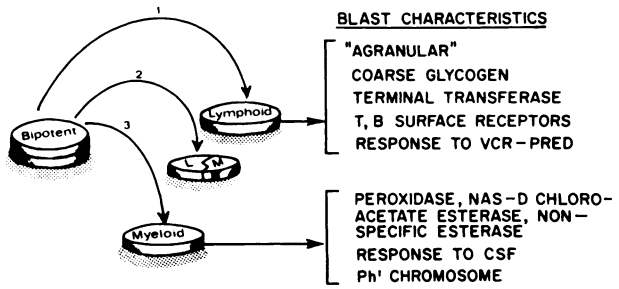

Fig. 9. Scheme incorporating possible differentiation expressions of leukemic blast cells along lymphoid, myeloid, and mixed lymphoid-myeloid lines (see text). CSF, colony-stimulating factor.

activity has been found in acute myeloblastic leukemia, CML in chronic phase, or normal circulating leukocytes.[9,20] Recently, McCaffrey et al.[21] have also reported that a specific antigenic property of leukemic lymphoblasts is present in the blasts of about one-third of CML blastic crisis patients. In addition, Moore has pointed out that 17% of patients in blast crisis of CML have blast cells that do not possess the ability to respond in vitro to colony-stimulating factor (a myeloid cell property). Instead, these blast cells show biophysical characteristics and behavior in vitro relating them to leukemic lymphoblasts rather than myeloblasts.[22]

Histochemical markers are generally accepted as useful adjunctive criteria for blast cell classification in acute leukemias.[23] Myeloblasts may possess peroxidase, Sudan black B reaction, or naphthol AS-D chloroacetate esterase activity, while lymphoblasts lack these properties, but may show glycogen in typical clumped distribution. Studies of blast cell histochemistry in CML blast crisis have demonstrated heterogeneity of blast cell populations in different patients. For example, some cases show only typical myeloid markers, while in others, coarse PAS positivity can also be demonstrated.[24]

Surface receptor site analysis has seldom been employed in the study of blast crisis cells, since the lymphoid-myeloid problem of cell identities has only recently been considered in any systematic fashion. In these cases, search for T lymphoid markers (E rosetting), or B lymphocyte receptors (EAC rosetting, immunoglobulin) may provide additional evidence to support lymphoid cell characteristics. In our second patient, E rosetting of the blast cells has been repeatedly demonstrated.[25]

Both of our Ph^1-positive CML patients showed more than one feature consistent with interpretation of blast cells as lymphoid or "lymphoid-myeloid" (Fig. 9). In our first patient (C.F.), there was clinical expression of two forms of leukemia (ALL and CML) at different times. The original diagnosis of ALL was based only on clinical and morphologic grounds, although a Ph^1 chromosome was demonstrated throughout the course. Her response to initial protocol therapy and sustained remission was consistent with lymphoid leukemia behavior. During this child's blastic crisis, more firm evidence of both lymphoid and myeloid cell lineage was demonstrated. The blast cells appeared morphologically myeloblastic and possessed myeloid cytochemical markers, but there was elevation of TdT activity and response to vincristine-prednisone treatment.

In the second case, initial blastic presentation was accompanied by clear-cut evidence of a myeloid leukemia (Ph^1 chromosome, extreme granulocytic and eosinophilic hyperplasia with dysplastic maturation, and marked splenomegaly). However, the blast cells showed no histochemical myeloid markers, demonstrated marked elevation of TdT, showed presence of T-lymphoid surface receptor properties, even in granulated cells with promyelocyte morphology, and repeatedly responded to vincristine and prednisone (Fig. 5). Our evidence was consistent with the hypothesis that the leukemic cells of these patients could express both lymphoid and myeloid properties, either simultaneously or sequentially.

In summary, two children with Ph^1 chromosomes and clinical and laboratory findings consistent with CML have shown evidence for simultaneous lymphoid-

myeloid properties of the blast cells. As a result of these experiences, we propose that some childhood leukemias (especially those presenting with 22q−) may present complex biological stem cell relationships. In these cases, routine laboratory studies fail to provide definitive characterization of the leukemic process. A comprehensive battery of cytogenetics, histochemistry, enzymatic properties, and surface receptor site analysis of the leukemic blast cells allows further definition of the atypical leukemic states. The ultimate implication of such characterization in terms of therapeutic responses is an important consideration which could be fruitfully investigated in controlled studies.

ACKNOWLEDGMENT

We wish to acknowledge Dr. Paul H. La Marche and Dr. Laurent J. Beauregard for their assistance in the cytogenetic studies.

REFERENCES

1. Boggs DR: Hematopoietic stem cell theory in relation to possible lymphoblastic conversion of chronic myeloid leukemia. Blood 44:449, 1974

2. Moorhead PS, Nowell PC, Mellman WJ, Battips DM, Hungerford PA: Chromosome preparation of leukocytes cultured from human peripheral blood. Exp Cell Res 20:613, 1960

3. Seabright M: A rapid banding technique for human chromosomes. Lancet 2:971, 1971

4. Caspersson T, Zeck L, Johnson C, Modest EJ: Identification of human chromosomes by DNA binding fluorescent agents. Chromosoma 30:215, 1970

5. Yam LT, Li CY, Crosby WH: Cytochemical identification of monocytes and granulocytes. Am J Clin Pathol 55:283, 1971

6. Williams WJ: Hematology. New York, McGraw-Hill, 1972, pp 1393, 1395

7. Barka T, Anderson PJ: Histochemistry. New York, Harper & Row, 1963, p 73

8. Steel CM, Evans J, Smith MA: The sheep-cell rosette test on human peripheral blood lymphocytes: An analysis of some variable factors in the technique. Br J Haematol 28:245, 1974

9. McCaffrey R, Harrison TA, Parkman R, Baltimore D: Terminal transferase activity in leukemic cells and normal thymocytes. N Engl J Med 292:775, 1975

10. Nathan DG, Oski FA: Hematology of Infancy and Childhood. Philadelphia, Saunders, 1974, pp 699-701

11. Rosen RR, Nishiyama H: Leukocyte alkaline phosphatase in chronic granulocytic leukemia of childhood. Ann NY Acad Sci 155: 992, 1968

12. Hardisty RM, Speed DE, Till M: Granulocytic leukaemia in childhood. Br J Haematol 10:551, 1964

13. Weatherall DJ, Edwards JA, Donohoe WTA: Haemoglobin and red cell enzyme changes in juvenile myeloid leukaemia. Br Med J 1:679, 1968

14. Newman DR, Pierre RV, Linman JW: Studies on the diagnostic significance of hemoglobin F levels. Mayo Clin Proc 48:199, 1973

15. Jenkins DW, Rivera HP, Coltman CA: Case report: Acute lymphatic leukemia followed by a clinical picture indistinguishable from chronic granulocytic leukemia in the same patient. Am J Med Sci 264:329, 1972

16. Tanzer J, Jacquillat C, Boiron M, Bernard J: A case of acute lymphoblastic leukemia presenting three years later with a chronic myelocytic leukemia with a Philadelphia chromosome. Lyon Med 233:311, 1975

17. Whang-Peng JW, Gralnick HR: Chronic granulocytic leukemia (CGL) during the course of chronic lymphocytic leukemia (CLL): Correlation of blood, marrow, and spleen morphology and cytogenetics. Blood 43:333, 1974

18. Jordan E, Lyman G, Rigby DJ, Henderson ES: Apparent conversions of lymphocytic to myelocytic leukemia. Proc Am Assoc Cancer Res 12th Mtg, 1976, p 285 (unpublished)

19. Marmont AM, Damasio EE: The treatment of terminal metamorphosis of chronic granulocytic leukaemia with corticosteroids and vincristine. Acta Haematol 50:1, 1973

20. Sarin PS, Anderson PN, Gallo RC: Terminal deoxynucleotidyl transferase activities in human blood leukocytes and lymphoblast cell lines: High levels in lymphoblast cell lines and in blast cells of some patients with chronic

myelogenous leukemia in acute phase. Blood 47:11, 1976

21. McCaffrey R, Greaves M, Harrison TA, Revezs T, Beard M, Baltimore D: Biochemical and immunological evidence for lymphoblastic conversion in chronic myelogenous leukemia. Blood 46:1043, 1975 (Abstr)

22. Moore MAS: Marrow culture—A new approach to classification of leukemias. Blood Cells 1:149, 1975

23. Hayhoe FGJ, Cawley JC: Acute leukaemia: Cellular morphology, cytochemistry and fine structure. Clin Haematol 6:49, 1972

24. Shaw MT, Bottomley RH, Grozea PN, Nordquist RE: Heterogeneity of morphological, cytochemical, and cytogenetic features in the blastic phase of chronic granulocytic leukemia. Cancer 35:199, 1975

25. Forman EN, Barker, BE, Farnes P: Evidence of lymphoid and myeloid properties of blast cells in chronic myelocytic leukemia in children. Blood 46:1013, 1975 (Abstr)

1977 49: 549-558

Ph1-positive childhood leukemias: spectrum of lymphoid-myeloid expressions

EN Forman, T Padre-Mendoza, PS Smith, BE Barker and P Farnes

Blood (print ISSN 0006-4971, online ISSN 1528-0020), is published weekly by the American Society of Hematology, 2021 L St, NW, Suite 900, Washington DC 20036.