CONCISE REPORT

Molecular Analysis of Clonality and \( bcr \) Rearrangements in Philadelphia Chromosome-Positive Acute Lymphoblastic Leukemia


Philadelphia chromosome (Ph\(^1\))-positive chronic myelogenous leukemia (CML) patients consistently show a rearrangement in a 5.8-kilobase length of chromosome 22, referred to as the breakpoint cluster region (\( bcr \)). In Ph\(^1\)-positive acute lymphoblastic leukemia (ALL), the breakpoint in chromosome 22 is more heterogeneous and, in some instances, does not occur within this region. In such cases the cell of origin of the neoplastic clone and the relationship of the disease to CML has remained obscure. We have analyzed the \( bcr \) rearrangement in the malignant cells from three patients who presented with Ph\(^1\)-positive ALL and who in cytogenetic studies had shown evidence of variable involvement of myeloid cells in the Ph\(^1\)-positive clone. Rearrangements in \( bcr \) typical of most cases of CML were detected in purified granulocyte preparations from two of the ALL patients (nos. 1 and 2) and in the blasts from patient 3 at the time of her terminal relapse. In the same analysis the simultaneously obtained granulocytes from patient 3, however, did not show any evidence of \( bcr \) rearrangement. Patient 3 was also heterozygous for the \( B_{am}H1 \) polymorphism in the X-linked hypoxanthine phosphoribosyltransferase (HPRT) gene, thus permitting a different method of clonal analysis based on methylation differences in active and inactive alleles. When DNA from her granulocytes that had shown no \( bcr \) rearrangement was hybridized to an HPRT probe, a pattern typical of a polyclonal population was seen. A similar pattern was exhibited by her marrow fibroblasts. In marked contrast, her simultaneously isolated blasts showed an unambiguous monoclonal pattern. These findings demonstrate the origin of the disease in the first two patients in a cell with myelopoietic as well as lymphopoietic potential and confirm the restricted lymphoid cell origin of the neoplastic clone in the third Ph\(^1\)-positive ALL patient. Furthermore, they indicate that different target cells for transformation within the hematopoietic system may be affected by very similar \( bcr \) rearrangements.

PHILADELPHIA chromosome (Ph\(^1\))-positive acute lymphoid leukemia (ALL) was first recognized in 1970. Since that time the relationship of this disease to chronic myeloid leukemia (CML) and the role of the translocation in the development and progression of malignant hematopoietic clones have been the subject of intense speculation and investigation. In some patients presenting with Ph\(^1\)-positive ALL, inclusion of myeloid cells within the neoplastic clone appears likely, as evidenced by the persistence of large numbers of Ph\(^1\)-positive cells in the marrow even after the blast population is reduced by chemotherapy. Such cases are generally viewed as examples of patients with CML whose chronic phase escapes diagnosis and who are thus clinically recognized first in a lymphoid blast crisis. In other patients, clinical remission may be associated with the disappearance of Ph\(^1\)-positive marrow metaphases, and the extent of lineage involvement in the original disease is more difficult to establish.

Studies of the breakpoint on chromosome 22 have revealed a heterogeneous pattern in patients presenting with Ph\(^1\)-positive ALL. In some, the rearrangement occurs in the same 5.8-kilobase (kb) region of the \( bcr-1 \) gene\(^6\) that is involved in most cases of CML. In others, the breakpoint may occur 5' of this region but still within \( bcr-1 \).\(^7\) According to the site of the breakpoint, two different \( c-abl \) proteins may be produced, a 210-kd \( bcr-abl \) fusion protein typical of CML cells\(^8\) and a 190-kd \( c-abl \) protein seen in some cases of Ph\(^1\)-positive ALL.\(^10,12\) This has raised the question as to whether these molecular differences might correlate with the cell type initially transformed.\(^5,11\)

In a recent study of five patients who presented with Ph\(^1\)-positive ALL, extensive cyogenetic analysis of direct marrow preparations and individually removed erythroid, granulopoietic, and mixed erythroid-granulopoietic colonies derived from marrow or peripheral blood progenitors suggested variability in the involvement of myeloid cells in different patients.\(^12\) Additional material was available from three of these patients for molecular studies of clonality\(^13,14\) and \( bcr \) rearrangement. The results of these studies provide additional evidence that one of these patients was a case of lymphoid-restricted, Ph\(^1\)-positive ALL. Further, they show that the breakpoint on chromosome 22 in this patient’s blasts occurred within the same region of \( bcr-1 \) that was involved in the two cases of multilineage Ph\(^1\)-positive ALL studies and in eight cases of CML also analyzed.

MATERIALS AND METHODS

Patient specimens. Heparinized peripheral blood and/or marrow cells were obtained with informed consent from all patients studied as part of their routine diagnostic and follow-up procedures. All three Ph\(^1\)-positive ALL patients in this study presented with a typical ALL picture, 50% to 98% lymphoblasts in the marrow that...
showed features consistent with a B lineage phenotype (patient 1, common ALL antigen-positive [CALLA +], DR +; patient 2, CALLA+, terminal deoxy-nucleotidyl-transferase—positive [TDT +], B4 +; patient 3, TdT +, CALLA +, DR +, B4 +, L12+, cytoplasmic IgM +), and all were treated as ALL patients. Direct marrow morphose preparations obtained at diagnosis showed the Ph' translocation in all three patients. After remission induction the proportion of normal metaphases increased initially in all cases; however, with time Ph'-positive metaphases became more prevalent in the marrow of ALL patients 1 and 2, and aspirate and biopsy specimens were judged to be consistent with an underlying diagnosis of CML.

Cytogenetic analysis of metaphases from cultured myeloid colonies (granulocytic, erythroid, and mixed granulocyte/erythroid) revealed the presence of the Ph' translocation in a readily detectable proportion of myeloid progenitors from both ALL patients 1 and 2 (22% to 49% and 87% to 100%, respectively). In contrast, Ph'-positive metaphases were not seen in direct preparations of marrow from ALL patient 3 except at diagnosis and later at the time of her terminal relapse and were at no time detectable in her myeloid colonies (a total of 136 metaphases examined from colonies cultured at presentation, in remission, and at the time of terminal relapse). A more detailed description of the clinical and cytogenetic findings on these patients appears elsewhere.12

Granulocytes were purified from peripheral blood samples by separation on Ficoll-Hypaque (1.077 g/mL) and NH4Cl lysis of the red cell pellet. Marrow cells were depleted of red cells by a similar lysis procedure. Blast cells from ALL patient 3 were obtained at the time of her terminal relapse when her marrow was packed with blasts and her peripheral WBC count was 80,000/μL. These were further purified by separation of the blood on Ficoll-Hypaque and removal of the light-density cell fraction. Granulocytes from this patient (no. 3) refer to the cells that copelleted with the red cells in this same separation. Fibroblasts were obtained by seeding marrow cells at ~2 x 10⁶ cells/mL in α medium plus 20% fetal calf serum and subculturing the fibroblasts as they became confluent at 2 to 3 week intervals for several passages.

Southern analyses. Southern blot analyses were performed essentially as described by others with minor modifications.14 For HPRT analyses, DNA was digested with BamHI alone or BamHI plus HpaII or HhaI. After agarose gel electrophoresis, the DNA was transferred to nylon filters (Zeta-probe, Bio-Rad Laboratories, Richmond, CA) by the alkaline transfer technique.15 Prehybridization and hybridization conditions were as described14 with the addition of 20% formamide and 10% dextran sulfate during hybridization. After hybridization at 60°C for 18 to 24 hours, the filters were washed three times in 0.1% sodium dodecyl sulfate, 0.1 x SSC, and 0.1% sodium pyrophosphate at 60°C for 30 minutes; this was followed by autoradiography. Blots were hybridized to a 600-base pair HPRT fragment isolated from a HPRT p600 plasmid14 and labeled with 32P by the oligolabeling method.16 Southern analyses of bcr rearrangements were performed by following a similar protocol after DNA digestion with BamHI, BglII, EcoRI, or HindIII. A 1.2-kilobase (kb) HindIII-BglII fragment from the 3’ end of bcr (probe 1, Oncogene Sciences, Mineola, NY) and a 2.1-kb BglII-HindIII fragment17 from the 5’ region of the bcr were used for hybridization.

RESULTS AND DISCUSSION

Evidence indicating a restricted distribution of malignant cells in ALL patient 3 to the B lineage was provided by taking advantage of a BamHI restriction fragment length polymorphism in one of her X-linked HPRT genes. Differences in the methylation status of the active and inactive alleles in individuals who are heterozygous for a BamHI site in this locus make it possible to distinguish monoclonal and polyclonal populations by using recombinant DNA techniques as described by Vogelstein et al.13,14 (Fig 1A). BamHI digestion of DNA from purified blasts and granulocytes obtained simultaneously at the time of terminal relapse and subsequent Southern blot analysis revealed the two characteristic 24-kb and 12-kb bands corresponding to the two HPRT alleles (fig 1B, lanes 1, 4, and 7). Further cleavage of the granulocyte DNA with either HpaII or HhaI (lanes 5 and 6, respectively) resulted in a reduction but not elimination of the intensities of hybridization in both bands, which is indicative of active and inactive copies of both HPRT alleles in the original DNA, as expected for a polyclonal cell population. Analysis of normal control DNA from her marrow fibroblasts similarly revealed a pattern typical of a polyclonal population after HhaI digestion (Fig 1B, lane 8). In contrast, combined digestion of DNA isolated from her blast cells with BamHI and HhaI completely eliminated the 24-kb allele, thus demonstrating their monoclonal origin (lane 3). Similarly, combined digestion of her blast cell DNA with BamHI and HpaII resulted in the disappearance of both the 24-kb and 12-kb alleles (lane 2), a pattern commonly

![Fig 1. (A) Clonality studies of bone marrow cells from two informative patients by using BamHI polymorphism of the HPRT gene. (B) Southern blot analysis of cells from patient 3 who was heterozygous for a BamHI polymorphism in the HPRT gene. BamHI, lanes 1, 4, 7; BamHI plus HpaII, lanes 2, 5; BamHI plus HhaI, lanes 3, 6, and 8.](image-url)
To investigate the type of \( bcr \) rearrangement present in the \( Ph^+ \)-positive cells of the three patients, we performed \( BamHI, BglII, EcoRI, \) and \( HindIII \) digests of DNA samples from their granulocytes (ALL patients 1, 2, and 3), blast cells (ALL patient 3), and fibroblasts (ALL patient 3) (Fig 2). Control DNA was obtained from marrow cells from a normal allogeneic transplant donor as well as from marrow or blood cells from eight patients with typical \( Ph^+ \)-positive CML. Southern blot analysis using the 1.2-kb genomic \( bcr \) probe from the 3' portion of the 5.8-kb region of \( bcr-1 \) typically involved in CML showed a rearrangement in \( bcr-1 \) for all eight CML patients studied but none in the normal transplant marrow sample (data not shown). \( Bcr \) rearrangements were similarly detected in \( BamHI \)- or \( BglII \)-digested granulocyte DNA from ALL patients 1 and 2 when using this probe (Fig 2A, lanes 1, 2, 5, and 6). In contrast, this probe did not reveal any rearranged bands in either the polyclonal granulocytes or the monoclonal blasts from ALL patient 3 after digestion of the DNAs with \( BamHI, BglII, EcoRI, \) or \( HindIII \) (Fig 2A, lanes 3, 4, 7, and 8). However, a second 2.1-kb probe from the 5' portion of the same 5.8-kb region of \( bcr-1 \) clearly demonstrated a rearrangement in the blast cells of ALL patient 3 but not in her simultaneously obtained granulocytes (Fig 2B, lanes 9 to 12). The pattern of rearrangement with \( EcoRI, BglII \) and \( BamHI \), but not \( HindIII \) is consistent with a translocation point in the middle (zone 2) of \( bcr \) and deletion of sequences 3' to the breakpoint.

Thus, the leukemic cells from all three ALL patients showed a rearrangement within the same 5.8-kb region of \( bcr-1 \) that is usually associated with CML. For the first two patients, the cytogenetic and clinical follow-up findings also suggested that these two cases might have had a disease very similar to CML that was characterized by a clone that originated in a stem cell with extensive myeloid as well as lymphopoietic potential. In contrast, ALL patient 3 showed no clinical evidence of preexisting CML, and neither clonal nor \( Ph^+ \)-positive myeloid cells were ever detectable by either
HPRT (Fig 1) or bcr (Fig 2B) analysis of her granulocytes and by cytogenetic analysis of her myeloid progenitors, even at the time when her leukemic clone was expanding uncontrollably 8 months after diagnosis. It therefore seems likely that in this patient the leukemic clone was confined to the B lymphoid lineage and arose in a cell already restricted to this differentiation pathway. Because her leukemic blasts showed a bcr rearrangement similar to that seen in CML clones of multilineage stem cell origin, our findings do not substantiate the prediction$^{5,11}$ that there is an association between the region of bcr-1 that is rearranged and the cell type in which the rearrangement occurs. Interestingly, a lack of specificity has also very recently been found for Ph'-positive multilineage disease,$^{6,12,13}$ which in some cases may be characterized by a breakpoint outside the 5.8-kb region of bcr-1 that results in the production of a 190-kd abl protein rather than the 210-kd bcr-abl fusion protein typical of CML.$^{6,9}$ Phenotypically, the blasts of patients presenting with Ph'-positive ALL and those seen in CML patients undergoing lymphoid blast crisis are also indistinguishable.$^{2}$ Our findings thus raise interesting questions about the significance of the different bcr-abl fusion proteins described in Ph'-positive ALL and emphasize the lack of tissue specificity of many transforming molecular genetic changes.

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