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Abstract

The rearrangement of breakpoint cluster region (ber) was examined in leukemic cells obtained from 3 patients initially diagnosed as having Ph+ acute leukemia, 2 with acute lymphocytic leukemia (ALL) and one with acute mixed leukemia. DNA was digested with Bgl II and BamH I. The ber rearrangement was present in the case of acute mixed leukemia (Case 1), but was absent in the 2 cases of ALL (Cases 2 and 3). These results suggest that Case 1 represented a type of blast crisis of chronic myelocytic leukemia which was unusual in the sense of the occurrence of a myeloid-lymphoid conversion and lack of an apparent chronic phase. Cases 2 and 3 appeared to be de novo Ph+ ALL.

KEYWORDS: Ph-positive acute leukemia, blast crisis with a silent chronic phase, myeloidlymphoid conversion, chronic myelocytic leukemia, bcr-rearrangement

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—Brief Note—

Rearrangement of the Breakpoint Cluster Region in Philadelphia Chromosome Positive Acute Leukemia

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The rearrangement of breakpoint cluster region *(bcr)* was examined in leukemic cells obtained from 3 patients initially diagnosed as having Ph$^+$ acute leukemia, 2 with acute lymphocytic leukemia (ALL) and one with acute mixed leukemia. DNA was digested with *Bgl* II and *Bam*H I. The *bcr* rearrangement was present in the case of acute mixed leukemia (Case 1), but was absent in the 2 cases of ALL (Cases 2 and 3). These results suggest that Case 1 represented a type of blast crisis of chronic myelocytic leukemia which was unusual in the sense of the occurrence of a myeloid-lymphoid conversion and lack of an apparent chronic phase. Cases 2 and 3 appeared to be *de novo* Ph$^+$ ALL.

Key words : **Ph-positive acute leukemia, blast crisis with a silent chronic phase, myeloid-lymphoid conversion, chronic myelocytic leukemia, *bcr*-rearrangement**

The Philadelphia chromosome (Ph) is found in most patients with chronic myelocytic leukemia (CML), and has been widely used for the diagnosis of this disease. Cytogenetic studies of hematological neoplasms have also revealed that Ph is present not only in CML, but also in various types of acute leukemia (1). Patients with CML mostly develop a blast crisis after a chronic phase lasting 3-4 years. The distinction between blast crisis of Ph$^+$ CML, particularly cases without an apparent chronic phase, and *de novo* Ph$^+$ acute leukemia is occasionally difficult.

It has been found that in Ph$^+$ CML the oncogene c-*abl* normally located on chromosome 9 band q34 is translocated to chromosome 22 band q11 in a 5.8-kilobase (kb) region named the breakpoint cluster region (*bcr*). An altered c-*abl* protein p210 is expressed as the consequence of the molecular rearrangement in leukemic cells (2-5). Previous studies from our laboratory have shown that *bcr* rearrangements were present in 28 of 30 patients with standard Ph$^+$ CML, and also in 2 patients with variant Ph$^+$ CML (6). Determination of whether or not the molecular rearrangement observed in Ph$^+$ GML is also observed in Ph$^+$ acute leukemia would aid in the distinction between these two diseases (7-9). The present paper describes the *bcr* rearrangement in leukemic

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cells obtained from 3 cases initially diagnosed as Ph^+ acute leukemia.

Case 1. A 54-year-old woman was admitted to the hospital in March 1987. Examination of the peripheral blood showed a red blood cell count (Rbc) of $327\times10^4/\mu l$, a platelet count (Plt) of $4.9\times10^4/\mu l$ and a white blood cell count (Wbc) of $251,000/\mu l$, with 73% blasts. Bone marrow aspirates showed a nucleated cell count (NCC) of $35.0\times10^4/\mu l$, with 82.6% blasts. Immunophenotyping of blast cells revealed the coexistence of lymphoid and myeloid antigen positive cells (Ia^+, TdT^+, $CALLA^+$, $My4^+$, $My7^+$ and $My9^+$). Cytogenetic analysis showed 46, XX, t (9; 22)(q34; q11)(9/9 cells). Case 2. A 45-year-old man was admitted to the hospital in December 1986 and diagnosed as having acute lymphocytic leukemia (ALL). Examination of the peripheral blood showed Rbc of $470\times10^4/\mu l$, Plt of $2.6\times10^4/\mu l$ and Wbc of $6.7000/\mu l$, with 1% promyelocytes, 4% myelocytes, 3% metamyelocytes, 21% neutrophils, 1% eosinophils, 2% monocytes, 60% lymphocytes and 8% lymphoblasts. Bone marrow examination showed NCC of $12.7\times10^4/\mu l$, with 94.6% lymphoblasts. Cytogenetic analysis revealed 46, XY, t (9; 22)(q34; q11) del (9)(p21)(10/10 cells). Immunophenotyping of blast cells was not done. Cases 3. A 59-year-old woman was admitted to the hospital in May 1987. Examination of the blood showed Rbc of $464\times10^4/\mu l$, Plt of $2.9\times10^4/\mu l$ and Wbc of $48,500/\mu l$, with 9% lymphoblasts. There were 93% lympho-

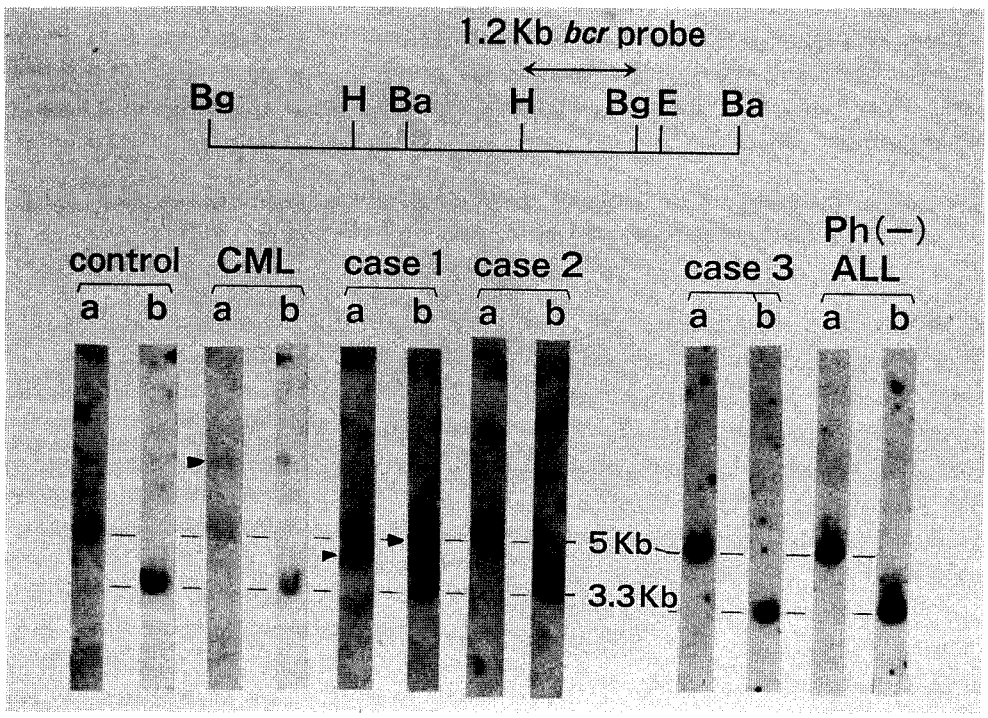

Fig. 1 Southern blots of DNA from Ph^+ acute leukemia patients. DNA obtaianed from patients with Ph^+ acute leukemia (Cases 1,2 and 3), a patient with Ph^+ chronic meylocytic leukemia (CML), a patient with Ph^- acute lymphocytic leukemia (ALL) and a normal individual was digested with *Bgl* II (Lane a) and *Bam*H I (Lane b). The 5.0 kb and 3.3 kb fragments are normal. Extra fragments, indicated by arrows, are recognized in Case 1 and Ph^+ CML, whereas only normal bands are recognized in Case 2, Case 3, Ph^- ALL and a normal control.

lasts in the bone marrow. The immunophenotypes of the blast cells indicated a null-cell ALL. Cytogenetic analysis revealed 46, XX, t(9; 22)(q34; q11)(4/19 cells) and 46, XX(15/19 cells).

Southern blotting was done as previously described(10). Briefly, leukemic cells were isolated from the peripheral blood or bone marrow aspirates, and high molecular weight DNA was prepared by the phenol-chloroform method. All samples of DNA of 10 μg were digested with the restriction enzymes *Bam*H I, *Bgl* II, *Hind* III and *Eco*R I. The digested DNA was separated on a 0.7% agarose gel (Sigma Type I) by electrophoresis, and transferred to Hybond-N (Amarsham) by Southern blotting. The filters were hybridized with radiolabeled (^{32}P) *bcr* probe in hybridization buffer [6× SSC (1× SSC= 150 mmol/l sodium chloride, 15 mmol/l sodium citrate), 5×Denhardt, 0.5% SDS, 250 μg/ml salmon sperm DNA] at 65℃ for 14-24 h. They were autoradiographed for 1-4 days at −70℃ after washing twice with 2× SSC for 15 min, once with 2× SSC and 0.1% SDS for 30 min and once with 0.1× SSC for 10 min at 65℃. The *bcr* probes (Oncogene Science, Mineola, NY) used are indicated in Fig 1.

The results of Southern blotting of DNA from Cases 1, 2 and 3 are demonstrated in Fig 1. The *bcr* rearrangement was observed in the DNA from Case 1 after either *Bgl* II or *Bam*H I cleavage. Only the normal restiriction enzyme fragments were present in DNA from Cases 2 and 3. CML patients mostly develop a blast crisis after a chronic phase lasting 3-4 years. At the time of blast crisis, patients usually exhibit basophilia, marked hepatosplenomegaly and additional nonrandom chromosomal aberrations such as +8, i(17q), +19, +17, −17 and a second Ph(1). Hematological and physical findings of the present cases were not consisted with blast crisis of CML. However, Case 1 was suspected to be a case of blast crisis with a silent chronic phase because of the positive *bcr* rearrangement (*bcr*$^+$) in the DNA. Immunophenotypically, blast crisis can be divided into two general forms: lymphoid and myeloid. Griffin *et al.* (11) determined the cell surface phenotypes of 30 patients, and found one case to have a mixed population of My7$^+$/CALLA$^+$ cells. Bettelheim *et al.* (12) found two cases of mixed myeloid and lymphoid cells in 45 cases of CML blast crisis. Case 1, therefore, was thought to represent a type of blast crisis which was unusual not only for the absence of an apparent chronic phase but also for the presence of a myeloid-lymphoid conversion. Chan *et al.* (9) reported a case of Ph$^+$ ALL associated with *bcr*$^+$ and p210$^+$, and proposed that Ph$^+$ ALL with *bcr*$^+$ and p210$^+$ is probably a lymphoid blast crisis following a clinically silent chronic phase of CML, whereas Ph$^+$ ALL with *bcr*$^-$ and p190$^+$ is *de novo* ALL. Further molecular analysis of the *bcr-abl* fused mRNA and altered *abl* protein in leukemic cells of Case 1 is in progress.

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