Ph1-positive, bcr-negative Acute Leukemias: Clustering of Breakpoints on Chromosome 22 in the 3' End of the BCR Gene First Intron

By Sai Juan Chen, Zhu Chen, Josette Hillion, David Grausz, Pascale Loiseau, Georges Flandrin, and Roland Berger

About 50% of the Philadelphia-positive acute leukemias undergo molecular rearrangements outside the now classical bcr sequence (or M-BCR-1 rearranged in chronic myeloid leukemia (CML). Most of the breakpoints on chromosome 22 have been shown to be clustered in a 10.8-kb region of the first intron of the BCR gene (called bcr2 or m-BCR-1). In this report we examined two cases of Ph1

THE PH1 CHROMOSOME, hallmark of chronic myeloid leukemia (CML), is also found in 5% to 20% of acute lymphoblastic leukemias (ALL) and in about 1% of acute nonlymphoblastic leukemias (ANLL).1 Recent studies showed that about half of the Ph1-positive ALLs exhibit molecular rearrangements in the 5.8-kb breakpoint cluster region (bcr, or M-BCR-1 according to a recently proposed nomenclature)2 of the BCR gene, as described in the great majority of CML,3 and produce a 210-Kd chimeric bcr-abl' protein translated from an 8.5-kb fusion messenger RNA (mRNA) and displaying a significant tyrosine kinase activity.2 In the remaining half of Ph1-positive ALLs, no bcr rearrangements are detected. However, in some of these, designated Ph1-positive, bcr-negative (Ph1' bcr') ALLs, a new bcr-abl' chimeric mRNA of 7.0 kb and a resultant protein of 190 Kd were found.2 Nucleic acid sequence analysis and immunoprecipitation showed that the new gene product of 190 Kd were found.2 Nucleic acid sequence analysis and immunoprecipitation showed that the new fusion molecular was composed of the first exon of the BCR gene and the common exons of c-abl', implying that the breakpoints on chromosome 22 should be localized in the first intron of the BCR gene.4 We have recently cloned 64 kb of the 5' part of the BCR gene, including an approximately 40-kb region derived from the first intron.5 Using a panel of genomic probes, the breakpoints on chromosome 22 in six out of seven Ph1' bcr' acute leukemias were localized in a region of 10.8 kb, designated bcr2 (or m-BCR-1 according to Gale and Goldman),5 which is 40 kb upstream of the classical bcr.5 During this study, we had one Ph1' bcr' case in which no rearrangement could be detected with bcr2 probes. Here we localize the rearrangement of the BCR gene in this case and show that it is very close to that observed in another acute lymphoblastic leukemia in adult patients that exhibited breakpoints in a 5-kb segment of the BCR gene first intron, 16 kb upstream of the previously described cluster, suggesting the possibility of a second minor breakpoint cluster. In addition, the breakpoints on chromosome 9 were located in a region just 5' of the c-abl exon 1a.

MATERIALS AND METHODS

Patients. The leukemic cells of two patients, 59 and 62 years old, were studied. The hematologic, immunophenotypical, and immunogenotypical data of these two ALL cases (L2 in the French-American-British [FAB] classification) indicate a pre-B origin for the leukemic clones (DR+, B4-, CALLA-). No myeloid markers were detected.

Cytogenetics. Chromosome studies were performed on bone marrow and blood cells as previously reported.6 They showed a mosaic 46,XY.t(9;22)(q34;q11)/47,XY.t(9;22)(q34;q11)+Ph1/46,XY in the first case (Na) and only Ph1-positive metaphases with the common t(9;22)(q34;q11) translocation in the second patient (Bo).

Molecular cloning of part of the bcr' gene first intron. The establishment of three genomic DNA libraries in phage EMBL3 was described elsewhere.7 The cloning of a 64-kb 5' BCR gene segment was performed using the chromosome-walking technique. A series of 13 clones was selected by successively screening the libraries with subcloned fragments from the 5' end of phage clones obtained in precedent experiments.

Probes. The following molecular probes were used: the "universal bcr" probe (bcr-u), kindly provided by Dr J. Groffen; the 0.7 PstI-PstI Ph1 cDNA probe, kindly provided by Dr O.N. Witte; a 0.6-kb KpnI-PstI and a 1.1-kb BamHI-BamHI (BB-1) probe covers the previously defined bcr2 region,8 a 1.5-kb BamHI-BamHI (BB-2) and a 0.9-kb EcoRI-EcoRI probe have recently been subcloned from phage clones that cover a region of approximately 16 kb upstream of bcr2; a 0.7-kb EcoRI-EcoRI genomic probe isolated from the second intron of the c-abl' gene (Fig 1B); a 1.8-kb c-abl' cDNA probe. The probes were 32P-labeled with the random primer method to obtain a specific activity of 1 to 2 x 106 cpm/μg DNA.

Southern and Northern analyses. The standard procedures were followed as previously described.8 Twenty micrograms total RNA were used in Northern analysis.

RESULTS AND DISCUSSION

In initial hybridization experiments, no rearrangements were detected with bcr-u and the 0.7-kb PstI-PstI cDNA probes in DNA digests from patients Na and Bo, nor with the two bcr2 KpnI-PstI and BB-1 probes (Figs 1A and 2A). Therefore the breakpoints on chromosome 22 could take place in a region either downstream or upstream of bcr. We have tried a probe that covers a region about 15 kb downstream to bcr in patient Na with no obvious rearrangement

From INSERM U 301 and Laboratoire d’Oncologie et d’Immunopathologie UM7 CNRS, Laboratoire Central d’Hematologie, and Laboratoire d’Immunologie et d’Histocompatibilité, Hôpital Saint-Louis, Paris; and from Shanghai Institute of Hematology, Shanghai Second Medical School, Shanghai, China.

Address reprint requests to Roland Berger, MD, INSERM U 301, Hôpital Saint-Louis, 4 Av Claude Vellefaux, 75475 Paris Cedex 10, France.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

© 1989 by Grune & Stratton, Inc.
detected. Northern blot analysis showed a strong 7.0-kb mRNA expression with almost the same intensity as that of the 8.5-kb bcr-abl mRNA in the K562 cell line. No normal 6.0-kb abl transcripts were visible (Fig 3). This result suggested that the breakpoint was within the BCR gene first intron similar to other Ph1 'bcr' ALL and not in the third other BCR-related loci, since according to Lifshitz et al10 the BCR first exon sequence exists only in the first identified BCR gene. The BCR first intron is extremely large. Although only about 60 kb were actually cloned,2 evidence has recently been obtained suggesting that this intron may be as large as 70 kb.11 We thus continued the chromosome walk to obtain 25 kb upstream to bcr2 in the first intron. When the probe BB-2 was used to hybridize DNA from patients Na and Bo, rearranged bands were detected in several DNA digests (Fig 2B). Since no rearrangement was seen in the BB-2 fragment, an EcoRI-EcoR1 probe just 5' to BB-2 was isolated. This probe further localized the breakpoints to a region adjacent to the breakpoints.14 Further information is needed before any general statement could be proposed.

As for the breakpoints on chromosome 9 in Ph1 ' acute leukemias, little information is available. In three recent reports,12,13,15 two for "fresh" cases of Ph1 'bcr' ALL and the other for the Ph1 'bcr' leukemic cell line SUP-B13, the rearrangements were localized to the second ABL intron (ie, a region between the exon Ia and exon II; (Fig 1B). Among our eight cases of Ph1 ' acute leukemias studied, only one showed a rearrangement in this intron (Fig 1B). Interestingly, the present two cases with a bcr3 rearrangement had the breakpoints in the same region of the ABL gene. As shown in Fig 2C, a rearranged band in the HindIII digest was recognized by both the 0.7-kb EcoR1-EcoR1 ABL probe and the BB-2 probe. Now that the breakpoints in the BCR gene have been localized, we can localize the breakpoint in the c-abl' gene. In both cases it is situated 5 to 6 kb upstream to c-abl' exon Ia (Figs 1B and 2C). Among six Ph1 'bcr' cases, one also had the breakpoint 5 to 6 kb 5' to ABL exon Ia (Fig 1B). The designation of this region as a hot spot of rearrangement requires further study. The polymerase chain reaction (PCR) has recently been employed to detect the hybrid bcr-abl mRNA in CML and in Ph1 'bcr' ALL using appropriate primers. In a Ph1 'bcr' ALL cell line (SUP-
B15) and in fresh cases a specific amplification reaction has been achieved. This still relatively difficult method is specific and highly sensitive, but it cannot provide information on the genomic location of the breakpoint.

Some authors reported that the bcr-ALL is observed mainly in adults, while the bcr-ALL is observed mainly in children. It is worth noting that the present two cases of Ph1-bcr-ALL are both adults. Among our 14 Ph1 acute leukemia cases, there are three adults and three children of the bcr- type, while six adults and two children are of the bcr- type. In addition, the difference in breakpoint localization in the BCR gene does not seem to be important in the determination of the stage of cell differentiation arrest in that both ALL and ANLL cases can show rearrangement in M-BCR-1 as well as in the first intron. However, since different abnormal abl' proteins may not play the same roles

Fig 2. DNA digests from leukemic cells of a Ph1-bcr2 ALL and of patients Na and Bo were hybridized with the bcr2 probe BB-1 (A), the bcr3 probe BB-2 (B), and the 0.7-kb EcoRI-EcoRI c-abl' intronic probe (C). ---, germline bands; ●, rearranged bands; ●, rearranged bands of 18 and 25 kb for Na and Bo, respectively, were recognized by both the BB-2 and the abl probes. The Southern analyses were performed in different experiments. Note that in Fig 2A there are additional HindIII (12.5 kb), EcoRI (11 kb), BglII (8.5 kb) bands due to the deletion/insertion polymorphism already documented by Rubin et al.

Fig 3. abl' mRNA expression in the HL-60 cell line, the K562 cell line, leukemic cells from a patient with Ph1-bcr2 ALL, patient Na, and a Ph1-bcr- ALL.
in the physiopathology of Ph1-related leukemias, use of BCR first intron probes in association with classical BCR probes should help the diagnosis and classification of the Ph1+ acute leukemias as well as the establishment of the possible clinical relevance of the types of hybrid proteins.

ACKNOWLEDGMENT

TcRβ and TcRγ probes were kindly provided by Drs J. Strominger and T. Rabbitts, the Phi cDNA probe by Dr O.N. Witte, and the bcr probe by Dr J. Groffen. We sincerely acknowledge the efficient photographic work of B. Boursin and the expert secretarial help of C. Czapek. We are also indebted to C-J Larsen for discussion of the manuscript.

NOTE ADDED IN PROOF

Heisterkamp et al (Nucleic Acids Res 1988, 16:10069) have recently shown that the size of the first bcr gene intron is 68 kb. They located the breakpoints of two Ph1+ bcr ALLS in the region we designated bcr3, and those in 3’ of this segment.

REFERENCES

Ph1-positive, bcr-negative acute leukemias: clustering of breakpoints on chromosome 22 in the 3′ end of the BCR gene first intron

SJ Chen, Z Chen, J Hillion, D Grausz, P Loiseau, G Flandrin and R Berger