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

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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-ALL recently studied in our laboratory. Since the distribution of breakpoints in these two cases falls within a 5-kb region that is about 16 kb upstream of bcr2, the existence of a new breakpoint cluster region may be questioned. The breakpoints on chromosome 9 are in both cases situated in a region just 5' to c-abl' exon 1a.

MATERIALS AND METHODS

Patients. The leukemic cells of two patients, 59 and 62 years old, were studied. The hematologic, immunophenotypic, and immunogenotypic 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.9 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.3

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 cover the previously defined bcr2 region,3 a 1.5-kb BamHI-BamHI (BB-2) and a 0.9-kb EcoR1-EcoR1 probe have recently been subcloned from phage clones that cover a region of approximately 16 kb upstream of bcr2; a 0.7-kb EcoR1-EcoR1 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 \times 10^6 cpm/\mug DNA.

Southern and Northern analyses. The standard procedures were followed as previously described.2 Twenty micrograms total RNA were used in Northern analyses.

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.

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ACUTE LEUKEMIAS: CLUSTERING OF BREAKPOINTS

Fig 1. (A) Partial restriction map of the BCR gene. The black boxes below the map indicate the positions of exons. The slashes indicate a gap in the first intron (noncloned region). Detailed restriction map for three clusters of rearrangements (bcr or M-BCR-1, bcr2 or m-BCR-2, and bcr3 or M-BCR-3) is magnified to indicate the precise location of breakpoints (arrows) in 14 Ph+ acute leukemia cases of our series. The probes used are represented by black boxes above the map (see also text). (B) Genomic organization of the c-abl gene on chromosome 9 and breakpoints (arrows) in Ph+ acute leukemias. The breakpoints in patients Bo and Na, as well as in a Ph+ bcr- case of ALL, are situated 5 to 6 kb upstream to exon Ia. A rearrangement has been localized in the second intron in a Ph+ bcr2 ALL case (see text). The position of the 0.7-kb EcoRI-EcoR1 probe is shown above the map (black box). In both (A) and (B), previously localized breakpoints are indicated by the letters F, R, and H in open arrows. Abbreviations: B, BamHI; Bg, BglII; E, EcoR1; H, HindIII; S, Sall.

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 Ph+ bcr+ ALL and not in the three other BCR-related loci, since according to Lifshitz et al 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, evidence has recently been obtained suggesting that this intron may be as large as 70 kb. 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 5-kb BamHI-BglII region 3' to BB-2 (Fig 1A).

Figure 1A illustrates the localization of breakpoints on chromosome 22 in our series of 14 Ph+ acute leukemia cases and shows that six exhibit bcr2 rearrangements at the 3' end of the first BCR intron. The region with rearrangements defined in the present study may be supposed to represent a new minor hot spot of breakpoints (here provisionally designated as bcr3 or m-BCR-2), although more cases should be studied to confirm this possibility. Analysis of restriction maps and/or breakpoints in the five Ph+ bcr- ALL cases or cell lines that have been mapped by others shows that these breakpoints also are situated within or very close to what we call bcr2 or m-BCR-1 (Fig 1A). Taken together, these results illustrate that in most, if not all, of Ph+ bcr- acute leukemias, the breakpoints on chromosome 22 are clustered in the 3' part of the first bcr intron. The clustering of the breakpoints, instead of their random distribution, suggests that there may be some particular sequences that favor the recombination process. Our subsequent work, cloning and sequencing the breakpoints from one case with a bcr2 rearrangement, has shown that indeed there are two pairs of Alu sequences in this region as well as on chromosome 9 adjacent to the breakpoints. Further information is needed before any general statement could be proposed.

As for the breakpoints on chromosome 9 in Ph+ acute leukemias, little information is available. In three recent reports, two for "fresh" cases of Ph+ bcr+ ALL and the other for the Ph+ 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 Ph+ 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 EcoRI-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 Ph+ 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 Ph+ bcr- ALL using appropriate primers. In a Ph+ 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-I as well as in the first intron. However, since different abnormal abl proteins may not play the same roles

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**Fig 2.** DNA digests from leukemic cells of a Ph1 bcr2 ALL1 and of patients Na2 and Bo3 were hybridized with the bcr2 probe BB-1 (A), the bcr3 probe BB-2 (B), and the 0.7-kb EcoRl-EcoRl 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), EcoRl (11 kb), BglI (8.5 kb) bands due to the deletion/insertion polymorphism already documented by Rubin et al.18

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**Fig 3.** abl' mRNA expression in the HL-60 cell line,1 the K562 cell line,2 leukemic cells from a patient with Ph1 bcr2 ALL, patient Na, and a Ph1 bcr ALL.8
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.

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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

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