A Possible Correlation Between the Type of bcr-abl Hybrid Messenger RNA and Platelet Count in Philadelphia-Positive Chronic Myelogenous Leukemia

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The Philadelphia (Ph1) chromosome, in which the hybrid bcr-abl gene is formed, is thought to be the initial event in chronic myelogenous leukemia (CML). The position of the breakpoint within the breakpoint cluster region (bcr) on Ph1 chromosome and the splicing pattern determine the species of the fused bcr-abl messenger RNA (mRNA). We tried to detect the two types of fused mRNAs in 57 chronic-phase cases of Ph1-positive CML using the polymerase chain reaction procedure (RT-PCR). The bcr exon 2/abl exon 2 fused mRNA (b2-a2) was detected in 17 patients, the bcr exon 3/abl exon 2 fused mRNA (b3-a2) was detected in 34 patients, and both types of mRNAs were detected in six patients. The platelet counts of patients who expressed b3-a2 mRNA or both types were significantly higher than those of patients who expressed only b2-a2 (841.5 x 10^9/L; P < .015), although there was no significant difference in the white blood cell counts or hemoglobin. This finding suggests a possibility that the type of bcr-abl mRNA may affect the thrombopoietic activity in CML.

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MATERIALS AND METHODS

Patient population. Blood and bone marrow samples were obtained from 57 Ph1-positive CML patients in the chronic phase of the disease. Most of the patients had not received therapy before RNA analysis. The clinical data of each patient are that at diagnosis.

RT-PCR for detection of bcr-abl fusion mRNA. The total RNA of mononuclear cells was extracted by the CaCl method. RT-PCR was performed using the methods described by Roth et al. and by Kawasaki et al with a slight modification. Approximately 2 μg of total RNA was used as the template with 10 U of avian myeloblastosis virus reverse-transcriptase (Boehringer-Mannheim, Indianapolis, IN) for 1 hour at 37°C in 50 mmol/L Tris-HCl, pH 8.3, 50 mmol/L KCl, 8 mmol/L MgCl₂, 10 mmol/L dithiothreitol, dNTPs at 500 μmol/L each in a total volume of 50 μL with 0.05 OD₂₆₀ units of oligo-primer (abl-RT: 5'-ACGAAAGGTTGGGGT-3', antisense strand). Ten microliters of the reverse transcriptase reaction mixture was adjusted to PCR conditions (10 mmol/L Tris-HCl, pH 8.3, 50 mmol/L KCl, 15 mmol/L MgCl₂, 2 mmol/L dithiothreitol, 200 μmol/L dNTPs) with 0.0125 OD₂₆₀ units of one of two sets of primers (the first set, A: 5'-AGATGGCCCTTCAGGCTGA-CAGCCCAACGCAGGCAA-3', coding strand, and B: 5'-TCAGGTCAGCAAGGAAGT-CCTTGGAGTT-3', antisense strand; the second set; C: 5'-GGAGCTGAGATGCTGACCA-3', coding strand and D: 5'-TCAGGACTCCCTGACGCTA-3', antisense strand) and 2 U of Taq polymerase (Thermus aquaticus DNA polymerase; Perkin Elmer Cetus, Norwalk, CT) in a 50 μL reaction volume (Fig 1). PCR of 40 cycles was performed, consisting of 30 seconds at 94°C (denaturation), 30 seconds at 55°C (annealing), and 1 minute at 75°C (extension). Finally, the PCR products (20 μL) were phenol-extracted, ethanol-precipitated, and electrophoresed through a 2% agarose gel. The PCR products (20 μL) were phenol-extracted, ethanol-precipitated, and electrophoresed through a 2% agarose gel. The PCR products were visualized directly in ethidium bromide-stained gels, photographed, and transferred onto Gene Screen Plus membranes (New England Nuclear, Boston, MA). Southern hybridization was performed using a 0.6HB probe corresponding to the bcr exon 2 (provided by Dr S. Hiraoswa, Tokyo Medical and Dental University), or two kinds of oligonucleotides (E: 5'-GCTGAAGGCGTTT-
**bcr-abl** mRNA (8.5 kb)

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**Fig 1.** Schematic representation of the **bcr-abl** mRNA with position of oligomers numbered A, B, C, and D used in the PCR reaction, and ab1-RT oligomer for making cDNA synthesis as described.\textsuperscript{14,19} (□) **bcr**-specific sequences; (■) **abl**-specific sequences.

TGAACCTCT\textsuperscript{3'}, specific for **bcr** exon3/abl exon 2; and F: 5'-GCTGAAGGGCTTCTCTTATTGATG-3', specific for **bcr** exon2/abl exon 2.\textsuperscript{11}

**RESULTS**

RNA samples from 57 CML patients were analyzed for specific **bcr-abl** mRNA. The **bcr-abl** mRNA junctions were amplified by the RT-PCR method using two pairs of primers. The two junctions, bcr2-ab12 and bcr3-ab12, were identified by their electrophoretic mobility and hybridization to **bcr** exon2 (0.6HB) probe or junction-specific oligoprobes. An example of the RT-PCR analysis in which a pair of primers (primers A and B) was used is shown in Fig 2. The RT-PCR products of RNA samples from K562 cells and five CML patients were electrophoresed and subsequently visualized by ethidium bromide staining and hybridization to the 0.6HB probe. The 319-bp RT-PCR product is the band specific for b3-a2 mRNA, while the 244-bp RT-PCR product is specific for b2-a2 mRNA. The RT-PCR analysis of 57 patients showed the presence of b3-a2 mRNA in 34 cases and b2-a2 mRNA in 17 cases, and coexpression of both junctions in six cases. The relationship between the type of **bcr-abl** mRNA and platelet count is shown in Fig 3. The platelet counts of patients expressing b3-a2 mRNA were significantly higher than those of patients expressing only b2-a2 mRNA (841.5 \( \pm \) 373.5 \( \times \) 10\(^7\)/L; \( P < .015 \)). We also examined the relationship with the white blood cell count and hemoglobin, but no significant correlations were found.

**DISCUSSION**

Ph1-positive CML is thought to be a clonal myeloproliferative disorder resulting from oncogenic transformation of a bone marrow stem cell. In the chronic phase, the disease phenotype is characterized by pronounced granulocytosis and thrombocytosis in peripheral blood and increased myeloid and megakaryocyte compartments in the hypercellular bone marrow.

Recently, our understanding of the pathogenesis of CML has been greatly advanced by the molecular studies. Ph1-positive CML is characterized by the presence of a **bcr** rearrangement. Attempts have been made to elucidate the relationship between the breakpoint site and the duration of survival or the other clinical features by Southern analysis at the DNA level. Shtalrid et al,\textsuperscript{9} Jaubert et al,\textsuperscript{10} and Morris et al\textsuperscript{11} reported that there was a significant correla-

**Fig 2.** Examples of Southern blot hybridization of RT-PCR products of the **bcr-abl** mRNA in Ph1-positive patients. Products from RT-PCR analyses were size-fractionated by 2.0% agarose gel electrophoresis, transferred to a nylon membrane, and hybridized to a 0.6HB probe. K562 cells and patients 1, 4, and 6 have the b3a2 junction. Patients 2 and 5 have the b2a2 junction only. Patient 3 produces both junctional products.

**Fig 3.** Correlation of platelet count with the type of **bcr-abl** mRNA. The platelet counts were obtained at diagnosis before the therapy. The normal range is indicated by the hatched box. The bars indicate the mean values. The significance of platelet counts in both types was tested with the Wilcoxon-2 sample test.
tion between the site of the bcr breakpoint and the duration of survival. CML patients have one of two bcr-ab1 mRNA types or both. Application of the RT-PCR method to determine the type of bcr-ab1 mRNA in CML patients has made it easier to identify the site of the bcr rearrangement. The studies using the RT-PCR method are now prevailing in this research area. Recently, we found that CML patients with a 3' breakpoint site within the bcr gene have a tendency to have higher thrombopoietic activity than those with a 5' breakpoint. Therefore, to confirm this preliminary observation, we extensively studied the relationship between the type of bcr-ab1 mRNA and the patient's platelet count in 57 Ph1-positive CML cases using the PCR method. As expected, the platelet counts were higher in the patients expressing b3-a2 mRNA.

A recent study reported similar finding in Ph1-positive essential thrombocytemia. The b3-a2 type mRNA was observed in five patients and the b2-a2 type mRNA was observed in only one patient. In our present study, 16 patients who had platelet counts of more than 900 x 10^9/L expressed only b3-a2 type mRNA, and almost all of the patients with b2-a2 type mRNA had a platelet count of less than 600 x 10^9/L. There were no apparent differences in hematologic findings between the patients with b3-a2 type mRNA and the CML patients with b2-a2 type mRNA, except for the platelet count. Thus, we speculate that the type of bcr-ab1 mRNA may affect the thrombopoietic activity in CML, although we are currently unable to explain why the platelet count correlates with the type of bcr-ab1 mRNA.

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REFERENCES

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