

Brief Report

SPONTANEOUS REMISSION IN A PATIENT WITH CHRONIC MYELOGENOUS LEUKEMIA

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IN chronic myelogenous leukemia (CML) the Philadelphia (Ph) chromosome originates from a reciprocal translocation, $t(9;22)(q34;q11)$,¹ that generates a chimeric *BCR-ABL* gene.² The disease is incurable without bone marrow transplantation. In some cases, intensive chemotherapy^{3,4} or treatment with interferon alfa can suppress the Ph-positive clone in the bone marrow,⁵⁻⁷ but the detection of residual disease by the polymerase chain reaction (PCR) in patients with a complete cytogenetic response suggests incomplete eradication of these cells.⁸

Few cases of Ph-positive CML have been reported in which a long-term remission occurred without treatment.⁹⁻¹¹ We describe a patient with Ph-positive CML who entered a lengthy remission, with a normal karyotype and no evidence of the *BCR-ABL* gene, without any therapy.

CASE REPORT

A 45-year-old man was referred to our hospital for evaluation of leukocytosis in January 1985. Three months previously, he had reported tarry stools. A peptic ulcer was diagnosed and treated with intravenous cimetidine. At that time, leukocytosis, thrombocytosis, and anemia were detected. A bone marrow aspirate showed marked myeloid hyperplasia. Cytogenetic analysis revealed Ph-positive cells in the bone marrow, and a diagnosis of CML was

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made. During the next month the leukocyte count decreased to 14,400 per cubic millimeter, but it subsequently gradually increased to 31,800 per cubic millimeter before admission to our hospital.

Physical examination on admission revealed anemia and mild hepatosplenomegaly. A complete blood count again showed leukocytosis and thrombocytosis. The neutrophil alkaline phosphatase score was 94 (normal range, 170 to 335). Plasma histamine and prostaglandin E concentrations were within the normal range. An endoscopic examination revealed an ulcer scar in the duodenal bulb.

Regular follow-up, without chemotherapy, was planned for the patient. In February 1985, the hepatosplenomegaly disappeared. The leukocyte count and platelet count returned to normal in April 1985. As of January 30, 1996, the patient had been well, without any signs of recurrence, for 11 years. Blood counts since June 30, 1994, have been normal. Table 1 summarizes the hematologic values.

METHODS

Chromosome preparations were made from bone marrow cells, as described previously,¹² and mounted on slides, which were then subjected to G and Q banding. Abnormal karyotypes were classified according to the International System for Human Cytogenetic Nomenclature.¹³

RNA was extracted from the bone marrow cells¹⁴ and reverse-transcribed to amplify the chimeric *BCR-ABL* complementary DNA (cDNA) with PCR.¹⁵ Southern blot analysis with a 1.3-kb 5'-*BCR* probe was performed as previously described,¹⁶ with genomic DNA extracted from the marrow cells.

RESULTS

Table 1 shows the results of eight chromosomal analyses carried out over a period of 10 years. All marrow cells in metaphase were Ph-positive on the first (November 8, 1984) and second (January 30, 1985) examinations. The abnormality was apparently due to a reciprocal translocation between chromosomes 9 and 22, giving a karyotype of 46,XY,t(9;22)(q34;q11) (data not shown). The proportion of Ph-positive cells in the marrow was decreased in July 1985, and no Ph-positive cells were detected in February 1987. Thereafter, all metaphases examined showed a normal karyotype.

Reverse-transcription PCR was expected to yield a chimeric product of 446 bp, a 371-bp fragment of the major *BCR-ABL* region, a 472-bp fragment of the minor *BCR-ABL* region, and a 253-bp fragment of *ABL* (which was used as an internal standard). However, no rearrangements of the 3' or 5' ends of the *BCR* gene were detectable by Southern blot analysis (data not shown), and chimeric cDNA was not found by reverse-transcription PCR in the bone marrow cells obtained on August 10, 1993 (Fig. 1), and June 30, 1994. No DNA or RNA from the bone marrow cells containing Ph-positive cells was available for analysis.

The serum level of interferon- α measured in July 1985 was within the normal range (<4 IU per milliliter), and the level of interferon- α produced by peripheral-blood mononuclear cells was also normal (1600 IU per milliliter).

TABLE 1. HEMATOLOGIC VALUES AND RESULTS OF CYTOGENETIC ANALYSIS IN THE PATIENT.

DATE	SPLENO- MEGALY*	HEMO- GLOBIN	PLATELET COUNT	WHITE- CELL COUNT	DIFFERENTIAL COUNT							NAP SCORE†	VITAMIN B ₁₂ ‡	No. OF Ph-POSITIVE CELLS/No. OF CELLS IN METAPHASE ANALYZED§
					BAND FORMS	SEG- MENTED CELLS	BASO- PHILS	EOSINO- PHILS	LYMPHO- CYTES	MONO- CYTES	OTHER			
10/12/84	?	9.0	488	46.4	4	68	5	2	7	1	13¶			
10/22/84	?	10.2	1010	35.0	6	64	4	1	14	2	9	84	>6400	
11/8/84	?	11.3	721	14.4	8	62	10	0	18	1	1**			25/25
11/20/84	?	12.7	965	28.5	8	76	8	1	4	2	1**			
12/13/84	?	12.9	810	31.8	11	62	6	3	6	3	9††			
1/9/85	+	13.4	514	17.3	5	56	1	3	32	3	0			
1/30/85	+	13.6	468	15.3	2	70	8	2	17	1	0	94	>3200	12/12
2/27/85	-	13.5	471	10.2	1	59	17	6	25	1	1**			
3/6/85	-	13.4	454	9.7	1	61	9	2	26	1	1**		>3200	
7/16/85	-	14.5	298	4.4	1	50	1	4	44	0	0			9/23
10/8/85	-	14.3	290	4.1	7	55	0	2	29	7	0			
4/4/86	-	14.3	320	4.9	5	56	1	3	32	3	0			
7/11/86	-	14.4	293	5.3	3	48	3	3	42	1	0			
2/17/87	-	15.7	301	4.7	3	45	1	3	44	4	0			0/20
10/27/87	-	14.8	301	5.7	2	50	0	3	41	4	0			
1/26/88	-	14.8	299	5.2	2	54	1	2	40	1	0		766	0/20
7/21/88	-	15.4	294	6.7	0	66	0	1	28	5	0	145	683	0/150
5/20/91	-	15.5	220	6.0	1	46	0	3	48	2	0			
8/10/93	-	16.1	304	6.7	2	72	0	1	24	1	0			0/20
6/30/94	-	14.8	232	5.7	2	56	1	8	31	2	0	158	758	0/20
4/24/95	-	15.7	299	8.3	5	57	0	4	29	5	0			
1/30/96	-	15.4	268	7.1	4	53	0	3	35	5	0			

*A minus sign indicates the absence of splenomegaly; a plus sign indicates the spleen was palpable at the left costal margin.

†The normal range for neutrophil alkaline phosphatase (NAP) is 170 to 335.

‡The normal range for vitamin B₁₂ is 300 to 900 pg per milliliter. To convert values to picomoles per liter, multiply by 0.7378.

§To identify the Ph chromosome, G- or Q-banding techniques were used, except for the analysis on July 21, 1988 (on that date only simple Giemsa staining was used).

¶Blasts made up 5 percent of the total, promyelocytes 3 percent, myelocytes 4 percent, and metamyelocytes 1 percent.

||Blasts made up 0.5 percent of the total, promyelocytes 2 percent, myelocytes 5.5 percent, and metamyelocytes 1 percent.

**The cells consisted of myelocytes.

††Promyelocytes made up 1 percent of the total, myelocytes 6 percent, and metamyelocytes 2 percent.

DISCUSSION

The striking feature of this case is the disappearance of Ph-positive cells from the marrow without any treatment. To our knowledge, only three other cases of hematologic and cytogenetic regression without chemotherapy have been reported.⁹⁻¹¹ In two of those cases, however, the remission was incomplete. Overt leukemia subsequently developed in one patient, with 100 percent Ph-positive marrow cells; in the other, a small percentage of Ph-positive cells persisted in the marrow.¹⁰ The third patient, who was followed for four years, was similar to ours.¹¹ All Ph-positive cells disappeared from his blood, and

Southern blot analysis revealed no rearrangements of *BCR* in the marrow cells.

In our patient, the absence of the chimeric *BCR-ABL* gene in bone marrow cells was demonstrated by Southern blot analysis and reverse-transcription PCR after the disappearance of cells. Because molecular analysis was not done before the disappearance of these cells, we cannot exclude the possibility that our patient may have had a unique breakpoint in the *BCR* gene that influenced his remission. However, the possibility of a relation between a *BCR* breakpoint and the duration of the chronic phase of CML is controversial.¹⁷

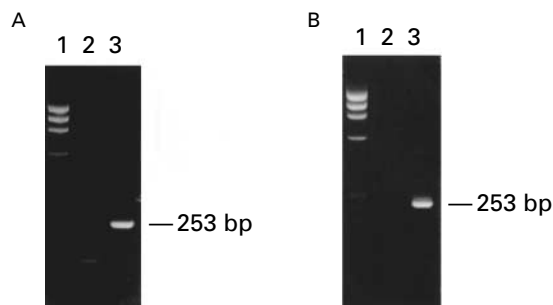


Figure 1. Analysis of Chimeric *BCR-ABL* Messenger RNA by Reverse-Transcription PCR.

Total RNA extracted from patient's bone marrow cells on August 10, 1993, was amplified with major *BCR-ABL* primers (lane 2 in Panel A), a minor *BCR-ABL* primer (lane 2 in Panel B), and a 253-bp *ABL* primer, which was used as an internal standard (lane 3 in Panels A and B). In each panel, lane 1 shows the size marker ϕ X174 digested with *Hae*III. No chimeric *BCR-ABL* products were detected.

Transient spontaneous regression of acute non-lymphocytic leukemia in adults is a rare event that is generally associated with pyogenic bacterial infection and lasts only weeks to months.¹⁸ In such cases, intrinsic cytokines, such as interferons, may have a role. However, our patient had no febrile episodes after the onset of the disease and did not receive interferon therapy. An influence of intrinsic interferon- α on his clinical course seems unlikely, since the serum interferon- α level and the level of interferon- α produced by mononuclear cells were normal in July 1985. The mechanism of the spontaneous remission in our case, as in most other examples of spontaneous regression of neoplasms, is unknown.

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REFERENCES

- Rowley JD. A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. *Nature* 1973;243:290-3.
- Heisterkamp N, Stephenson JR, Groffen J, et al. Localization of the *c-abl* oncogene adjacent to a translocation break point in chronic myelocytic leukaemia. *Nature* 1983;306:239-42.
- Smalley RV, Vogel J, Huguley CM Jr, Miller D. Chronic granulocytic leukemia: cytogenetic conversion of the bone marrow with cycle-specific chemotherapy. *Blood* 1977;50:107-13.
- Cunningham I, Gee T, Dowling M, et al. Results of treatment of Ph⁺ chronic myelogenous leukemia with an intensive treatment regimen (L-5 protocol). *Blood* 1979;53:375-95.
- Talpaz M, McCredie KB, Mavligit GM, Gutterman JU. Leukocyte interferon-induced myeloid cyto-reduction in chronic myelogenous leukemia. *Blood* 1983;62:689-92.
- Talpaz M, Kantarjian HM, McCredie K, Trujillo JM, Keating MJ, Gutterman JU. Hematologic remission and cytogenetic improvement induced by recombinant human interferon alpha₂ in chronic myelogenous leukemia. *N Engl J Med* 1986;314:1065-9.
- Talpaz M, Kantarjian H, Kurzrock R, Gutterman JU. Interferon alpha in the therapy of CML. *Br J Haematol* 1991;79:Suppl 1:38-41.
- Opalka B, Wandl UB, Becher R, et al. Minimal residual disease in patients with chronic myelogenous leukemia undergoing long-term treatment with recombinant interferon alpha-2b alone or in combination with interferon gamma. *Blood* 1991;78:2188-93.
- Canellos GP, Whang-Peng J. Philadelphia-chromosome-positive preleukemic state. *Lancet* 1972;2:1227-8.
- Smadja N, Krulik M, Audebert AA, de Gramont A, Debray J. Spontaneous regression of cytogenetic and haematologic anomalies in Ph1-positive chronic myelogenous leukaemia. *Br J Haematol* 1986;63:257-62.
- Provan AB, Majer RV, Herbert A, Smith AG. Spontaneous remission of chronic myeloid leukaemia with loss of the Philadelphia chromosome. *Br J Haematol* 1991;78:578-9.
- Sandberg AA, Abe S. Cytogenetic techniques in haematology. *Clin Haematol* 1980;9:19-38.
- Mitelman F, ed. *ISCN (1995): an International System for Human Cytogenetic Nomenclature*. Basel, Switzerland: S. Karger, 1995.
- Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 1987;162:156-9.
- Sawyers CL, Timson L, Kawasaki ES, Clark SS, Witte ON, Champlin R. Molecular relapse in chronic myelogenous leukemia patients after bone marrow transplantation detected by polymerase chain reaction. *Proc Natl Acad Sci U S A* 1990;87:563-7.
- Groffen J, Stephenson JR, Heisterkamp N, de Klein A, Bartram CR, Grosfeld G. Philadelphia chromosomal breakpoints are clustered within a limited region, *bcrl*, on chromosome 22. *Cell* 1984;36:93-9.
- Mills KI, Benn P, Birnie GD. Does the breakpoint within the major breakpoint cluster region (*M-bcr*) influence the duration of the chronic phase in chronic myeloid leukemia? An analytical comparison of current literature. *Blood* 1991;78:1155-61.
- Wiermick PH. Spontaneous regression of hematologic cancers. In: Lewison EF, ed. *Conference on spontaneous regression of cancer*. National Cancer Institute monograph 44. Washington, D.C.: Government Printing Office, 1976:35-8. (DHEW publication no. (NIH) 76-1038.)