

Preferential Involvement of Chromosomes No. 8 and No. 21 in Acute Leukemia and Preleukemia

By Kiyomi Yamada and Shimpei Furusawa

Chromosome analyses were performed by a direct method on bone marrow cells of 147 patients with acute leukemia and preleukemia; in 53 chromosomally abnormal cell lines were found. Chromosome abnormalities due to structural alterations were observed in 48% of the aneuploid patients. Using the ASG banding technique, the exact identification of the abnormal chromosomes was successfully made in 22 aneuploid patients. Even though variability between patients ex-

isted in the chromosome changes; the non-random occurrence of some chromosome abnormalities was revealed, involving most frequently chromosomes No. 8 and No. 21. Abnormalities of chromosome No. 22 were not encountered, contrasting sharply with the frequent involvement of this chromosome in chronic myelogenous leukemia. The significance of the preferential involvement of No. 8 and No. 21 chromosomes is discussed in relation to leukemogenesis.

THE EXACT IDENTIFICATION of individual human chromosomes could not be established with certainty until recently. However, it is now possible to identify rigorously with newly developed chromosome banding techniques each chromosome pair, parts of chromosomes, and those involved in chromosomal rearrangements. A rapid expansion of our knowledge in chromosome cytology has been brought about by these new techniques. In chronic myelocytic leukemia (CML) it has been demonstrated that the Philadelphia chromosome (Ph¹) is a deleted No. 22 chromosome and that the deleted segment most often is translocated onto chromosome No. 9.¹ These methods also make possible a detailed analysis of the chromosomes in acute leukemia and related states, particularly regarding the random or nonrandom involvement of certain chromosomes in the karyotypic abnormalities of these disease states. We wish to describe chromosome studies in patients with acute leukemia or preleukemic disorders, based on the identification of abnormal chromosomes with banding techniques, and to demonstrate a possible nonrandomness of the chromosome abnormalities in the leukemic and preleukemic cells.

MATERIALS AND METHODS

Chromosome analysis was performed on cells from bone marrow aspirates. The preparation of cells was made according to the direct method of Tjio and Whang² with slight modifications. The procedures for chromosome banding were essentially the same as those described by Sumner et al.,³ referred to as the acetic/saline/Giemsa (ASG) technique, except for longer incubation of the slides in $2 \times$ SSC. Fresh slides were incubated in $2 \times$ SSC (0.3 M sodium chloride and 0.03 M sodium citrate) for 6–18 hr at 60°C. Afterwards, the slides were rinsed with deionized water, stained

From the Department of Cytogenetics, Medical Research Institute, and the First Department of Internal Medicine, Faculty of Medicine, Tokyo Medical and Dental University, Tokyo, Japan.

Submitted May 30, 1975; accepted October 26, 1975.

Supported by Grant 957279-1974 for Scientific Research from the Ministry of Education, Japan.

Address for reprint requests: Kiyomi Yamada, D. Sc., Department of Genetics, Clinical Research Institute, National Medical Center Hospital, 1-Toyamacho, Shinjuku-ku, Tokyo 162, Japan.

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for 1.5 hr with 2% Giemsa solution (Gurr's Giemsa R66 and pH 6.8 buffer), rinsed with deionized water and air dried. When necessary, slides were stained with 0.005% quinacrine mustard for 20 min, according to the method of Caspersson et al.⁴ and studied by means of fluorescence microscopy, especially for detection of the Y chromosome. The identification of individual chromosomes by their banding patterns and the description of karyotypes were made according to the report of the Paris Conference (1971).⁵

Since 1967, karyotypic information has been obtained on a total of 147 hematologic patients: 74 with acute myelocytic leukemia (AML), 26 with acute lymphocytic leukemia (ALL), and 47 with preleukemic myeloid disorders which included well-defined chronic myeloproliferative disorders (excepting CML), aplastic or sideroblastic anemia, and other myelodysplasias. Among the 147 patients, 38 with acute leukemia and 15 with preleukemia had aneuploid cell lines and the remaining 94 had only diploid cells. Chromosome analysis in each patient was made on at least 30 cells and more than two metaphases were karyotyped. With the introduction of the banding techniques in 1972, the 28 aneuploid cases seen since then were further subjected to study with these techniques.

RESULTS

The exact identification of abnormal chromosomes, based on their banding patterns, was successfully made in 22 of the 28 cases studied. Failure in the six cases was due to ambiguous banding patterns: three cases of AML with karyotypes of 47,XY,+C, 46,XY,-C,+D, and 78 chromosomes, respectively; and one case each of ALL, polycythemia vera, and sideroblastic anemia with karyotypes of 47,XY,+C, 45,XY,-C, and 47,XX,+C, respectively.

Table 1. Karyotypes Identified by Banding Techniques in 22 Cases

Case No.	Date of Marrow Sampling	Age (yr)	Karyotype of Aneuploid Cells	Clinical Diagnosis
1	12/14/72	34	47, XY, +8	AML
2	1/25/73	53	47, XX, +8	Aplastic anemia
3	10/24/73	58	47, XY, +8	AML
4	1/16/74	55	47, XY, +8	Pancytopenia, converted later to possible AML
5	4/18/74	36	47, XY, +8	Aplastic anemia, converted later to possible EL
6	2/9/73	40	49, XY, +8, +12, +17, t(17;21) (p11;q22)	EL, converted from PNH
7	2/26/73	24	45, X, -Y, t(8;21) (q22;q22)	AML
8	6/15/73	44	47, XX, +21, i(17q)	ALL
9	11/13/72	26	45, XY, -21, 18p+	AML
10	10/2/73	19	45, XY, -21, t(4;9) (p16;q22)	AML
11	1/14/74	8	45, XX, -21/52, unknown	ALL
12	1/17/74	12	44, X, -Y, -21/45, XY, -21/46, XY, 1q+	Sideroblastic anemia
13	6/11/74	60	44, XY, -18, -21	Myelodysplasia
14	7/21/72	22	46, XY, t(1;6;11) (q12;q23;p15)	AML
15	12/22/72	32	47, XX, +16	ALL
16	6/5/73	34	46, XY, 1q+	ALL
17	6/28/73	49	45, XY, -7	AML
18	9/11/73	23	45, X, -X	ALL
19	9/28/73	50	45, XX, -2, 5q+	AML
20	12/7/73	33	46, XY, del (11) (q23), del (12) (p11)	AML
21	1/22/74	38	44, XY, -3, -18	EL
22	6/28/74	41	45, XX, -18	EL

AML, acute myelocytic leukemia; ALL, acute lymphocytic leukemia; EL, erythroleukemia; PNH, paroxymal nocturnal hemoglobinuria.

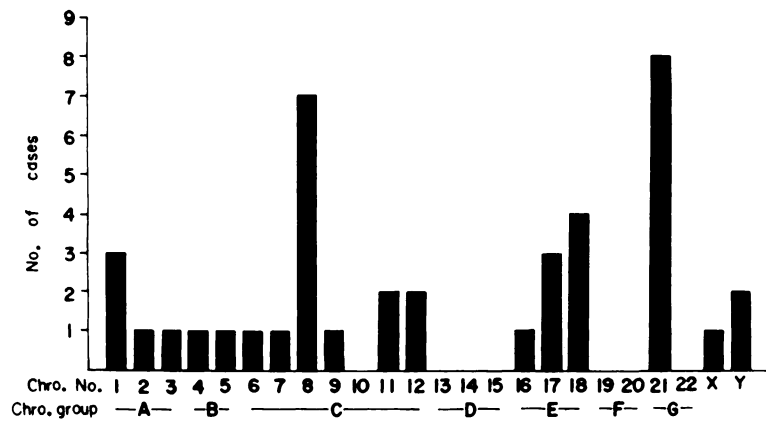


Fig. 1. Frequency distribution of individual chromosomes involved in numerical and structural changes in 22 cases.

The detailed karyotypes of the modal cells and the clinical diagnoses of the 22 identified patients (12 AML, 5 ALL, and 5 preleukemia) are shown in Table 1. The frequency of individual chromosomes involved in abnormalities is graphically shown in Fig. 1. A range of chromosome changes among these patients was observed, even in those with precisely identified karyotypes. However, it was found that the frequency of the chromosomes involved in numerical and structural changes was nonrandom, showing two peaks of frequency of chromosomes No. 8 and No. 21. The following are the descriptions of the chromosome abnormalities most frequently observed in the present study.

No. 8-Trisomy

The karyotypes were identified to be 47,XY or XX,+8 in five patients (Cases 1-5, and Fig. 2); except for Case 1, the patients had a mixed population of normal and 8-trisomic cells in the bone marrow.

Case 1. A 35-yr-old man with purpura since 1969 and anemia since 1971

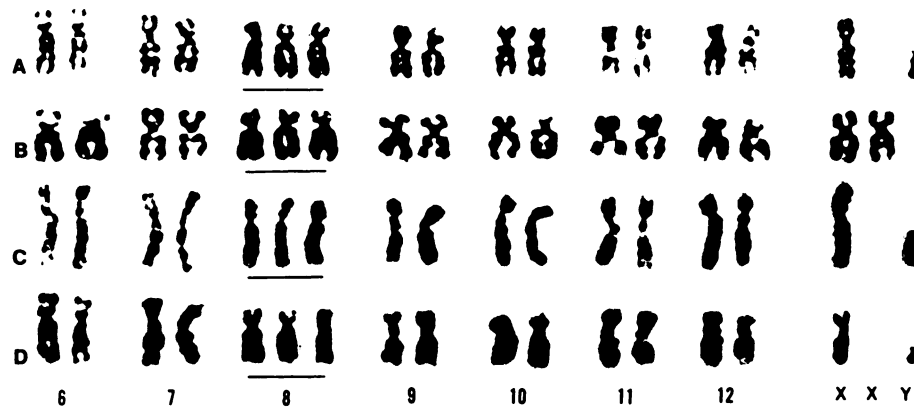


Fig. 2. Trisomy of No. 8. Partial karyotypes of one cell each from cases 1-4 (A-D) showing banding patterns of group C (6-12) and sex chromosomes.

was admitted to the hospital in November 1972. He had moderate anemia and thrombocytopenia, and mild leukocytosis with a few immature granulocytes. Bone marrow investigation revealed proliferation of all the three marrow elements and a left-sided granulocyte shift, giving the impression of preleukemia. Five months later, the hematologic picture was shown to be compatible with that of myelocytic leukemia. The patient died of respiratory infection in January 1974. The proportion of 47,XY,+8 cells was 95% in December 1972, and, subsequently, 100%, 92%, 86%, and 88% on four different occasions during a period of 7 mo. All of the analyzed cells including the polyploid ones were aneuploid.

Case 2. A 52-yr-old woman had repeated episodes of high fever and multiple ulcers of the mucous membranes, for which the administration of prednisolone was markedly effective. A diagnosis of aplastic anemia was made in 1958, when pancytopenia was found. The pancytopenia gradually progressed and blood transfusions were occasionally necessary. Bone marrow examination revealed normocellularity with increased erythropoiesis and decreased granulo and megakaryocytopoiesis. Ferrokinetic study showed a pattern compatible with aplastic anemia. The proportion of 47,XX,+8 cells decreased gradually with improvement of the anemia; 94% in 1969, 66% and 57% in 1971, 45% and 7% in 1973, and 23% in 1974. Pancytopenia still persists at present.

Case 3. A 58-yr-old man was diagnosed as having acute myelomonocytic leukemia in October 1973. He obtained partial remission after chemotherapy but 6 mo later died of sepsis.

Case 4. A 54-yr-old man with pancytopenia, who had received blood transfusions 3 mo before, was admitted to the hospital in January 1974 because of purpura and serum hepatitis. On admission, blood cell counts showed neutropenia, monocytosis, and marked thrombocytopenia. Bone marrow examination revealed slight hypocellularity with a moderate increase of monocytoïd cells and atypical megakaryocytes. Preleukemia was suspected. The first chromo-

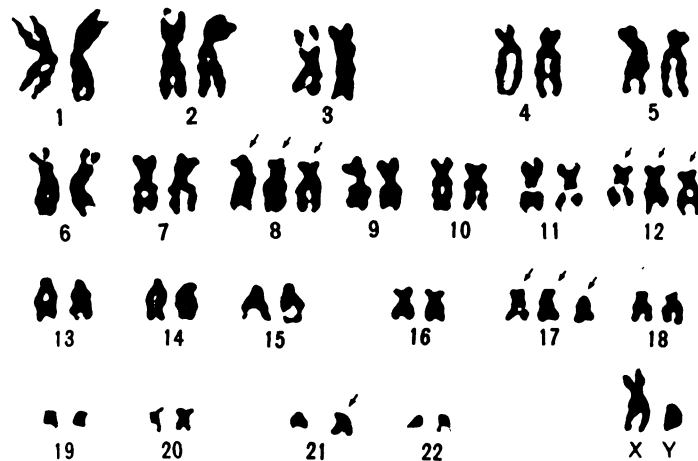


Fig. 3. The 49,XY,+8,+12,+17,t(17;21)(p11;q22) karyotype of case 6, a patient with erythroleukemia.

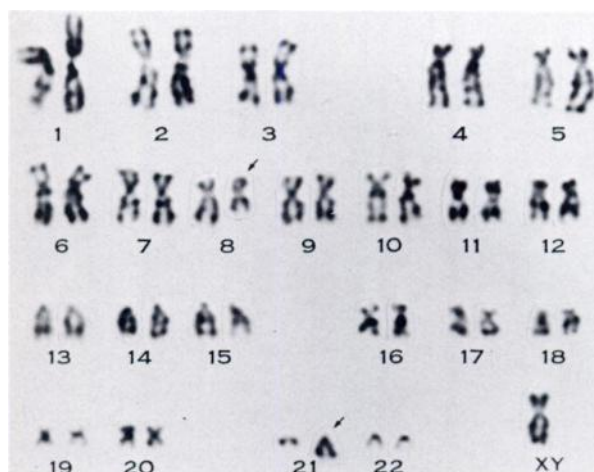


Fig. 4. The 45,X,-Y,t(8;21)(q22;q22) karyotype of case 7, a patient with acute myelocytic leukemia.

some study was done on this marrow specimen. Ferrokinetic study showed an almost normal pattern. In November 1974 bone marrow investigation revealed a marked increase of myeloblasts and monocytoïd cells, compatible with acute myelomonocytic leukemia. The proportion of 47,XY,+8 cells increased gradually from 11% to 60% during an untreated period from January to September in 1974.

Case 5. A 35-yr-old man was admitted to the hospital in April 1974. On admission blood cell counts showed marked pancytopenia. Bone marrow investigation revealed a marked increase of erythropoiesis with megaloblastoid traits and a moderate increase of megakaryocytopoiesis with atypical features; the findings were suggestive of preleukemia. Ferrokinetic study showed ineffective erythropoiesis. Five months later, the hematologic picture appeared to be that of erythroleukemia. He died of cerebral bleeding soon thereafter. The proportion of 47,XY,+8 cells was 86%, 85%, and 79% on three different occasions over a period of 5 mo.

No. 21 Abnormality

Abnormalities of chromosome No. 21 were observed in eight patients, but the types were different; No. 21-monosomy was present in five patients (cases 9-13), translocation with other chromosomes in two patients (cases 6 and 7; Figs. 3 and 4), and No. 21-trisomy in one patient (case 8).

Case 12. A 12-yr-old boy was admitted to the hospital in December 1973 because of anemia and hepatomegaly. He had a 6-yr history of aplastic anemia, which had been partially controlled with anabolic steroid therapy. On admission, he had marked normochromic anemia with normal leukocyte and platelet counts. Bone marrow examination revealed normoblastic hyperplasia with a number of ringed sideroblasts. Ferrokinetic study showed a pattern of ineffective erythropoiesis. Liver function tests were suggestive of acute hepatitis. No familial history of anemia was noted. Chromosome examination revealed aneuploidy in 50% of the analyzed cells. A diagnosis of sideroblastic anemia (acquired ?) was made. His anemia appeared to be partially responsive to vitamin B₆ therapy. A moderate anemia still persists at present.

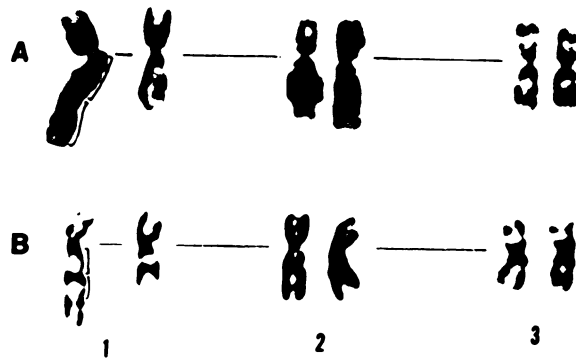


Fig. 5. The banding patterns of group-A chromosomes in a cell: (A) from case 12; (B) from case 16. The long arm in a No. 1 chromosome is probably partially duplicated in the segment between 1q12 and 1q44 in (A), and in the segment between 1q12 and 1q25 in (B).

Case 13. A 60-yr-old male became aware of fatigue and palpitation in May 1974 and was admitted to the hospital a short time later. Physical examination revealed marked anemia and slight hepatomegaly without splenomegaly or lymphadenopathy. He had marked pancytopenia but no immature myeloid cells in the blood. Bone marrow examination revealed a hypercellular marrow with 28% monocytoid cells and no increase of the immature myeloid cells. Chromosome examination revealed aneuploidy in 93% of the analyzed cells. A tentative diagnosis of myelodysplasia, highly suggestive of preleukemia, was made. Four months later he developed AML and died suddenly of sepsis.

No. 1q+ Marker

A large submetacentric marker chromosome with a banding pattern suggestive of a partial duplication of the long arm of chromosome No. 1 was observed in the modal cells from two patients (cases 12 and 16, Fig. 5).

DISCUSSION

From the results of the exact identification of abnormal chromosomes by banding techniques, it was concluded that the frequency of individual chromosomes involved in abnormalities was clearly nonrandom, showing a preferential involvement of chromosomes No. 8 and No. 21. In previous studies^{6,7} it was pointed out that the chromosomes belonging to groups C and G were frequently involved in karyotypic abnormalities in acute leukemia. In the present study these were revealed to be probably No. 8 in the C group and No. 21 in the G group.

We found five patients who had an No. 8-trisomy cell line in the bone marrow. The detailed clinical features of patients with No. 8-trisomy have been described in seven patients in the literature: two with sideroblastic anemia^{8,9} and one patient each with pancytopenia,¹⁰ granulocytopenia and thrombocytopenia,¹⁰ polycythemia vera,¹¹ CML without a Ph¹,¹¹ and AML.⁹ The clinical features of these 12 patients were somewhat different, but ineffective erythropoiesis in the bone marrow was commonly observed. Recently, the frequent occurrence of No. 8-trisomy with or without other chromosome abnormalities was reported in AML and in the acute phase of CML.¹² Therefore, No. 8-trisomy seems to be a common abnormality in nonlymphocytic cells in pa-

tients with various hematologic disorders. We would like to postulate that some genes responsible for the control of hematopoiesis may be located on chromosome No. 8.

Abnormalities of chromosome No. 21 were found in the cells from eight patients of the present study consisting mostly of No. 21-monosomy and rearrangements involving chromosome No. 21. G-monosomy and rearrangements of G-group chromosomes have been frequently seen in cells transformed *in vitro* by viruses.^{13,14} Recently, evidence suggesting that the gene for antiviral protein is located on chromosome No. 21 has been presented.¹⁵ In Down's syndrome, a disease due to No. 21-trisomy, an increased incidence of leukemia in affected patients is well known.^{16,17} These two lines of evidence suggest some relationship between chromosome No. 21 abnormalities and leukemia development.

Among the abnormal karyotypes of leukemic cells, particularly interesting is the 45,X,t(8;21)(q22;q22) karyotype found in a male patient (case 7, and Fig. 4). The same karyotype has been reported in two female¹⁸ and one male patient,¹⁹ and a 46,XX,t(8;21)(q22;q22) karyotype in a female patient.¹⁹ Therefore, this type of t(8;21)(q22;q22) translocation is the first example of a relatively specific karyotypic change in acute leukemia revealed by banding techniques. Another interesting finding is that this translocation was frequently accompanied by a missing sex chromosome, an X in females and the Y in males. The most likely interpretation for this phenomenon is that the loss of a sex chromosome occurred in connection with the presence of the t(8;21) translocation.¹⁹ The concept that the loss and/or excess of certain autosomes or sex chromosomes in leukemic cells is associated with the presence of a translocation in the same cell may help us to understand the complicated chromosomal changes observed in many karyotypes in the present study.

A correlation between the karyotypic profiles and the clinical pictures can be established only after a large number of patients is studied with banding techniques. For the present, it is premature to speculate on the similarities in clinical features of patients with similar chromosome abnormalities. Recent results indicate that chromosome abnormalities induced by x-ray and chemical substances are nonrandom.^{20,21} The possibility that diseases with similar karyotypic characteristics are caused by a common etiologic agent has been recently proposed for human tumors and leukemias.^{12,22} An analysis of the correlation between specific karyotypes and the clinical features, responsiveness to therapeutic agents, and possible etiologic agents, remains an important area to be examined in the future.

ACKNOWLEDGMENT

The authors wish to express sincere thanks to former Professor Masafumi Komiya and Assistant Professor Takeo Nomura, First Department of Internal Medicine, Faculty of Medicine, Tokyo Medical and Dental University; Dr. Hisatoshi Miyata, Hematological Department, Japan Red Cross Medical Center, Tokyo; Dr. Tatsuya Ōhashi and Dr. Masahide Kamakura, First Department of Internal Medicine, Faculty of Medicine, University of Tokyo, for valuable criticism on the clinical data of the patients and for supplying bone marrow materials. The authors are very thankful to Dr. Avery A. Sandberg, Roswell Park Memorial Institute, Buffalo, N.Y., for reading the manuscript.

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