Partial Deletion of the Long Arm of Chromosome 16 and Bone Marrow Eosinophilia in Acute Nonlymphocytic Leukemia: A New Association

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Recently, several specific chromosomal abnormalities have been associated with distinctive clinical and/or morphological subtypes of acute nonlymphocytic leukemia (ANLL). To further investigate the clinical utility of karyotype analysis in ANLL, we have examined G-banded metaphase chromosomes at diagnosis in 61 consecutive patients. Of the 60 patients who had adequate mitoses, 47 (78%) had a clonal chromosome abnormality. The sole karyotypic abnormality found in 5 patients was a del(16)(q22). The unique pretreatment characteristic of these 5 patients was marrow eosinophilia ranging from 8% to 54%. No other patient had more than 4% marrow eosinophils. Among the patients with eosinophilia, all had Auer rods, serum muramidase was elevated in the 4 tested, and 4 had hepatomegaly at presentation. Both patients who survived initial treatment remain in complete remission at 23+ and 33+ mo. The data suggest that we have identified a new cytogenetic-clinical subtype of ANLL defined by the del(16)(q22).

BANDED CHROMOSOME studies have been done with considerable success in ANLL in the past decade.[1-4] Recent improvements in methodology have made it possible to identify clonal chromosome abnormalities in a majority, perhaps all, of these patients.[5-7] Clonal chromosome abnormalities have been found to correlate with specific subtypes of ANLL, such as the t(8;21) in M2 and the t(15;17) in M3 and M3 variant subgroups, and with prognosis.[1,2,6,8-16] In this article we report a new cytogenetic-clinicopathologic association in ANLL: partial deletion of the long arm of one chromosome 16 and bone marrow eosinophilia. Patients with this subtype of ANLL who achieve a complete remission may have prolonged disease-free survival.

MATERIALS AND METHODS

All 61 newly diagnosed ANLL patients admitted to the Medical and Pediatric Oncology Services at the University of Minnesota Hospitals between January 1980 and April 1982 were included in this study. Eight of the patients were children ages 7 mo to 14 yr, and 53 were adults ages 20–80 yr. Eight patients (all adults) had been previously treated with chemotherapy and/or radiation for other malignancies. The diagnosis of ANLL was made on the basis of morphology and cytochemical staining of the initial bone marrow aspirate and biopsy, and all were classified according to the French-American-British (FAB) system.[17]

Bone marrow cells from all 61 patients were obtained from heparinized posterior iliac crest aspirates, and the specimens were processed within 1 hr of aspiration. Metaphase chromosomes from direct preparations and short-term cultures were harvested according to previously described methods.[18] G-banding was done using the Wright's technique of Sanchez.[19] In all cases we attempted to analyze a minimum of 20 metaphases, and this was possible in 44 patients. No mitoses were obtained in one patient, and less than 10 metaphases were present in 5 additional patients. Twelve to 19 mitoses were analyzed in the remaining 11 patients. Photographs were taken on high contrast S0115 film, and multiple photokaryotypes were constructed in each case.

Heparinized peripheral blood was also received from 54 patients. Direct preparations and/or short-term cultures were done. Chromosomes were harvested and G-banded using the same methods as for bone marrow. No mitoses were present for analysis in 24 patients. In 19 patients, the findings in peripheral blood confirmed those from bone marrow. In 7 patients, the bone marrow contained only abnormal metaphases, but normal cells were found in peripheral blood. In the remaining 4 patients, abnormal clones were found in peripheral blood that were not seen in bone marrow.

RESULTS

Of the 60 patients who had adequate mitoses for analysis, 47 patients (78%) were found to have a clonal chromosome abnormality as defined by the Second International Workshop on Chromosomes in Leukemia.[6] Five patients had as their only abnormality a partial deletion of the long arm of one chromosome 16 (Fig. 1). The cytogenetic studies of these 5 patients are summarized in Table 1. A mixture of normal and abnormal metaphases was found in direct preparations of bone marrow from patients 1, 3, 4, and 5. Bone marrow cultures yielded additional normal and abnormal metaphases in patients 3 and 4. Short-term cultures of blood had a mixture of normal and abnormal metaphases in patients 1 and 2. In the case of patient 2, the chromosome abnormality was found in blood and not in bone marrow. Follow-up cytogenetic studies of bone marrow in remission in patients 3 and 4 have shown a disappearance of the del(16)(q22) clone.

The clinical features of the 5 patients with the del(16)(q22) are shown in Table 2. Three patients

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Supported in part by the Coleman Leukemia Research Fund.

Submitted August 27, 1982; accepted November 23, 1982.

This paper was presented in part at the American Society of Clinical Oncology meeting, in St. Louis, MO, April 1982.

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0006-4971/83/6105-0027$01.00/0

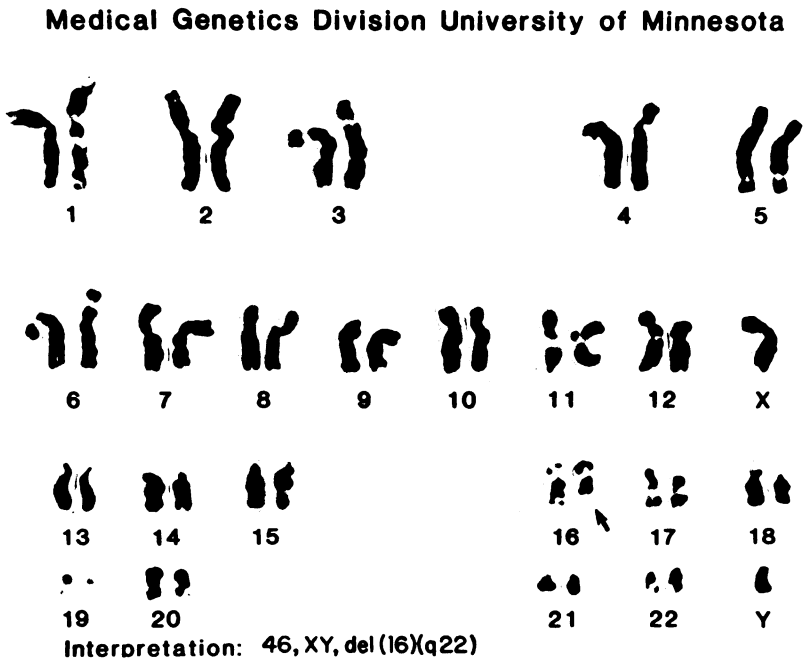

Fig. 1. G-banded karyotype showing the partial deletion of the long arm of one chromosome 16 in patient 1.

were males, ages 22 mo, 53 and 79 yr, and two were females, ages 14 and 50 yr. None of the 5 patients had received treatment for a previous malignancy. All patients had blasts with monocytoid morphological features; however, three patients had cytochemical stains most consistent with a diagnosis of M2 leukemia. Thus, three patients were designated as having M2 and two M4 leukemia using the FAB classification. Four patients had hepatomegaly at diagnosis. None had gingival hypertrophy, skin infiltrates, or central nervous system leukemia. Anemia was present in all cases, with hemoglobin values ranging from 5.2 to 11.5 g/dl. Leukocyte counts ranged from 2.8 to 80.0 $\times$ 10^9/liter, and platelet counts from 9 to 158 $\times$ 10^9/liter. Percentages of bone marrow blasts ranged from 24% to 69% and peripheral blood blasts from 32% to 77%. Auer rods were found in all 5 patients. Serum muramidase was elevated in the 4 patients in whom it was measured. The unique pretreatment feature of these 5 patients was bone marrow eosinophilia. Eosino-

Table 1. Cytogenetic Studies

Patient	Time of Study	Tissue	Method	No. of Cells Studied			No. of Cells Banded	Karyotype
				Total	Normal	Abnormal		
1	Diagnosis	Marrow	Direct	17	7	10	8	46,XY/46,XY,del(16)(q22)
			Culture	No mitoses				—
		Blood	Culture	3	1	2	3	46,XY/46,XY,del(16)(q22)
2	Diagnosis	Marrow	Direct	9	9	0	0	46,XX
			Culture	No mitoses				—
		Blood	Culture	20	13	7	14	46,XX/46,XX,del(16)(q22)
3	Diagnosis	Marrow	Direct	23	17	6	11	46,XX/46,XX,del(16)(q22)
			Culture	8	5	3	6	46,XX/46,XX,del(16)(q22)
		Blood	Culture	No mitoses				—
	Remission (6 mo)	Marrow	Direct	12	12	0	0	46,XX
			Culture	9	9	0	3	46,XX
	Remission (11 mo)	Marrow	Direct	1	1	0	0	46,XX
			Culture	29	29	0	8	46,XX
4	Diagnosis	Marrow	Direct	16	13	3	16	46,XY/46,XY,del(16)(q22)
			Culture	8	7	1	1	46,XY/46,XY,del(16)(q22)
		Blood	Culture	No mitoses				—
	Remission (12 mo)	Marrow	Direct	5	5	0	0	46,XY
			Culture	7	7	0	2	46,XY
5	Diagnosis	Marrow	Direct	21	17	4	6	46,XY/46,XY,del(16)(q22)
			Culture	No mitoses				—
		Blood	Culture	No mitoses				—

Table 2. Clinical Characteristics at Diagnosis and Response to Therapy

Patient	Sex	Age (yr)	FAB*	Hb (g/dl)	WBC ($\times 10^9$/liter)	Platelets ($\times 10^9$/liter)	Percent Blasts BM	Percent Blasts BL	Auer Rods	Percent Eos BM	Percent Eos BL	Serum Muram.	Other Findings	Duration of Initial Remission (mo)	Survival (mo)
1	M	1 9/12	M2	10.2	54.2	127	24	32	+	18	3	44	Fever Lymphadenopathy Hepatomegaly	Induction death	0.5
2	F	14	M2	5.2	80.0	9	69	66	+	13	2	58	Weakness Hepatomegaly Splenomegaly Bleeding gums	3.5	5.5†
3	F	50	M4	9.8	35.5	41	33	77	+	9	0	ND	Weakness	22+	23+
4	M	53	M2	11.5	2.8	158	26	50	+	54	6	17	Hepatomegaly	32+	33+
5	M	79	M4	9.9	40.3	72	61	65	+	8	0	84	Weakness Hepatomegaly Splenomegaly	No treatment	1

*FAB, French-American-British classification; Hb, hemoglobin; WBC, white blood cell count; BM, bone marrow; BL, blood; Eos, eosinophils; ND, not done; Serum Muram, serum muramidase, normal values = 2–12.

†Died in first remission of infectious complications of bone marrow transplantation.

phils and precursors comprised 8%, 9%, 13%, 18%, and 54% of the bone marrow cells.

Patient 5 refused therapy and died 1 mo after diagnosis. Patients 1 and 2 were treated with daunorubicin and cytosine arabinoside according to the Children's Cancer Study Group Protocol no. 251. Patient 1 died suddenly and unexpectedly on day 14 of induction chemotherapy, and autopsy failed to reveal a cause of death. Patient 2 achieved a complete remission and underwent allogeneic bone marrow transplantation according to Protocol no. 251. She died in remission of complications of transplantation (infection) 5.5 mo after diagnosis. Patients 3 and 4 were treated with a combination of adriamycin, cytosine arabinoside, 6-thioguanine, prednisone, and vincristine.[20] Both patients achieved a complete remission after one cycle of chemotherapy, and they presently continue in remission 23+ and 33+ mo after diagnosis.

DISCUSSION

Using the G-banding technique of staining metaphase bone marrow and peripheral blood chromosomes at diagnosis of ANLL in 61 consecutive patients, we have found a subgroup of 5 patients (8%) whose only karyotypic abnormality was a del(16)(q22). This abnormality was clonal, but normal metaphases were also found in bone marrow from all 5 patients and blood from 2 patients. Although the del(16)(q22) was not restricted to a single subtype of ANLL using the FAB classification, all patients had blasts with monocytoid morphological features, and the serum muramidase was elevated in all 4 patients with the del(16)(q22) who were tested. In contrast only 49% (24 of 47 tested) of the other ANLL patients had elevated serum muramidase levels. Auer rods were identified in the initial bone marrow aspirates of all 5 of these patients, but in only 28 of the other 55 patients (51%). Hepatomegaly was present at diagnosis in 4 of these 5 patients (80%) as compared to 22 (40%) of the remaining 55 patients who had adequate material for cytogenetic studies.

The unique pretreatment clinical finding identified in these 5 patients was bone marrow eosinophilia. None of the other 55 patients had marrow eosinophilia. All of them had less than 4% marrow eosinophils, and 51 patients had less than 2%. These data suggest that we have identified a new subtype of ANLL characterized by marrow eosinophilia and a partial deletion of the long arm of one chromosome 16. To our knowledge, this association has not been previously reported.

In all 5 of our patients with the del(16)(q22), the primary leukemic process was clearly ANLL, not eosinophilic leukemia or the hypereosinophilic syndrome.[21,22] Eosinophilia was prominent in each case in the bone marrow but not in peripheral blood. The eosinophils appeared to be part of the leukemic process, both granulation and differentiation being abnormal. Interestingly, these patients appear to morphologically resemble a subgroup of ANLL recently reported by Keating.[23] He has found that eosinophilia (>4%) in the initial bone marrow of patients with ANLL is an independent prognostic factor predictive of prolonged remission. Indeed, both of our patients who survived induction and intensification remain in remission at 23+ and 33+ mo. Among the 25 patients treated in 1980 (i.e., the same period as patients 3 and 4), 14 achieved a complete remission with initial therapy. Of these 14 patients, only 3 are currently surviving in first remission: the 2 patients described above, and 1 other.

The remaining 11 patients had all relapsed by 19 mo after diagnosis.

The del(16)(q22) has not been noted as a specific recurring chromosome abnormality in the previously published large series of patients with ANLL.[1-6,13] A del(16)(q22?) has been found as part of a complex karyotype in a patient who developed ANLL 2 yr after diagnosis of non-Hodgkin's lymphoma.[24] The percent of eosinophils was not indicated in this case. Although a number of different chromosome abnormalities have been reported in patients with the hypereosinophilic syndrome, eosinophilic leukemia, and myeloproliferative syndromes with eosinophilia, none of them included a deletion 16q.[25-29]

As banded chromosome studies have been done with increasing success, several specific karyotypic abnormalities have been found to correlate with particular subtypes of ANLL. A t(8;21)(q22;q22), with or without a missing sex chromosome, has thus far been found exclusively in patients with M2 morphology.[6,8,9,13] Likewise, the t(15;17) has been found only in patients with M3 or M3 variant ANLL.[6,10-13] A new association of a balanced translocation t(9;11)(p21;q23) and acute leukemia of the M5 type has recently been proposed.[30] And finally, partial deletion of the long arm or absence of chromosomes 5 and/or 7 have been associated with treatment-induced ANLL and with prior exposure to carcinogens in patients with de novo ANLL.[2,13] Among our series of 61 consecutively diagnosed ANLL patients, we have found 5 patients whose only karyotypic abnormality was a partial deletion of the long arm of one chromosome 16, del(16)(q22). These patients are unique in that at diagnosis, prior to therapy, they all had marrow eosinophilia, whereas none of the remaining 56 patients had greater than 4% marrow eosinophils. Other similar clinical findings among these 5 patients were Auer rods, elevated serum muramidase, and hepatomegaly at presentation. The two patients who were examined in remission showed disappearance of the eosinophilia and the del(16)(q22) clone. We believe that we have identified a new cytogenetic-clinical subtype of ANLL. Based on our patients 3 and 4 and those previously reported by Keating,[23] we speculate that this subgroup of patients may have prolonged disease-free survival.

ADDENDUM

Since the submission of this manuscript, the morphology of the bone marrow from these five patients with the del(16)(q22) has been reviewed at the Fourth International Workshop on Chromosomes in Leukemia held in Chicago, IL, September 1982. In the absence of special stains, some felt all cases could be classified as M4.

ACKNOWLEDGMENT

We are indebted to Sharla Schmidt for her expert technical assistance.

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1983 61: 994-998

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Blood (print ISSN 0006-4971, online ISSN 1528-0020), is published weekly by the American Society of Hematology, 2021 L St, NW, Suite 900, Washington DC 20036.