Chromosome Studies in Acute Leukemias Developing in Patients with Multiple Myeloma

Dieter K. Hossfeld, James F. Holland, Richard G. Cooper, and Rose R. Ellison


SUMMARY

Chromosomal findings are reported in three patients with acute myelomonocytic leukemia and in one with reticulosa-
oma leukemia who had been treated for multiple myeloma with melphalan and X-ray. All four patients had striking chromosomal abnormalities. An iatrogenic causation of aneu-
ploydy is suggested. This is supported by chromosomal findings in patients with acute leukemia following polycy-
themia vera and Hodgkin's disease; practically all of the leukemias have been aneuploid. A comparison is made of such "secondary" acute leukemias with "primary" acute leukemias that are aneuploid in only 40% of the cases. Chromosomal changes are not considered to be the initial event in leukemogenesis.

INTRODUCTION

In 1970 several papers were published describing the development of acute leukemia in patients who had received long-term treatment with alkylating agents, particularly phenylalanine mustard (melphalan), for the treatment of multiple myeloma. The possibility of a causal relationship between the development of acute leukemia and such treatment was suggested (1, 9, 12, 15). Since 1970 many more cases of acute leukemia have been reported following treatment of multiple myeloma (3, 17, 18, 26), other hematological diseases, and solid tumors with alkylating agents (for review, see Ref. 21). Several authors have referred to the chromosome-damaging radiomimetic effect of alkylating agents, which might play a critical role in the genesis of such leukemias. The natural history of plasma cell dyscrasias might predispose to the acquisition of acute leukemia because of immunodeficiency.

We report here the results of chromosome analyses in 4 cases of acute leukemia following treatment of multiple myeloma with alkylating agents and X-rays that suggest that such leukemias are different from "spontaneous" leukemias.

MATERIALS AND METHODS

Patients

Case 1. This 60-year-old woman had back pain for 15 months, leading to a diagnosis of multiple myeloma in March 1968. X-rays revealed a compression fracture of D12 and osteolytic lesions in the left scapula and the proximal part of the left humerus. Hemoglobin was 11 g/100 ml; WBC count was 5300/μl with a normal differential. Plate-
lets were 195,000/μl. Total protein in the serum was 7.0 g/100 ml; albumin, 3.36 g/100 ml; α1-globulin, 0.31 g/100 ml; α2-globulin, 0.87 g/100 ml; β-globulin, 0.84 g/100 ml; and γ-globulin, 1.62 g/100 ml. IgM was normal in amount and IgA was slightly decreased, but IgG was greatly increased and moved on electrophoresis as a mono-
clonal spike. Melphalan (2 mg/day) and prednisone (10 mg/day) were instituted in April 1968. Melphalan was stopped in September 1969 because of thrombocytopenia (94,000 platelets/μl) but was reinstituted in October 1969. In January 1970, melphalan was discontinued because of leukocytes (1800 platelets/μl) and anemia (7.0 g/100 ml). The platelet count at that time was 222,000 platelets/μl. For several months the patient received only prednisone or dexamethasone and vitamins.

On September 28, 1970, a rise of WBC from a previous range of 3000 to 5000 up to 9800/μl and a fall of hemoglobin from 7 to 9 g/100 ml to 5.9 g/100 ml were noted. A blood differential leukocyte count on October 9, 1970, revealed 83% myeloblasts, 2% myelocytes, 8% nor-
moblasts, and 7% lymphocytes. The platelet count at that time was 222,000 platelets/μl. For several months the patient received only prednisone or dexamethasone and vitamins.

Received December 23, 1974; accepted May 29, 1975.
Case 2. This man was found to have multiple myeloma in April 1967, at the age of 57. The diagnosis was based on the presence of over 90% plasma cells in the marrow, Bence-Jones proteinuria and proteinemia by immunoelectrophoresis, multiple punched-out areas in the skull, and osteolytic and destructive lesions in the vertebral bodies. Treatment was started on April 28, 1967, with melphalan (10 mg/day) for 10 days. In June 1967, he was found to have pathological rib fractures, and he was given X-ray therapy to the right lateral chest wall with a total depth dose of 1640 rads. He received melphalan intermittently through 1967. In September 1967, he was found to be uremic with a maximum blood urea nitrogen of 128 mg/100 ml. He was treated with prednisone, with a fall of his blood urea nitrogen, but never to normal levels. He received additional radiotherapy to a 10- x 15-cm field on his neck to a total of 800 rads. During 1968, 1969, and early 1970, he was asymptomatic and received melphalan (2 mg/day) intermittently.

In August 1970, after several months of bone pain, increasing weakness, and 1 week of dyspnea, he was found to have a hemoglobin of 5.2 g/100 ml; WBC count of 1,900/µl, with 2% myelocytes and 8% metamyelocytes; and 130,000 platelets/µl, falling to 40,000 platelets/µl in several days. Bone marrows in August and September 1970 showed marked erythroid hyperplasia with megaloblastoid changes and a shift to the left in the granulocytic series. He was admitted to Roswell Park Memorial Institute in October 1970, at which time he was febrile, pale, and seriously ill. His liver was enlarged 2 cm below the costal margin. His leukocytes were 2,400/µl, falling to 800/µl without treatment. His platelets were 35,000/µl and fell to 2,500/µl. His hemoglobin was maintained with transfusions. His blood urea nitrogen was 55 mg/100 ml; uric acid, 11.3 mg/100 ml; total protein, 6.1 g/100 ml; albumin, 2.9 g/100 ml; and, on quantitative immunodiffusion assay, IgG was 750 mg/100 ml, IgA was 95 mg/100 ml, and IgM was 58 mg/100 ml. In November 1970, his marrow was 1+ cellular and contained 16% blasts, 12% promyelocytes, 42% more mature granulocytic elements, 27% lymphocytes, and 2% erythrocytes. Some cells appeared monocytoid. A diagnosis of acute myelomonocytic leukemia was made, and he started on cytosine arabinoside and thioguanine in low doses. His platelet count had risen to 118,000/µl, and she was changed to cyclophosphamide (15 mg/kg/week), which was cut in half in July and discontinued in August 1970 because of leukopenia and thrombocytopenia. She then was given prednisone (10 mg) and fluoxymesterone (5 mg) daily. In October 1970, she had a WBC count of 6,600/µl, with 98% "lymphocytes," and 12,000 platelets/µl.

Bone marrow and chromosome analyses in October 1970 were compatible with acute myeloblastic leukemia. The bone marrow differential was: myeloblasts, 85.0%; promyelocytes, 3.0%; myelocytes, 0.5%; bands, 0.5%; lymphocytes, 8.0%; plasma cells, 2.0%; normoblasts, 1.0%. The specimen was hypercellular, and megakaryocytes were severely decreased. The myeloblasts were somewhat abnormal, with deep blue cytoplasm and folded nuclei. Liver and spleen were not palpable. She was treated with cytosine arabinoside, cyclophosphamide, vincristine, and prednisone, without response, and she died on November 12, 1970. Autopsy revealed leukemia infiltration of lungs, liver, pancreas, spleen, adrenals, kidneys, bladder, uterus, lymph nodes, and bone marrow (report by Dr. D. E. Scott, Boise, Idaho). Sections of vertebral marrow we reviewed showed different findings on different slides: there was clear-cut evidence of myeloma on 1 slide, of acute myeloblastic leukemia on another, and the 3rd slide showed cells from both diseases.

Case 4. This woman was 51 years old when, in May 1968, she was found to have IgG multiple myeloma. The diagnosis was based on the presence of 35% abnormal plasma cells in the marrow and lytic lesions of the skull. There was a vertebral fracture and diffuse osteoporosis. Total protein was 9.1 g/100 ml, with 5.5 g/100 ml globulin. Immunoelectrophoresis revealed IgG myeloma. Bence-Jones protein was negative.

In May 1968, she was started on melphalan (4 mg/day), which was given through October 1970. On October 29, 1970, it was noted that the WBC count had dropped from a usual 5,900 to 7,000/µl range to 3,200/µl, and the platelets had dropped from a usual 150,000 to 200,000/µl range to 30,000/µl. The bone marrow was infiltrated with reticulocarcinoma cells. The marrow differential on the same specimen from which the 1st chromosome analysis was done was: myeloblasts, 1.0%; promyelocytes, 7.4%; myelocytes, 15.2%; juveniles, 2.2%; stabs, 22.0%; bands, 13.8%; lymphocytes, 9.0%; pronormoblasts, 0.4%; normoblasts, 13.0%; reticulocarcinoma cells, 16.0%. The marrow had a normal cellularity. The M:E ratio was 4.7. Reticulocarcinoma cells were also seen in the peripheral blood. Melphalan was stopped, and oxy methylene, which had been given between 1969 and 1970, was increased to 40 mg/day. She then was treated with vincristine and prednisone for 4 consecutive OCTOBER 1975 2809
weeks. Under therapy the bone marrow showed more infiltration with reticulosarcoma cells. The bone marrow differential, obtained in January 1971 at the time of the 2nd chromosome analysis, was: myelocytes, 1.8%; metamyelocytes, 0.5%; juveniles, 0.7%; filaments, 0.5%; lymphocytes, 6.5%; reticulosarcoma cells, 89.5%; normoblasts, 0.5%.

She developed gram-negative sepsis and died on February 1, 1971. No autopsy was allowed. The patient never had received X-ray therapy.

The exact identification of the cells that we call reticulosarcoma cells was difficult. Cytochemically, they were characterized by negative Sudan black and peroxidase reactions and a positive periodic acid-Schiff reaction. Serum lysozyme was 13.5 µg/ml; the urine was negative for lysozyme.

Direct chromosome preparations from bone marrow cells were performed according to standard techniques (25).

RESULTS

In all 4 cases, the great majority of metaphases derived from bone marrow cells were numerically and structurally abnormal. Numerical findings are summarized in Table 1.

In Case 1, a bimodal chromosome distribution was found, with a minor peak at 43,XX,-B,-17(18?),-G, and a major peak at 44,XX,-B,-17(18?) (Fig. 1). Besides lacking B, E, and G chromosomes, other cells with apparent modes of 42 were missing different chromosomes, thus suggesting artificial loss. None of the 40 metaphases analyzed was normal. Many hypotetraploid metaphases were seen. Using a low power magnification, the number of near tetraploid metaphases was estimated to be 10% (normally 3 to 4% in our laboratory).

Case 2 also revealed a bimodal chromosome distribution. One mode was characterized by 45,XY,-3,-B,+C, the other by 44,XY,-3,-B,-16,+C? (Fig. 2). In metaphases with a mode of 43, chromosomes were also missing in D or F groups. Fifteen of 29 metaphases with either 44 or 45 chromosomes contained a large acrocentric marker chromosome, replacing a D-group member. Among the 40 metaphases analyzed, only 2 appeared to be normal. Upon screening under low power, 9% hypotetraploid cells were found.

In Case 3, the chromosome number ranged between 48 and 60. Forty metaphases were analyzed, and 7 of them were karyotyped. None of the metaphases studied had a normal chromosome complement. Although 55% of the metaphases had 56 chromosomes, karyotypic analysis revealed them to have different abnormalities. Except for Group E, all chromosome groups contributed to hyperdiploidy, Groups B and C being particularly and consistently involved. Between 1 and 3 marker chromosomes were present in all metaphases (Fig. 3). The morphology and size of the marker chromosome varied in the same metaphase and among different metaphases between acrocentric and submetacentric; in some metaphases the marker appeared to be a small ring chromosome.

In Case 4, the chromosome number varied between 36 and 46. In the bone marrow specimen obtained in 1970, 50 metaphases were counted, and 47 were counted in the 1971 specimen. A total of 12 metaphases was karyotyped. Whereas in 1970 no definite mode was apparent, a suggestive mode was demonstrable in 1971, characterized by 42,XX,-D,-D,-17(18?),-F,+mar. In metaphases with less than 42 chromosomes, additional members of Groups D, E, and F were missing. On both occasions the marker was detected in 80 and 92% of the metaphases, respectively. Two markers of similar morphology were found in 15% of the metaphases (Fig. 4). In some metaphases the marker presented as a small chromatin clump; in the majority of metaphases, however, it was seen as a small metacentric chromosome.

DISCUSSION

The 4 cases presented share several features. Because of multiple myeloma, all patients had been treated with alkylating agents, and 3 of them had received X-ray therapy in addition.

Three developed acute myelomonocytic leukemia and 1 developed reticulosarcoma-leukemia 2 to 3 years after multiple myeloma had been diagnosed.

Chromosome analysis of bone marrow cells, performed at a time when the marrow consisted predominantly of myeloblasts or reticulosarcoma cells, revealed striking abnormalities; 1 had a chromosome mode of 56; 2 had a bimodal, hypodiploid distribution (43 and 44 and 45 chromosomes, respectively); and the patient with reticulosarcoma leukemia had a mode of 42 chromosomes. Marker chromosomes were found in 3 of the 4 cases. Clearly, the interpretation of the chromosomal findings is hampered by the lack of chromosome banding patterns. Unfortunately,
banding techniques were not available when the chromo-
some studies were performed.

To our knowledge, only 2 comparable cases have been
published in the literature. Nowell (19) described a patient
who had multiple myeloma for 2.5 years and had been
treated with melphalan (dose not started) for over a year
when he was found to have atypical acute leukemia.
Chromosome analysis of bone marrow cells revealed 100% of
the metaphases to belong to 3 closely related abnormal
stem lines with 44, 45, and 46 chromosomes. Bennett (3)
reported a patient who had received X-ray therapy and 800
mg of melphalan over an 18-month period because of
multiple myeloma and who was diagnosed to have erythro-
leukemia 1 year later. Dividing marrow cells were shown to
have 43 and 44 chromosomes, with missing chromosomes of
Group C.

Thus, in all 6 acute leukemias developing in patients who
had been treated for multiple myeloma with melphalan (and
with radiotherapy in 4 cases), significant chromosome
anomalies were present in all, or almost all, of the
metaphases. Since spontaneous acute leukemia in adults
appears to be chromosomally normal in about 60% of cases,
it seems improbable that one would find chromosomal
anomalies in all 6 cases by chance. Moreover, the extent of
chromosomal abnormalities, although not unique, certainly
appears unusual. On review of chromosomal data of 388
acute leukemias that were contributed by 5 different labora-
tories (10, 11, 13, 24, 33), it was found that only 14 cases
had chromosome modes below 45, and not more than 7
cases had modes above 50. All the acute leukemias herein
presented are among these extremes, a highly improbable
event by chance alone.

In addition, marker chromosomes occur in less than 20%
of ordinary acute leukemias. We found marker chromo-
somes in 3 of 4 cases, and Nowell's case apparently was
marker positive also.

The unusual degree of aneuploidy in these cases of acute
leukemia in patients with multiple myeloma supports the
interpretation that they are different from spontaneous
acute leukemia cases. Presently, it appears difficult to
understand the mechanism that causes aneuploidy and to
define its role in the pathogenesis of neoplasia. In view of
the melphalan and radiotherapy history, it is tempting to
speculate on iatrogenic causes for aneuploidy. This is
supported by chromosomal findings in acute leukemias of
patients who had received chemotherapy and/or radiation
therapy because of Hodgkin's disease or polycythemia vera.
To our knowledge, all acute leukemias with a prior history
of treated Hodgkin's disease have been aneuploid (6, 8, 28).
Of 16 patients with acute leukemia and a history of treated
polycythemia vera, 15 have been aneuploid (7, 14, 16, 31).
Marker chromosomes were frequently found, particularly
among the leukemias developing after polycythemia.

It is of great interest that, in 1 single case of myelomatosis
developing acute leukemia before any exposure to alkylat-
ing agents or X-ray therapy, no chromosomal anomalies
were detected (20).

Following the application of radiomimetic drugs or
X-rays, stable chromosome anomalies of clonal character
are rarely observed in human or animal cells. As a rule, cells
with visible chromosome anomalies do not survive or do not
proliferate, and the percentage of chromosomally abnormal
bone marrow metaphases drops to normal levels within a
few days after cessation of therapy (2, 5, 27, 32). However,
in some respects the situation may be different in patients
with a history of treated myeloma, Hodgkin's disease, or
polycythemia vera. All these patients had received their
treatment for a long time, and a relationship appears to
exist between the manifestation of stable chromosome
anomalies and the duration of application of radiomimetic
drugs (23). Impaired host defenses, due either to the
disease per se or induced by long-term chemotherapy and/
or X-ray therapy, might allow chromosomally abnormal
cells to proliferate.

Our contention that the chromosomal anomalies are
probably related to previous treatment does not mean that
we assume that they represent the primary event in the
leukemogenesis of such leukemias. Considering the strong
mutagenic activity of alkylating agents and X-rays (4, 22),
we believe that mutations on the gene level could more
likely represent the primary event, with chromosomal
anomalies only serving as a reflection of increased instabil-
ity of the genome, impaired repair mechanisms, or disorder
of the mitotic apparatus.

It is unfortunate that, in our cases as well as in the 2 found
in the literature, no chromosome studies of marrow cells
were done during the myeloma phase. Although about 50%
of multiple myeloma specimens appear to be chromosome-
ally normal, chromosome modes between 43 and 45 and
between 52 and 62 can be encountered in 6 and 20% of the
cases, respectively (25). Thus, it cannot be excluded that the
anomalies observed in the cases under discussion were
already present in the myeloma phase. Lawler et al. (16)
presented evidence in cases changing from polycythemia
vera to acute leukemia that either completely new or
additional anomalies appeared during the transition. Serial
chromosome studies in multiple myeloma would be of great
value in clarifying the question whether plasma cells may
transform into myeloblasts as suggested by Thijis et al. (29)
and Videbaek (30) or whether an entirely new cell popula-
tion emerges.

ACKNOWLEDGMENTS

The generous cooperation of Dr. S. A. Aitchley, Jr., Boise, Idaho, and
Dr. J. E. Kostinas, Portsmouth, Va., is gratefully acknowledged.

REFERENCES

1. Andersen, E., and Videbaek, A. Stem Cell Leukaemia in Myelomato-
Cytogenetic and Morphologic Abnormalities in Human Bone Marrow
3. Bennett, J. M. Plasma Cell Myeloma Terminating in Erythremic
5. Datta, P. K., and Schleiermacher, E. The Effects of Cytosan on the

OCTOBER 1975 2811

Chromosomes in Secondary Acute Leukemia

Downloaded from cancerres.aacrjournals.org on November 13, 2017. © 1975 American Association for Cancer Research.
Figs. 1 to 4. Giemsa, × 1000.
Fig. 1. Karyotype of a bone marrow cell from Case 1, with 43,XX, -B,-17(18?), -G.
Fig. 2. Karyotype of a bone marrow cell from Case 2, with 45,XY, -3, -B,+C,-mar. Arrow, marker.
Fig. 3. Karyotype of a bone marrow cell from Case 3, with 60,XX, +B, +B, +C, +C, +C, +C, +D, +D, +F, +F, +mar, +mar, +mar.
Fig. 4. Karyotype of a bone marrow cell from Case 4, with 44,XX, -D, -D, -D, -17(18?), +mar, +mar.
Chromosome Studies in Acute Leukemias Developing in Patients with Multiple Myeloma


Updated version  Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/35/10/2808

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, use this link http://cancerres.aacrjournals.org/content/35/10/2808. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.