Chromosome Studies in Acute Leukemias Developing in Patients with Multiple Myeloma

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SUMMARY

Chromosomal findings are reported in three patients with acute myelomonocytic leukemia and in one with reticulosarcoma leukemia who had been treated for multiple myeloma with melphalan and X-ray. All four patients had striking chromosomal anomalies. An iatrogenic causation of aneuploidy is suggested. This is supported by chromosomal findings in patients with acute leukemia following polycythemia 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. Platelets 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, 0.38 g/100 ml; α6-globulin, 0.71 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 monoclonal 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% normoblasts, and 7% lymphocytes. The platelet count was 8000 platelets/μl. The bone marrow used for chromosome analysis contained 51% myeloblasts and promyelocytes, with occasional Auer rods and 40% abnormal plasma cells; erythro- and megakaryocytopenia were severely decreased. Results of electrophoresis were: total protein, 8.5 g/100 ml; albumin, 2.14 g/100 ml; α1-globulin, 0.44 g/100 ml; α2-globulin, 0.87 g/100 ml; β-globulin, 0.84 g/100 ml; and γ-globulin, 4.8 g/100 ml. Elevation of IgG persisted. A diagnosis was made of acute myelocytic leukemia complicating multiple myeloma. Chemotherapy with cytosine arabinoside and thioguanine was started on October 10, 1970. No remission was obtained. A bone marrow specimen on November 11, 1970, revealed 31% myeloblasts plus promyelocytes and 16% plasma cells. The patient developed pneumonia and died on November 27, 1970. No autopsy was performed.

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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; WBC count of 1,900/μl; and 500/μl. Urinary tract infection and coagulopathy developed, and he died on November 19, 1970, on the 18th day of antileukemic chemotherapy.

In August 1970, after several months of bone pain, increasing weakness, and 1 week of dyspnea, he was found to have a hemoglobin of 8 g/100 ml; WBC count of 1,200/μl; and 6% lymphocytes. 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 20,000/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 i+ cellular and contained 16% blasts, 12% promyelocytes, 42% more mature granulocytic elements, 27% lymphocytes, and 2% erythroid cells. Some cells appeared monocytoid. A diagnosis of acute myelomonocytic leukemia was made, and he was started on cytosine arabinoside and thioguanine in low doses. His leukocyte count fell to 150/μl and his platelets fell to 500/μl. Urinary tract infection and coagulopathy developed, and he died on November 19, 1970, on the 18th day of antileukemic chemotherapy.

At autopsy, there were no microscopic changes characteristic of either acute myelogenous leukemia or of multiple myeloma. The pathological diagnosis was reticular hyperplasia of spleen, nodes, and marrow, with marked erythropagocytosis and aplasia of the marrow. A chromosome analysis was performed 3 weeks prior to death; the results are presented below.

Case 3. This woman was 69 years old when, in July 1967, she presented with a painless swelling over the left pectoral area that was biopsied and found to contain sheets of plasma cells. Bone marrow examination confirmed the diagnosis of multiple myeloma with 73% large immature plasma cells. Serum protein showed a high spike in the γ-globulin fraction. X-ray studies revealed generalized demineralization and destructive metastatic lesions of the pelvis, right femur, and vertebral. In August 1967, a total of 3,720 R was given to a right inguinal and middorsal field, and she received melphalan (1 mg/day) for 17 weeks. From January 1968 to April 1970, she received melphalan (7 mg/day) for 7 consecutive days every 6th week. In April 1970, melphalan was discontinued because of thrombocytopenia (77,200 platelets/μl). At the end of May 1970, her 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. Immunelectrophoresis 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 reticulosarcoma 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%; reticulosarcoma cells, 16.0%. The marrow had a normal cellularity. The M:E ratio was 4:7. Reticulosarcoma cells were also seen in the peripheral blood. Melphalan was stopped, and oxymetholone, 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
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,−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 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,

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Table 1

Summary of chromosomal findings

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banding techniques were not available when the chromosome 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 laboratories (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 chromosomes 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 (6, 8, 28). Marker chromosomes were frequently found, particularly among the leukemias developing after polycythemia vera. It is of great interest that, in 1 single case of myelomatosis developing acute leukemia before any exposure to alkylating 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 instability 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 chromosomally 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 Thijs et al. (29) and Videbaek (30) or whether an entirely new cell population emerges.

ACKNOWLEDGMENTS

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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, +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, +mar.
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