Probable Clonal Origin of Acute Myeloblastic Leukemia Following Radiation and Chemotherapy of Colon Cancer

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A 64-yr-old female developed acute myeloblastic leukemia following radiation and drug therapy for colon carcinoma. The patient was heterozygous for glucose-6-phosphate dehydrogenase (G-6-PD) and displayed types A and B isoenzymes in nonhematopoietic tissue. In contrast, only type B G-6-PD was observed in peripheral blood white cells. In addition, a karyotypic abnormality was found in peripheral blood and marrow cells but not in skin fibroblasts. The data are consistent with a clonal origin of this leukemia.

ACUTE LEUKEMIA is a recognized complication of radiation exposure, alkylating agent treatment of multiple myeloma, ovarian carcinoma, and other tumors, and combined modality therapy of Hodgkin disease. In such cases the leukemia may be the result of direct damage to genetic material or of the activation of a latent oncogenic virus within normal cells. In addition, immunosuppression of the host may also have a pathogenetic role in the emergence of leukemia.

Previous studies of the spontaneously occurring neoplasms chronic myelocytic leukemia and polycythemia vera using glucose-6-phosphate dehydrogenase (G-6-PD) isoenzyme markers showed that at the time of study these diseases very probably have unicellular (clonal) origin. G-6-PD isoenzyme studies have not been reported in cases of leukemia that developed after treatment with radiation or cytotoxic drugs. In the patient reported here, acute myeloblastic leukemia developed after radiation and drug therapy of disseminated colon cancer. Chromosome and G-6-PD marker studies strongly suggest a clonal origin for the leukemia.

CASE REPORT

A 55-yr-old black female underwent a right hemicolectomy in July 1967 for a grade-2 penetrating adenocarcinoma of the right colon (Duke’s stage B2). In 1969 a pulmonary recurrence was treated by a left lower lobe resection. She was again asymptomatic until November 1973, when a recurrence in the left supraclavicular region was treated with 60Co radiation (3500 rads). Pulmonary metastases recurred and were treated with parenteral 5-fluorouracil on an intermittent weekly schedule from February 1974 to June 1975. After initial stabilization the disease pro...
gressed, and in August 1975 the patient received 4200 rads to a 12.5 x 13.5-cm port over the left lung followed by a 4-mo course of 5-fluorouracil, methyl-CCNU (total dose 400 mg), and vincristine. Despite this therapy and a 3-wk trial of streptozotocin, the pulmonary metastases progressed.

On September 2, 1976, the liver span was 13 cm and the spleen was palpable 4 cm below the left costal margin. The hematocrit was 24.5%; white blood cell count 33,500/mm³; platelet count 75,000/mm³. The differential showed 7% segmented forms, 8% bands, 45% metamyelocytes, 13% myelocytes, 2% promyelocytes, 5% blasts, 18% monocytes, and 2% small lymphocytes. Anisopoikilocytosis of the red cells and a pseudo-Pelger-Huet anomaly in the granulocytic series were noted. No Auer rods were seen in the blast cells. Marrow aspirate and biopsy revealed hypercellular marrow with approximately 50% myeloblasts and a decrease in megakaryocytes and red blood cell precursors. The blast cells showed variation in size, prominent nucleoli, and occasional clefts in the nucleus (Fig. 1); they were peroxidase positive. Marrow iron stores were normal; ringed sideroblasts were not present. Leukocyte alkaline phosphatase score was 0 (normal 15-85); serum vitamin B₁₂ 1400 pg/ml (normal 200-900), and vitamin B₁₂ binders 2749 pg/ml (normal 475-1600).

A diagnosis of acute myeloblastic leukemia was made, and the patient was treated with vincristine and prednisone without response. Subsequent treatment with hydroxurea controlled the white blood cell count for 2 mo, but the patient died shortly thereafter from progression of lung metastases and pulmonary insufficiency. At time of death, the peripheral white count was 100,000/mm³ with 20% blasts. Permission for autopsy was not granted.

Fig. 1. Bone marrow aspirate with a predominance of myeloblastic cells. Wright-Giemsa. x 400.
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MATERIALS AND METHODS

On September 9, 1976, before any therapy for leukemia was instituted, blood was drawn and sent to Seattle on ice via air freight for chromosome and G-6-PD studies. At that time the hematocrit was 24%, and the peripheral blood white cell count was 32,000/mm³ with 10% blast cells. The patient had last been transfused 3 wk earlier with 3 units packed red cells. A marrow aspirate was sent to Seattle in a similar manner on September 15, 1976. Extracts of blood cells, skin, and fibroblasts cultured from skin were prepared and tested for G-6-PD starch gel electrophoretic patterns as previously described. The relative activity of the isoenzyme bands was estimated visually. This technique allows detection of a minor enzyme component contributing at least 5% of the total activity.

For chromosome studies, marrow and peripheral blood white cells obtained in preservative-free heparin were examined after 24 and 48 hr in culture with and without phytohemagglutinin (PHA) stimulation. Karyotypes were prepared using the acetic-saline-Giemsa banding technique.

RESULTS

G-6-PD analysis. Approximately equal amounts of B and A types of G-6-PD were found in skin and cultured skin fibroblasts. Thus the patient was presumed to be heterozygous for G-6-PD. In marked contrast, only type B enzyme was detected in peripheral blood white cells and erythrocytes.

Chromosomes. No karyotypic abnormalities were detected in 30 metaphases studied at the third transfer of cultured skin fibroblasts. In contrast, each of 90 blood cell metaphases cultured for 24 and 48 hr in the absence of PHA was missing approximately two-thirds of the short arms of a chromosome 12 (46,XX, 12p-). The same abnormality was detected in 18 of 24 marrow cells cultured for 24 hr in the absence of mitogen; no abnormalities were detected in the other 6. Of 38 blood cells cultured for 48 hr in the presence of PHA, 32 had the partial deletion of the short arm of a chromosome 12 and the other 6 appeared normal (46,XX). The Philadelphia chromosome was not detected in any of the blood or marrow cells.

DISCUSSION

While the occurrence of acute leukemia in a patient who has been exposed to radiation and cytotoxic drugs may be a coincidental association, it is reasonable to postulate that this patient’s leukemia was causally related to her previous treatment. The occurrence of acute leukemia 2–3 yr after radiation exposure is in accord with earlier observations on patients with ankylosing spondylitis treated with radiation of the spine and on survivors of atomic bomb radiation in Japan. Our patient was also treated with cytotoxic drugs in the 2 yr prior to the diagnosis of leukemia, including intermittent courses of 5-fluorouracil throughout that time, and in the year preceding the diagnosis with the nitrosoureas, methyl-CCNU (4 mo), and streptozotocin (3 wk). Antimetabolites such as 5-fluorouracil are not generally regarded as human carcinogens. Leukemia following treatment with cytotoxic drugs is usually associated with alkylating agents after a treatment period of about 30–90 mo. A review by Sotrel et al. noted that the shortest reported time interval between the onset of alkylating agent treatment and diagnosis of leukemia was 13 mo. Thus the time interval of under 1 yr between nitrosourea treatment and appearance of acute
leukemia seems too short to ascribe a causal factor in this patient’s leukemia, although the drugs could have influenced the final transformation of radiation-damaged cells into neoplastic cells.

Since only one of the two X chromosomes is active in somatic cells of females, subjects heterozygous at the X-linked G-6-PD locus for the common Gd⁰ gene and a variant such as Gd⁴ or Gd⁺ have two populations of cells, one synthesizing type B enzyme and the other type A. Normal tissues from G-6-PD heterozygotes almost always contain a mixture of cells and manifest both B and A enzymes, while clonal proliferations display only one enzyme type, either B or A.²² For example, G-6-PD heterozygotes with chronic myelogenous leukemia, polycythemia vera, and agnogenic myeloid metaplasia with myelofibrosis have both enzymes in their normal tissues, but only one type has been found in their blood granulocytes, erythrocytes, and platelets.¹⁴¹⁶²³ These results strongly suggest clonal origin for the myeloproliferative disorders and provide formal evidence that they arise from pluripotential hematopoietic stem cells.

The present patient was a G-6-PD heterozygote. Both type B and type A enzymes were found in her skin and cultured skin fibroblasts, but only type B G-6-PD was detected in the abnormal white blood cells. This is consistent with a leukemia of clonal origin. The chromosome studies provide additional evidence of unicellular origin. Cultured cells from normal tissues were 46,XX, but all studied unstimulated blood cells had a characteristic chromosomal abnormality.

These findings add a case of acute myeloblastic leukemia following radiation and cytotoxic drug treatment to the list of myeloproliferative disorders that may arise from the progeny of a single cell. Despite the exposure of many marrow cells to carcinogenic influences, it may be that only one cell actually underwent transformation. Alternatively, many cells may have been transformed, but only one population proliferated to the point of expressing clinical disease. The present data provide no information about the number of neoplastic events or the number of cells initially involved, but the G-6-PD results suggest strongly that by the time of diagnosis a single clone of leukemic cells had evolved.

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