Second malignancies occur with a greater frequency in patients with chronic lymphatic leukemia than in the general population. Previously, 20 patients having CLL have been reported as developing acute leukemia, but no cases of development of chronic granulocytic leukemia have been observed. In the present report, we will describe patients with Ph\(^1\) chromosome positive CGL associated with previously described CLL. One patient received total body radiation treatment for his CLL, while the second was not treated. It is apparent that previous therapy may not necessarily be a prerequisite for the development of a second malignancy in CLL. Cytogenetics offers a useful tool to confirm morphologic criteria in establishing the diagnosis of a second neoplasm.

SECOND MALIGNANCIES OCCUR with a greater frequency in patients with chronic lymphocytic leukemia (CLL) than in the general population.\(^2\) The association of CLL and acute leukemia has been reported in 20 cases.\(^3\)-\(^8\) Each of the 20 patients received cytotoxic or radiation therapy. No cases of chronic granulocytic leukemia (CGL) were observed in these series. We would like to report two patients with Ph\(^1\) chromosome positive CGL associated with previously diagnosed CLL. One patient was treated with total body radiation for CLL. Three years later he developed CGL, which later transformed to the blastic phase. The second patient was untreated and has developed typical CGL two yr after the initial diagnosis of CLL.

MATERIAL AND METHODS

Chromosome preparations of the mitotic cells in the bone marrow were studied without prior in vitro culture by using the air-dried method.\(^9\) Peripheral blood was cultured for 24 and 72 hr, with or without PHA stimulation, and preparations made according to the technique of Moorhead et al.\(^10\) The cells in metaphase were scored, analyzed, and karyotyped according to the Denver
nomenclature. Peripheral blood, bone marrow aspirate smears, and bone marrow sections were examined as previously described.

Case 1

A 62-yr-old male (P.S.) developed fatigue and lost 25 lb over a 3-mo period in the fall of 1967. Physical examination disclosed a palpable spleen and lymphadenopathy. The white cell count was 30,000 per cu mm, with a predominance of lymphocytes; the platelet count was 176,000 per cu mm, the hemoglobin was 16.1 g/100 ml, and the hematocrit was 48%. The bone marrow aspirate showed that over 90% of the cells present were mature lymphocytes. The bone marrow biopsy was hypercellular, and the marrow particles were virtually replaced by mature lymphocytes (Fig. 1). The diagnosis of CLL was made. In 1968, because of increased symptomatology, he received 137 rads total body radiation. At the end of the treatment, his hemoglobin was 13.6 g/100 ml, the platelet count was 91,000 per cu mm, and the WBC was 5600 per cu mm, with 63% mature lymphocytes. No palpable adenopathy or splenomegaly were noted.

He felt well until November 1970, but at this time he again developed fatigue and weight loss. The bone marrow was moderately hypercellular, with an increase in cells of the myeloid series, and megakaryocytes were decreased. The cause of the granulocytic hyperplasia was unknown.

Table 1. Chromosome Analysis, Case 1 (P.S.)

| Date      | Sample | Total | < 45 | 46 | 47 | < 46 | 46 | 47 | 48 | 49 | Frag. and | Ring, Pulv. |
|-----------|--------|-------|------|----|----|------|----|----|----|----| Breaks    |             |
| 11-30-71  | BM     | 25    | 25   |    |    |      |    |    |    |    |           |             |
| 01-15-71  | PB-3d  | 5     | 5    |    |    |      |    |    |    |    |           |             |
| 02-09-71  | Spleen | 49    | 42   | 39 | 4  |     | 6   |    |    |    |           |             |
| 05-18-71  | BM     | 50    | 50   |    |    |      |    |    |    |    |           |             |
|           | PB-1d  | 0     |      |    |    |      |    |    |    |    |           |             |
|           | PB-3d  | 54    | 1    | 50 | 1  | 16%  |    |    |    |    |           |             |
| 06-29-71  | BM     | 50    | 48   |    |    |      |    |    |    |    |           |             |
| 12-14-71  | BM     | 50    | 44   | 4  |    | 2%   | 2%  |    |    |    |           |             |
|           | PB-1d  | 17    | 15   |    |    |      |    |    |    |    |           |             |
|           | PB-3d  | 3     | 3    |    |    |      |    |    |    |    |           |             |
| 12-22-71  | BM     | 52    | 2    |    | 48*| 2    |    |    |    |    |           |             |
| 02-24-72  | BM     | 33    | 2    | 14*| 17 |      |    |    |    |    |           |             |
| 03-07-72  | BM     | 40    | 8    | 32 |    |      |    |    |    |    |           |             |
|           | PB-1d  | 0     |      |    |    |      |    |    |    |    |           |             |
|           | PB-2d  | 0     |      |    |    |      |    |    |    |    |           |             |
|           | PB-3d  | 50    | 2    | 2  | 46†| 8%   | 18% |    |    |    |           |             |

*100% dicentric marker.
†Four per cent long acrocentric marker.
although the possibility of early granulocytic leukemia was considered. At this time, all analysable metaphases of the bone marrow preparation for chromosome analysis had 46 chromosomes with a male karyotype and contained one Ph' chromosome. Subsequent bone marrow examinations in late 1970 were consistent with a diagnosis of CGL.

In January 1971 he developed splenomegaly. After 700 rads to the spleen, the spleen size decreased; however, splenectomy was performed in February 1971. Microscopically, the spleen had moderate prominence of red pulp with splenic sinuoids and cords exhibiting a marked hyperplasia of mature granulocytes. The white pulp was prominent with variable large and irregular malphighian corpuscles, some of which were confluent and seen to fade imperceptibly into the surrounding red pulp. The lymphoid cells exhibited mild pleomorphism and variable maturity. Scattered mitotic figures were identified. The spleen showed more granulocytic hyperplasia than evidence of lymphocytic leukemia. A liver biopsy at this time showed no evidence of CGL, but infiltrates consistent with CLL were present. The results of chromosome preparations of the spleen are shown in Table 1: 78% of the metaphases had 48 chromosomes with an extra chromosome each in groups C and F, and no Ph' chromosome was seen (Fig. 2). The results of sequential chromosome analysis obtained from both marrow aspirates and peripheral blood cultures are seen in Table 1.

In December 1971, he was readmitted to the hospital after a sudden rise in the white cell count. The vital signs were normal, but physical examination revealed that the liver was palpated 4 cm
below the right costal margin. A complete blood count showed a hemoglobin of 14.5 g/100 ml, hematocrit of 41.5%, platelet count of 120,000 per cu mm, and a WBC of 101,000 per cu mm. The differential showed polys 20%, bands 9%, metamyelocytes 5%, myelocytes 13%, myeloblasts 41%, lymphocytes 4%, monocytes 5%, eosinophils 1%, and basophils 2%. The bone marrow section was moderately hypercellular with 90% myeloblasts and decreased megakaryocytes. The diagnosis of blast transformation of CGL was made.

A bone marrow in March 1972 showed more than 80% blasts in both the section and smear. All of the metaphases of the chromosome preparation contained one Ph\(^1\) chromosome, with 80% of them having 45 chromosomes. These cells were missing one chromosome each in groups C and E, and had one extra dicentric marker chromosome (Fig. 3). The patient was not responsive to chemotherapy and expired in April 1972.

**Case 2**

The second patient is a 74-yr-old male (W.M.), who had been periodically followed by his private physician as a result of a diagnosis of chronic lymphocytic leukemia made in 1970. At that time, he had an elevated white count of 80,000 per cu mm with a predominance of lymphocytes. The bone marrow smear was slightly hypercellular with 60% - 70% of the cells mature lymphocytes (Fig. 4A). The megakaryocytes were normal in number and appearance. There was no significant evidence of enlargement of the lymph nodes or the spleen, and he was not treated. The white cell count declined, but in March 1972, it rose to levels of 160,000 per cu mm. In April
In 1972, he was hospitalized for further evaluation. At this admission, he had a normal platelet count and normal red cells. The uric acid was normal but the leukocyte alkaline phosphatase was decreased. The spleen tip was barely palpable, and the liver was slightly enlarged, but the patient was otherwise clinically well. A bone marrow examination on April 28, 1972 showed (Fig. 4B) a markedly hypercellular marrow with marked granulocytic hyperplasia and eosinophilia. The megakaryocytes were increased. Cells of the lymphocytic series comprised only 6% of the marrow population.

In May 1972 the hemoglobin was 13.8 g/100 ml, and the white cell count 22,250 per cu mm. The differential showed polys 45%, bands 10%, metamyelocytes 20%, myelocytes 12%, blasts 1%, lymphs 12%. There were two nucleated red cells per 100 WBC, and the platelet count was 315,000 per cu mm. The metaphases of a 1-day peripheral blood culture all contained one Ph1 chromosome. The metaphases of the 3-day culture showed six cells with a normal male karyotype, two cells with 48 chromosomes with two extra chromosomes in group C, one cell with 46 chromosomes with four fragments, two cells with 45 chromosomes and one Ph1 chromosome, and three cells with 46 chromosomes and one Ph1 chromosome. The chromosome preparation of the bone marrow showed 20 metaphases, all of which contained one Ph1 chromosome. Two cells had 44 chromosomes, 15 cells had 45 chromosomes (missing the Y), and three cells had 46 chromosomes. The patient was then diagnosed as CGL and placed on myleran. At the time of this report the patient is doing well.

DISCUSSION

In this report we have described two patients with CLL; both have subsequently developed classical Ph1 positive CGL. Case I received total body radiation for CLL, while the second patient received no treatment and had no history of previous radiation exposure.
After development of the CGL in case 1, there were entirely different clones of cells, both cytogenetically and morphologically, in the various organs. The bone marrow and peripheral blood cells consisted primarily of Ph\(^+\) positive myeloblasts. The spleen was principally infiltrated with hyperdiploid Ph\(^-\) negative lymphoid cells with intermediate maturation. These findings indicate a coexistence of both CLL and CGL. Involvement of the bone marrow and peripheral blood was indicative of CGL, while the involvement of the liver seemed related to CLL. Whether or not both forms of leukemia will eventually occupy the same site is not known. Only one cell with 48 chromosomes, supposedly from the lymphoid series, was seen in the bone marrow sample immediately following the splenectomy and disappeared after that sampling. Whether the CGL cells are a more invasive clone, and will eventually replace both the normal and abnormal CLL cells is not clear, but the evidence provided by the first patient seems to favor this viewpoint. In the second case, the bone marrow and peripheral blood are consistent with the criteria established for classical Ph\(^+\) positive CGL.

One other case has suggested the coexistence of CLL and another leukemic process. In that case the peripheral lymphocyte count rose and lymph nodes enlarged at the time that an acute myelomonocytic leukemia was evolving. The peripheral blood lymphocytes in that case responded to phytohemagglutinin in a normal manner.

In the previously reported 20 cases of acute leukemia associated with CLL, 17 patients were treated with radiophosphorus, one patient with total body radiation and chlorambucil, and two patients were treated solely with chlorambucil. Our second patient with CGL and CLL did not receive any chemotherapy or radiotherapy.

Chromosome analysis was of great value in establishing the diagnosis of a second form of leukemia and in establishing the coexistence of CLL and CML in different organs in the first patient.

Patients with CLL have an increased incidence of a second neoplasm. At this time it is not clear whether or not a second leukemic or lymphomatous process in these patients represents a therapy-induced disease (complication) or if it simply represents an increased susceptibility to a second malignancy (superimposed disease).

Since one of our patients was not treated for CLL, it is our observation that previous therapy is not necessarily a prerequisite for the development of CGL.

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Chronic Granulocytic Leukemia (CGL) During the Course of Chronic Lymphocytic Leukemia (CLL): Correlation of Blood, Marrow, and Spleen Morphology and Cytogenetics

Jacqueline Whang-Peng, Harvey R. Gralnick, Ralph E. Johnson, Elaine C. Lee and Arnold Lear

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