CLONAL ORIGIN OF CHRONIC MYELOCYTIC LEUKEMIA IN MAN*

BY PHILIP J. FIALKOW, STANLEY M. GARTLER, AND AKIRA YOSHIDA

DEPARTMENTS OF MEDICINE AND GENETICS, UNIVERSITY OF WASHINGTON, SEATTLE

Communicated by Curt Stern, August 7, 1967

Knowledge of whether a tumor is unicellular or multicellular in origin may provide important insight into its pathogenesis. Information relevant to this point can be obtained by studying neoplasms arising in individuals with two or more genetically different cell populations, such as females heterozygous for the X-linked glucose-6-phosphate dehydrogenase (G-6-PD) locus. In these subjects, a given cell or clone of cells shows only one of the two enzyme types seen in a mixture of their cells. For example, electrophoretic analyses of mass cultures of fibroblasts derived from females heterozygous for the A and B genes at the G-6-PD locus show both type A and type B enzyme, whereas analyses of clones originating from single cells derived from the mass cultures show either type A or type B enzyme but never both A and B. Thus, neoplasms arising from a single cell in heterozygous females should exhibit only one enzyme type, while those with a multicellular origin should contain both enzymes.

In the first application of this system to the study of neoplasms, Linder and Gartler found only type A or type B enzyme in leiomyomas whereas both A and B were found in adjacent normal uterine tissue. This observation strongly suggests a unicellular origin of these tumors. Subsequent studies of this kind provided evidence suggesting a multicellular origin of hereditary trichoepitheliomas, carcinoma of the colon, the liver, and the breast and perhaps also of chronic lymphocytic leukemia, in a case of lymphoma, unicellular origin was suggested. In this paper we report the application of this system to chronic myelocytic leukemia (CML). In addition to indicating a clonal origin of this malignancy, our results provide strong support for the hypothesis that erythrocytes and granulocytes have a common stem cell.

Patients.—Three Negro females heterozygous for the common (A) electrophoretic variant of G-6-PD were studied. They had typical clinical and hematological features of CML. The Philadelphia chromosome was demonstrated in the first two cases, but cytogenetic studies were not done in the third case. Case 1 was a 15-year-old girl who had not yet been treated. At the time of study the peripheral white blood count was 700,000 cells per mm³ of which 6% were myeloblasts, 1% promyelocytes, 1% myelocytes, 44% metamyelocytes, 13% band forms, 31% mature polymorphonuclear cells, and 4% were mononuclear cells. Case 2 was an 86-year-old woman who was being treated with busulfan. At the time of study the peripheral white blood count was 18,000 cells per mm³ of which 3% were metamyelocytes, 7% band forms, 69% mature polymorphonuclear cells, and 21% were mononuclear cells. Case 3 was a 22-year-old woman who had been treated with busulfan for two months. Two weeks before the time of study the peripheral white blood count was 31,500 cells per mm³ of which 3% were myeloblasts, 35% myelocytes, 13% metamyelocytes, 23% neutrophils, 5% basophils, 5% eosinophils, and 16% were mononuclear cells.

Methods.—Blood was drawn in heparin for preparation of granulocytes and in
acid-citrate-dextrose for preparation of hemolysates of red blood cells (RBC's). Attempts at preparing lymphocytes were unsuccessful due to the relative paucity of those cells. Cells of the granulocytic series comprised 90 and 91 per cent of the total cells in the white cell preparations used for enzyme analysis in Cases 1 and 2, respectively. In the third case cellular morphology was not sufficiently preserved to allow accurate counts. The granulocytes were disrupted by ultrasound and extracts were prepared. Small skin biopsies were obtained from the patients for tissue culture.\textsuperscript{8} After the third transfer, the fibroblasts derived from the skin biopsies were harvested, washed in saline, resuspended in 1 ml of 1 $\times$ $10^{-4}$ M NADP in distilled water, and exposed to repeated freezing and thawing. Aliquots of the erythrocyte, granulocyte, and fibroblast preparations were subjected to starch gel electrophoresis\textsuperscript{4} and in Case 1 also to CM-Sephadex column chromatography.\textsuperscript{10}

**Results.**—After starch gel electrophoresis, both A and B type enzymes were found in preparations of fibroblasts from the cell cultures of each of the three patients (see Fig. 1), indicating that the patients were heterozygous at the G-6-PD locus. In contrast, only one enzyme type (type A) was demonstrable in erythrocyte and granulocyte preparations from the three patients. Since the high peripheral white blood count in Case 1 made it likely that freshly prepared RBC hemolysates were significantly contaminated by granulocytes, hemolysates were also prepared from washed erythrocytes that had been stored in the refrigerator in acid-citrate-dextrose for six weeks. Under these circumstances the great majority of granulocytes had undergone lysis and the washed RBC's were relatively pure. Again, only type A enzyme was demonstrable in these cells.

Column chromatography\textsuperscript{10} was done with material from Case 1 and, as with starch gel electrophoresis, two enzyme types were found in skin cells, but only one enzyme type was found in erythrocytes and granulocytes.

**Discussion.**—The presence of a single G-6-PD type in the blood cells of our three patients who are heterozygous for the G-6-PD locus is compatible with the suggestions of a clonal origin of the tumor and of a common stem cell for red blood cells and granulocytes. However, several alternative explanations for these findings should be mentioned. For example, it is conceivable that the leukemic process alters type B G-6-PD so that it cannot be distinguished from type A enzyme by our methods. In order to explore this possibility two patients with CML and type B enzyme in their fibroblasts were studied. In both cases typical type B enzyme was demonstrable in erythrocyte and granulocyte preparations.

Another possible explanation for the presence of only one enzyme type in the RBC's and granulocytes of our patients with CML who are heterozygous for the G-6-PD locus is that during the process of random X-chromosome inactivation in embryogenesis the same X chromosome in every hematopoietic precursor cell was

---

**Fig. 1.**—Starch gel electrophoresis of specimens from Case 2. From left: slot 1, red blood cells (A); slot 2, granulocytes (A); slot 3, B control; slot 4, fibroblasts from skin culture (AB); slot 5, B control.
inactivated. Occasionally only one type of G-6-PD is seen in RBC's from normal individuals who are genetically known to be heterozygous for the G-6-PD locus. The available data suggest that this event is unusual and probably occurs with a frequency less than 0.05. Thus, the chance that the RBC's in our three patients had only one type of G-6-PD before they contracted CML (and, therefore, independently of CML) is very small.

Still another possibility is that malignant transformation occurs in several or many cells and the cells producing type A enzyme then selectively overgrow those producing B enzyme. Since A and B cells coexist indefinitely in the great majority of heterozygotes, there seems to be no a priori reason for assuming that a malignant transformed A cell will outgrow a similarly transformed B cell. Thus, it is more probable that the finding of only one type of G-6-PD in preparations derived from millions of granulocytes from patients with chronic myelocytic leukemia who are heterozygous for the G-6-PD locus indicates a clonal origin of CML. It seems likely that the leukemia arises as a result of a rare event (e.g., somatic mutation) in a single cell.

Studies utilizing another kind of marker, the Philadelphia chromosome,\textsuperscript{11} also suggest a clonal origin of CML. This small marker chromosome which is consistently seen in bone marrow cells of almost all typical cases of CML\textsuperscript{12-14} is not seen in other cells such as lymphocytes and fibroblasts.

In many cases of CML almost 100\% of dividing marrow cells have the Philadelphia chromosome.\textsuperscript{12-14} This finding has been interpreted as evidence for the hypothesis that erythrocytes and granulocytes have a common stem cell.\textsuperscript{15} The demonstration in our patients that erythrocytes and granulocytes have the same single enzyme type while two cell types are found in skin cells provides strong support for this hypothesis. Furthermore, it indicates with virtual certainty that the malignant transformation occurs in a stem cell common to both the erythrocytic and granulocytic series.

CML is not considered morphologically to be a stem cell leukemia. The predominant picture in the bone marrow is an overgrowth of myelocytes and the ensuing more mature granulocytic cells with little evidence of stem cell proliferation. The single G-6-PD type persists in peripheral RBC's and granulocytes in patients in remission and in such patients the Philadelphia chromosome is still present in almost all dividing marrow cells.\textsuperscript{13-14} To bring these genetic data together with the morphological observations it seems necessary to postulate that the CML stem cell replaces the normal stem cell population and that it gives rise to a myelocytic series which is the source of abnormal proliferation into both intra- and extramedullary spaces.

Summary.—The G-6-PD types of erythrocytes, granulocytes, and skin cells in three patients with CML were determined. The patients were heterozygous at the G-6-PD locus, so that two enzyme types were found in skin cells. The fact that only one type was found in erythrocytes and granulocytes was interpreted as strong evidence that erythrocytes and granulocytes have a common stem cell and that CML has a clonal origin. It appears most likely that this malignancy arises as a consequence of a rare event in a single stem cell.
We thank Dr. Rouben M. Jiji, Department of Medicine, University of Maryland, and Dr. Carlos Manso, Faculty of Medicine, University of Mozambique, for their interest and for providing material from these cases for study. We are indebted to Mrs. B. Burt and L. Steinmann for skilled technical assistance.

* This work was supported by grants from the United States Public Health Service HD 1682, HD 02497, GM 15253, and the National Science Foundation B-5937.