

## CONCISE REPORT

## Chronic Myelocytic Leukemia: Eosinophils Involved in the Malignant Clone

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Chronic myelocytic leukemia (CML) is a clonal disorder involving neutrophil, monocyte, erythrocyte, and platelet precursors. In order to determine if the eosinophils are also involved in the leukemic clone, we purified the eosinophils from a woman heterozygous for the common electrophoretic variants of the G6PD gene. Only type B enzyme was demonstrable in the eosinophils, neutrophils, and red cells, but both A and B enzymes were found in the fibroblasts. The data provide evidence that the eosinophil is involved in the malignant clone.

**M**OST PATIENTS with chronic myelocytic leukemia (CML) have a specific chromosomal abnormality known as the Philadelphia (Ph<sup>1</sup>) chromosome that usually results from the translocation of a portion of the long arm of chromosome 22 onto the long arm of chromosome 9. This chromosomal change has been found in neutrophil, monocyte, erythrocyte, platelet, and possibly basophil precursors.<sup>1-5</sup> The Ph<sup>1</sup> chromosome has not been found in fibroblasts nor in most of the peripheral blood lymphocytes.<sup>3,6,7</sup> The clonal nature of the disease is further suggested by isozyme studies, especially those using the X-linked enzyme G6PD.<sup>8-10</sup> In human females, one of the X chromosomes is partially or totally inactive in each cell.<sup>11,12</sup> Women heterozygous for the common electrophoretic variants of the glucose-6-phosphate dehydrogenase (G6PD) gene have two populations of cells: one produces type B enzyme, and the other produces type A. Tumors with clonal origin from a single cell in a G6PD heterozygote should have tumor cells composed of all B or A type enzyme.<sup>13</sup> Previous studies by Fialkow have shown that chronic myelocytic leukemia (CML) has a clonal origin in the stem cell common to the granulocyte, monocyte, platelet, and erythrocyte.<sup>10</sup> Recent investigations by Fialkow and colleagues<sup>14</sup> suggest that some of the B and T lymphocytes are also involved in the clonal disease. We report here a G6PD

heterozygote with CML whose eosinophils exhibit only B enzyme, suggesting involvement of this cell type in the clonal development.

### CASE REPORT

A 34-yr-old black woman was found to have chronic myelocytic leukemia (CML) in November 1978. The patient had no splenomegaly and a white cell count of 52,000/ $\mu$ l with 43% neutrophils, 18% bands, 17% myelocytes and younger granulocyte precursors, 15% lymphocytes, 5% eosinophils, and 2% basophils. The Ph<sup>1</sup> chromosome was identified by chromosome banding analysis in 37 of 39 metaphases from blood that was cultured for 24 hr without phytohemagglutinin. The patient had never received a blood transfusion nor any other therapy previous to this study.

Eosinophils from the patient were purified by a series of isolation steps modified from the technique of Parrillo and Fauci.<sup>15</sup> Heparinized venous blood (60 ml) was centrifuged on a Ficoll-Hypaque gradient (900 g, 45 min, 20°C). The cell button was resuspended in alpha-media (Flow Laboratories, Oxnard, Cal.) and dextran-sedimented (3% dextran in normal saline) at normal gravity. The leukocyte-rich fraction was lysed with ammonium chloride in Tris buffer to remove remaining erythrocytes. This neutrophil-eosinophil-basophil mixture was suspended in 3 ml alpha-medium containing 20% heat-inactivated fetal calf serum and run through a nylon-wool column (3 g of nylon wool, Leukopak, Fenwal Laboratories, Deerfield, Ill., packed into a 35-ml syringe). The column was primed with 100 ml alpha-medium and 20% heat-inactivated fetal calf serum. The cells were passed through the column twice and then incubated in the column for 15 min at 37°C. The neutrophils adhered to the nylon wool, and the eosinophils were eluted with 50-100 ml of alpha-medium at 37°C. The granulocytes were collected by placing the nylon-wool column at 4°C for 30 min and eluting the cells with 100 ml of cold alpha-medium. Eosinophil and granulocyte viability, as determined by trypan blue exclusion, was greater than 95%. Aliquots of the final cell suspensions were used for making cytocentrifuge slides, which were stained with Wright-Giemsa and luxol blue. The eosinophil-rich fraction contained  $3.1 \times 10^6$  cells with 84% eosinophils, 9% basophils, 6% neutrophil bands, 1% lymphocytes, and a rare nucleated red cell. The neutrophil-rich fraction contained 98% neutrophils and bands.

Extracts of the whole blood erythrocytes, cultured skin fibroblasts, and purified eosinophils and neutrophils were tested for G6PD electrophoretic patterns.<sup>16</sup> Both A and B enzymes were found in fibroblasts, but only type B enzyme was demonstrable in eosinophils, neutrophils, and red cells.

### DISCUSSION

Both B and A G6PD enzymes were found in skin fibroblasts of a black woman with CML, but only a

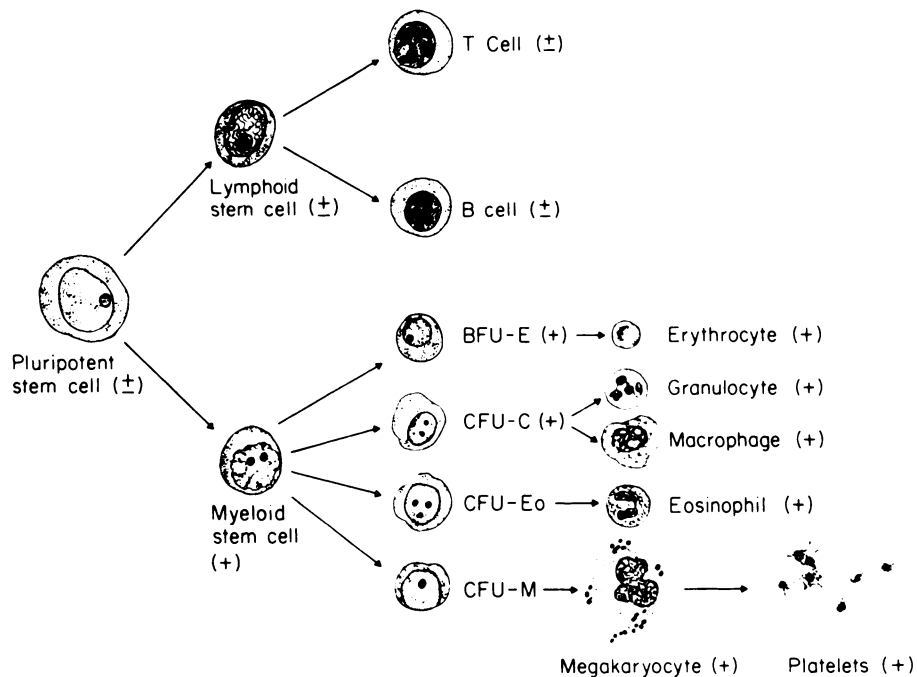
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**Fig. 1.** Clonal origin of human hematopoietic cells in chronic myelogenous leukemia. These data represent accumulated isozyme and chromosome studies. A recent study<sup>14</sup> suggests that a subset of T and B lymphocytes are involved in the CML clone. BFU-e, CFU-c, CFU-ee, CFU-m refer to erythroid, granulocyte-monocyte, eosinophil, and megakaryocyte colony-forming cells, respectively.

single G6PD type was observed in the CML eosinophils, granulocytes, and red cells. The fact that single G6PD expression occurs in CML eosinophils strongly favors the eosinophil involvement in the malignant clone.

Additional evidence for the eosinophil involvement in the malignant clone is provided by studies of the Ph<sup>1</sup> chromosome. If the greater percentage of dividing CML cells are eosinophil precursors, and all the metaphases analyzed contained the Ph<sup>1</sup> chromosome, then the eosinophils probably arose from the abnormal stem cell. Six patients with marked eosinophilia and the Ph<sup>1</sup> chromosome have been described.<sup>17-20</sup> There is controversy over the cytogenetic data<sup>21-23</sup> because all the patients were male and the studies were performed before the development of banding techniques and a short male Y chromosome might be confused with the Ph<sup>1</sup> chromosome.

Our electrophoretic assay can detect a cell population if it contributes approximately 5% of the total G6PD activity. The basophils represented about 10% of the eosinophil-rich cell population. Because this cell fraction demonstrated only the B enzyme, the basophils are probably also involved in the leukemic clone. This confirms a recent in vitro study suggesting the basophil precursors contained the Ph<sup>1</sup> chromosome.<sup>5</sup>

Because the red cells, granulocytes, eosinophils, and probably basophils from our G6PD heterozygote with CML displayed only one enzyme, the data provide evidence that eosinophils, and possibly basophils, share a stem cell common with the CML erythrocyte

and granulocyte. Figure 1 summarizes present understanding of the clonal origin of CML.

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