Rapid Publication

Pancytopenia as a Clonal Disorder of a Multipotent Hematopoietic Stem Cell

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Abstract. Hematopoiesis was investigated in a 14-yr-old girl who had a 2-yr history of stable asymptomatic pancytopenia and who was also heterozygous at the structural locus for glucose-6-phosphate dehydrogenase (G-6-PD). There was no morphologic or cytogenetic evidence for preleukemia and no suggestion of Fanconi anemia. In the skin and sheep erythrocytes-rosetted T lymphocytes, the ratio of G-6-PD A/B activities was 1:1. However, only type B activity was found in peripheral blood erythrocytes, granulocytes, and platelets. Most erythroid bursts and all granulocyte/macrophage colonies formed in methylcellulose culture were derived from the abnormal clone. These findings demonstrate that (*a*) some cases of pancytopenia are stem cell diseases that apparently develop clonally; (*b*) circulating differentiated cells originate from this clone; (*c*) despite a hypoproliferative anemia, the in vivo expression of presumably normal (nonclonal) progenitors is suppressed. In this patient, the relationship between clonal dominance and possible malignancy may be assessed prospectively.

Introduction

Studies of patients heterozygous for glucose-6-phosphate dehydrogenase (G-6-PD)[1] suggest that many hyperproliferative hematologic disorders result from clonal expansion of a multipotent stem cell (1–3). In this report, we document for the first time that clinically stable pancytopenia may also result from such a process.

The structural locus for G-6-PD is on the portion of the X chromosome that is randomly inactivated early in embryogenesis in somatic cells of females (1). Therefore, in females heterozygous for the usual G-6-PD gene (Gd^B) and a variant such as Gd^A, some cells produce type B enzyme while others synthesize type A. A neoplasm arising in a single cell in a Gd^B/Gd^A heterozygote will manifest a single-enzyme type (e.g., B or A, but not both), whereas normal tissues will show both enzyme types.

Methods

Subject. A 12.5-yr-old black girl was found to have moderately severe pancytopenia in February 1981. There was no significant past medical or family history. Growth and development were normal (80th percentile for height; menarche at age 11). Physical examination showed short, slightly broad thumbs and great toes, clinodactyly of both fifth fingers, a bifid uvula, a submucous cleft palate, and mild ocular hypertelorism, anomalies not specifically associated with Fanconi anemia (4). There were no café-au-lait or hypopigmented spots. Radiographic examinations of hands and feet demonstrated no osseous lesions and normal bone age. Laboratory values were: hematocrit 14; hemoglobin 4.8 g/dl; mean corpuscular volume 103 fl; reticulocyte count 4.4% (index 1.5); leukocyte count 1,900/μl with 610 neutrophils and 950 lymphocytes; and platelets 19,300/μl. Serum level of vitamin B_{12} was 730 pg/ml and of folate 8.1 mg/ml. Serum immunoglobulins were IgG 1,430 mg/dl (normal 680–1,493); IgM 148 mg/dl (normal 45–237), and IgA 125 mg/dl (normal 81–232). A marrow aspirate and biopsy showed normoblastic erythropoiesis with granulocytic and megakaryocytic hypoplasia. No excess blasts were seen. No ringed sideroblasts were present on marrow iron stain.

Sucrose hemolysis, obtained 2 mo following erythrocyte transfusion, was negative. A serum erythropoietin (Ep) level was >1,000 mU/ml (normal 18.7±4.7) by radioimmunoassay (5). A plasma iron turnover was 1.08 mg iron/100 ml whole blood per d (normal = 0.6–0.8) and

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1. *Abbreviations used in this paper:* BFU-E, erythroid burst-forming unit; CFU-E, erythroid colony-forming unit; CFU-GM, granulocyte/macrophage colony-forming unit; Ep, erythropoietin; E-rosetted T cells, T cells rosetted with neuraminidase-treated sheep erythrocytes; G-6-PD, glucose-6-phosphate dehydrogenase.

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utilization was 49% (normal mean 80%) (6). As a three- to sixfold increase in erythropoiesis would be expected with anemia and elevated Ep (6), the ferrokinetic values confirm the hypoplastic nature of the disease. Hemoglobin electrophoresis showed 35% hemoglobin S. The hemoglobin F level, determined by alkali denaturation, was elevated at 6.9% and there were 28% F cells (7). Though consistent with aplastic anemia and preleukemic syndromes (8), these findings may also reflect a heterocellular type of hereditary persistence of fetal hemoglobin in this patient with sickle cell trait (9). Blood from parents and siblings was not available for study.

No chromosomal gaps or breaks were seen in 60 RHG-banded and 7 GTG-banded metaphases of phytohemagglutinin-stimulated peripheral blood lymphocytes. In 20 additional nonbanded Giemsa-stained metaphases, a single chromatid gap was detected in both patient and control cultures. No increased chromosome breakage was noted when lymphocytes were treated with diepoxybutane as a provocative test for Fanconi anemia (10). 11 marrow karyotypes prepared with GTG-banding, following overnight incubation without mitogen, were also normal.

Over 2 yr, the patient has remained asymptomatic and peripheral blood counts and marrow morphology have not changed. Therapy has been limited to erythrocyte transfusions at 1–3-mo intervals to maintain her hematocrit > 20. A trial of prednisone did not change hematologic parameters. Informed consent was obtained from the patient and her guardian for experimental studies.

G-6-PD analysis. The patient was determined to be heterozygous for G-6-PD by electrophoretic analysis of a full-thickness skin biopsy. Lymphocytes, granulocytes, erythrocytes, and platelets were separated (11) from serial peripheral blood samples obtained 2–3 mo following previous transfusions. The ratio of G-6-PD A/B activities was visually estimated following enzymatic staining of lysates separated by cellulose acetate (12) and starch gel electrophoresis (11). A minor component contributing 5% to the total G-6-PD activity can be detected by this method (13).

Cell culture. Mononuclear marrow cells were cultured for granulocyte/macrophage colony-forming units (CFU-GM), erythroid burst-forming units (BFU-E), and erythroid colony-forming units (CFU-E) in the presence of 2.5% phytohemagglutinin-stimulated human lymphocyte-conditioned media and 1 unit/ml of partially purified human urinary Ep. CFU-GM were also cultured with 7.5% lymphocyte-conditioned media without Ep. At day 7, erythroid colonies were enumerated. At day 12–14 erythroid bursts and GM colonies were counted and individually harvested for G-6-PD analysis (14, 15).

Lymphocyte preparation. T lymphocytes were rosetted with neuraminidase-treated sheep erythrocytes (E-rosetted T cells). Relative proportions of T helper and suppressor cells were assessed by indirect immunofluorescence following exposure to antibodies 66.1 (helper/inducer, equivalent to OKT-4) and 51.1 (cytotoxic/suppressor, equivalent to OKT-8) (16). T cell subsets were separated on a fluorescence-activated cell sorter for G-6-PD analysis. E-rosette negative cells, which did not adhere to plastic after 2-h incubation, were considered B lymphocytes. 1–2% of this cell population were monocytes by Wright-Giemsa and nonspecific esterase stains.

Results

G-6-PD analysis of blood cells (Table I). Equal activities of G-6-PD types A and B were found in skin and E-rosetted T cells. In contrast, only type B activity was detected in preparations of erythrocytes, granulocytes, and platelets on six separate occasions. Although the helper/inducer to cytotoxic/suppressor T cell ratio in this patient was reversed (0.74; normal 1.75 [17]), T cell samples sorted positively and negatively with antibodies 66.1 and 51.1 had equal activities of G-6-PD types A and B. The G-6-PD activities of E-rosette negative, nonadherent cells were skewed (20% A and 80% B).

Table I. G-6-PD Analysis

	G-6-PD	
	Type A	Type B
	%	%
Skin	50	50
Peripheral blood		
Erythrocytes	0	100
Granulocytes	0	100
Platelets	0	100
E-rosette positive (ER[+]) mononuclear cells	50	50
ER (+), 66.1 (+)	50	50
ER (+), 66.1 (−)	50	50
ER (+), 51.1 (+)	50	50
ER (+), 51.1 (−)	50	50
ER (−) nonadherent cells	20	80

Marrow culture. Marrow-derived colony-forming cells grew poorly in culture. Two to four erythroid colonies (normal = 40–100), 6–10 erythroid bursts (normal = 40–130), and 0–1 GM colonies (normal = 20–110) formed per 10^5 mononuclear cells. In May 1982, five erythroid bursts tested were G-6-PD type A and nine were type B, confirming the presence of normal stem cells in the marrow and suggesting that only 30% of marrow BFU-E were derived from the aberrant clone (15) (Table II). 3 mo later, repeat studies showed 1 burst with G-6-PD type A and 28 with G-6-PD type B. In November 1982, one burst tested was type A and nine, type B. Each of 14 CFU-GM-derived colonies tested in August and November was G-6-PD type B.

Table II. G-6-PD Analysis of Hematopoietic Colonies

	Number G-6-PD	
	Type A	Type B
May, 1982		
BFU-E	5	9
August, 1982		
BFU-E	1	28
CFU-GM	0	2
November, 1982		
BFU-E	1	9
CFU-GM	0	12

Discussion

Cellular mosaicism for G-6-PD in this patient with hematologically stable pancytopenia offers a unique opportunity to study the mechanism of marrow failure. Circulating erythrocytes, granulocytes, and platelets had a single G-6-PD type (B) and, by inference, were derived from a single clone. Most BFU-E and CFU-GM also originated from this clone, suggesting that the pancytopenia was secondary to the clonal expansion of a myeloid stem cell. This mechanism is conceptually similar to that in the myeloproliferative diseases (1) and refractory acquired sideroblastic anemia (3). Yet, in contrast to these diseases, proliferation and differentiation of the abnormal clone in this patient were insufficient to support normal peripheral blood counts.

Initially, presumably normal (G-6-PD type A) BFU-E matured in vitro. However, despite a hypoproliferative marrow, confirmed by morphologic and iron kinetic studies, and an elevated Ep level, circulating nonclonal erythrocytes were not seen. Since the electrophoretic techniques detect 5% of a minor enzyme component of a sample (13), the failure to detect nonclonal erythrocytes could be explained if the in vivo differentiation of normal erythroid progenitors were suppressed. Alternatively, the patient may be an A^-/B heterozygote. A^- erythrocytes lose G-6-PD activity with age (13) and therefore might not contribute to the erythrocyte G-6-PD determination.

No presumably normal GM colonies were detected in culture. However, by analogy with chronic myelogenous leukemia, it is likely that normal multipotent stem cells exist. In five reported G-6-PD heterozygotes with chronic myelogenous leukemia only 2 of 1,308 GM colonies tested derived from the normal cell population (14). Yet, in one patient, following aggressive chemotherapy, many nonclonal CFU-GM and BFU-E were detected (15). If the clonal disorder in our patient represents a neoplastic transformation of a myeloid stem cell, then the neoplastic process directly inhibits the growth of normal stem cells.

There are two possible mechanisms, other than neoplasia, for the apparent clonal nature of this patient's disorder. Following marrow damage inhibiting normal hematopoiesis, a cell, resistant to the environmental insult, may be able to proliferate, but unable to support normal blood cell levels. A similar mechanism has been hypothesized to explain the development of paroxysmal nocturnal hemoglobinuria in patients recovering from aplastic anemia (18).

Alternatively, hypoplasia and apparent clonality may have resulted from stem cell depletion or stem cell loss through differentiation. At a given time, only a few of the small number of residual pluripotent cells might be actively cycling. The two or three active stem cells might by chance all have G-6-PD type B. As this model assumes that stem cells have a limited capacity for proliferation in vivo and that clones, exhausted through terminal differentiation, are replaced by residual stem cells, it predicts that over time cells with G-6-PD type A might dominate in this patient.

Circulating lymphocytes were also studied to determine if they originated from the dominant clone. E-rosette-positive T cells were not clonal. Though E-rosette negative, nonadherent cells were skewed towards G-6-PD type B, suggesting that some B-lymphocytes may derive from the dominant clone, it is likely that contamination of the sample by small numbers of monocytes, which have an extremely high G-6-PD activity per cell, distorted these results.

Of interest is the reversal of the helper/inducer to cytotoxic/suppressor T cell ratio in this patient. As each T cell subset had equal activities of G-6-PD A and B, the imbalance of these populations did not result from direct clonal expansion. Whether the reversed T cell ratio in our patient is an epiphenomenon, is responsible for the suppression of normal stem cell growth (19, 20), facilitated the clonal expansion of an abnormal myeloid stem cell, or represents a physiologic response controlling neoplastic expansion (21) is unclear. This latter possibility raises a theoretical problem, however, when one considers antithymocyte globulin therapy for patients with aplastic anemia.

Serial study will establish if the clonal disorder in this patient represents a neoplastic change, perhaps preleukemic, of a myeloid stem cell, or is a reflection of stem cell suppression or depletion following an unknown injury.

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