Evidence That Essential Thrombocythemia is a Clonal Disorder With Origin in a Multipotent Stem Cell

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Essential thrombocythemia is characterized by proliferation of hematopoietic tissue predominantly involving megakaryocytes and resulting in marked thrombocytosis. The disorder has some clinical and laboratory features that resemble those seen in the clonal multipotent stem cell disorders chronic myelogenous leukemia, polycythemia vera, and agnogenic myeloid metaplasia. It has been argued that essential thrombocythemia should be classified together with those disorders as a myeloproliferative syndrome. However, without knowledge of the numbers and types of cells that are involved in essential thrombocythe

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The X-linked glucose-6-phosphate dehydrogenase (G6PD) locus is a particularly good marker to use in investigations of this kind. Since only one of the two X-chromosomes is active in XX somatic cells, a woman who is heterozygous at this locus for the common gene, GaB (which determines B-type G6PD) and a variant allele such as GaA (which determines type A enzyme) has two distinct cell populations, one producing B-type G6PD and the other A-type enzyme. All progeny of a neoplastic stem-cell clone will manifest type B or A enzyme, whereas a tumor that arises from many cells may show both B and A enzymes.

CASE REPORTS

The three patients with thrombocythemia and heterozygous at the G6PD locus were identified with the assistance of the National Polycythemia Vera Study Group.

Endometrial Carcinoma

Patient 1, a 66-yr-old woman, was admitted in June 1976 to the Surgical Service at the Medical College of Georgia with massive upper gastrointestinal bleeding. She had been taking Coumadin for 5 mo for deep vein thrombophlebitis. At the time of admission, the hematocrit was 15%; platelet count, 1,760,000/cu mm; and white cell count, 36,000 with 80% polymorphonuclear cells, 1% metamyelocyte, 1% myelocyte, 1% eosinophil, 1% basophil, 4% lymphocytes and 3% monocytes.
The patient was referred to the Hematology Service. Marrow biopsy showed hypercellularity with increased megakaryocytes, myeloid hyperplasia, and mild erythroid hypoplasia. There was no fibrosis; iron stores were absent. Leukocyte alkaline-phosphate score was 115 (normal range 10–90). Values for simultaneously evaluated controls were 1 for a patient with chronic myelogenous leukemia and 193 for a patient with reactive leukocytosis. Serum vitamin B12 level was 1053 pg/ml and B12 binders were 3590 pg/ml. Liver-spleen radionuclide scan revealed no organomegaly. Attempts to study marrow chromosomes were unsuccessful.

Diagnoses of peptic ulcer and myeloproliferative syndrome were made. The patient was transfused to a normal hematocrit and discharged on iron therapy. Repeat leukocyte alkaline-phosphatase score was 98 (values for simultaneously studied control patients with chronic myelogenous leukemia and reactive leukocytosis were 6 and 160, respectively). Several attempts to study chromosomes in marrow and peripheral blood cells were unsuccessful. After 3 mo of iron therapy, when the patient showed evidence of having been replenished with iron (serum iron, 115; total iron binding capacity, 255; and iron stores present in marrow) and the hematocrit stabilized between 30% and 35%, $^{51}$Cr red blood cell mass was 18 ml/kg (normal value in females, 25 ± 5 ml/kg). During this time, platelet counts ranged between 1,200,000 and 4,000,000.

The diagnosis of essential thrombocythemia was made, the patient was entered on the Thrombocytosis Protocol of the Polycythemia Vera Study Group, and was randomized to $^{32}$P treatment, which was instituted in April 1977. The platelet count decreased from 4,000,000 to 538,000. Four subsequent treatments with $^{32}$P were given between May 1977 and November 1978 to maintain platelet counts below 600,000. White cell counts and hematocrits ranged between 7000 and 12,000 and 35% and 45%, respectively.

At the time of study of G6PD (July 10, 1979), the hematocrit was 45%; white cell count, 10,900; and platelet count 682,000. In vitro platelet aggregation studies showed no second wave in response to epinephrine and ADP, but there was a normal response to collagen. Platelet adhesiveness was 29% (normal greater than 90%). The patient had remained asymptomatic since diagnosis. However, she died unexpectedly in August 1979. No autopsy was obtained.

Patient 2, a 39-yr-old woman, was admitted in January 1976 to the Neurology Service at Georgetown University Hospital with blurred vision and right-sided weakness. The hematocrit was 41%; white cell count 6,800; and platelet count, 1,403,000. The neurologic symptoms resolved spontaneously over the next 5 days.

The patient remained well until 1978 when she developed numbness of the hands and feet and myalgia. In July 1978, the platelet count was 2,000,000 and she was referred to the Hematology Division. There was no history of bleeding or thrombotic episodes. On physical examination, the spleen tip was palpable just below the left costal margin. The hematocrit was 43%; white cell count, 7500; and platelet count, 1,793,000. Bone marrow aspirate revealed megakaryocytic hyperplasia. Iron stores were present. Leukocyte alkaline phosphatase score was 120 (normal range 20–120). Serum vitamin B12 level was 475 pg/ml. Serum iron was 84, with a total iron binding capacity of 345. Liver-spleen radionuclide scan showed no organomegaly.

Busulphan therapy was discontinued and supplemental iron was given. Three months later the white cell count was 5400 and the hematocrit was 39%. The platelet count had risen from 675,000 to 1,070,000. Hydroxyurea therapy was begun, and the patient remains free of symptoms with platelet counts ranging between 400,000 and 600,000. At the time of first study of G6PD in November 1979, the hematocrit was 35%; platelet count, 529,000; and white cell count, 3600 with a normal differential. Two months later, the patient was restudied. Marrow biopsy showed normal cellularity with no fibrosis. Iron stores were present in a marrow aspirate. Of 35 marrow cell metaphases studied after 12 hr in culture without mitogen stimulation, 2 had 45, and 33 had 46 chromosomes. Ph1 or other abnormalities were not detected in banded karyotypes of 15 cells prepared with a Giemsa-banding technique.

**MATERIALS AND METHODS**

Samples of skin, blood, and marrow were sent to Seattle for G6PD studies. Extracts of skin cells and blood granulocytes, erythrocytes, and platelets were prepared and G6PD assayed as described previously. The relative activities of the enzyme bands were estimated visually. This technique allows detection of a minor enzyme component contributing at least 5% of the total activity.

**RESULTS AND DISCUSSION**

The ratio of B:A-type G6PD activity was approximately 1:1 in the skin from each patient. The observation of both types of G6PD in these normal tissues indicates that the patients were heterozygous at the G6PD locus. In marked contrast to these findings in skin, only a single-enzyme type was demonstrable in blood erythrocytes, granulocytes, and platelets in patients 1 and 3 (A-type G6PD in patient 1 and B-type enzyme in patient 3). Marrow cells from patient 3 also showed only B-type enzyme. When first studied, about 95% type-B enzyme was found in granulocytes and platelets from patient 2. Four months later, only B-type G6PD was detected in blood plate-
cells, granulocytes, and red cells and in marrow red cells and nucleated cells.

In previous studies of over 50 nonleukemic Gd 
Gd 
 heterozygotes, whenever both enzyme types were detected in skin, they were also found in blood granulocytes and red cells (Fialkow PJ, unpublished data). Thus, the results in our patients suggest strongly that at the time of study, a single multipotent stem-cell clone with abnormal proliferative properties was present and resulted in the clinical picture of essential thrombocythemia. A much less likely possibility is that chemotherapy has selected for a single clone in each patient. The data also indicate that remission induced with 32P or chemotherapy is not accompanied by return to the marrow of expression of normal stem cells.

Essential thrombocythemia is frequently an evolving condition in which some clinical and laboratory features resemble those found in other myeloproliferative disorders. Because patients with chronic myelogenous leukemia or polycythemia vera may have marked thrombocytosis, it is necessary to exclude these diseases before a firm diagnosis of essential thrombocythemia can be made. The data in patients 1 and 2 definitively excluded polycythemia vera as the correct diagnosis. Although chromosome studies were not successful in patient 1, the clinical features and high leukocyte alkaline phosphatase scores weighed heavily against the presence of chronic myelogenous leukemia. Ph 
 was not found in marrow cells from patient 2. Thus, we consider these patients to have had essential thrombocythemia.

In patient 3, the conclusions based on the G6PD results, that the hematopoietic cell proliferation was clonal and involved a multipotent stem cell, indicate that she has a myeloproliferative disorder. The presence of chronic myelogenous leukemia in this patient is very unlikely in view of the clinical course, high leukocyte alkaline phosphatase scores, and normal marrow cell chromosomes. However, she was not evaluated definitively for polycythemia vera before chemotherapy was begun. Nonetheless, the findings that at a time when the patient was not receiving chemotherapy, her hematocrit rose to only 39% while the platelet count increased to over 1,000,000, suggest that she did not have polycythemia vera. Thus, we consider the most likely diagnosis in this patient to be essential thrombocythemia.

The clonal nature and multipotent stem-cell involvement in essential thrombocythemia in these three patients suggest strongly that the disorders do not result from proliferation of normal stem cells in response to a myeloproliferative factor. Furthermore, G6PD can now be used to study in vitro regulation of hematopoiesis in essential thrombocythemia as has been done in other clonal myeloproliferative disorders. For example, G6PD studies of hematopoietic cell colonies grown in vitro suggest important differences in the pathogenesis, or rapidity of progression, or both, between chronic myelogenous leukemia and polycythemia vera. It is of interest to determine if regulatory dysfunctions studied in vitro in essential thrombocythemia resemble those found in polycythemia vera more closely than those detected in chronic myelogenous leukemia.

Although Ph 
-positive and Ph 
-negative chronic myelogenous leukemia, polycythemia vera, agnogenic myeloid metaplasia, and essential thrombocythemia all involve multipotent hematopoietic stem cells and perhaps even the same stem cell, there are marked variations in the manifestations and natural histories of the diseases. A likely possibility to explain these differences is that the pathogeneses of these diseases involve induced alterations in the genomes of somatic cells, that these changes are different in each disorder, and that they govern the responses of proliferating cells to regulatory factors and thereby determine which cell type will predominate—granulocytic cells in chronic myelogenous leukemia, erythrocytic cells in polycythemia vera, and platelet precursors in essential thrombocythemia.

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