**32P and Acute Leukemia: Development of Leukemia in a Patient With Hemoglobin Yakima**

By Grover C. Bagby, Jr., Kathryn Richer-Boe, and Robert D. Koler

In 1954 a then 31-yr-old male was found to have erythrocytosis. Over the ensuing decade he received 72 mCi 32P. In 1964 his daughters were found to have erythrocytosis. Further investigation led to the discovery of hemoglobin Yakima, a variant with high oxygen affinity. He received no further therapy and was well until 1975, when he developed the preleukemic syndrome. Within 12 mo he developed acute nonlymphocytic leukemia accompanied by fetal erythropoiesis. Because the initial discovery of this type of hemoglobinopathy came 27 yr after the introduction of 32P for use in the treatment of polycythemia vera, and because there are now known to be more than 39 different high-oxygen-affinity hemoglobins, we anticipate that more patients such as ours have been exposed to 32P. The exposed population should be closely followed, since this will likely permit assessment of the risk of 32P-induced leukemia in a nonneoplastic condition.

Although the leukemogenicity of ionizing radiation is widely accepted, the real leukemogenic potential of 32P in polycythemia vera (PV) has been difficult to assess. Since the first report of acute leukemia complicating 32P-treated polycythemia vera, the issue has remained controversial and was recently the subject of a comprehensive review. The essential features of the debate have related to the inadequacy of diagnostic criteria in older studies, conflicting observations on the relationship of risk to cumulative dose, the known risk of leukemia in patients with PV who have been treated with phlebotomy alone, and an understandable dearth of patients who received 32P for nonneoplastic conditions.

In 1967 we reported familial erythrocytosis due to a hereditary high-oxygen-affinity hemoglobinopathy (Hb Yakima) in a family whose propositus has been treated with 32P over the previous decade. We report herein the recent development of the preleukemic syndrome followed by acute nonlymphocytic leukemia (ANLL) in this same man.

**CASE REPORT**

In 1954 an asymptomatic 31-yr-old male was found to have an elevated PCV during a routine medical examination. No other abnormalities were found. He was referred to the University of Oregon Medical School, where a 51Cr red cell mass measured 71 ml/kg (normal, 26-32 ml/kg) and the diagnosis of PV was made. He was followed without treatment until April 1955, when therapy with 32P was initiated. He received 32P at 6-24-mo intervals over the next 10 yr to a cumulative dose of 72 mCi by January 1965 (Fig. 1). During 32P therapy, PCV and WBC ranged...
Fig. 1. Clinical course of the patient from time of diagnosis to June 1977. $^{32}$P therapy discontinued in 1965. In 1975 he developed pancytopenia and progressive increase in HbF (alkaline denaturation, ---; Betke-Kleihauer stain, - - - -). In January 1976 peripheral blood and bone marrow samples supported the diagnosis of the preleukemic syndrome. One year later the patient developed acute nonlymphocytic leukemia and was treated successfully with combination chemotherapy.

From 0.43 and 1.8 x 10$^9$/liter to 0.56 and 6.0 x 10$^9$/liter, respectively. In 1964 the patient's two daughters were found to have erythrocytosis. Further investigation led to the discovery of a new hemoglobin variant with high oxygen affinity, designated hemoglobin Yakima.§ Hemolysates from the propositus and both daughters gave comparable results for HbA$_2$ (2.5% - 3.1%), Yakima (36.9% - 38.5%), and A (45.8% - 51.1%).

The patient was followed without treatment and remained stable except for persistent leukopenia (3.0 - 5.0 x 10$^9$/liter) until September 1975, when he reported symptoms of fatigue, palpitations, and dizziness. Hb was 9.9 g/dl, PCV 0.28, reticulocytes 1.2%, WBC 2.9 x 10$^9$/liter, and platelets 89.5 x 10$^9$/liter. He was given red cell transfusions totaling 5 units between October 1975 and January 1976 (see Fig. 1). A bone marrow examination performed in September 1975 showed moderate hypocellularity with no apparent maturation abnormality, but in January 1976 there were megaloblastoid erythroid and megakaryocytic elements in a hypercellular marrow. Serum B$_12$ and folate levels were normal. During the following year the hemoglobin electrophoretic pattern showed progressively decreasing amounts of Hb Yakima and increasing amounts of HbF (Fig. 1, Table 1). Betke-Kleihauer stains indicated the presence of an increasing number of eryth-

<table>
<thead>
<tr>
<th>Date</th>
<th>HbA$_2$</th>
<th>HbF*</th>
<th>HbA$_1$</th>
<th>Hb Yakima</th>
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<tr>
<td>9/66</td>
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<tr>
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<td>35.7</td>
<td>26.5</td>
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<td>1.8</td>
<td>1.9</td>
<td>55.3</td>
<td>39.8</td>
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*Alkaline denaturation.
†DEAE-Sephadex chromatography.
§HbA$_1$ pH 8.3 on cellulose acetate, Hb Yakima and hybrids of Hb Yakima with HbA and Hb Yakima with HbF migrate behind HbA and cannot always be resolved into separate bands. The estimates of total Hb Yakima were made by subtracting the amount of HbF (as determined by alkaline denaturation) from the total Hb eluted from this region. This overestimates the actual amount of Hb Yakima by the amount of the HbA/Yakima hybrid running in this region.
‡Values for Hb Yakima and HbA/Yakima hybrid were 28%, and 27%, allowing for calculation of the amount of Hb Yakima.
Erythrocytes containing HbF, parallel to the rise in HbF as measured by alkaline denaturation. In October 1976 erythrocyte i/I antigen ratio was 1:1. Erythrocyte carbonic anhydrase B was normal (11-14 mg/g Hb) in September 1976 but levels fell to 2 and 6 mg/g Hb in December 1976 and January 1977, respectively.

In January 1977 the patient developed fever due to cellulitis of the arm and was admitted to a Yakima hospital, where leukoerythroblastos is discovered on a peripheral blood smear. He was transferred to the University of Oregon Health Sciences Center in February 1977. His hemoglobin was 9.5 g/dl, PCV 0.28, WBC 64.7 x 10^9/liter with 88% blasts. Bone marrow aspirate was markedly hypercellular with an E/G ratio of 1:50, markedly decreased megakaryocytes, and greater than 90% myeloblasts.

He was treated with Adriamycin, vincristine, cytosine arabinoside (ara-C), 6-thioguanine, prednisone, and broad-spectrum antibiotics. His hospital course was complicated by the development of intracranial hemorrhage, *Enterobacter* sepsis, maxillary sinusitis, staphylococcal cellulitis, oral candidiasis, and prolonged marrow suppression lasting 5 wk. Marrow recovery occurred in the sixth posttreatment week, at which time Hb Yakima increased and HbF decreased (Fig. 1). The patient remained in complete remission until this writing (25 wk).

**Cytogenetic studies.** A banded chromosome study of aspirated marrow cells obtained during the acute leukemic phase was performed according to methods previously described.8,9

**Agar bone marrow culture.** Double layer agar cultures were performed according to a modification of methods previously described.10-12 Cultures were performed once during overt disease and twice during remission.

**RESULTS**

**Cytogenetics.** All 35 metaphases examined were normal (46,XY).

**Agar culture.** Colonies and clusters were decreased (10 and 21 per 2 x 10^5 nucleated marrow cells plated, respectively) at the time the patient's marrow was diagnostic of acute leukemia. Four weeks after remission, neither cluster nor colony growth occurred. Nine weeks after remission induction, mean colony growth and cluster growth were 9 and 16 per 2 x 10^5 cells, respectively. In our laboratory normal agar colony growth ranges from 49 to 124 colonies per 2 x 10^5 nucleated marrow cells plated.

**DISCUSSION**

For a number of reasons the leukemogenic potential of 32P has been difficult to assess. For example, the natural course of untreated PV is one that has eventuated in acute leukemia. Therefore the aim of studies attempting to measure the risk of leukemia in a PV population must deal with risk enhancement rather than pure causality. Previously described nonpolycythemic 32P-treated reference groups have been less than optimal. Osgood4 reported that the risk of leukemia in 32P-treated patients with chronic lymphocytic leukemia (CLL) was less than the risk in patients with PV similarly treated. However, the median survival was less in CLL than in PV; hence the treated CLL population may not have survived long enough to develop leukemia of the nonlymphocytic variety. Modan and Lilienfeld3 noted that patients with “questionable polycythemia” treated with 32P had a 14% incidence of acute leukemia, yet because the diagnostic category of “questionable polycythemia” probably included patients with early forms of PV,2 the observed incidence may not reflect 32P leukemogenicity per se. However, they also reported two patients with acute leukemia who had been treated with 32P for erythrocytosis secondary to cardiopulmonary disease.5 That their erythrocytosis represented a normal physiologic
response to tissue hypoxia and not a manifestation of a myeloproliferative disorder suggests that $^{32}$P was a leukemogen in these patients. Patients treated with $^{32}$P for "secondary erythrocytosis" represent what we consider to be the best population available for studies of $^{32}$P-induced leukemia. Patients with high-oxygen-affinity hemoglobinopathies are part of this population.

Hereditary hemoglobin abnormalities characterized by increased oxygen affinity were first described in 1966. Unlike PV, these are benign disorders associated with isolated erythroid hyperplasia without concomitant granulocytic or megakaryocytic hyperplasia and are not thought to be inherently associated with an increased risk of leukemia. At least 39 different high-oxygen-affinity hemoglobins have been reported since 1966. Because the discovery of these hemoglobin variants came 27 yr after the introduction of $^{32}$P for use in the treatment of PV, we suspect that more patients such as ours have been exposed to $^{32}$P. Such patients may represent a unique population, perhaps the first group permitting assessment of the risk of $^{32}$P-induced leukemia in a nonneoplastic disorder. Nevertheless until the nature of radiation-induced leukemia is better understood, we recognize that even this group of patients may represent an imperfect reference group. For example, if the incidence of radiation-induced mutation is higher in more highly proliferative stem cell populations, and if the proliferative activity of the target stem cells is abnormally high in patients with secondary erythrocytosis, then even this group may be unusually susceptible to $^{32}$P-induced leukemia.

This patient's initial marrow abnormality met our criteria for the diagnosis of the preleukemic syndrome, which should always be considered in a cytopenic patient who has received cytotoxic therapy in the past. An unusual feature of our patient's illness was the progressive reversion to a pattern of fetal erythropoiesis during the preleukemic and overtly leukemic phases. Although occasional modest elevations of fetal hemoglobin are found in adults with acute leukemia, the marked increase of HbF production in our patient is unusual. Furthermore, in our patient there was a progressive decrease in erythrocyte carbonic anhydrase B levels as well as an abnormally high i/I antigen ratio. Such a reversion to a fetal pattern of erythropoiesis is characteristic of chronic granulocytic leukemia of the juvenile type but to our knowledge has not been reported in adult acute leukemia. That Hb Yakima decreased as HbF increased suggests that fetal erythropoiesis occurred in the emerging leukemic clone, but we cannot exclude the possibility that the reversion of fetal erythropoiesis occurred in the normal (nonleukemic) clone. The mechanism of fetal erythropoiesis might be elucidated using erythroid cloning techniques, particularly if the leukemic clone is marked by a chromosome abnormality.

Although we are familiar with only one other report of a myeloproliferative disorder in a patient with a high-oxygen-affinity hemoglobinopathy, we anticipate similar reports of $^{32}$P-related leukemia in patients with high-oxygen-affinity hemoglobinopathies. The unusual process of fetal erythropoiesis might prove to be a common feature and may afford opportunities to analyze the pathophysiologic mechanisms of fetal erythropoiesis in the leukemic disorders.

Note added in proof: Since preparation of this manuscript, the patient had a relapse 44 wk after induction that was presaged by increasing numbers of fetal
erythrocytes. He responded to reinduction antileukemic therapy, has been in remission for 6 wk, and has had normal levels of HbF.

REFERENCES

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