Three Patients With Structurally Abnormal X Chromosomes, Each With Xq13 Breakpoints and a History of Idiopathic Acquired Sideroblastic Anemia

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Structural abnormalities of the X chromosome are rarely found in neoplastic disorders. We describe three patients with a history of idiopathic acquired sideroblastic anemia (IASA); each one had an abnormal clone of cells in the bone marrow, characterized by a structurally abnormal X chromosome. In two of these patients, the predominant karyotype was 47,X,2idic(X)(q13); in the other patient, it was 46,X,t(X;11)(q13;p15). Inasmuch as all three of these cases involved chromosome band Xq13, as did two previously published cases, we suggest that band Xq13 may be more prone to structural rearrangement than other X chromosome bands in hematologic disorders. The common Xq13 chromosome breakpoint and clinical presentation (IASA) among these three patients and the occurrence of an X-linked type of sideroblastic anemia may suggest that an association exists between X chromosome abnormalities and IASA. Perhaps alteration of a gene or chromosome structure in or near band Xq13 predisposes to development of IASA. The fact that two of these patients had preleukemia and the third had overt acute leukemia may imply that patients with IASA and X chromosome abnormalities have a poor prognosis. Cases of IASA without associated X chromosome abnormalities are known; thus, if an association between IASA and an abnormal X chromosome does exist, most likely it involves only some patients with IASA.

CASE REPORTS

Case 1

A 77-yr-old white woman was referred to the Mayo Clinic for evaluation and treatment of a sideroblastic anemia. She had suffered from back pain for 30 yr and had taken multiple analgesics. In January 1977, anemia was diagnosed. A bone marrow specimen revealed erythroid hyperplasia, increased iron stores, and abundant ringed sideroblasts. She was treated with pyridoxine, but no response was evident. Blood transfusions were required to maintain satisfactory hemoglobin levels.

When examined at the Mayo Clinic in May 1977, she was found to have scoliosis and pallor but no hepatosplenomegaly. She had a hemoglobin level of 7.1 g/dl and an erythrocyte count of 2,260,000/cu mm. A leukocyte differential showed slight immaturity, including 8.5% band cells, 2% metamyelocytes, and 2% myelocytes, and dimorphic red blood cells (oval macrocytes and hypochromic microcytes) were noted. Direct Coombs' test was strongly positive, and multiple antibodies, including anti-K, anti-D, and anti-C, were present. During the next 5 mo she was supported with transfusions and treated with varying doses of corticosteroids but showed no apparent improvement.

She was reexamined at the Mayo Clinic in November 1977; her chief complaint was back pain. The patient was febrile and had pallor, echymoses, and scoliosis. The following determinations were recorded: hemoglobin level 9.7 g/dl, erythrocyte count 2,620,000/cu mm, leukocyte count 5,100,000 with a normal differential leukocyte count, and platelet count 181,000/cu mm. Direct Coombs' test was weakly positive, and the same antibodies were present as in May. The bone marrow was hypercellular with megaloblastoid erythropoiesis and bizarre multinucleated erythroid precursors. Sizable iron was increased, and ringed sideroblasts were abundant. Megakaryocytes were atypical and granulopoiesis was abnormal, characterized by bizarre pseudo-Pelger-Huet granulocytic precursors and 5% myeloblasts. The patient was found to have urine and blood cultures positive for Escherichia coli, and she experienced rapid defervescence after parenteral ampicillin therapy. Cytogenetic studies revealed an abnormal clone of cells (see Cytogenetic Results). IASA with evolving preleukemia was diagnosed. She was subsequently treated with azathioprine, 150 mg/day, and prednisone, 20 mg/day, but had no apparent response.
Case 2

A 33-yr-old white woman was initially examined at the Mayo Clinic for evaluation of headaches and fatigue. She was found to have cholelithiasis. She had a normal hemoglobin level of 15.4 g/dl and a leukocyte count of 4700/cu mm. She was reexamined here at age 55 yr for low back pain, at which time her hemoglobin level was 12.2 g/dl.

In 1979, at age 76 yr, she underwent surgical repair of a right inguinal hernia. She was noted to be anemic and was treated with orally administered iron and vitamin B12 injections after a barium enema and upper gastrointestinal series revealed normal findings. She was then referred to the Mayo Clinic for further evaluation of her anemia.

The patient had a history of increased bruisability during the preceding year. She was obese and had scattered ecchymoses and petechiae of the legs. Her hemoglobin level was 11.1 g/dl, erythrocyte count was 3,600,000/cu mm, and leukocyte count was 2200/cu mm with 41% neutrophils, 50% lymphocytes, 4% monocytes, and 5% atypical lymphocytes. Her platelet count was 60,800/cu mm. The leukocyte alkaline phosphatase score was 113 (normal range for our laboratory, 40–100). A peripheral blood smear showed a dimorphic erythrocyte population (oval macrocytes and hypochromic microcytes). The erythrocyte sedimentation rate was 45 mm in 1 hr. Her bone marrow was hypercellular with megaloblastoid erythroid hyperplasia and rare ringed sideroblasts. The serum vitamin B12, folic acid, total thyroxine, and uric acid values were within normal limits, as were results of liver function tests and renal function tests.

Because the patient was thought to have an evolving myeloproliferative disorder, folic acid and pyridoxine therapy was instituted. Follow-up examination 3 mo later showed that her condition was unchanged.

At a follow-up examination in May 1979, she complained of fatigue and tiredness. She continued to notice increased bruisability and had aching pains in the rib cage. She was still obese and had a systolic ejection murmur. Her hemoglobin level was 9.8 g/dl, erythrocyte count was 3,480,000/cu mm, leukocyte count was 2500/cu mm, and platelet count was 24,000/cu mm. The bone marrow was hypercellular with a left-shifted granulopoiesis including 10% myeloblasts, megaloblastoid erythroid hyperplasia, and occasional ringed sideroblasts. Cytogenetic studies revealed an abnormal clone of cells (see Cytogenetic Results). The diagnosis was IASA that had evolved to acute meylomonocytic leukemia.

Case 3

A 62-yr-old white woman was first seen at the Mayo Clinic for a routine physical examination. She had no specific complaints but had taken vitamins and iron for the preceding 8 yr because she feared becoming anemic. Her blood pressure was 170/94 mm Hg, and she had cystic mastitis in the left breast and varicose veins. Her hemoglobin level was 13.0 g/dl, erythrocyte count was 4,010,000/cu mm, leukocyte count was 6100/cu mm, and a differential leukocyte count was normal. Her serum iron level was elevated at 233 μg/dl.

The patient was next examined at age 67 yr. She was still taking vitamins and an iron supplement. Her blood pressure was 150/84 mm Hg, and she had early cataracts in both eyes and varicose veins. Her hemoglobin level was 13.5 g/dl, erythrocyte count was 4,160,000/cu mm, leukocyte count was 5800/cu mm, and a differential leukocyte count was normal.

She remained in good health until March 1979, when at age 74 yr she was noted to be neutropenic. Her leukocyte count was 2100/cu mm and her hemoglobin level was 12.3 g/dl. Three months later, her hemoglobin level was 8.5 g/dl and her leukocyte count was 1700/cu mm. Her bone marrow showed erythroid hyperplasia and 4+ iron stores with multiple ringed sideroblasts. Pyridoxine therapy was instituted, and 3 mo later she had a hemoglobin level of 7.3 g/dl, leukocyte count of 6900/cu mm with 34% myeloblasts, macrocytic erythrocyte indices, and a mean corpuscular volume of 127 fl.

She was reexamined at the Mayo Clinic 1 mo later, at which time she had bilateral cataracts, a hyperpigmented postinflammatory scar of the left calf, and a grade 2 (on the basis of 1–6) systolic ejection murmur but no hepatosplenomegaly or bone tenderness. She had a hemoglobin level of 7.3 g/dl, erythrocyte count of 1,730,000/cu mm, mean corpuscular volume of 130 fl, leukocyte count of 48,000/cu mm with 86% myeloblasts, and platelet count of 213,000/cu mm. A peripheral blood smear showed oval macrocytes and hypochromic microcytes. The leukocyte alkaline phosphatase score was 0, and serum iron was 233 μg/dl with 100% saturation of total iron-binding capacity. Serum glutamic-oxalacetic transaminase and uric acid levels were mildly increased. The bone marrow was markedly hypercellular with decreased erythropoiesis, 50%–69% myeloblasts, and rare ringed sideroblasts. Cytogenetic studies revealed the presence of an abnormal clone of cells (see Cytogenetic Results). The diagnosis was IASA that had evolved to acute meylomonocytic leukemia.

CYTOGENETIC METHODS

About 1 ml of bone marrow from each of these three patients was processed for chromosome analysis by a modified method of Lampley and Po-Tang. The specimen was collected in a 20-ml saline solution containing 0.1 ml (0.5 μg/ml) of demecolcine and 0.2 ml (0.5 μg/ml) of actinomycin D. After 1 hr of incubation at 37°C, the specimens were harvested with hypotonic potassium chloride (0.075 M) and cold methanol-glacial acetic acid (3:1) fixative. Microscope slides were prepared from about 75% of each specimen; the remainder was stored at 4°C in fixative. The slides were air-dried for 24 hr and analyzed by Giemsa nonbanding and GTG-banding with trypsin. One patient (case 2) was also studied by C-banding with sodium hydrosixide.

In two patients (cases 2 and 3), cytogenetic studies were also done on peripheral blood lymphocytes grown in Difco chromosome medium with phytohemagglutinin for 72 hr. These cultures were harvested with demecolcine (0.5 μg/ml) for 1 hr. hypotonic potassium chloride (0.075 M), and cold methanol-glacial acetic acid (3:1) fixative. The slides were air-dried and analyzed by GTG-banding with trypsin.

CYTOGENETIC RESULTS

Case 1: 47,X,2idic(X)(q13)

Thirty-three metaphases were analyzed from a bone marrow specimen: 24 nonbanded and 9 GTG-banded. The initial study was done with use of a nonbanding method. Among 24 nonbanded metaphases, 20 had 47 chromosomes and 1 each had 44, 45, 46, and 78 chromosomes. Later we studied the refrigerated reserve specimen using GTG-banding to establish the nature of the extra chromosome. We located 9 metaphases; 8 contained 2 structurally abnormal chromosomes that had the band pattern and morphological features of an isodicentric X [idic(X)] chromosome.

ABNORMAL X CHROMOSOMES
The break and fusion point appeared to be at Xq13. The ninth metaphase had 48 chromosomes and included 3 idic(X) chromosomes. We reevaluated the nonbanded metaphases and noted the presence of the dicentric chromosomes in the cells with 47 chromosomes; thus, the bone marrow metaphases were predominantly 47,X,2idic(X)(q13). A representative GTG-banded karyotype and the two idic(X) chromosomes from 3 different metaphases are shown in Fig. 1.

The bone marrow specimen was insufficient for adequate C-banding, but each abnormal chromosome had two definite primary constrictions (Fig. 1). The single metaphase with three idic(X) chromosomes may be evidence that these morphologically dicentric chromosomes have two functional centromeres and consequently on occasion encounter difficulty in cell division. Unfortunately, this patient died before other tissues could be studied.

**Case 2: 46,X,idic(X)(q13) and 47,X,2idic(X)(q13) Clones**

Twenty-six GTG-banded metaphases were analyzed from a bone marrow specimen: 6 had the karyotype 46,X,idic(X)(q13) and 20 were 47,X,2idic(X)(q13). A representative karyotype from the cell line with 47 chromosomes and the 2 idic(X) chromosomes from 3 metaphases are shown in Fig. 2. In Fig. 2, one idic(X) chromosome is broken in the intercentromeric region, perhaps from maldvision of an anaphase bridge. In 10 C-banded metaphases, there were two distinct C-bands, each associated with a primary constriction on each of the idic(X) chromosomes (Fig. 3). Chromosome analysis on 30 GTG-banded metaphases from a peripheral blood lymphocyte culture showed only a normal 46,XX karyotype. Thus, most likely this abnormal cell line is not constitutional but rather is related to the hematologic disorder.

**Case 3: 46,X,t(X;11)(q13;p15)**

Only 10 suitable GTG-banded metaphases were observed from the bone marrow specimen, but each had a translocation involving an X and an 11 chromosome (Fig. 4). The breakpoint on the X chromosome appeared to be Xq13, the same site as in cases 1 and 2. The breakpoint on chromosome 11 was at 11p15. The t(X;11) chromosomes and their structurally normal homologues from two metaphases are shown in Fig. 4. Cytogenetic analysis on 30 GTG-banded metaphases
Fig. 3. C-banded partial metaphases from patient 2, showing two blocks of centromeric constitutive heterochromatin on the two isodicentric chromosomes (arrows).

from a peripheral blood specimen showed a normal 46,XX karyotype. Thus, the abnormal cell line is most likely associated only with the hematologic disorder.

DISCUSSION

Cytogenetic

Among the few reports of structural abnormalities of X chromosomes in neoplastic disorders, band Xq13 is the most frequently cited breakpoint. Band Xq13 may contain a gene or have a certain structure that predisposes it to breakage in the presence of some carcinogen, or perhaps a cell with alteration in band Xq13 has more of a selective advantage than other breakpoints.

Two of our patients and one of Philip et al. not only had Xq13 breakpoints but also very likely had the same kind of structural abnormality. Our patients 1 and 2 had idic(X) chromosomes. The case of idic(X;X) reported by Philip et al. was probably not a translocation dicentric chromosome, as is implied, but rather also an isodicentric chromosome. In the literature, translocation dicentric and isodicentric chromosomes have often been confused.

The confusion derives from the fact that the band pattern is similar on both arms of an idic(X) chromosome, analogous to the band pattern of an X-X translocation if the break and fusion points are identical on both homologues. The observation of a normal X chromosome and a dicentric X in the same metaphase would be good evidence that the abnormal X did not arise as a translocation. The karyotype of our patients 1 and 2 and the case of Philip et al. had a normal X in addition to an idic(X). Thus, the abnormal Xs most likely arose as isodicentric chromosomes (Fig. 5).

Quite likely, the ancestral karyotype of the abnormal cell lines in patients 1 and 2 was 46,X,idic(X). The cells with two idic(X) chromosomes probably arose from the abnormal segregation of a dicentric chromosome during mitosis in at least one cell with the ancestral karyotype. A single maldivision of an idic(X) chromosome could produce a cell with two idic(X) chromosomes and hence, through mitosis, a cell line. The biologic conditions may have favored the 47,X,2idic(X) cells over others. A few cells with the ancestral karyotype were noted in patient 2 but not in patient 1. The presence of two active centromeres may also explain the occasional breakage of the idic(X) chromosome in patient 1 (Fig. 1) and the metaphase with three idic(X) chromosomes in patient 2.

In patients 1 and 2, the predominant cell line contained two idic(X) chromosomes. Thus, most of the time the idic(X) chromosomes may be segregating normally in mitosis. An isodicentric chromosome can segregate normally if one centromere loses its ability to function or if the intercentromeric distance is small. The intercentromeric distance in patients 1 and 2 was relatively small, and in a small proportion of the metaphases there was evidence of centromere inactivation (Figs. 1, 2, and 3). A dicentric chromo-

Fig. 4. GTG-banded 46,X,t(X;11) (q13;p15) from patient 3 (left) and the structurally rearranged X and 11 chromosomes and their normal homologues from two metaphases (right).
Daughter
cells

Fig. 5. During the S phase, it is possible for the DNA to break at Xq13 and reunite in a manner that leads to fusion of sister chromatids. Then, normal separation of the centromere produces an idic(X). The idic(X), along with a normal X, segregates into only one daughter cell; the other daughter cell contains only the normal homologue. The acentric fragment is lost.

some with only one functional centromere is characterized by two C-bands, but only one is associated with a primary constriction. Whatever biologic forces give the predominant cell line a selective advantage may also contribute to the stability of the 47,X,2idic(X) cell lines by continually eliminating those cells with other karyotypes.

Clinical

Physical findings were sparse. Patient 1 had a sideroblastic anemia that was refractory to hematinsics and was thought to be an evolving preleukemia. Patient 2 had a slowly evolving myeloproliferative disorder with pancytopenia. There was evidence of a mild bleeding diathesis, as manifested by mild bruising. Patient 3 had a very short prodromal or preleukemia phase before frank ANLL developed. None of these patients had a history of exposure to carcinogens.

All three patients exhibited a sideroblastic anemia, which is a general term indicating an anemia characterized by iron overload, pathologic ringed sideroblasts, and hypochromic microcytic erythrocytes. Sideroblastic anemia may be hereditary or acquired, but the former is uncommon. An X-linked form is well characterized and, as expected, most frequently affects males. Because these three patients each had X chromosome abnormalities, we attempted to compare their conditions with X-linked sideroblastic anemia. Female heterozygotes with X-linked sideroblastic anemia may have two populations of mature erythrocytes: normal erythrocytes thought to derive from erythrocytic precursors with the normal X chromosome active, and hypochromic microcytic erythrocytes from precursor cells with the defective X chromosome. Each of our patients had two populations of red blood cells, oval macrocytes and hypochromic microcytes, but none had a normal erythrocyte population.

In acquired forms of sideroblastic anemia, if the cause is unknown the condition is called primary or idiopathic; if the cause is known it is called secondary. The most common causes of secondary sideroblastic anemia are deficiencies of folate, vitamin B12, or pyridoxine or intoxication with ethanol or heavy metals (such as lead or arsenic). None of these causes was present in our patients.

Approximately 10% of cases of IASA progress to ANLL, and many of these patients have clonal chromosomal abnormalities. IASA has been included among the dysmyelopoietic syndromes or preleukemic states. Patients with IASA may also exhibit defects in vitro cell cultures similar to those observed in other preleukemic states and in overt ANLL. Whether cytogenetic studies can be used to predict which patients with IASA have a true preleukemic disorder has not been established.

Cytogenetic and Clinical Correlations

We believe that the abnormal cytogenetic findings in the bone marrow from these three patients were associated with their hematologic disorders and were not constitutional. All three patients were elderly women who had previously enjoyed good health. Patient 2 had had three children, whereas the other two had had no pregnancies. Patient 3 had desired children and had had normal menstrual periods; the cause of her infertility was unknown. Patient 1 was single and apparently had had normal menses. We were unable to do extensive cytogenetic studies in patient 1, but peripheral blood lymphocytes were 46,XX in patients 2 and 3.

It is not known whether it is fortuitous that these three patients all had IASA or whether a definite association exists between IASA and abnormal X chromosomes with Xq13 breakpoints. The existence of an X-linked hereditary type of sideroblastic anemia indicates that a gene associated with this condition is located on the X chromosome. This gene may be located in or near Xq13, and certain modifications of this site may predispose to IASA. Unlike patients with X-linked sideroblastic anemia, patients with structurally abnormal X chromosomes acquire this anomaly sometime during their lifetime. As a consequence, only a small portion of the cells of such patients possess the abnormal chromosomes. In these cases, the degree of sideroblastic anemia may depend on the proportion of bone marrow cells represented by the clone with an abnormal X chromosome and may account for the variability in frequency of ringed sideroblasts in our three patients.
Philip et al.\(^3\) did not specify whether their two patients with X chromosome abnormalities had a history of IASA, only that they had ANLL. Thus, we assume that no one has previously noted an association between IASA and abnormal X chromosomes. There are cytogenetic reports involving sideroblastic anemia in which structurally abnormal X chromosomes have not been mentioned.\(^22\) Thus, if an association exists between IASA and abnormal X chromosomes, it may involve only a small proportion of patients with sideroblastic anemia. During 1979 and 1980, 19 Mayo Clinic patients with IASA were referred for chromosome analysis; 2 of the cases in this report were among them. We believe our sample size is too small, however, to serve as either proof of such an association or an indication of the actual incidence. Thus, we must await further confirmation from other laboratories. If such an association exists, this will provide an important clue to the cause of a certain type of IASA. Furthermore, this group of patients may have a poor prognosis—two of our patients were thought to have preleukemia and one had acute leukemia. Thus, we believe it would be worthwhile for all patients with sideroblastic anemia to undergo cytogenetic analysis.

REFERENCES

Three patients with structurally abnormal X chromosomes, each with Xq13 breakpoints and a history of idiopathic acquired sideroblastic anemia

GW Dewald, RV Pierre and RL Phyliky