Acute Megakaryoblastic Leukemia in Early Childhood

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Two girls, each less than 2 yr of age, developed acute megakaryoblastic leukemia (malignant myelosclerosis). Both presented with anemia, severe thrombocytopenia, and a low percentage of blasts in their peripheral blood. Their marrow showed marked reticulin fibrosis with an increase in blasts and immature megakaryocytes. The blasts stained negatively for myeloperoxidase and Sudan Black B, but showed acid phosphatase (ACP) and α-naphthyl acetate esterase (ANAE) activity inhabitable by sodium fluoride. They were identified as megakaryoblasts by the platelet peroxidase reaction. Cytogenetic studies showed multiple chromosomal abnormalities in both cases. Chemotherapy with vincristine, prednisone, and L-asparaginase was without effect, while daunorubicin and cytosine arabinoside induced a complete remission in one case. The second case responded to a combination of cytosine arabinoside, daunorubicin, and 6-thioguanine. This article documents that acute megakaryoblastic leukemia occurs in early childhood and describes its clinical, pathologic, and cytogenetic features. Previous reports of childhood “myelofibrosis” are reviewed, and their possible relationship with acute megakaryoblastic leukemia is discussed.

THE TERM “malignant myelosclerosis” was used by Lewis and Szur to describe five patients who presented with pancytopenia and marrow fibrosis and died within a year from admission to the hospital. Their peripheral blood showed only mild red cell morphological abnormalities and a variable, but small, percentage of blasts. Spleen and lymph nodes were not palpable clinically. Their marrow showed a pronounced increase in reticulin fibers, mononuclear cells, and prominent giant cells resembling megakaryocytes. Bearman et al. reviewed the literature on this disorder and considered it a distinct clinicopathologic entity, provided strict clinical and pathologic criteria are used for diagnosis. Recently, Bain and coworkers reported four cases in which the blasts were characterized as megakaryoblasts using the platelet peroxidase reaction. They postulated that most cases of malignant myelosclerosis (acute myelofibrosis) are in fact cases of acute megakaryoblastic leukemia and that the myelofibrosis is probably secondary to the megakaryoblastic proliferation.

Myelofibrosis is rare in early childhood, and most reported cases have a rapidly fatal course. Few cases are reported in sufficient detail to assess the clinical and pathologic features of malignant myelosclerosis, aside from short survival. In none of the reported cases were the blasts studied by the platelet peroxidase reaction. We report two children, less than 2 yr of age, who had the typical clinical and morphologic features of malignant myelosclerosis. Platelet peroxidase activity was clearly demonstrated in their circulating blasts. This indicates that at least some cases of early childhood myelofibrosis represent acute megakaryoblastic leukemia.

CASE HISTORY

Case 1

The patient presented at the age of 15 mo with a 3-mo history of easy bruising, which worsened 2 wk before admission. She had no other symptoms. There was no family history of malignancy or bleeding problems.

Physical examination showed a well developed and well nourished afebrile baby. She had a few petechiae and small ecchymoses on the trunk and extremities. No lymphadenopathy or hepatosplenomegaly were noticed. Her physical examination was otherwise normal. The routine serum chemistry profile, chest x-ray, intravenous pyelogram, and skeletal survey were all normal.

Results of her hematologic investigation were as follows: hemoglobin (Hb), 9.4 g/dl; platelets, 13,000/cu mm; white blood cells (WBC), 14,000/cu mm with 54% lymphocytes, 3% monocytes, 13% segmented neutrophils, 5% bands, 1% metamyelocytes, 3% myelocytes, 2% promyelocytes, and 19% blasts. Red cells showed mild anisopoikilocytosis. Most of the blasts (Fig. 1) were between 10 and 14 μ in diameter. Their nuclei were round or oval, with uniform finely stippled chromatin. Nucleoli were not prominent. The cells had only a narrow rim of basophilic cytoplasm, with some showing protrusions or blebs. Cytoplasmic granules were not seen. An adequate bone marrow aspirate could not be obtained, even after repeated attempts at different sites. The trephine biopsy (Figs. 2 and 3) showed a cellular marrow of variable composition. In some areas, the myeloid and erythroid series were well preserved with only scattered or small clusters of blasts. Other areas contained lymphocytes and blasts mixed with many immature megakaryocytes. Mature megakaryocytes were markedly decreased. There was moderate to marked reticulin fibrosis.

The blasts were negative for myeloperoxidase activity by light microscopy. Many of the cells showed moderately strong alpha-naphthyl acetate esterase (ANAE) and acid phosphatase (ACP)
The blasts generally have round nuclei with a stippled chromatin pattern and inconspicuous nucleoli. Some of them have cytoplasmic blebs and small excrescences (Wright's stain, ×1,000).

Fig. 2. A trephine biopsy section showing a cluster of blasts (arrow), scattered lymphocytes, myeloid and erythroid elements [Hematoxylin and eosin (H and E), ×1,000].

Fig. 3. Other areas of the biopsy showing proliferation of atypical early megakaryocytic cells with large irregular nuclei and abundant eosinophilic cytoplasm (arrows) (H and E, ×1,000).

activity. Alpha-naphthyl butyrate esterase (ANBE) was not demonstrated, while ANAE activity was completely inhibited by sodium fluoride. Some blasts had finely granular periodic acid-Schiff (PAS) positivity. They were negative for terminal deoxynucleotidyl transferase.

Electron microscopy (Fig. 4) showed undifferentiated blasts. Demarcation membranes and specific granules could not be identified. Using ultrastructural cytochemistry, platelet peroxidase activity (Fig. 5) could be demonstrated along the nuclear envelope and in strands of endoplasmic reticulum. This was completely inhibited by prior fixation with glutaraldehyde. No ultrastructural myeloperoxidase activity was demonstrated in any of the blasts, but the few myelocytes present in the preparation showed strong activity.

Cytogenetic studies showed a modal number of 50 chromosomes in 75% of metaphases examined, and exhibited a consistent spectrum of abnormalities: 50XX, +2, +8, −1, +1q−, +1p−, +1p− (Fig. 6). In addition, several metaphases demonstrated the loss of one X-chromosome.

The patient was treated with prednisone (60 mg/sq m) and vincristine (1.5 mg/sq m) weekly. Bone marrow aspiration was attempted after 2 wk and 4 wk of treatment. Marrow remained difficult to obtain, and most of the cells aspirated were blasts. L-Asparaginase was given for 10 days without any improvement.

Seven weeks after the initial visit, the patient was readmitted and received daunomycin 23 mg i.v. for 3 days and cytosine arabinoside
Fig. 4. Electron micrograph showing a primitive cell with no morphological evidence of differentiation (uranyl acetate and lead citrate, x10,200).

Fig. 5. Platelet peroxidase activity can be seen along the nuclear envelope and in strands of rough endoplasmic reticulum. The reaction product is also seen in mitochondria due to the presence of cytochrome oxidase (diaminobenzidine, x10,200).

Fig. 6. Cytogenetic study of case 1 showing 50XX, +2, +8, −1, +1q−, and +1p− karyotype.
50 mg daily by continuous intravenous infusion over 18 hr for 7 days. Her blood counts improved gradually, and 25 days after the induction therapy with daunomycin and cytosine arabinoside, she had a WBC of 40,000/cu mm with 80%-85% neutrophils and a platelet count of 400,000-600,000/cu mm. The last bone marrow aspirate done 5 wk after induction showed that she was in complete remission. A moderate degree of reticulin fibrosis was still present. She is at present on maintenance treatment with subcutaneous cytosine arabinoside at 100 mg/sq m and 6-thioguanine at 200 mg/sq m every 12 hr for 10 doses, alternating every 4 wk with cytosine arabinoside, given as before, together with intravenous cyclophosphamide at 1,000 mg/sq m on day 1. She is still in remission 12 mo after her induction therapy.

Case 2

An 18-mo-old white female was admitted with a 1-mo history of spiking fever and petechial skin hemorrhages. She was a well developed, well nourished child with a fever of 101°F. There were a few bruises and petechiae. Physical examination was otherwise unremarkable. No lymphadenopathy or hepatosplenomegaly was noted. Hematologic examination showed a hemoglobin of 7.2 g/dl, platelet count of 8,000/cu mm, and white blood count of 5,100/cu mm with 28% neutrophils, 71% lymphocytes, and 1% eosinophils. Occasional blasts were noted, and their morphology was similar to that seen in case 1. Bone marrow aspiration at multiple sites was unsatisfactory, but there was an increase in blasts. The blasts were myeloperoxidase and Sudan Black B negative, but showed strong ACP and fluoride-sensitive ANAE activity. A few of the blasts were also weakly positive for ANBE activity. Ultrastructural cytochemistry again demonstrated the presence of primitive blasts, which were positive for platelet peroxidase activity. The posterior iliac crest trephine biopsy (Fig. 7) showed a slightly hypocellular marrow, with prominent reticulin fibrosis. There was a marked lymphohistiocytic infiltration with abundant mature and immature megakaryocytes. Both the myeloid and erythroid series were hypoplastic and showed a left shift in maturation. Focal clusters of blasts were seen. Cytogenetic studies (Fig. 8) showed one or more chromosomal abnormalities in 78% of the metaphases with cells containing 49, 51, 52, or 56 chromosomes. A translocation between chromosomes 11 and 22 was present in all abnormal metaphases. Trisomy and quadrasomy 21 were the next most frequent finding (72%), followed by trisomies 19 and 12 (67%). Some of the cells had one or more of the following additional chromosomes: 6, 7, and 18.

She was treated with daunomycin, 14 mg each day for 3 days, cytosine arabinoside, 45 mg/day for 7 days, and 6-thioguanine, 40 mg daily for 7 days. She is in complete remission 10 mo after the induction therapy.

MATERIALS AND METHODS

Isolation of Peripheral Blood Mononuclear Cells

Peripheral blood and marrow mononuclear cells were isolated by Ficoll-Hypaque gradient separation. Cells at the interface of the gradient were washed 3 times in Hanks’ balanced salt solution (HBSS) before ultrastructural cytochemical studies.

Blood Smears and Cytochemical Studies

Blood films were stained with Wright’s stain. Cytochemical reactions for alpha-naphthyl acetate esterase (ANAE) with and without fluoride inhibition, alpha-naphthyl butyrate esterase (ANBE), acid phosphatase (ACP), myeloperoxidase, and the periodic acid-Schiff (PAS) reaction were performed.

Ultrastructural Cytochemistry and Electron Microscopy

For the demonstration of platelet peroxidase (PPO) activity, mononuclear cells prepared as described above were incubated unfixed with a reaction mixture containing 20 mg dianisobenzidine tetrahydrochloride (DAB) in 10 ml of 0.5 M Tris-Ringer buffer containing 10 μl of 3% hydrogen peroxide at pH 7.3. After an hour of incubation at room temperature, the cells were washed 3 times in Tris-Ringer buffer and then fixed in 1.25% glutaraldehyde in Millonig’s buffer. The cells were then embedded in 3.0% agar, cut into small cubes, postfixed in 1.25% osmium tetroxide, and embedded in Maraglas for transmission electron microscopy without prior staining. Myeloperoxidase (MPO) activity was demonstrated at an ultrastructural level by fixing the cells in 1.25% glutaraldehyde in Millonig’s buffer for 1 hr, with subsequent incubation in DAB solution and processing as described for the PPO reaction. Cells for standard transmission electron microscopic study were fixed in glutaraldehyde and processed without incubation with the DAB reaction mixture. Thin sections were stained with uranyl acetate and lead citrate.

Determination of Terminal Deoxynucleotidyl Transferase (TdT)

Cytocentrifuge preparations of peripheral blood mononuclear cells were stained for the presence of TdT using an indirect immunofluorescence method (Bethesda Research Lab., Rockville, MD).

Cytogenetic Studies

Cytogenetic evaluation was performed on both peripheral blood and bone marrow specimens, with each being subjected to short-
term (1-4 hr) and long-term (24-48 hr) culture without phytohemagglutinin (PHA). Colcemid (10 μg/ml) was added during the final hour of culture, followed by treatment with 0.075 M potassium chloride solution, and final fixing in 3:1 methanol/acetic acid fixative. Metaphase chromosomes were banded according to the method of Sun et al.22 Representative metaphases were then karyotyped in accordance with the Paris Conference on human chromosomes.23

DISCUSSION

Our two patients demonstrated most of the features typical of malignant myelosclerosis.1,2 They presented with anemia, thrombocytopenia, and a low or marginally normal neutrophil count. There was a small percentage of blasts in the peripheral blood, and the red blood cells showed a mild to moderate degree of anisocytosis and poikilocytosis. No hepatosplenomegaly was detected. The marrow was only partially infiltrated with blasts. In both cases, there were well preserved myeloid and erythroid series in many areas, with a left shift in myeloid maturation. Mature megakaryocytes were rare in case 1, but more abundant in case 2. Both cases, however, showed a marked increase in immature megakaryocytes. Reticulin fibrosis was prominent.

Most cases of malignant myelosclerosis have been reported in adults or older children.1,3,24-27 The blasts in this condition have been considered by some authors to be of myeloid origin1,25 on light microscopic examination. Recently, after a detailed study of this condition, Bain and den Ottolander1,24 suggested that these cells are megakaryoblasts and that acute myelofibrosis represents a leukemic proliferation of the megakaryocytic series. In our cases, the light microscopic morphology and cytochemistry of the blasts were similar to their findings.1,24 The presence of PPO was demonstrated in the blasts using ultrastructural cytochemistry confirming their megakaryoblastic origin.

Primary childhood myelofibrosis is very rare. Almost all reported cases have survived less than a year from presentation, unlike the more chronic course of adult myelofibrosis with myeloid metaplasia. These cases are summarized in Table 1 and probably represent a number of different clinicopathologic entities. Only the second case reported by Evans12 and those by Hamazaki13 and Hillman9 have features of acute megakaryoblastic leukemia. Cytochemical studies, however, were not performed and the origin of the blasts remains uncertain. Recently, Pui et al.29
described a 3-yr-old boy with clinical and pathologic features typical of malignant myelosclerosis. The blasts expressed an antigen that reacted with a rabbit anti-rat platelet antiserum and were considered to be megakaryoblasts. Our cases and the case reported by Pui et al. demonstrate clearly that malignant myelosclerosis can occur in early childhood and accounts for at least a portion of the cases of so-called "childhood myelofibrosis."

Multiple chromosomal abnormalities were detected in our patients. The karyotype of case 1 is fully consistent with the chromosomal abnormalities described in acute nonlymphocytic leukemias. In particular, trisomy 8 is the most frequently occurring chromosomal addition, with trisomy 2 and deletion of an X-chromosome recorded on a consistent basis.\(^{10-12}\) Abnormalities of chromosome 1 are often reported in adults with myelofibrosis with myeloid metaplasia and in polycythemia vera.\(^{13-15}\) Both of these disorders are characterized at some stage by abnormal megakaryocytic proliferation and reticulin fibrosis. Case 2 showed a translocation of part of the long arm of chromosome 22 to chromosome 11 in all abnormal metaphases. In addition, one or more extra no. 21s were observed in 72% of the metaphases. Abnormalities in chromosome 21 may have a more than random relationship to acute megakaryoblastic leukemia. Two of the three patients with features suggestive of acute megakaryoblastic leukemia\(^{9,12}\) had Down's syndrome, while Pui's case\(^{39}\) had a ring 21 chromosome. Trisomy 21 may predispose patients not only to acute lymphoblastic and myeloid leukemia, but also to other hematopoietic disorders, including acute megakaryoblastic leukemia.

Most of the childhood cases of myelofibrosis were treated with supportive therapy. The case reported by Marino\(^{36}\) was treated with vincristine, prednisone, and later doxorubicin without response. Norden\(^{37}\) reported a case with good response using a chemotherapeutic protocol for acute lymphocytic leukemia (ALL) (protocol ALB 7611). It is not clear whether his case represents ALL with fibrosis or megakaryoblastic leukemia. All adult cases treated with single drugs including steroid, 6-mercaptopurine, vincristine, or combination chemotherapy, such as prednisone and vincristine with or without the addition of cyclophosphamide or cytosine arabinoside, failed to respond.\(^{1,3,4,24,25}\) Complete remissions, however, have been reported after treatment with a combination of cytosine arabinoside and daunorubicin or Adriamycin.\(^{3,29}\) We have also found this combination to be effective, and it may be the treatment of choice. Recently, intensive chemotherapy with irradiation followed by bone marrow transplantation has been used for treating patients with acute myelofibrosis.\(^{27}\) One of the four patients was reported to be in complete remission 1 yr after transplantation, and the marrow fibrosis appeared to be reversible. Bone marrow transplantation may offer an alternative primary therapy or may be useful when chemotherapy fails if there is an HLA-matched sibling donor.

Acute megakaryoblastic leukemia is probably a better term than malignant myelosclerosis or acute myelofibrosis for cases similar to the two we describe. Despite the fact that the marrow is not completely replaced by blasts, as often seen in acute leukemias, the presence of marrow failure with peripheral cytope-
nias, an increased proportion of early hematopoietic precursors, proliferation of megakaryoblasts, and marked chromosomal abnormalities point toward a leukemic process. This concept also has important implications in the selection of therapy, as drug combinations effective for acute nonlymphocytic leukemias appear to be the treatment of choice.

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