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Megakaryoblastic termination of myeloproliferative disorders is rare. The morphology of megakaryoblastic transformation can be subtle and is often mistaken for myeloid or lymphoid proliferations. Previously reported observations suggest a relatively poor prognosis for this category of patients, making precise diagnosis imperative. A multifaceted approach using morphology, ultrastructure, cytochemistry, and immunological membrane analysis may be helpful. We present two cases of myeloproliferative disorder with aggressive megakaryoblastic phases (myelofibrosis with agnogenic myeloid metaplasia and chronic myeloid leukemia with blast crisis). The clinical course is described and the results of the morphological, cytochemical, ultrastructural, and cytogenetic studies of both cases are presented. In addition, immunochemical studies (flow cytometry) and platelet function studies (aggregation, beta-thromboglobulin, and platelet factor IV release) were done for one of these patients. (Henry Ford Hosp Med J 1992;40:122-6)

Acute leukemia with a predominant megakaryoblastic proliferation was first reported by Von Boros in 1931 and in North American scientific literature by McDonald in 1948 (1). Most of the early reported cases of megakaryoblastic proliferation were of patients who demonstrated obvious megakaryocytic differentiation. Less differentiated cases may have gone unrecognized. The advent of cytochemistry, electron microscopy, and immunochemistry (immunohistochemistry and flow cytometry) has led to precise identification of these conditions.

Neoplastic megakaryocytic/blastic proliferation may arise de novo, be secondary to myeloproliferative disorders and myelodysplastic syndromes, or may occur as complications of congenital chromosomal abnormalities (chromosome 21). We present two cases of myeloproliferative disorders, one of chronic myeloid leukemia (CML) and the other of primary myelofibrosis, with development of an accelerated phase comprised predominantly of megakaryoblasts and megakaryocytes.

Case Reports

Case 1

This 63-year-old male was known to have myelofibrosis for three years. At diagnosis, the spleen was palpable 10 cm below the left costal margin. Peripheral blood morphology revealed a leukoerythroblastic anemia which was associated with marrow fibrosis. Results of cytogenetic studies were normal. The patient was treated with oral testosterone, followed by nandrolone decanoate (Deca-Durabolin™) intramuscularly and oral prednisone, to which he responded partially. Two years later, marrow biopsy revealed increased fibrosis.

Three years after the diagnosis, there was a gradual increase in hepatosplenogmegaly associated with increasing transfusion-dependent anemia. A red cell survival scan demonstrated destruction and shortened half-life. The patient underwent a splenectomy without complications.

One month postsplenectomy, the patient developed pedal edema and massive hepatomegaly (20 cm below the costal margin). The hemoglobin was 89 g/L, WBC count 36×10^9/L, and platelet count 1.3×10^9/L. A bone marrow biopsy showed 89% blasts, some of which resembled megakaryoblasts (Figs 1-3). Megakaryocytic differentiation was confirmed by electron microscopy and membrane antigen phenotyping by flow cytometry and by cytochemistry (Table 1, also see Figs 4-6). Cytogenetic studies revealed an abnormality of the long arm of chromosome 17: 46,XY/46,XY,i(17q) (Fig 7). The patient was started on a daily dose of 100 mg of hydroxyurea (Hydrea™) which was later increased to 4 g. Buffy coat preparation with separation of leukocytes and platelets (leukapheresis and thrombocytopheresis) was performed. Interferon, 10 million units daily, was administered subcutaneously for two days only. The WBC and platelet counts decreased to 19×10^9/L and 0.6×10^9/L, respectively. Over the next two months the patient became progressively pancytopenic and eventually died of sepsis. No autopsy was performed.

Case 2

This 33-year-old male had CML which had been well controlled with intermittent busulfan (Myleran™) for approximately four years. Blast crisis was manifested by left upper quadrant pain, dyspnea, and fever. The spleen was strikingly enlarged and there was cervical and axillary lymphadenopathy. Hemoglobin was 7.2 g/L, WBC count 261×10^9/L, and platelet count 0.6×10^9/L. Most (90%) of the nucleated cells in the marrow and blood were blasts. The megakaryocyte differentiation was supported by morphology (light and ultrastructural) and cyto-

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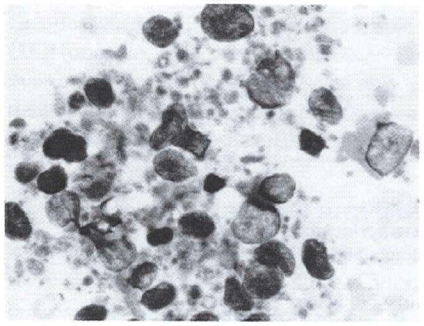

Fig 1—Numerous blasts with marked thrombocytosis (Leishman stain, X400).

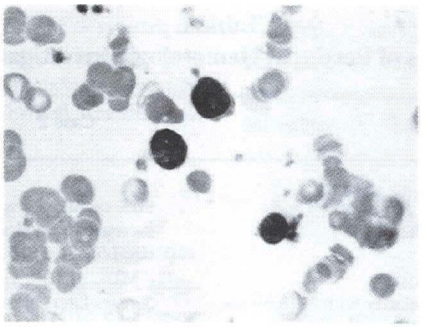

Fig 2—Platelets budding from blasts which otherwise resemble lymphoblasts (Leishman stain, X400).

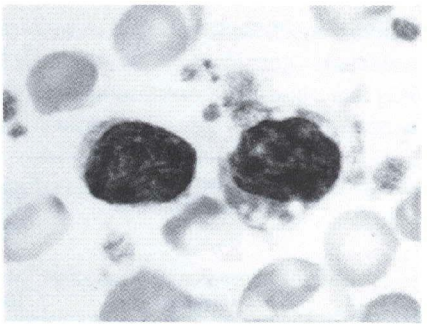

Fig 3—Megakaryoblasts with multiple nucleoli, vacuolated cytoplasm, and budding platelet forms (Leishman stain, X2000).

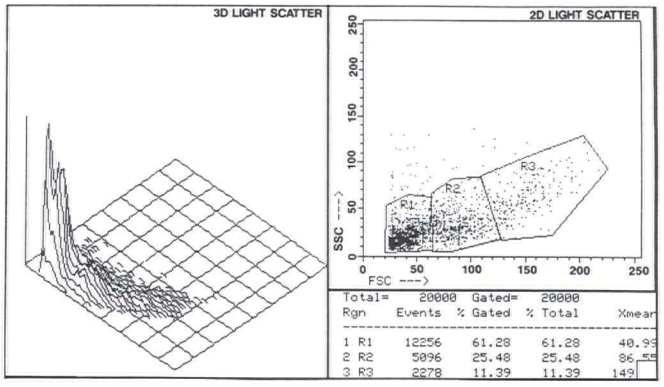

Fig 4—Light scatter of low density marrow mononuclear cells. Right: dot plot of forward (size) versus side light scatter (granularity). R1 = small "lymphoid"/RBCs; R2 = large "lymphoid"; R3 = blasts. Fractions of cells in each area are indicated in box below dot plot. Left: Three-dimensional plot of light scatter.

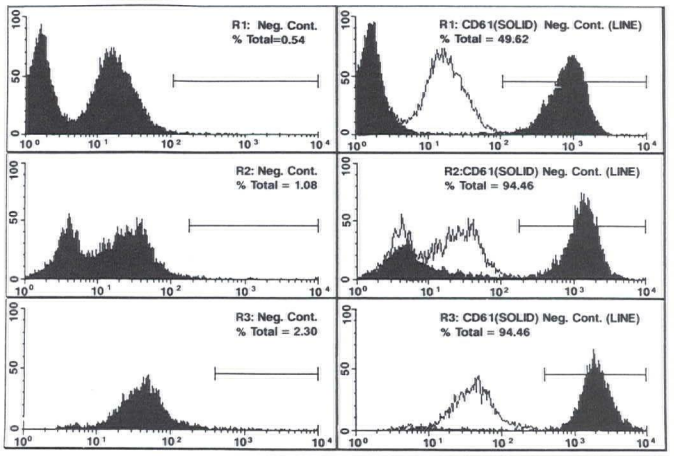

Fig 5—Expression of CD61/GPIIIa by marrow mononuclear cells. Left upper, middle, lower: Nonspecific fluorescence staining by FITC-conjugated negative control for the R1, R2, and R3 populations, respectively. Right upper, middle, lower: Fluorescence staining by FITC-conjugated anti-CD61 (solid histograms) as compared to nonspecific fluorescence staining by FITC-conjugated negative control (line histograms) for the R1, R2, and R3 populations, respectively.

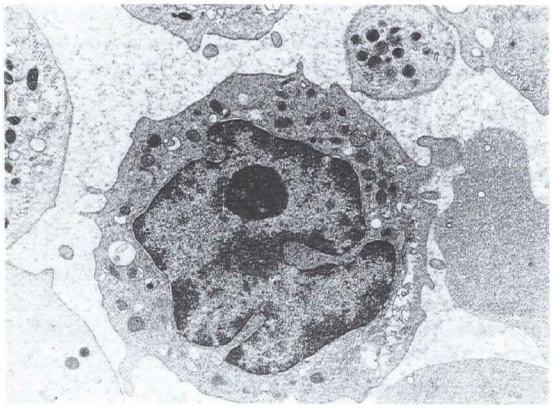

Fig 6—Electron microscopy of peripheral blood showing a megakaryoblast (X20000).

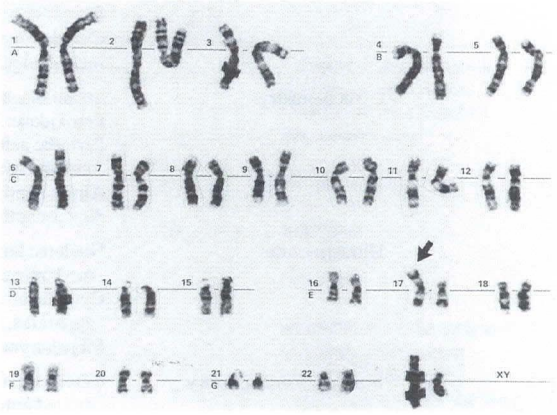

Fig 7—Cytogenetics 46XY/46XY, i(17q).

Table 1
Results of Pertinent Hematologic Investigations

	Case 1	Case 2
Bone Marrow:		
Initial presentation	Trilineage proliferation with 90% cellularity	90% cellularity with shift to left, increase in myelocytes, metamyelocytes, band forms and mature granulocytes
Impression	Myelofibrosis	CML
Postsplenectomy	100% cellularity with 89% blasts resembling megakaryoblasts (Figs 1-3)	5 years later: blast crisis with 90% blasts showing megakaryoblastic morphology
Cytochemistry:		
PAS	++	++
Specific esterase	+ (< 5%)	+ (few)
Myeloperoxidase	+ (< 5%)	+ (few)
Sudan black B	–	+ (few)
Membrane Analysis (Flow Cytometry) (Figs 4,5):		
CD61 (GP IIIa)	90%	Not done
CD14	0%	
CD33	< 1%	
CD3	2%	
CD13	10% (dim)	
CD19/20	< 1%	
TdT	Negative (immunocytochemistry)	
Electron Microscopy (Case 1 and Case 2): Large blasts with prominent nucleoli and cytoplasmic organelles similar to adjacent platelets. Morphology consistent with megakaryoblasts (Fig 6).		
Cytogenetics:		
Initial	(MF) Normal	(CML) Philadelphia chromosome t(9;22)(q34,q11)
In blast crisis	i(17q) (Fig 7)	No further abnormality developed
Platelet Function:		
Aggregation	ADP, epinephrine, arachidonic acid, ristocetin, and collagen were all defective	Not done
BTG	125 IU/mL (normal 10-40 IU/mL)	
PD4	4-15 IU (normal 0-5 IU)	

CML = chronic myeloid leukemia, MF = myelofibrosis.

Table 2
Identification of Megakaryoblasts

Morphology:	Undifferentiated blasts (lymphoid or myeloid morphology) Blasts with cytoplasmic budding Circulating micromegakaryocytes Increased platelets, large platelets with dysplastic forms
Cytochemistry:	Sudan black B: Negative Peroxidase: Negative Periodic acid-Schiff: Localized, frequently positive (nonspecific) Acid phosphatase: Localized, frequently positive (nonspecific) Alpha-napthyl acetate esterase: Positive Alpha-napthyl butyrate esterase: Negative
Ultrastructure:	Nucleus: large, prominent nucleolus, irregular nuclear membrane, moderate chromatin condensation Cytoplasm: mitochondria, rough endoplasmic reticulum, small Golgi apparatus, few granules. Platelet peroxidase reaction positive (granules) Megakaryocytes: Alpha-granules, demarcation membranes
Immunohistochemistry:	CD42 (Ib), CD41 (IIb), CD61 (IIIa) (surface glycoproteins), factor VIII related antigen: Positive Lymphoid antigen negative (TdT, CD2,5,7,19,20) Monocyte antigen negative (CD13,14)

chemical evidence. There was diffuse marrow fibrosis. Six courses of OAP (oncovin, ara-C, and prednisone) with supplemental hydroxyurea over a four-month period produced only transient responses. The patient eventually died of sepsis. No autopsy was performed. The diagnosis was megakaryoblastic crisis of CML.

Material and Methods

Fresh peripheral blood and bone marrow smears and imprints were stained by Leishman stain. Cytochemical stains also were performed: peroxidase, periodic acid-Schiff, specific esterase, and Sudan black B. Reticulin stain was done on biopsy specimens (case 1).

Antigen phenotyping was performed by flow cytometry and immunocytochemistry on peripheral blood in case 1. Monoclonal antibody reagents were obtained from Coulter (CD33, CD13), Becton Dickinson (CD14,19,20), Dako (GPIIIa), and our institution's Molecular Genetics department (TdT). Membrane immunofluorescence utilized both direct and indirect staining techniques. Positive/negative fluorescence gating was determined using fluorochrome and isotype matched monoclonal negative controls. Immunocytochemistry utilized a standard avidin biotin technique on acetone-fixed cytopreparations.

Cells from peripheral blood were fixed in 30% cacodylate-buffered glutaraldehyde, pH 7.4. They were postfixed in 1% osmic acid and embedded in Araldite, stained with uranilacetate and lead citrate, and viewed with a transmission electron microscope (Zeiss model 109).

Bone marrow aspirate and peripheral lymphocytes were cultured for 24 hours in medium RPMI. Cells were arrested in metaphase by Colcemid (D-acetyl methyl cholchicine), and after hypotonic treatment in 0.075 M KCL the material was fixed in 3:1 methanol:acetic acid. Metaphase cells were GTG banded by standard procedures.

Platelet function tests done in case 1 included aggregation in response to adenosine diphosphate, epinephrine, arachidonic acid, ristocetin, and collagen. Beta-thromboglobulin and platelet factor IV levels were determined using enzyme immunoassay techniques.

The results are summarized in Table 1.

Discussion

Megakaryoblastic transformation of myeloproliferative disorders is rare. It can occur in CML (2-8), myelofibrosis (2,5-7,9), and essential thrombocythemia (10), thus reemphasizing the stem cell defect present in these conditions.

The morphology of megakaryoblastic transformation may be subtle and often mistaken for myeloid or lymphoid morphology (1,4,7,11-13). Routine Romanowsky stains usually reveal undifferentiated blasts with occasional clues towards megakaryocytic differentiation, such as blasts with cytoplasmic budding, micromegakaryocytes, or large platelets with dysplastic forms (6,7,9,13). Routine cytochemistry, when done, can be helpful but not diagnostic (13). The periodic acid-Schiff and acid phosphatase reactions are frequently positive with a localized reaction. Alpha-naphthyl acetate esterase may be positive but the reaction is inhibited by the addition of fluoride.

The megakaryocytic differentiation of the blasts can be confirmed by electron microscopy (3,4,10-13) and/or immunohistochemistry (1,9,12). Electron microscopy reveals a large nucleus, with a single large nucleolus or multiple nucleoli, a slightly irregular outline, and moderate chromatin condensation (12). Cytoplasm shows numerous mitochondria, large profiles of rough endoplasmic reticulum, a small Golgi apparatus, and a few granules. Circulating micromegakaryocytes will reveal alpha-granules and/or demarcation membranes. However, the

Table 3
Megakaryoblastic Transformation of Myelofibrosis

Study (Reference)	Age/Sex	Primary Diagnosis	Splenectomy	Development of Blast Crisis Postsplenectomy (months)	Survival Post Blast Crisis (months)	Chromosomal Abnormality
Efrati et al (5) (Case 1)	45/F	MF	Yes	24	1	Trisomy 9 and balanced translocation between chromosomes 12 and 13
Egner et al (6) (Case 1)	61/M	MF	Yes	34	6	No abnormality
Jacobs et al (7) (Case 1)	59/M	MF	Yes	28	31	No abnormality
Jacobs et al (7) (Case 2)	60/F	MF	Yes	12	18	Not done
Marcus et al (9) (Case 1)	36/M	MF	Yes	3 (approximately)	12	Not done
Akahoshi et al (2) (Case 2)	60/F	MF	Not done		3	Duplication of the long arm of 1 and translocation involving band 3q26
Present Study (Case 1)	63/M	MF	Yes	1	3	Isochromosome for the long arm of 17

MF = myelofibrosis.

most reliable confirmation of the megakaryocytic nature is demonstration of platelet peroxidase activity of the granules (4,11-14).

Recently, antigenic analysis by immunochemical procedures has received much attention (1,9,12,13). The expression of platelet surface glycoproteins Ib (CD42), IIb (CD41), IIIa (CD61) (11), and factor VIII-related antigens in the absence of other lymphoid TdT, CD2/7, and myelomonocytic (CD14/13) antigens strongly support the diagnosis of a megakaryocytic proliferation (15). Thus a multifaceted approach using morphology, cytochemistry, ultrastructure, and immunochemistry may be necessary for precise identification (Table 2).

Platelet functions done as in case 1 to characterize their nature in a megakaryoblastic proliferation showed defective aggregation to various stimuli. In addition, BTG and PF4 levels were elevated. These have been correlated with increased turnover with myeloproliferative disorder (16,17).

There is limited cytogenetic data on megakaryoblastic blast crisis. Most cases with CML as a primary diagnosis had the same changes which are characteristic of typical blast crisis, i.e., +8, second Philadelphia chromosome (3,5,8). Cases with myelofibrosis as a primary diagnosis had various abnormalities such as trisomy 9, balanced translocation between chromosomes 12 and 13 (5), and rearrangements involving chromosome band 3q26 (2). Abnormalities of 3q26 have been associated with dysmegakaryocytopoiesis (18).

Cytogenetic studies in case 1 showed progression from normal (at time of diagnosis of myelofibrosis) to that showing an isochromosome for the long arm of 17:(17q). This is one of the characteristic changes of blast crisis of CML and has been reported occasionally in patients with myelofibrosis. The observation of i(17q) as the sole, de novo abnormality is infrequent. Case 2 showed standard Philadelphia translocation t(9;22) (q34;q11). During blast crisis there were no other cytogenetic changes. About 25% of CML cases have Philadelphia translocation as the sole abnormality throughout their disease.

The prognosis of patients with megakaryoblastic transformation is uniformly poor. In a review of the literature, Williams and Weiss (8) observed that seven cases of reported CML with megakaryoblastic transformation were all fatal, with survival ranging from a few days to 15 months after the transformation. Reported cases of myelofibrosis with similar transformations were also all fatal, with survival ranging from less than one month to 31 months (Table 3). In patients with myelofibrosis, all except one case (Table 3) had undergone splenectomy which was followed by development of megakaryoblastic transformation from one month to 34 months postoperatively. Since splenectomy is often performed on patients with myelofibrosis, and the vast majority of them do not develop megakaryoblastic transformation, the relationship between splenectomy and megakaryoblastic transformation in these cases may be merely coincidental.

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