Chronic Myeloid Leukemia: Manifesting as Spontaneous Splenic Rupture and Terminating in Megakaryoblastic Transformation

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We report a case of chronic myeloid leukemia with spontaneous splenic rupture as the initial presenting feature; there was a successful surgical outcome, but this case terminated in megakaryoblastic transformation. Results are reported based on morphological, immunological, cytochemical, ultrastructural, immunocytochemical studies, and in vitro liquid culture studies. The megakaryocytic nature of the blast cells was identified through the demonstration of platelet peroxidase activity by ultrastructural cytochemistry and the presence of platelet and megakaryocyte-specific antigen using monoclonal antibody, as well as the anti-factor VIII antibody by immunocytochemical technique.

Key words: splenic rupture, megakaryoblast, micromegakaryocyte, platelet peroxidase, immunocytochemistry

INTRODUCTION

Spontaneous splenic rupture is exceedingly rare in chronic myeloid leukemia (CML) and occurs mostly during the course of treatment [1-4]. There were only two reported cases with spontaneous rupture of the spleen as the presenting feature of CML in the English literature [5,6]. In the acute transformation of CML, the vast majority of patients terminate in myeloblastic or lymphoblastic crisis [7-9]; only rarely does megakaryoblastic (MKB) transformation occur. We had the opportunity to observe one patient with Ph1-positive CML who initially presented with spontaneous splenic rupture and later terminated in MKB transformation. The remarkable feature of this report is the concurrence of both the unusual initial presentation and the unique terminal outcome of CML in a single patient.

CASE REPORT

A 36-year-old Chinese male was admitted to our hospital because of severe abdominal pain. He gave no history of trauma and had been well until the day prior to admission, when he was awakened in the midst of his sleep by the sudden onset of severe left upper abdominal pain. On the next day, the pain grew excruciatingly worse, prompting him to report our emergency room. On physical examination, he was pale and looked acutely ill with a pulse rate of 96/min, blood pressure of 130/90 mm Hg and body temperature of 38°C. Diffuse abdominal tenderness was present and a huge tender spleen was palpated in the left abdomen. Bowel sounds were hypoactive. Initial blood count showed the hemoglobin at 9.5 g/dl, the white blood cell count (WBC) at 160.9 × 10^9/liter with a differential of blasts 1.25%, promyelocytes 6.5%, myelocytes 4.25%, metamyelocytes 4.25%, band forms 15.25%, segmented 63%, eosinophils 0.75%, basophils 0.75%, and lymphocytes 4%. The platelet count was 865 × 10^9/liter. Bleeding time, prothrombin time, and activated partial thromboplastin time were all within normal limits. He was observed intensively under the assumption of CML with splenic infarction. Twenty-four hours later, peritoneal signs became more pronounced. The abdominal tapping easily withdrew uncoagulated blood. The apparent internal bleeding due to probable splenic rupture necessitated urgent surgical exploration. At the laparotomy, there was about 1,000 ml of blood in the peritoneal cavity. The spleen was markedly enlarged with a laceration of 4 by 1.5 cm in the capsule at the lower pole. The excised spleen weighed 1,500 gm. Light microscopy disclosed the red pulps to be dilated and filled with mature and immature myeloid cells. The splenic capsule was infiltrated with leukemic cells. There was no splenic infarction or perisplenitis.

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The immediate postoperative course was rather smooth. Bone marrow examination showed extreme hypercellularity with megakaryocytic and granulocytic hyperplasia; there was no increase of reticulin fibers. Leukocyte alkaline phosphatase score was 9 (normal 80–200). A chromosomal analysis revealed presence of Philadelphia chromosome. Following splenectomy, the platelet count rapidly increased up to $1,800 \times 10^9$/liter. Busulfan was initiated postoperatively and was discontinued 2 months later, when blood counts returned to normal. During the ensuing year, he remained free of symptoms and signs except for two episodes of marked thrombocytosis (platelet 1,150 and $1,460 \times 10^9$/liter respectively) with mild leukocytosis, which resolved by reinstitution of busulfan. Fourteen months after presentation, the hemoglobin was 12.9 g/dl, the platelet count was $513 \times 10^9$/liter with many giant dysplastic forms (Fig. 1A), and the nucleated cell count was $46.2 \times 10^9$/liter with 35% blasts. The blasts showed heterogeneity in cell size with the majority having a high nucleocytoplasmic ratio, round or oval nuclei, fine chromatin with or without nucleoli, agranular cytoplasm, and, frequently, cytoplasmic blebs (Fig. 1B). The rapid rise of blasts in the blood was associated with the appearance of 50% micromegakaryocytes (mMK) and nuclear fragments of megakaryocytes (MK) (small lymphocyte-like cells with cytoplasmic features of platelets or of apparently bare nuclei) (Fig. 1C); they often appeared in groups. A bone marrow aspiration showed absence of normal MK with the presence of many atypical small MK and numerous aggregates of MK fragments surrounded by groups of platelets. There was an increase of undifferentiated blasts (37%) having the same appearance as in the peripheral blood. Focal reticulin fibrosis was evident on biopsy section. The patient was then treated with cytarabine and 6-thioguanine, which afforded only transient partial response; the blasts and mMK rapidly repopulated shortly afterward. Subsequently, repeated bone marrow aspirations yielded dry taps, while sections from four consecutive trephine biopsy specimens showed hypercellularity with an increasing number of undifferentiated blasts within a dense reticulin network (Fig. 2). The number of blasts and the degree of reticulin fibrosis continued to increase as the disease advanced. Five months after blast crisis, the nucleated cell count was $57.1 \times 10^9$/liter with 53% blasts and 30% mMK or fragments thereof. The ultrastructural study of the peripheral blood was performed. The serum lactic dehydrogenase was 630 units/liter (normal 47–140 units/liter) with an isomorphic isoenzyme pattern. Seven months after blast transformation, the nucleated cells exceeded $100 \times 10^9$ with 65% blasts and 23% mMK. The immunocytochemical staining was carried out at that time. Despite the continuation of chemotherapy with cytarabine, 6-thioguanine, and doxorubicin, the patient’s condition subsequently deteriorated. Eleven months after the acute transformation, he developed massive gastrointestinal bleeding and died. Postmortem examination was not obtained.

**MATERIALS AND METHODS**

**Cytochemical Studies**

The following cytochemical staining on blood smears for myeloperoxidase, Sudan black B, periodic acid-Schiff (PAS), chloroacetate esterase (CAE), alpha naphthyl acetate esterase (ANAE) with and without sodium fluoride, alpha naphthyl butyrate esterase (ANBE), and acid phosphatase (AP) were performed by standard methods [10].

**Immunological Marker Studies**

Studies were carried out on samples of heparinized peripheral blood. Ficoll-Hypaque separated mononuclear
cells (MNC) were examined for surface immunoglobulin (SIg) (Dako), E-rosette formation, and intranuclear terminal deoxynucleotidyl transferase (TdT) by indirect immunofluorescence using rabbit anti-calf TdT (Bethesda Research Laboratories, MD). Indirect immunofluorescence with monoclonal antibodies was performed with OKIa, OKM1 (Ortho Diagnostic), CALLA (Becton Dickinson), and HP1-1D (specific for human platelet membrane glycoprotein IIb/IIIa, kindly provided by Dr. C.Y. Li and Dr. W.L. Nichols, Mayo Clinic, Rochester, MN). Immunocytochemical stain with HP1-1D was also performed by indirect immunoalkaline phosphatase staining on peripheral blood smears [11]. Immunohistochemical stain for factor VIII antigen using rabbit anti-human factor VIII (Dako) was performed on paraffin-embedded bone marrow biopsy sections [12].

Cytochemical Ultrastructural Study

Buffy coat cells of the peripheral blood were examined for platelet peroxidase (PPO) activity using three separate procedures as described by Breton-Gorius et al [13–15]. Briefly, aliquots of the cells were 1) incubated unfixed in diaminobenzidine (DAB) medium containing H2O2 and afterward fixed in glutaraldehyde, 2) fixed in a tannic acid-aldehyde mixture and then incubated in DAB medium with H2O2, and 3) fixed with 1.25% glutaraldehyde followed by incubation in DAB medium. After fixation, the cells were processed as for routine electron microscopy. Cells incubated in medium without DAB or H2O2 served as controls.

In Vitro Liquid Culture Studies

Suspension cultures were performed on two occasions, 10 × 10⁶ peripheral blood MNC were suspended in 25-cm² tissue culture flasks in 10 ml alpha medium containing 15% fetal calf serum (FCS). The flasks were incubated at 37°C in a humidified 5% CO₂-95% air atmosphere. The culture medium was removed at intervals of 4 days, and fresh medium containing FCS was added. The morphological findings of the culture cells at each feeding were examined with modified Romanowsky-stained cytocentrifuge preparations.

RESULTS

Cytochemistry

The blasts and mMK-appearing cells did not stain with peroxidase, Sudan black B, CAE, or ANBE. The leukemic cells were positive for PAS reaction with a diffuse granular pattern as well as for AP activity. They showed positive ANAE activity, which was partially inhibited by sodium fluoride.

Immune Marker Studies

The leukemic cells were negative for SIg and E-rosette formation. They were TdT negative and unreactive with monoclonal antibodies of CALLA, OKM1, and OKIa. Sixty-four percent of the leukemic cells were found to be positively stained with HP1-1D by indirect immunofluorescence method. Indirect immunoalkaline phosphatase stain with HP1-1D monoclonal antibody demonstrated positive reddish granular cytoplasmic staining in platelets and in the majority of leukemic cells, including blasts, mMK, and MK fragments (Fig. 3). Immunohistochemical technique using anti-factor VIII antibody revealed positive cytoplasmic staining in blast cells and mMK of the marrow sections.

Ultrastructural Cytochemistry

The majority of blasts displayed positive reaction for PPO which was observed in both the unfixed and tannic
Fig. 3. Indirect immunoalkaline phosphatase stain for platelet-specific surface antigen showing positive granular reaction in blast, micromegakaryocytes, and platelet. ×1,000.

acid-fixed preparations in the perinuclear space and endoplasmic reticulum (Figs. 4,5). The cytoplasmic granules were negative for PPO activity, but mitochondrial membranes were frequently positive owing to the presence of cytochrome enzymes. PPO reaction was absent when either DAB or H₂O₂ was omitted from the incubation medium.

Liquid Culture Studies

After 4 days in liquid culture, almost all of the cells were blast forms. The blast cells exhibited considerable variation in cell size with distinct nucleoli. The cytoplasm displayed agranularity, frequent vacuolation, and prominent pseudopods (Fig. 6). There were many polyploid cells showing several nuclei. Mitotic features were frequently observed. After 8 days in culture, cells showed no further differentiation. At day 12, most cultured cells disintegrated, but one or two MK could be seen (Fig. 6, inset), and a few macrophages appeared.

DISCUSSION

Spontaneous splenic rupture is a rare complication in hematologic malignancies. Of the 53 cases with splenic rupture reviewed by Bauer et al in 1981 [1], only six patients had CML, and most occurred after the leukemia had been diagnosed and treatment had commenced. There were only two cases with spontaneous splenic rupture as the initial manifestation of CML as seen in our patient [5,6]. Among those cases with CML, the splenic rupture had been the fatal event with one exception [1,2,5,6]. The correct preoperative diagnosis and prompt surgical intervention accounted for the successful outcome in the present case. Three mechanisms of spontaneous rupture of the spleen in leukemia have been suggested: 1) the mechanical effect of leukemic infiltration, especially if the capsule is invaded, 2) splenic infarction, and 3) defects in blood coagulation [2]. In our patient, pathological examination revealed no evidence of splenic infarction or of coagulation defect; thus leukemic infiltration of the spleen with capsular invasion appeared to be responsible for the rupture.

MKB transformation of CML was observed to be very rare, as shown by a recent review of Williams and Weiss in 1982 [16]. They found a total of less than 15 cases. In addition, in most of these cases the blast cells were not fully characterized. However, they did not note the reports by Huhn and Ascher [17] of one case and by Efrati et al [18] of another case. Recently, another two cases were reported by Jacobs et al [19] and Lingg et al [20], respectively, and Hanada et al added one child case [21].

We have documented ultrastructurally and immunocytochemically an additional case of MKB transformation of CML. His leukemic cells in blastic phase fulfilled the diagnostic criteria of acute leukemia of megakaryocyte lineage proposed by the French-American-British (FAB) group [22].

In the current case the morphological features of the undifferentiated blasts, concomitant with the presence of large number of mMK and fragments thereof, suggested the MK lineage of the disordered cells. Both immune marker and conventional cytochemical studies failed to show a lymphoid phenotype or a granulocytic/monocytic differentiation. The results of positive PAS and AP reactions, combined with the positive ANAE activity, which was partially inhibited by sodium fluoride and a negative reaction to ANBE were almost identical with the one observed in normal MK [23]. These findings were also consistent with the cytochemical features of megakaryoblastic leukemia described by the FAB group [22] and similar to those observed by Bain et al [24] in a case of MKB crisis of CML. Recently, MK origin of the cells can be identified definitely by the demonstration of 1) PPO, a specific enzyme marker of normal or neoplastic MK and their precursors, at the electron microscopic level [13–15,25], 2) platelet- and MK-specific membrane glycoproteins by reaction with monoclonal antibodies [11,26,17], and 3) factor VIII antigen [12, 28]. In this case, the vast majority of the circulating blast cells possessed PPO activity in the perinuclear space and endoplasmic reticulum by ultrastructural cytochemistry, thus confirming their MKB nature. Using platelet-specific monoclonal antibody (HP1-1 D) and polyclonal anti-factor VIII antibody in conjunction with immunofluorescence, immunocytochemical, or immunohistochemical staining techniques provided additional evidence that the MKB and mMK almost made up the entire population of the marrow and blood of our patient during blastic crisis. It has been shown that human marrow MK produce a
fibroblast-stimulating factor [29,30]. In this case, progressive increase in marrow fibrosis during the acute transformation stage was attributed to the neoplastic MK proliferation.

To our knowledge, there were only three reports utilizing the in vitro culture studies for the MKB crisis of CML [18,20,31]. The findings of in vitro liquid culture in the present case were similar to those of Efrati et al [18], except that we observed a few mature MK at day 12. Our results supported the observation by Vainchenker et al [31] and Lingg et al [20] that the block in the maturation during the MKB transformation of CML can be overcome in vitro.

In summary, the clinical course of a case with Ph1-positive CML and its interesting features are described. We used three methods for identification and confirma-
tion of the MK origin of the leukemic cells. With the aid of ultrastructural cytological and immunocytochemical studies, MKB transformation of CML may be recognized more frequently than previously thought.

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