Blastic Transformation of Essential Thrombocythemia: Dual Expression of Myelomonoblastic/Megakaryoblastic Phenotypes

By Debra Frei-Lahr, James C. Barton, Ronald Hoffman, Luther L. Burkett, and Josef T. Prchal

Three patients developed blastic transformation of essential thrombocythemia (tET). Morphological studies in all patients showed that the majority of blasts had either myeloblastic or myelomonoblastic differentiation. Immunologic assays of hematopoietic cells were performed in two patients. In patient 1, 86% of peripheral blood mononuclear cells (predominantly blasts) reacted with a monoclonal antibody specific for granulocytes and monocytes (MMA), and 15% of mononuclear cells reacted with Tab, a monoclonal antibody specific for megakaryocyte-platelet glycoproteins (PGP) IIb and IIIa. In patient 2, 41.5% of peripheral blood mononuclear cells (predominantly blasts) were MMA-positive, 22.5% were Tab-positive, and 40% reacted with rabbit anti-human PGP. These results suggest either that two subpopulations of blast cells exist in tET, or that blast cells simultaneously express surface markers of myeloblastic/monoblastic and megakaryoblastic differentiation. In these three and in nine previously reported cases of tET, neither age, sex, nor previous therapy were obvious etiologic factors. tET occurred 24.2 ± 14.4 mo after diagnosis of essential thrombocythemia, and a majority of patients had hepatomegaly and/or splenomegaly, anemia, leukocytosis, and thrombocytopenia. Leukemic cell morphology was myeloblastic and/or monoblastic in 12/12 patients; 5/12 had marrow fibrosis. Despite various treatments, death occurred in 3.6 ± 2.7 mo; one patient had a brief complete remission.

ESSENTIAL THROMBOCYTHEMIA (ET) is an uncommon myeloproliferative disorder characterized by megakaryocytic hyperplasia, thrombocytosis, hemorrhagic and/or thrombotic diathesis, splenomegaly, and hepatomegaly. This disease has been demonstrated to be a clonal disorder of the myeloid stem cell. Acute blastic transformation of ET (tET) is rare, with only nine patients having been reported previously. We present the clinical course of three patients with tET whose blast cells were studied by cytochemical and immunologic methods. Cytogenetic analysis of hematopoietic cells was successful in one case. Further, clinical and laboratory features of the previously described cases are reviewed.

CASE HISTORIES

Two patients came from our clinical practice, over a 7-yr interval, at the University of Alabama in Birmingham (UAB). The third patient was treated by two of us at the University of Tennessee Hospitals in Memphis. The Division of Hematology/Oncology at UAB treats approximately 90 new patients per year for adult acute leukemia, including blastic transformation of chronic myeloproliferative disorders.

Patient 1

A 69-yr-old black female presented with fatigue and myalgias, and was found to have ET (Table 1). Five months prior to presentation, pulmonary emboli had been suspected because of an abnormal ventilation-perfusion scan. She was treated at presentation with an infusion of cytosine arabinoside and with 5 mCi of 32P; this therapy was associated with return of blood counts to normal for 7 mo. Thereafter, she developed a leukocyte count of 23.7 × 10⁹/liter, hemoglobin concentration of 10.4 g/dl, and a platelet count of 2,200 × 10⁹/liter. The platelet count remained elevated above 1,000 × 10⁹/liter despite the administration of melphalan and busulfan and additional 32P. Pack red blood cell transfusions were complicated by the development of antieythrocyte alloantibodies. Thirteen months after presentation, she developed fever, confusion, acalculous cholecystitis, azotemia, hypercalcemia, and tET (Table 2). Peripheral blood blast cells rapidly decreased in number following treatment with methotrexate, vincristine, and prednisone, but the patient died of an unexplained cardiac arrest 5 days after initiation of therapy for tET.

Patient 2

An asymptomatic 72-yr-old white male was referred for evaluation of thrombocytosis and was found to have ET (Table 1). Two years prior to presentation, an amputation of his right leg had been performed for an unexplained arterial occlusion. The platelet count decreased with platelet apheresis and with cytosine arabinoside infusion, and remained normal with busulfan administration for 18 mo. Thereafter, the patient developed malaise, weight loss, pulmonary congestion, and tET (Table 2). Peripheral blood blasts decreased in number for 19 days following treatment with methotrexate, vincristine, and prednisone, but the patient expired with refractory hypotension and fever, despite antibiotic treatment: multiple cultures for bacteria were negative.

Patient 3

A 66-yr-old black male was referred because of prolonged bleeding from a tongue laceration and was found to have ET (Table 1). Platelet count fell following the administration of 32P and busulfan. The patient did well for 2 yr after presentation, when he developed fatigue, dyspnea, fever, and mild pedal edema. He was found to have congestive heart failure due to arteriosclerotic heart disease and...
calcific aortic stenosis and tET (Table 2). He was treated with daunorubicin, cytosine arabinoside, and 6-thioguanine, but developed progressive renal failure, which was possibly obstructive in etiology. He expired during cystoscopy, following an unexplained cardiorespiratory arrest.

MATERIALS AND METHODS

Peripheral blood and bone marrow films were stained with Wright’s, periodic acid-Schiff (PAS), and Sudan black B stains, as well as for naphthol AS-D chloroacetate and ct-naphthyl butyrate. Bone marrow aspirate smears or touch preparations were fixed in neutral buffered formalin, decalcified in citrate-buffered formic acid, and routinely embedded in paraffin. Sections 4 μm in thickness were stained with hematoxylin and eosin and PAS technique. Ultrastructural studies were kindly performed by Dr. Richard T. Parmley; heparinized peripheral blood and bone marrow aspirate smears were stained with uranyl acetate and lead citrate, as previously described.12

Routine chromosome analysis and Giemsa banding studies (using the method of Seabright)13 were performed using unstimulated peripheral blood and bone marrow cells. Immunologic assays performed on tET peripheral blood mononuclear cell preparations (predominantly blasts) were: sheep E rosettes, slgM, slgD, slgG, staining for surface IgM (slgM) and cytoplasmic IgM (clgM) (clgM) for pre-B cell identification (slgM−·clgM+), anti-B, slgM, anti-slgD, anti-κ/λ ratio, HLA-DR, natural killer antigen, and myelomonocytic antigen (MMA).14–16 Anti-MMA is nonreactive with platelets,16 and the extensive experience of Dr. Carlo Grossi at UAB with this antibody has failed to reveal its reactivity with human megakaryocytes and their morphologically identifiable precursors.17 Anti-MMA is commercially available (LeuM-1; Becton-Dickinson Monoclonal Center, Mountain View, CA). These assays were kindly performed in the Clinical Laboratory of the Cellular Immunobiology Unit of Dr. Max D. Cooper, Comprehensive Cancer Center, UAB. Anti-B and anti-natural killer antigen antibodies were the generous gift of Dr. Toru Abo.

Antibodies used to identify cells of megakaryocytic lineage were rabbit anti-human platelet glycoprotein (PGP)18 and the monoclonal antibody Tab, which is specific for PGP IIb and IIIa.19 The Tab antibody was kindly provided by Dr. Roger McEver, University of Texas, San Antonio. The specificity of rabbit anti-PGP and Tab has not only been assured by testing against purified normal cells,18–20 but also in pathologic situations.18–24 In addition, to date 9 cases of acute myelofibrosis have been examined, and in only 2 has evidence of megakaryoblastic differentiation reflected by reactivity with anti-PGP and Tab antibodies been found.25 Rabbit anti-PGP and Tab were diluted with phosphate-buffered saline (PBS) to a concentration of 1:200 and were layered on smears of mononuclear cells, incubated at room temperature in 100% humidified air for 60 min, washed 3 times with PBS, and reincubated for 60 min with fluorescein-conjugated goat anti-mouse or anti-rabbit IgG as appropriate. After washing with PBS, specimens were counterstained with uranyl acetate and lead citrate, as previously described.26

RESULTS

At the onset of tET in patient 1, Wright’s stain and cytochemical morphology of a majority of blast cells
was that typical of myeloblasts (M2, FAB classification) (Table 2). Less than 1% of blast cells had megakaryoblastic morphology (abundant basophilic cytoplasm organized in buds, blunt pseudopodia, and platelet-like structures). Cytogenetic analysis of peripheral blood cells was unsuccessful. Immunologic studies on peripheral blood (Table 3) revealed that 86% of the mononuclear cells reacted with M MA, 15% with Tab, and none with rabbit anti-PGP. Increased numbers of mononuclear cells were positive for HLA-DR (a marker of proliferating cells irrespective of lineage, as well as of resting B lymphocytes and monocytes). Multiple immunologic assays did not demonstrate a B cell lineage of blasts.

The blasts of patient 2 were predominantly myelomonoblastic by Wright’s stain morphology and cytochemical reactions (M5, FAB classification) (Table 2). Less than 1% of blasts had megakaryoblastic features. Transmission electron microscopy confirmed the presence of myeloblasts and monoblasts, although platelet peroxidase staining was not performed. The karyotype obtained from peripheral blood cells revealed an abnormal hypodiploid chromosome complement in 20 of 21 metaphases examined. Giemsa banding studies demonstrated that, in addition to several missing chromosomes, a majority of cells exhibited a number of rearrangements and markers that could not be identified completely. A majority of cells had deletions of chromosomes 7, 11, 17, and 22 and had extra material on 4p, 13q, and 21q. A single cell with an apparently normal male chromosome complement (46,XY) was observed. Immunologic studies on peripheral blood (Table 3) revealed that 41.5% of mononuclear cells reacted with M MA, 40% with rabbit anti-PGP, and 22.5% with Tab. A majority of cells were HLA-DR-positive and a subnormal number of cells were of either T or B cell lineage.

In patient 3, the blasts had myeloblastic morphology
(M2, FAB classification), and Auer rods were present (Table 2). No megakaryoblastic cells were seen. The peripheral blood blasts were positive with Sudan black B staining (60%) and negative for myeloperoxidase and with PAS stain. Marrow trephine biopsy specimen was diffusely hypercellular due to primitive hematopoietic cell infiltration and fibrosis, with a marked decrease in the numbers of normal marrow elements. Cytogenetic analysis was not performed; immunologic studies were not available because this patient was seen in 1975.

Clinical and laboratory observations in previously reported cases of tET from literature are summarized in Table 4.

**DISCUSSION**

Essential thrombocythemia is a rare myeloproliferative disorder of unknown etiology that occurs most frequently in the sixth and seventh decades. In older series of cases, 80% of treated patients with ET were alive 1 yr after diagnosis, but most expired within 4 yr, usually because of thrombosis and/or hemorrhage or unrelated disease. A recent report describing thio-TEPA and chlorambucil therapy for ET suggests that improved longevity is possible: a median survival of 36 mo in 25 patients, of whom 22 were living. In this series, only one patient thus far developed tET.

Only nine patients have been described previously to have tET (Table 4), yet blastic transformation of other chronic myeloproliferative diseases, particularly chronic granulocytic leukemia (CGL), polycythemia rubra vera, and idiopathic myelofibrosis, is well appreciated. Including our cases, the mean age at diagnosis of ET among patients who developed tET is 54.2 ± 20.7 yr (range 14–73 yr); the male:female ratio is 7:5, which is not distinctly different from that predicted in large series of patients with ET. The interval from ET to development of tET is 24.2 ± 14.4 mo (range 12–64 mo). Those whose therapy for ET included 32P developed tET in 24.6 ± 18.1 mo (range 12–64 mo, n = 7) after diagnosis of ET. Patients whose therapy for ET did not include 32P developed tET in 23.6 ± 8.9 mo (range 18–36 mo, n = 5). Those whose treatment for ET included chemotherapy developed tET in 19.0 ± 7.8 mo (range 12–36 mo, n = 8) after diagnosis of ET. Patients whose therapy for ET did not include cytotoxic drugs developed tET in 34.5 ± 20.1 mo (range 20–64 mo, n = 4). These data suggest that neither age at diagnosis of ET, sex, nor 32P therapy of ET are obvious etiologic factors in the development of tET. They also suggest that treatment of ET with alkylating agents may hasten the development of tET. Alternatively, patients with more aggressive ET may be more likely to be treated with chemotherapy, yet treatment results in ET with thioTEPA and chlorambucil seem to be superior to those with other well-documented therapies. These conclusions must be tempered, however, by the small number of patients with tET reported.

Blast morphology in previously reported cases of tET has been myeloblastic. In our cases, morphology was also myeloblastic in patients 1 and 3 and myelomonoblastic in patient 2. Blast cells in patient 3 exhibited Auer rods. Myelomonoblastic phenotype was further confirmed by cytochemical studies, the results

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<table>
<thead>
<tr>
<th>Surface Marker Assay</th>
<th>Patient</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>E rosettes</td>
<td>9</td>
<td>55-73</td>
</tr>
<tr>
<td>slgM - slgM* (pre-B)</td>
<td>&lt;1</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td>slgM</td>
<td>0.5</td>
<td>5.7-15.3</td>
</tr>
<tr>
<td>slgD</td>
<td>&lt;0.2</td>
<td>4.0-13.4</td>
</tr>
<tr>
<td>slgG</td>
<td>&lt;0.2</td>
<td>0.7-2.5</td>
</tr>
<tr>
<td>slgA</td>
<td>&lt;0.2</td>
<td>0.5-1.9</td>
</tr>
<tr>
<td>Anti-B</td>
<td>&lt;0.2</td>
<td>4-21</td>
</tr>
<tr>
<td>k/λ</td>
<td>1</td>
<td>1.5-2.9</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>Positive</td>
<td>11-46</td>
</tr>
<tr>
<td>Natural killer antigen</td>
<td>6</td>
<td>4.2-20</td>
</tr>
<tr>
<td>Myelomonocytic antigen</td>
<td>86</td>
<td>8-32</td>
</tr>
<tr>
<td>Rabbit anti-PGP</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tab</td>
<td>22.5</td>
<td>0</td>
</tr>
</tbody>
</table>

*No studies performed on patient 3.
†Not performed.
‡All blasts and monocyteid cells positive, although no positively staining lymphocytes as control cells could be identified.
Table 4. Blast Transformation of Essential Thrombocythemia (ET): Clinical and Laboratory Findings in Previously Reported Cases

<table>
<thead>
<tr>
<th>Author</th>
<th>Duration of ET (mo)</th>
<th>Therapy of ET</th>
<th>Signs, Symptoms</th>
<th>Leukocyte Count (g/dL)</th>
<th>Platelet Count (x 10^9/Liter)</th>
<th>Bone Marrow</th>
<th>Therapy of ET</th>
<th>Survival tET (mo)</th>
<th>Postmortem Examination</th>
</tr>
</thead>
<tbody>
<tr>
<td>McCabe (1954)²</td>
<td>24</td>
<td>³²P, total body irradiation</td>
<td>Malaise, weight loss, bone pain, lymphadenopathy, cutaneous nodules, hepatosplenomegaly</td>
<td>11.8</td>
<td>39.0 (2)</td>
<td>500</td>
<td>³²P</td>
<td>2</td>
<td>Leukemic infiltrates in abdominal, paratracheal, peribronchial lymph nodes and in skin, liver, spleen, kidneys, lungs, brain</td>
</tr>
<tr>
<td>Ozer (1960)⁶</td>
<td>20</td>
<td>³²P</td>
<td>Weakness, leg aches, hepatosplenomegaly</td>
<td>16</td>
<td>15.0 (0)</td>
<td>3,500</td>
<td>³²P</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Lewis (1972)⁵</td>
<td>64</td>
<td>³²P</td>
<td>Tiredness</td>
<td>17</td>
<td>15.0 (7)</td>
<td>20</td>
<td>None</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Herrmann (1973)⁶</td>
<td>15</td>
<td>³²P, busulfan</td>
<td>Fever, backache, lymphadenopathy, hepatosplenomegaly</td>
<td>9.4</td>
<td>29.2 (5)</td>
<td>105</td>
<td>None</td>
<td>1</td>
<td>“Acute myeloblastic leukemia”</td>
</tr>
<tr>
<td>Fickers (1974)⁷</td>
<td>12</td>
<td>ThioTEPA, ³²P</td>
<td>Cutaneous hemorrhage, splenomegaly</td>
<td>10.2</td>
<td>67.0 (35)</td>
<td>430</td>
<td>Blasts positive with Sudan black B; Auer rods</td>
<td></td>
<td>9</td>
</tr>
<tr>
<td>Fickers (1974)⁷</td>
<td>18</td>
<td>ThioTEPA, busulfan</td>
<td>Hepatosplenomegaly</td>
<td>12.8</td>
<td>1.2 (few)</td>
<td>106</td>
<td>Blast infiltration, Auer rods; cytogenetic analysis normal</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Raman (1981)⁸</td>
<td>16</td>
<td>Busulfan (minimal)</td>
<td>Epistaxis</td>
<td>8.6</td>
<td>3.4 (7)</td>
<td>9</td>
<td>Cellularity 95%, 39.5% blasts + promyelocytes, megakaryocytes markedly decreased</td>
<td></td>
<td>4+</td>
</tr>
<tr>
<td>Geller (1982)⁹</td>
<td>30</td>
<td>None</td>
<td>Multiple ecchymoses</td>
<td>7.6</td>
<td>8.4 (0)</td>
<td>104</td>
<td>Undifferentiated granulocytic cells; fibrosis, increased reticulum</td>
<td>Vincristine, prednisone</td>
<td>7</td>
</tr>
<tr>
<td>Case (1982)¹⁰¹¹</td>
<td>36</td>
<td>ThioTEPA, chlorambucil</td>
<td>Weakness, petechiae</td>
<td>8.4</td>
<td>15.4 (23)</td>
<td>2</td>
<td>Acute myelomonoblastic leukemia, megakaryocytic hyperplasia and dysplasia, fibrosis; blasts in blood positive for myeloperoxidase and with chloroacetate esterase</td>
<td>Daunorubicin, cytosine arabinoside, m-AMSA</td>
<td>3</td>
</tr>
</tbody>
</table>

*LYmph node and skin infiltration with blasts.
of which are similar to findings in previously published cases. The results of transmission electron microscopy in patient 2 confirmed that the majority of blasts in tET morphologically resemble myeloblasts and/or monoblasts.

Cytogenetic studies are usually normal in ET, as was the case in one of our patients with ET (patient 1) in whom analysis was successful. In a minority of patients with ET, various chromosomal abnormalities have been reported. Although a normal karyotype has been reported in one patient with tET,7 one of our patients in whom chromosome analysis was successful had hypodiploidy and other chromosomal anomalies (patient 2).

Antibodies specific for antigens of hematopoietic cell differentiation also permit phenotypic characterization of leukemic cells. Anti-MMA reacts with adherent mononuclear cells and granulocytes by indirect immunofluorescence and complement-mediated lysis assays. Most of the mononuclear cells (blasts) in the two patients we studied reacted with anti-MMA. Rabbit anti-PGP reacted strongly with the mononuclear cells in one of these cases, and significant numbers of Tab-positive mononuclear cells were found in both cases. These results could be explained by the adherence of either platelets or platelet fragments to blast cells. However, light microscopy in patients 1 and 2, and ultrastructural studies in patient 2, did not reveal any evidence of this phenomenon. A “continuous” pattern of blast surface immunostaining with anti-PGP and with Tab was observed, not a “discontinuous” pattern, which would be expected due to platelet fragment adherence. Further, both patients were thrombocytopenic at the time immunologic assays were performed. The cytochemical and immunochemical markers of these blasts suggest involvement of a stem cell capable of aberrant and incomplete differentiation along myelomonoblastic and megakaryocytic-platelet pathways. Such aberrant differentiation can alter antigen expression on neoplastic cells. This could explain the recognition of blasts in patients 1 and 2 by the monoclonal antibody Tab and the failure of polyclonal rabbit anti-PGP to react with blasts from patient 1. Conversely, however, K562 cells are reactive with anti-PGP and nonreactive with monoclonal markers for megakaryoblastic differentiation. A different monoclonal antibody, AN51, permitted the identification of a minor population of megakaryoblasts (5%–16%) in 4 cases of blastoid transformation of CGL. A combination of immunologic, ultrastructural, and cytochemical methods has demonstrated the differentiation of blasts along multiple cell lines in a patient with acute myelofibrosis. Further, in a previously reported case of tET, primitive hematopoietic cells that had infiltrated a lymph node were positive for chloroacetate esterase, yet peripheral blood blasts in the same patient were unexpectedly positive for terminal deoxynucleotidyl transferase. The presence of individual blasts simultaneously expressing characteristics of more than one lineage has been reported to occur in murine leukemia, and similarly, the human leukemia cell line K562 expresses phenotypic markers of erythroid, myeloid, and megakaryocytic differentiation. Studies in our own patients, however, have not demonstrated with certainty whether two or more differentiation lines occur in tET, or whether a single population of cells exists, since these cells simultaneously bear phenotypic characteristics of two or more lineages. Thus far, resolution of this issue has not come forth by double staining of blast cells with anti-PGP and anti-MMA (one labeled with fluorescein and the other with rhodamine), due to our inability to find a goat anti-rabbit immunoglobulin antibody that retains its reactivity after extensive absorption with mouse immunoglobulin.

The appropriate therapy for tET is unknown. In our first two patients and in two others, treatment with vincristine and prednisone with/without methotrexate was associated with substantial reductions in the numbers of peripheral blood and/or bone marrow blasts. However, in none of these patients did a complete remission occur. Two patients were treated with radio-phosphorus alone for tET, but they expired shortly after the occurrence of acute leukemia. A complete hematologic remission lasting approximately 4 mo was achieved in one patient with four courses of cytosine arabinoside and 6-thioguanine, followed by maintenance therapy with methotrexate, cytosine arabinoside, and daunorubicin. At relapse of tET, this patient achieved a partial remission with daunorubicin and cytosine arabinoside, but died 9 mo after the development of tET. Our third patient had a reduction in blast cell numbers with daunorubicin, cytosine arabinoside, and 6-thioguanine, but died unexpectedly during therapy. The common proximate causes of death among patients with tET, as in other patients with acute leukemia, appear to be infection and/or hemorrhage. These observations suggest that tET may be treated effectively, at least for brief intervals, with a variety of antileukemic drugs. Because of the rarity of tET, cooperative group trials investigating etiologic, morphological, cytogenetic, immunologic, and therapeutic aspects of tET probably will be necessary to define this syndrome clearly and to predict the most effective available treatment, similar to that now possible in blastoid transformation of CGL.
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