Myelomastocytic Leukemia: Evidence for the Origin of Mast Cells from the Leukemic Clone and Eradication by Allogeneic Stem Cell Transplantation

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Abstract

Purpose: Myelomastocytic leukemia is a term used for patients with advanced myeloid neoplasms, in whom elevated numbers of immature atypical mast cells are found, but criteria for a primary mast cell disease are not met. The origin of mast cells in these patients is presently unknown.

Patient and Methods: We have analyzed clonality of mast cells in an 18-year-old patient suffering from acute myeloid leukemia with a complex karyotype including a t(8;21) and mastocytic transformation with a huge increase in immature mast cells and elevated serum tryptase level, but no evidence for a primary mast cell disease/mastocytosis.

Results: As assessed by in situ fluorescence hybridization combined with tryptase staining, both the tryptase-negative blast cells and the tryptase-positive mast cells were found to contain the t(8;21):specific AML1/ETO fusion gene. Myeloablative stem cell transplantation resulted in complete remission with consecutive disappearance of AML1/ETO transcripts, decrease of serum tryptase to normal range, and disappearance of neoplastic mast cells.

Conclusion: These data suggest that mast cells directly derive from the leukemic clone in patients with myelomastocytic leukemia.

Myelomastocytic leukemia is a term used for patients with advanced myeloid neoplasms, in whom a substantial increase in immature atypical mast cells is found, but the criteria for a primary mast cell disease (mast cell leukemia or systemic mastocytosis) are not fulfilled (1–5). Typically, patients with myelomastocytic leukemia exhibit an increase in blast cells as well as >10% metachromatic cells in peripheral blood and/or bone marrow smears (3–5). Blast cells are myeloblasts by morphologic and immunophenotypic criteria. These patients are thus diagnosed to have an advanced myelodysplastic syndrome with excess of blasts, a myeloproliferative disease, or an acute myeloid leukemia (AML; refs. 1–4). Myelomastocytic leukemia is a rare disease. In fact, <5% of all patients with myelodysplastic syndrome, myeloproliferative disease, or AML are considered to develop a “mastocytic transformation” (3, 4).

Metachromatic cells in myelomastocytic leukemia are immature, often exhibit a blast-like morphology, and are mast cell lineage cells by electron microscopic and immunophenotypic criteria (1–6). In contrast to “true” mast cell disease (i.e., mast cell leukemia or systemic mastocytosis; refs. 8–11), Metachromatic cells in myelomastocytic leukemia are immature, often exhibit a blast-like morphology, and are mast cell lineage cells by electron microscopic and immunophenotypic criteria (1–6). In contrast to “true” mast cell disease (i.e., mast cell leukemia or systemic mastocytosis; refs. 8–11), Metachromatic cells in myelomastocytic leukemia are immature, often exhibit a blast-like morphology, and are mast cell lineage cells by electron microscopic and immunophenotypic criteria (1–6). In contrast to “true” mast cell disease (i.e., mast cell leukemia or systemic mastocytosis; refs. 8–11), Metachromatic cells in myelomastocytic leukemia are immature, often exhibit a blast-like morphology, and are mast cell lineage cells by electron microscopic and immunophenotypic criteria (1–6). In contrast to “true” mast cell disease (i.e., mast cell leukemia or systemic mastocytosis; refs. 8–11), Metachromatic cells in myelomastocytic leukemia are immature, often exhibit a blast-like morphology, and are mast cell lineage cells by electron microscopic and immunophenotypic criteria (1–6). In contrast to “true” mast cell disease (i.e., mast cell leukemia or systemic mastocytosis; refs. 8–11), Metachromatic cells in myelomastocytic leukemia are immature, often exhibit a blast-like morphology, and are mast cell lineage cells by electron microscopic and immunophenotypic criteria (1–6). In contrast to “true” mast cell disease (i.e., mast cell leukemia or systemic mastocytosis; refs. 8–11), Metachromatic cells in myelomastocytic leukemia are immature, often exhibit a blast-like morphology, and are mast cell lineage cells by electron microscopic and immunophenotypic criteria (1–6). In contrast to “true” mast cell disease (i.e., mast cell leukemia or systemic mastocytosis; refs. 8–11), Metachromatic cells in myelomastocytic leukemia are immature, often exhibit a blast-like morphology, and are mast cell lineage cells by electron microscopic and immunophenotypic criteria (1–6). In contrast to “true” mast cell disease (i.e., mast cell leukemia or systemic mastocytosis; refs. 8–11), Metachromatic cells in myelomastocytic leukemia are immature, often exhibit a blast-like morphology, and are mast cell lineage cells by electron microscopic and immunophenotypic criteria (1–6). In contrast to “true” mast cell disease (i.e., mast cell leukemia or systemic mastocytosis; refs. 8–11), Metachromatic cells in myelomastocytic leukemia are immature, often exhibit a blast-like morphology, and are mast cell lineage cells by electron microscopic and immunophenotypic criteria (1–6). In contrast to “true” mast cell disease (i.e., mast cell leukemia or systemic mastocytosis; refs. 8–11), Metachromatic cells in myelomastocytic leukemia are immature, often exhibit a blast-like morphology, and are mast cell lineage cells by electron microscopic and immunophenotypic criteria (1–6). In contrast to “true” mast cell disease (i.e., mast cell leukemia or systemic mastocytosis; refs. 8–11), Metachromatic cells in myelomastocytic leukemia are immature, often exhibit a blast-like morphology, and are mast cell lineage cells by electron microscopic and immunophenotypic criteria (1–6). In contrast to “true” mast cell disease (i.e., mast cell leukemia or systemic mastocytosis; refs. 8–11), Metachromatic cells in myelomastocytic leukemia are immature, often exhibit a blast-like morphology, and are mast cell lineage cells by electron microscopic and immunophenotypic criteria (1–6). In contrast to “true” mast cell disease (i.e., mast cell leukemia or systemic mastocytosis; refs. 8–11), Metachromatic cells in myelomastocytic leukemia are immature, often exhibit a blast-like morphology, and are mast cell lineage cells by electron microscopic and immunophenotypic criteria (1–6). In contrast to “true” mast cell disease (i.e., mast cell leukemia or systemic mastocytosis; refs. 8–11), Metachromatic cells in myelomastocytic leukemia are immature, often exhibit a blast-like morphology, and are mast cell lineage cells by electron microscopic and immunophenotypic criteria (1–6). In contrast to “true” mast cell disease (i.e., mast cell leukemia or systemic mastocytosis; refs. 8–11), Metachromatic cells in myelomastocytic leukemia are immature, often exhibit a blast-like morphology, and are mast cell lineage cells by electron microscopic and immunophenotypic criteria (1–6). In contrast to “true” mast cell disease (i.e., mast cell leukemia or systemic mastocytosis; refs. 8–11), Metachromatic cells in myelomastocytic leukemia are immature, often exhibit a blast-like morphology, and are mast cell lineage cells by electron microscopic and immunophenotypic criteria (1–6).
Thus far, little is known about the pathogenesis of myelomastocytic leukemia (3, 4). In all cases reported thus far, a complex karyotype (without specific or recurrent cytogenetic aberrations) has been described (1–5). However, many questions concerning the origin and uncontrolled growth of mast cells remain open. One most important hitherto unresolved question was whether mast cells in these patients belong to the leukemic (myeloid) clone. In the present article, we provide evidence for the clonal origin of mast cells from the leukemic clone in a patient with AML and myelomastocytic transformation.

**Patient and Methods**

**Case report.** In February 2001, a 17-year-old male patient presented with fever, an abscess in his left thigh, anemia, thrombocytopenia, and a white blood count of 62,400 × 10^9//L. The differential count showed 1% segmented neutrophils, 1% basophils, 7% lymphocytes, 2% monocytes, 2% metamyelocytes, and 87% blasts. The bone marrow smear revealed 87% myeloblasts as well as an increase in immature mast cells (range, 7–11%). In the bone marrow trephine biopsy, a diffuse infiltration with leukemic blasts and immature mast cells (~10%), but no focal dense mast cell infiltrates, was found. Karyotyping of bone marrow mononuclear cells revealed a complex pattern including a variant t(8;21) involving chromosomes 8, 10, and 21. The reported karyotype was 46,XY, t(8;10;21)(q22;q21;q22), t(11;19)(q13;13) [30 of 50 metaphases]; 46,XY, t(8;10;21)(q22;q21;q22), t(11;19)(q13;13), del(9)(q22) [20 of 50 metaphases]. Reverse transcriptase-PCR confirmed the presence of the t(8;21)-specific fusion gene AMN1/ETO. The c-kit point mutation OD816V, typically found in systemic mastocytosis (6), was not detected. The diagnosis myelomastocytic leukemia arising in AML with a complex karyotype including t(8;21) was established. The serum tryptase level was markedly elevated (745 ng/mL; normal range, <15 ng/mL).

**Staining techniques and fluorescence in situ hybridization.** Immunohistochemistry and flow cytometry were done to characterize neoplastic cells and to discriminate between AML blasts and immature mast cells. Expression of surface antigens was analyzed by multicolor flow cytometry on a FACScan (Becton Dickinson, San Diego, CA) using monoclonal antibodies against CD117 (YB5.B8), CD34 (581), CD2, CD3, CD10, and CD19 (purchased from Vysis, Inc., Downers Grove, IL) were applied on Ficoll-separated bone marrow mononuclear cells that had been dropped onto cytospin slides and then were stained with anti-tryptase monoclonal antibodies (Chemicon, Temecula, CA; refs. 17–19). For interphase fluorescence in situ hybridization (FISH), probes hybridizing to AML1 or ETO (purchased from Vysis, Inc., Downers Grove, IL) were applied on Ficoll-separated bone marrow mononuclear cells that had been dropped onto cytospin slides and then were stained with anti-tryptase monoclonal antibody G3 and an AMCA-labeled anti-mouse antibody (Vector Laboratories, Burlingame, CA). Hybridization was done as described (20, 21). Tryptase-positive and tryptase-negative cells were evaluated separately. Slides were examined under an Axiosplan-2 immunofluorescence microscope (Zeiss, Jena, Germany) equipped with appropriate filters to visualize green, red, and blue immunofluorescence either separately or simultaneously. Images were captured with a cooled charge-coupled device camera (Photometrics, Tucson, AZ) mounted on the microscope and linked to an Apple Macintosh computer. Prints were obtained with a Phaser 440 Tektronix color printer (Tektronix, Wilsonville, OR).

**Monitoring of disease during therapy using disease-related markers.** Disease-related markers were measured serially before and after therapy to define and monitor the response to therapy. AML/ETO was analyzed in bone marrow and peripheral blood mononuclear cells by reverse transcriptase-PCR as described (22). Serum tryptase levels were determined by fluoroenzyme immunoassay (Pharmacia, Uppsala, Sweden; ref. 17).

**Results**

**Morphologic and immunophenotypic delineation of mast cells and acute myeloid leukemia blasts.** As assessed by morphology and immunophenotyping, two distinct populations of neoplastic cells were detected in the bone marrow (i.e., AML blasts and immature mast cells). AML blasts showed a prominent nucleus with fine nuclear chromatin and a basophilic cytoplasm without metachromatic granules (Fig. 1A). Pheno-typically, blasts were CD34+, CD117+, and tryptase negative (Table 1). Mast cells were small to medium sized with a pale cytoplasm containing few or numerous metachromatic granules and a round or bilobed nucleus (Fig. 1A). By morphologic examination alone, it was impossible to define whether these cells were mast cells or basophils. As assessed by flow cytometry, these cells were found to express tryptase and CD117 confirming the mast cell lineage but did not express CD34, CD2, or CD25 (Fig. 1B; Table 1). Identical results were obtained by immunohistochemistry. Figure 1C shows expression of tryptase in diffusely spread mast cells. Compact infiltrates of tryptase-positive cells (typically found in systemic mastocytosis) were not detected in bone marrow sections.

**Demonstration that mast cells and acute myeloid leukemia blasts belong to the same clone.** To study clonality of blast cells and mast cells, FISH analysis with probes specific for the t(8;21) were done on tryptase-stained bone marrow cells. In these experiments, AML1/ETO cohybridization signals indicating a t(8;21) were observed in 61% of all tryptase-negative blast cells examined (100 cells evaluated). In addition, AML1/ETO cohybridization signals were detected in 94% (47 of 50) of all tryptase-positive mast cells analyzed (Fig. 2). Thus, the leukemia-specific chromosomal aberration was present both in AML blasts and the immature tryptase-positive mast cells.

**Response to therapy and monitoring of disease-related variables.** In response to induction chemotherapy (daunorubicin, 45 mg/m^2//d, days 1-3; etoside, 100 mg/m^2//d, days 1-5, and cytarabine, 100 mg/m^2//d, days 1-7; “3 + 5 + 7 protocol”), the patient entered a hematologic CR. However, tryptase levels remained positive (Fig. 3). In August 2001, the patient relapsed. In response to induction chemotherapy (daunorubicin, 45 mg/m^2//d, days 1-3; etoside, 100 mg/m^2//d, days 1-5, and cytarabine, 100 mg/m^2//d, days 1-7; “3 + 5 + 7 protocol”), the patient entered a hematologic CR. However, tryptase levels remained positive (Fig. 3). In August 2001, the patient relapsed. In response to salvage therapy (FLAG: fludarabin, 30 mg/m^2//d, days 1-5; total body irradiation: 2 Gy on day 0). After transplantation, the tryptase level still remained elevated (73 ng/mL), immature atypical mast cells were still detectable in bone marrow smears. In addition, the AML1/ETO fusion gene product was detectable by PCR. Because of pulmonary aspergillosis requiring lobectomy and antymycotic therapy, no high-dose consolidation was administered at that time. In June 2001, a nonmyeloablative stem cell transplantation (sibling donor) was done in CR (conditioning regimen: fludarabin, 30 mg/m^2//d, days 4 to 2; total body irradiation: 2 Gy on day 0). After transplantation, the tryptase level still remained elevated (73 ng/mL), immature atypical mast cells were still detectable in bone marrow smears, and the AML1/ETO PCR remained positive (Fig. 3). In August 2001, the patient relapsed. In response to salvage therapy (FLAG: fludarabin, 30 mg/m^2//d, days 1-5; cytarabine, 2,000 mg/m^2//d, days 1-5; granulocyte colony-stimulating factor, 300 μg/m^2//d) was administered in September 2001 and led to a second CR. Then, myeloablative stem cell transplantation (same donor; conditioning regimen: busulfan,
4 × 0.8 mg/kg/d, days −7 to −4; endoxan, 2 × 60 mg/kg/d, days −3 to −2) was done (November 2001). After transplantation, the patient entered continuous CR (thus far, 742 days after transplantation). In addition, all disease-related variables (AML1/ETO, atypical bone marrow mast cells, elevated serum, and tryptase) disappeared (Fig. 3).

Discussion

Myelomastocytic leukemia is a term used for patients with advanced myeloid neoplasms (AML, myelodysplastic syndrome with excess of blasts, and chronic myelogenous leukemia blast phase), in whom elevated numbers of atypical immature mast cells are found, but the criteria for systemic mastocytosis or mast cell leukemia are not fulfilled (1–6). The current study was done to clarify whether mast cells in myelomastocytic leukemia are derived from the leukemic clone. As assessed by combined FISH and tryptase staining, both the tryptase-negative AML blasts and tryptase-positive mast cells were found to contain the t(8;21) in a patient with myelomastocytic leukemia. In response to chemotherapy and stem cell transplantation, all disease-related markers (blasts, atypical mast cells, elevated serum, and tryptase) disappeared. These results suggest that mast cells were derived from the leukemic clone.

In previous reports describing myelomastocytic leukemia, the monoclonal origin of leukemic blasts and mast cells has already been discussed (3–5). The hypothesis was based on the histology and (immature) morphology of mast cells in these patients (3–5). Concerning the histology, the bone marrow always shows diffusely scattered mast cells infiltrating the bone marrow without dense focal infiltrates (1–5). Morphologically, mast cells are immature (blast like) or are more mature with a “promastocyte morphology” (i.e., bilobed or polynucleated; refs. 1–5, 23). In our patient, mast cells were also found to be “rather mature” metachromatic cells many of which exhibited bilobed nuclei. All in all, the morphology of mast cells in our case was closely resembling the promastocyte stage of mast cell differentiation (23). Therefore, it was quite easy to differentiate between mast cells and blast cells in bone marrow smears and cytospin slides. The identity of mast cells was also confirmed by immunophenotyping.

A number of criteria have become available to differentiate between myelomastocytic leukemia and a primary mast cell disease (systemic mastocytosis or mast cell leukemia). These criteria include the histology, morphology, expression of CD2 and CD25, and the c-kit mutation D816V. In primary mast cell diseases, mast cells typically form dense infiltrates in the bone marrow histology, often are spindle-shaped cells, express CD2 and/or CD25, and exhibit c-kit D816V (5, 6, 24–26). In our patient, none of these criteria were fulfilled, so that a primary mast cell disease could be excluded. This is of particular importance, because patients with AML may also have an associated primary mast cell disease (i.e., systemic...
mastocytosis) and t(8;21) is frequently detected in these systemic mastocytosis/AML patients (27–29).

In primary mast cell disorders (systemic mastocytosis, mast cell leukemia), monoclonality of mast cells has been documented by the recurrent somatic c-kit mutation D816V (24, 25). It has also been described that mast cells and leukemic cells may both contain the c-kit mutation D816V and thus are of monoclonal origin in systemic mastocytosis with concordant myelomonocytic leukemia (30, 31). However, the clonality status of mast cells in patients with myelomastocytic leukemia has not been analyzed thus far. In the current study, we show by combined FISH and tryptase staining that tryptase-negative AML blasts and tryptase-positive mast cells both contain the t(8;21) in a patient with myelomastocytic leukemia. These data show that mast cells and blasts were derived from the same clone. Whether mast cells and blast cells are of monoclonal origin in all patients with myelomastocytic transformation remains at present unknown. Because of the immaturity of mast cells in most of these patients (1–5), however, the monoclonality concept seems most likely.

Thus far, only a few clinical reports have alluded to treatment options for patients with myelomastocytic leukemia. Patients receiving cytoreductive treatment and supportive care had a short survival (1). In one case with myelomastocytic leukemia, intensive polychemotherapy was administered and resulted in CR (2). Based on this knowledge and the age of our patient, we decided to apply intensive chemotherapy and stem cell transplantation. Interestingly, whereas nonmyeloablative transplantation did not result in continuous CR, myeloablative transplantation (with stem cell from the same donor) resulted in a continuous CR. The early decrease in blast cells as opposed to the persistence of mast cells after transplantation may have several explanations. First, blasts may be more sensitive to the chemotherapy applied compared with mast cells (12–15). The second possibility would be that immature mast cell progenitors but not more mature mast cells were targets of therapy. If so, any effect of chemotherapy on mast cell numbers must be expected to occur after a certain latency period because of the extremely long differentiation and life span of mast cells (months to years; ref. 32).

The serum tryptase concentration has recently been introduced as a novel marker of minimal residual disease in AML (17, 33). In the current study, the tryptase level was also employed to monitor the disease and disease response to therapy. One interesting observation was that tryptase levels showed a good correlation with “AML1/ETO positivity” and with the numbers of atypical mast cells in the bone marrow. This observation suggests that the serum tryptase level may be a reliable variable to monitor the disease in patients with myelomastocytic leukemia.

As mentioned above, mast cells are considered relatively insensitive to conventional chemotherapies (12–14). This may apply especially to primary mast cell diseases, including aggressive systemic mastocytosis and mast cell leukemia, but may apply to a degree also to myelomastocytic leukemia (12–14, 27). Still, however, there may be an important difference. In fact, based on this case and several other observations (2, 12–14, 27), it may be concluded that compared with mast cell leukemia and aggressive systemic mastocytosis, patients with myelomastocytic leukemia have a (much) better chance to be cured by aggressive chemotherapy and stem cell transplantation.

In summary, our results provide evidence that mast cells directly derive from the AML clone in myelomastocytic leukemia thereby contrasting patients with AML with coexisting systemic mastocytosis. In line with this notion, neoplastic mast

### Table 1. Expression of mast cell–related markers

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<th>Marker</th>
<th>Patient Mast cell leukemia*</th>
<th>Normal mast cells*</th>
<th>Normal basophils*</th>
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<tbody>
<tr>
<td>CD2</td>
<td>-</td>
<td>+</td>
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<tr>
<td>CD25</td>
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<tr>
<td>CD117</td>
<td>-</td>
<td>+</td>
<td>+</td>
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<tr>
<td>c-kit D816V</td>
<td>-</td>
<td>+</td>
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*Data from Valent et al. (15) and Schernthaner et al. (16).
cells disappeared together with AML blasts in our patient after allogeneic stem cell transplantation, which is not seen in patients with systemic mastocytosis/AML or true mast cell leukemia. Based on these notions, we believe that it is of importance to differentiate among myelomastocytic leukemia, mast cell leukemia, and systemic mastocytosis/AML.

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References


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