Kit Ligand/Mast Cell Growth Factor-Independent Differentiation of Mast Cells in Myelodysplasia and Chronic Myeloid Leukemic Blast Crisis


Autonomous, factor-independent growth and differentiation of malignant cells in preleukemic and leukemic disease states is a well-recognized phenomenon and is often associated with a poor prognosis. Mast cells are distinct hematopoietic cells and express a unique profile of antigens. Growth and differentiation of normal mast cells is dependent on mast cell growth factor (MGF), the ligand of the c-kit proto-oncogene product. In this study, we screened for mast cell-lineage involvement in 52 patients suffering from myeloid leukemias, myelodysplastic syndromes (MDS), systemic mastocytosis, or other diseases by probing for mast cell-related molecules (c-kit, tryptase, histamine, and MGF) and by analyzing kit ligand/MGF-independent growth of mast cells in long-term suspension culture. Of the 52 patients tested, 2 patients with refractory anemia with excess of blast cells in transformation and 1 patient suffering from chronic myeloid leukemia blast crisis (CML-BC) were diagnosed as mastocytic disease. These patients were characterized by complex chromosomal abnormalities, splenomegaly, high percentages of circulating metachromatic cells (5% to 25%), high levels of cellular tryptase (>10 ng/10⁶ peripheral blood mononuclear cells/mL) and a tryptase/histamine (ng:ng) ratio greater than 1. The metachromatic cells expressed the mast-cell-related surface antigen c-kit, but not basophil-related antigens (CD11b, CDw17). Furthermore, in these 3 patients, spontaneous, MGF-independent growth of mast cells along with spontaneous synthesis of tryptase was demonstrable in long-term culture. No autocrine production, paracrine production, or overproduction of MGF was found. The spontaneous growth of mast cells could neither be abrogated by addition of monoclonal antibodies (MoAbs) to c-kit nor by MoAbs against MGF (<5% inhibition), whereas factor (MGF)-dependent differentiation of mast cells in these patients could be abrogated by MoAbs to c-kit or MoAbs to MGF (>70% inhibition, P < .001). In addition, serum MGF levels in these patients were within the normal range and MGF could not be detected in cell-free culture supernatants.

Of 3 patients who showed rapid progression of disease and had a survival time of less than 1 year. In conclusion, we describe a unique form of transformation in MDS and CML-BC characterized by mast cell lineage involvement and factor-independent differentiation of mast cells. This form of leukemic transformation has to be delineated from chronic myeloid leukemia with basophilia or basophil crisis, from primary mast cell leukemia, and from mastocytic leukemias and myelodysplastic disorders associated with basophilia.

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receptor gene(s) in experimental animals may be associated with a mast-cell–deficient phenotype. MGF/SCF acts on its target cells via the c-kit proto-oncogene product, the tyrosine kinase receptor for MGF. SCF receptors (c-kit) are expressed on normal hematopoietic progenitors, leukemic myeloid cells, and mast cells. Other mature hematopoietic cells, including basophils, do not express substantial amounts of c-kit.

Although mast cells originate from uncommitted myeloid progenitor cells, involvement of the mast cell lineage in myelodysplastic and leukemic disease states has rarely been described. This rarity may be caused by the fact that reliable mast cell markers have only been identified very recently and/or may be because mast cell lineage involvement in leukemic disease states is a rather infrequent event. In fact, only a few reports on primary mast cell leukemias exist.

In the present study, we screened for mast cell lineage involvement and factor (MGF/SCF)-independent differentiation/growth of mast cells in myeloid leukemias, MDS, and other disease states by probing for immunologic and biochemical mast cell markers.

**PATIENTS AND METHODS**

**Patients' Characteristics and Case Reports**

Fifty-two patients suffering from various hematologic disorders as well as 5 normal donors were enrolled in the study after informed consent was given. Patients suffered from refractory anemia with ringed sideroblasts (RAS; n = 3), refractory anemia with excess of blasts (RAEB/RAEBIT; n = 11), chronic myelomonocytic leukemia (CMML; n = 3), acute myeloid leukemia (AML; n = 16), CML (n = 11), severe aplastic anemia (SAA; n = 1), systemic mastocytosis (SM; n = 5), and piebaldism (n = 2). The patients were aged between 1 and 94 years; the mean age was 53.6 years and the median age was 58 years. Diagnoses were established according to French-American-British (FAB) criteria, histologic examination (mastocytes), and/or typical clinical findings (piebaldism and urticaria pigmentosa). The patients' diagnoses and characteristics are summarized in Table 1. In 3 patients (1 patient suffering from CML in blast crisis [CML-BC] and 2 patients suffering from refractory anemia with an excess of blasts in transformation [RAEB/IT]), mast cell lineage involvement was evidenced.

**Case no. 1 (patient no. 28).** A 64-year-old woman suffering from Ph-chromosome positive CML was admitted at the Vienna University Hospital in January 1987 because of disease acceleration with increase in blast cells and thrombocytopenia. The diagnosis of CML had been established in 1981 (increased white blood cell [WBC] counts, Ph chromosome, and 3% basophils). The patient had been treated with busulfan. At admission (in 1987), her WBC count was 96.4 x 10^9/L, the hemoglobin level was 9.8 g/dL, and the platelet count was 80.0 x 10^9/L. The differential blood cell count showed 29% neutrophils, 5% eosinophils, 10% metachromatic cells (recorded as atypical basophils), 9% monocytes, 9% lymphocytes, 10% promyelocytes, 6% myelocytes, 5% promyelocytes, and 17% blast cells. Peripheral blood colony-forming unit–granulocyte-macrophage (CFU-GM) levels were within the normal range (83 ± 2 per 10^5 mononuclear cells [MNC]). The mononuclear blood cells expressed CD13, HLA-DR antigen, and CD33, but did not express CD14, CD15, or lymphoid marker antigens. The metachromatic cells expressed c-kit (70%) and some of them (15% to 20%) expressed CD11b and CD17, suggesting the presence of mast cells together with a small population of basophils. Mast cell lineage involvement was confirmed by elevated levels of cellular tryptase and histamine. A bone marrow smear showed signs of transformation, with 20% blasts and many metachromatic cells (16% of nucleated cells). Chromosomal analysis confirmed the t(9;22) and showed multiple additional abnormalities. Within 2 months, the patient developed a progressive increase in circulating blast cells (up to 63%), an increase in metachromatic cells (17%), and severe thrombocytopenia. The patient died 5 months after the onset of blast crisis.

**Case no. 2 (patient no. 29).** A 65-year-old man was first observed in October 1991. He suffered from refractory anemia, mild thrombocytopenia, splenomegaly, and erosive duodenitis. The WBC count was 4.2 x 10^9/L, the hemoglobin level was 8.8 g/dL, and the platelet count was 124.0 x 10^9/L. A differential count showed 36% neutrophils, 7% eosinophils, 25% metachromatic cells (atypical basophils), 1% monocytes, 26% lymphocytes, 1% myelocytes, 2% promyelocytes, and 2% blast cells. The blast cells expressed the HLA-DR antigen, c-kit, CD13, and CD33, but did not express CD14, CD15, or T- or B-cell antigens. The metachromatic cells expressed the c-kit antigen, but not CD11b, CD17, or Bsp-1 and the increased levels of histamine and tryptase in both bone marrow and peripheral blood cell lysates confirmed the presence of mast cell lineage cells. A bone marrow smear showed signs of dyserythropoiesis and dysgranulopoiesis, 15% blasts, and increased numbers of metachromatic mast cell–like cells (11% of nucleated cells). Cytogenetic and molecular (Southern blot for bcr-ab1) analyses showed a complex chromosomal pattern and absence of the Ph chromosome. Because the blast cell and WBC counts initially remained at a low level, no cytoreductive therapy was administered. However, after 3 months, he developed progressive disease with severe thrombocytopenia and splenomegaly and a blast cell increase and died in another hospital after an observation period of 6 months.

**Case no. 3 (patient no. 30).** An 81-year-old woman was first observed at the Vienna University Hospital in September 1989 because of refractory anemia, thrombocytopenia, leukocytosis, and gastric ulcer. The WBC count was 22.3 x 10^9/L, the hemoglobin level was 8.4 g/dL, and the platelet count was 68.0 x 10^9/L. CFU-GM were 50/10^3 blood MNC. The differential blood cell count showed 37% neutrophils, 3% metachromatic cells (atypical basophils), 33% monocytes, 14% lymphocytes, 3% metamyelocytes, 3% myelocytes, 1% promyelocytes, and 6% blast cells. A bone marrow smear showed 10% blast cells and signs of dyserythropoiesis, together with an increase of atypical metachromatic mast cell–like cells (6%). Chromosomal analysis showed a complex pattern and absence of the Ph chromosome. Correspondingly, no bcr-ab1 fusion gene could be detected in Southern blot analysis. The patient was treated with cytoreductive therapy (busulfan). In April 1990, rapid progression of disease with severe thrombocytopenia and an increase in circulating blast cells (63% blasts) and metachromatic cells (9%) was observed. The malignant cells expressed c-kit, the HLA-DR antigen, and CD33, but not CD14 or CD15. The metachromatic cells expressed c-kit, but not CD11b or CD17, as assessed by immunofluorescence analyses. Mast cell involvement was confirmed by cellular tryptase levels. The patient died shortly after admission.

**Preparation of Leukocytes**

Leukocytes were obtained from the peripheral blood (venipuncture) and (or) from bone marrow aspirates. MNCs were prepared (from heparinized samples) by Ficoll density gradient centrifugation. After washing, cells were either stored frozen in liquid nitrogen until use or were used immediately as fresh cells. Cell viability was more than 90% in all cases as assessed by dye (trypan blue) exclusion criteria. The human embryonic lung fibroblast cell line CCD-11Lu was obtained from American Type Culture Collection (Rockville,
Monoclonal Antibodies (MoAbs)

The following MoAbs were used in this study. The MoAbs VIM12 (CD11b), VIM-2 (anti-sialo-oligofucosyl-lactosamine), VIM-13 (CD14), VIM-D5 (CD15), VIB-C5 (CD24), and VID-1b (anti-HLA-DR) were kindly provided by O. Majdic and W. Knapp (Institute of Immunology, University of Vienna). The anti-kit MoAb YB5.84 was a kind gift from L.K. Ashman (University of Adelaide, Adelaide, Australia) and the basophil-reactive MoAb Bsp-1 was kindly provided by M. Bodger (Christchurch Hospital, MD) and was cultured in RPMI 1640 medium with 10% fetal calf serum (FCS).

### Table 1. Patients’ Diagnoses and Characteristics at the Time of Blood Sampling

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<th>Patient No.</th>
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<th>Hb (g/dL)</th>
<th>Platelets (10^9/L)</th>
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Abbreviations: MCS, metachromatic cells; MMA, patients with myeloid disease and mastocytic involvement.

**Monoclonal Antibodies (MoAbs)**

The following MoAbs were used in this study. The MoAbs VIM12 (CD11b), VIM-2 (anti-sialo-oligofucosyl-lactosamine), VIM-13 (CD14), VIM-D5 (CD15), VIB-C5 (CD24), and VID-1b (anti-HLA-DR) were kindly provided by O. Majdic and W. Knapp (Institute of Immunology, University of Vienna). The anti-kit MoAb YB5.84 was a kind gift from L.K. Ashman (University of Adelaide, Adelaide, Australia) and the basophil-reactive MoAb Bsp-1 was kindly provided by M. Bodger (Christchurch Hospital, MD) and was cultured in RPMI 1640 medium with 10% fetal calf serum (FCS).
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Christchurch, New Zealand). The CD17 MoAb T5A7 (antilactosylceramide) was obtained from the Fourth International Conference on Human Leukocyte Differentiation Antigens (Vienna, Austria 1990). The MoAbs MY-7 (CD13) and MY-9 (CD33) were purchased from Becton Dickinson (Sunnyvale, CA). Neutralizing anti-MGF MoAb was purchased from Genzyme (Boston, MA).

Indirect Immunofluorescence Staining Techniques

Flow cytometry. MNC were evaluated for cell surface marker expression by indirect immunofluorescence and flow cytometry as described.22 In brief, cells were incubated with MoAbs for 30 minutes (4°C), washed twice in phosphate-buffered saline (PBS; 4°C), and exposed to a fluorescein-labeled goat F(ab')2 anti-IgG, IgM antibody for 30 minutes. Thereafter, cells were analyzed by flow cytometry (FACS Star; Becton Dickinson).

Combined toluidine blue/immunofluorescence staining technique. To assess expression of cell surface antigens on circulating or bone marrow-derived, metachromatic cells, a combined toluidine blue/immunofluorescence staining technique was applied as described.22 In brief, MNC were stained with MoAbs and fluorescein isothiocyanate (FITC) conjugate (see above). Cells were then fixed in 0.025% glutaraldehyde for 1 minute. Cells were again washed and then exposed to toluidine blue (0.0125%) for 8 minutes at room temperature. Cells were washed and analyzed under bright field and fluorescence light.

Measurement of Mediators

Histamine and tryptase were measured in cell lysates after freeze-thawing by radioimmunoassay (histamine RIA [Immunotech, Marseille, France] and tryptase RIA [Pharmacia, Uppsala, Sweden]), as described previously.22 Mast cell growth factor (MGF/SCF) was measured in serum samples as well as in cell-free culture supernatants by enzymelinked immunoassay (ELISA), using MGF/SCF (Genzyme, Minneapolis, MN). The ELISA system for MGF showed a detection limit of 10 pg MGF/mL. No cross-reactivities with other cytokines (IL-1 through IL-12, tumor necrosis factors [TNFs], interferons [IFNs], transforming growth factors [TGFs], and colony-stimulating factors [CSFs]), tryptase, heparin, or histamine were observed.

Mast Cell Differentiation/Maturation Assay

Spontaneous and factor-dependent growth/differentiation in vitro of mast (precursor) cells was analyzed in long-term suspension culture, as described.23 In brief, peripheral blood or bone marrow-derived MNC were cultured in the presence (induced, factor-dependent growth) or absence (spontaneous mast cell growth) of recombinant human SCF (rhSCF; 100 ng/mL; Genzyme, Cambridge, MA). Cells were cultured in 24-well microculture plates (Costar, Cambridge, MA) with 10% FCS at 5°C to 37°C. Cells were fed weekly by careful removing the supernatants (50% of volume) and replacing the volume by fresh medium (plus FCS) and rhSCF/SCF or control medium. After 42 days of culture, cells were harvested and analyzed for the presence of mast cell (Giemsa staining) and mast cell tryptase. In inhibition experiments, cells were cultured with anti-MGF MoAb (5 µg/mL) or anti-c-kit MoAb YB5.B8 (5 µg/mL) in the presence or absence of rhSCF (100 ng/mL). Antibodies were added on day 0 and on the days when cells were fed (1-week intervals).

Karyotyping

Karyotyping was performed on unstimulated (24 hours) bone marrow cells according to standard techniques.25 Chromosomes were R-banded with acridine orange after denaturation with phosphate buffer. Karyotypes were classified according to criteria provided by the International System for The Human Cytogenetic Nomenclature (ISCN).11

Statistical Evaluation

Differences in mediator content and expression of mast cell markers were evidenced by statistical analyses. Standard tests, including the paired Student's t-test, were performed. Differences were considered significant when P was less than .05.

RESULTS

Clinical Findings and Course of Disease

Of the 52 patients tested, 3 were identified as having mast cell lineage involvement. These patients were characterized by expression of mast cell-related marker antigens on their circulating metachromatic cells and spontaneous, factor-independent differentiation of mast cells in vitro (Tables 2 and 3). Clinical characteristics were a progressive form of disease, splenomegaly, high percentages of metachromatic cells, and complex chromosomal abnormalities (Table 4). Two of the three patients suffered from RAEBIT and 1 patient suffered from blast crisis after a stable phase (lasting 6 years) of Ph chromosome-positive CML. No case history of a preceding systemic mast cell disease was evident. During the short course of disease, the blast cell counts rapidly increased and an elevation of metachromatic cells was observed. The survival time of the 3 patients (nos. 28, 29, and 30) was 4, 5, and 8 months, respectively, and were thus not significantly different from those of other patients with RAEBIT or CML-BC without mast cell lineage involvement (survival time of <2 years). Apparent clinical signs of mast cell activation were not noted in the 3 patients with mast cell lineage involvement. Two patients suffered from gastrointestinal erosions or ulcer and 1 patient had a transient urticaria-like exanthema and headache. However, these symptoms could not be definitively linked to mastocytic disease.

As differential diagnoses, CMMML with basophilia and basophil (blast) crisis of CML disease were considered at initial presentation. However, cell marker analyses of malignant cells excluded these diagnoses (Table 3). Monocytic leukemia was excluded because the malignant clone did not express monocytic-related antigens (CD14 and CD15) and basophil markers were not expressed (Table 3). Primary mast cell leukemia seems unlikely because of a secondary increase in mast cells after a prephase of myeloid disease and the apparent signs (complex karyotype and multilineage involvement) of a long lasting underlying myeloproliferative (patient no. 28) or myelodysplastic (patients nos. 29 and 30) process.

Morphology of Malignant Cells

The morphology of the leukemic cells in the 3 patients with mast cell involvement was studied on Giemsa-stained bone marrow and peripheral blood smears (Fig 1). The blast cells showed an immature myeloid phenotype with prominent nuclei and fine nuclear chromatin. Nucleoli could be detected in blast cells of all 3 patients. Some of the immature myeloblasts as well as the maturing cells exhibited mono-
Table 2. Biochemical Data in Patients With Kit Ligand-Independent Differentiation of Mast Cells

<table>
<thead>
<tr>
<th>Patient No./Diagnosis</th>
<th>% MCS</th>
<th>% c-kit&lt;sup&gt;+&lt;/sup&gt; PB MCS</th>
<th>Tryptase (ng/10&lt;sup&gt;5&lt;/sup&gt; PB MNC/mL)</th>
<th>Histamine (ng/10&lt;sup&gt;5&lt;/sup&gt; PB MNC/mL)</th>
<th>T/H Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>28/CML-BC</td>
<td>17</td>
<td>70</td>
<td>19</td>
<td>7.4</td>
<td>2.6</td>
</tr>
<tr>
<td>29/RAEBIT</td>
<td>25</td>
<td>95</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>30/RAEBIT</td>
<td>9</td>
<td>95</td>
<td>11.4</td>
<td>10.4</td>
<td>1.1</td>
</tr>
<tr>
<td>RAEBIT except 29/30 (n = 6)</td>
<td>&lt;5</td>
<td>&lt;10</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>—</td>
</tr>
<tr>
<td>CML-BC except 28</td>
<td>&lt;5</td>
<td>NT</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>—</td>
</tr>
<tr>
<td>CML-BC/basophilia*</td>
<td>5-29</td>
<td>-5</td>
<td>&lt;1</td>
<td>1-4.1</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>CML, stable (n = 4)</td>
<td>1-12</td>
<td>&lt;5</td>
<td>&lt;1</td>
<td>3-20</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>Normal donors (n = 5)</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>—</td>
</tr>
<tr>
<td>AML (n = 5)</td>
<td>&lt;3</td>
<td>NT</td>
<td>&lt;5</td>
<td>0.9-8.8</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

Abbreviations: MCS, metachromatic cells; NT, not tested.

* Patients with CML-BC exhibiting 5% to 29% basophils and no signs of mast cell lineage involvement.

cyte-like features with lobulated nuclei (Fig 1B), although, by immunophenotyping, monocyte markers were not expressed on leukemic cells (Table 3). However, the bilobulated or multilobulated monocytoid nuclei have recently been described for mast cell progenitors. The metachromatic cells had a lobulated or compact round nucleus. The chromatin pattern in these cells varied in shape and structure. By morphologic criteria alone it was impossible in most cases to decide whether a given cell would belong to the basophil or mast cell lineage. Figure 1A shows metachromatic cells with mast (precursor) cell features in patient no. 29. Mast cells (with typical morphologic characteristics of tissue mast cells) could not be detected. However, in patient no. 28 (CML-BC), eosinophilic and basophilic granulocytes could be detected together with mast cell-like cells. Electron microscopy was also performed in these patients. The ultrastructure of the cells showed immature myeloid progenitor cells and mast cell-like features (pro-granules, mast cell granules, and scrolls) in a subset of cells (manuscript in preparation).

Expression of Mast Cell-Related Mediators in Leukemic Cells

Mast cells express a unique composition of antigens, including tryptase (a mast cell marker enzyme) and histamine. Basophils, in contrast, express histamine, but almost no detectable amounts of tryptase. In this study, patients with mast cell lineage involvement were characterized by high levels of cellular tryptase in their circulating MNC (>10 ng/10<sup>5</sup> MNC/mL) and high levels of cellular histamine. The tryptase/histamine ratio (ng:ng basis) in patients with mast cell lineage involvement was greater than 1. By contrast, in all hematologic disease states with elevated numbers of basophils, the tryptase/histamine ratio was clearly less than 1 (0.16 ± 0.19) and the tryptase levels were less than 5 ng per 10<sup>5</sup> cells/mL (see Table 2).

Measurement of c-kit Ligand/MGF by ELISA

To assess whether mast cell involvement could by a secondary phenomenon caused by overexpression of mast cell growth factor MGF (c-kit ligand), we measured serum MGF/

Table 3. Cell Surface Marker Expression on PB MNC and on Metachromatic PB Cells (MCS)

<table>
<thead>
<tr>
<th>MoAb</th>
<th>CD</th>
<th>AG</th>
<th>% Reactivity of PB MNC/MCS</th>
<th>With MoAbs</th>
</tr>
</thead>
<tbody>
<tr>
<td>VIM-12</td>
<td>11b</td>
<td>C3bi R</td>
<td>62/20</td>
<td>NT/5</td>
</tr>
<tr>
<td>VIM-13</td>
<td>14</td>
<td>LPSRAg</td>
<td>02/5</td>
<td>01/5</td>
</tr>
<tr>
<td>VIM-D5</td>
<td>15</td>
<td>3-FAL</td>
<td>11/5</td>
<td>12/5</td>
</tr>
<tr>
<td>T5A7</td>
<td>17</td>
<td>Lactoceramid</td>
<td>NT/5</td>
<td>NT/5</td>
</tr>
<tr>
<td>IgE/algE</td>
<td>NC</td>
<td>IgE R I</td>
<td>04/50</td>
<td>&lt;5/20</td>
</tr>
<tr>
<td>VID-1b</td>
<td>NC</td>
<td>HLA-DR</td>
<td>67/5</td>
<td>NT/5</td>
</tr>
<tr>
<td>YB5.BB</td>
<td>117</td>
<td>c-kit R</td>
<td>32/70</td>
<td>71/90</td>
</tr>
</tbody>
</table>

MCS were analyzed by combined toluidine blue/immunofluorescence staining (see text). MNC were analyzed by flow cytometry. Abbreviations: NT, not tested; NC, not yet clustered.
Fig 1. Morphology of meta-chromatic cells. (A) A bone marrow smear in patient no. 29. (B and C) Metachromatic cells obtained from patient no. 30. Giemsa staining showed a sub-population of immature metachromatic cells with a mast cell-like phenotype. These cells were mononuclear or had lobulated nuclei. Note that lobulated nuclei are also present in immature, agranular myeloid cells (B).

SCF levels in patients with mast cell involvement as well as in other patients and in healthy controls. Serum SCF levels in normal individuals (n = 5) ranged between 900 and 2,100 pg/mL, corresponding to the data published by Langley et al. In the patients with mast cell lineage commitment, serum MGF levels were 1,350 pg/mL (patient no. 28), 1,070 pg/mL (patient no. 29), and 1,120 pg/mL (patient no. 30), respectively. Serum MGF levels in systemic mastocytosis, in myeloid leukemias, and in myelodysplastic disorders were within or below the normal range (manuscript in preparation). To further exclude a reactive process leading to mast cell differentiation in vitro, we also measured MGF levels in cell-free culture supernatants in long-term culture (LTC). However, MGF could neither be detected in LTC established with MNC obtained from CML-BC/RAEBIT patients with mast cell involvement nor in control LTC (Table 5), whereas significant levels of MGF (>100 pg/mL) were detected in human lung fibroblast (CCD-11Lu) supernatants.

Expression of Cell Surface Antigens on Leukemic Cells

Expression of cell surface antigens was examined on MNC and blast cells as well as on the metachromatic cell fractions. In the patients with mast cell lineage involvement, the MNC were found to express HLA-DR antigen and a subset of cells (30% to 71%) expressed c-kit, as assessed by flow cytometry (Table 3). In contrast, we were unable to detect substantial amounts of CD14 or CD15 on these cells, nor any lymphoid marker antigens. Metachromatic cells were analyzed by a combined toluidine blue/immunofluorescence staining technique, as described. In this study, mast cell-related antigens were found to be expressed on circulating metachromatic cells obtained from our MDS/CML patients with mast cell involvement. In particular, these cells expressed the c-kit antigen, and a subset of cells expressed IgE binding sites (Table 3). In the 2 RAEBIT patients with mast cell disease, basophil-related antigens (Bsp-1, CD11b, and CD17) could not be detected. In the CML-BC patient with mastocytic involvement, basophil antigens were expressed on a small subset of metachromatic cells (15% to 20%). In CML (stable phase) and in 5 normal donors, all the circulating metachromatic cells (>95%) expressed CD11b and CD17 and the majority expressed Bsp-1, whereas these cells did not react with c-kit MoAb YB5.B8.
Immunophenotypic analyses and respective data are summarized in Table 3.

**Chromosomal Pattern**

All 3 patients with mastocytic involvement had a complex chromosomal pattern with near diploid karyotypes. Patient no. 28 had a typical Ph chromosome translocation, whereas patients no. 29, and 30 had not. Chromosomal abnormalities were expressed in multiple clones and frequently involved chromosomes 5, 7, 8, 9, 12, 13, 17, and 18. Loss of 5q14-q23, 7q32–qter, 17p, and 18q21–qter and overexpression of chromosomes 8 and 21 were noted. However, no consistent chromosomal abnormality could be detected. Chromosomal regions coding for the c-kit proto-oncogene were expressed in multiple clones and frequently involved chromosomes 4q11–q13 and for the c-kit ligand SCF/MGF (12q22–q24) were not affected. The karyotypes of the 3 patients (nos. 28, 29, and 30) suffering from mastocytic disease are depicted in Table 4. Other patients with RAEBIT or CML-BC (without mast cell lineage involvement) also displayed complex chromosomal abnormalities and/or the Ph chromosome (CML-BC patients; see also Table 1).

**Factor (c-kit ligand/MGF)-Dependent and -Independent Formation of Mast Cells In Vitro**

To show factor-dependent growth/differentiation of mast cells in vitro, the patients’ MNC were exposed to rhMGF or control medium in long-term (day 42) suspension culture. In almost all patients tested (with the exception being phebaldism and aplastic anemia), rhMGF/SCF induced formation of mast cells and cellular tryptase in LTC. A pronounced increase in cellular tryptase (measured on day 42) in response to rhMGF was found in patient no. 28 (1,515 ng/mL) and patient no. 29 (4,890 ng/mL) when compared with other disease states or controls (<1,000 ng/mL; see Table 5).

In the majority of patients, spontaneous differentiation of mast cells could not be detected. However, in all 3 patients diagnosed as having mast cell lineage involvement, factor-independent formation of mast cells in vitro was demonstrated (Fig 2 and Table 5). In particular, the total number of metachromatic cells and the total amount of cellular tryptase increased in culture over the 42-day culture period. The increase in tryptase in patients with mastocytic involvement was sevenfold (patient no. 28) and 1.4-fold (patient no. 30). Day 0 values for patient no. 29 were not available. The number of metachromatic cells increased by 8.8-fold (patient no. 28), 11-fold (patient no. 29), and 1.7-fold (patient no. 30). In patients no. 28 and 30, MGF-independent growth of mast cells was tested at two different time points (at least 1-month interval) and the data showed a reproducible increase of cellular tryptase in culture (data not shown). Factor-independent mast cell growth could neither be inhibited by addition of MoAb to c-kit nor by MoAb against SCF/MGF (Fig 3A), whereas factor (SCF)-dependent growth was inhibited by addition of antibodies to either c-kit or SCF (Fig 3B).

**DISCUSSION**

Factor-independent growth and/or differentiation of malignant cells in preleukemic and leukemic disease states has been described and often is associated with a poor prognosis. In this study, we have defined a unique form of myeloblastic transformation in MDS and CML. This type of transformation is characterized by spontaneous, factor (MGF)-independent growth/differentiation of mast cells, a complex chromosomal pattern, elevated numbers of circulating metachromatic cells expressing c-kit, elevated levels of cellular tryptase, and a tryptase/histamine ratio greater than 1. These patients suffered from rapid progression of disease (thrombocytopenia and increase in blast cells) and had a survival time of less than 1 year. It is important to know this type of progression in MDS and CML and to delineate it from stable-phase CML with basophilia, basophil crisis of CML, or CMML with basophilia. Using mast cell-related markers, the diagnosis of mast cell lineage involvement in CML-BC or RAEBIT can be established rapidly.
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suggesting the presence of mast cell-lineage cells. In contrast, basophil-related myeloid antigens (CD11b, CD17, and Bsp-1) could not be detected in the 2 RAEBIT patients with mastocytic involvement. However, in the CML-BC patient with mast cell involvement, a small subset of metachromatic cells (20%) expressed CD11b and CD17. This finding suggests that basophils and mast cells were present which may be caused by the pluripotent stem cell origin of the CML clone, capable of giving rise to both types of cells (basophils and mast cells). The IgE receptor was only expressed on a subset of metachromatic cells in our patients. This might be due to the immature stage of differentiation of cells and extremely low levels of expressed receptors. Interestingly, mast cells grown from their precursor cells in rhMGF in LTC, as well as HMC-1 cells (mast cell leukemia cell line), also lack substantial amounts of IgE receptors.

A well-established mast cell marker is the enzyme tryptase. Although the amount of tryptase (per cell) may vary between immature and mature mast cells, substantial amounts of this enzyme are only found in mast cell lineage cells but neither in blood basophils nor in any other circulating blood cell. In this study, mast cell lineage involvement was confirmed for all 3 patients by elevated levels of cellular tryptase and histamine and a tryptase/histamine ratio greater than 1. In contrast, patients suffering from CML or MDS with basophilia expressed only low or undetectable levels of cellular tryptase in their peripheral blood MNC and a tryptase/histamine ratio of less than 1 (ie.0.16 ± 0.19). How-

So far, mast cell lineage involvement in preleukemic or leukemic myeloid disorders has rarely been described. This rarity might be caused by the fact that reliable mast cell markers have only been described very recently. A problem in most cases is the difficulty to delineate the basophil from the mast cell lineage. Mast cells and basophils are metachromat cells, express IgE-binding sites, and produce histamine. However, mast cells differ from blood basophils in their biochemical and immunologic phenotype and clearly form a unique cell lineage within the hematopoietic cell system. In this study, the metachromatic cells in the 3 patients with mastocytic disease expressed the c-kit antigen suggesting the presence of mast cell-lineage cells. In con-

Fig 2. Spontaneous formation of tryptase in LTC. Peripheral blood MNC (pb) of 2 normal donors and of 44 patients as well as bone marrow (bm) of 5 patients were cultured in RPMI 1640 medium plus 10% FCS and antibiotics at 37°C for 42 days. On day 42, cells were lysed and, after freeze thawing, the total amount of the mast cell-specific enzyme tryptase was determined by ELISA. The figure shows the total tryptase amounts on day 42. Bone marrow (bm) of patient no. 30 is indicated as RAEBIT/MMA (bm), MMA, myeloid leukemia disease with mastocytic involvement. Systemic mastocytosis/urticaria pigmentosa patients (SM/UP) were analyzed for peripheral blood (pb) precursor cell growth.

Fig 3. Effects of anti-MGF and anti-kit MoAb on MGF-dependent and -independent formation of mast cells. The patients' MNC were cultured in the presence or absence of rhMGF (100 ng/mL), anti-c-kit MoAb YB5-B8 (5 μg/mL) or anti-SCF MoAb (5 μg/mL) for 42 days, as described in the text. Thereafter, total tryptase values were measured. (A) Tryptase values obtained in cultures (patient no. 28) grown in the presence of control medium (CO; without SCF). (B) Values obtained in cultures grown with rhSCF/MGF in comparison with control medium (CO).
ever, despite an increase in (intra)cellular mediators, no severe clinical signs of mast cell activation were noted.

A potential association between mastocytosis and myelo-
dysplastic or myeloproliferative disorders has recently been described.55-59 Horny et al55 reported a simultaneous occurrence of (malignant) mastocytosis and myeloproliferative disorders. Rothenberg et al56 described two patients suffering from chronic myelogenous leukemia with biochemical features (heparin and PGI2 production) suggesting involvement of the mast cell lineage. Other investigators have described ultrastructural signs of mast cell lineage involvement in CML blast crisis.57 In this study, we have used a number of different mast cell markers and have accumulated evidence that indeed mast cell lineage involvement and spontaneous differentiation of mast cells in MDS or CML-BC can occur. The accurate frequency of mast cell lineage involvement in acceleration/transformation of MDS or CML disease remains to be determined. However, in the light of our data, this may be a more frequent event than has so far been assumed. We believe that in our cases, mast cell lineage involvement was a secondary event after progenitor cell deregulation, because mast cell lineage cells increased in number after a prephase of myeloid disease. Indeed, the complex chromosomal pattern found in myeloid precursor cells suggests a long-lasting prephase of deregulation. On the other hand, it cannot totally be ruled out that these patients suffered from asymptomatic mastocytosis (long) before the onset of the leukemic disease. Indeed, myeloblastic transformation in mastocytosis patients has recently been reported.58

Autonomous growth and/or differentiation of cells in leu-
kemic disease states is often associated with a poor prognosis.7,8 For example, AML patients with factor-independent proliferation of leukemic blast cells have a shorter survival time compared with AML patients without autonomous growth.7 In CML, the (factor-independent) increase of basophils indicates progression of disease and short duration of stable phase of leukemia.8-10 In this study, an increase of circulating metachromatic cells together with spontaneous growth of mast cells was found. The short survival time in these patients would be in agreement with the above hypothesis and a poor prognosis. It also would be in agreement with the finding that primary mast cell leukemia patients have a very unfavorable prognosis.8,9 However, so far, the number of cases is too small to predict that mast cell lineage involvement in CML-BC or RAEBIT is invariably associated with a poor prognosis.

The mechanisms underlying factor-independent differenti-
ation of mast cells in our patients remain unknown. Endoge-
nous overproduction of mast cell growth factor MGF (eg, by leukemic cells) seems rather unlikely. Thus, MGF serum levels in the 3 patients were within the normal range compared with other myeloproliferative diseases and with controls. Furthermore, MGF could not be detected in cell-free supernatants in long-term suspension cultures (established from patients’ cells). Finally, neutralizing anti-MGF or anti-c-kit antibodies did not affect spontaneous formation of mast cells but significantly blocked MGF-induced differentiation of mast cells in LTC. This finding points to a cellular, KL/ MGF-independent defect and evolution of mast cells from the malignant clone.

Factor (MGF)-dependent differentiation of mast cells and tryptase formation in vitro in patients with mast cell lineage involvement was enhanced compared with other disease states and controls. This difference might be caused by hyperresponsiveness of progenitor cells to MGF, as recently described for other growth factors.63,64 Alternatively, the increase in mast cells in culture was caused by an increased number of (normal responsive) mast cell (committed) progenitor cells. A third possibility could be that differentiated mast cells in these patients had a longer survival time as compared with normal mast cells in vitro.

The genes coding for MGF and MGF receptor (c-kit) are located on human chromosomes 12 and 4, respectively.50,54 The karyotypes of the blast cells in patients with mastocytic disease showed a complex pattern and a Ph chromosome in patient no. 28. However, no consistent karyotype abnormality was found and chromosomes 4 and 12 were not involved or affected in a specific manner. Recently, point mutations in the tyrosine kinase domain of the c-kit receptor (RTK family, subclass III) in myelodysplasia and in a mast cell leukemia cell line have been described.65-67 Whether point mutations in the MGF or MGF receptor (c-kit) genes or other defects in the MGF-c-kit pathway were responsible for mast cell lineage involvement in our patients is currently under investigation. Alternatively, spontaneous differentiation of mast cells was caused by other cellular defects involving less well-defined regulators of mast cell lineage commitment.

In summary, we have defined a unique form of transforma-
tion in CML or MDS/RAEB/T, characterized by a complex chromosomal pattern, splenomegaly, high numbers of circulat-
ing metachromatic cells, and mast cell lineage involve-
ment. This form of transformation has to be delineated from CML with basophilia, basophil blast crisis, primary mast cell leukemia, and MDS/CMLL with basophilia.

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