MASTOCYTOSIS is a rare disorder with manifestations either in the skin, as urticaria pigmentosa, or as a generalized form, systemic mastocytosis, or even as mast cell leukemia. Anemia, leukocytosis, thrombocytopenia, or eosinophilia may be present. An increased number of mast cells in the bone marrow and lesions with varying numbers of lymphocytes, eosinophils together with mast cells, may be found.

Certain patients with mastocytosis can initially be diagnosed as having or may later develop a myeloproliferative disorder. Many patients with myeloproliferative diseases have chromosome abnormalities in bone marrow cells; however, cytogenetic analysis has been reported in only a few cases with mastocytosis and normal karyotypes were then observed. The in vitro colony-forming capacity of bone marrow granulocytic-macrophage stem cells can also show an abnormal growth pattern in myeloproliferative disorders. Animal cell culture studies indicate that the mast cell may be of bone marrow origin. Due to these findings it would be of interest to investigate patients with mastocytosis with techniques that have revealed abnormalities in the myeloproliferative disorders. This paper reports on cytogenetic studies and bone marrow colony growth in 13 patients with mast cell disease. To our knowledge, no systematic studies have previously been carried out.

MATERIAL AND METHODS

Patients. Thirteen adult patients (median age 40 years) with mastocytosis participated, after informed consent, in the present study. Clinical data are presented in Table 1. The six patients (four males, two females) with systemic mastocytosis were 30 to 69 years of age (median 58 years) with a disease duration varying from 2 to 27 years (median 5.5 years). The age ranged between 19 and 60 years (median 28 years) for the seven patients (two males, five females) with urticaria pigmentosa and was lower than for patients with systemic mastocytosis. The disease duration was also shorter varying from 1 to 5 years (median 3 years).

The diagnosis of the skin disease was based on clinical criteria for urticaria pigmentosa, ie, multiple red-brown macules that urticate after rubbing. The extension of the skin disease was calculated from the number of macular skin lesions, which was estimated as small, moderate, or large. The severity of the pruritus was estimated by each patient. None of the patients had a history of flush attacks, headache, or decreasing BP.

The clinical diagnosis was confirmed in all the patients by histological examination of punch biopsies from hyperpigmented macular spots from the abdominal skin. The diagnosis of systemic mastocytosis was based on direct demonstration of an increased number of mast cells in the bone marrow section and an increase in the urinary excretion of methylimidazole acetic acid. Patient no. 6 with systemic mastocytosis had an increased number of mast cells in the bone marrow but normal excretion of methylimidazole acetic acid in urine. Splenomegaly was found in three patients (Table 1).

Control group. For comparison, the in vitro bone marrow colony growth capacity for granulocytic-macrophage stem cells was studied in nine subjects (seven males and two females) aged 23 to 70 years with a median age of 39 years, after informed consent. These healthy subjects had normal peripheral blood values and bone marrow morphology.

Sampling procedure. Bone marrow (1 to 2 mL) from the sternum or iliac crest was aspirated. The cells were suspended in 3 mL McCoy’s medium (Flow Laboratories) containing 125 IU heparin (Kabi Vitrum) for colony growth and the marrow was suspended in 6 mL McCoy’s medium for cytogenetic analysis.

The assay of colony formation was measured by using a two-layered semisolid, agar culture system described by Pike and Robinson. Placenta extract was used as source of the colony-stimulating factor, according to Burgess et al, instead of using mononuclear cells in the feeder layer. The top layer was seeded with 1 x 10^6 mononuclear cells per milliliter per dish. The cells were isolated on Isopaque-Ficoll. The cultures were incubated at 37°C, in a humidified atmosphere of 5% CO2 in air. Four dishes for each culture were used as for unstimulated controls, the mean value was given. After seven and ten days the dishes were examined with an inverted microscope. Aggregates of more than 40 cells were defined as colonies, 21 to 40 cells as macroclusters, and three to 20 cells as microclusters. Under these conditions the highest value for colonies was attained on day 10 and the colony growth reached a plateau in the amount of placenta extract used. Growth was linear for up to ten times the cell concentration used. The reproducibility was good and tested from two sequential cultures in six subjects.

In the last six patients examined, the dishes were fixed and stained with May-Grünwald-Giemsa to evaluate the morphology of the blood values. Two patients with systemic mastocytosis had clones with chromosome abnormalities and some abnormal hematological values. The proportion of patients with chromosome abnormalities and an abnormal growth pattern was higher among these patients with mastocytosis than in healthy control subjects. These results may be of interest when discussing the origin of mast cell disorders and indicate an association with the myeloproliferative disorders.

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CHROMOSOMES AND COLONY GROWTH IN MASTOCYTOSIS

Table 1. Clinical Findings in 13 Patients With Mastocytosis

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age at Examination (y)/Sex</th>
<th>Duration of Disease (y)</th>
<th>No. of Macular Skin Lesions</th>
<th>Pruritus</th>
<th>Spleen Area Scintigraphy (cm²)</th>
<th>Increased No. of Mast Cells in Bone Marrow Section</th>
<th>U-MeImAA (mg/24 h)</th>
<th>Clinical Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>69/M</td>
<td>2</td>
<td>Small</td>
<td>Mild</td>
<td>195</td>
<td>Yes</td>
<td>58.2</td>
<td>SM</td>
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<tr>
<td>2</td>
<td>63/F</td>
<td>27</td>
<td>Large</td>
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<td>Yes</td>
<td>48.4</td>
<td>SM</td>
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<tr>
<td>3</td>
<td>44/F</td>
<td>20</td>
<td>Large</td>
<td>Moderate</td>
<td>Normal</td>
<td>Yes</td>
<td>22.8</td>
<td>SM</td>
</tr>
<tr>
<td>4</td>
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<td>5</td>
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<td>7.1</td>
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<tr>
<td>5</td>
<td>56/M</td>
<td>6</td>
<td>Large</td>
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<td>110</td>
<td>Yes</td>
<td>4.9</td>
<td>SM</td>
</tr>
<tr>
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<td>60/M</td>
<td>3</td>
<td>Moderate</td>
<td>Mild</td>
<td>104</td>
<td>Yes</td>
<td>3.3</td>
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<tr>
<td>7</td>
<td>39/F</td>
<td>1</td>
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<td>No</td>
<td>Normal</td>
<td>No</td>
<td>2.5</td>
<td>UP</td>
</tr>
<tr>
<td>8</td>
<td>40/F</td>
<td>3</td>
<td>Small</td>
<td>Moderate</td>
<td>Normal</td>
<td>No</td>
<td>1.9</td>
<td>UP</td>
</tr>
<tr>
<td>9</td>
<td>19/F</td>
<td>5</td>
<td>Small</td>
<td>No</td>
<td>Normal</td>
<td>No</td>
<td>1.9</td>
<td>UP</td>
</tr>
<tr>
<td>10</td>
<td>60/F</td>
<td>4</td>
<td>Moderate</td>
<td>No</td>
<td>Normal</td>
<td>No</td>
<td>1.8</td>
<td>UP</td>
</tr>
<tr>
<td>11</td>
<td>25/M</td>
<td>5</td>
<td>Large</td>
<td>Moderate</td>
<td>Normal</td>
<td>No</td>
<td>1.8</td>
<td>UP</td>
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<tr>
<td>12</td>
<td>28/M</td>
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<td>-</td>
<td>1.6</td>
<td>UP</td>
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<tr>
<td>13</td>
<td>19/F</td>
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<td>No</td>
<td>Normal</td>
<td>No</td>
<td>1.0</td>
<td>UP</td>
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</table>

Abbreviations: SM, systemic mastocytosis; UP, urticaria pigmentosa.
*Scintigraphic examination of the spleen was performed according to Larsson and Neip in 1966. Reference value <81 cm².†See Ridell et al. The upper limit of mast cells for reference subjects is 71/mm².
‡U-MeImAA (urine methylimidazole acetic acid) was measured by high performance liquid chromatography as described by Granerus. Reference values = 1.2 to 3.8 mg/24 h.

The enumerated colonies and clusters included neutrophils, macrophages, eosinophils, and mast cells, as determined by the morphological appearance with this staining.

Chromosomes from bone marrow cells were prepared with conventional techniques. When no evaluable metaphases could be obtained in direct preparations, marrow cells were also studied after 48 hours in culture. G- and Q-banding was used. Chromosome identification and karyotypes were classified in accordance with the International System for Human Cytogenetic Nomenclature, ie, a clone was defined as at least two cells with the same numerical and or structural abnormality.

Routine laboratory techniques were used for the determination of peripheral blood parameters. Plasma cobalamines (B₁₂) and folates concentrations were assayed with microbiological techniques.

RESULTS

The laboratory results are presented in Table 2. The hemoglobin concentration was normal in all patients except two (cases no. 1 and 2) with systemic mastocytosis (Table 2). The leukocyte counts were normal in all patients. Differential leukocyte count showed no significant abnormality; however, slight eosinophilia was found in patients no. 2 and 3. Patient no. 1 exhibited an increased platelet number, while all other patients had normal levels. All patients had serum iron and total iron binding capacity within reference values. Patients no. 1 and 2 exhibited an increased plasma B₁₂ concentration and none of the patients had been given drugs containing B₁₂. Plasma and blood folates levels were normal in all patients.

The cytogenetic analysis of the bone marrow cells revealed clones with chromosome abnormalities in five patients (Table 3, Fig 1). Clones were present only to a minor extent, except in patient no. 8, and were revealed in three patients after a 48-hour culture. The type of abnormality was 5q- , 9p+ , 11q- , +13 and -16 , +mar in each patient. In patient no. 1 a loss of one no. 16 was seen and it cannot be excluded that the marker derives from no. 16. We regard the deletions in patients no. 2 and 6 as interstitial deletions. Patient no. 8 had some extra unidentified material on the short arm of...
Table 3. Results of Cytogenetic Analyses and In Vitro Cultures for Granulocytic-Macrophage Progenitor Cells
From Bone Marrow in Patients With Mastocytosis

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Bone Marrow Karyotype</th>
<th>No. of Cells Karyotyped</th>
<th>Proportion of Abnormal Clone (%)</th>
<th>Number of Clusters and Colonies for CFU-GM/10^6 Cells</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Microcluster</td>
</tr>
<tr>
<td>1</td>
<td>46,XY/46,XY,-16,+mar</td>
<td>28</td>
<td>21</td>
<td>208</td>
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<tr>
<td>2</td>
<td>46,XX/46,XX,del(5)(q21q31)</td>
<td>25*</td>
<td>13</td>
<td>252</td>
</tr>
<tr>
<td>3</td>
<td>46,XX</td>
<td>13</td>
<td>0</td>
<td>140</td>
</tr>
<tr>
<td>4</td>
<td>46,XY</td>
<td>11</td>
<td>0</td>
<td>359</td>
</tr>
<tr>
<td>5</td>
<td>46,XY</td>
<td>14</td>
<td>0</td>
<td>373</td>
</tr>
<tr>
<td>6</td>
<td>46,XY/46,XY,del(11)(q13q21)</td>
<td>11*</td>
<td>18</td>
<td>1029</td>
</tr>
<tr>
<td>7</td>
<td>46,XX</td>
<td>14</td>
<td>0</td>
<td>268</td>
</tr>
<tr>
<td>8</td>
<td>46,XX,9p+/46,XX</td>
<td>24</td>
<td>83</td>
<td>362</td>
</tr>
<tr>
<td>9</td>
<td>46,XX/46,XX+G/47,XX,+13</td>
<td>11*</td>
<td>27/18</td>
<td>231</td>
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<tr>
<td>10</td>
<td>46,XX</td>
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<td>0</td>
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<td>13</td>
<td>46,XX</td>
<td>14</td>
<td>0</td>
<td>361</td>
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</tbody>
</table>

Patients (n = 13), median (range) 356 (140-1029) 220 (13-343) 75 (0-438) 80 (4-242)

Control group (n = 9), median (range) 344 (173-907) 126 (76-267) 54 (1-220) 47 (22-168)

*Prepared after 48-h culture.

chromosome No. 9, which did not seem to be a pericentric inversion, however C banding was not performed. The extra G chromosome in patient no. 9 bore the closest resemblance to a no. 22. The other eight patients exhibited normal karyotypes. All patients had a normal karyotype in phytohemagglutinin stimulated peripheral blood cells.

Patients no. 1 and 2 with an abnormal karyotype had both anemia and elevated plasma histamine-concentrations.

The colony growth capacity in all the patients examined showed a median value of 356 (range 140 to 1,029) on day 7 for microclusters, 220 (range 13 to 343) for macroclusters, and 75 (range 0 to 438) for colonies (Table 3). On day 10, the median value was 80 for colonies (range 4 to 242). No obvious difference could be observed between the patients with systemic mastocytosis and those with urticaria pigmentosa. In comparison with the control group the number of colonies was nearly twice as numerous while the cluster counts were of the same order of magnitude. The largest number of clusters was reached after a seven-day culture. An increased number of clusters was found in patients no. 4, 6, 8, and 13. The number of colonies reached a peak after seven days of culture for seven patients, four of whom showed an increased number in comparison with the control group. Six patients had a larger number of colonies on day 10, but all had values within the reference values. Only patient no. 11 had defective colony growth.

The morphology in May-Grünwald-Giemsa stained clusters and colonies could be evaluated in patients no. 1, 3, 4, 7, 9, and 13. All patients exhibited cells with features similar to mast cells, especially with regard to the cell size and the...
heavy content of basophilic granules; however, further evaluation is necessary. Three of the five patients with chromosome abnormalities showed an abnormal in vitro growth pattern for granulocytic-macrophage stem cells.

**DISCUSSION**

Disorders with mast cell proliferation include a wide variety of manifestations, from benign infiltration of the skin, urticaria pigmentosa, to the malignant course seen in mast cell leukemia. An intriguing connection between both myeloid- and lymphoproliferative disorders has been discussed in case reports. This is the first report on a small series of patients with mastocytosis investigated by cytogenetic analysis. Chromosome abnormalities in bone marrow cells were found in 36% of the patients, which is a higher proportion than in healthy subjects. In myeloproliferative disorders the incidence of abnormalities varies from 85% in patients with chronic granulocytic leukemia with the Ph1 chromosome to ~15% in patients with polycythemia vera close to the diagnosis. Some patients with chronic myeloid leukemia develop basophilia, and in a series of patients with chronic myeloid leukemia the chromosome abnormality isol(7q) in addition to the Ph1 abnormality, was related to high values of basophils. This abnormality, however, was not found in the present series of patients with mastocytosis. The abnormalities 5q- and 11q- found in two patients are also common abnormalities in myeloid disorders and in myelodysplastic syndromes.

In seven patients, the in vitro colony growth pattern of bone marrow cells showed an increased number of clusters and/or colonies similar to findings recently reported by Denburg et al. They found an increased number of colony-forming units in peripheral blood from patients with systemic mastocytosis. This pattern is more like the pattern found in chronic myeloid leukemia, than that found in myelodysplastic syndrome with defective or no colony forming capacity. An increased number of histamine positive granulocytic colonies from blood has been found in patients with systemic mastocytosis and chronic myeloid leukemia. It would have been desirable to identify the cell type that contained the chromosome abnormalities. However, techniques for such identification were not available to us.

The occurrence of chromosome aberrations or increased growth capacity was similar in patients with urticaria pigmentosa and systemic mastocytosis. There was no obvious relationship between the cytogenetic results and the colony growth seen in this small series. However, three patients had both an abnormal karyotype and abnormal growth capacity, yet they still exhibited normal peripheral blood values.

All the patients with urticaria pigmentosa had normal hemoglobin, leukocyte, and platelet concentrations, as has been described in earlier reports. In the group with systemic mastocytosis, one patient had anemia and thrombocytosis, and another had anemia and eosinophilia. These findings are in agreement with those in the reports on larger numbers of patients. These two patients also had clones with chromosome abnormalities, as well as an increased plasma B12 concentration, a common finding in myeloproliferative disorders.

Bone marrow cells from our patients with mastocytosis showed chromosome aberrations and an abnormal in vitro colony growth similar to findings in myeloproliferative disorders and thus supports the idea of an association between these disorders. The hypothesis that mast cells derive from a hematopoietic origin is supported by observations obtained with bone marrow mast cells, grown in long-term culture from a patient with mastocytosis. An increased number of bone marrow mast cells has been reported in preleukemic syndromes. Experimental data suggest that the production of granulocyte-macrophage colony stimulating factor can be activated in tumorigenic mast cell lines derived from Abelson murine leukemia virus-transformed hematopoietic cells. This observation may be one of the possibilities to explain the increased growth capacity seen in some of our patients.

We intend to follow-up this group of patients with repeated examinations, both with regard to their cytogenetic evolution and their colony growth pattern. The preliminary observation, in particular that mast cells grow from the bone marrow of patients both with systemic mastocytosis and urticaria pigmentosa, needs to be confirmed with other methods.

**REFERENCES**

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Cytogenetic studies and in vitro colony growth in patients with mastocytosis

B Swolin, S Rodjer and G Roupe