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A Follow-up Study of Bone Marrow Chromosomes and *In vitro* Colony Growth in Patients with Mastocytosis

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Proliferation of mast cells can give rise to many clinical manifestations in patients, and an association with hematological disorders has been pointed out. The study was initiated to determine whether patients with mastocytosis show a clinical progression in relation to bone marrow cell parameters analyzed. During a median follow-up period of 5.5 years, 10 patients with mastocytosis were re-examined with regard to clinical symptoms, urine histamine metabolites (U-MeImAA), bone marrow cells examined with chromosome analyses, and in vitro stem cell growth for CFU-GM. Seven patients showed a clinical progression with increase of either symptoms, bone marrow infiltrates of mast cells or U-MeImAA. One patient with a myeloproliferative bone marrow morphology had a malignant course. Four of the 7 patients showed an increased colony growth, while 3 showed a decreased growth, in the second examination compared with the first examination. One patient had a persistent clone with the chromosome aberration 9p+. A variable pattern was observed in the other patients, in resemblance with findings in chronic myeloproliferative disorders. Our conclusion is that mastocytosis belongs to the myeloproliferative disorders. ***Key words: cytogenetics; CFU-GM; urticaria pigmentosa.***

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Proliferation of mast cells can give rise to a wide spectrum of clinical manifestations in patients, generally called mastocytosis (1, 2). Earlier a borderline was drawn between localized skin infiltration, i.e. urticaria pigmentosa, and more generalized infiltration, i.e. systemic mastocytosis. During the last few years this simple classification has been questioned and other classifications have been proposed, taking into consideration both the clinical course and the extent of the mast cell infiltration, and an association with hematological disorders has been pointed out (3–6).

The origin of the mast cell is still somewhat doubtful but the cell is probably bone marrow-derived. The relation to myelopoiesis is under debate (7). Other groups and ourselves have earlier reported mast cell findings in bone marrow biopsies (8–10), and also results from chromosome analyses and in vitro stem cell growth for colony forming units granulocyte-macrophage (CFU-GM) in patients with mastocytosis (11, 12). In these studies, chromosome aberrations and an increase of the number of CFUs were found in some patients, and the similarity between these findings and what can be found in chronic myeloproliferative disorders was discussed. A follow-up study was therefore performed in patients with mastocytosis to see whether progression of this disease could be observed in terms of clinical findings, cytogenetic analysis and, in vitro stem cell growth pattern, in conformity with what can be seen in chronic myeloproliferative syndromes.

MATERIAL AND METHODS

Ten adult patients with the diagnosis of systemic mastocytosis or urticaria pigmentosa were reinvestigated, including bone marrow examination with chromosome analysis and in vitro colony growth for CFU-GM, after giving their informed consent. The group consisted of 6 women and 4 men with a median age of 40 years (range 19 to 69 years) at the time of the first investigation, see Table I.

The diagnoses of urticaria pigmentosa and systemic mastocytosis were based on clinical, histological and laboratory criteria described earlier (10, 12). The disease duration before the second examination varied between 5 and 24 years. The time between the first and the second investigation was 2.5 years in one patient with a malignant course and 4 to 6 years in the other patients, with a median time of 5.5 years.

During this follow-up time 7 patients (1, 3, 5, 7–10) were untreated. Patient 2, who had severe pruritus and general symptoms, was treated with H-1 blockade together with sodium cromoglycate for one year. This patient also had two periods of treatment with chlorambucil 5–10 mg per day, altogether 13 months. The first marrow examination was performed before and the second 2 months after chlorambucil therapy. Patient 4 received H-1 blockade. Patient 6 had pruritus and was treated with UVA light together with oral 8-methoxypsoralen (PUVA) in periods of 6 to 8 weeks.

Control group

Sixteen healthy volunteers with a median age of 36 years (range 23 to 71 years) were investigated, after informed consent, for the in vitro bone marrow colony growth capacity for CFU-GM. The healthy subjects had normal peripheral blood values and bone marrow morphology. Eight subjects were investigated at the time of the first examination of patients with mastocytosis and a further 8 subjects at the time of the second examination.

Sampling procedure

Bone marrow, 1 to 2 ml was obtained by sternal or iliac crest aspiration. The cells were suspended in 3 ml McCoy's medium (Flow Laboratories, UK) containing 125 IU heparin (Kabi, Vitrum, Sweden) for colony growth and in 6 ml medium for cytogenetic analysis.

Assay for CFU-GM

The colony formation was measured using a two-layered semisolid agar culture system (13), modified so that placenta extract was used as a source of the colony stimulating factors (14) instead of mononuclear cells. The bone marrow cells were isolated on Hypaque-Ficoll (Pharmacia, Sweden) and then seeded in the top layer with 1×10^5 cells per ml per dish. The cultures were incubated at 37°C in a humidified atmosphere at 5% CO_2 in air. The cultures were set up in four dishes and the mean value was given. The dishes were examined and counted with an inverted microscope on day 7 and day 10. The largest number of clusters was reached on day 7 and the largest number of colonies on day 10. The colony growth reached a plateau at the concentration of placenta extract used. The growth was linear up to ten times the used cell concentration. Aggregates of more than 40 cells were defined as

colonies, 21 to 40 cells as macroclusters and 3 to 20 cells as microclusters. A normal number of colonies was in the following defined as >16 colonies and less than 168 on day 10, based on the results from the healthy control group. The method was not changed during the follow-up period.

Cytogenetic analysis

Chromosomes from bone marrow cells were prepared with conventional techniques. When no evaluable metaphases could be obtained in direct preparations, cells were also studied after 48 h in culture. G- and Q-banding was used (15, 16). Chromosome identification and karyotypes were classified according to the International System for Human Cytogenetic Nomenclature (ISCN 1985) (17). An abnormal clone was defined as two or more cells with the same structural aberration or supernumerary chromosome, a loss of chromosome found in at least three cells.

Table I. *Clinical and laboratory findings in 10 patients with mastocytosis*

U-MeImAA = urine methylimidazole acetic acid, reference values 1.2–4.1 mg/24 h (18, 19); UP = urticaria pigmentosa; SM = systemic mastocytosis. Spleen size: reference values 57 $cm^2 \pm 12$ (M ± SD) (20).

Pat No.	Diagnosis at examination		Spleen size, cm^2		U-MeImAA (mg/24 h)	
	1st	2nd	1st	2nd	1st	2nd
1	SM	SM	55	84	22.5	77.4
2	SM	SM	195	197	29	51.4
3	SM	SM	50	62	10.5	10.9
4	SM	SM	110	77	4.9	5.5
5	UP	SM	–	60	3.7	7.5
6	UP	SM	50	–	1.8	–
7	UP	UP	45	43	1.9	6
8	UP	UP	56	76	1.9	5.4
9	UP	UP	25	33	1.8	2.5
10	UP	UP	50	45	1.3	2.1

RESULTS

Clinical course

During the follow-up period, a clinical progression of the disease was manifested in 7 patients (1–3, 5–8), evaluated as an increase in one or more of the parameters' symptoms, mast cell infiltrations in bone marrow and excretion of U-MeImAA in the urine.

Four patients (1–3, 5) showed a progressive disease in the skin with increasing number of maculae. Patients 5 and 6 were reclassified from urticaria pigmentosa to systemic mastocytosis, based on the infiltration of mast cells in the bone marrow biopsies. Two patients, 1 and 2, showed severe, progressive extradermal symptoms. Patient 1 had gastrointestinal symptoms with diarrhoea including hepatosplenomegaly. Patient 2, with a myeloproliferative bone marrow and anemia, also had hepatosplenomegaly and ascites and died. Patients 4 and 8 also had splenomegaly, estimated by scintigraphic examination, see Table I. Patient 7 showed progression in the excretion of U-MeImAA in urine. Nine patients were still alive 2 years after the second examination.

The peripheral blood values were unchanged during the follow-up. In patient 2, the serum vitamin B_{12} level was elevated at both examinations and a morphological picture of myeloproliferation was seen at the follow-up examination of the bone marrow section.

In vitro growth for CFU-GM

The results are presented in Table II. The number of macroclusters and colonies decreased between the two examinations, from a median value of 203 to 126 clusters, and from 70 colonies at the first examination to only 28 at the second. However, 4 patients grew more colonies, while 6 patients had a decrease in the number, in comparison with the first investigation. Three patients had less than 16 colonies at the second investigation, while none grew more colonies than the control group. The number of microclusters increased in 8 patients, and for all patients it increased from a median value of 358 clusters at the first examination to 797 at the second. However, none of the patients showed values over the range of the control group. In patients 3, 4, 5, and 9 with decreased numbers of colonies, an increased number of microclusters was observed.

Cytogenic analysis

The results are presented in Table II. At the first investigation patients 2, 3, 7 and 8 showed a cytogenetically abnormal clone. Patient 2 had been treated for severe symptoms during the follow-up period and the abnormal clones disappeared while random losses still persisted in 42% of the cells. Patient 3 had a clone with loss of chromosome No. 15 and about 30% cells with variable losses at the first examination. The clone disappeared but the variable losses persisted at the second examination. Patient 7 had a clone with extra material on the short arm of chromosome 9 at the first examination in 83% of the cells, and this clone persisted and increased to 100% at the second investigation. Patient 8 showed cells with extra small chromosomes at the first examination, which disappeared at the second examination. Patient 5 showed a loss of chromosome No. 22 as a minor clone at the second investigation, while the karyotype was normal at the first investigation.

Single cells with extra chromosomes or structural rearrangements were seen in 4 patients at the first examination, and the abnormal cells persisted at the second examination in 2 patients but disappeared in the other 2 patients. Variable losses of chromosomes in more than 30% of the examined cells were observed in 2 patients at the first examination, which persisted in the second examination. A further 3 patients with losses of chromosomes were found at the follow-up. Patients 5 and 6, with a clinical change from urticaria pigmentosa to systemic mastocytosis, showed addition of a pathological clone and persistence of variable losses, respectively, at the second investigation.

All control subjects had normal karyotypes.

DISCUSSION

During the last few years the different clinical manifestations of mast cell proliferation have been discussed and the association with hematological disorders has been pointed out (2, 3, 6, 8, 9, 12). A new classification system separating a clinical indolent form of mastocytosis from a more proliferative form with an

Table II. *Results of cytogenetic analyses and in vitro culture for granulocyte-macrophage progenitor cells from bone marrow in patients with mastocytosis*

Pat No.	Duration of disease before 2nd examination (years)	Time between examinations (years)	Bone marrow karyotype*	No. of cells/ No. of total karyotyped cells	CFU-GM/1 × 10^5 cells No. of colonies and clusters		
					7 days		10 days
					Micro	Macro	Colonies
1	24	3.9	1. 46,XX/47,XX,+G	12/1/13	140	177	54
			2. 46,XX/46,XX,1q+/47,XX,+?G	8/1/1/10	433	234	80
2	5	2.5	1. 46,XY/46,XY,−16,+mar/46,XY,+3,−6+varied changes	20/6/2/28	208	102	14
			2. 46,XY/42-45,XY	7/5/12	1201	320	26
3	14	6.3	1. 46,XX/45-41,XX,−15/45-42,XX	7/4/5/16	363	16	8
			2. 46,XX/46,XX,−18+G/45-42,XX	8/1/4/13	972	12	2
4	19	5.7	1. 46,XY/43-40,XY	11/3/14	373	260	169
			2. 46,XY/45-41,XY	8/5/13	617	36	28
5	8	4.0	1. 46,XY	11/11	292	332	290
			2. 46,XY/45,XY,−22	12/3/15	939	318	28
6	11	5.3	1. 46,XY/45-43,XY	4/7/11	427	13	4
			2. 46,XY/45-40,XY	4/6/10	656	126	33
7	7	5.6	1. 46,XX,9p+/46,XX	20/4/24	363	339	92
			2. 46,XX,9p+	13/13	1169	1154	144
8	8	5.5	1. 46,XX/46,XX,+G/46,XX,+?13,−16/48,XX,+?13,+G	6/3/1/1/11	231	229	240
			2. 46,XX	14/14	216	326	169
9	19	5.7	1. 46,XX/47,XX,+G	7/1/8	356	122	44
			2. 46,XX/46,XX,7q−/47,XX,+mar	12/1/1/14	1248	44	2
10	7	5.5	1. 46,XX	14/14	361	331	86
			2. 46XX/45-42,XX	8/6/14	102	12	9
Control group ($n = 16$)			All normal	Mean	518	163	53
				Median	395	141	38
				Range	173–1404	37–289	16–168

* / = separates clones and cells; figures refer to chromosome no.; p = short arm of chromosome; q = long arm of chromosome; + = additional; − = loss; mar = not identified chromosome. The clones are ordered according to size.

associated hematological disorder was recently presented (3). In the indolent group, both patients with localized skin involvement (urticaria pigmentosa) and patients with systemic engagement (bone marrow or gastrointestinal) are included.

According to the proposed classification scheme (3), the patients in this follow-up study can be considered indolent with one exception (patient 2). Seven of our patients showed progression of one or more of the parameters (Table I), indicating that even the indolent form of mastocytosis can be a slowly progressive disorder. Two patients (5, 6) changed clinically from the diagnosis of urticaria pigmentosa to the systemic form with mast cell infiltration of the bone marrow. Patient 2 had a myeloproliferative picture in the bone marrow and a rapid clinical deterioration. This explains the short time between the two bone marrow examinations in this patient.

In chronic myeloproliferative diseases, both the number of patients with and the number of chromosomal abnormalities increase with the duration of the disease (21, 22). To determine whether patients with mastocytosis showed a similar chromosome pattern, we re-examined 10 patients after about 5 years. To our knowledge, a prospective study of bone marrow chromosome findings in patients with mastocytosis has not yet been carried out. Patient 1 had persistence of single cell chromosome abnormalities. Patient 2 had meanwhile been treated and the chromosomally abnormal clone had disappeared, but an increased tendency to chromosome losses was noted at the follow-up. Patient 6 had the same tendency to variable losses in 60% of the cells at both examinations. Patient 7 had an abnormal clone in both examinations. Thus, the bone marrow cytogenetic findings showed a variable pattern with unchanged, appearance and disappearance of clones similar to that which can be found in polycythemia vera (21). This pattern may be an expression of cytogenetic instability or vulnerability of the bone marrow cells. At present there are only a few reports of cytogenetic findings in mastocytosis. In one study 11 patients were examined and 5 had clonal aberrations; 4 of these patients had a concomitant

hematological disorder (6). A patient with malignant mastocytosis was shown to have chromosome abnormalities (23). Our patient 2 with a myeloproliferative picture in the bone marrow showed abnormalities at the first examination which were not detectable after cytostatic treatment at the second examination. However, cytogenetic changes were also seen in the patients with indolent mastocytosis, and even in patients 7 and 8, classified from a clinical viewpoint as urticaria pigmentosa.

In vitro colony growth for CFU-GM was also performed in this study. A decreased in vitro growth for CFU-GM for macroclusters and colonies was seen in patients 3, 4, 5, and 8. In patient 5, an abnormal clone in the cytogenetic analysis appeared. Patient 8 showed a slight decrease in the growth towards a normal level and her chromosome abnormalities were not detectable at the second investigation, although her U-MeImAA excretion increased above the reference value at the second investigation. The cluster growth at day 7 had increased at the follow-up examination in 8 patients. However, 6 patients had a decreased number of colonies at the second examination (Table II). The change of the growth pattern with increased clusters and reduced colonies can be seen in patients with myelodysplastic syndromes and leukemia (24, 25). The size of the clusters and colonies reflects stem cells of varied differentiation; thus a changed growth pattern is a sign of a disturbed stem cell function (26). The sum of the number of clusters and colonies was increased in 7 of the patients at the follow-up examination. An increased number of progenitors has also been found in myeloproliferative disorders (27). The findings in the in vitro growth of CFU-GM in patients with mastocytosis may point to a disturbed stem cell. However, we have not identified mast cells in the cultures.

The origin of the mast cell is still not fully elucidated in man but most findings point to the pluripotent hematopoietic stem cell (28–30). The cytogenetic and the in vitro findings in the patients with mastocytosis are similar to those that can be found in the myeloproliferative diseases. Recently, a patient with mastocytosis was reported who showed a good reponse to treatment with interferon-α 2b (31), similar to what has been seen in chronic myeloproliferative disorders.

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