

The presence of membrane-bound stem cell factor on highly immature nonmetachromatic mast cells in the peripheral blood of a patient with aggressive systemic mastocytosis

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Background: Systemic mastocytosis is characterized by mast cell infiltration of bone marrow and tissues in the absence of identified circulating bone marrow-derived progenitors. A 58-year-old man was first seen with aggressive systemic mastocytosis manifested by urticaria pigmentosa, hepatosplenomegaly, generalized bone lesions, anemia, thrombocytopenia, monoclonal gammopathy, and increased urine histamine levels.

Objectives and Methods: A rapidly progressive anemia and thrombocytopenia dictated a splenectomy. We sought to identify the mast cell progenitors in the peripheral blood and to provide evidence of their maturation in tissues with immunohistochemical and ultrastructural analyses.

Results: The peripheral blood contained 1% to 3% nonmetachromatic mononuclear cells with eccentric nuclei that expressed the mast cell proteases, tryptase and carboxypeptidase A, along with c-kit, stem cell factor (SCF), and high-affinity IgE receptor (FcεRI), but not chymase. Similar mononuclear cells colocalized in the spleen and lymph nodes with mature, metachromatic mast cells that expressed tryptase, chymase, carboxypeptidase A, c-kit, SCF, and FcεRI. Electron microscopy disclosed, at each site, a mature mast cell population with electron-dense, scroll-poor granules.

Conclusions: The peripheral blood of a patient with aggressive systemic mastocytosis contained immature mononuclear cells of the mast cell lineage that express c-kit, SCF, tryptase, carboxypeptidase A, and FcεRI. These cells were also found in the skin, spleen, and lymph nodes where they presumably expand, differentiate, and mature, assuming the mast cell phenotype for those tissues characterized by metachromasia, expression of a full range of mast cell-related secretory granule proteases, and ultrastructural appearance. The presence of SCF on the surface membrane of the circulating, highly immature mast cells suggests an autocrine regulation of the c-kit-SCF interaction. (*J Allergy Clin Immunol* 1996;98:831-40.)

Key words: Systemic mastocytosis, tryptase, chymase, CPA, c-kit, SCF, mast cell progenitors, peripheral blood

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Mastocytosis is defined by the presence of increased numbers of mast cells in one or more tissues and is characterized by the effects of local mast cell mass, the tissue response to that mass, and the release of bioactive substances acting at both distal and local sites.¹ The various presentations of systemic mastocytosis are classified by the absence or presence of an associated dysmyelopoiesis or leukemic state and by the ongoing clinical course of the disease.² Type 1 or indolent systemic mastocytosis is the most common presentation and has a generally benign prognosis.³ Patients with indolent systemic mastocytosis may have intractable

Abbreviations used

CPA: Carboxypeptidase A

SCF: Stem cell factor

ble ulcers, diarrhea and malabsorption, hepatosplenomegaly, bone lesions, and flushing, sometimes with syncope⁴; however, these patients have no hematologic abnormalities and have a normal life span.⁵ In type 2 systemic mastocytosis, the features of indolent disease are accompanied by the presence of either dysmyelopoiesis or frank leukemia,⁶ and the prognosis is dictated by these abnormalities.⁷ Type 3 or aggressive systemic mastocytosis is a progressive form of the disease in which mast cells rapidly infiltrate organs. Unlike type 2 systemic mastocytosis, in type 3 systemic mastocytosis neither hematopoietic dysplasia nor neoplasia is found. Type 4 systemic mastocytosis is a mast cell leukemia in which patients have circulating, atypical, granulated, metachromatically staining mast cells.⁵ The diagnosis of systemic mastocytosis requires tissue evidence of mast cell infiltrates, and the bone marrow biopsy specimen usually reveals focal aggregates of metachromatically staining mast cells.⁸ Although the cause of mastocytosis is unknown, in one study of the cutaneous accumulation of mast cells, an excess of soluble stem cell factor (SCF) was demonstrated by immunohistochemical analysis.⁹

Unlike other cells of bone marrow origin, mast cells do not mature in the marrow and do not circulate in a recognized mature form. In tissue they are recognized as mature populations with tissue-related phenotypes. On the basis of the immunodetection of secretory granule neutral proteases, mast cells in the lung parenchyma and intestinal mucosa selectively express tryptase; those in the intestinal and airway submucosa, skin, lymph nodes, and breast parenchyma express tryptase, chymase, and carboxypeptidase A (CPA)¹⁰; and occasional mast cells in intestinal submucosa express chymase and CPA but not tryptase.¹¹ It is thus assumed that unrecognized mast cell progenitors enter the tissue and undergo regulated proliferation, differentiation, and maturation.

In this study, nonmetachromatic mononuclear cells have been defined as circulating highly immature members of the mast cell lineage. SCF at the membranes of these circulating immature mast cells and in mature tissue mast cells may represent

a marker for aggressive systemic mastocytosis, and its presence suggests a mechanism for uncontrolled proliferation.

CASE REPORT

A 58-year-old Caucasian man who was previously in good health noted a diffuse, erythematous, nonpruritic, maculopapular rash on his trunk in January 1992. Physical examination revealed the patient to be free of symptoms except for 3 to 4 mm reddish-brown, oblong macules, which were occasionally papular over his trunk and extremities, and splenomegaly. The hematocrit was 34.8%, the platelet count was 100,000 cells/mm³, the white blood cell count was 8000 cells/mm³, the alkaline phosphatase level was 681 U/L, and the erythrocyte sedimentation rate was 50 mm/hr. X-ray films of the spine and chest demonstrated diffuse osteosclerosis involving all the dorsal vertebral bodies and the ribs. The urine histamine level was elevated (315 ng/24 hr; normal range = 0 to 118 ng/24 hr). A skin biopsy specimen, obtained in June 1992, confirmed the diagnosis of urticaria pigmentosa.

A splenectomy was performed in March 1994 to alleviate hypersplenism. At that time, the hematocrit was 18.5%, the platelet count was 54,000 cells/mm³, and the white blood cell count was 9200 cells/mm³. After surgery, both the hematocrit and the platelet count rose dramatically, and the patient resumed his regular professional activities. In November 1994, the patient had pleuritic pain in the left side. The hematocrit was 26.5%, the platelet count was 105,000 cells/mm³, and the white blood cell count was 16,700 cells/mm³. A computed tomographic scan disclosed pericardial and bilateral pleural effusions. Analysis of the pleural fluid by smear, cell count, cytologic examination, and culture was not diagnostic. SCF levels in serum were 840 ng/ml and 750 ng/ml in two separate determinations (R&D Systems Inc., Minneapolis, Minn.; range from 40 healthy donors, 1164 to 1912 ng/ml). A bone marrow biopsy specimen revealed diffuse infiltrates of small-to-medium-sized cells with oval and spindle-shaped nuclei and moderate amounts of pink granular cytoplasm, consistent with mast cells. Erythroid elements were markedly decreased, and the myeloid elements, although moderately decreased, exhibited normal maturation without dysplastic or neoplastic transformations. Over the next 4 weeks, the pleuropericardial effusions resolved partially, and the patient resumed his professional activities.

METHODS**Tissue specimens**

One set of peripheral blood smears was obtained 1 week before splenectomy and monthly for 8 months afterward. A fresh 18 × 8 × 8 cm spleen with intact capsule and three small lymph nodes from the spleen hilum were obtained at surgery. Separate surgical skin biopsy specimens (0.5 × 0.5 cm) of a reddish-brown lesion and of an uninvolved neighboring skin site were

obtained from the patient's back 5 months after the splenectomy. Multiple attempts to obtain enough bone marrow tissue or smears for immunochemistry studies were unsuccessful.

Histopathologic examination

Peripheral blood smears were routinely fixed in Carnoy's solution or isotonic formaldehyde acetic acid,¹² and tissues were fixed in 4% paraformaldehyde. Tissues were dehydrated and embedded in JB4-glycolmethacrylate in accordance with instructions from the manufacturer (Polysciences, Inc., Worthington, Pa.)¹³ and were sectioned and stained in a routine fashion. Tissue area was determined with a computer-controlled digitizing board with a Sigma Scan digitizing program (Jandel Scientific, San Rafael, Calif.), and mast cells were counted with a Leitz optical microscope (Leica, Inc., Rockleigh, N.J.). The results were expressed as the number of mast cells per square millimeter.

For immunochemistry, slides were incubated for 1 hour at room temperature and then overnight at 4°C with one of the following antibodies: mouse monoclonal anti-tryptase (1:50; Chemicon International, Temecula, Calif.), rabbit polyclonal anti-chymase (1:200, gift of Dr. N. Schechter), mouse monoclonal anti-CPA (1:800; Sigma Chemical Co., St. Louis, Mo.), mouse monoclonal anti-CPA (gift of Dr. S. Goldstein), sheep monoclonal anti-cathepsin G (1:500; Biotest, Kennebunkport, Maine), sheep polyclonal anti-SCF (1:10; Genzyme, Cambridge, Mass.), mouse monoclonal anti *c-kit* (YB5B8) (1:80, gift of Dr. K. Nocka), or mouse monoclonal anti-FcεRIα (1:20, gift of Dr. J. P. Kinet). Immunocytochemical procedures were carried out as previously described with alkaline phosphatase¹⁴ as the chromogenic reporter. The standard immunohistochemical technique with a chromogenic reporter yields a somewhat particulate reaction product, which does not reflect cellular granulation. Optimal concentrations of the primary antibodies were defined by serial dilutions. Control preparations consisted of sections of tissue treated with isotype-matched IgG (tryptase, CPA, and *c-kit*), irrelevant IgG (chymase, cathepsin G, SCF, and FcεRI), or no primary antibody (tryptase, chymase, CPA, cathepsin G, SCF, *c-kit*, and FcεRI). For staining of FcεRI, slides were incubated with human myeloma IgE before being stained with anti-IgE (rabbit polyclonal coupled to fluorescein isothiocyanate; Dako Corporation, Temecula, Calif.).

Double immunofluorescent staining of peripheral blood cells in fixed smears with anti-tryptase or anti-CPA and the anti-SCF primary antibodies was developed with secondary antibodies labeled with fluorescein isothiocyanate (for tryptase and CPA) and rhodamine (for SCF). Double staining of FcεRI and CPA was done with fluorescein isothiocyanate anti-human IgE and rhodamine-conjugated secondary antibody for CPA. For electron microscopy, tissues were fixed and processed in a standard fashion¹⁵ and were examined on a JEOL 100 CX transmission

electron microscope (JEOL, Peabody, Mass.) operating at 80 kV.

Reverse-transcription polymerase chain reaction analysis of SCF messenger RNA

The SCF transcript from the mRNA of spleen from the patient and that of spleen from an accident victim were each analyzed with primers corresponding to specific sequences in the human SCF complementary DNA (upstream primer: 5'-GGGCTGGATCGCAGCGC-3' and downstream primer: 5'-CAGTGTGATACAAGCACA-3').⁹ DNA blot analysis of the membrane containing the transferred, amplified product of the reverse-transcription polymerase chain reactions was carried out with a phosphorous 32-labeled 900-nucleotide probe containing the entire human SCF coding region (Immunex R & D, Seattle, Wash.).

RESULTS

Peripheral blood

Before and after splenectomy, the only peripheral blood cells from the patient that stained metachromatically with Wright's and Giemsa stains or with toluidine blue were basophils with polylobed nuclei (<1%). A population of atypical mononuclear cells with eccentric nuclei and clear cytoplasm, similar in size to typical monocytes, accounted for $2.2\% \pm 1.4\%$ (mean $\pm$ SD, $n = 8$) of the total nucleated cells before and in each of eight monthly studies after splenectomy. That cell population was $6.4\% \pm 3.2\%$ ($n = 8$) of the total mononuclear cell population that was consistently elevated ($33.7\% \pm 11.3\%$) ($n = 8$). Cells expressing tryptase, CPA, and *c-kit* (Fig. 1) were present in peripheral blood smears in the same proportion and with the same morphologic characteristics as the agranular mononuclear cells. As demonstrated by immunofluorescence double labeling, the mononuclear cell population expressing tryptase and CPA was also found to express SCF and FcεRI (Fig. 2). No cells that expressed chymase were found (Fig. 1). Mononuclear cells expressing cathepsin G were present in proportion to the agranular mononuclear population and consistent with their structure (Fig. 1). Smears from two nonallergic normal individuals and from three patients with indolent systemic mastocytosis did not have mononuclear cells that stained with any of the primary antibodies applied.

Spleen

The adherent cells in touch preparations of the incised spleen stained metachromatically with toluidine blue and immunocytochemically for the mast cell-specific proteases—CPA, tryptase, and chy-

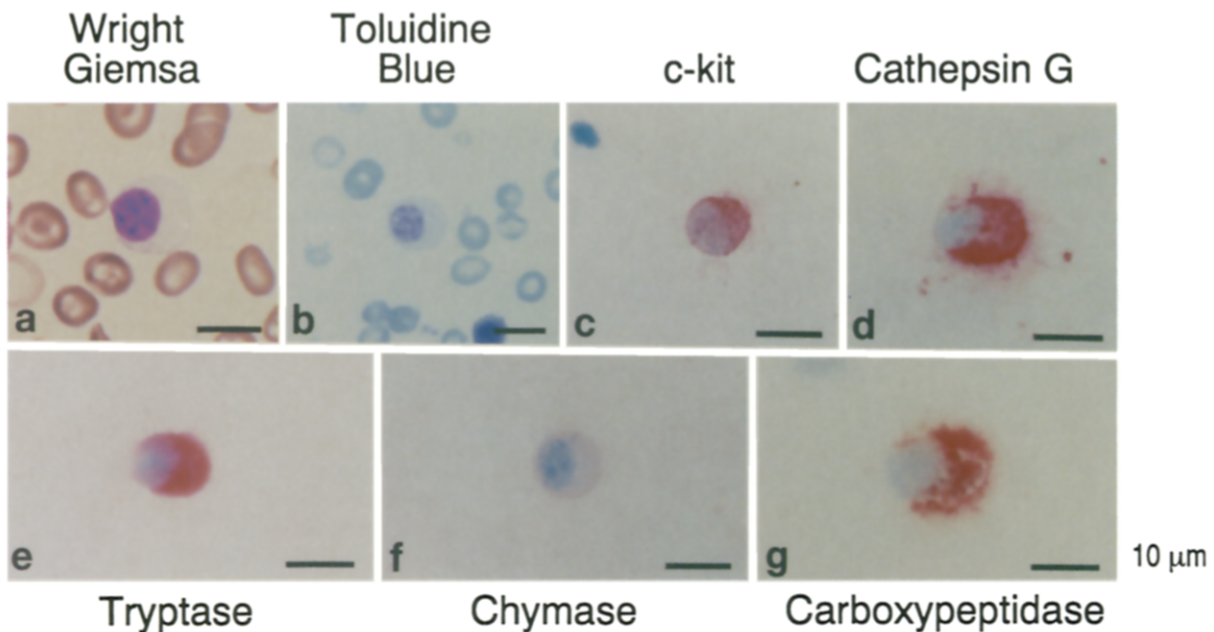


FIG. 1. Composite assembly of mononuclear cells with eccentric nuclei made from peripheral blood smears. Cells stained with Wright's and Giemsa stains (**a**) are toluidine blue-negative (**b**) and are positive for *c-kit* (**c**), cathepsin G (**d**), and mast cell-specific markers tryptase (**e**) and CPA (**g**). Cells on smears taken on 8 consecutive occasions were chymase-negative (**f**), including two smears fixed in isotonic formaldehyde acetic acid.¹² Carnoy's fixation Vectastain (Vector Laboratories, Inc., Burlingame, Calif.) red immunoreaction product (**c** to **g**).

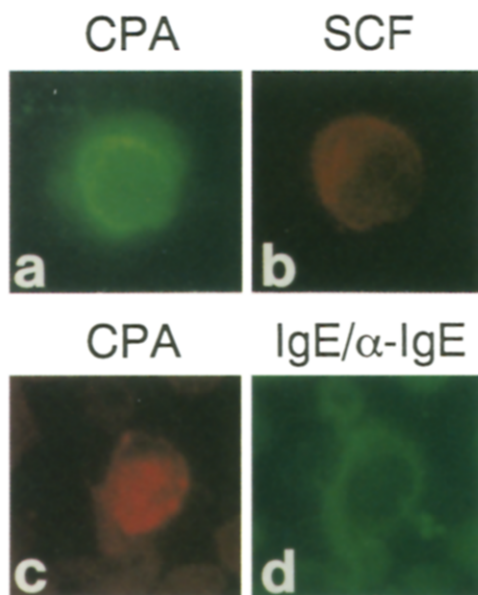


FIG. 2. Mononuclear cells similar to those shown in Fig. 1. **Panel a** and **b**, Double immunofluorescently labeled with anti-CPA (fluorescein) (**a**) and with anti-SCF (rhodamine) (**b**). **Panel c** and **d**, Double immunofluorescently labeled with anti-CPA (rhodamine) (**c**) and IgE/anti-IgE (fluorescein) (**d**).

mase (data not shown). In tissue sections stained with hematoxylin-azure-eosin, the splenic architecture was extensively altered as a result of perivascular and parenchymal fibrosis. There was marked attenuation of the white pulp and considerable hemosiderin deposition. Extramedullary multifocal hematopoiesis was evident by the presence of myeloid and erythroid elements, with occasional megakaryocytes. Mast cells diffusely infiltrated the remaining red and white pulp and formed focal aggregates in the fibrotic trabeculae and stroma, where numerous eosinophils were also observed. Mast cells also surrounded blood vessels and often intercalated into their smooth muscle wall. Various degrees of mast cell metachromasia and granulation were revealed with toluidine blue. By morphometric analysis of JB-4 methacrylate sections prepared with methylene blue, the spleen contained 401 ± 32 mast cells/mm² (mean $\pm$ SD, $n = 6$).

Mast cells staining metachromatically with toluidine blue or methylene blue expressed CPA, tryptase, chymase, cathepsin G, FcεRIα (Fig. 3), and *c-kit*. Mononuclear cells similar to those found

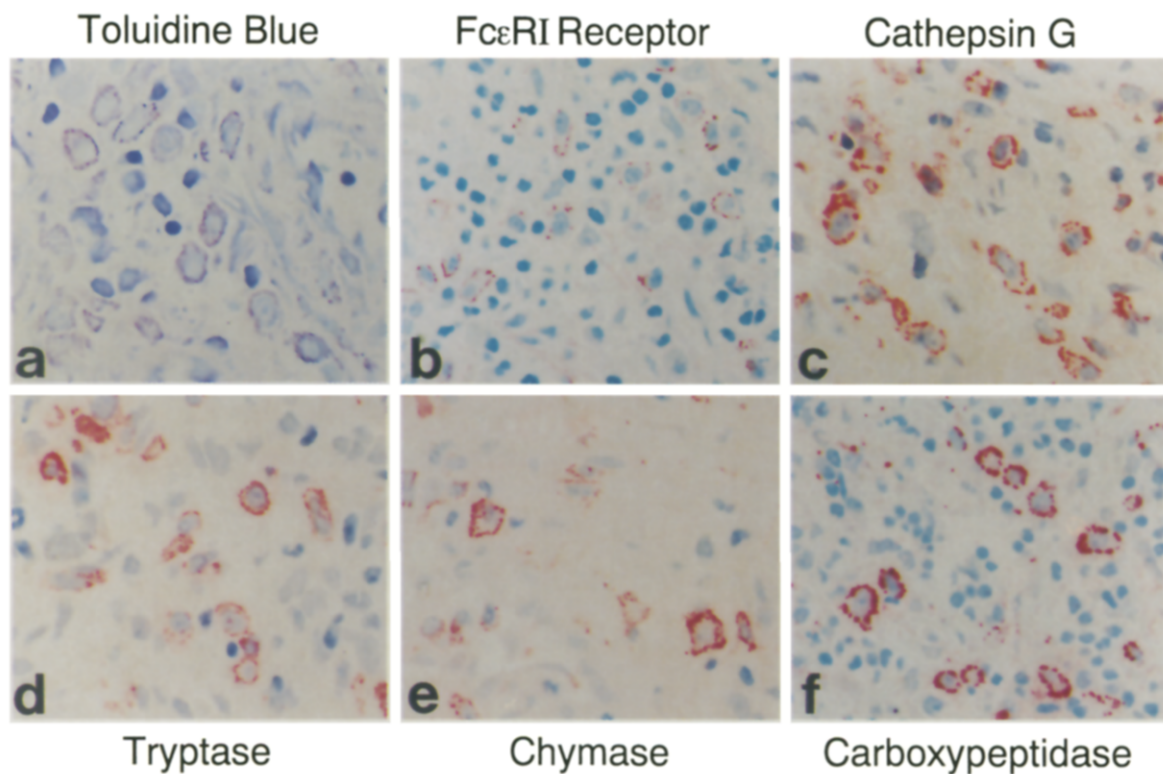


FIG. 3. In the spleen mast cells that stain with toluidine blue (**a**) are immunoreactive for FcεRI (**b**) and cathepsin G (**c**) and for the mast cell-specific proteases, trypsinase (**d**), chymase (**e**), and CPA (**f**).

in the peripheral blood were identified in spleen tissue at a frequency of about 0.5 per 100 metachromatic mast cells. These mononuclear non-metachromatic cells also expressed trypsinase. SCF reacted at the surface membrane of about one half of the mast cells that expressed chloroacetate esterase. No cellular source for SCF other than the mast cell itself could be identified with certainty.

Electron microscopy revealed that the mast cell had an average of 23 granules per thin section of the cell. These granules were preponderantly of the scroll-poor variety, containing less than 10% complete scrolls, and were filled with amorphous material. Some of the granules had clear halos between the amorphous granule content and the granule-limiting membrane.

Lymph node

Perifollicular mast cell infiltration was predominant, but many follicles were clear of mast cell infiltration in the sections stained with hematoxylin-azure-eosin. There were 526 ± 40 metachromatic mast cells/mm² ($n = 6$), as determined by methylene blue staining, in the sinusoids. As defined by staining of adjacent sections, metachro-

matic mast cells expressed trypsinase, chymase, CPA, *c-kit*, cathepsin G, and FcεRIα. Mononuclear cells that stained with trypsinase but not with methylene blue were observed at a frequency of about 1 per 100 metachromatically staining mast cells (Fig. 4). Serial sections with immunohistochemical analysis demonstrated that these cells expressed trypsinase. In Fig. 5 a circulating mast cell that expresses *c-kit* is also seen inside a vein. SCF staining of the lymph nodes revealed one third of the mast cells to be positive, as shown by serial sections with antibodies to SCF and CPA staining the same cells (Fig. 5). No cellular source of SCF other than mast cells could be identified.

Skin

Lesional skin had 314 ± 46 metachromatic mast cells/mm² in the dermis compared with 64 ± 11 metachromatic mast cells/mm² in adjacent nonlesional skin. Metachromatic skin mast cells expressed trypsinase, chymase, CPA, cathepsin G, and *c-kit* in adjacent serial sections. SCF staining of skin mast cells was more uniform than that in spleen or lymph node tissue, coating most mast cells (Fig. 6). Comparable staining of keratinocytes

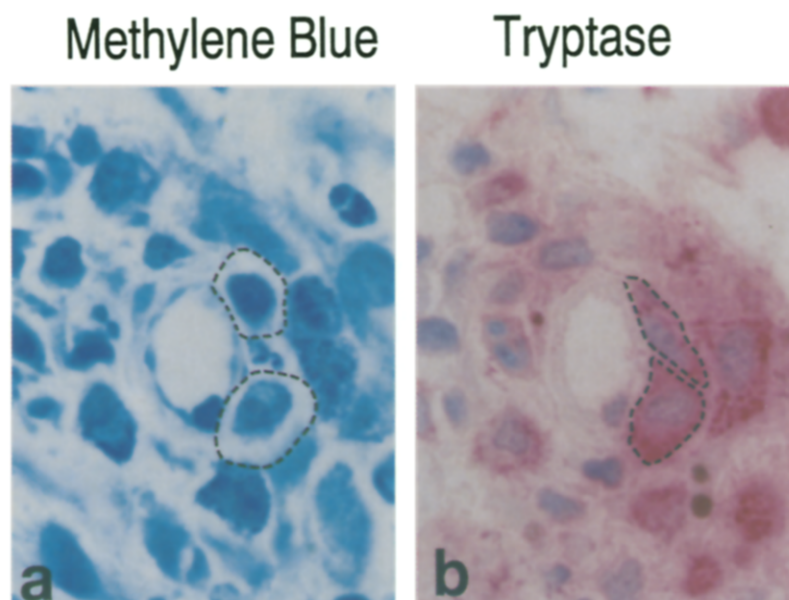


FIG. 4. Tryptase-positive, methylene/toluidine blue-negative cells were present in the spleen and lymph nodes. Two such mast cells in serial sections of lymph node tissue are marked as dotted lines. The serial section procedure separates portions of cells presented in panels **a** and **b** by 1.5 μm .

was not observed. No mast cells were observed in the stratified squamous epithelium.

By electron microscopy, the lesional skin mast cells exhibited a preponderance ($97\% \pm 8\%$) of amorphous granules with incomplete scrolls (Fig. 7). The remaining granules were scroll-rich. Among the scroll-poor granules, 6% had crystalline lattices and gratings, which were not observed in splenic mast cell granules. The periodicity of gratings, lattices, and scrolls was similar to that reported for lung mast cells.¹⁶ Mast cells from nonlesional tissue were also scroll-poor, with the same distribution of granule types as in the lesional skin.

SCF transcripts in spleen tissue

Transcripts for SCF, detected after reverse-transcription polymerase chain reaction of spleen RNA from the patient and the control subject by DNA blot analysis, exhibited two species of similar intensity in both preparations when hybridized with the full-length nucleotide probe of human SCF (data not shown).

DISCUSSION

Nonmetachromatically staining mononuclear cells in blood have been identified as highly immature members of the mast cell lineage in a patient with progressive systemic mastocytosis without dysmyelopoiesis. Similar immature mast cells were

also observed at the tissue level to colocalize with extensive populations of mature, differentiated, metachromatically staining mast cells of normal phenotype. Thus the link between the bone marrow origin and the tissue-localized mast cell is recognized as an atypical mononuclear cell that is identified immunohistochemically by the expression of mast cell-specific secretory granule proteases, tryptase and CPA, and membrane *c-kit*, SCF, and Fc ϵ RI (Figs. 1 and 2).

In a recent study nonmetachromatically staining mononuclear cells were recognized in the spleen and intestinal mucosa of recipient mice at 3 to 6 days after adoptive transfer of v-abl-transformed, bone marrow-derived immature mast cells. These v-abl-transformed mast cells matured and differentiated at each tissue site into a phenotype, defined immunohistochemically by protease and abl expression, consistent with the resident mast cell population. These findings in a mouse model of systemic mastocytosis were interpreted as demonstrating that a lineage-specific, nonmetachromatically staining mononuclear cell gave rise to mature mast cells with a phenotype regulated by the tissue of residence.¹⁷

The elevated leukocyte alkaline phosphatase level in the patient is compatible with a leukemoid reaction accompanying the intense mast cell hyperplasia. The bone marrow biopsy specimen revealed up to one half occupancy by diffuse mast cell

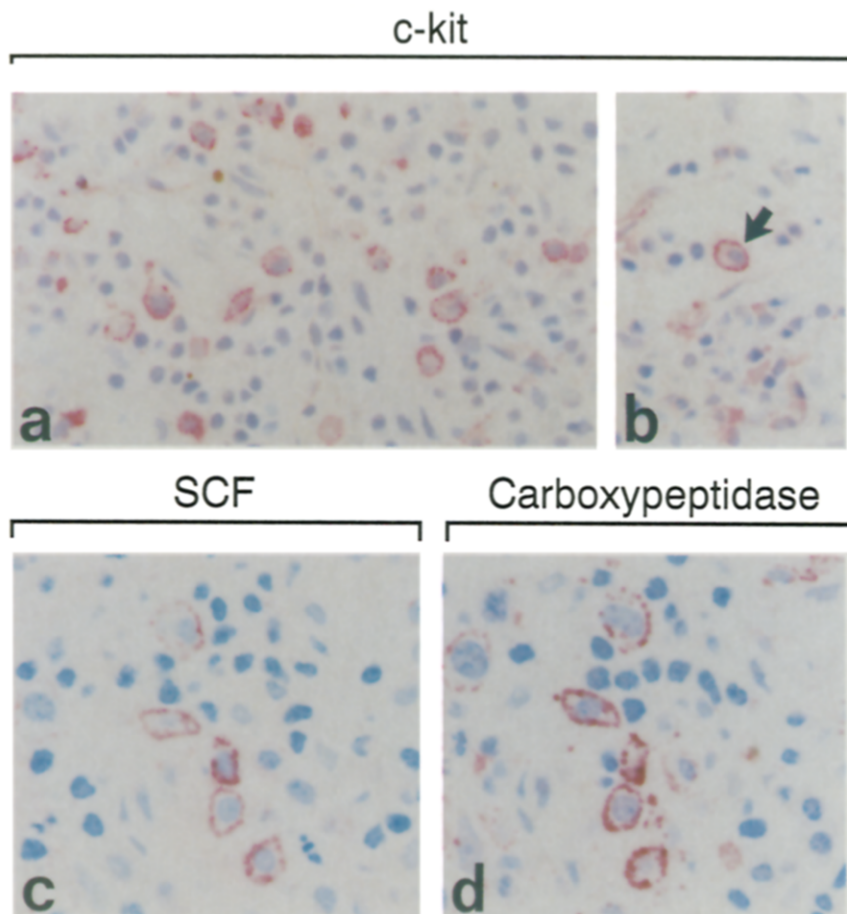


FIG. 5. In a lymph node, surfaces of mast cells are positive for *c-kit* (parenchyma [a] and lumen of a vein [b, arrow]) and SCF (c). Mast cells were immunoreactive for SCF (c) and mast cell-specific proteases, as illustrated for CPA (d) in serial sections.

populations of characteristic histochemical appearance with oval or spindle-shaped nuclei and pink, granular cytoplasm as demonstrated by hematoxylin-azure-eosin stain and metachromasia with toluidine blue staining. The absence of a dysplastic or neoplastic change is central to the view that the circulating, nonmetachromatically staining mononuclear cells with mast cell-specific proteins are immature progenitors increased in numbers, presumably because of autocrine stimulation by SCF (Figs. 1 and 2).

The proportion of total leukocytes containing secretory granule tryptase or CPA ranged from 1% to 3% of the total white blood cells and was similar to the proportion of cells expressing *c-kit*, SCF, and FcεRI at the membrane. Mast cells have been distinguished from basophils by the presence of *c-kit* along with their distinct nuclear structure,¹⁸ inasmuch as both cell types are metachromatic and express FcεRI. Tryptase has been found in trace amounts in mature human basophils but would not

be detected with the antibody dilutions used to characterize the mast cell progenitors.¹⁹ An analysis of normal hematopoietic cells for steady-state levels of mast cell CPA transcripts with total and/or poly(A)⁺ RNA was positive only for partially purified human lung mast cells, thereby delineating the specificity of this probe.²⁰ The proportion of peripheral blood leukocytes expressing the three individual mast cell-specific probes—tryptase, CPA, and *c-kit*—was comparable to that for the unselective marker cathepsin G when the values were corrected for neutrophils. Immature myeloid elements were not present. Similar non-metachromatically staining cells expressing tryptase (Fig. 4) colocalized with the granulated, mature mast cells at a frequency of 0.5% to 1% in the spleen and lymph nodes.

The absence of immunodetectable chymase in the circulating progenitors, which express tryptase and CPA (Fig. 1), is compatible with the analyses of human mast cells developed in cultures of

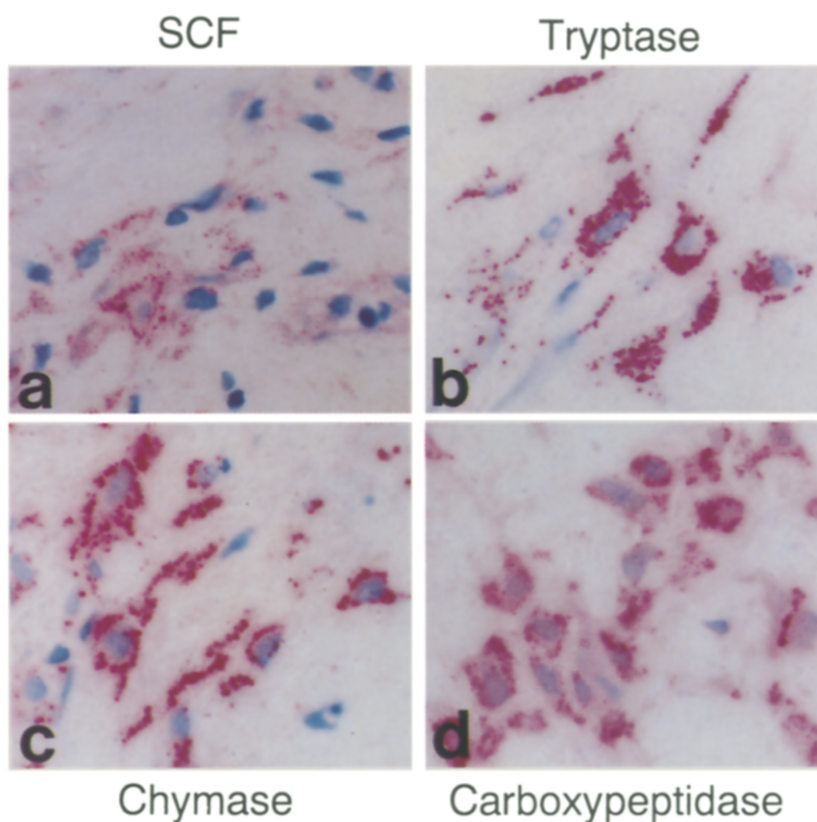


FIG. 6. Mast cells in an urticaria pigmentosa lesion show surface staining for SCF (**a**) and granular staining with antibodies to tryptase (**b**), chymase (**c**), and CPA (**d**).

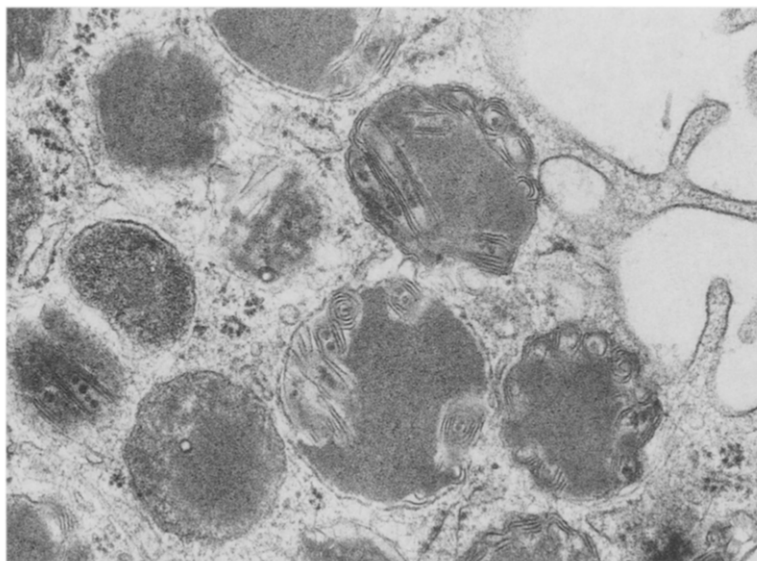


FIG. 7. Electron micrographs of skin mast cell granules showing the variety of patterns in scroll-poor granules.

CD34-positive cord blood mononuclear cells.²¹ In two recent studies with SCF and supporting cytokines, tryptase was expressed early in mast cell development and was expressed by the entire mast

cell population at a time when less than one third of the cells expressed chymase.^{21, 22} Importantly, tryptase has even been detected before the appearance of metachromatically staining cells in a cord

blood culture system,²³ as also observed in this study for the circulating highly immature mast cells (Fig. 1) and for the similar population colocalizing with metachromatically staining mast cells in spleen and lymph node tissue (Fig. 4). The earliest mast-cell-lineage-related peripheral blood cells identified by their in vitro differentiation are CD34⁺, *c-kit*⁺, FcεRI⁻ before the expression of tryptase, and then FcεRI^{+24, 25}; whereas the non-metachromatic, *c-kit*⁺, tryptase⁺, CPA⁺ mononuclear progenitors in our study were also weakly FcεRI⁺. This difference most likely reflects the in vivo presentation of a progenitor population in this patient with SCF-driven type III systemic mastocytosis, as compared with harvest of less mature progenitors and observation of their differentiation in vitro.

The circulating immature mononuclear mast cells that express tryptase and CPA are also found by double immunofluorescent labeling to demonstrate staining for membrane-bound SCF (Fig. 2). Mast cells have not been reported as a source of mRNA for SCF, but SCF protein has been detected bound to skin mast cells in lesions of patients with urticaria pigmentosa.⁹ In that study, SCF in skin lesions was attributed to its release from keratinocytes. The surface expression of both the *c-kit* receptor protein and its ligand, SCF, has been recently reported in normal human tissues such as breast, parotid gland, sweat gland, and epithelial and myoepithelial cells and at low levels in thyroid follicle cells.²⁶ Our study suggests that immature mast cells that already contain tryptase and CPA can express SCF on their surface membranes. Studies of transfected cell lines have revealed that SCF can drive proliferation either as a membrane-associated protein or as a released ligand by interaction with *c-kit* on the target cell population.²⁷ The stringent conditions applied during the immunochemical studies, particularly the wash steps, suggest that the SCF on the committed progenitors and mature tissue mast cells (Figs. 2, 5, and 6) was membrane-associated and likely autocrine.

That the mast cells in the spleen, lymph nodes, and skin of the patient were of the normal phenotype for those tissue sites was revealed by the expression of tryptase, chymase, and CPA (Figs. 3 and 6). Their normal appearance was also apparent from electron microscopy in which the granule structure was largely amorphous with some crystalline lattices and scrolls of the same periodicity (Fig. 7). There was no evidence of the tryptase-only protease phenotype or of the scroll-rich ultra-

structural phenotype, which defines mast cells localized to the lung parenchyma or intestinal mucosa.¹⁰ The proliferation of mast cells in spleen, lymph node, and skin tissue with an immunohistochemical (Figs. 3, 5, and 6) and ultrastructural phenotype (Fig. 7) (skin and spleen) characteristic for these locations supports the interpretations of an autocrine mast cell hyperplasia that is still subject to tissue-directed differentiation and maturation.

A mast cell line developed from a patient with mast cell leukemia and circulating metachromatic mast cells has revealed a point mutation of *c-kit* tyrosine kinase²⁸ identical to that of the growth factor-independent rat basophilic leukemia²⁹ and mouse P-815 mast cell lines.³⁰ Although the patient with type 3 systemic mastocytosis described in this report had normal levels of circulating SCF and lacked the myeloid dysplasia or leukemia observed to occur in type 2 or 4, both the highly immature circulating mast cells and the fully mature tissue mast cells expressed an immunochemically detectable ligand for the *c-kit* receptor. Splenic RNA from this patient contained two transcripts for SCF as determined by reverse-transcription polymerase chain reaction and DNA blot analysis, as also observed with the RNA from lesions of urticaria pigmentosa in other studies⁹ and normal spleen tissue, which suggests that the abnormality is post-transcriptional. We conclude that nonmetachromatic mononuclear cells are bone marrow-derived highly immature mast cells and that in this patient with aggressive systemic mastocytosis, the aggressive proliferation with characteristic tissue differentiation and maturation of mast cells may be due to their autocrine elaboration of SCF.

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