

Levels of mast-cell growth factors in plasma and in suction skin blister fluid in adults with mastocytosis: Correlation with dermal mast-cell numbers and mast-cell tryptase

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Background: Mast-cell accumulation has been observed in the skin and other organs of patients with systemic indolent mastocytosis (SM). The basis for this pathologic increase is not fully understood.

Objective: We sought to determine levels of mast-cell growth factors in the skin and plasma of patients with SM, patients with atopic dermatitis (AD), and healthy individuals and to correlate these levels to dermal mast-cell numbers and levels of mast-cell tryptase.

Methods: Skin suction blister fluid and plasma levels of stem-cell factor, IL-3, IL-4, IL-6, vascular endothelial growth factor, and total mast-cell tryptase were analyzed by means of ELISA. The number of mast cells was determined in a biopsy section taken from adjacent skin.

Results: Mast-cell numbers in the dermis were higher in patients with SM compared with numbers in patients with AD ($P < .001$) or in healthy control subjects ($P < .0001$) and correlated with tryptase levels in both skin blister fluid ($P < .0001$) and plasma ($P < .0001$). Stem-cell factor and vascular endothelial growth factor levels in the skin blister fluid and plasma of patients with SM were not significantly different from those in patients with AD or healthy control subjects. IL-3 and IL-4 levels were below the limit of detection. IL-6 levels were significantly increased in the plasma of patients with SM compared with in plasma of patients with AD ($P < .002$) and healthy control subjects ($P < .0001$) and correlated with plasma tryptase levels ($P < .05$) and dermal mast-cell numbers ($P < .02$).

Conclusion: Because elevated levels of IL-6 could contribute to the fever, fatigue, and osteoporosis observed in patients with SM and because IL-6 is antiapoptotic for mast cells, IL-6 could potentiate the biologic consequences of this disease. (*J Allergy Clin Immunol* 2002;109:82-8.)

Key words: Mastocytosis, atopic dermatitis, mast cells, tryptase, stem-cell factor, IL-6

Abbreviations used

AD: Atopic dermatitis
SCF: Stem-cell factor
SM: Systemic indolent mastocytosis
VEGF: Vascular endothelial growth factor

Systemic indolent mastocytosis (SM) in adults is characterized by an excessive accumulation of mast cells in the skin, bone marrow, gastrointestinal tract, lymph nodes, liver, and spleen.¹ Normal mast-cell development requires interaction between stem-cell factor (SCF) and KIT, the receptor for SCF on the surface of mast cells.² In vitro, IL-3 and IL-6 may increase mast-cell numbers; IL-4 has been reported to have either a growth-enhancing or growth-limiting effect.³ Furthermore, dermal mast-cell numbers have been reported to be increased in vascular endothelial growth factor (VEGF)-transfected mice,⁴ and patients with cutaneous mastocytosis have signs of increased vascularization (telangiectasia macularis eruptiva perstans).⁵

Theoretically, either a neoplastic process involving an activating mutation in KIT or hyperplasia caused by local or systemic overproduction of SCF, IL-3, IL-4, IL-6, or VEGF or a combination of the above could be responsible for the mast-cell proliferation seen in SM.⁶⁻⁸ The discovery of somatic mutations in KIT that cause constitutive activation of the receptor has now provided evidence of one mechanism promoting the clonal expansion of mast cells in SM.⁶ Others, however, have also reported increased immunohistochemical staining for SCF between keratinocytes in cutaneous mast-cell lesions, although local levels of SCF were not measured.⁹ The possibility that overproduction of soluble SCF could lead to SM is supported by the observation that intradermal injection of SCF results in mast-cell hyperplasia and hyperpigmentation.¹⁰

To further examine the possibility that local or systemic overproduction of mast-cell growth factors could play a role in the genesis of SM, we investigated soluble SCF, IL-3, IL-4, IL-6, and VEGF levels in plasma and in skin of patients with SM, patients with atopic dermatitis (AD), and healthy volunteers. Cytokine levels were cor-

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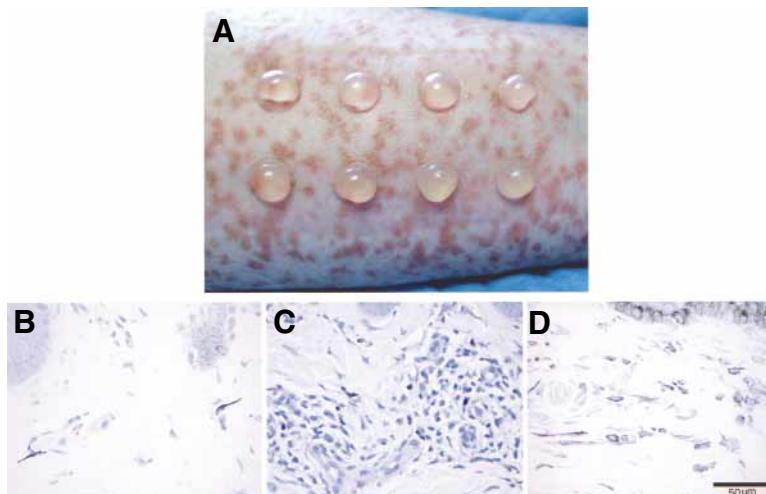


FIG 1. Skin blisters are induced by means of suction on the volar aspect of the forearm; a patient with SM is shown in **A**. Urticaria pigmentosa lesions can be seen through the blisters, which are filled with clear fluid. Toluidine blue-stained sections of skin (magnification 400 $\times$) from a normal control subject (**B**), a patient with AD (**C**), and a patient with SM (**D**) illustrate the mast-cell hyperplasia (toluidine blue positive) that characterizes dermal involvement in SM (compare **B** or **C** with **D**).

related with dermal mast-cell numbers and levels of mast-cell tryptase. As will be shown and in contrast to mast-cell tryptase levels, SCF, IL-3, IL-4, and VEGF levels were not elevated in skin blister fluid or in the plasma of adult patients with SM. IL-6 levels were elevated in the plasma of patients with SM and correlated with plasma tryptase levels and dermal mast-cell numbers.

METHODS

Adult subjects

After informed consent and approval by the National Institute of Allergy and Infectious Diseases Institutional Review Board, 10 patients with SM confirmed by means of bone-marrow biopsy, 7 patients with AD according to the criteria of Hanifin and Rajka, and 9 healthy control subjects participated in this study. Six patients with SM had an extent of skin involvement with urticaria pigmentosa of more than 50%, and in 4 patients less than 50% of the skin was involved. In all 6 patients with more than 50% skin involvement, the bone-marrow biopsy showed a diffuse pattern of mast-cell infiltration; in the other 4 patients, a focal distribution of mast cells was seen. At the time of the study, 2 patients with AD had disseminated erythematous and edematous lesions with some involvement of the blister site, and 5 patients had minimal localized disease without involvement at the blister site but with xerosis and some lichenification.

Suction skin blister technique

A sterile, 8-well skin suction chamber (Neuro-Probe, Inc, Rockville, Md) was secured on the volar forearm. A vacuum of 350 mm Hg was applied with an aspirator pump (Boeckel Industries Inc, Foesterville, Pa) for 1.5 to 2 hours. A heating pad warmed to 43°C was wrapped around the chamber to promote blister formation. When the blisters had developed (Fig 1, A), the pooled blister fluid was aspirated with tuberculin syringes and centrifuged at 300g for 5 minutes, with the supernatant stored at -80°C . Blood was collected in EDTA and centrifuged at 500g for 10 minutes, and then plasma was stored at -80°C .

Microscopy

Four-millimeter punch biopsy specimens were obtained adjacent to the blister sites to correlate skin blister fluid findings with mast-cell numbers in the dermis. In patients with SM and clearly defined urticaria pigmentosa lesions on the volar forearm next to the blister site, the biopsy specimen was taken from lesional skin. The biopsy specimens were immediately fixed in 10% phosphate-buffered formaldehyde and embedded in paraffin. Four- μm sections were stained with acidic 0.1% toluidine blue (Merck, Darmstadt, Germany) for 20 minutes and examined in a blinded fashion with a Zeiss Universal Microscope under 400 $\times$ magnification. An eyepiece graticule with a 10×10 matrix of 0.04-mm squares (Graticules LTD, Tonbridge, England) was used for quantification. Scoring of upper dermal mast-cell numbers was accomplished by starting at the upper left corner of the epidermis and systematically quantifying each field directly underlying the epidermis until the entire width of the section was examined.

Analysis

The following cytokines in blister fluid and plasma were determined by means of ELISA, as described by the manufacturers. A mouse monoclonal antibody was used for capture and a goat polyclonal antibody for detection, with the exception of IL-3, for which a mouse monoclonal antibody was also used for detection (lower detection limits are shown): IL-4, 0.25 pg/mL; IL-6, 0.15 pg/mL; SCF, 31.3 pg/mL; VEGF, 15.6 pg/mL (R&D Systems Inc, Minneapolis, Minn); and IL-3, 7.8 pg/mL (Biosource International Inc, Camarillo, Calif). All of these limits are well below the concentration of these growth factors required to influence mast-cell proliferation in vitro.¹¹ The VEGF assay is designed to measure VEGF₁₆₅, the biologically active isoform in physiologic, as well as pathologic, angiogenesis. Results obtained for naturally occurring human VEGF and recombinant human VEGF₁₂₁ showed linear curves that were parallel to the standard curves, as determined with the ELISA kit standards. Samples were diluted in appropriate calibrator diluent when exceeding the upper limit of detection of the assay.

Levels of total mast-cell tryptase were determined by means of immunoassay with the UNICAP system (Pharmacia-Upjohn, Pea-

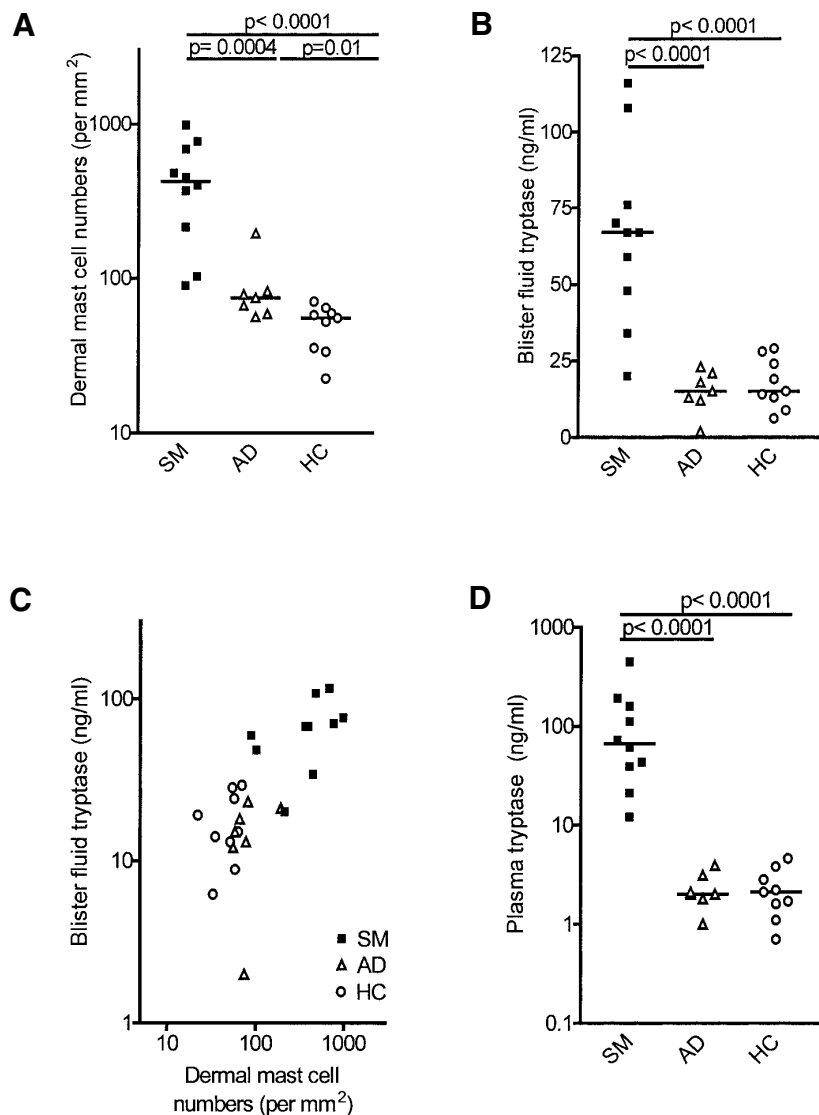


FIG 2. Dermal mast-cell numbers and mast-cell tryptase levels in the blister fluid and plasma. Mast-cell numbers in the dermis (**A**) and levels of blister fluid tryptase (**B**) are elevated in patients with SM when compared with values obtained in patients with AD and in healthy control subjects, and dermal mast-cell numbers correlate with blister fluid tryptase levels (**C**; $r = 0.75$, $P < .0001$). **D**, Plasma tryptase levels were also elevated in patients with SM when compared with plasma levels in patients with AD and in healthy control subjects. HC, Healthy control subjects.

pack, NJ), which uses the mouse IgG₁ mAb B12 for capture and mouse IgG₁ β -galactosidase-G4 mAb for detection. The total protein level was measured with a BCA protein assay kit, with BSA as standard (Pierce Inc, Rockford, Ill).

Values are given as medians and ranges. For statistical evaluation of differences between subject groups, the Wilcoxon rank sum (Mann-Whitney *U*) test was used. Correlations between parameters were analyzed with the Spearman correlation coefficient. *P* values of less than .05 were considered significant.

RESULTS

Ten patients with SM (median age, 51 years; range, 23-69 years), 7 patients with AD (median age, 35 years; range, 19-48 years), and 9 healthy control subjects (median age, 35 years; range, 29-43 years) were enrolled in this

study. Patient and control groups were comparable, with the exception that the age of patients with SM was higher compared with that of the other groups ($P < .007$). The total serum IgE levels in patients with SM (median, 10.5 kU/L; range, 6-17 kU/L) were lower ($P < .0001$) than in patients with AD (median, 470 kU/L; range, 41-24980 kU/L) and in healthy control subjects (median, 38 kU/L; range, 17-464 kU/L), as has been reported.¹²

As expected, patients with SM had increased dermal mast-cell numbers compared with patients with AD ($P = .0004$) and healthy control subjects ($P < .0001$; Fig 1, B-D, and Fig 2, A).^{13,14} There was no difference in total protein levels in the blister fluid among patients with SM (median, 1.7 mg/dL; range, 1.1-2.7 mg/dL), patients with

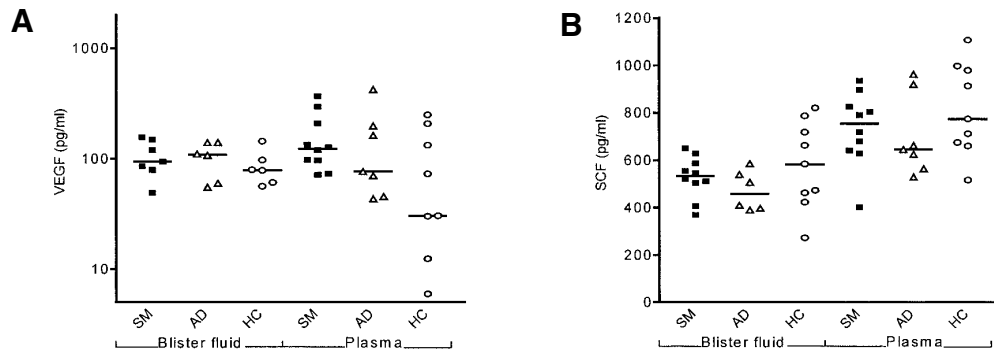


FIG 3. Blister fluid and plasma VEGF and SCF levels. Blister fluid and plasma VEGF (A) and SCF (B) levels were not significantly increased in patients with SM compared with those in patients with AD and in healthy control subjects. HC, Healthy control subjects.

AD (median, 1.5 mg/dL; range, 0.9-2.6 mg/dL), and healthy control subjects (median, 1.8 mg/dL; range, 1.3-3.1 mg/dL; data not shown). Levels of mast-cell tryptase in the blister fluids were elevated in patients with SM compared with those in patients with AD ($P < .0001$) and those in healthy control subjects ($P < .0001$, Fig 2, B). Blister fluid tryptase levels correlated with dermal mast-cell numbers ($r = 0.75$, $P < .0001$; Fig 2, C). Elevated tryptase levels were also observed in the plasma of patients with SM compared with levels in patients with AD ($P < .0001$) and in healthy control subjects ($P < .0001$; Fig 2, D). Values of plasma tryptase correlated with dermal mast-cell numbers ($r = 0.75$, $P < .0001$) and with blister fluid tryptase levels ($r = 0.77$, $P < .0001$).

Levels of the angiogenic growth factor VEGF were neither elevated in the blister fluid nor in the plasma in any group of patients or control subjects (Fig 3, A), including plasma VEGF levels in patients with SM compared with those in healthy control subjects. There was no correlation between levels of VEGF and tryptase either in the blister fluid or plasma, or to dermal mast-cell numbers.

The principle known growth factors for mast cells and their precursors are IL-3 and SCF. All blister fluid and plasma samples analyzed contained less than 7.8 pg/mL of IL-3, the lowest IL-3 standard. Blister fluid and plasma SCF levels were not different between patient and control groups and did not correlate with tryptase levels in the blister fluid and plasma or with dermal mast-cell numbers (Fig 3, B).

IL-4 levels in the blister fluid and plasma were below the detection limit of the assay (0.25 pg/mL) in all samples analyzed. Blister fluid levels of IL-6 were elevated in patients with AD ($P < .05$) but not in patients with SM in comparison with those in normal control subjects (Fig 4, A). In plasma, IL-6 levels were elevated in patients with SM compared with levels measured in normal control subjects ($P < .0001$) and in patients with AD ($P < .002$; Fig 4, A). There also was a correlation between plasma IL-6 and plasma tryptase levels ($r = 0.66$, $P < .05$; Fig 4, B) and between plasma IL-6 levels and dermal mast-cell numbers ($r = 0.75$, $P < .02$; data not shown) in patients with SM. In patients with SM only, blister fluid IL-6 levels correlated with levels of blister fluid tryptase

($r = 0.73$, $P < .02$; Fig 4, C) but not with dermal mast-cell numbers ($r = 0.60$, $P < .07$; Fig 4, D).

DISCUSSION

There is more speculation about the role of SCF in the pathogenesis of SM than there are data.¹⁵ This study in adults with SM, adults with AD, and adult healthy control subjects demonstrates that soluble SCF in the suction blister fluid (Fig 1, A) of the skin does not correlate with mast-cell numbers in the dermis (Fig 1, B-D) nor with skin blister total tryptase levels. Furthermore, SCF is not increased, either in skin blister fluid or in plasma of adult patients with SM compared with levels found in patients with AD and in healthy control subjects (Fig 3, B). This is the first report measuring production of mast-cell growth factors in skin blister fluid to assess soluble SCF levels of the skin in vivo, and these results supplement immunohistochemistry data, in which SCF in the extracellular compartment was judged either to be increased^{9,16} or to be no different from that found in normal control subjects.¹⁷ Our results do not thus support the hypothesis that local or systemic overproduction of soluble SCF in adults has a role in the pathogenesis of SM. Although the conclusion has been drawn that blister fluid resembles interstitial fluid,¹⁸ consideration must be given to a possible dilution during the procedure, which could prevent detection of small increases in local concentrations of growth factors. It is unlikely that the injury of the blister altered local levels of growth factors because growth factor levels were comparable among patients with SM, patients with AD, and healthy control subjects. Our findings are compatible with the concept that the mast-cell hyperplasia observed in adult patients with SM is, at least in part, caused by activating mutations in KIT.^{19,20}

The ELISA used in this study measures free SCF and SCF complexed with soluble KIT in the blister fluid and in the plasma. The possibility remains that there could be overproduction of membrane-bound SCF. However, other studies with immunohistochemistry have found no evidence that there is an increase in membrane-bound SCF in the dermis of patients with SM.^{9,17}

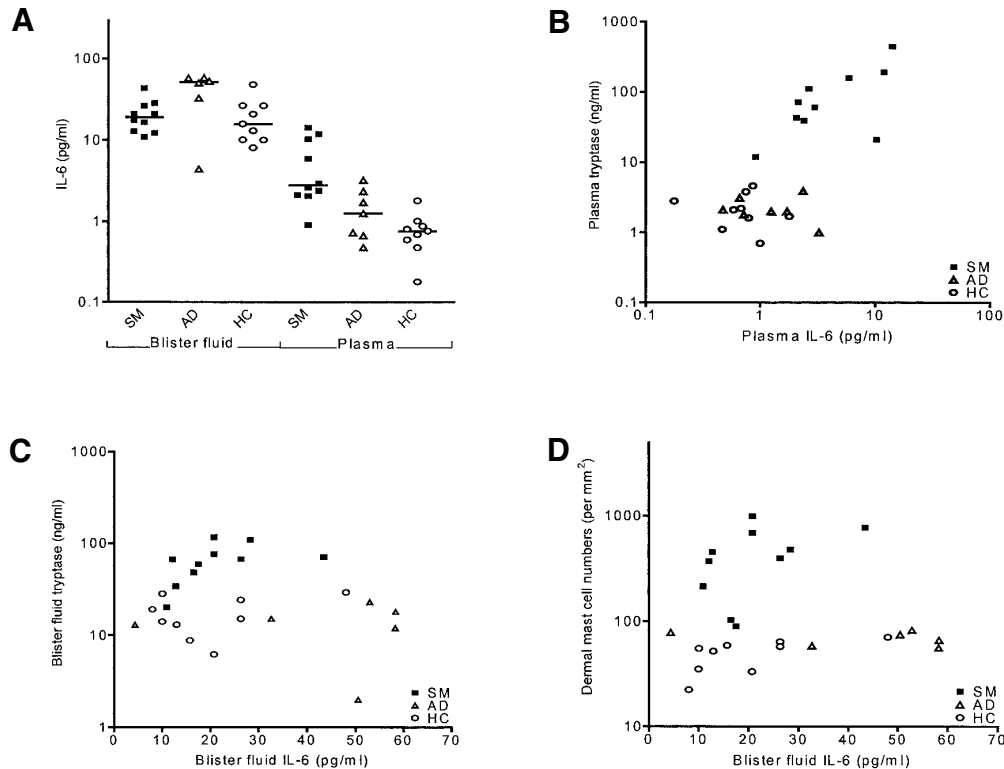


FIG 4. Blister fluid and plasma IL-6 levels and correlation to dermal mast-cell numbers and to blister fluid and plasma tryptase levels. **A**, Blister fluid IL-6 levels are elevated in patients with AD compared with values obtained in healthy control subjects ($P < .05$), and plasma IL-6 levels are elevated in patients with SM compared with those in patients with AD ($P < .002$) and in healthy control subjects ($P < .0001$). **B**, Plasma IL-6 levels are correlated with levels of plasma tryptase in patients with SM ($r = 0.66$, $P < .05$). **C**, There is a significant correlation between levels of blister fluid IL-6 and blister fluid tryptase values in patients with SM ($r = 0.73$, $P < .02$). **D**, There was no correlation between blister fluid IL-6 levels and dermal mast-cell numbers in patients with SM ($r = 0.60$, $P < .07$).

We were unable to detect IL-3 and IL-4 at levels higher than the detection limits in skin blister fluid and plasma of all patients analyzed. These limits are well below the concentration of these growth factors required to influence mast-cell proliferation *in vitro*.¹¹

Patients with AD were chosen to be a control group because they have been reported to have mildly elevated mast-cell numbers,²¹ which we confirmed in this study (Fig 2, A). However, SCF was not elevated in the blister fluid or plasma of this patient group, and SCF levels here also did not correlate with dermal mast-cell numbers. Lower IgE levels were observed in patients with SM compared with values obtained in patients with AD and in healthy control subjects and may reflect binding of IgE by an increased number of mast cells.¹² The most notable finding in patients with AD was an elevation of IL-6 in skin blister fluid in comparison with values observed in healthy control subjects (Fig 4, A). Increased serum IL-6 levels have also been reported in a larger group of 26 patients with AD compared with those in normal control subjects.²²

Levels of IL-6 were highly elevated in the plasma obtained from patients with SM (Fig 4, A) and correlated with plasma tryptase levels (Fig 4, B) and with dermal mast-cell numbers. IL-6 is produced by inflamma-

tory cells, such as monocytes-macrophages, mast cells, activated T cells, fibroblasts, and neutrophils, and has been found to be highly expressed by keratinocytes in psoriatic skin.²³ Skin, especially when injured or inflamed, has also been reported to show elevated IL-6 levels.²⁴ The high elevation of plasma IL-6 levels in patients with SM may relate to systemic involvement. Mean IL-6 levels appear to be more than 10-fold elevated in certain malignancies, such as lymphoma, whereas in patients with autoimmune inflammatory disorders, such as rheumatoid arthritis, systemic lupus erythematosus, or Sjogren syndrome, mean levels of plasma IL-6 are generally about 5- to 10-fold increased compared with those found in healthy control subjects,²⁵⁻²⁷ as is the case for patients with SM (mean plasma IL-6 levels of patients with SM, patients with AD, and healthy control subjects are 5.5, 1.5, and 0.8 pg/mL). Plasma IL-6 levels also increase with age at approximately 0.016 pg/mL per year in patients aged between 23 and 85 years,²⁸ which cannot account for the differences found in this study. The sources of IL-6 in SM may not directly relate to mast cells but rather to the inflammatory cells, including monocytes, mentioned above, which increase in patients with SM.¹

There was no significant increase of VEGF in the skin blister fluid or plasma of patients with SM (Fig 3, A). VEGF has been reported to stimulate mast-cell migration, and transgenic mice are reported to have higher numbers of dermal mast cells.^{4,29} VEGF is also reportedly produced by mast cells,³⁰ but mast-cell numbers and blister fluid tryptase levels were not correlated with VEGF levels. Thus this study provides no evidence for a role for VEGF in the pathogenesis of SM.

Skin blister fluid and plasma total tryptase levels were elevated in patients with SM. Skin blister fluid levels of tryptase were higher than plasma levels and correlated with mast-cell numbers in the dermis (Fig 2). Blisters were large enough to cover lesional and nonlesional areas in patients with SM, both areas of which, to a greater or lesser degree, show an increase in mast cells.¹³ Thus mast-cell numbers under the blister site would have, on average, approximated those in the biopsy specimens, which accounts for the statistically significant correlation. Skin blister fluid tryptase levels are also known to be elevated in patients with urticaria but not in patients with AD or psoriasis.³¹ In our study dermal mast-cell numbers correlated with plasma tryptase levels, which suggests that the skin is a major production site for plasma tryptase.

In conclusion, this study found no evidence to support the hypothesis that a local overproduction of mast-cell growth factors in the dermis contributes to the mast-cell hyperplasia observed in the skin of patients with SM. The study did, however, find that IL-6 levels are elevated in plasma obtained from patients with SM. This observation is of potential importance because some of the pathologic features of SM, such as fever, fatigue, and osteoporosis, are possible consequences of the overproduction of IL-6.³² Finally, IL-6 in the presence of SCF prevents mast-cell apoptosis.³³ Thus although mutations in KIT may be a principle initiating factor in the pathogenesis of SM (and were present in PBMCs in 3 patients studied [data not shown]), elevated IL-6 levels as a secondary event could potentiate the biologic consequences of these mutations. Strategies to inhibit the actions of elevated levels of IL-6 or strategies directed to inhibiting IL-6 synthesis could, in some cases, possibly have a role in treating patients with SM.

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