

Cutaneous Biology

Decreased cutaneous expression of stem cell factor and of the p75NGF receptor in urticaria

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Summary

Background Mast cells, the main effector cells in urticaria, have been reported to be increased in number in lesional and nonlesional skin of urticaria patients, but the underlying mechanisms have so far not been studied. Serum NGF has however, been reported to be increased in urticaria.

Objectives We have therefore explored the potential involvement of known mast cell growth modulating factors in urticaria.

Methods Tissue sections from patients with different types of urticaria and healthy controls were studied for the immunohistochemical expression of known mast cell growth factors (stem cell factor, SCF; nerve growth factor, NGF), of the inhibitory granulocyte-macrophage colony-stimulating factor (GM-CSF), and of the corresponding receptors, using the alkaline phosphatase–antialkaline phosphatase technique.

Results Compared to skin of normal controls, staining for SCF, but not for NGF and GM-CSF, was significantly decreased in epidermis, endothelium and perivascular cells in lesional and nonlesional skin of all urticarias. On separate analysis of urticaria subtypes, decreased expression reached significance only in delayed pressure urticaria. Expression of the p75NGF receptor (p75NGFR) was also significantly decreased on endothelium and on perivascular cells of lesional and nonlesional skin in all urticarias. On evaluation of serial sections, p75NGFR expression was also decreased on c-Kit positive dermal mast cells. In contrast, expression of the NGF receptor tyrosine kinase and of the SCF and GM-CSF receptors was unchanged.

Conclusions These findings show that SCF and p75NGFR are selectively and systemically down-regulated in the skin of urticaria patients and may represent a negative feedback to increased mast cell reactivity and proliferation.

Key words: c-Kit, growth factors, mast cells, nerve growth factor, stem cell factor, urticaria

Urticaria is a mast cell dependent disease that varies widely in its clinical presentation, in the stimuli that elicits it, and in the intensity and types of inflammatory infiltrate. Furthermore, inflammation and the associated disease seem to be in part self-perpetuating.¹ Irrespective of the type of urticaria, mast cell numbers, e.g. based on extended toluidine blue staining at low pH of a large number of formalin-fixed sections, have

been shown to be increased by at least 100% in lesional and nonlesional patient skin,^{2,3} but the mechanisms involved have so far not been studied.

In recent years, major advances have been made in the understanding of mast cell ontogeny and turnover in the tissue, specifically showing that mast cells arise from myeloid stem cells in the bone marrow, that they circulate as precursors within the monocytic fraction of the blood, and that they emigrate from there into the tissue where specific growth and chemotactic factors regulate their growth and survival.^{4,5}

Stem cell factor (SCF) is a pluripotent haematopoietic factor that also induces mast cell growth, differentiation,

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survival, secretion and chemotaxis (reviewed in⁶). In the skin, SCF is produced by keratinocytes, fibroblasts, endothelial cells and mast cells. Its biological effects are mediated via a transmembrane tyrosine kinase type III receptor named c-Kit which, in the skin, is expressed only on mast cells and melanocytes. In humans, SCF has been shown to induce mast cell proliferation, differentiation, survival, chemotaxis and secretion both *in vitro* and *in vivo*.^{7–10}

Nerve growth factor (NGF) is comparable to SCF with regard to its *in vitro* effects on human mast cell differentiation,^{11,12} but it exerts no chemotactic or antiapoptotic activities on human mast cells.^{8,13} Its biological activities are mediated via two receptors: a 75-kDa (p75) receptor glycoprotein that is expressed by many cell types including keratinocytes, melanocytes and nerve fibres in the skin, and a 140-kDa tyrosine kinase (TrkA) NGF receptor that has been identified primarily in nervous tissue, but also on keratinocytes, melanocytes, various myeloid cells, and on immature and mature human mast cells (reviewed in¹³).

GM-CSF, a multipotent haematopoietic growth factor, induces proliferation of primitive mast cell progenitors, but interferes with further steps of mast cell differentiation and maturation.^{14–16} The factor binds to a GM-CSF-specific, low affinity 45-kDa α -subunit and a 120-kDa β -subunit that together form a high affinity binding site that is expressed on most mature leucocytes, dendritic and endothelial cells.^{17–19}

In the past, we have found that expression of SCF and both NGF receptors are increased in human cutaneous scars at protein and/or mRNA level, suggesting a possible pathogenetic impact of these growth factors or their receptors on mast cell increase and function during the process of scar formation.²⁰ In the present study, we have examined the above factors and their receptors for their expression in urticaria lesions by immunohistochemistry. The data show an unexpected decrease of SCF as well as of p75NGF receptor (p75NGFR) expression, suggesting a distinctive regulation of these molecules in urticaria.

Materials and methods

Tissue

Biopsies were taken after informed consent from test sites of patients with delayed pressure urticaria (DPU), from fresh spontaneous lesions of unselected acute and chronic urticaria, irrespective of the underlying aetiology, and from contralateral nonlesional skin of the

same patients, as well as from skin of normal controls ($n =$ five each). Clinical characteristics of the patients have been reported previously.²¹ Briefly, biopsies were taken from skin anaesthetized circumferentially with 1% lidocaine without added vasoconstrictives, preferably from the trunk. In DPU, lesions were elicited by application of weighted pressure rods to the back skin, as described,²² and lesions appearing 4–6 h later were biopsied at this time point. Biopsy specimens were snap frozen and stored at -80°C until analysis. Approval for the study was obtained from the local ethics committee.

Determination of growth factor and receptor proteins

Immunohistochemistry was done on cryostat sections fixed in 10% acetone, using the alkaline phosphatase–antialkaline phosphatase (APAAP) methodology, as described before,²³ after prior titration and appropriate blocking to ensure specificity of staining. The following antibodies were studied: monoclonal antibodies against SCF, NGF, GM-CSF, TrkA, p75NGFR (Boehringer, Mannheim, Germany) [all immunoglobulin (Ig) G1], against the SCF receptor c-Kit (YB5B8, kindly provided by Dr L.K.Ashman, Adelaide, Australia), and against the α -chain of the GM-CSF receptor (GM-CSFR; Cdw116) (IgG2a) (Genzyme, Cambridge, MA U.S.A.). A murine monoclonal antibody against human desmin (IgG1) (Dianova, Hamburg, Germany) and a nonspecific antibody of the IgG2a isotype (Immunotech, Marseille, France) served as negative controls. Sequential sections stained with the c-Kit antibody YB5B8 and NGF receptor specific antibodies were studied for coexpression on mast cells.

Sections were evaluated as previously described.²¹ Briefly, cells were evaluated at $\times 400$ magnification in a microgrid covering 6.25 nm epidermal length/field or 6.25 nm^2 in the dermis by three independent observers. Reactivity was classified on a 4-point scale as: 0 = no staining; 1 = weak staining, rare positive cells; 2 = more intense staining, more cells; 3 = intense staining, many cells; 4 = very intense staining, all cells.

Statistical significance was calculated with the two- and one-tailed Student's *t*-test samples.

Results

Epidermal reactivity

SCF reactivity was prominent throughout the epidermis, with statistically significant less intense and extensive staining in lesional and nonlesional skin of

Table 1. Stem cell factor reactivity scores in urticaria biopsies vs. normal controls

	Urticaria		Normal controls
	Lesional skin	Nonlesional skin	
Epidermis	1.1 ± 0.8 ^a	1.4 ± 0.5 ^c	2.1 ± 0.2
Endothelium	2.4 ± 0.5 ^b	2.3 ± 0.59 ^c	3.0 ± 0.1
Perivascular and interstitial cells	2.4 ± 0.5 ^d	2.4 ± 0.5 ^f	3.1 ± 0.5

Data are shown as means ± SD. ^a*P* = 0.0001, ^b*P* = 0.0005, ^c*P* = 0.004, ^d*P* = 0.0021, ^e*P* = 0.01, ^f*P* = 0.06, compared with normal controls.

urticaria, as summarized in Table 1 and illustrated in Figure 1(a–c). On analysis of subtypes of urticaria (not shown), significance compared to controls was, however, only reached for lesional skin of DPU (*P* = 0.014). In contrast, immunoreactivity to NGF and GM-CSF was weak and detectable in only a few scattered epidermal cells, with no differences between skin from controls and urticaria patients (not shown).

Investigations of the receptors for these growth factors showed staining for c-Kit only in the basal layer of the epidermis (Fig. 1d), corresponding to the location of melanocytes,²⁴ with no differences between urticaria and control skin. The TrkA was expressed throughout the epidermis (Fig. 2a,b), but no differences in reactivity were noted for normal skin (mean intensity 3.6) vs. lesional and nonlesional skin in urticaria (mean 3.8 and 3.9, respectively). For the p75NGFR, expression was scarce in basal epidermal cells, again with no differences between urticaria and normal skin (Fig. 2c,d). Epidermal reactivity for GM-CSF and its receptor was not noted at all (not shown).

Dermal reactivity

In the dermis, SCF immunoreactivity was seen particularly on endothelial, perivascular, periappendageal and interstitial cells, with lower reactivity in lesional and nonlesional urticaria vs. normal skin (Fig. 1a–c; Table 1). Epithelial structures located in the dermis, such as hair follicles and sweat glands, were stained as described above for the epidermis, with reduced reactivity in urticaria lesional and nonlesional skin (not shown). Only rare dermal cells showed reactivity for NGF and GM-CSF, with no differences in urticaria patients and controls (not shown).

Both NGF receptors were expressed at moderate intensity on endothelial and perivascular dermal cells. For the TrkA, mean values on endothelial cells were

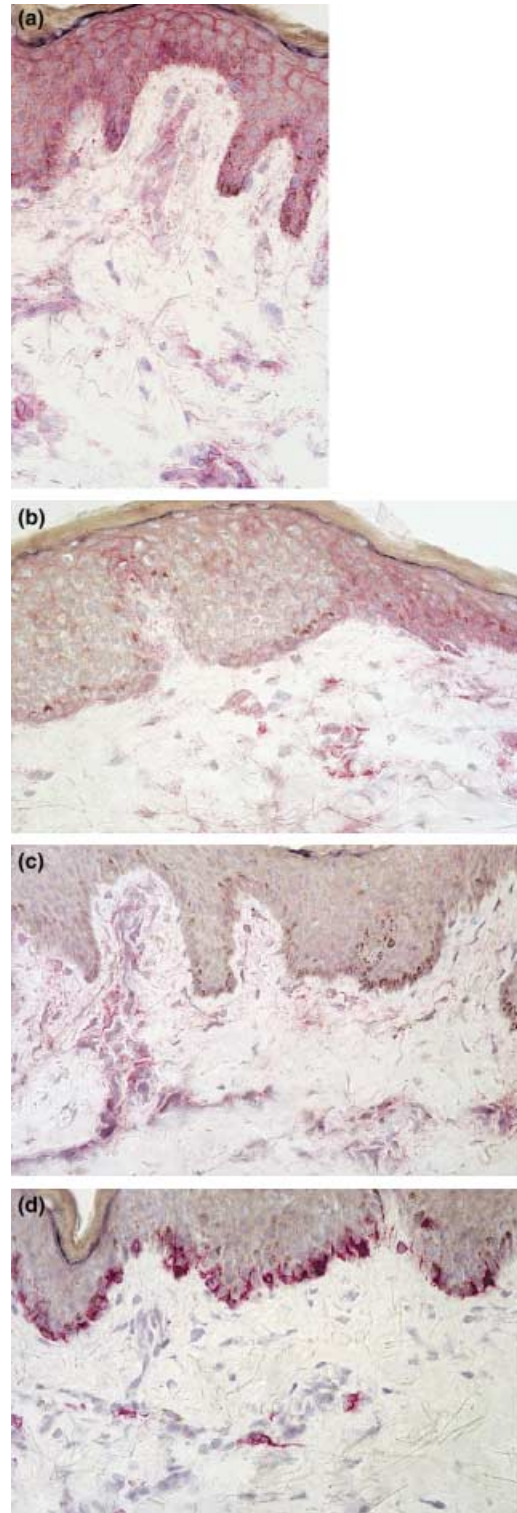


Figure 1. Immunoreactivity towards stem cell factor (SCF) and its receptor in urticaria. SCF in (a) normal skin, (b) acute urticaria, and (c) delayed pressure urticaria lesions and (d) SCF receptor c-Kit in delayed pressure urticaria lesion. Immunohistochemistry using the alkaline phosphatase–antialkaline phosphatase technique (magnification × 400).

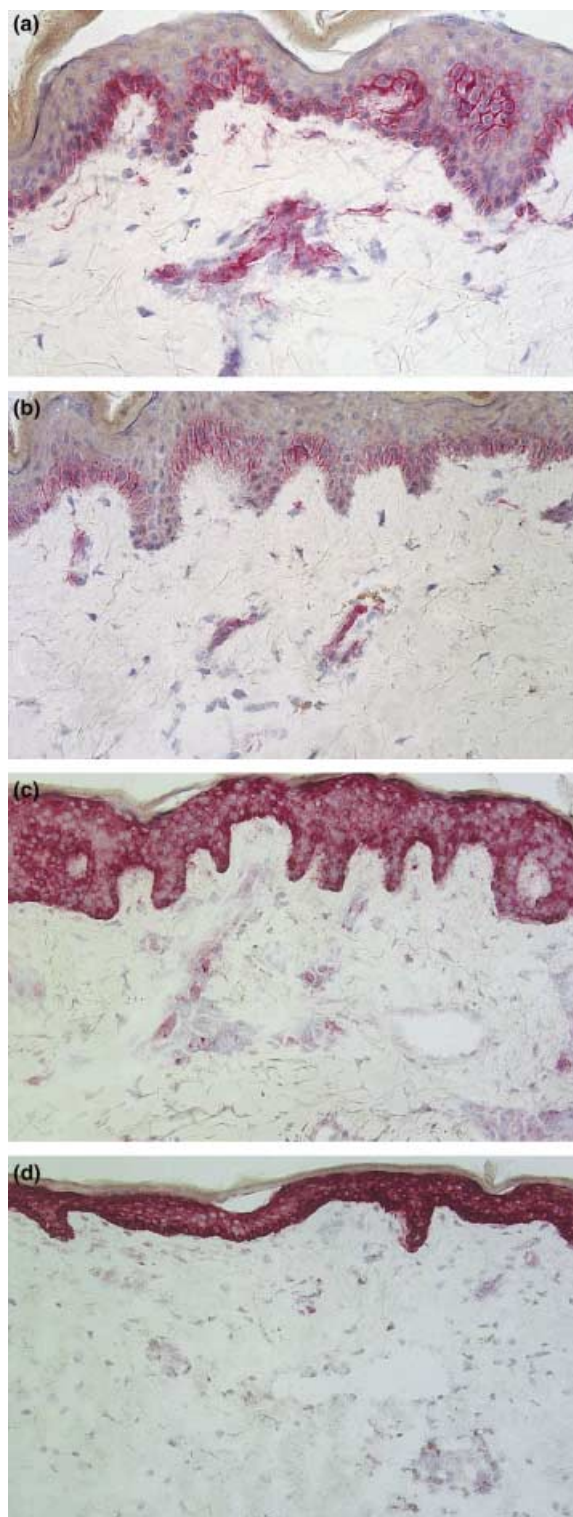


Figure 2. Immunoreactivity towards nerve growth factor (NGF) receptors in normal skin vs. urticaria lesions. p75NGF receptor (a) normal skin, (b) delayed pressure urticaria lesional skin; both at magnification $\times 400$; tyrosine kinase (c) normal skin, (d) chronic urticaria lesion; both at magnification $\times 200$.

Table 2. NGF-p75 reactivity in lesional skin of different types of urticaria and normal skin

	Endothelium	Perivascular cells
Acute urticaria	1.6 ± 0.6	0.2 ± 0.4^a
Chronic recurrent urticaria	1.4 ± 0.5	0.2 ± 0.5^b
Delayed pressure urticaria	1.6 ± 0.6	1.2 ± 0.44
All urticarias	1.5 ± 0.5^a	0.5 ± 0.6^c
Normal control skin	2.0 ± 0.1	1.2 ± 0.6

Data are shown as means $\pm$ SD. ^a $P = 0.002$, ^b $P = 0.008$, ^c $P = 0.03$, compared with normal controls.

1.6 vs. 1.8 and on perivascular cells 1.0 vs. 1.4, comparing all urticaria vs. normal skin specimens, respectively. In epithelial structures located in the dermis, TrkA reactivity was as strong as in the epidermis (mean 3.2 for both normal skin and urticaria) (Fig. 2c,d). In contrast, reactivity for the p75NGFR was weaker in endothelial and/or perivascular cells in lesional skin of acute and chronic, but not in delayed pressure urticaria (Table 2) and in nonlesional patient skin (not shown). Staining was also noted on sweat glands and nerves, with no alterations in reactivity of lesional vs. nonlesional vs. normal skin (Fig. 2a,b and not shown).

As expected, dermal c-Kit reactivity was only observed in perivascular and periappendageal cells, corresponding to the location of dermal mast cells (Fig. 1d). Values were 2.4 for normal skin, 2.17 for lesional and 1.92 for uninvolved patient skin, with no statistically significant differences. This held also for urticaria subtypes (not shown).

In contrast to the reactivity described so far, staining for GM-CSF and its receptor was weak and scarce in perivascular dermal cells only (not shown).

Nerve growth factor receptor expression on c-Kit positive mast cells

We have previously observed that in scar tissue, expression of the p75NGFR as well as of c-Kit is decreased in resident dermal mast cells, based on cells stained with avidine.^{20,25} We therefore analysed the expression of these molecules on sequential sections stained for c-Kit and the NGF receptors. The GM-CSFR was not quantified because overall reactivity was weak, with no apparent differences between normal skin and urticaria sections. In all urticaria lesional and nonlesional skin sections, p75NGFR reactive c-Kit-positive mast cells were reduced by a mean of 36.2%, compared with control skin, and no TrkA reactivity was observed on c-Kit positive cells in the dermis of normal or diseased skin.

Discussion

The present findings demonstrate for the first time alterations in the expression of mast cell growth factors and/or their receptors in skin biopsies of involved and uninvolved skin in different types of urticaria, including acute urticaria. They suggest furthermore that GM-CSF, a haematopoietic growth factor known to inhibit mast cell differentiation, most likely plays no role in urticaria.

The decreased expression of SCF was found in all cells known to synthesize and release this potent mast cell growth and activating factor in the skin.^{6,26,27} Its demonstration in urticaria is in line with a recent report showing a similar picture in actively progressing mastocytosis,²⁸ but it stands in stark contrast to increased SCF expression in human scar tissue, as reported by us recently using the same APAAP methodology as well as polymerase chain reaction.²⁰ Increased vs. decreased SCF expression may reflect divergent mechanisms of inflammation which determine the fate of SCF in the tissue. SCF is known to be cleaved by mast cell-derived chymase to its active, soluble product,²⁹ but this should not affect immunodetection of the molecule. The decreased expression of SCF in urticaria reflects instead most likely a decreased production of this factor or its decreased storage in the cells. Acutely released mast cell mediators during anaphylactic release from the cells may be involved. In fact, not only histamine, but also leukotrienes, interleukin-3 and tumour necrosis factor α are all up-regulated in the skin of urticaria patients,^{1,22} and these molecules might well affect mediator production and receptor expression on mast cells.

The increase of SCF expression, as observed in cutaneous scars,²⁰ may instead reflect pathogenetic events associated with more chronic, reparative processes. Alternatively, SCF decrease in urticaria tissue might be due to uptake of receptor-bound SCF into the cell. This fits with the observation that c-Kit-positive cells, as observed here, are slightly decreased in number, compared to the at least 100% increase of toluidine-blue positive mast cells in lesional and non-lesional skin in urticaria, as reported previously.^{2,3} No changes in mast cell numbers in urticaria were reported in studies where mast cell enzymes (tryptase, chymase, myeloperoxidase) were used as markers for the cells,^{30,31} probably due to their secretion from mast cells during urticarial reactions, making the cells less readily detectable (ghost mast cells). In view of the abundant source of SCF in the skin and the restriction

of its receptor to mast cells and melanocytes,⁶ it is tempting to assume that in this situation, receptor–ligand complex internalization by mast cells explains the pronounced decrease of SCF in epidermis and dermis in urticaria, together with the moderate decrease of c-Kit positive mast cells, but proof for this awaits further studies.

NGF is comparable in its potency to SCF during human *in vitro* mast cell differentiation,^{12,13} and might act like SCF as an autocrine growth factor for mast cell proliferation.³² NGF has been reported to be elevated in the serum of patients with diverse human allergic diseases, particularly in asthma, with moderately increased levels also in urticaria.³³ In contrast to these findings and the decreased SCF immunohistochemical reactions observed here, staining for NGF was weak and about the same in control and urticaria sections. This may be due to the basic reactivity of the NGF antibody, as NGF was also only poorly expressed in scar tissue using the same methodology.²⁰ Furthermore, the ability of cells from different organs to produce NGF has been shown to be highly variable³⁴ and in part modulated by other mediators of inflammation.³⁵

Even minor amounts of NGF might nevertheless exert some effects via increased expression of their receptors. In both urticaria sections and normal skin, immunoreactivity for both types of NGF receptors was distinct and strong on structures and cells known to express these receptors (reviewed in^{13,36}), including nerve bundles and melanocytes, lymphocytes, keratinocytes and Merkel cells. The selective reduction of the p75NGFR in different cell types in urticaria lesions, including mast cells, suggests increased NGF binding and increased internalization of the NGF-p75 complex. This fits with the decrease of the p75NGFR on endothelial cells and inflammatory cells both of which are exposed to increased NGF levels in serum³³ and therefore also dermal tissue fluid. In contrast to the p75NGFR, TrkA was neither expressed on mast cells nor was its expression significantly changed in urticaria sections. These findings stand in contrast to findings in scar tissue where this receptor is up-regulated,²⁰ and to those in immature human mast cells where TrkA has been identified,^{12,37} underlining again differences in the expression of this molecule independent of the type of inflammation and the microenvironment of the cells studied.

Taken together, the present findings show that SCF and the NGF receptor p75 expression is decreased in urticaria, even in uninvolved skin, and is thus most

likely induced via systemic factors. The underlying mechanisms, specifically those due to the acutely released mast cell mediators that regulate these molecules, remain to be determined. In pathological terms, this down regulation of mast cell growth factors and growth factor receptors may represent an attempt of the tissue to counteract mechanisms associated with mast cell precursor recruitment, proliferation, maturation and survival resulting in a further increase of mast cell numbers and increased releasability in the skin of urticaria patients.

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