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ORIGINAL ARTICLE **Basic Mechanisms in Allergic Disease**

Heterogeneity in the responses of human lung mast cells to stem cell factor

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Summary

Background Stem cell factor (SCF) is a growth factor that is involved in mast cell differentiation and proliferation. SCF primes human lung mast cells for enhanced responses to IgE-directed activation but is not generally recognized as a direct activator. SCF mediates its effects through c-kit.

Objective The aim of this study was to reappraise the effects of SCF on human lung mast cells.

Methods Mast cells were isolated from human lung. Mast cells were challenged with anti-IgE or SCF and the generation of histamine, cysteinyl-leukotrienes (cys-LTs) and prostaglandin D_2 (PGD_2) was assessed as was expression of the activation marker, CD63. The effects of c-kit inhibitors on mediator release were evaluated.

Results Stem cell factor (10 ng/mL) alone was unable to induce mediator release but primed mast cells for enhanced IgE-dependent secretion. At higher concentrations ($\geq$ 30 ng/mL), SCF had more varied effects and even when used alone was able to drive substantial levels of histamine release in about a third of all preparations studied. Similarly, SCF (100 ng/mL) alone was effective in stimulating the generation of cys-LTs in half of the preparations studied. SCF (100 ng/mL) was even more effective at stimulating PGD_2 generation as almost all preparations generated substantial quantities of the prostanoid. Mediator release induced by SCF was accompanied by the up-regulation of the activation marker, CD63. There was a positive correlation between the extent of mediator release induced by SCF and c-kit receptor expression. The effects of SCF on mediator release from mast cells were reversed by the c-kit inhibitor imatinib.

Conclusions and clinical relevance These data demonstrate that the responses of mast cells to SCF are heterogeneous. SCF can drive much greater levels of mediator release from mast cells, especially of PGD_2, than hitherto appreciated and this could be important in the context of respiratory diseases.

Keywords CD63, c-kit, cysteinyl-leukotrienes, dasatinib, histamine, human lung mast cell, imatinib, nilotinib, prostaglandin D_2, stem cell factor

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Introduction

The mast cell has long been recognized as central to allergic disease [1]. This association has spawned a large body of work that has been directed at understanding all aspects of mast cell biology including mast cell development, proliferation and activation.

A key molecule that plays an essential role in supporting mast cell differentiation and proliferation is stem cell factor (SCF). SCF, also known as c-kit ligand, is a growth factor that drives mast cell differentiation by interacting with the c-kit receptor (CD117) [2]. The cytoplasmic region of c-kit expresses intrinsic protein tyrosine kinase activity, and this enzymatic activity is stimulated by SCF binding to the receptor [3]. Mutations in c-kit, leading to constitutively active receptors, are associated with mastocytosis [4]. Mast cells express high levels of c-kit and CD117 can be used as a marker

to distinguish mast cells in a mixed population of cells [5]. These findings indicate that the SCF/c-kit receptor axis plays a critical role in regulating mast cell development.

Stem cell factor has also been studied as a potential activator of mast cells. Reports demonstrate that while SCF is relatively ineffective by itself in most mast cell types, it can act as a co-stimulus to enhance IgE-dependent mediator release from mast cells [6–9]. As the principal mechanism by which mast cells are activated is by allergens in an IgE-dependent fashion, SCF could play a role in exaggerating responses of mast cells to allergens *in vivo*. This could be of particular importance in the context of asthma. IgE-dependent activation of pulmonary mast cells leads to the rapid release of a wide variety of autacoids such as histamine, cysteinyl-leukotrienes (cys-LTs) and prostaglandin D_2 (PGD_2) and over the longer term leads to the generation of cytokines and pro-inflammatory chemokines [10–13]. The release and generation of these mediators causes bronchoconstriction, inflammation and airway remodelling [14–16]. As high levels of SCF have been identified in asthma [17, 18], it is probable that SCF may potentiate IgE-triggered lung mast cell responses and so heighten pathological processes.

In this study, we have investigated the effects of SCF on a number of measures of human lung mast cell activation. Our data show that SCF is a more effective activator of human lung mast cells than has hitherto been appreciated. These findings indicate that SCF may play an important role in activating mast cells *in vivo* and this could influence the development of certain respiratory diseases.

Methods

Buffers

Phosphate buffered saline (PBS) was employed in these studies. PBS contained (mM): NaCl 137; $Na_2HPO_4.7H_2O$ 8; KCl 2.7; KH_2PO_4 1.5. PBS-HSA was PBS additionally supplemented with: $CaCl_2.2H_2O$ 1 mM; $MgCl_2.6H_2O$ 1 mM; glucose 5.6 mM; human serum albumin (HSA) 30 μg/mL. The pH of PBS buffers was titrated to 7.3.

Preparation of compounds

Imatinib, nilotinib and dasatinib were prepared as stock solutions (10 mM) in dimethyl sulphoxide and stored at − 20°C in appropriate aliquots. SCF was prepared as a stock solution (100 μg/mL) in water and stored frozen in appropriate aliquots. Monoclonal anti-human IgE antibody (2 mg/mL stock) was stored frozen over the long-term but a working stock was kept at 4°C for up to a month.

Lung tissue

Non-lesional tissue from lung resections of patients was obtained following surgery. Most of the patients were undergoing surgery for carcinoma. The male to female split was two to one and 90% of the patients were white caucasians. The provision of lung tissue and the use of the tissue in this study were approved by the National Research Ethics' Committee (REC reference:10/H1010/50). Informed consent was obtained from those who permitted us to use tissue in this study.

Cell isolation

Mast cells were isolated from human lung tissue by a modification of the method described by Ali and Pearce [19]. Macroscopically normal tissue from lung resections was chopped into small fragments with scissors in a small volume of Rosswell Park Memorial Institute (RPMI) -1640 buffer containing Foetal bovine serum (FBS) (10%) and DNase (15 μg/mL). The chopped tissue was washed over a nylon mesh (100 μm pore size; Incamesh, Warrington, UK) with ~ 250 mL of the same RPMI buffer to remove lung macrophages. The tissue was reconstituted in the same buffer (5 mL per g of tissue) containing collagenase Ia (800 Units per g of tissue) and placed in a shaking water bath set at 37°C for 90 min. The supernatant (containing some mast cells) was separated from the tissue by filtration over nylon mesh. The collagenase-treated tissue was then reconstituted in a small volume of the FBS and DNase supplemented RPMI buffer and disrupted mechanically with a syringe. The disrupted tissue was then washed over nylon gauze with the supplemented RPMI buffer (300–600 mL). The pooled filtrates were sedimented (480 × g, room temperature, 10 min), the supernatant discarded and the pellets reconstituted in the same RPMI buffer (100 mL). The pellet was washed a further two times. Mast cells were visualized by microscopy using an alcian blue stain [20]. Of the total cells, 3–13% were mast cells. This method generated approximately 6×10^5 mast cells per g of tissue. Mast cells of enhanced purity (15–60% purity) were used in this study and were prepared by flotation over discontinuous Percoll gradients [21].

Mediator release

Mediator release experiments were performed in PBS-HSA buffer. Either anti-IgE or SCF was used to activate the cells. Human lung mast cells express IgE endogenously and so respond to anti-IgE without the need for a passive sensitization. Moreover, the response is not affected by purification [22]. Stimulus-induced secretion was allowed to proceed for 25 min at 37°C after which time the cells were pelleted by centrifugation (480 × g,

room temperature, 4 min). When imatinib, nilotinib or dasatinib was used, mast cells were incubated for 15 min with a c-kit inhibitor before challenge with a stimulus. Histamine released into the supernatant was determined by the modified [23] automated fluorometric method of Siraganian [24]. PGD_2 and cys-LT content in the supernatants were determined using commercially available kits (Cayman Chemical Company, Ann Arbor, MI, USA). Total histamine content was determined by lysing aliquots of the cells with perchloric acid at a final concentration of 1.6%. Cells incubated in buffer alone served as a measure of spontaneous histamine release which ranged from 2 to 8% of the total histamine content. Histamine release was thus expressed as a percentage of the total histamine content after subtracting the spontaneous histamine release. All experiments were performed in duplicate.

When overnight incubations were performed, RPMI-1640 buffer supplemented with penicillin (10 Units/mL), streptomycin (10 μg/mL), gentamicin (50 μg/mL) fungizone (1 μg/mL) and FBS (10%) was employed. Cells were incubated overnight at a density of 0.1×10^6 mast cells per mL in six well plates with or without SCF. After overnight incubation the cells were washed twice and reconstituted in PBS-HSA for mediator release experiments as described above.

Flow cytometry

Flow cytometry experiments were performed on mast cells of enhanced purity (20–60%) following flotation over Percoll. Mast cells were mostly identified as the c-kit (CD117) positive population using a monoclonal (IgG_1) anti-c-kit-PE (A3C6E2) or the same antibody tagged with Allophycocyanin (APC). Alternatively, mast cells were identified as the FcεRIα positive population using a monoclonal (IgG_{2b}) anti-FcεRIα-FITC (AER-37). FcεRIα expression in the $CD117^+$ population and CD117 expression in the $Fc\varepsilon RI\alpha^+$ population were determined. On occasion, IgE density in the $CD117^+$ population was also determined using a monoclonal (IgG_1) anti-IgE-FITC (BE5). Expression of CD63 was determined using a monoclonal (IgG_1) anti-CD63-FITC (AHN16.1/46-54-5).

Mast cells were first fixed with Medium A (Fix & Perm kit; Invitrogen, Carlsbad, CA, USA) for 20 min at room temperature. The cells were then washed in PBS. All subsequent incubations with antibodies were performed in a FACS buffer (PBS, 1% FBS, 5 mM ethylene diamine tetraacetic acid (EDTA), 0.02% sodium azide). Cells were incubated with conjugated antibodies (1 : 5–1 : 20) on ice in the dark for 1 h after which the cells were washed twice with FACS buffer and then re-suspended in FACS buffer for analysis. Samples were run on an LSR II flow cytometer (BD Biosciences, Oxford, UK) and data captured using DIVA software (BD Biosciences). The flow cytometer was optimized for use by setting up compensation samples using anti-mouse CompBeads (BD Biosciences). Analysis of data was performed using FlowJo software (Tree Star, Ashland, OR, USA). Briefly, debris and dead cells were gated out and the remaining viable cells were gated as either $CD117^+$ positive or $Fc\varepsilon RI\alpha^+$ positive populations. The amount of fluorescence in the gated population was quantified as net mean fluorescence index, which was calculated as the geometric mean of the specific antibody signal subtracted by the geometric mean of the relevant isotype control signal.

In some experiments, the time-dependent expression of CD63 following activation with a stimulus was determined. In this situation, mast cells were activated for discrete time intervals (1, 3, 6 or 9 min) and the reaction stopped by adding ice-cold PBS buffer containing EDTA (5 mM). The cells were then recovered by centrifugation and the cells incubated with the relevant antibodies as described in the previous paragraph.

Materials

The following were purchased from the sources indicated; collagenase, DNase, HSA, Percoll, mouse-IgG_1-FITC (Sigma, Poole, UK); gentamicin, penicillin/streptomycin, fungizone, RPMI 1640, mouse IgG_1-PE, mouse-IgG_1-APC, mouse IgG_{2b}-FITC (Invitrogen, Paisley, UK); imatinib (Cayman Chemical Company); nilotinib, dasatinib, (LC Laboratories, Woburn, MA, USA); anti-human IgE, clone HP6061 (Stratech Scientific Ltd, Newmarket, UK); SCF (Peprotech, Rocky Hill, NJ, USA); IL-6, IL-10 (R&D Systems, Oxford, UK); FBS (PromoCell, Heidelberg, Germany); anti-c-kit-PE, anti-c-kit-APC (Miltenyi Biotec, Woking, UK); anti-FcεRIα-FITC (eBioscience, Hatfield, UK); anti-CD63-FITC, anti-CD81-FITC (Santa Cruz Biotechnology, Heidelberg, Germany); anti-IgE-FITC (Abcam, Cambridge, UK). All other reagents were from Sigma.

Data analysis

Potencies (EC_{50}) and maximal responses (E_{max}) were determined by non-linear regression analysis (GraphPad Prism, version 5.0d, La Jolla, CA, USA). Statistical significance was assessed utilizing either repeated measures ANOVA or Student's *t*-test.

Results

Stem cell factor enhances mediator release

Previous studies have shown that SCF enhances IgE-dependent histamine release from mast cells [6–8]. In accord with these findings, SCF (10 ng/mL), which had little effect on histamine release from human lung mast cells by itself, was found to enhance release induced by a suboptimal concentration (0.2 μg/mL) of anti-IgE

(Fig. 1a). In addition, under the same experimental conditions, the IgE-dependent generation of cys-LTs (Fig. 1b) and PGD_2 (Fig. 1c) from mast cells was potentiated by SCF.

Stem cell factor stimulates mediator release

In further studies, the effects of higher concentrations (> 10 ng/mL) of SCF on mast cells were investigated (Fig. 2). These studies demonstrate that in two-thirds of the preparations (6 out of 9 preparations), SCF (0.1–300 ng/mL) hardly affected the release of histamine but in a third of the preparations, quite substantial levels of histamine release were observed at higher concentrations ($\geq$ 30 ng/mL) of SCF.

These studies were extended and in a total of 56 mast cell preparations, the release of histamine in response to either SCF (100 ng/mL) or a maximal releasing (2 μg/mL) concentration of anti-IgE was evaluated. The extent of release was highly variable among preparations (Fig. 3a). Although, overall, SCF induced levels of histamine release that were lower (11 ± 2%, $n = 56$) than release induced by anti-IgE (31 ± 2%, $n = 56$), it was noticeable that 18 of 56 mast cell preparations were found to release sizeable levels of histamine (16 to 53%) in response to SCF. There was a reasonably good positive correlation ($r = 0.65$, $P < 0.0001$) between the response of mast cells to anti-IgE and to SCF (Fig. 3b). Hence, there was a tendency that mast cells that responded well to anti-IgE would also respond effectively to SCF.

In a subset of these mast cell preparations, the effects of SCF (100 ng/mL) and anti-IgE (2 μg/mL) on cys-LT generation from mast cells were also investigated. The generation of cys-LT induced by either stimulus was variable. Although, overall, SCF induced levels of cys-LT generation that were lower (6 ± 1 ng cys-LT/10^6 mast cells, $n = 25$) than that induced by anti-IgE (15 ± 3 ng cys-LT/10^6 mast cells, $n = 25$), it was noticeable that 13 of 25 mast cell preparations generated sizeable amounts of cys-LT (5–19 ng cys-LT/10^6 mast cells) in response to SCF alone (Fig. 3c). There was a positive correlation ($r = 0.41$, $P < 0.05$) between the

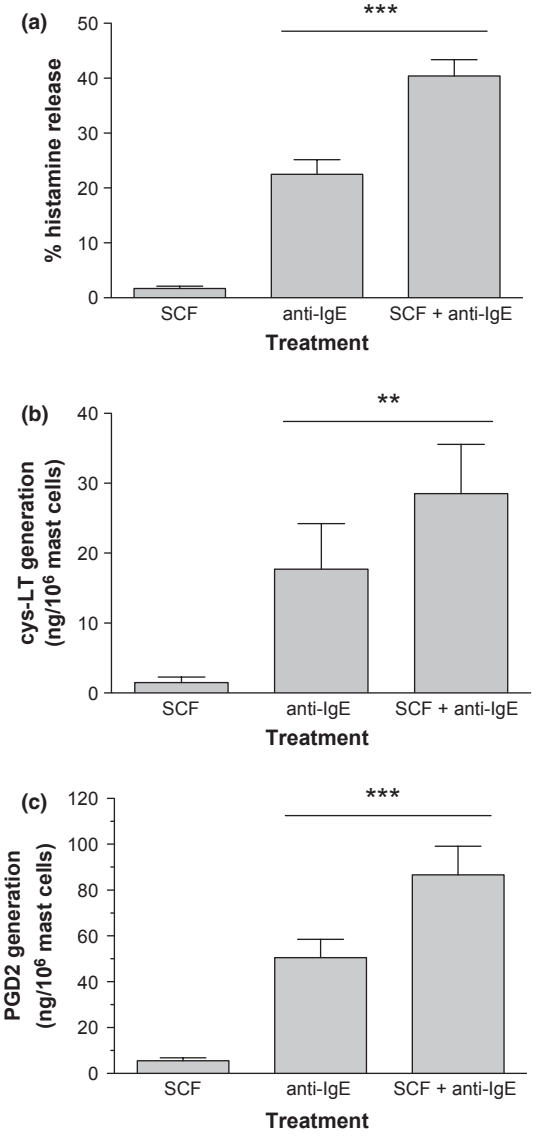

Fig. 1. Effects of stem cell factor (SCF) and anti-IgE on mediator release. Mast cells were treated for 2 min with or without SCF (10 ng/mL) and then with or without anti-IgE (0.2 μg/mL) for a further 25 min and the release of (a) histamine, (b) cysteinyl-leukotrienes and (c) prostaglandin D_2 in the supernatants was measured. Values are means ± SEM for (a) 26, (b) 11 and (c) 15 experiments. In all instances, the presence of SCF increased mediator release induced by anti-IgE to a statistically significant (**$P < 0.005$, ***$P < 0.0005$) extent.

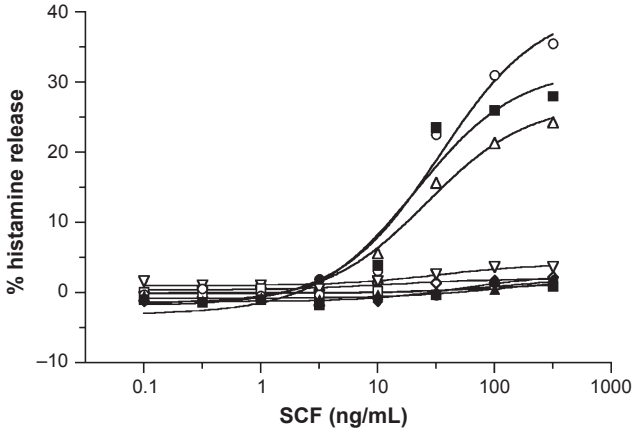

Fig. 2. Effect of stem cell factor (SCF) on histamine release. Mast cells were exposed to increasing concentrations of SCF for 25 min after which supernatants were recovered and assessed for histamine content. Results are expressed as the % histamine release. Each curve represents an individual mast cell preparation and data for a total of 9 different preparations are shown.

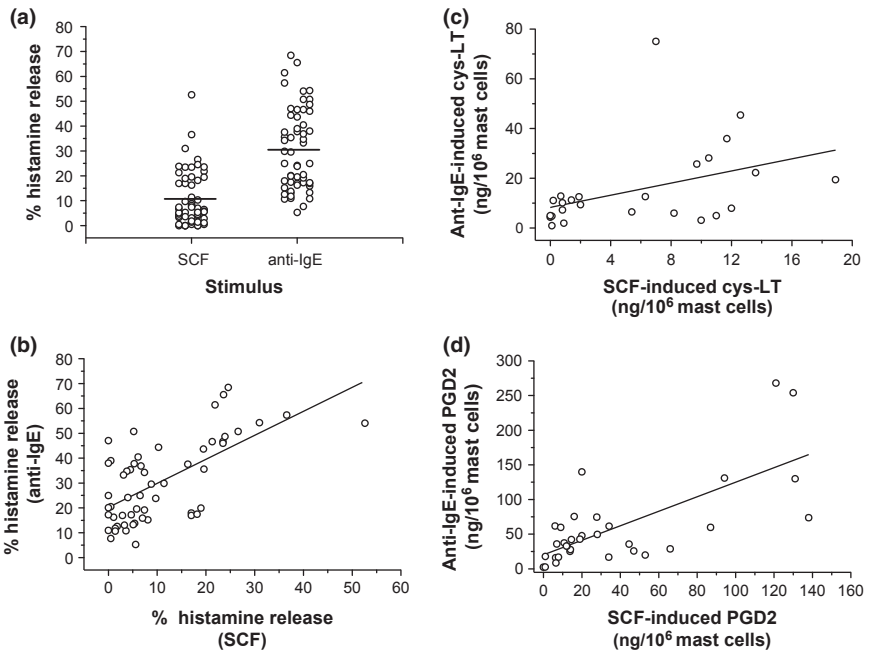

Fig. 3. Variability in mediator release among mast cell preparations. (a) Cells were incubated either with stem cell factor (SCF) (100 ng/mL) or anti-IgE (2 μg/mL) for 25 min after which histamine (a and b), cysteinyl-leukotrienes (cys-LT) (c) or prostaglandin D_2 (PGD_2) (d) released into the supernatants was evaluated. (a) Each point represents histamine release from a different mast cell preparation of a total of 56 that were studied. Horizontal solid lines represent mean data. (b) The histamine release data in (a) were reworked and a correlation ($r = 0.65$, $P < 0.0001$) was found between histamine release induced by SCF and anti-IgE. (c) There was a correlation between cys-LT generation induced by SCF and anti-IgE ($r = 0.41$, $P < 0.05$, $n = 25$). (d) There was a correlation between PGD_2 generation induced by SCF and anti-IgE ($r = 0.70$, $P < 0.0001$, $n = 33$).

response of mast cells to anti-IgE and to SCF in the context of cys-LT generation.

The effects of SCF (100 ng/mL) and anti-IgE (2 μg/mL) on PGD_2 generation from mast cells were also investigated and again, as with other mediators, the extent of PGD_2 generation was variable. SCF induced levels of PGD_2 generation that were lower (37 ± 7 ng $PGD_2/10^6$ mast cells, $n = 33$) than that induced by anti-IgE (59 ± 11 ng $PGD_2/10^6$ mast cells, $n = 33$). However, it was evident that in the vast majority of mast cell preparations (30 out of 33 preparations), sizeable quantities of PGD_2 (6–138 ng $PGD_2/10^6$ mast cells) were generated in response to SCF (Fig. 3d). There was a good positive correlation ($r = 0.70$, $P < 0.0001$) in the extent of PGD_2 generation from mast cells induced by anti-IgE and SCF. It was also noteworthy that most mast cell preparations that failed to release histamine or cys-LT in response to SCF, generated at least some PGD_2.

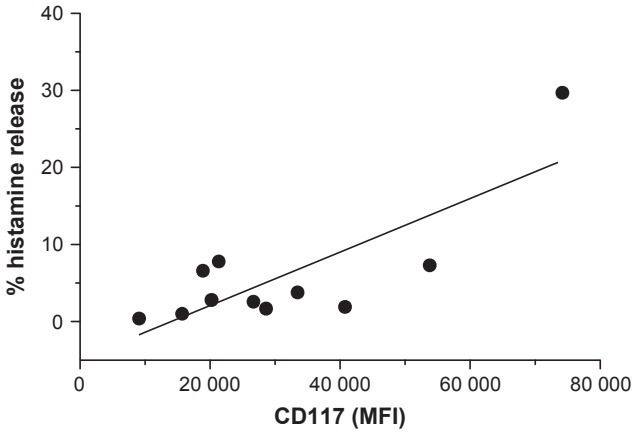

Fig. 4. Correlation between c-kit (CD117) expression and release induced by stem cell factor (SCF). Mast cells from a given preparation were either activated with SCF (100 ng/mL) for histamine release or assessed for CD117 expression by flow cytometry. There was a correlation between histamine release induced by SCF and CD117 expression ($r = 0.79$, $P < 0.005$, $n = 11$).

Stem cell factor responses correlate with c-kit receptor expression

In further experiments, we investigated whether the wide variability in the responses of mast cells to SCF might be explained by differences in c-kit expression. Our studies indicated that there was a positive correlation ($r = 0.79$, $P < 0.005$, $n = 11$) between the extent of histamine release induced by SCF and c-kit expression (Fig. 4). In contrast, there was no correlation between the extent of histamine release induced by anti-IgE and either FcεRIα expression ($r = 0.06$, $P = 0.83$, $n = 14$) or IgE density ($r = 0.08$, $P = 0.78$, $n = 14$).

Stem cell factor up-regulates CD63 expression

Up-regulation of the tetraspanin CD63 has been identified as a molecular marker of activation of mast cells and basophils [25–27]. Human lung mast cells were activated (1–9 min) with either SCF (100 ng/mL) or anti-IgE (2 μg/mL) and changes in CD63 expression assessed by flow cytometry. Following activation with either anti-IgE (Fig. 5, upper panel) or SCF (Fig. 5, lower panel), there was a time-dependent increase in CD63 expression. The extent of CD63 expression was linked to the extent of histamine release induced by the stimuli. Indeed, when taking the data as a whole there was a very good correlation ($r = 0.87$, $P < 0.005$, $n = 10$) between the extent of histamine release and the up-regulation of CD63 induced by both stimuli. In these same experiments, the expression of CD81, another tetraspanin linked to degranulation but not known to be up-regulated during activation [28, 29], was also monitored. Mast cells expressed little or no CD81 constitutively and there was no change in the levels of CD81 following activation with either anti-IgE or SCF (data not shown).

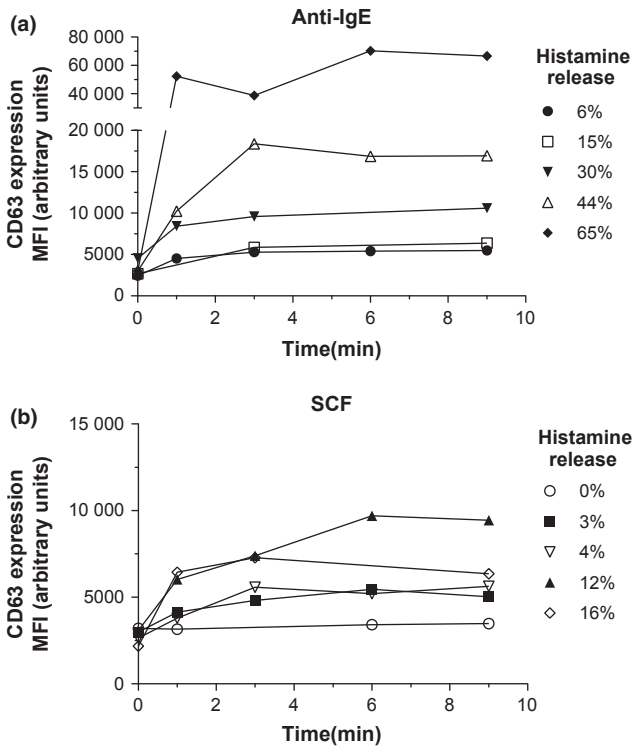

Fig. 5. Up-regulation of CD63 following mast cell activation. Mast cells were activated for discrete time intervals with either (a) anti-IgE (2 μg/mL) or (b) stem cell factor (SCF) (100 ng/mL). Reactions were terminated by the addition of ice-cold PBS buffer containing EDTA (5 mM). The cells were pelleted and processed to determine CD63 expression by flow cytometry. Following anti-IgE challenge (upper panel), CD63 was determined in the $CD117^+$ population and with SCF challenge (lower panel), CD63 was determined in the $Fc\varepsilon RI^+$ population. Each curve represents a time course of CD63 expression for an individual mast cell preparation. A total of ten different mast cell preparations were studied. The extent of histamine release induced by anti-IgE (upper panel) or SCF (lower panel) following 25 min activation of a given preparation is provided alongside the symbol legend.

Imatinib inhibits Stem cell factor responses

Imatinib can act as an inhibitor of the c-kit receptor [3]. In preliminary studies, the effects of imatinib on the release of histamine and eicosanoids from mast cells were assessed. Imatinib (10 μM) was effective at attenuating histamine, cys-LT and PGD_2 generation from mast cells activated with SCF but had very little effect on histamine, and eicosanoid generation induced by anti-IgE (data not shown, $n = 4$). In further studies, the effects of imatinib over a larger concentration range (10^{-10}–10^{-5} M) on SCF induced PGD_2 generation and histamine release were determined. The data demonstrate that imatinib inhibited PGD_2 generation and histamine release in a concentration-dependent fashion and with an EC_{50} of 254 and 130 nM respectively (data not shown, $n = 4$).

As well as investigating the effects of imatinib, alternative purported inhibitors of c-kit, dasatinib and nilotinib, were also studied. Incubation (15 min) of mast cells with these inhibitors (10^{-5} M) completely abolished both PGD_2 generation (Fig. 6b) and histamine release (Fig. 6a) induced by SCF (100 ng/mL) although it should be noted that, in these experiments, the levels of unblocked histamine release were low. The effects of these inhibitors on PGD_2 generation and histamine release induced by anti-IgE (2 μg/mL) were investigated. Whereas imatinib was ineffective, both dasatinib and nilotinib inhibited mediator release induced by anti-IgE (Fig. 6c and 6d).

Stem cell factor enhances mediator release over the longer term

When isolated mast cells are maintained in culture or developed from $CD34^+$ precursor cells, SCF along with IL-6 and IL-10 are usually present in the culture medium. To establish what effect SCF might have over the longer term, mast cells were incubated with or without SCF (100 ng/mL) for 24 h, the cells washed and then stimulated with increasing concentrations of anti-IgE (0.2–2000 ng/mL). There was a statistically significant ($P < 0.01$, $n = 5$) enhancement in the maximal response to anti-IgE following SCF treatment (E_{max}, 50 ± 5%) compared with the control response (E_{max}, 29 ± 2%). The effect of overnight treatment with SCF (100 ng/mL) on eicosanoid generation induced by anti-IgE was also investigated (Table 1). The SCF treatment enhanced the IgE-dependent generation of cys-LTs, but not PGD_2, to

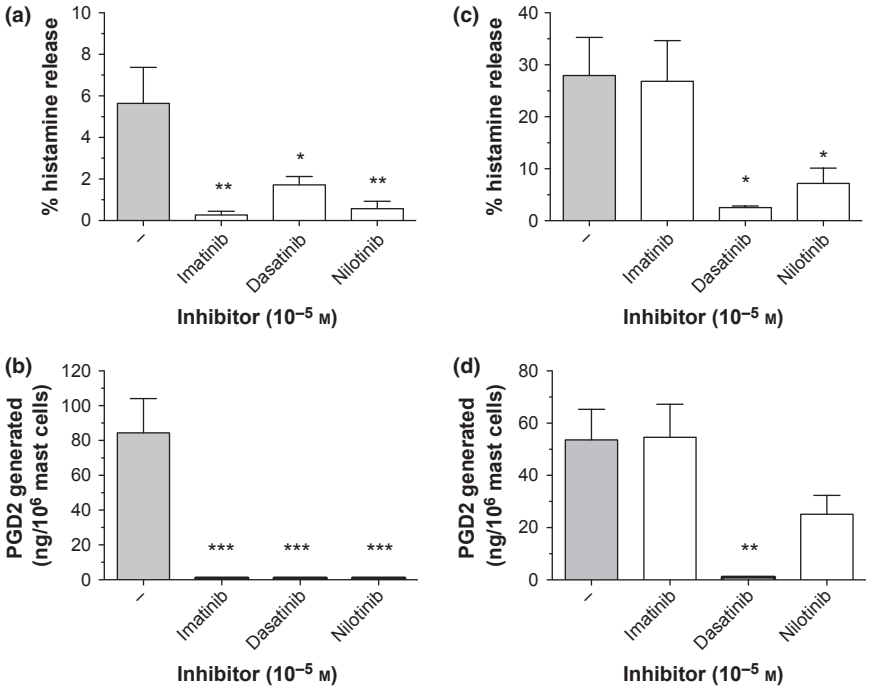

Fig. 6. Effects of c-kit inhibitors on mediator release. Mast cells were incubated for 15 min without or with an inhibitor and then challenged with (a and b) stem cell factor (100 ng/mL) or (c and d) anti-IgE (2 μg/mL) for 25 min. Supernatants were harvested and (a and c) histamine and (b and d) prostaglandin D_2 content was determined. Values are means ± SEM for 4 (a and b) or 6 (c and d) experiments. Statistically significant inhibition compared with the unblocked control is indicated by asterisks (*$P < 0.05$, **$P < 0.005$, ***$P < 0.0005$).

Table 1. Effect of overnight treatment with SCF on mediator release from mast cells

	Anti-IgE induced mediator release		
Treatment	% Histamine release	cys-LT (ng/10^6 mast cells)	PGD_2 (ng/10^6 mast cells)
Buffer	25 ± 3	55 ± 24	214 ± 23
SCF	49 ± 5**	80 ± 30*	180 ± 48

Mast cells were incubated (24 h) either in buffer or SCF (100 ng/mL). After these treatments, the cells were washed extensively and then activated with a maximal releasing concentration of anti-IgE (2 μg/mL) for 25 min and histamine, cys-LT and PGD_2 content in the supernatants assessed. The overnight SCF treatment caused statistically significant increases.(*$P < 0.05$, **$P < 0.01$) in the extent of histamine release and cys-LT generation compared with control. Values are means ± SEM for 4 experiments. SCF, Stem cell factor; cys-LT, cysteinyl-leukotrienes; PGD_2, prostaglandin D_2.

a statistically significant ($P < 0.05$) extent. In further studies, the effects of overnight treatment with IL-6 (50 ng/mL), IL-10 (10 ng/mL), SCF (100 ng/mL) and all three cytokines together on the response of mast cells to anti-IgE were evaluated. Neither IL-6 nor IL-10 had any effect on IgE-dependent histamine release, whereas treatment with all three cytokines enhanced histamine release to the same extent as treatment with SCF alone (data not shown, $n = 4$).

Discussion

SCF is known to act as a critical growth factor for mast cells and can also promote mast cell survival [2]. Whether SCF acts as an activator of mast cells appears to depend on the type of mast cell studied and the output measured [6–8, 13, 30, 31]. In human lung mast cells, SCF is not generally recognized as a direct activator, but SCF has been shown to enhance IgE-dependent histamine release [6, 7, 32]. This was confirmed in this study as a submaximal concentration of SCF was shown to enhance not only histamine release but also to potentiate the generation of cys-LTs and PGD_2 from human lung mast cells activated with a submaximal concentration of anti-IgE.

In further studies, it became evident that SCF not only acted to potentiate human lung mast cell responses but could also activate mast cells directly. The response of mast cells to SCF displayed a great deal of heterogeneity, whereas a majority of preparations did not release a great deal of histamine in response to SCF which is in keeping with some previous studies [6, 7], about a third of all preparations released sizeable quantities of histamine which was a surprising finding. A similar situation existed for the generation of cys-LTs from mast cells challenged with SCF, wherein about half of all the preparations studied released sizeable quantities of cys-LTs. Most striking however was the biosynthesis of PGD_2 by

mast cells in response to SCF, where essentially all mast cell preparations generated PGD_2 at least to some extent and in some instances to very high levels. Although the releasability of mast cells to SCF was lower than that to anti-IgE, there was a correlation in the extent to which the stimuli induced mediator release suggesting that releasability is an intrinsic property of the cell. Moreover, it is noteworthy that essentially all mast cells responded to SCF in some capacity with, at the very least, some generation of PGD_2 and in some instances with very profound levels of mediator release. This property of SCF to generate mediators directly from human lung mast cells, especially PGD_2, could be important in the clinical context [33, 34].

In further studies, we wondered whether the wide-range in responses to SCF might be explained by c-kit expression in human lung mast cells. A positive correlation between c-kit expression and the extent of histamine release induced by SCF was established. This suggests that c-kit expression limits the extent of mast cell responses to SCF. This contrasts with the situation for anti-IgE where no correlation was seen between the extent of histamine release and FcεRI/IgE expression in mast cells. This lack of correlation between anti-IgE and histamine release probably reflects the fact that IgE density in mast cells is in excess of what is needed for stimulation [22, 35].

Activation of mast cells by anti-IgE or SCF led to the up-regulation of the tetraspanin, CD63. These findings indicate that CD63 could be used as a molecular marker of human lung mast cell activation as has been suggested for cord-blood derived mast cells [25]. Indeed, there was a very good correlation between CD63 up-regulation and the extent of histamine release in human lung mast cells, suggesting that CD63 expression may serve as an indicator of degranulation. This is consistent with studies demonstrating that CD63 is found associated with histamine-containing granules [26]. Up-regulation of CD63 may also be indicative of the type of degranulation taking place. In human basophils, different stimuli can induce different types of degranulation. These can be broadly characterized as anaphylactic degranulation, which involves the fusion of granules with the plasma membrane and piecemeal degranulation, which involves the shuttling of small histamine-containing vesicles from granules to the plasma membrane. CD63 up-regulation is linked to anaphylactic degranulation in human basophils [26], so it is possible that both SCF and anti-IgE mediate anaphylactic degranulation in human lung mast cells.

In further studies, we investigated whether SCF mediates its effects through the c-kit receptor. To this end, we studied the effects of the c-kit inhibitor imatinib [3]. Imatinib was effective at inhibiting mediator release from mast cells activated by SCF, but was completely ineffective at preventing IgE-dependent mediator release. This inhibitory effect of imatinib against SCF-driven, but not IgE-dependent degranulation, is in general keeping with findings reported for mouse bone-marrow-derived mast cells and cultured human mast cells [36]. This suggests that imatinib acts selectively to prevent SCF/c-kit signalling in a number of mast cell systems not just human lung mast cells.

Two other c-kit inhibitors were also studied, nilotinib and dasatinib. Both compounds were effective at preventing mediator release from mast cells induced by both SCF and anti-IgE. Dasatanib has been shown to be an effective inhibitor of IgE-triggered histamine release from human basophils [37], and a recent study has shown that nilotinib attenuates mast cell activation both *in vitro* and *in vivo* in rodents [38]. Dasatinib as well as acting at c-kit is known to act at multiple kinases including src kinases and Bruton tyrosine kinases which have been implicated in IgE-dependent signalling, whereas nilotinib is thought to display a profile of inhibitory activity similar to imatinib [3, 39, 40]. Although an expectation might have been that nilotinib would have behaved similarly to imatinib, this was not borne out by the data. Overall, these studies indicate that of the compounds studied, imatinib shows superior selectivity for the c-kit-dependent signalling pathway.

In addition to investigating acute effects on mast cells, the potential influence of an overnight incubation with SCF on the subsequent response of human lung mast cells to IgE-directed activation was evaluated. There was a substantial enhancement in the response to anti-IgE following the overnight treatment demonstrating that SCF can have longer term effects on mast cell responses. It is possible therefore that long-term exposure to SCF may lead to alterations in the functional phenotype of the cell creating a population of cells with increased reactivity. Alternative growth factors, IL-6 and IL-10, which are frequently used to maintain mast cells in culture did not alter mast cell responses. Indeed, reports suggest that the anti-inflammatory cytokine, IL-10, inhibits mast cell responses [41], but there was no evidence for this in the present system.

Overall, these data demonstrate that SCF is an important multifaceted modulator of human lung mast cell activity. As well as enhancing IgE-dependent mediator release, SCF can directly activate mast cells to release mediators especially PGD_2. In addition to acute effects, SCF can also enhance mast cell responses over the longer term.

Clinical relevance

As high concentrations of SCF have been reported in the asthmatic lung [16, 17], direct and indirect activation of mast cells by SCF could be important in asthma.

These studies suggest that targeting SCF/c-kit might be a valuable way to prevent mast cell activation and allay the symptoms of asthma.

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