

Effect of cytokines on mediator release from human dispersed lung mast cells

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To evaluate the contribution of human lung mast cells (HLMC) to allergic inflammation, we investigated whether or not cytokines, including stem-cell factor (SCF), monocyte chemotactic and activating factor (MCAF), and RANTES, activate HLMC. SCF induced histamine release from dispersed HLMC in a dose-dependent fashion ($P < 0.01$). The release was $7.8 \pm 1.0\%$ at 500 ng/ml SCF ($n = 9$). This response was also observed in chopped lung tissue. HLMC from which surface IgE molecules had been removed by treatment with lactic acid responded to SCF, while these cells lost their response to anti-IgE. The process was relatively rapid and reached a maximum in 5 min. This response required extracellular calcium, and it was observed at 37°C , but not at 4°C or 20° . A brief preincubation (10 min) with lower concentrations of SCF, which were ineffective in releasing histamine, enhanced anti-IgE-induced histamine release ($P < 0.05$), while its enhancing effect was lost by the longer preincubation (30 min). SCF did not prime basophils to enhance stimulated-histamine release. Interleukin (IL)- 1α , IL- 1β , IL-3, IL-4, IL-5, granulocyte/macrophage-colony stimulating factor (GM-CSF), MCAF, and RANTES neither induced histamine release nor enhanced the release stimulated by anti-IgE after a 10- or 30-min preincubation. The combination of IL-3 and IL-4 showed no effect on histamine release from HLMC. Leukotriene (LT) $\text{C}_4/\text{D}_4/\text{E}_4$ production by SCF was negligible, as compared with anti-IgE-induced LT production. SCF at 1.5 ng/ml augmented anti-IgE-induced LT generation significantly ($536 \pm 117 \text{ pg}/10^5$ mast cells and $1569 \pm 258 \text{ pg}/10^5$ mast cells; $P < 0.01$). These results provide further evidence that numerous aspects of the phenotype of mast cells and basophils are heterogeneous, including structure, relevant secretagogues, and pharmacologic control.

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Basophils and mast cells which have receptors for IgE on their cell surfaces release chemical mediators through IgE-receptor bridging, and participate in immediate hypersensitivity. Basophils also respond to histamine-releasing factors (HRFs) which might play a role in the pathogenesis of the late-phase reaction (LPR). HRFs have been shown to be produced by mononuclear cells (30), lymphocytes (1), monocytes (28), alveolar macrophages (27), neutrophils (33), and platelets (25), and to be present in nasal washings (19) and in blister fluids of cutaneous LPR (32). Fifty percent of the HRF activity of the peripheral blood mononuclear cells (PBMC) supernatant was attributed to monocyte chemotactic and activating factor (MCAF), a recently cloned chemokine (15).

Recently, MCAF was reported to cause histamine release from basophils (2, 6, 15, 17). Several laboratories including ours showed that IL-3, IL-5, and granulocyte/macrophage-colony stimulating factor (GM-CSF) directly induce or enhance histamine release stimulated by secretagogues including anti-IgE from basophils (3, 11–13, 20).

We recently showed that stem-cell factor (SCF), which is a newly identified mast-cell growth factor, activates rat peritoneal mast cells to induce the secretion of histamine (22). A preliminary experiment suggests that SCF does not cause histamine release from basophils.

It has been widely accepted that mast cells and basophils are heterogeneous in morphologic, func-

tional, and biochemical characteristics (10). Therefore, we investigated whether or not these cytokines interact with human lung mast cells (HLMC).

Material and methods

Cytokines

Human recombinant (r)SCF (21) was donated by Dr K. Zsebo (Amgen Center, CA, USA). rIL-1 α and rIL-1 β (23) were acquired from Otsuka Pharmaceutical Co (Osaka, Japan). rIL-3 (36) was donated by Dr G. G. Wong (Genetics Institute, Cambridge, MA, USA). rIL-4 (37) was purchased from Genzyme Corporation (Boston, MA, USA). rIL-5 (4), produced by the Suntory Institute of Biomedical Research (Mishima, Osaka, Japan), was donated by Dr T. Honjo (University of Kyoto, Kyoto, Japan). rGM-CSF (7) was donated by Dr J. F. DeLamarter (Biogen, Geneva, Switzerland). MCAF (9) and RANTES (24) were acquired from Pepro Tech, Inc (Rochy Hill, NJ, USA).

Buffer

We used PIPES A buffer containing 25 mM PIPES, 119 mM NaCl, 5 mM KCl, and 0.03% human serum albumin (pH 7.4). For the histamine-release assay, PIPES A containing 2 mM Ca²⁺ and 0.5 mM Mg²⁺ (PIPES ACM) was used.

Preparation of cell suspension

HLMC were prepared by a slight modification of the method of Lowman et al. (18). Briefly, macroscopically normal lung tissue from surgical specimens removed for bronchogenic carcinoma was cut into fragments with a chopper for enzymatic dispersion. Tissue fragments were washed and stored at 4°C overnight in RPMI 1640 containing 15% heat-inactivated fetal calf serum (FCS) and 4 mM glutamine and antibiotic-antimycotic mixture (RPMI 1640/15% FCS). On the following day, fragments were washed and then incubated with collagenase (1 mg/ml) (Wako Pure Chemical Industries, Osaka, Japan), hyaluronidase (type 1) (0.5 mg/ml) (Sigma Chemical Co, St Louis, MO, USA), and deoxyribonuclease (bovine pancreas) (10 μ g/ml) (Calbiochem-Behring Corporation, La Jolla, CA, USA) in RPMI 1640 with 2% FCS for 60 min at 37°C. Dispersed cells were separated from undissociated tissue by filtration through nylon gauze (100 μ m) and were washed twice with RPMI containing 2% FCS. The residual tissue was subjected to a second digestion and cell separation. Cells obtained from each digestion were pooled, and dispersed mast cells were passively sensitized with human IgE myeloma serum (donated by Dr H. Tomioka, School of Medicine,

Toho University, Chiba, Japan) in RPMI 1640/15% FCS by overnight incubation at 37°C. The cells were washed twice with PIPES A, and finally resuspended in PIPES ACM for mediator release assays.

Mediator release

To examine the mediator-releasing effect of cytokines on HLMC, we incubated cells with various concentrations of cytokines at 37°C for 30 min unless otherwise indicated. When the effect of cytokines on IgE-mediated mediator release was investigated, mast cells were stimulated by 1/100, 1/1000, or 1/10000 dilution of goat antihuman IgE (36 μ g protein/ml; Medical Biologic Laboratory, Nagoya, Japan) after preincubation with cytokines for 10 or 30 min. The incubation was continued for 30 min at 37°C. Supernatants were recovered by centrifugation and stored until mediator assay. Experimental tubes for each concentration of cytokines were set up in duplicate. The effects of four or five cytokines on histamine release were investigated on the same lung sample.

Mediator assay

Histamine was determined by an automated fluorometric method (29). Spontaneous release was consistently less than 5% of the total histamine content. Percent histamine release was calculated by the following formula: $100 \times (\text{histamine released by a stimulus or a cytokine} - \text{spontaneously released histamine}) / (\text{total histamine content} - \text{spontaneously released histamine})$. Enhancement of histamine release by cytokines was calculated by the following formula: $100 \times (\text{histamine released by a stimulus plus a cytokine} - \text{histamine released by a stimulus}) / (\text{total histamine content})$.

The released LTC₄/D₄/E₄ was quantified with an ELISA kit (LTC₄/D₄/E₄ ELISA system, Amersham, UK). The cross-reactivity of the antibody was 100% to LTC₄/D₄ and 70% to LTE₄, according to the manufacturer's information. Data were presented as mean $\pm$ SEM.

Statistical analysis

The data were submitted to an analysis of variance. Statistical significance was determined with Bonferroni's test.

Results

Effect of cytokines on histamine release from HLMC

Cell suspension contained mast cells (purity: $2.3 \pm 0.5\%$). HLMC were recognized as cells stained with Alcian blue.

Neither IL-1 α (3 pM to 3 nM), IL-1 β (3 pM to 3 nM), IL-3 (1 pM to 1 nM), IL-4 (10 pM to 1 nM), IL-5 (1 pM to 1 nM), GM-CSF (1 pM to 1 nM), nor the combination of IL-3 (1 nM) and IL-4 (10 pM-1 nM) directly activated HLMC to induce histamine release or to enhance anti-IgE-evoked histamine release from HLMC after 10- or 30-min preincubation (data not shown). Moreover, MCAF (5.50 nM) and RANTES 5.50 nM) neither directly induced histamine release nor augmented IgE-mediated release in HLMC (data not shown).

Among the cytokines tested, only SCF activated HLMC. SCF caused histamine release from HLMC in a dose-dependent fashion (Fig. 1; $P < 0.01$). Percent histamine release was $7.8 \pm 1.0\%$ (mean $\pm$ SEM; range: 4.4–12.0%, $n = 9$) at 500 ng/ml SCF. However, SCF was found to be a relatively weak secretagogue, because 1/00 of anti-IgE and 0.2 μ g/ml of ionophore A23187 induced higher percentages of histamine release from HLMC with percent release of $22.8 \pm 8.2\%$ and $41.3 \pm 7.8\%$, respectively ($n = 9$). This response was also observed in mast cells partially purified by the combination of elutriation and discontinuous Percoll gradient centrifugation (purity: 50–60%) (26) (data not shown), indicating that the action of SCF on HLMC was probably not exerted via other contaminating cells.

To rule out the possibility that enzymatic treatment had damaged cells, we examined the effect of SCF on chopped lung tissue without enzymatic treatment. A volume of 500 ng/ml SCF induced histamine release from chopped lung tissue as well as dispersed HLMC with percent releases of $8.4 \pm 1.5\%$ and $8.2 \pm 3.2\%$, respectively (Table 1). To explore the role of surface IgE molecules on HLMC in SCF-induced histamine release, we stripped off IgE from

Table 1. Histamine release induced by anti-IgE or SCF from chopped lung, dispersed HLMC, and dispersed and IgE-stripped HLMC

	Chopped lung	Dispersed HLMC	Dispersed and IgE-stripped HLMC ^a
Anti-IgE ^b			
1/100	9.0 ± 1.8^c	8.0 ± 2.6	1.1 ± 1.6
SCF			
500 ng/ml	8.4 ± 1.5	8.2 ± 3.2	7.0 ± 0.8

^a Cells were treated with 0.01 M lactic acid solution containing 140 mM NaCl and 5 mM KCl (pH 3.9) for 3.5 min at 23°C before stimulation.

^b Cells or tissue fragments were incubated with a stimulus for 30 min at 37°C.

^c Percent histamine release; means $\pm$ SEM of four experiments.

cells by treatment with lactic acid, and examined histamine release with SCF. HLMC devoid of IgE released histamine in response to 500 ng/ml of SCF to the same extent as HLMC with IgE, while the former cells lost their response to anti-IgE (Table 1).

As shown in Fig. 2, the process of SCF-induced histamine release was as rapid as that of anti-IgE-evoked histamine release, which reached a maximum in 5 min. Histamine release stimulated with SCF was temperature-dependent and was not observed at 4° or 20° (Table 2). SCF did not induce the reaction without extracellular calcium (Table 3). This complete dependence on extracellular calcium was the same as that of the anti-IgE-induced response.

The priming effect of SCF on HLMC was investigated by using lower concentrations of this factor, which failed by itself to cause histamine release. As shown in Table 4, the preincubation of cells with SCF for 10 min enhanced anti-IgE-induced histamine release from HLMC (control release compared with release with 1.5 ng/ml of SCF; $P < 0.05$), although its effect was lost after a longer preincubation exceeding 30 min (data not shown).

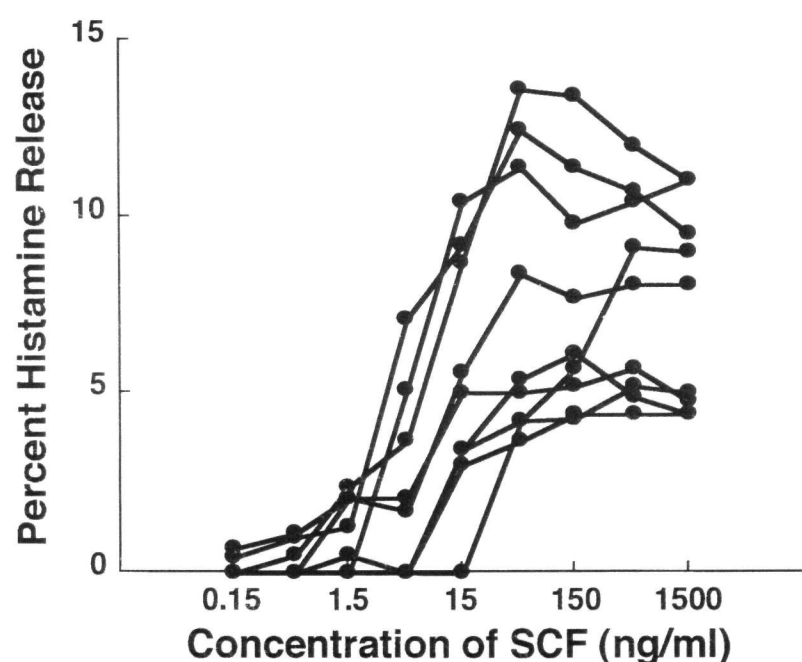


Fig. 1. SCF-induced histamine release from HLMC. Mast cells were incubated with various concentrations of SCF for 30 min at 37°C ($n = 9$).

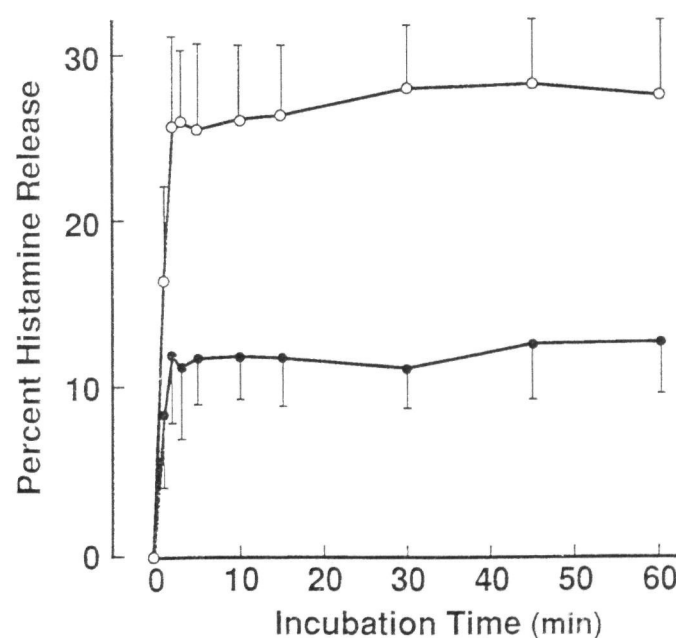


Fig. 2. Process of SCF- or anti-IgE-induced histamine release from HLMC. HLMC were stimulated with 500 ng/ml of SCF (●) or 1/100 of anti-IgE (○) for indicated periods ($n = 4$).

Table 2. Temperature dependence of SCF- and anti-IgE-induced histamine release from HLMC

Temperature	Anti-IgE 1/100	SCF 500 ng/ml
4 °C	0.3±0.3 ^a	0.3±0.3
20 °C	0.2±0.2	0.3±0.3
37 °C	10.9±4.1	7.8±3.1

^a Percent histamine release; means±SEM of three experiments.

Table 3. Calcium dependence of SCF- or anti-IgE-induced histamine release from HLMC

Ca ²⁺	Anti-IgE 1/100	SCF 500 ng/ml
0 mM	0.7±0.5 ^a	0
0.1 mM	2.5±2.3	0
0.3 mM	8.3±5.6	0.4±0.3
1.0 mM	19.1±6.1	5.3±1.5
2.0 mM	20.9±6.2	11.1±2.9

^a Percent histamine release; means±SEM of four experiments.

Table 4. Effect of SCF on histamine release evoked by anti-IgE from HLMC

	Anti-IgE		
	1/10000	1/1000	1/100
SCF ^a			
0.15 ng/ml	7.6±0.8 ^b	6.5±3.0	8.9±2.6
1.5 ng/ml	11.2±1.6	10.4±1.8	11.2±1.4

^a Cells were preincubated with SCF for 10 min at 37 °C, and then stimulated with anti-IgE for 30 min.

^b Percent enhancement of histamine release; means ± SEM of four experiments.

Effects of SCF on LT release

HLMC released only a small amount of LT by exposure to 150 and 500 ng/ml of SCF. A 10-min preincubation of HLMC with 1.5 ng/ml of SCF caused a remarkable augmentation of anti-IgE-induced LT production (Fig. 3) (536 ± 117 pg/10⁵ mast cells and 1569 ± 258 pg/10⁵ mast cells; $P < 0.01$).

Discussion

In this study, we showed that SCF activates HLMC to induce histamine release at higher concentrations, and enhances anti-IgE-mediated histamine release at lower concentrations. As for newly generated mediators, LT generation by SCF itself was found to be negligible, while the remarkable augmentation of anti-IgE-induced LT generation by SCF was observed. Bischoff & Dahinden examined the effect of SCF on HLMC and reported that SCF itself did not induce mediator release, but enhanced the release of histamine and LTC₄ in response to IgE-receptor

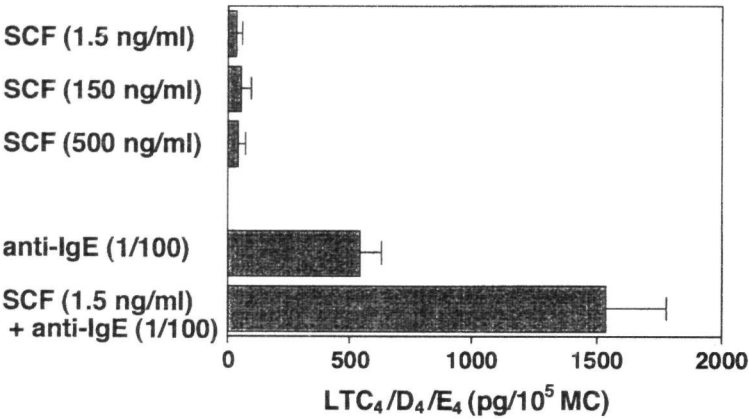


Fig. 3. Effect of SCF on LTC₄/D₄/E₄ production of HLMC. HLMC were preincubated with various concentrations of SCF for 10 min and stimulated with 1/100 of anti-IgE for 30 min ($n = 4$).

cross-linking (5). Although the reason for this discrepancy between two results is not known at present, the finding that SCF induced histamine release from chopped lung tissue argues against the possible influence of enzymes on the histamine-releasing effect of SCF. Histamine-releasing and histamine-release-enhancing effects of SCF on human skin mast cells have been also reported (8).

SCF has been shown not to induce histamine secretion but to augment IgE-mediated histamine release in basophils (8). Another report found that SCF does not enhance basophil histamine release (5). Therefore, we tried to resolve the issue. SCF neither caused the release of histamine nor enhanced IgE-mediated release. The discrepancy of the data remains to be resolved in further studies.

Histamine release by SCF was extracellular calcium- and temperature-dependent, but independent of the presence of surface IgE molecules. We investigated the SCF-induced signaling pathway with rat peritoneal mast cells, and found that the activation of phosphatidylcholine-specific phospholipase D is involved in SCF-induced mediator release (14).

IL-3 and GM-CSF can directly induce mediator release, and also promote release evoked by secretagogues from basophils (3, 11, 12, 20). IL-5 has been shown to augment basophil secretion by secretagogues (13). Moreover, these cytokines have been observed to enhance the survival of basophils (34), and to attract basophils (35). Therefore, we investigated whether or not cytokines, such as IL-3, IL-5, and GM-CSF, interact with HLMC. We showed that IL-3 does not act on HLMC to cause or modulate histamine release. The failure of IL-3 to affect HLMC is supported by a recent report that IL-3 binding sites were not detected on HLMC (31). IL-5 and GM-CSF also did not interact with HLMC. We also examined the effect of other cytokines, such as IL-1 α , IL-1 β , and IL-4 on HLMC. However, these cytokines did not show any effect on HLMC. MCAF and RANTES also did not affect histamine release, although both chemokines were shown to induce

basophil histamine release (2, 6, 15–17). Because the response to anti-IgE, ionophore A23187, or SCF stimulation was observed with HLMC after dispersion with enzymes and chopped lung tissue, the lack of response to those cytokines is believed to be due not to damage to cells by enzymatic treatment, but to the lack of receptors on HLMC.

In conclusion, IL-3, IL-5, GM-CSF, MCAF, and RANTES neither induced nor modulated the release of histamine in HLMC. In contrast, SCF induced histamine release, or augmented histamine and LT release from HLMC. These results provide further evidence that numerous aspects of the phenotype of mast cells and basophils are heterogeneous, including structure, relevant secretagogues, and pharmacologic control.

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