

Stem Cell Factor Has a Suppressive Activity to IgE-Mediated Chemotaxis of Mast Cells¹

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Stem cell factor (SCF), which is well known as a cytokine capable of amplifying development and functions of mast cells, is mainly released from fibroblasts in the peripheral tissue. To investigate whether SCF controlled chemotactic migration of mast cells induced by IgE-specific Ag, murine bone marrow-derived cultured mast cells (BMCMC) and human cord blood-derived cultured mast cells (HuCMC) were preincubated with SCF. Although BMCMC and HuCMC sensitized with IgE directly moved toward specific Ag, preincubation for even 1 h with an optimal dose of SCF suppressed the IgE-mediated chemotactic movement. No or little inhibitory effect of SCF was detected in BMCMC derived from *c-kit* receptor-defect WBB6F₁-W/W^v mice. In contrast, preincubation of BMCMC and HuCMC with SCF enhanced β -hexosaminidase release and Ca²⁺ mobilization in response to Ag after sensitization with IgE. Using the real-time record of chemotactic migration, BMCMC preincubated with SCF manifested motionless without degranulation. These results suggest that locally produced SCF may have an inhibitory effect on chemotaxis of mast cells, contributing to their accumulation and enhancement of functions at the peripheral site in allergic and nonallergic conditions. *The Journal of Immunology*, 2005, 174: 3626–3632.

Mast cells, which accumulate at local tissues the process of inflammation, play a central role in allergic reactions and resistance of host defense responses against helminth parasites, ectoparasites (1–3), and bacterial infection (4, 5). The increase in the number of mast cells at the local tissue may result from their proliferation and directional movement. There are various kinds of cytokines (6–11) and chemoattractants (12–19) which not only induce chemotactic movement of mast cells but also promote their other functional abilities. Much is known that cross-linking of IgE bound to Fc ϵ RI by specific Ag mediates rapid release of biologically active chemical mediators and production of proinflammatory cytokines. Recently, IgE binding to the mast cell surface has been reconfirmed to mediating directional migration toward IgE-specific Ag (20, 21). Moreover, monomeric IgE up-regulates Fc ϵ RI expression and induces survival and cytokine production of mast cells (22–24). However, the details of regulation with regard to their migration and multiple functions are not fully understood.

Stem cell factor (SCF),³ which is an indispensable factor for mast cell proliferation (25) and differentiation (26, 27), is secreted in abundance by fibroblasts, especially at the local site of inflammation (28), and inflammatory cytokines induce fibroblasts to enhance SCF production (29). In general, SCF functions as an up-regulator of mast cells. For example, SCF enhances mast cell adhesion (30, 31), promotes innate immunity (32), and directly induces degranulation and migration (6, 7, 33, 34). SCF drastically increases IgE-mediated release of histamine, leukotriene C₄, prostaglandin D₂, and serotonin, resulting in strong induction of inflammatory reactions (35–38). In addition to these potentiating effects, SCF has a potential to control mast cell numbers at the peripheral tissues. Daily injection with SCF is capable of inducing mast cells hyperplasia (39), which is reversed when the administration of SCF is discontinued (40). The large amount of soluble SCF compensates for the reduction of mast cell numbers caused by the low expression of *c-kit*, the receptor of SCF (41). These experimental findings give rise to a possibility that SCF manipulates mast cell migration and functions.

Recently, we have found that SCF inhibits the production of matrix metalloproteinase-9 (MMP-9) from murine bone marrow-derived cultured mast cells (BMCMC) (42), suggesting that SCF has not only the ability to induce considerable proliferation of mast cells (25, 43, 44), but also the ability to down-regulate their motility. In the present study, we demonstrated that chemotaxis of BMCMC and human cord blood-derived cultured mast cells (HuCMC) toward IgE-specific Ag was suppressed by an optimal dose of SCF, while β -hexosaminidase release and Ca²⁺ mobilization were enhanced by the preincubation with SCF.

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³ Abbreviations used in this paper: SCF, stem cell factor; MMP-9, matrix metalloproteinase-9; BMCMC, murine bone marrow-derived cultured mast cell; HuCMC, human cord blood-derived cultured mast cell; NGF, nerve growth factor; BPO, benzylpenicilloyl; NP, 4-hydroxy-3-nitrophenylacetyl-BSA; PMC, peritoneal mast cell; TNP, trinitrophenyl; CCD, charge-coupled device.

Materials and Methods

Mice and rats

WB-W/+ mice and C57BL/6-W⁺/+ mice originated from The Jackson Laboratory were maintained in our laboratory with food and water ad libitum. Male genetically *c-kit* receptor-defect WBB6F₁-W/W⁺ mice and their control *c-kit* receptor-normal WBB6F₁-+/+ mice were generated and used at 8–12 wk of age in this study. Female Wistar rats (8–16 wk of age) were purchased from Charles River Japan (Kanagawa, Japan) and were kept for >1 wk before they were sacrificed.

Reagents

Mouse rIL-3 (spec. act., >1 × 10⁹ U/mg) and human rIL-6 (spec. act., >1 × 10⁷ U/mg) were provided from Kirin Brewery. Rat rSCF and human rSCF were supplied from Amgen. Nerve growth factor (NGF) was provided from Wako Pure Chemical. DNP-BSA and benzylpenicilloyl-BSA (BPO-BSA) were purchased from LSL and 4-hydroxy-3-nitrophenylacetyl(NP)-BSA was purchased from Biosearch Technology. Mouse anti-DNP IgE (clone SPE-7), mouse anti-TNP IgE (clone C38-2), and NP-specific chimeric mouse F(ab) and human Fc-IgE (anti-NP IgE) were obtained from Sigma-Aldrich, BD Pharmingen, and Serotec, respectively. Unless otherwise indicated, all chemicals were purchased from Sigma-Aldrich.

BMCMC, HuCMC, and rat peritoneal mast cells (rat PMC)

BMCMC were obtained according to the technique described previously (5). Briefly, bone marrow cells of WBB6F₁-+/+ mice were cultured in α -MEM containing 10% FCS and 100 U/ml IL-3. More than 98% of cells obtained 4 wk after the initiation of the culture were identified as toluidine blue-positive mast cells, and BMCMC aged at 5–8 wk were used in all experiments. HuCMC were obtained by culture of human cord blood CD34⁺ cells with 100 ng/ml SCF and 50 ng/ml IL-6 as previously described (45), and cells aged over 8 wk were used in experiments. All human subjects in this study provided written informed consent, which were approved by the Human Studies Internal Review Board at Kyoto University (No. 322). Rat PMC were purified by Percoll solution (Pharmacia Biotech) as described previously (9). Sedimented PMC (>98% purity) were washed twice and used for experiments.

Chemotaxis assay

To increase the expression of Fc ϵ RI (22, 46), BMCMC were treated with 5 μ g/ml anti-DNP IgE and 100 U/ml IL-3 for 4 days, and the cells were preincubated in the presence or absence of various doses of SCF for 2 additional days. Before the chemotaxis assay, BMCMC were sensitized with 5 μ g/ml anti-DNP IgE for 50 min at 4°C and washed three times with α -MEM containing 1% BSA. HuCMC were treated with 1 μ g/ml anti-NP IgE in the presence of 100 ng/ml SCF and 50 ng/ml IL-6 for 4 days. For the last 12 h of the treatment, HuCMC were washed out and incubated only with 1 μ g/ml anti-NP IgE. To investigate an effect of SCF, HuCMC were preincubated with various concentrations of SCF for 1–3 h. A chemotactic activity was examined by using a modification of the method described previously (8, 9). Briefly, 24-well Transwell plates with a 3- μ m pore size polycarbonate filter (Costar) for BMCMC and an 8- μ m pore size filter for HuCMC, and Nunc Cell Culture Inserts with an 8- μ m pore size polycarbonate filter (Nunc) for rat PMC were used for a regular chemotaxis assay. α -MEM containing 1% BSA supplemented with DNP-BSA, BPO-BSA, NP-BSA, or NGF was placed in the lower side of the chamber and 5 × 10⁴ cells were loaded in the upper side of the chamber. The plates were incubated for 3 h at 37°C and then the migrated cells collected from the lower chamber were suspended in 500 μ l of PBS. To determine cell numbers, 25 μ l of a flow-count fluorosphere solution (Becton Coulter) was added to each sample tube, then cells were counted by using a flow cytometer (EPICS XL; Becton Coulter) and analyzed by an EPICS XL System II software. The total number of migrated cells in each tube equaled the number of counted cells multiplied by the total number of fluorospheres divided by fluorospheres counted (cells = (counted cells/counted fluorospheres) × total fluorospheres). In some experiments, after a chemotaxis assay, mast cells on the surface of the membrane were stained with 0.05% toluidine blue (pH 4.1).

Expression of Fc ϵ RI on BMCMC

Expression of Fc ϵ RI on BMCMC was measured by a flow cytometric analysis as described previously (22). BMCMC were preincubated with 10 μ g/ml anti-CD16/CD32 mAb (2.4G2; BD Pharmingen) for 15 min to block nonspecific binding of IgE to Fc γ RII/III and then incubated with 5 μ g/ml anti-DNP-IgE for 50 min at 4°C. After treatment with 5 μ g/ml FITC-

conjugated anti-mouse IgE (BD Pharmingen) for 30 min at 4°C, expression of Fc ϵ RI on the cells was measured by a flow cytometric analysis.

β -Hexosaminidase release assay

A total of 2 × 10⁵ BMCMC or HuCMC was sensitized with 5 μ g/ml anti-DNP IgE or 1 μ g/ml anti-NP IgE in 1 ml of Tyrode's buffer (10 mM HEPES buffer (pH 7.4), 130 mM NaCl, 5 mM KCl, 1.4 mM CaCl₂, 1 mM MgCl₂, 5.6 mM glucose, and 0.1% BSA) for 50 min at 4°C, and then cells were suspended with 10 ng/ml DNP-BSA or 10 ng/ml NP-BSA and incubated for 90 min at 37°C. β -Hexosaminidase in supernatants and cell lysates was quantified by hydrolysis of *p*-nitrophenyl-*N*-acetyl- β -D-glucopyranoside (Sigma-Aldrich) in 0.1 M sodium citrate buffer (pH 4.5) for 40 min at 37°C. The percentage of β -hexosaminidase release was calculated as described previously (47).

Ca²⁺ mobilization

Intracellular Ca²⁺ mobilization was calculated as described elsewhere (5). Briefly, BMCMC or HuCMC (1 × 10⁶ cells/200 μ l) were incubated under the condition of darkness with 5 μ g/ml anti-DNP IgE for BMCMC or 1 μ g/ml anti-NP IgE for HuCMC in the presence of 5 μ M fura 2-AM (Dojindo Laboratories) for 30 min at 37°C. Cells were washed with an excess amount of cold Tyrode's buffer and then resuspended in Tyrode's buffer containing 1 mM CaCl₂ and 0.6 mM MgCl₂ at the concentration of 5 × 10⁵ cells/ml. After stimulation with 10 ng/ml DNP-BSA or 10 ng/ml NP-BSA at 37°C, intracellular Ca²⁺ mobilization of cells was monitored at a 510 nm emission wavelength excited by 340 and 380 nm using a fluorescence spectrophotometer F2500 (Hitachi).

Horizontal chemotaxis assay

The KK chamber (Effector Cell Institute, Tokyo, Japan) was used to detect real-time horizontal chemotaxis and to examine whether BMCMC were degranulated. The KK chamber consists of an etched silicon substrate and a flat glass plate, both of which form two compartments with a 5- μ m-deep microchannel (48). In some experiments, Thermanox coverslips (Nalge Nunc International) were placed onto the glass plates. BMCMC (1 μ l of 10⁶ cells/ml) were put into the one hole with which the device is held together with a stainless steel holder, and 1 μ l of 500 ng/ml DNP-BSA was put into another contra-hole. The KK chamber was incubated for 1 h at 37°C. A charge-coupled device (CCD) camera was used to record the migration of BMCMC toward the high concentration of DNP-BSA on the microchannel where the gradient of DNP-BSA was. In some experiments, coverslips were stained with 0.05% toluidine blue (pH 4.1) after the incubation.

Statistical analysis

Statistical analysis was performed using one-way unpaired ANOVA, and *p* < 0.05 was taken as the level of significance.

Results

Migration of BMCMC and HuCMC in response to IgE-specific Ag

BMCMC were treated with or without 5 μ g/ml anti-DNP IgE in the presence of 100 U/ml IL-3 for 4 days, and then the cells were sensitized with anti-DNP IgE before a chemotaxis assay. Various concentrations of IgE-specific Ag, DNP-BSA, or nonspecific Ag, BPO-BSA, were placed into the lower compartments of Transwell plates, and a chemotactic activity of BMCMC was assayed for 3 h. IgE-treated BMCMC migrated toward DNP-BSA and the activity reached maximal levels at 10 ng/ml DNP-BSA (Fig. 1A); the directional migration of BMCMC was increased in a time-dependent manner (Fig. 1B). In contrast, IgE-sensitized BMCMC exhibited no migration toward unrelated Ag, BPO-BSA, and nontreated BMCMC did not move toward DNP-BSA (Fig. 1A). HuCMC treated and sensitized with 1 μ g/ml anti-NP IgE, migrated toward 10 ng/ml NP-BSA, IgE-specific Ag (Fig. 1C), but not toward BPO-BSA, IgE-nonspecific Ag, or medium alone. Nontreated HuCMC did not move toward NP-BSA (Fig. 1, C and D).

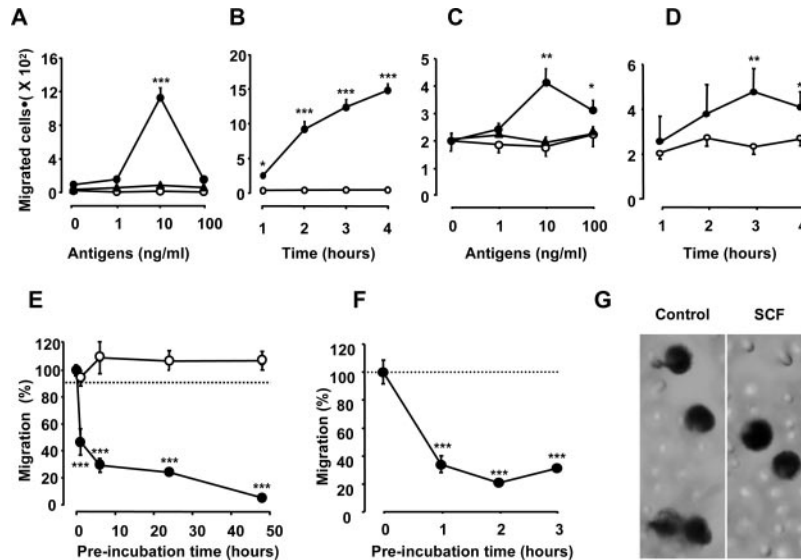


FIGURE 1. Chemotactic movement toward IgE-specific Ag and suppressive activity of SCF. *A* and *B*, BMCMC were treated with 5 μ g/ml anti-DNP IgE and 100 U/ml IL-3 (●, ○) or with IL-3 alone (▲) for 4 days, and then BMCMC were sensitized with 5 μ g/ml anti-DNP IgE. *A*, A chemotactic activity toward various concentrations of DNP-BSA (●, ▲) and BPO-BSA (○) was assayed as described in *Materials and Methods*. ***, $p < 0.001$, when compared with 0 ng/ml DNP-BSA. *B*, A time course of a chemotaxis assay of BMCMC toward 10 ng/ml DNP-BSA (●) or medium alone (○) was identified. *, $p < 0.05$; ***, $p < 0.001$, when compared with medium alone. *C* and *D*, HuCMC were treated with (●, ○) or without (▲) 1 μ g/ml anti-NP IgE in the presence of 100 ng/ml SCF and 50 ng/ml IL-6 as described in *Materials and Methods*. *C*, A chemotactic activity toward various concentrations of NP-BSA (●, ▲) or BPO-BSA (○) was assayed. *, $p < 0.05$; **, $p < 0.01$, when compared with 0 ng/ml NP-BSA. *D*, A time course of a chemotaxis assay of HuCMC toward 10 ng/ml NP-BSA (●) or medium alone (○) was identified. *, $p < 0.05$; **, $p < 0.01$, when compared with medium alone. *E*, After treatment of BMCMC with anti-DNP IgE and IL-3 for 4 days, 100 ng/ml SCF (●) or 50 ng/ml NGF (○) was added and cells were incubated for various hours. BMCMC sensitized with anti-DNP IgE were assayed for chemotaxis toward 10 ng/ml DNP-BSA. Data are presented as the percentages of control migration on BMCMC preincubated without SCF or NGF. Each point represents the mean $\pm$ SE of five to eight separate experiments. ***, $p < 0.001$, when compared with or without SCF or NGF. *F*, HuCMC treated and sensitized with anti-NP IgE were incubated with 100 ng/ml SCF for various hours after depletion of cytokines, then a chemotactic activity toward 10 ng/ml NP-BSA was assayed. ***, $p < 0.001$, when compared with the condition without SCF. Each point represents the mean $\pm$ SE of three separate experiments. *G*, After a chemotaxis assay, cells on the membrane were rinsed with medium and stained with toluidine blue. A polarized shape is observed in BMCMC preincubated without SCF (left), but BMCMC preincubated with SCF show no polarized shape change (right).

Inhibition of BMCMC and HuCMC chemotaxis toward IgE-specific Ag by preincubation with SCF

To determine the effect of SCF on IgE-mediated chemotaxis of mast cells, BMCMC were treated with 5 μ g/ml anti-DNP IgE and 100 U/ml IL-3 for 4 days and then preincubated further for various hours with or without 100 ng/ml SCF. BMCMC sensitized with IgE were assayed for their chemotactic activity toward 10 ng/ml DNP-BSA. When BMCMC were preincubated with SCF, chemotactic migration toward IgE-specific Ag was significantly inhibited (Fig. 1*E*). The marked effect of SCF was noted even after preincubation for 1 h and most BMCMC preincubated for 48 h remained in the upper compartment. NGF, which is produced by fibroblasts as SCF, promotes differentiation and chemotaxis of mast cells (9, 49). Therefore, we examined its effect on the IgE-mediated chemotaxis of BMCMC. Preincubation with an optimal dose of NGF for various hours had no effect on the directional movement of BMCMC (Fig. 1*E*). HuCMC treated and sensitized with anti-NP IgE were preincubated with 100 ng/ml SCF for various hours, then the chemotactic activity toward 10 ng/ml NP-BSA was assayed (Fig. 1*F*). SCF inhibited migration of HuCMC toward IgE-specific Ag as well as BMCMC; the drastic inhibitory effect was observed in cells preincubated for even 1 h. Since SCF promotes adhesion of mast cells to the extracellular matrix (30, 31), we validated whether SCF gave mast cells a strong adhesion to the membrane substrate. After a chemotaxis assay, cells on the membrane were rinsed with medium and stained with toluidine blue. Although a few BMCMC remained, there was no difference in their number between preincubation with and without SCF (the

total number of cells in five fields under the condition with SCF was 59.7 ± 9.4 and under the condition without SCF was 63.7 ± 16.4). In contrast, a marked shape change with polarized morphology was observed in BMCMC on the membrane when cells were preincubated without SCF (Fig. 1*G*). BMCMC preincubated with 100 ng/ml SCF for 48 h showed little or no shape change. We concluded that SCF significantly inhibited chemotactic movement of IgE-sensitized BMCMC and HuCMC toward IgE-specific Ag.

Effect of SCF on BMCMC derived from *c-kit* receptor-defect *WBB6F₁-W/W^v* mice

We conducted experiments to determine whether the inhibitory effect of SCF on IgE-mediated chemotactic movement of BMCMC was mediated through *c-kit* receptors. BMCMC derived from *c-kit* receptor-defect *WBB6F₁-W/W^v* mice and *c-kit* receptor-normal *WBB6F₁-+/+* mice were preincubated with 100 ng/ml SCF for 48 h and sensitized with 5 μ g/ml anti-DNP IgE, and their directional migration toward 10 ng/ml DNP-BSA was examined for 3 h. Although the inhibitory effect of SCF was detected on BMCMC derived from *WBB6F₁-W/W^v* mice, >70% of BMCMC derived from *WBB6F₁-W/W^v* mice revealed the directional movement (Fig. 2*A*).

Effect of various doses of SCF on chemotactic movement

After preincubation with various concentrations of SCF (1, 10, 50, and 100 ng/ml), BMCMC sensitized with anti-DNP IgE were applied to a chemotactic assay toward 10 ng/ml DNP-BSA (Fig. 2*B*). The directional migration of BMCMC was significantly inhibited

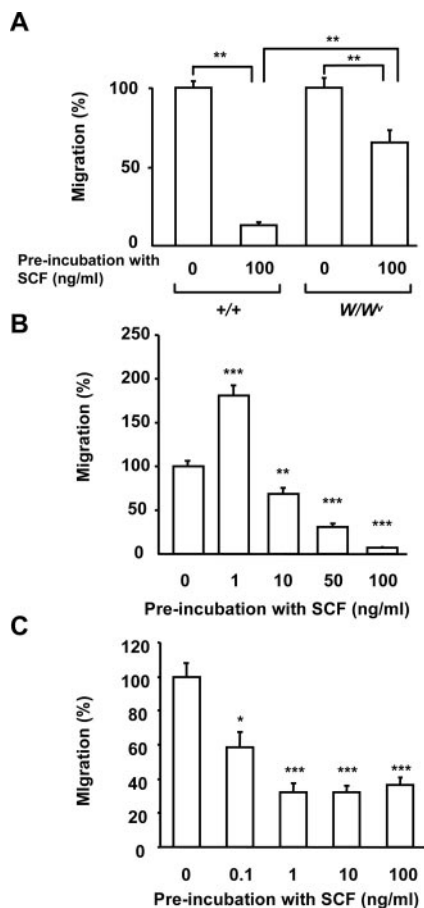


FIGURE 2. Effect of SCF on BMCMC derived from WBB6F1-W/W^v mice and dose effect of SCF on chemotactic movement of BMCMC and HuCMC. *A*, BMCMC derived from WBB6F1-W/W^v mice and derived from its littermate WBB6F1-+/+ mice were preincubated with or without 100 ng/ml SCF. After sensitization with anti-DNP IgE, the chemotactic activity toward DNP-BSA was assayed. The percentage of the migration of BMCMC preincubated without SCF was calculated. Each value represents the mean $\pm$ SE of three separate experiments. **, $p < 0.01$. *B*, After treatment with anti-DNP IgE and IL-3 for 4 days, BMCMC were preincubated with various concentrations of SCF for 2 days and sensitized with anti-DNP IgE. Migrated cells toward 10 ng/ml DNP-BSA for 3 h were counted by a flow cytometric analysis. As a control, the percentage of migration of BMCMC preincubated without SCF was calculated. Each value represents the mean $\pm$ SE of four separate experiments. **, $p < 0.01$; ***, $p < 0.001$, when compared with 0 ng/ml SCF. *C*, HuCMC treated and sensitized with anti-NP IgE were incubated with various concentrations of SCF for 3 h and the chemotactic activity toward 10 ng/ml NP-BSA was assayed after depletion of cytokines. Each value represents the mean $\pm$ SE of two separate experiments. *, $p < 0.05$; ***, $p < 0.001$, when compared with 0 ng/ml SCF.

in a dose-dependent manner by preincubation with 10–100 ng/ml SCF. In contrast, preincubation with 1 ng/ml SCF drastically enhanced chemotactic movement of BMCMC. HuCMC treated and sensitized with anti-NP IgE were incubated with various concentrations of SCF for 3 h and assayed for a chemotactic activity toward 10 ng/ml NP-BSA (Fig. 2C). SCF also inhibited HuCMC chemotaxis toward IgE-specific Ag in a dose-dependent manner.

β -Hexosaminidase release and calcium mobilization enhanced by preincubation with SCF

Since SCF is a promoter for IgE-mediated mast cell reactions (35–38), we conducted some experiments to examine the possible ef-

fect of SCF on IgE-mediated β -hexosaminidase release and intercellular Ca^{2+} mobilization. As shown in Fig. 3, when BMCMC and HuCMC were preincubated with various concentrations of SCF, both β -hexosaminidase release and Ca^{2+} mobilization in response to IgE-specific Ag were increased in a dose-dependent manner. Thus, in contrast with the inhibitory effect on IgE-mediated chemotactic movement of BMCMC and HuCMC, SCF enhanced both β -hexosaminidase release and Ca^{2+} mobilization in response to IgE-specific Ag.

Internalization of Fc ϵ RI

Following the binding of ligands to their specific receptors, cellular responses rapidly attenuate and receptors undergo desensitization and recycling. These processes can serve as an important mechanism by which leukocytes maintain their ability to sense a chemoattractant gradient (50, 51). Because Fc ϵ RI also internalizes following cross-linking of IgE (52), there was a possibility that SCF inhibited the internalization of Fc ϵ RI, eventually resulting in inhibition of directional migration and up-regulation of β -hexosaminidase release and Ca^{2+} mobilization. To clarify this hypothesis, expression of Fc ϵ RI on BMCMC treated or nontreated with 10 ng/ml DNP-BSA was determined by flow cytometric analysis.

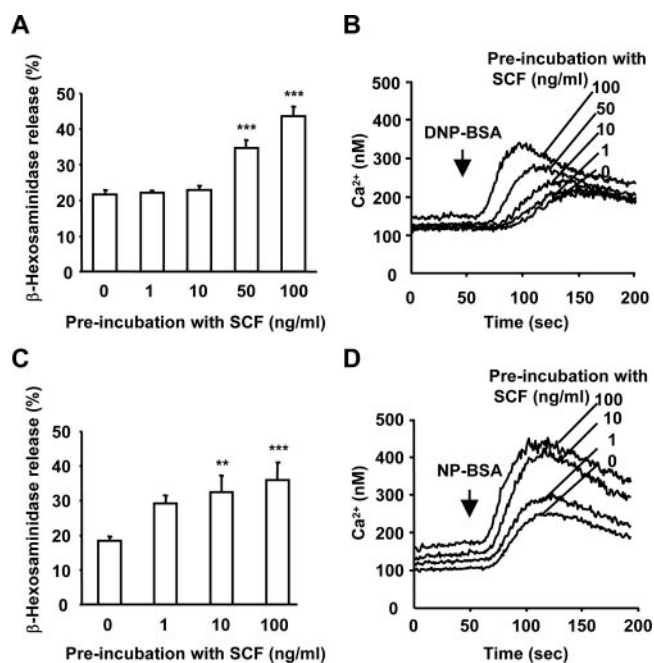
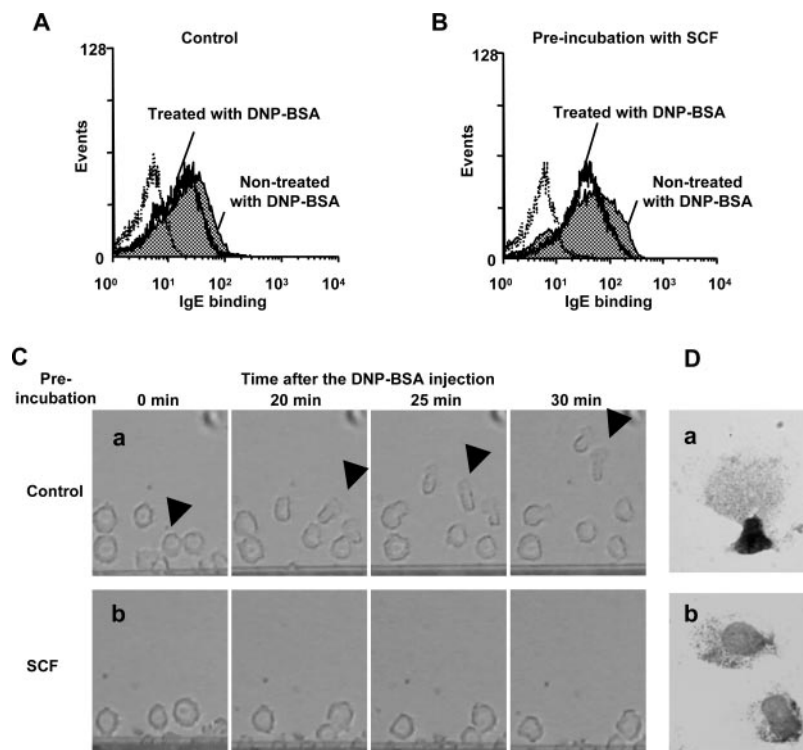


FIGURE 3. Dose-dependent effect of SCF on β -hexosaminidase release and Ca^{2+} mobilization. *A* and *B*, BMCMC were preincubated with various concentrations of SCF for 48 h and sensitized with anti-DNP IgE. *A*, After incubation with 10 ng/ml DNP-BSA, β -hexosaminidase in the supernatant was measured. Each value represents the mean $\pm$ SE of three separate experiments. ***, $p < 0.001$, when compared with 0 ng/ml SCF. *B*, BMCMC were preincubated with various concentrations of SCF for 48 h. After incubation with fura 2-AM and 5 μ g/ml anti-DNP IgE, 5×10^5 /ml BMCMC were assayed for Ca^{2+} mobilization. Arrows indicate the time point when DNP-BSA was applied. *C* and *D*, HuCMC treated and sensitized with anti-NP IgE were incubated with various concentrations of SCF for 3 h after depletion of cytokines. *C*, After incubation with 10 ng/ml NP-BSA, β -hexosaminidase in the supernatant was measured. Each value represents the mean $\pm$ SE of three separate experiments. **, $p < 0.01$; ***, $p < 0.001$, when compared with 0 ng/ml SCF. *D*, HuCMC were incubated with fura 2-AM, then Ca^{2+} mobilization was assayed as described in *Materials and Methods*. Arrows indicate the time point of application with IgE-specific Ag.

FIGURE 4. Internalization of FcεRI on BMCMC stimulated with DNP-BSA and horizontal chemotaxis assay by the KK chamber. BMCMC were preincubated with (B) or without (A) 100 ng/ml SCF and incubated with 5 μg/ml anti-DNP-IgE. After stimulation with 10 ng/ml DNP-BSA for 30 min at 37°C, BMCMC were stained with FITC-conjugated anti-mouse IgE. Expression of FcεRI was analyzed by a flow cytometer. The histograms with bold lines represent the expression of FcεRI on BMCMC treated with DNP-BSA, the shaded area represents without treatment with DNP-BSA, and a dashed line represents a negative control. C and D, BMCMC were preincubated with (b) or without (a) SCF and sensitized with anti-DNP IgE. Then, 1 μl of 1×10^6 BMCMC was put into the hole of the chamber and 1 μl of 500 ng/ml DNP-BSA was put into another contra-hole. C, Migrating BMCMC under the gradation of DNP-BSA was recorded by a CCD camera. Arrows represent one of the migrating cells. These images are a part of individual frames.⁴ D, After the horizontal chemotaxis assay for 1 h, BMCMC on the coverslip of the KK chamber were stained with toluidine blue.



Although the expression of FcεRI on BMCMC treated with DNP-BSA was decreased irrespective of preincubation, there was no difference in the FcεRI expression between these conditions (Fig. 4, A and B). Thus, preincubation with SCF did not affect the internalization of FcεRI induced by IgE-specific Ag.

Real-time records of migration

To further evaluate the inhibitory effect of SCF, we used the KK chamber, which is capable of detecting the real-time horizontal migration of cells. After preincubation with or without 100 ng/ml SCF for 48 h, 1 μl of BMCMC (1×10^6 cells/ml) was put into the one hole of the KK chamber which consists of an etched silicon substrate and a flat glass plate, both of which form two compartments with a 5-μm-deep microchannel in between. After the 1 μl of 500 ng/ml DNP-BSA was added, the KK chamber was incubated for 1 h at 37°C and the migration of BMCMC under the gradient of DNP-BSA was recorded with a CCD camera (Fig. 4C). When BMCMC were preincubated without SCF, some of the BMCMC started to migrate toward the high concentration of DNP-BSA in 10 min. These migrating cells spread lamellipodium widely toward the high concentration of DNP-BSA (Fig. 4Ca).⁴ In contrast, when BMCMC were preincubated with 100 ng/ml SCF, no directional migration was observed (Fig. 4Cb).⁴ Moreover, motionless cells expressed neither morphological change nor degranulation. To determine whether degranulation of BMCMC suppressed chemotactic movement, we placed a coverslip on the glass plates of the KK chamber and recorded the real-time reaction in relation to the DNP-BSA gradient. After the migration assay for 1 h, the coverslip was stained with toluidine blue (Fig. 4D). Migrating cells, which were detected in the control condition, spread their cytosol protuberantly with their granules toward the high concentration side of DNP-BSA. Cells preincubated with SCF expressed no extreme shape change and retained their round shape. Degranulation of BMCMC was observed in very few BMCMC

preincubated with or without SCF (data not shown). Thus, we concluded that inhibition of IgE-mediated chemotaxis by SCF was not involved in degranulation of BMCMC.

Effect of SCF on chemotaxis induced by NGF and IgE-specific Ag

We next attempted to examine whether the inhibitory effect of SCF was specific to IgE-mediated chemotaxis. Since NGF is a chemoattractant for rat PMC (9), rat PMC were preincubated with various concentrations of SCF for 1 h, then chemotaxis toward 50 ng/ml NGF or 10 ng/ml DNP-BSA was assayed (Fig. 5). Interestingly, preincubation with 100 ng/ml SCF increased the number of mast cells migrating toward NGF by 2-fold, whereas at the same dose of SCF, the number of IgE-sensitized mast cells migrated toward Ag was decreased by one-half.

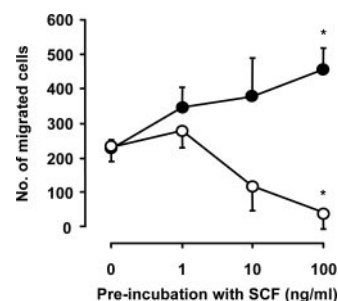


FIGURE 5. Effect of SCF on chemotaxis induced by NGF and IgE-specific Ag. Rat PMC were treated with various concentrations of SCF for 1 h at 37°C in the presence (for the chemotaxis toward IgE-specific Ag) or absence (for the chemotaxis toward NGF) of 5 μg/ml anti-TNP IgE, then chemotactic activity toward 50 ng/ml NGF (●) or 10 ng/ml DNP-BSA (○) was assayed. Each point represents the mean $\pm$ SE which was subtracted from the number of nonspecific migrating cells in medium alone from the number of migrating cells toward NGF or DNP-BSA of two separate experiments. *, $p < 0.05$, when compared with the condition of 0 ng/ml SCF.

⁴ The on-line version of this article contains supplemental material.

Discussion

In the present study, we succeeded in detecting a novel inhibitory effect of SCF on mast cell chemotaxis caused by IgE-specific Ag. Preincubation with an optimal dose of SCF drastically suppressed chemotactic movement of BMCMC, HuCMC, and rat PMC toward IgE-specific Ag. The chemotactic activity of rat PMC induced by NGF was enhanced by preincubation with SCF, supporting the observation that the inhibitory effect of SCF may be specific to the motility induced by IgE-specific Ag. We previously reported that the production of MMP-9, which is engaged in degeneration of matrix components and associated with migration, was inhibited by SCF. Therefore, SCF may have a suppressive effect on mast cell motility by direct inhibition of chemotaxis and indirect inhibition corresponding to suppression of their MMP-9 production.

Interestingly, despite the inhibitory effect of a high dose of SCF on mast cells, preincubation with a low dose of SCF enhanced chemotactic movement of BMCMC caused by IgE-specific Ag, but neither HuCMC nor rat PMC showed such an enhancement effect. Therefore, the effect observed at a low dose of SCF might derive from mast cell heterogeneity in species. Although SCF alone induced chemotactic movement at a low concentration, this chemotactic activity was attenuated at a high concentration, which is the optimal dose for proliferation (6, 43). Considering these data, mast cell chemotaxis might be amenable to a slight amount of SCF, whereas a large amount of SCF may not only suppress motility of mast cells but also enhance proliferation and release of chemical mediators. In fact, preincubation with suboptimal and optimal doses of SCF enhanced β -hexosaminidase release and intracellular Ca^{2+} mobilization in response to IgE-specific Ag. Because epithelial cells, in addition to fibroblasts (28), produce SCF at the local site in patients with allergic rhinitis (53) and the serum levels of SCF are enhanced in patients with atopic dermatitis in correlation with the severity of dermatitis (54), local levels of SCF might change in response to the peripheral condition. Thus, SCF may control mast cells functions: particularly motility and degranulation, corresponding to the peripheral levels of SCF.

SCF and IgE-specific Ag activate many common signal molecules. Up-regulation of IgE-mediated reactions by SCF is considered to be the result of the amplification of these common molecules. Therefore, we suspected that there might be suppressive mechanisms apart from the control by intracellular signal molecules. To define the mechanisms of suppression on mast cell chemotaxis, the following experiments were conducted. First, we analyzed the Fc ϵ RI expression to clarify a speculation that SCF might change the expression of Fc ϵ RI, resulting in inhibition of migration. There was no change in the expression of Fc ϵ RI between BMCMC preincubated with and without SCF (data not shown), similar to the result obtained of the previous study (38). Second, although we examined the internalization of Fc ϵ RI, no marked change in the internalization was detected between BMCMC preincubated with and without SCF. Third, to investigate whether an increase in degranulation by SCF resulted in inhibition of chemotaxis, we recorded chemotaxis of BMCMC real time with the KK chamber. Because a very few degranulated BMCMC were observed, suppression of motility may not be a direct result of degranulation. Finally, we checked the possibility that the strong adhesion of cells to the membrane substrate was induced by SCF, resulting in the inhibition of chemotaxis. We could not find any difference in adhesion to the membrane substrate between cells preincubated with and without SCF. Therefore, although SCF enhances adhesion to the extracellular matrix (30, 31), there was no or little possibility that adhesion to the membrane substrate influ-

enced the inhibitory effect of SCF. These results suggest that SCF had this inhibitory effect on BMCMC mediated by intracellular mechanisms rather than due to association with changes in the expression and internalization of Fc ϵ RI, degranulation, or adhesion.

Recently, SCF and IgE-specific Ag have been reported to differently regulate common signal molecules (55–57). In neutrophils, although either fMLP or IL-8 induces chemotaxis by itself, fMLP has an inhibitory effect on migration of neutrophils caused by IL-8. This inhibition is ascribed to the activation of p38 MAPK in response to fMLP and inhibition of phosphoinositide 3-kinase/Akt. Moreover, Ca^{2+} flux is not related to this change in chemotaxis (58). Therefore, there is a possibility that suppression of chemotaxis and amplification of degranulation might be differently controlled by common signal molecules in mast cells. Although further evaluation might be required with respect to molecular mechanisms, we clearly demonstrated that SCF suppressed IgE-mediated chemotactic movement of mast cells. Thus, SCF, which orchestrates chemotaxis and degranulation, is a cyclopedic regulator of mast cell functions.

Disclosures

The authors have no financial conflict of interest.

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