

Stem cell factor stimulates the chemotaxis, integrin upregulation, and survival of human basophils

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Background: Little is known about the mechanisms that regulate the selective recruitment of basophils to sites of allergic inflammation.

Objective: Here we examine the role of stem cell factor (SCF) in the regulation of basophil function.

Methods: Human basophils were isolated from peripheral blood, and their migration was investigated in chemotaxis assays. Apoptosis was detected by means of annexin V and propidium iodide staining. The expression of cell-surface molecules was measured by means of flow cytometry.

Results: SCF amplified the chemotactic responsiveness of human peripheral blood basophils to the chemoattractants eotaxin, monocyte chemoattractant protein 2 and macrophage inflammatory protein 1 α , and C5a, without being chemotactic or chemokinetic by itself. SCF synergized with chemoattractants in causing basophil upregulation of the integrin CD11b, and this effect was inhibited by a c-kit antibody, the tyrosine kinase inhibitor imatinib mesylate (STI-571), and a phosphatidylinositol 3 kinase inhibitor but not by inhibitors of p38 mitogen-activated protein kinase or mitogen-activated protein kinase/extracellular signal-regulated kinase kinase. Basophils bound fluorescence-labeled SCF and expressed its receptor, c-kit, which was markedly upregulated in culture for 24 to 48 hours in the presence of IL-3. Moreover, SCF prolonged basophil survival in concert with IL-3 by delaying apoptosis. These effects of SCF were selective for basophils because chemotaxis and CD11b upregulation of eosinophils or neutrophils were unchanged.

Conclusion: SCF might be an important selective modulator of basophil function through a phosphatidylinositol 3 kinase-dependent pathway. (J Allergy Clin Immunol 2005;116:820-6.)

Key words: Basophils, chemoattractants, allergy, stem cell factor, c-kit

Basophils are known to be key effector cells in host defense responses to ectoparasitic ticks, prominent in late-phase cutaneous allergic reactions, and constituents of the cellular infiltrate in the asthmatic lung¹ and are thought to be one of the key cell types involved in biasing an immune response toward a T_H2 phenotype.² Basophils, along with eosinophils and T_H2 lymphocytes, are rapidly recruited to the skin, lung, or nasal mucosa after allergen challenge³ directed by chemoattractants, which are released at sites of inflammation. However, the magnitude and kinetics of basophil influx after allergen challenge parallel neither eosinophil recruitment nor generation of known chemoattractants at the inflammatory site,⁴ thus pointing to selective recruitment of basophils by yet undefined mechanisms. The present study suggests that stem cell factor (SCF), an important growth factor for mast cells, might be a selective modulator of basophil recruitment.

METHODS

Reagents

All reagents, if not otherwise stated, were from Sigma (Vienna, Austria). PBS, RPMI 1640, and HEPES were purchased from Invitrogen (Vienna, Austria). The assay buffer used was PBS with 0.9 mM Ca²⁺ and 0.5 mM Mg²⁺ supplemented with 0.1% BSA, 10 mM HEPES, and 10 mM glucose, pH 7.4. Phycoerythrin (PE)- or Cy5-conjugated anti-human CD123 and CD16 antibodies, PE-conjugated anti-human c-kit antibody (clone YB5.B8), FACSFlo, and CellFix were obtained from BD Biosciences (Vienna, Austria). Fluorescein isothiocyanate (FITC)-conjugated anti-human CCR3 antibody and blocking anti-human c-kit antibody (clone 47233) were from R&D Systems (Wiesbaden, Germany). All cytokines were from Peprotech (London, United Kingdom). The p38 mitogen-activated protein (MAP) kinase inhibitor SB-202190, the MAP kinase/extracellular signal-regulated kinase (MEK) kinase inhibitor U-0126, and the phosphatidylinositol 3 (PI-3)-kinase inhibitor LY-294002 were purchased from Biomol (Hamburg, Germany). STI-571 (imatinib mesylate) was kindly provided by Novartis (Basel, Switzerland).

Preparation of human leukocytes

Leukocytes from citrated whole blood were separated by means of dextran sedimentation and centrifugation on Histopaque 1077 into a polymorphonuclear cell fraction (eosinophils and neutrophils) and a mononuclear cell fraction (basophils, monocytes, and lymphocytes).^{5,6} For chemotaxis experiments, basophils and eosinophils were further purified by means of negative magnetic selection with

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Abbreviations used

FITC: Fluorescein isothiocyanate
MAP: Mitogen-activated protein
MCP: Monocyte chemotactic protein
MEK: MAP kinase/extracellular signal-regulated kinase
MIP-1 α : Macrophage inflammatory protein 1 α
PE: Phycoerythrin
PI: Phosphatidylinositol
SCF: Stem cell factor

antibody cocktails from StemCell Technologies (Vancouver, Canada), with purity of greater than 95% and viability of greater than 98%.⁵

Chemotaxis

Cells, 2×10^4 , resuspended in assay buffer were applied to the top of a 48-well microBoyden chamber with 5- μ m pore-size polycarbonate filters (NeuroProbe Inc, Gaithersburg, Md) and agonists or buffer placed in the bottom wells. The plate was incubated at 37°C in a humidified CO₂ incubator for 1 hour, and cells that had migrated into the lower chamber were counted by using flow cytometry.⁶

Surface marker expression and apoptosis assay

Changes in surface expression of CD11b or CCR3 were recorded by means of flow cytometry after samples of whole blood anticoagulated with heparin or polymorphonuclear or mononuclear cell preparations had been stimulated with chemokines for 30 minutes at 37°C and then stained with anti-CD11b-FITC (1:20) or anti-CCR3-FITC (1:20). C-kit expression was investigated in samples of whole blood stained with PE-conjugated anti-human c-kit antibodies (clone YB5.B8) and IgG₁ control antibodies (at 1:20 dilution) on ice for 30 minutes. The binding of fluorescence-labeled SCF to leukocytes was investigated in polymorphonuclear and mononuclear cell preparations by incubating the cells with biotinylated SCF, unlabeled SCF, or biotinylated control protein (15 nM, R&D Systems) for 60 minutes on ice, followed by a 30-minute incubation with avidin-fluorescein. Red blood cells were lysed with ammonium chloride where applicable. Basophils were detected by means of flow cytometry as CD123⁺/HLA-DR⁻ cells, monocytes as CD14⁺ cells, neutrophils as CD16⁺ cells with low side scatter, and eosinophils as CD16⁻ cells with high side scatter.

Purified basophils were cultured in RPMI 1640, 100 U/mL penicillin, and 100 mg/mL streptomycin in the absence or presence of cytokines for up to 48 hours to record changes in c-kit expression and apoptosis. C-kit expression was measured as described above. Apoptotic basophils were identified by staining with annexin V-FITC (1:100) and propidium iodide (1:50) by means of flow cytometry.⁷

Calcium flux

Mononuclear cells were loaded with 2 μ M of the acetoxymethyl ester of the calcium-sensitive fluorescent dye Fluo-3 in the presence of probenecid and pluronic F-127 for 20 minutes at room temperature. Basophils were detected as CD123⁺/HLA-DR⁻ cells, and calcium mobilization was recorded by using flow cytometry as increased fluorescence intensity in the FL-1 channel.⁸

Statistical analysis

Data are shown as means $\pm$ SEM. Comparisons of groups were performed with the Mann-Whitney *U* test, the Wilcoxon signed-rank

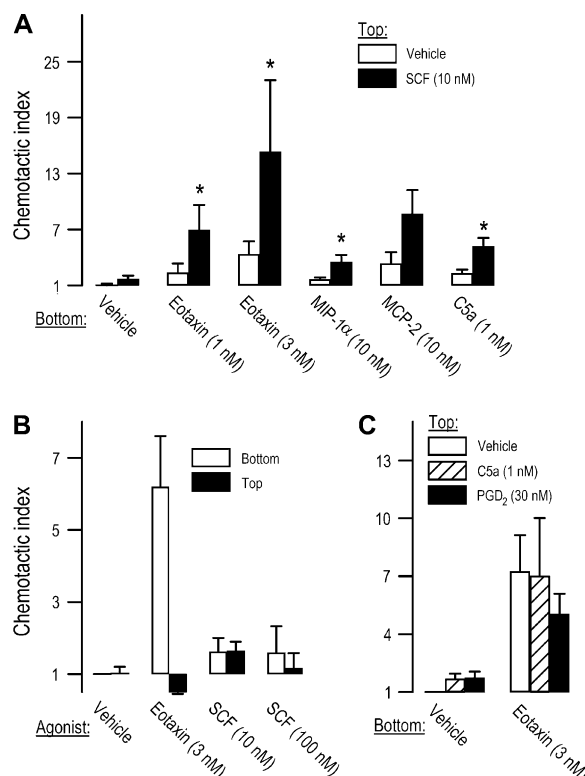


FIG 1. SCF enhances the migration of basophils. Basophils were mixed with vehicle or agonists (top wells) and were allowed to migrate toward agonists in the bottom wells. The amount of migrated cells was expressed relative to nonstimulated migration (chemotactic index). **P* < .05, SCF versus vehicle (*n* = 6-8). PDG₂, Prostaglandin D₂.

test, or 2-way ANOVA with the Bonferroni post test. *P* values of less than .05 were considered statistically significant.

RESULTS

The chemotactic responsiveness of basophils to the chemoattractants eotaxin (1-3 nM), macrophage inflammatory protein 1 α (MIP-1 α ; 10 nM), monocyte chemotactic protein 2 (MCP-2; 10 nM), and C5a (1 nM) was enhanced 4-fold after a 5-minute pretreatment at room temperature with SCF (10 nM; Fig 1, A). Of interest, MIP-1 α induced significant basophil migration only in the presence of SCF. In contrast, SCF alone in the bottom or top wells at concentrations up to 100 nM did not cause significant migration, indicating that SCF was not chemotactic or chemokinetic for basophils (Fig 1, B). When basophils were mixed with the chemoattractants C5a (1 nM) or prostaglandin D₂ (30 nM) instead of SCF, their migration toward eotaxin was not enhanced (Fig 1, C). SCF did not facilitate the migratory response of eosinophils to eotaxin or neutrophils to IL-8 (*n* = 4, data not shown), which suggests that SCF selectively amplifies the chemotactic responsiveness of basophils.

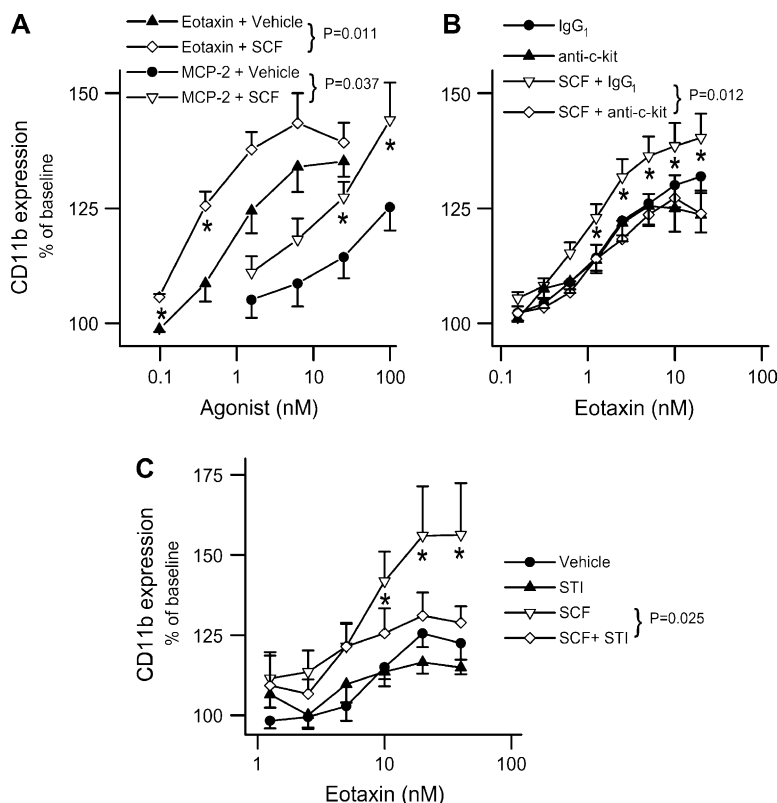


FIG 2. SCF enhances the chemoattractant-induced CD11b upregulation in basophils through c-kit tyrosine kinase. In **A** and **B**, PBMCs were pretreated with anti-human c-kit antibody (clone 47233, 20 μ g/mL) or IgG₁ and then mixed with vehicle or SCF (10 nM) and stimulated with eotaxin or MCP-2. In **C**, samples of whole blood were pretreated with the tyrosine kinase inhibitor STI-571 (1 μ M) and then mixed with vehicle or SCF (10 nM) and stimulated with eotaxin. CD11b upregulation in basophils was quantified as a percentage of the baseline value. The *P* values show the levels of probability for differences between the indicated treatments (2-way ANOVA). **P* < .05, vehicle versus SCF pretreatment (*n* = 6–8).

Purified basophils rapidly underwent apoptosis because the total number and the number of nonapoptotic (ie, annexin V/propidium iodide negative) basophils decreased after 48 hours to $25\% \pm 4\%$ and $1.5\% \pm 0.4\%$, respectively, of the starting population (ie, 100%; *n* = 8). In the presence of IL-3 (10 pM), however, the total number and the number of nonapoptotic basophils after 48 hours was $91\% \pm 27\%$ and $32\% \pm 13\%$, respectively. SCF (10 nM) alone had no significant effect on basophil apoptosis compared with vehicle (data not shown) but further augmented the antiapoptotic effect of IL-3 because the total number and the number of nonapoptotic basophils increased to $126\% \pm 30\%$ and $63\% \pm 25\%$, respectively (*P* < .05, IL-3 vs SCF/IL-3).

In the presence of SCF (10 nM), CD11b upregulation induced by eotaxin, MCP-2 (Fig 2, A), and MCP-1 (data not shown, *n* = 6) was enhanced, and the sensitivity of basophils to chemoattractants increased, as indicated by a 4-fold shift of the concentration response curve to the left (Fig 2, A). SCF alone did not induce considerable changes in CD11b expression (data not shown, *n* = 6). In contrast to basophils, CD11b upregulation induced by eotaxin and IL-8 in eosinophils and neutrophils, respectively, was not enhanced by SCF (data not shown, *n* = 6). Pretreatment

of basophils with a blocking anti-human c-kit antibody (clone 47223, 20 μ g/mL) for 10 minutes at 37°C completely reversed the effect of SCF (10 nM) to facilitate the CD11b upregulation in response to eotaxin, whereas the isotype-matched control antibody (clone MOPC21) had no effect (Fig 2, B). The ability of SCF to amplify chemoattractant-induced CD11b upregulation in basophils was also evident in whole blood (Fig 2, C), in which eotaxin had a 10-fold lower potency at activating basophils compared with isolated cell, probably because of binding of the chemokine to Duffy antigen on erythrocytes.⁹ A 20-minute pretreatment with the tyrosine kinase inhibitor STI-571 (1 μ M)¹⁰ prevented the facilitating effect of SCF (Fig 2, C). The eotaxin-induced CD11b upregulation in the absence of SCF was not altered by the blocking c-kit antibody or STI-571 (Fig 2, B and C). These data indicate that SCF amplifies the CD11b response of basophils through c-kit tyrosine kinase activation.

Among leukocytes, basophils and monocytes were stained positive in flow cytometry with the c-kit antibody (clone YB5.B8) compared with the isotype control, whereas c-kit was not detectable on neutrophils or eosinophils (Fig 3, A). Similar findings were obtained with a second c-kit antibody (clone 47223, *n* = 3). Moreover,

binding assays demonstrated specific binding of SCF to basophils: after addition of fluorescence-labeled SCF (15 nM), the fluorescence of basophils and monocytes increased by a factor of 4.3 ± 0.7 and 8.3 ± 2.8 , respectively ($n = 8$), whereas no significant changes were observed in eosinophils or neutrophils, with fold increases of 1.1 ± 0.0 and 1.2 ± 0.2 , respectively ($n = 6$). Fluorescence-labeled irrelevant control protein or unlabeled SCF did not result in increased fluorescence in basophils or monocytes, suggesting specific binding of SCF. When basophils were cultured in the presence of 300 pM of IL-3, a concentration that completely prevented basophil apoptosis for at least 48 hours (data not shown, $n = 6$), their levels of c-kit expression further increased. Fig 3, B, is a representative plot of c-kit staining of purified basophils showing a marked increase in c-kit expression after 48 hours of IL-3 treatment compared with c-kit expression at baseline. Fig 3, C, shows the corresponding time course of the IL-3-induced upregulation of c-kit in isolated basophils, which peaked at 24 hours with a 100% increase in c-kit levels. C-kit expression did not change during culture in the absence of IL-3 (Fig 3, C).

Although eotaxin elicited a calcium flux in basophils, SCF did not induce calcium flux at concentrations of up to 100 nM (data not shown, $n = 5$). A 5-minute pretreatment at 37°C of the cells with SCF (10 nM) did not significantly alter the calcium flux in response to eotaxin (1–10 nM; data not shown, $n = 6$).

The enhancement of basophil responsiveness to chemoattractants by SCF did not involve reduced internalization of their receptors because a 30-minute incubation of cells with eotaxin (1–100 nM) caused similar decreases in CCR3 expression in the absence and presence of SCF (10 nM). For instance, 6.25 nM of eotaxin reduced the CCR3 expression by $34\% \pm 8\%$ in the absence and $45\% \pm 5\%$ in the presence of SCF ($n = 3$). Basophil CCR3 expression was not altered by SCF (10 nM) in the absence of eotaxin ($94\% \pm 7\%$ of control CCR3 expression, $n = 3$).

The pathways of intracellular signal transduction that underlie the SCF enhancement of chemoattractant-induced CD11b upregulation in basophils were also investigated by using inhibitors of p38 MAP kinase (SB-202190), MEK kinase (U-0126), and PI-3 kinase inhibitor (LY-294002) by preincubating the cells for 20 minutes at 37°C at concentrations reported to be both effective and selective.^{11–13} The eotaxin-induced CD11b upregulation in the absence of SCF was not altered by the PI-3 kinase inhibitor LY-294002 (20 μ M) but was significantly reduced by the p38 MAP kinase inhibitor SB-202190 (10 μ M; $P = .01$, $n = 6$) and the MEK kinase inhibitor U-0126 (2 μ M; $P = .003$, $n = 6$). In contrast, SB-202190 or U-0126 did not prevent SCF from facilitating the eotaxin-induced CD11b upregulation (data not shown, $n = 6$), whereas SCF was unable to enhance the eotaxin-induced CD11b upregulation after pretreatment with the PI-3 kinase inhibitor LY-294002 (Fig 4, A). Similarly, pretreatment of basophils with LY-294002 largely abolished the ability of SCF

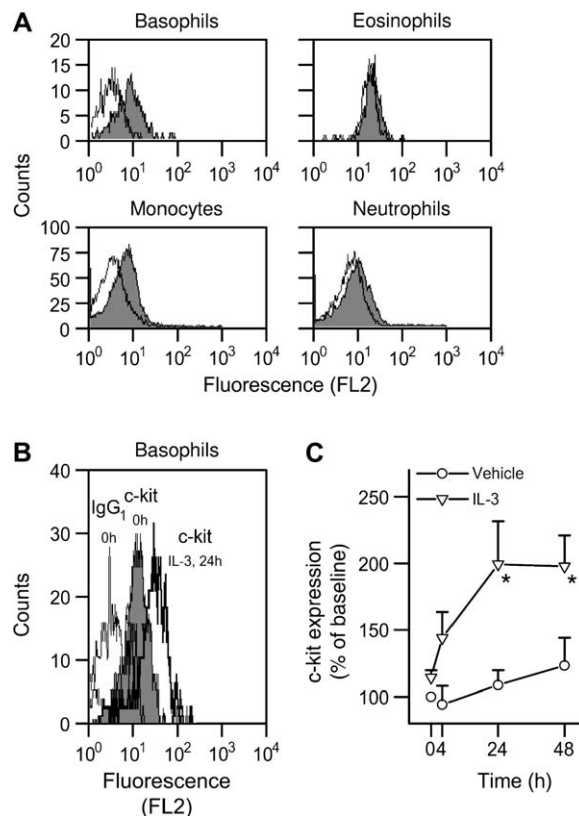


FIG 3. Basophils express c-kit, which is upregulated by IL-3. In **A**, samples of whole blood were stained with the anti-c-kit antibody YB5.B8 (filled histograms) or the IgG₁ control antibody (open histograms). The tracings are representative of 9 donors. In **B** and **C**, isolated basophils were cultured in the presence (bold histogram) or absence (filled histogram) of IL-3 (300 pM) for up to 48 hours, and cells were stained with the anti-human c-kit antibody (clone YB5.B8, bold and filled histograms) or IgG₁ (open histogram). A representative staining and means $\pm$ SEM of 4 experiments are shown. * $P < .05$ versus baseline.

to enhance basophil migration toward eotaxin or C5a (Fig 4, B).

DISCUSSION

Our data suggest that the chemotactic effect of chemoattractants can be biased from eosinophils toward basophils in the presence of SCF, thus constituting a novel mechanism of selective basophil recruitment. We observed that a short pretreatment with SCF markedly enhanced the basophil chemotaxis to eotaxin, MIP-1 α , MCP-2, and C5a. Other hemopoietic growth factors, such as IL-3 or GM-CSF, have been shown to amplify basophil chemotaxis through a chemokinetic action.¹⁴ However, we did not observe significant basophil migration when SCF alone was present in the top wells or bottom wells at concentrations of up to 100 nM, which shows that SCF is not chemotactic or chemokinetic for basophils. In contrast, SCF has been reported to be directly chemotactic for mast cells.¹⁵ When basophils were activated

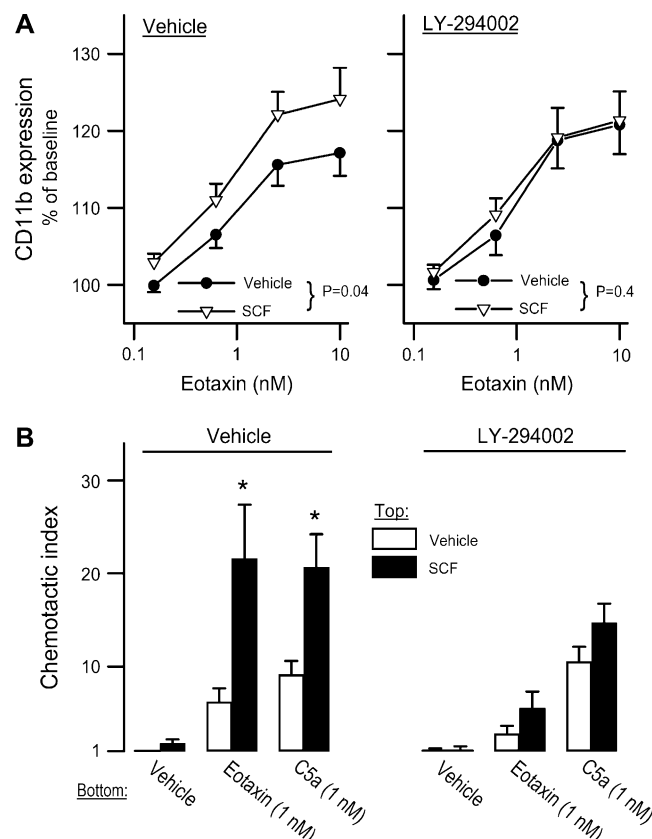


FIG 4. PI-3 kinase mediates the SCF-induced enhancement of basophil CD11b upregulation and chemotaxis. In **A**, PBMCs were pretreated with the PI-3 kinase inhibitor LY-294002 (20 μ M) or vehicle and then mixed with vehicle or SCF (10 nM) and were finally stimulated with eotaxin. CD11b upregulation in basophils was quantified as a percentage of the baseline value. In **B**, basophils pretreated with the PI-3 kinase inhibitor LY-294002 (20 μ M) or vehicle and mixed with vehicle or SCF (10 nM, top wells) were allowed to migrate toward eotaxin or C5a (bottom wells). The amount of migrated cells was expressed relative to nonstimulated migration (*chemotactic index*). The *P* values denote significant overall differences between vehicle and pretreatment (2-way ANOVA). **P* < .05, SCF versus its vehicle (*n* = 6-8).

by C5a or prostaglandin D₂ instead of SCF before chemotaxis, this did not result in enhanced migration toward eotaxin. SCF-induced upregulation of chemotactic responsiveness is restricted to basophils because SCF did not alter the chemotaxis of eosinophils or neutrophils. Accordingly, we could detect c-kit on basophils but not on neutrophils or eosinophils. Similarly, fluorescence-labeled SCF revealed the binding of the cytokine to basophils but not to eosinophils or neutrophils. In contrast, isolated eosinophils had been described to be weakly positive for c-kit previously,¹⁶ suggesting that c-kit might be induced on eosinophils during the isolation procedure or might be expressed only by a subgroup of donors.

SCF also enhanced the chemoattractant-induced CD11b upregulation of basophils. The β_2 -integrin CD11b/CD18 (also referred to as CR3, Mac-1, or $\alpha_m\beta_2$) is a complement receptor that binds multiple ligands, including C3bi, intercellular adhesion molecule 1, fibrinogen, and β -glucan. Apart from adhesion, CD11b/CD18 is an important mediator, modulator, or both of leukocyte responses, including migration,¹⁷ phagocytosis,¹⁸ mediator release,¹⁹ and apoptosis.²⁰ In basophils CD11b is

involved in the priming effect of IL-3 for increased histamine release.²¹ Therefore it is conceivable that excessive CD11b upregulation in the presence of SCF might have a profound effect on other basophil functions, such as migration to sites of allergic reactions, mediator release, or apoptosis.

Intracellular signaling of c-kit involves p38 MAP kinase, PI-3 kinase, protein kinase C, and MEK kinase.²²⁻²⁵ Using specific inhibitors of the respective kinases, we have shown that eotaxin-induced CD11b upregulation in the absence of SCF is mediated by p38 MAP kinase and MEK kinase but not by PI-3 kinase. In contrast, the SCF-induced enhancement of eotaxin-stimulated CD11b upregulation was abolished by inhibition of PI-3 kinase but was left unaltered by inhibitors of p38 MAP kinase or MEK kinase, and inhibition of PI-3 kinase also prevented SCF from amplifying basophil migration toward eotaxin or C5a. The effects of SCF were also inhibited by the tyrosine kinase inhibitor STI-571 and a blocking anti-human c-kit antibody. Therefore we could demonstrate that the additional stimulation of PI-3 kinase by SCF through c-kit tyrosine kinase accounts for the

increased responsiveness of basophils to chemoattractants. The antiapoptotic effect of IL-3 has been shown to depend on PI-3 kinase,²⁶ and we have observed that SCF enhances the antiapoptotic effect of IL-3. Thus it is likely that in concert with IL-3, an additional activation of PI-3 kinase by SCF can significantly contribute to prolonged basophil survival in tissue.

Exogenous SCF can induce long-term airway hyperactivity,²⁷ SCF expression is upregulated in allergic inflammation, and neutralizing antibodies and SCF antisense oligonucleotides ameliorate the allergic response in animal models.^{28,29} Genetic or pharmacologic inactivation of PI-3 kinase in mice inhibits the responses to SCF and protects them also against allergic inflammation.³⁰ SCF is present and partially accounts for mast cell chemotactic activity in bronchoalveolar and nasal lavage fluid collected from atopic patients,^{31,32} hence suggesting a role for SCF also in human pathology. Although c-kit expression on basophils has been demonstrated,³³ the role of SCF in basophil function has remained controversial: although some authors could demonstrate that SCF enhances basophil histamine release, others did not.^{33,34} In the present study we have shown that SCF very effectively enhances the chemotaxis, the upregulation of adhesion molecules, and the survival of human basophils through a c-kit-, tyrosine kinase-, and PI-3 kinase-dependent pathway. These effects of SCF are restricted to basophils compared with eosinophils or neutrophils. Basophil c-kit expression had been suggested to be increased in atopic patients.³⁵ Here we observed that IL-3, which is known to be increased in atopic disease,³⁶ potentially increased c-kit expression in basophils. During the allergic response, this would presumably also result in enhanced responsiveness of basophils to SCF, hence underscoring the importance of SCF as a driving force of basophil function by enhancing their influx to and activation and survival at sites of inflammation. With respect to the important role of basophils in allergic inflammation, the SCF-basophil axis might be of therapeutic relevance.

REFERENCES

- Falcone FH, Haas H, Gibbs BF. The human basophil: a new appreciation of its role in immune responses. *Blood* 2000;96:4028-38.
- Schroeder JT, Lichtenstein LM, Roche EM, Xiao H, Liu MC. IL-4 production by human basophils found in the lung following segmental allergen challenge. *J Allergy Clin Immunol* 2001;107:265-71.
- Liu MC, Hubbard WC, Proud D, Stealey BA, Galli SJ, Kagey-Sobotka A, et al. Immediate and late inflammatory responses to ragweed antigen challenge of the peripheral airways in allergic asthmatics. Cellular, mediator, and permeability changes. *Am Rev Respir Dis* 1991;144:51-8.
- Macfarlane AJ, Kon OM, Smith SJ, Zeibecoglou K, Khan LN, Barata LT, et al. Basophils, eosinophils, and mast cells in atopic and nonatopic asthma and in late-phase allergic reactions in the lung and skin. *J Allergy Clin Immunol* 2000;105:99-107.
- Heinemann A, Hartnell A, Stubbs VE, Murakami K, Soler D, LaRosa G, et al. Basophil responses to chemokines are regulated by both sequential and cooperative receptor signaling. *J Immunol* 2000;165:7224-33.
- Stubbs VE, Schratl P, Hartnell A, Williams TJ, Peskar BA, Heinemann A, et al. Indomethacin causes prostaglandin D(2)-like and eotaxin-like selective responses in eosinophils and basophils. *J Biol Chem* 2002;277:26012-20.
- Yoshimura C, Miyamasu M, Nagase H, Iikura M, Yamaguchi M, Kawanami O, et al. Glucocorticoids induce basophil apoptosis. *J Allergy Clin Immunol* 2001;108:215-20.
- Heinemann A, Ofner M, Amann R, Peskar BA. A novel assay to measure the calcium flux in human basophils: effects of chemokines and nerve growth factor. *Pharmacology* 2003;67:49-54.
- Bryan SA, Jose PJ, Topping JR, Wilhelm R, Soderberg C, Kertesz D, et al. Responses of leukocytes to chemokines in whole blood and their antagonism by novel CC-chemokine receptor 3 antagonists. *Am J Respir Crit Care Med* 2002;165:1602-9.
- Ueda S, Ikeda H, Mizuki M, Ishiko J, Matsumura I, Tanaka H, et al. Constitutive activation of c-kit by the juxtamembrane but not the catalytic domain mutations is inhibited selectively by tyrosine kinase inhibitors STI571 and AG1296. *Int J Hematol* 2002;76:427-35.
- Kampen GT, Stafford S, Adachi T, Jinquan T, Quan S, Grant JA, et al. Eotaxin induces degranulation and chemotaxis of eosinophils through the activation of ERK2 and p38 mitogen-activated protein kinases. *Blood* 2000;95:1911-7.
- Favata MF, Horiuchi KY, Manos EJ, Daulerio AJ, Stradley DA, Feeser WS, et al. Identification of a novel inhibitor of mitogen-activated protein kinase kinase. *J Biol Chem* 1998;273:18623-32.
- Vlahos CJ, Matter WF, Hui KY, Brown RF. A specific inhibitor of phosphatidylinositol 3-kinase, 2-(4-morpholinyl)-8-phenyl-4H-1-benzopyran-4-one (LY294002). *J Biol Chem* 1994;269:5241-8.
- Yamaguchi M, Hirai K, Shoji S, Takaishi T, Ohta K, Morita Y, et al. Haemopoietic growth factors induce human basophil migration in vitro. *Clin Exp Allergy* 1992;22:379-83.
- Meininger CJ, Yano H, Rottapel R, Bernstein A, Zsebo KM, Zetter BR. The c-kit receptor ligand functions as a mast cell chemoattractant. *Blood* 1992;79:958-63.
- Yuan Q, Austen KF, Friend DS, Heidtman M, Boyce JA. Human peripheral blood eosinophils express a functional c-kit receptor for stem cell factor that stimulates very late antigen 4 (VLA-4)-mediated cell adhesion to fibronectin and vascular cell adhesion molecule 1 (VCAM-1). *J Exp Med* 1997;186:313-23.
- Rainger GE, Buckley C, Simmons DL, Nash GB. Cross-talk between cell adhesion molecules regulates the migration velocity of neutrophils. *Curr Biol* 1997;7:316-25.
- Graham IL, Gresham HD, Brown EJ. An immobile subset of plasma membrane CD11b/CD18 (Mac-1) is involved in phagocytosis of targets recognized by multiple receptors. *J Immunol* 1989;142:2352-8.
- Couturier C, Haeflner-Cavaillon N, Weiss L, Fischer E, Kazatchkine MD. Induction of cell-associated interleukin 1 through stimulation of the adhesion-promoting proteins LFA-1 (CD11a/CD18) and CR3 (CD11b/CD18) of human monocytes. *Eur J Immunol* 1990;20:999-1005.
- Reid DM, Perry VH, Andersson PB, Gordon S. Mitosis and apoptosis of microglia in vivo induced by an anti-CR3 antibody which crosses the blood-brain barrier. *Neuroscience* 1993;56:529-33.
- Watanabe A, Tominaga T, Tsuji J, Yanagihara Y, Koda A. Effects of anti-CD11a, anti-CD11b and anti-CD18 on histamine release from human basophils primed with IL-3. *Int Arch Allergy Immunol* 1992;98:308-10.
- Kapur R, Chandra S, Cooper R, McCarthy J, Williams DA. Role of p38 and ERK MAP kinase in proliferation of erythroid progenitors in response to stimulation by soluble and membrane isoforms of stem cell factor. *Blood* 2002;100:1287-93.
- Nagai S, Kitani S, Hirai K, Takaishi T, Nakajima K, Kihara H, et al. Pharmacological study of stem-cell-factor-induced mast cell histamine release with kinase inhibitors. *Biochem Biophys Res Commun* 1995;208:576-81.
- Ueda S, Mizuki M, Ikeda H, Tsujimura T, Matsumura I, Nakano K, et al. Critical roles of c-Kit tyrosine residues 567 and 719 in stem cell factor-induced chemotaxis: contribution of src family kinase and PI3-kinase on calcium mobilization and cell migration. *Blood* 2002;99:3342-9.
- Sui X, Krantz SB, You M, Zhao Z. Synergistic activation of MAP kinase (ERK1/2) by erythropoietin and stem cell factor is essential for expanded erythropoiesis. *Blood* 1998;92:1142-9.
- Zheng X, Karsan A, Duronio V, Chu F, Walker DC, Bai TR, et al. Interleukin-3, but not granulocyte-macrophage colony-stimulating factor and interleukin-5, inhibits apoptosis of human basophils through

- phosphatidylinositol 3-kinase: requirement of NF-kappaB-dependent and -independent pathways. *Immunology* 2002;107:306-15.
27. Oliveira SH, Hogaboam CM, Berlin A, Lukacs NW. SCF-induced airway hyperreactivity is dependent on leukotriene production. *Am J Physiol Lung Cell Mol Physiol* 2001;280:L1242-9.
 28. Lukacs NW, Strieter RM, Lincoln PM, Brownell E, Pullen DM, Schock HJ, et al. Stem cell factor (c-kit ligand) influences eosinophil recruitment and histamine levels in allergic airway inflammation. *J Immunol* 1996;156:3945-51.
 29. Finotto S, Buerke M, Lingnau K, Schmitt E, Galle PR, Neurath MF. Local administration of antisense phosphorothioate oligonucleotides to the c-kit ligand, stem cell factor, suppresses airway inflammation and IL-4 production in a murine model of asthma. *J Allergy Clin Immunol* 2001;107:279-86.
 30. Ali K, Bilancio A, Thomas M, Pearce W, Gilfillan AM, Tkaczyk C, et al. Essential role for the p110delta phosphoinositide 3-kinase in the allergic response. *Nature* 2004;431:1007-11.
 31. Nilsson G, Hjertson M, Andersson M, Greiff L, Svensson C, Nilsson K, et al. Demonstration of mast-cell chemotactic activity in nasal lavage fluid: characterization of one chemotaxin as c-kit ligand, stem cell factor. *Allergy* 1998;53:874-9.
 32. Olsson N, Rak S, Nilsson G. Demonstration of mast cell chemotactic activity in bronchoalveolar lavage fluid collected from asthmatic patients before and during pollen season. *J Allergy Clin Immunol* 2000;105:455-61.
 33. Columbo M, Horowitz EM, Botana LM, MacGlashan DW Jr, Bochner BS, Gillis S, et al. The human recombinant c-kit receptor ligand, rhSCF, induces mediator release from human cutaneous mast cells and enhances IgE-dependent mediator release from both skin mast cells and peripheral blood basophils. *J Immunol* 1992;149:599-608.
 34. Frenz AM, Gibbs BF, Pearce FL. The effect of recombinant stem cell factor on human skin and lung mast cells and basophil leukocytes. *Inflamm Res* 1997;46:35-9.
 35. Li L, Li Y, Reddel SW, Cherrian M, Friend DS, Stevens RL, et al. Identification of basophilic cells that express mast cell granule proteases in the peripheral blood of asthma, allergy, and drug-reactive patients. *J Immunol* 1998;161:5079-86.
 36. Kay AB, Ying S, Varney V, Gaga M, Durham SR, Moqbel R, et al. Messenger RNA expression of the cytokine gene cluster, interleukin 3 (IL-3), IL-4, IL-5, and granulocyte/macrophage colony-stimulating factor, in allergen-induced late-phase cutaneous reactions in atopic subjects. *J Exp Med* 1991;173:775-8.

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