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)

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Summary

We evaluated mature peripheral blood eosinophils for their expression of the surface tyrosine kinase, c-kit, the receptor for the stromal cell-derived cytokine, stem cell factor (SCF). Cytofluorographic analysis revealed that c-kit was expressed on the purified peripheral blood eosinophils from 8 of 8 donors (4 nonatopic and 4 atopic) (mean channel fluorescence intensity 2.0–3.6-fold, average 2.8 ± 0.6-fold, greater than the negative control). The uniform and selective expression of c-kit by eosinophils was confirmed by immunohistochemical analysis of peripheral blood buffy coats. The functional integrity of c-kit was demonstrated by the capacity of 100 ng/ml (5 nM) of recombinant human (rh) SCF to increase eosinophil adhesion to 3, 10, and 30 μg/ml of immobilized FN 40, a 40-kD chymotryptic fragment of plasma fibronectin, in 15 min by 7.7 ± 1.4-, 5.3 ± 3.3-, and 5.4 ± 0.2-fold, respectively, and their adhesion to 0.1, 0.5, and 1.0 μg/ml vascular cell adhesion molecule-1 (VCAM-1), by 12.7 ± 9.2-, 3.8 ± 2.5-, and 1.7 ± 0.6-fold, respectively. The SCF-stimulated adhesion occurred without concomitant changes in surface integrin expression, thereby indicating an avidity-based mechanism. rhSCF (100 ng/ml, 5 nM) was comparable to rh eotaxin (200 ng/ml, 24 nM) in stimulating adhesion. Cell adhesion to FN 40 was completely inhibited with antibodies against the α4 and β1 integrin subunits, revealing that the SCF/c-kit adhesion effect was mediated by a single integrin heterodimer, very late antigen 4 (VLA-4). Thus, SCF represents a newly recognized stromal ligand for the activation of eosinophils for VLA-4-mediated adhesion, which could contribute to the exit of these cells from the blood, their tissue localization, and their prominence in inflammatory lesions.

Eosinophils are bone marrow–derived granulocytes with a dominant extravascular distribution primarily in mucosal tissues (1, 2). Eosinophils have been implicated beneficially in host defense against helminthic parasitic infection (3–6), in anti-tumor cytotoxicity (7–9), and in wound healing (10, 11). Conversely, the abundant eosinophils in the respiratory mucosal tissues from patients with asthma or rhinitis are believed to contribute to the inflammatory process by releasing preformed, highly cationic granule proteins with cytotoxic effects (12) and by generating lipid mediators, in particular the cysteinyl leukotriene, leukotriene C4, with attendant vascular and bronchial smooth muscle constrictor action (13). Eosinophils at the foci of tissue inflammation bear membrane markers of activation such as CD69 (14, 15) and exhibit extended survival, which is attributed to the attenuation of apoptosis by hematopoietic cytokines, particularly IL-5, and GM-CSF (16, 17).

Integrins, heterodimeric cell surface receptors, participate in the regulation of leukocyte endothelial cell adhesion, transendothelial cell/basement membrane migration, and localization in inflammatory tissues. Eosinophils express the very late antigen (VLA)-1 (α4β1) and VLA-6 (α5β1) as well as α6β4 (18–20). VLA-4 mediates leukocyte attachment to VCAM-1 on activated endothelial cells (18, 21). Anti-α4 antibodies block eosinophil recruitment and prevent antigen-induced bronchial hyperreactivity in several animal models, suggesting a critical role for the α4 integrins.

Abbreviations used in this paper: BCECF-AM, 2',7'-bis(2-carboxyethyl)-5-(and 6)-carboxyfluorescein acetoxymethyl ester; BMMC, bone marrow–derived mast cells; CS-1, connecting segment-1; HSA, human serum albumin; MACS, magnetic cell separation; MFI, mean fluorescence intensity; rh, recombinant human; SCF, stem cell factor; VCAM, vascular cell adhesion molecule; VLA, very late antigen.
in the tissue recruitment, activation, and/or accumulation of eosinophils in allergic disease (22–26). The VLA-4 integrin also binds to fibronectin through an alternatively spliced connecting segment-1 (CS-1) region of fibronectin (27). The interaction between VLA-4 and fibronectin results in prolonged eosinophil survival in culture by inducing the autocrine generation of GM-CSF and IL-3 (28). Inasmuch as a subpopulation of eosinophils in nasal polyps (29) and bronchoalveolar lavage fluid from individuals with asthma undergoing allergen challenge (30) expresses GM-CSF protein and/or mRNA, it is possible that in situ VLA-4-fibronectin interaction prolongs eosinophil retention and viability through an autocrine mechanism.

Stem cell factor (SCF, also known as steel factor) is a bone marrow stromal cytokine central to hematopoiesis (31–33). It is also a peripheral tissue product of fibroblasts and endothelial cells (34–37). SCF exists in two different forms, soluble and membrane bound, and is the ligand for the c-kit receptor that is found on primitive hematopoietic cells (38). Among hematopoietic cells, c-kit is believed to be retained only by mature tissue mast cells, and thus is a commonly used marker for the latter (39, 40). Interaction of the c-kit receptor with SCF stimulates the growth and early differentiation of hematopoietic cells (38) and sustains mast cell growth and differentiation in cultures of mouse bone marrow (41, 42) and human cord blood (43, 44). In response to cross-linking of the high affinity IgE receptor, FcεRI, SCF primes mature dispersed human lung mast cells for both augmented exocytosis of secretory granules (45) and cytokine production (46) and primes mouse bone marrow-derived mast cells (BMMC) for enhanced generation of membrane-derived eicosanoids (47). Additionally, SCF is a direct activator of BMMC, stimulating both exocytosis and eicosanoid generation with the same biochemical steps and kinetics as activation by FcεRI (47). SCF is also a direct activator of BMMC, stimulating both exocytosis and eicosanoid generation with the same biochemical steps and kinetics as activation by FcεRI (48).

We now demonstrate by cytofluorographic and immunohistochemical analyses the surface expression of c-kit receptor in freshly isolated peripheral blood human eosinophils. That recombinant human (rh)SCF augments eosinophil adhesion to the VLA-4 ligands fibronectin, and VCAM-1, establishes the functional integrity of the eosinophil-expressed c-kit. Thus, SCF represents an abundant stromal ligand with direct activating effects on eosinophils as well as mast cells.

Materials and Methods

R agents and Antibodies. rhSCF (catalogue no. 1833-01, lot no. B6326; Genzyme, Cambridge, MA), rh eotaxin (Endogen, Inc., Boston, MA), 2′,7′-bis-(2-carboxyethyl)-5′-(and-6′)-carboxyfluorescein acetoxyethyl amid (BCECF-AM) (Molecular Probes, Eugene, OR), human serum albumin (HSA) (Sigma Chem. Co., St. Louis, MO), purified anti-human c-kit Y.B.S.B (PharMingen, San Diego, CA), purified anti-human c-kit (5SC3) (Coulter, Miami, FL), purified anti-human integrin β1 (CD29) (4B4) (Coulter), purified anti-human integrin α4 (CD49d) (A4-PU1) (Upstate Biotechnology, Lake Placid, NY), purified anti-human CD3 (HIT3a) (PharMingen), mouse laminin and the 40-kD chymotryptic fragment of human fibronectin (FN40) (GIBCO BR L, Gathersburg, MD) were purchased. The preparation of purified SCF was performed by Dr. M.E. Hemler (Dana-Farber Cancer Institute, Harvard Medical School). Purified anti-human c-kit mAb SR-1 (ascites) (58) was a gift from Dr. V. Broyd (University of Washington, Seattle, WA).

Isolation of Eosinophils from Peripheral Human Blood. Blood was collected into sterile, heparinized syringes from the peripheral veins of nonatopic and atopic volunteer donors who gave informed consent. After the erythrocytes were sedimented with dextran for 45 min at 37°C, the granulocyte fraction was obtained by centrifugation through a cushion of Ficoll-Hypaque (1.77 g/ml; Pharmacia, Uppsala, Sweden) of the buffy coat at 350 g for 30 min. After the hypotonic lysis of residual erythrocytes, eosinophils were separated from neutrophils by negative immunomagnetic selection with a magnetic cell separation (MACS) column (Miltenyi Biotec, Sunnyvale, CA) (59). In brief, the erythrocyte-depleted granulocyte pellet was incubated for 45 min at 4°C with anti-CD16 mAb bound to immuno-magnetic beads. When the mixture was applied to a steel wire column in a strong magnetic field, the CD16+ neutrophils were retained, whereas the CD16- eosinophils were highly purified in the fraction that flowed through the column. Contaminating T lymphocytes and monocytes were further removed by incubating the CD16- fraction for another 15 min at 4°C with saturating concentrations of anti-CD3 and anti-CD14 magnetic beads, respectively (Miltenyi). Cyto centrifugation slides of the eosinophils stained with Wright’s and Giemsa stains showed that the purity of the isolated eosinophils was greater than 95% in all experiments.

Cytofluorographic and Immunohistochemical Analyses of Surface c-kit Expression on Eosinophils. Cytofluorographic analyses of surface epitopes expressed by human peripheral blood eosinophils with or without rhSCF stimulation were performed by established procedures (52). Freshly isolated human peripheral blood eosinophils were resuspended in RPMI 1640 medium containing 10% FCS at a concentration of 10⁶ cells/ml and were divided into two identical fractions. rhSCF was added to one fraction to a final concentration of 100 ng/ml (50 nM). Alternatively, rh eotaxin was used at a final concentration of 24 nM and both fractions were incubated for 15 min at 37°C. The cells were washed and then incubated in cold PBS containing 5.0% HSA and 0.02% sodium azide (FACS buffer). Samples of 5 × 10⁶ cells were then incubated for 1 h on ice with primary antibodies (purified mAbs at a final concentration of 10 μg/ml or ascites at a final dilution of 1:500 or P3 culture supernatant at 1:4 dilution). The cells were washed once with FACS buffer and incubated in the dark for 1 h on ice with fluorescein isothiocyanate-conjugated goat anti–mouse IgG (GIBCO BR L) at a final dilution of 1:100. The cells were washed again with the FACS buffer, resuspended in 0.25 ml of PBS, and analyzed on a FACSort® machine (Becton Dickinson, Oxford, CA). For c-kit expression (Fig. 1), the results are presented as overlaid histograms and the fold increase of mean fluorescence intensity (MFI). The fold increase of c-kit expression was calculated by dividing the MFI units of SR-1 staining by the MFI units of P3 control mAb staining in each donor.

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For immunohistochemical analysis of the surface c-kit expression, peripheral blood buffy coats were prepared after dextran sedimentation of erythrocytes. Two fractions of the buffy coat, each containing $10^8$ white blood cells, were incubated on ice for 1 h with SR-1 (1:500 diluted ascites) and control P3 antibody (1:4 diluted hybridoma culture supernatant) in a total volume of 100 µl of FACS buffer, respectively. Cells were washed once with 3 ml of FACS buffer, and cytocentrifugation slides were prepared, with 4 × 10^4 cells per slide. The slides were fixed in 4% paraformaldehyde (Polysciences, Inc., Warrington, PA) in PBS for 10 min at room temperature. After washing with PBS for 3-4 times, the slides were blocked with 2% chicken egg albumin (Sigma) for 30 min at room temperature, and were incubated with 1:10 diluted goat anti-mouse IgG(Fc)-conjugated gold particles (average size 10 nm) (Amersham International, Buckinghamshire, England) for 1 h at room temperature. The slides were washed first with PBS 2–3 times and then with distilled water 2–3 times. A silver enhancement procedure was carried out for 8 min at room temperature following the manufacturer's manual (AuroProbe®; Amersham). The slides were then counterstained with hematoxylin and eosin, mounted with Permount® (Fisher Scientific, Pittsburgh, PA), and analyzed with a Leica microscope (Model Dialux 20).

R~esults were photographically recorded using Kodak Royal Gold film ASA 25.

Cell Adhesion Assay. Cells were attached in triplicate to FN 40, VCAM-1, and laminin as described (52). FN 40 and laminin at concentrations of 3 µg/ml, 10 µg/ml, and 30 µg/ml were coated onto non-tissue culture grade 96-well microtiter plates (Nunc-Immuno Plate) in 0.1 M NaHCO$_3$ (pH 8.3) (100 µl/well) for 16 h at 4°C. The plates were washed twice with PBS (150 µl/well), and the non-specific binding sites were blocked by incubation with 5% HSA in PBS (100 µl/well) for 45 min at 37°C. After two washes with PBS (150 µl/well), the plates were ready to be used. For the VCAM-1 assay, plates were precoated with goat anti-mouse κ (GIBCO BRL) at a 1:1,000 final dilution in 0.1 M NaHCO$_3$ (100 µl/well) for 16 h at 4°C. After two washes with PBS, the plates were coated with VCAM-1-κ at concentrations of 0.1, 0.5, and 1.0 µg/ml in 0.1 M NaHCO$_3$ (100 µl/well) for 2 h at 4°C. The plates were washed with PBS and blocked with HSA as mentioned above. The freshly isolated eosinophils were incubated with BCECF-AM (5 µM/ml) in RPMI 1640 containing 10 mM Hepes and 0.1% HSA (RPMI-I-HSA) for 30 min at 37°C, washed twice with PBS, and resuspended in RPMI-I-HSA at 4 × 10^6 cells/ml. Samples (50 µl) of the cell suspension containing 2 × 10^6 cells were added to each well of the ligand-coated plate; each well had been preloaded with either 50 µl of RPMI-I-HSA or 50 µl of RPMI-I-HSA containing defined concentrations of rhSCF (generally 10 nM) or defined concentrations of rh eotaxin (generally 48 nM). The plates were incubated for 15 min at 37°C, and the fluorescence of total input cells was quantitated by a fluorescence analyzer (Idexx Laboratories, Westbrook, ME). Unbound cells were removed by washing the plates with RPMI/10 mM Hepes 4 to 5 times at 150 µl/well, and the fluorescence of the cells remaining after the final wash divided by the fluorescence of the total starting cells × 100, and assay results are presented as means ± SD of three independent experiments, each performed in triplicate.

For antibody-blocking experiments, cells were resuspended in cold RPMI-I-HSA at a concentration of 4 × 10^6 cells/ml and divided into four identical lots. Purified mAbs were added to each lot to a final concentration of 10 µg/ml. The mAbs used in this study were anti-CD 3 (negative control), anti-α4 (A4-PUJ1), anti-α5β1 (Act-1), and anti-β1 (4B4). Cells were incubated with the mAbs for 10 min on ice and added to each well of a FN40 (30 µg/ml) coated plate. During the 15-min incubation at 37°C, the mAb concentration in each well was reduced to 5 µg/ml by preloading the wells with RPMI-I-HSA with or without rhSCF or rh eotaxin. The remainder of the assay procedure was as described above.

Statistical Analysis. The statistical significance of differences between sample means for each set of cells was based on comparison as determined by the Student's t test for matched pairs. Results are presented as means ± SD.

Results

Cytofluorographic and Immunohistochemical Analysis of Surface c-kit Expression on Eosinophils. To determine whether freshly isolated, peripheral human blood eosinophils expressed the c-kit receptor, the purified cells from eight sep-
lyzed by cytofluorography for the expression of c-kit. Three mouse mAbs against three independent epitopes of the human c-kit (58, 60) gave virtually identical expression for two of the donors (Fig. 1 A), with mean log fluorescence intensities of 2.0- and 3.3-fold over control, respectively, with mAb SR-1. The surface c-kit expression was subsequently confirmed in six additional donors with one of the three mAbs, SR-1 (Fig. 1 B). In every case, the c-kit expression was readily detectable, with a mean log fluorescence intensity of 2.0 to 3.6-fold greater than the negative control (IgG control mAb P3) (mean 2.8 ± 0.6 fold, n = 8).

To confirm c-kit expression by peripheral blood eosinophils and determine its potential expression by other circulating leukocytes, peripheral blood buffy coats from two separate donors (1 atopic and 1 nonatopic) were incubated with either SR-1 or control P3 antibody, and were subjected to immunohistochemical analysis using secondary antibody-conjugated gold particles and a silver enhancement procedure, followed by counterstaining with hematoxylin and eosin. As indicated by the counterstaining and shown for the nonatopic donor (Fig. 2), 100% of the eosinophils in the buffy coats were positive for c-kit, and eosinophils were the only cells displaying a signal for c-kit receptor. Identical results were obtained for the atopic donor (data not shown). A similar positive signal was also detected on freshly isolated human peripheral blood eosinophils after MACS column purification (data not shown).

Effect of rhSCF on Eosinophil Adhesion to FN40 and rVCAM-1. To determine the functional integrity of the expressed c-kit receptor on eosinophils, the ability of rhSCF to augment their adhesion to ligands selective for integrin α4 and α6 was evaluated in a static adhesion assay. The 15-min time course for the adhesion assay was selected because a kinetic study showed that augmented adhesion peaked at 15 min, persisted for 30 min, and decreased to baseline by 45 min (data not shown). Adhesion to FN40 increased at all three inputs of 3, 10, and 30 μg/ml of immobilized FN40. In the absence of rhSCF, the adhesion of FN40 was limited (specific binding of 4.3 ± 3.5% at 30 μg/ml FN40). Concomitant stimulation with rhSCF (5 nM) augmented adhesion to 3, 10, and 30 μg/ml FN40 by 7.7 ± 1.4-fold, 5.3 ± 3.3-fold, and 5.4 ± 0.2-fold, respectively, compared with baseline (Fig. 3). Adhesion to rVCAM-1 also increased in relation to the input of ligand. In the absence of rhSCF, rVCAM-1 supported greater adhesion than FN40 (specific binding of 9.3 ± 9% and 22.5 ± 10% at 0.5 μg/ml and 1.0 μg/ml of rVCAM-1, respectively). Stimulation with rhSCF augmented adhesion to 0.1, 0.5, and 1.0 μg/ml rVCAM-1 by 12.7 ± 9.2-fold, 3.8 ± 2.5-fold, and 1.7 ± 0.6-fold, respectively, compared with baseline (Fig. 3).

Higher baseline adhesion mediated by VCAM-1 compared with fibronectin has been previously reported (22). Cell adhesion to laminin also increased in a dose-dependent fashion with respect to ligand input but did not increase further with stimulation by rhSCF (Fig. 3). Treatment with rhSCF did not change the surface expression of integrin α4, α6, β1, α6β1, or c-kit receptor as evaluated by cytofluorographic analysis (Fig. 4), indicating that the increases in adhesion to the α4 ligands were not due to increased receptor expression.

Effect of Antibody Neutralization on rhSCF-stimulated Adhesion of Eosinophils. To determine c-kit receptor expression on eosinophils, VLA-4 (α4β1) and α6β1, or c-kit receptor as evaluated by cytofluorographic analysis (Fig. 4), indicating that the increases in adhesion to the α4 ligands were not due to increased receptor expression.}

**Figure 2.** Immunohistochemical analysis of c-kit receptor expression on fresh human peripheral blood buffy coats. Peripheral blood buffy coats were incubated with either negative control mAb P3 (IgG matched) (a), or anti-human c-kit mAb SR-1 (b), and cytocentrifugation slides were prepared. After application of secondary antibody-conjugated gold particles and a silver enhancement procedure, the slides were counterstained with hematoxylin and eosin, and analyzed with a Leica microscope. Arrows indicate the eosinophils. Other leukocytes, as shown in the same field, were negative for surface c-kit expression. Higher magnification views of individual eosinophils are shown in the upper right corners.
in the absence or presence of rhSCF (5 nM) was also completely blocked by anti-α4 and anti-β1 mAbs (data not shown). Therefore, rhSCF–c-kit ligation specifically augmented the adhesion of VLA-4 to its ligands, fibronectin, and VCAM-1.

Dose Effect of rhSCF on Eosinophil Adhesion to FN40. The effect of rhSCF at concentrations ranging from 12.5 ng/ml (0.625 nM) to 200 ng/ml (10 nM) on eosinophil adhesion to 30 μg/ml FN40 was studied. Enhancement of VLA-4 binding to FN40 by rhSCF was significant at 2.5, 5, and 10 nM as compared with the unstimulated replicate eosinophils (P < 0.05) (Fig. 6). Each fourfold increment in rhSCF concentration produced a statistically significant gain in binding (P < 0.05) except for the final increment between 2.5 and 10 nM, suggesting that a plateau was reached.

Comparison of the Effects of rhSCF and rh Eotaxin on Eosinophil Binding to FN40. Eotaxin, a selective eosinophil chemoattractant (61, 62), belongs to the CC chemokine family. Other members of this family are known to be stimuli for eosinophil adhesion via integrin α4 (63). Preliminary dose-dependence experiments indicated that the effect of 24 nM rh eotaxin was similar to the effect of 5 nM rhSCF for augmenting eosinophil adhesion to 30 μg/ml FN 40. Thus, the effects of these concentrations on eosinophil adhesion were compared over three concentrations of FN40 (Fig. 7). Eotaxin at 24 nM increased eosinophil binding to FN40 to the same extent as 100 ng/ml (5 nM) rhSCF at each static input of FN40 ligand. Eotaxin did not change the surface expression of c-kit, α4, α6, β1, and α4β7 by cytofluorographic analysis (data not shown), and the augmented adhesion of these cells to FN40 produced by eotaxin was completely blocked by mAbs against α4 and β1 (Fig. 8).

The dose-related effect of rh eotaxin on human eosino-

![Figure 3](image-url)
phil adhesion to FN 40 (30 μg/ml) was analyzed in the absence and presence of 1.25 nM rhSCF. The approximate EC₅₀ for rh eotaxin-augmented adhesion occurred at the lowest concentration studied, 3 nM, and the plateau was reached at 12 nM (20.8 ± 13-fold, n = 3). The effect of the concomitant presence of rhSCF at slightly less than its EC₅₀ (see Fig. 6) was generally somewhat additive and did not extend the maximum reached with the plateau doses of rh eotaxin alone (Fig. 9).

Discussion

The finding that mature human peripheral blood eosinophils express a functional c-kit receptor for SCF reveals that...
a stromal ligand can activate effector cells conventionally linked to allergic inflammation. Although originally appreciated for its central role in the hematopoiesis of all lineages, SCF is elaborated in the peripheral microenvironment by diverse cell types, including endothelial cells and fibroblasts (34–37). In addition to the functional implications for eosinophil- and mast cell-directed inflammation, the study also uncovers a potential limitation to the use of c-kit detection as a marker of mature mast cells.

In the initial experiments, the surface expression of the c-kit receptor was established by cytofluorographic analysis of freshly isolated human peripheral blood eosinophils. The expression was similar among the cells of all eight donors tested (Fig. 1), irrespective of the presence of donor atopy by history. To exclude an unsuspected specificity, three mAbs directed against separate epitopes of the human c-kit were shown to yield nearly identical fluorescence signals. The positive surface c-kit expression was also demonstrated by immunohistochemical analysis of peripheral blood buffy coats (Fig. 2). Eosinophils were uniformly positive for c-kit expression and were the only cell type expressing c-kit in these preparations. The presence of c-kit was then confirmed by the functional, dose-related response of the eosinophils to SCF signal in static adhesion assays (Fig. 6). An earlier study did not detect a c-kit signal on peripheral blood eosinophils, and functional assays with SCF were not performed (64). However, this same study did demonstrate low level c-kit expression by human peripheral blood basophils and a priming effect of SCF on their IgE-dependent release of histamine (64). Because eosinophils and basophils are closely related and arise from a common progenitor (65), the expression of c-kit and their activation via SCF can now be added to the list of their shared characteristics. Moreover, a triad of hematopoietic allergic effector cells, mast cells, basophils, and eosinophils, would appear to share the expression of c-kit.

rhSCF–c-kit stimulated eosinophil adhesion to FN 40 and VCAM-1, but not to laminin (Fig. 3), implying a re-
of VCAM-1 expression (71). Alternatively, exposure of eosinophils to SCF in the extracellular matrix may mediate their transient attachment to fibronectin, altering their migration speed and influencing their tissue localization. Indeed, Palecek and colleagues (72) have demonstrated that at low concentrations of ligand, cell migration speed increased as integrin binding affinity increased. Thus, SCF is a candidate for participating in the tissue distribution of eosinophils under normal physiologic conditions.

The interaction of VLA-4 and VCAM-1 plays a key role in lymphocyte and eosinophil adhesion and extravasation in both in vitro and in vivo models of allergic inflammation (18, 22–26). VCAM-1 expression is upregulated in the eosinophil-rich, inflamed airway tissue of individuals with asthma (73, 74). VLA-4 is expressed on eosinophils and lymphocytes, but not neutrophils; and the c-kit receptor expression is limited to eosinophils among these three cell types. Thus, under conditions of allergic inflammation, local SCF could contribute both by priming mast cells to elaborate IL-4 (46, 75) and TNF-α (75) with consequent upregulation of endothelial cell VCAM-1 expression (76), and by mediating a transiently increased avidity for that ligand by eosinophil VLA-4. This possibility is supported by a recent finding in mice of the association of allergen challenge-induced eosinophilic airways inflammation with increased levels of histamine and SCF in bronchoalveolar lavage fluid and of SCF in serum (70). In that study, the administration of antibody to SCF before allergen challenge markedly decreased histamine release and pulmonary eosinophil infiltration. The inflammatory response induced by allergen challenge also increases the production of the eosinophil-selective chemoattractant, eotaxin (61), which is a product of a number of cell types including epithelial cells, endothelial cells, fibroblasts, and even eosinophils (62). Therefore, increased concentrations of eotaxin and SCF could provide a coordinated signal for the selective recruitment of eosinophils, beginning with transiently enhanced eosinophil adhesion to endothelial cells within the vasculature through VLA-4–VCAM-1 interaction, followed by directed tissue movement through adhesion/deadhesion in a chemotactic gradient, and final eosinophil tissue retention through regulated integrin–matrix interaction. Such a matrix interaction may also prolong eosinophil survival through autocrine production of IL-3 and GM-CSF initiated by VLA-4–fibronectin interaction (28).

We have provided evidence that SCF is an agonist for eosinophil adhesion. These findings have potential relevance for physiologic eosinophil trafficking and for allergic inflammation, in which mast cell activation and eosinophil accumulation are both prominent features.

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