Regulatory effects of stem cell factor and interleukin-4 on adhesion of human mast cells to extracellular matrix proteins

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Mast cells are inflammatory and immunoregulatory cells resident in tissues. They develop from bone marrow-derived progenitor cells that enter the tissue through the blood circulation. The specific localization and migration of mast cells in tissues is dependent on their interaction with extracellular matrix (ECM) proteins. Adhesion of human mast cells isolated from intestinal mucosa and cultured in the presence of stem cell factor (SCF) to ECM proteins is analyzed. It was observed that SCF is a unique cytokine enhancing mast cell adhesion to all tested ECM proteins (fibronectin, laminin, collagen I, III, IV, VI, XIV) up to 5-fold, particularly to fibronectin (54% ± 12% of mast cells) and to denatured collagens (40% ± 12% on cyanogen bromide-cleaved peptides of collagen I). Most noteworthy, preculture of mast cells with interleukin-4 (IL-4), in addition to SCF, reduced their potency to adhere to ECM proteins to one third compared to mast cells cultured with SCF alone. Mast cell adhesion was preferentially mediated by β1 integrins, and most cells expressed the ECM-binding integrins α2β1, α3β1, α4β1, α5β1, and αVβ3. SCF-induced mast cell adhesion was totally blocked by wortmannin and apigenin, indicating an involvement of phosphatidylinositol 3-kinase and mitogen-activated protein kinase, and it was related to an up-regulation of the HUTS-21 β1 epitope, which is associated with an activated conformation of β1. In conclusion, these data indicate that SCF induces the adhesion of cultured mast cells to ECM proteins, whereas IL-4 may promote detachment from the ECM. (Blood. 2002;99:966-972)

Introduction

Mature mast cells are thought to be involved in physiological processes, such as host defense against bacteria and tissue remodeling.[1,2] Apart from these functions, which are not understood in detail, mast cells are known to be of particular importance in the pathophysiology of immediate-type allergic reactions and of other chronic inflammatory diseases.[3] Humoral effector functions of human mast cells—for example, the release of histamine, heparin, proteases, and cytokines such as tumor necrosis factor (TNF)–α, transforming growth factor (TGF)–β, basic fibroblast growth factor, and interleukin-5 (IL-5)—are regulated by cross-linking of surface-bound immunoglobulin (Ig)E with antigen, by IgE-independent stimuli that are still poorly defined for human mast cells, and by cytokines such as stem cell factor (SCF) and IL-4.[1-6] Mast cells develop from bone marrow-derived progenitor cells that enter the tissue through the blood circulation. Mast cell maturation is dependent on SCF, IL-4, nerve growth factor (NGF), and probably other less-defined factors.[5,7-9]

The specific localization and migration of mast cells in tissues is dependent on their interaction with extracellular matrix (ECM) proteins.[10] Cell adherence to ECM proteins is mediated by specific cell adhesion receptors, mainly cell surface receptors of the integrin family. Integrins are heterodimers of noncovalently linked α- and β-chains that mediate cell binding to ECM proteins such as laminins, fibronectin, and various collagens.[11] ECM proteins are expected to play a crucial role for the homing, maturation, and function of mast cells in tissue. It was reported that bone marrow-derived mouse mast cells adhere to laminin and fibronectin and that this adherence is induced by FcεRI-mediated signals and SCF.[12,13] Studies in humans showed that mast cells from uterus and lung express the β1 integrins α4β1 and α5β1, known as receptors for fibronectin, and that skin mast cells adhere to fibronectin and laminin.[14-16] The immature human mast cell line (HMC)–1 also expresses α2β1, α3β1, α6β1, αVβ1, αVβ5, and CD44.[17] Interestingly, adhesion of HMC-1 to ECM proteins was shown to provide a costimulatory signal for cytokine expression and secretion.[18]

No information is available, however, on the adhesion of human mast cells derived from mucosal tissue to ECM proteins and its regulation by cytokines. A major reason for that is the difficulty in obtaining sufficient amounts of purified mast cells isolated from human mucosal tissue. Using recently developed methods for the isolation and culture of human mast cells derived from intestinal mucosa, we could show that SCF and IL-4 regulate mast cell proliferation and mediator release.[5,19] Moreover, we found that mast cells survive in coculture with human umbilical vein endothelial cells dependent on membrane-bound SCF and on adhesion molecules such as vascular cell adhesion molecule-1 on endothelial cells, suggesting that additional molecules (eg, adhesion receptors) may be important for mast cell regulation.[20] In the current study, we examined the capacity of human mast cells derived from intestinal tissue and cultured for 2 to 3 weeks to adhere to different ECM proteins, such as collagens, laminin and fibronectin, and the

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regulation of mast cell adhesion and integrin expression by SCF, IL-4, and other factors.

Materials and methods

Isolation and culture of human intestinal mast cells

Human mast cells were isolated from intestinal mucosa under sterile conditions by tissue dissection followed by a 4-step enzymatic tissue dispersion method described in detail previously.[19] Human tissue was obtained from surgical specimens (macroscopically normal border sections free of tumor cells, as determined by histologic examination) of patients who underwent bowel resection because of intestinal cancer. Permission to conduct the study was obtained from the local ethics committee of the Medical School of Hannover. Purification of mast cells was achieved by positive selection of c-*kit*–expressing cells using magnetic cell separation (MACS system; Miltenyi Biotec, Bergisch Gladbach, Germany) and the monoclonal antibody (mAb) YB5.B8 (PharMingen, Hamburg, Germany) directed against human c-*kit* and by culture of the selected cells for 14 to 21 days in the presence of 25 ng/mL (13.6 nM) human recombinant SCF (PeproTech, Rocky Hill, NJ) as described.[5,6] In some experiments, mast cells were cultured with human recombinant IL-4 (Novartis, Vienna, Austria) at 2 ng/mL (1.3 nM) in addition to SCF, as indicated in "Results." After preculture, mast cell purity was 98% or greater in all experiments. Mast cells were harvested, resuspended in fresh medium, and incubated for one day without cytokine supplementation before use in the adhesion assay.

Mast cell adhesion assay

Ninety-six–well plates (Becton Dickinson, Lincoln Park, NJ) were coated for 2 hours at 37°C with 100 μL phosphate-buffered saline (PBS) containing 2 μg of the following ECM proteins: human fibronectin (Serva, Heidelberg, Germany), human collagens I, III, IV, VI, and XIV, purified and characterized as described elsewhere, mouse laminin-1, or the cyanogen bromide (CNBr)–cleaved peptides and the chains α1 and α2 of human collagen type I.[21-23] The wells were rinsed twice with 100 μL PBS before use. Then 100 μL cell suspension containing 5×10^3 mast cells was added to each well; this was followed by incubation at 37°C for 90 minutes and the removal of nonadherent cells by washing the plates twice with PBS. Adherent cells were lysed by adding 100 μL H_2O to each well and 2 subsequent freeze-thaw cycles. Lysates were transferred to 1.5 mL tubes and centrifuged (5 minutes, 10 000*g*). Histamine was measured in supernatants using a commercially available radioimmunoassay (Coulter-Immunotech, Krefeld, Germany). The histamine concentration in these samples was expressed as a percentage of total histamine content determined in wells from which nonadherent cells were not removed. This parameter was used for quantification of mast cell adherence. Background binding was determined by measurement of histamine content of mast cells that bound to PBS–3% bovine serum albumin (BSA)–coated plates (<3% total histamine).

Stimulation and inhibition of mast cell adhesion

During adhesion, mast cells were challenged with SCF (PeproTech) at various concentrations (0.1-1000 ng/mL [0.05-540 nM]), with IL-3 (R&D Systems, Wiesbaden, Germany), IL-4 (Novartis), IL-6, IL-10, IL-13 (PeproTech), NGF (R&D Systems), TNF-α (Becton Dickinson, San Jose, CA) or IFN-γ (PharMingen, San Diego, CA), each at 100 ng/mL (30-65 nM), with TGF–β (PeproTech) at 10 ng/mL (4 nM), with IL-8 (PeproTech), the complement cleavage products C3a and C5a (gifts from C. A. Dahinden, Bern, Switzerland), each at 10^{-7} M, or by cross-linking of the high-affinity IgE receptor (FcεRI) using the mAb 29C6 at 100 ng/mL (6.5 nM) (provided by Hoffman-La Roche, Nutley, NJ). In another set of experiments, adhesion blocking mAbs directed against β1-integrins (CD29, clone P4C10; Biomol, Hamburg, Germany), β2-integrins (CD18, clone MHM23; DAKO, Glostrup, Denmark), and αvβ3 (CD51, clone 23C6; Southern Biotechnology, Birmingham, AL) were assessed. Mast cells were preincubated at 37°C for 10 minutes with an isotype control, anti-CD18 or anti-CD51, each at a final concentration of 3 μg/mL, or 1:100 dilution of anti-CD29 according to the specification sheets. To inhibit specific signaling pathways, cells were incubated with apigenin, Gö6976, or wortmannin (all from Calbiochem, La Jolla, CA) for 1 hour before stimulation at the concentrations indicated.

Immunocytochemistry

Immunocytochemistry was performed using antibodies (overnight incubation) against human tryptase (mAb, 230 ng/mL [15 nM]; Chemicon, Temecula, CA) and human chymase (mAb, 100 ng/mL [6.5 nM]; Chemikon) as primary antibodies and the LAB-SA detection system (Histostain-Plus kit; Zymed Laboratories, San Francisco, CA) as described.[5,19]

Scanning electron microscopy

Microscopic coverslips were coated with ECM proteins, and adhesion assays were performed as described above. Adherent cells were fixed in freshly prepared Karnovsky fixative (2.5% glutaraldehyde and 4% paraformaldehyde in 0.1 M cacodylate-HCl buffer, pH 7.4) for 2 hours and rinsed in 0.1 M cacodylate buffer overnight. Thereafter, cells were dehydrated in a graded series of ethanol (30%, 50%, 70%, 90%, and 100%) and were air-dried at room temperature. The cells were coated with gold-palladium before examination using a scanning electron microscope (Philips 505, Eindhoven, The Netherlands).

Reverse transcription–polymerase chain reaction

Total RNA was prepared from human intestinal mast cell preparations, and genomic DNA was removed as described.[6] Complementary DNA was synthesized using Superscript reverse transcriptase (Life Technologies, Eggenstein, Germany) and polymerase chain reaction (PCR) was carried out for 35 cycles (60 seconds at 94°C, 80 seconds at 60°C, 70 seconds at 72°C) using Taq DNA polymerase (Life Technologies) and specific primer pairs (sense; antisense) for the integrin subunits β1 5′-TGTTCAGTGCAGAGCCTTCA-3′; 5′-CCTCATACTTCGGATTGACC-3′), β2 5′-AGGCTCTGATCCACCTGAGC-3′; 5′-TCACCAACCTCAAGCCCTCC-3′), β3 5′-GGGGACTGCCTGTGTGACTC-3′; 5′-CTTTTCGGTCGTGGATGGTG-3′), α1 5′-CTACAAGATGGAGGATGGG-3′; 5′-GCCTCAGTGAATCAATCAAGGG-3′), α2 5′-CTGCTTCAGTGCAAAGTTCA-3′; 5′-CCCGTTCCAAATTCTGGTAG-3′), α3 5′-GTGGTCAGGGTCAGAAGACC-3′; 5′-GGAGAAGAAGCCGTGGAAGA-3′), α4 5′-CCACCTTGGTCCTCATGTCAT-3′; 5′-CATGCGCAACATTCTGATCCT-3′), α5 5′-AACAGGATGGCTAGGATGAT-3′; 5′-ACAAGTTGCTGACTCCATTG-3′), α6 5′-GTGTTGCCAACCAGAATGGC-3′; 5′-CAGTCACTCGAACCTGAGTG-3′), αV 5′-GGATCTTGCCCTCAGTGAAG-3′; 5′-CATGAGGTTGAAGCTGCTCC-3′). PCR products were separated on a 1% agarose gel containing ethidium bromide (500 ng/mL [1.3 μM]) and then were photographed.

Flow cytometry

For each labeling, 1×10^5 mast cells (purity, 98% or greater) were washed twice (4 minutes, 400*g*) and resuspended in PBS supplemented with 0.1% BSA, 0.1% sodium azide, and 250 μg/mL rabbit IgG. Cells were labeled using the primary mAbs directed against CD11a (Coulter-Immunotech), CD11b (Coulter-Immunotech), CD11c (PharMingen), CD18 (Coulter-Immunotech), CD29 (Coulter-Immunotech), activated conformation of CD29 (clone HUTS-21, PharMingen), CD49a (Coulter-Immunotech), CD49b (PharMingen), CD49c (PharMingen), CD49d (Coulter-Immunotech), CD49e (Coulter-Immunotech), CD49f (PharMingen), or CD61 (PharMingen), respectively. Appropriate isotype controls were performed. After incubation at 4°C for 45 minutes, cells were washed and labeled with the secondary antibody goat anti–mouse IgG fluorescein isothiocyanate (FITC) or goat anti–mouse IgG1 FITC (Southern Biotechnology). Flow cytometric analysis was performed using the FACSCalibur system (Becton Dickinson).

Results

Stem cell factor promotes adhesion of human intestinal mast cells to extracellular matrix proteins

Human mast cells were isolated from intestinal tissue and cultured in the presence of SCF. Adhesion to laminin-1, fibronectin, collagens, and denatured collagens (α1 and α2 chains and CNBr-cleaved peptides of collagen type I) was tested using a mast cell adhesion assay with and without the addition of 100 ng/mL (54 nM) SCF. In the absence of SCF, mast cell adhesion to fibronectin, but not to collagens and almost never to laminin-1, was observed. Addition of SCF to the adhesion assay caused a 2- to 5-fold increase in mast cell adhesion to all tested ECM proteins. In the presence of SCF, mast cell adhesion was again highest (up to 74%) to fibronectin, but substantial adhesion was also observed to laminin-1, the α1 and α2 chains of collagen I, and, even more pronounced, to CNBr peptides of collagen I (Figure 1A). Figure 1B shows that mast cell adhesion to fibronectin and to CNBr peptides of collagen type I is enhanced by SCF in a dose-dependent manner, reaching maximum at 10 to 1000 ng/mL (5.4-540 nM) SCF (ED_{50} = 0.5 ng/mL [0.27 nM] SCF in each case).

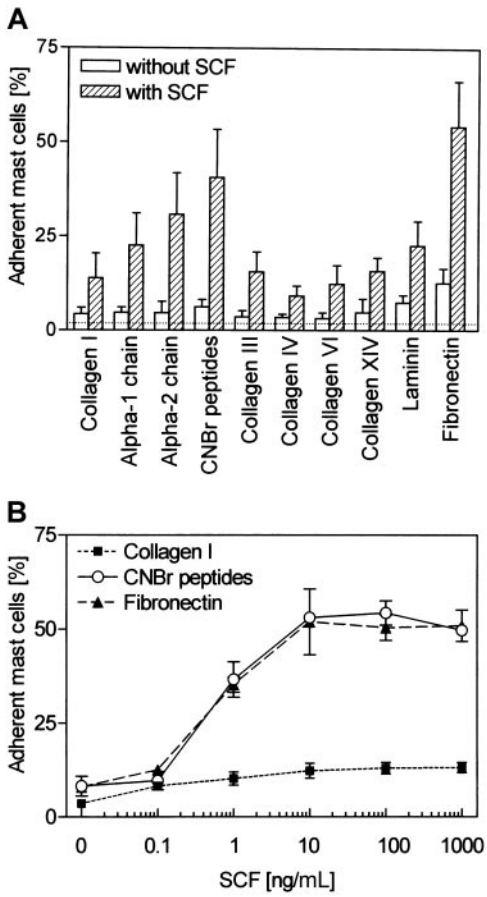

Figure 1. Adhesion rates of purified human intestinal mast cells to ECM proteins. (A) Mast cells precultured with SCF (purity, 98% or greater) were incubated for 90 minutes in wells coated with different ECM proteins indicated on the x-axis. Adhesion rates of mast cells, in the presence or absence of 100 ng/mL (54 nM) SCF, were determined by the measurement of histamine content of bound cells (expressed in percentage of total histamine of all cells). The dotted line indicates the background (binding of mast cells to BSA) (n = 5). (B) Dose dependency of the effect of SCF. Mast cells were challenged for 90 minutes with various concentrations of SCF indicated on the x-axis. Results represent the mean ± SD of 3 experiments.

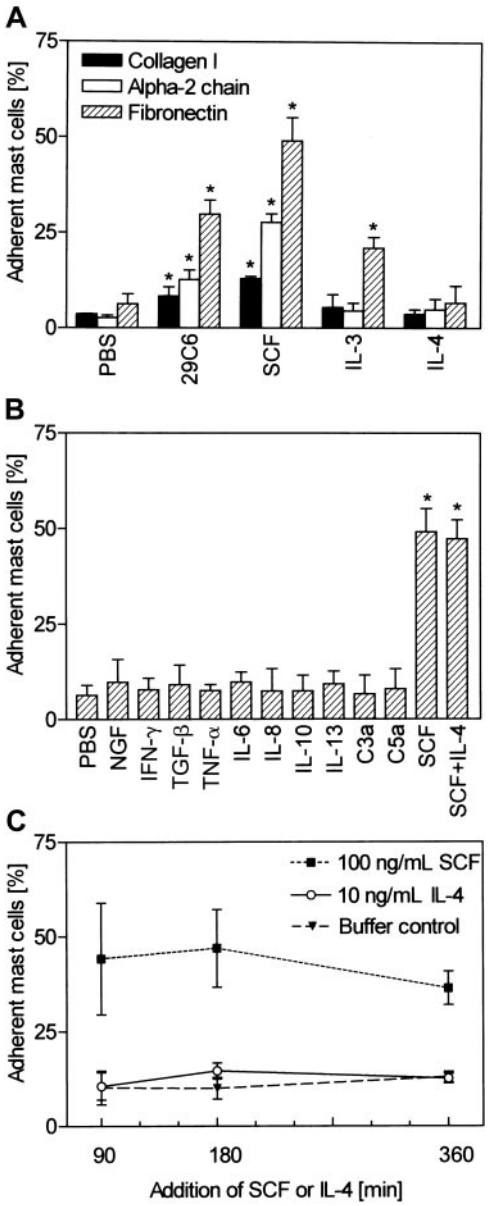

Figure 2. Effect of IgE receptor cross-linking and cytokines on mast cell adhesion to ECM proteins. (A) Mast cells were precultured with SCF before the adhesion assay was performed in the presence of mAb 29C6, inducing IgE receptor cross-linking, SCF, IL-3, or IL-4. Results represent mean ± SD of 4 experiments. *$P < .05$ for significant difference (2-tailed paired *t* test) compared to the PBS control. (B) Mast cell adhesion to fibronectin in the presence of NGF, IFN-γ, TGF-β, TNF-α, IL-6, IL-8, IL-10, IL-13, C3a, C5a, SCF, and SCF together with IL-4. Results represent mean ± SD of 3 experiments. *$P < .05$ for significant difference (2-tailed paired *t* test) compared to the PBS control. (C) Time course of mast cell adhesion to fibronectin in response to SCF or IL-4. Mast cells were precultured with SCF. Mast cell adhesion was determined in the presence of SCF, IL-4, or buffer control, respectively, after 90, 180, and 360 minutes. Results represent mean ± SD of 3 experiments.

IL-3 enhances mast cell adhesion to fibronectin

Apart from SCF, a broad spectrum of other cytokines and growth factors was tested for their capacity to modulate mast cell adhesion to collagen I, the α2 chain of collagen I, and fibronectin. Only IL-3 caused a significant increase in mast cell adhesion to fibronectin (21% compared with 10% without cytokines). However, adhesion rates to collagen I and the α2 chain of collagen I were not changed by IL-3 (Figure 2A). Moreover, we found that the stimulation of mast cells by FcεRI cross-linking leads to a 2.5-fold enhancement of mast cell adhesion to collagen I, the α2 chain of collagen I, and

fibronectin. In contrast, the addition of C3a, C5a, NGF and other cytokines for 90 minutes failed to modulate mast cell adhesion to fibronectin (Figure 2B). Treatment of mast cells with IL-4, in addition to SCF, did not alter SCF-induced mast cell adhesion (Figure 2B). IL-4 also did not have a significant effect on mast cell adhesion after prolongation of the adhesion assay up to 6 hours (Figure 2C).

Preincubation with IL-4 strongly reduces mast cell adhesion

Recently, we showed that the addition of IL-4 to the culture medium strongly enhances proliferation and mediator release of human intestinal mast cells.[5] Furthermore, IL-4 enhances the production of Th2-type cytokines in mast cells, but it does not affect or even down-regulate proinflammatory cytokines.[6] To analyze the effect of IL-4 preincubation on adhesion, mast cells cultured for 2 weeks with SCF were compared with mast cells cultured for the same time with IL-4 in addition to SCF. Mast cell adhesion was again tested in the presence or absence of SCF. When mast cells had been cultured with IL-4, adhesion to all tested ECM proteins was clearly reduced, particularly if SCF was added to the adhesion assay (Figure 3A). The IL-4–dependent reduction of mast cell adhesion occurred in a dose-dependent fashion—the ED_{50} of IL-4 was approximately 500 pg/mL (Figure 3B).

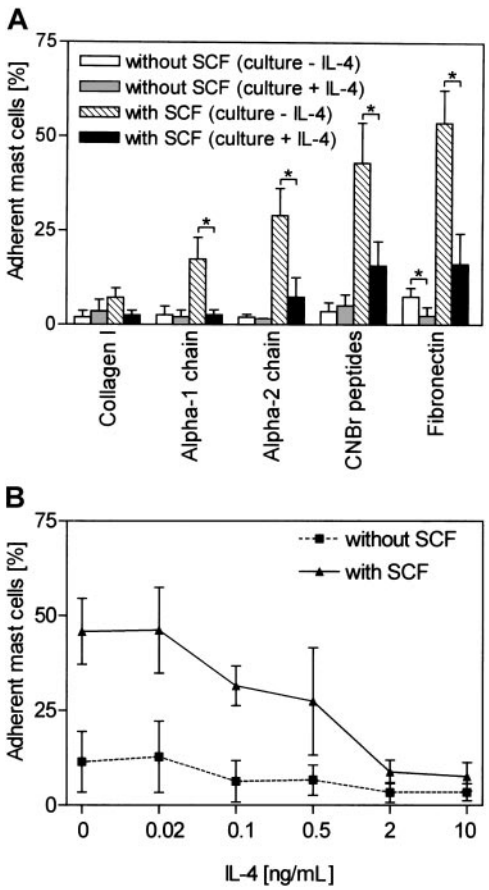

Figure 3. Effect of preincubation with IL-4 on mast cell adhesion. (A) Adhesion of mast cells precultured with SCF alone (as in Figure 1A) compared with mast cells precultured with IL-4 in addition to SCF (indicated in brackets). Adhesion assays were carried out in the presence or absence of 100 ng/mL (54 nM) SCF. Means ± SD are shown (n = 4). *$P < .05$ for significant difference (2-tailed paired *t* test) between mast cells precultured with SCF alone or with SCF and IL-4. (B) Dose dependency of the effect of IL-4. Mast cells were precultured for 14 days with various concentrations of IL-4 (in addition to SCF) indicated on the x-axis. Adhesion assays were carried out in the presence or absence of 100 ng/mL (54 nM) SCF. Results represent the mean ± SD of 3 experiments.

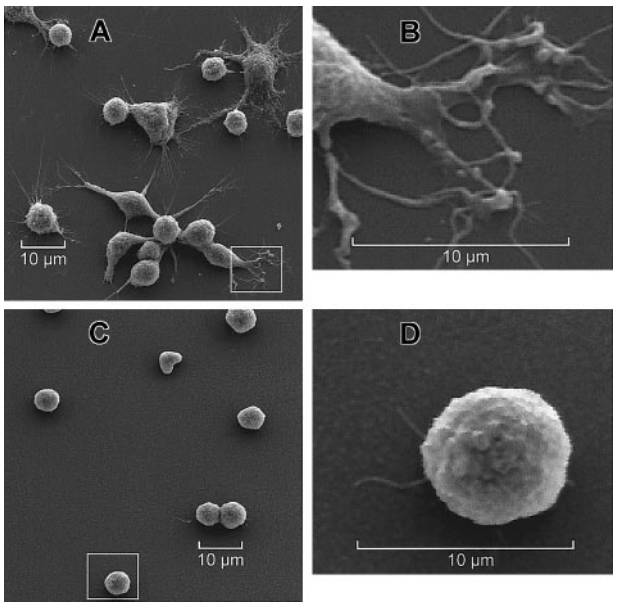

Figure 4. Scanning electron microscopy of human intestinal mast cells adhered to coated coverslips. Although mast cells adhering to fibronectin in the presence of SCF (A,B) show a pronounced spreading and formation of pseudopodia, few such protrusions are found for cells adhering to collagen I (C,D).

Adhesion is not restricted to a mast cell subtype

Mast cell subtypes that bind to fibronectin were studied by immunocytochemistry using antibodies against human tryptase and chymase. In all experiments, the adherent mast cells were always tryptase-positive. We found 54% ± 6% (mean ± SD, n = 3) chymase-positive cells (also called connective tissue-type mast cells) if cells were cultured with SCF alone. Confirming recent data, we found more chymase-negative cells (mucosal-type mast cells) if cells were cultured with SCF and IL-4 (36% ± 6% chymase-positive cells).[5] Analysis of adherent mast cells after 90 minutes revealed that the percentage of chymase-positive cells was not changed after adhesion, suggesting that interaction with ECM proteins is not restricted to a particular mast cell subclass.

Adhesion to fibronectin is accompanied by pronounced cell spreading

The ultrastructural morphology of adherent mast cells was analyzed by scanning electron microscopy. After mast cell adhesion in the presence of SCF, we observed differences in mast cell morphology depending on the kind of matrix protein. Particularly on fibronectin (Figure 4A-B), a pronounced spreading of mast cells was observed. Cell spreading was accompanied by the formation of pseudopodia. In contrast, mast cells adhering to collagen type I showed no or only thin pseudopodia (Figure 4C-D).

Human intestinal mast cells express a number of integrins

Using mast cells directly after their isolation (purity, 95%) or after culture in the presence of SCF for 14 days (purity, 99% or greater), we found messenger RNA (mRNA) expression for the integrin receptor subunits β1, β2, β3, α2, α3, α4, α5, and αV by reverse transcription (RT)–PCR (Figure 5A). These findings were confirmed for cultured mast cells by flow cytometry analysis. Almost

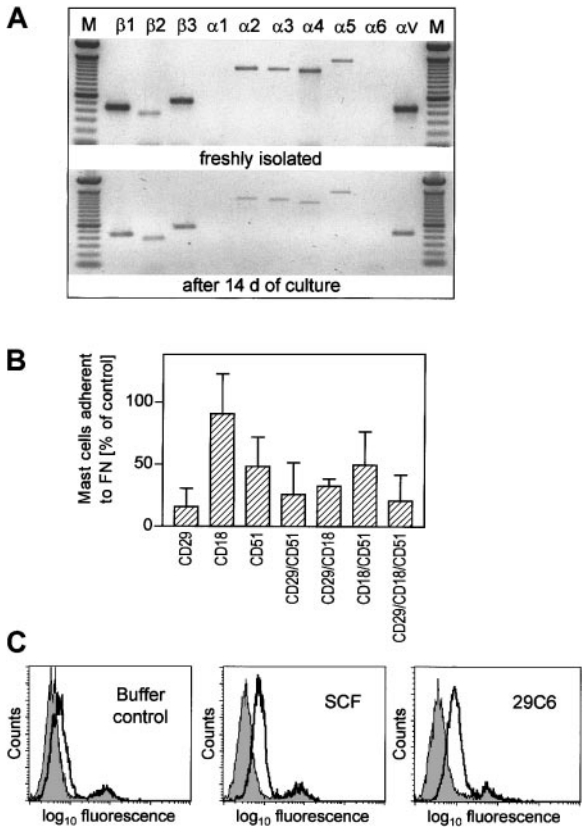

Figure 5. Involvement of integrins in human intestinal mast cell adhesion. (A) Integrin mRNA expression. RT-PCR was performed with RNA derived from mast cells directly after their isolation (purity, 95%) or with RNA from mast cells after culture in the presence of SCF (purity, 99% or greater). DNA amplifications with specific primer pairs were separated on a 1% agarose gel. M indicates 100-bp DNA ladder. (B) Blocking of integrin subunits. Cells were preincubated for 10 minutes at 37°C with an isotype control or blocking mAbs directed against the integrin subunits β1(CD29), β2 (CD18), and αVβ3 (CD51). Adhesion of mast cells to fibronectin (FN) is expressed as percentage of control, whereas adhesion of mast cells preincubated with isotype control was set at 100% (means ± SD, n = 3). (C) Effect of SCF and mAb 29C6 on HUTS-21 β1 epitope expression. Cells were incubated with SCF or 29C6, each at 100 ng/mL (54 nM or 6.5 nM), for 60 minutes or with buffer control before analysis of the expression of the HUTS-21 epitope presented on an activation-dependent confirmation of β1. Isotype control staining is shown in gray. Full lines show antibody staining (right peak). Results of 1 of 3 representative experiments are shown. Mast cell purity was 98% or greater.

all mast cells (98% to 100% positive cells) expressed the β1 integrins α4β1 (VLA-4) and α5β1 (VLA-5). Approximately 84% to 87% of the cells were positive for α2β1 (VLA-2) and α3β1 (VLA-3). The β2 integrins αLβ2 (LFA-1), αXβ2, and αMβ2 (Mac-1) were found on 90%, 55% or 17%, respectively, of the cells. Vitronectin receptor αVβ3 was detected on 80% of the cells. Interestingly, neither challenge of the mast cells with SCF in the adhesion assay nor culture of mast cells with IL-4 affected the expression of integrins on the mRNA or on the protein level (not shown).

Mast cell adhesion is mainly mediated by β1 integrins

To assess the functional relevance of the β integrin subunits detected on human intestinal mast cells, we incubated the cells with blocking mAbs directed against β1, β2, and αVβ3 before examining adhesion to fibronectin. Results shown in Figure 5B demonstrate that pretreatment of cells with anti-β1 (anti-CD29) inhibited mast cell adhesion to fibronectin by 83%, compared to 42% with anti-αVβ3 (anti-CD51). In contrast, pretreatment with anti-β2 (anti-CD18) did not affect mast cell adhesion to fibronectin. The combined addition of the mAbs caused no further inhibition of mast cell adhesion, indicating that mast cell adhesion to fibronectin is mainly mediated by β1 integrins.

Stem cell factor and FcεRI cross-linking changes β1 conformation to a high-affinity state

To evaluate further the mechanism underlying the enhancing effect of mast cell adhesion to ECM proteins, we quantified the expression of the HUTS-21 β1 epitope on mast cells. The HUTS-21 β1 epitope is recognized by a specific antibody after a change of the conformation of the β1 subunit of VLA integrins. Expression of the HUTS-21 β1 epitope correlates with the ligand-binding activity of VLA integrins.[24] We found by flow cytometric analysis a constitutive expression of the HUTS-21 β1 epitope on mast cells at low level. Stimulation of the cells with 100 ng/mL (54 nM) SCF or by FcεRI cross-linking using 100 ng/mL (6.5 nM) mAb 29C6 induced a clear up-regulation of the HUTS-21 β1 epitope, suggesting that SCF and FcεRI cross-linking increases the affinity of β1 integrins to ECM proteins (Figure 5C).

Treatment with apigenin or wortmannin inhibits stem cell factor–induced mast cell adhesion

To look for signaling pathways involved in mast cell adhesion, cultured mast cells isolated from human intestinal tissue were treated with specific inhibitors before stimulation with SCF. Gö6976, a specific inhibitor of protein kinase C, had no effect on mast cell adhesion.[25] In contrast, SCF-induced mast cell adhesion was totally blocked by apigenin, which inhibits mitogen-activated protein kinase (MAPK)–associated signaling pathways, and wortmannin, a specific inhibitor of phosphatidylinositol 3-kinase (PI3K) (Figure 6A).[26,27] Inhibitory effects of apigenin and wortmannin on mast cell adhesion were reflected by their effects on HUTS-21 β1 epitope expression. Apigenin and wortmannin inhibited the up-regulation of HUTS-21 β1 in response to stimulation with SCF, whereas Gö6976 did not (Figure 6B). These findings suggest an involvement of PI3K and MAPK, but not protein kinase C, in SCF-induced mast cell adhesion and an involvement of a change of the conformation of β1.

Discussion

Tissue factors involved in mast cell regulation have been poorly defined. Here, we provide evidence that mature human mast cells isolated from intestinal tissue and cultured in the presence of SCF interact with and are modulated by selected ECM proteins such as fibronectin and denatured collagens. These cells bind spontaneously to fibronectin, as reported earlier for human cutaneous mast cells and for the immature human mast cell line HMC-1.[16,17] The adhesion rate was similar to that described for cutaneous mast cells (approximately 14%), whereas HMC-1 cells showed a spontaneous adherence of up to 80%.[17] We found that the adhesion of intestinal mast cells increased up to 5-fold in response to stimulation with SCF, the ligand of c-*kit*. These data strongly suggest that the high spontaneous adhesion rate of HMC-1 is attributed to the point mutations in the coding sequence of the proto-oncogene c-*kit*, causing ligand-independent activation of c-*kit* in these cells.[28] Murine bone marrow-derived mast cells also adhere to fibronectin if they are challenged with SCF.[13] We observed a similar increase of mast cell adhesion to different types of collagen, strongly

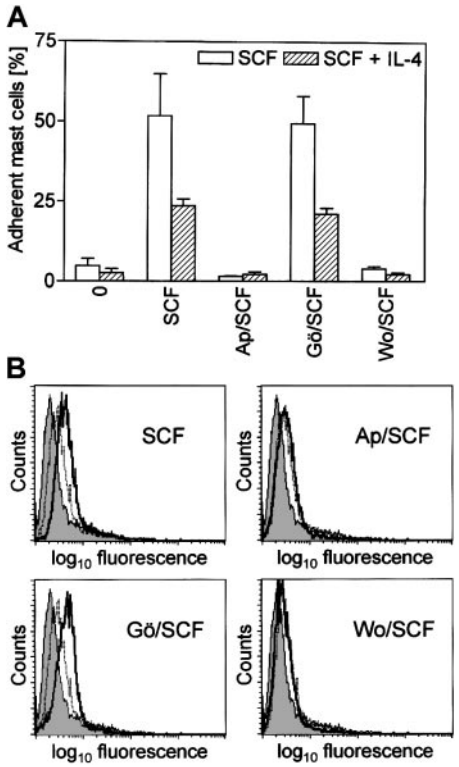

Figure 6. Effect of apigenin and wortmannin on mast cell adhesion and HUTS-21 β1 expression. (A) Cells precultured with SCF alone or with SCF and IL-4 were treated for 60 minutes with 20 μM apigenin (Ap), 2 μM Gö 6976 (Gö), 100 nM wortmannin (Wo), or buffer control. Adhesion rates of mast cells stimulated with 100 ng/mL (54 nM) SCF compared to buffer control (0) were determined on fibronectin (means ± SD, n = 4). (B) Cells precultured with SCF alone were treated for 60 minutes with 20 μM apigenin (Ap), 2 μM Gö6976 (Gö), 100 nM wortmannin (Wo), or buffer control and then were incubated with buffer control or stimulated with 100 ng/mL (54 nM) SCF for 30 minutes before the expression of the HUTS-21 epitope was analyzed. Isotype control staining is shown in gray. Dotted lines show the HUTS-21 β1 staining of unstimulated mast cells, full lines after stimulation with SCF. Results of 1 of 3 representative experiments are shown. Mast cell purity was 98% or greater.

suggesting that SCF has a general capacity for promoting mast cell adhesion to ECM proteins. Mast cell adhesion was also up-regulated after IgE receptor cross-linking and, to a lesser degree, by IL-3, whereas a number of other factors (NGF, IFN-γ, TGF-β, TNF-α, IL-6, IL-8, IL-10, IL-13, C3a, and C5a) failed to alter human mast cell adhesion. Most noteworthy, preincubation of the cells with IL-4 strongly reduced mast cell adhesion to all tested ECM proteins. This inhibitory effect of IL-4 was observed if cells were preincubated with IL-4 for approximately 2 weeks, but IL-4 had no effect if added to the adhesion assay for only 90 minutes. These findings suggest that SCF, IL-3, and FcεRI cross-linking could be involved in the short-term regulation of mast cell adhesion to ECM proteins, whereas IL-4 may enhance mast cell mobility in the long-term by suppressing adhesion. IL-4 is synthesized and released during inflammatory conditions on activation of T-helper lymphocytes and basophils, whereas SCF is thought to be expressed constitutively by tissue cells such as endothelial cells and fibroblasts, similar to ECM proteins.[29-31] Because of a permanent local interaction between mature mast cells and tissue factors such as ECM proteins and SCF, we hypothesized that SCF primarily regulates resident mast cell survival and adhesion to the ECM. In contrast, IL-4 may promote local proliferation and detachment of mast cells from the ECM and thus support their migration. This IL-4 effect could be of particular importance in Th2-driven inflammatory reactions such as allergic disease.

An increasing body of evidence indicates that mast cells are also involved in chronic inflammatory reactions such as Crohn disease and are thought to be Th1-driven and typically associated with tissue fibrosis.[32,33] In previous studies, we also found evidence for increased mast cell activation in patients with inflammatory bowel disease, including Crohn disease, compared to healthy controls.[34-36] Little is known, however, about the regulation of mast cells in Crohn disease and of the role of mast cells in the pathogenesis of intestinal fibrosis. Gelbmann et al[33] reported an accumulation of mast cells in the muscularis propria of strictures in Crohn disease. Interestingly, these mast cells were colocalized with ECM proteins such as laminin, but not with fibronectin or vitronectin, whereas collagens were not examined in this study. We found that SCF, which is known to be produced mainly by activated fibroblasts, strongly enhances mast cell adhesion not only toward fibronectin and laminin but also toward denatured or fragmented ECM proteins, such as α1 and α2 chains and CNBr peptides of collagen I. This SCF-triggered adhesion of mast cells to primary collagen sequences could be of importance for the chemotaxis of mast cells toward sites of ECM destruction. These data further support the hypothesis that mast cells are involved in the pathogenesis of chronic inflammatory conditions and of repair processes.[37]

Cell adhesion to ECM proteins is predominantly mediated by integrins.[11] Previous studies showed that human mast cells from uterus, lung, and skin express the β1 integrins α4β1 and α5β1.[14-16] In addition, human skin mast cells express α3β1.[16] We confirmed these findings for human intestinal mast cells. In addition, we found that 87% of human intestinal mast cells express α2β1, an integrin not detected on skin, lung, and uterine mast cells. These data clearly show that the expression pattern of β1 integrins and the percentages of integrin-expressing cells vary depending on the mast cell origin within the human body. For example, Columbo et al[16] reported that only 8.0% and 6.9% of human skin mast cells express the β1-integrins α4β1 and α5β1, respectively. In contrast, almost all mast cells obtained from uterine tissue or intestinal mucosa were positive for these integrins.[14] Moreover, our inhibition studies clearly showed that mast cell adhesion to fibronectin is mediated mainly through β1 integrins. Therefore, the high expression of α4β1 and α5β1, known to bind to fibronectin, could explain our finding that up to 70% of human intestinal mast cells bind to fibronectin after stimulation with SCF.[11] The SCF-mediated increase of mast cell adhesion to ECM proteins is not related to an up-regulation of ECM receptors because we did not detect any change of integrin expression after stimulation with SCF, confirming earlier findings in murine mast cells.[38] By contrast, SCF stimulation immediately induced the up-regulation of the activated β1 epitope HUTS-21 on mast cells.[24] HUTS-21 expression was also induced by FcεRI cross-linking. These findings strongly suggest that SCF and FcεRI cross-linking promote a change in the conformation of β1 to a high-affinity state. Thus, increased mast cell adhesion in response to SCF stimulation or FcεRI cross-linking could be explained by an increase in integrin affinity. Surprisingly, we failed to detect significant differences in integrin expression or HUTS-21 β1 epitope expression between mast cells cultured with SCF alone and mast cells cultured with SCF and IL-4 (data not shown), though the latter decreased matrix adhesion. The mechanisms causing reduced mast cell adhesion in response to long-term IL-4 challenge remains unclear but may be related to integrin conformation changes that cannot be assessed by measurement of HUTS-21 β1 epitope expression.

The inhibition of SCF-induced mast cell adhesion by wortmannin and apigenin suggest an involvement of PI3K and MAPK.

PI3K is though to be involved in the adhesion of bone marrow-derived mouse mast cells. Kinashi et al[39,40] reported a role for PI3K in receptor tyrosine kinase-stimulated adhesion and in affinity modulation of VLA-5. In contrast, involvement of the MAPK pathway in the induction of mast cell adhesion is a novel observation. MAPK is found to be involved in the suppression of integrin activation in Chinese hamster ovary cells, but the elements of this pathway are not yet identified.[41] Of note, the inhibition of PI3K and MAPK pathways resulted in the inhibition of the SCF-induced HUTS-21 β1 epitope expression. These findings suggest an involvement of PI3K and MAPK in SCF-induced mast cell adhesion and an involvement of a change of the conformation of β1 in the regulation of mast cell adhesion.

In conclusion, our data demonstrate that SCF and IL-4 regulate the adhesion of cultured human intestinal mast cells to ECM proteins. These findings may be of particular relevance for the regulation of mast cell migration, proliferation, and effector functions under normal conditions and in the course of inflammatory reactions.

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References

1. Galli SJ, Wershil BK. The two faces of the mast cell. Nature. 1996;381:21-22.
2. Metcalfe DD, Baram D, Mekori YA. Mast cells. Physiol Rev. 1997;77:1033-1079.
3. Galli SJ. New concepts about the mast cell. N Engl J Med. 1993;328:257-265.
4. Bischoff SC, Dahinden CA. C-kit ligand: a unique potentiator of mediator release by human mast cells. J Exp Med. 1992;175:237-244.
5. Bischoff SC, Sellge G, Lorentz A, Sebald W, Raab R, Manns MP. IL-4 enhances proliferation and mediator release in mature human mast cells. Proc Natl Acad Sci U S A. 1999;96:8080-8085.
6. Lorentz A, Schwengberg S, Sellge G, Manns MP, Bischoff SC. Human intestinal mast cells are capable of producing different cytokine profiles: role of IgE-receptor cross-linking and IL-4. J Immunol. 2000;164:43-48.
7. Toru H, Eguchi M, Matsumoto R, Yanagida M, Yata J, Nakahata T. Interleukin-4 promotes the development of tryptase and chymase double-positive human mast cells accompanied by cell maturation. Blood. 1998;91:187-195.
8. Welker P, Grabbe J, Grützkau A, Henz BM. Effects of nerve growth factor (NGF) and other fibroblast-derived growth factors on immature human mast cells (HMC-1). Immunology. 1998;94:310-317.
9. Welker P, Grabbe J, Gibbs B, Zuberbier T, Henz BM. Nerve growth factor-beta induces mast-cell marker expression during in vitro culture of human umbilical cord blood cells. Immunology. 2000;99:418-426.
10. Hamawy MM, Mergenhagen SE, Siraganian RP. Adhesion molecules as regulators of mast cell and basophil function. Immunol Today. 1994;15:62-66.
11. Hynes RO. Integrins: versatility, modulation, and signaling in cell adhesion. Cell. 1992;69:11-25.
12. Thompson HL, Thomas L, Metcalfe DD. Murine mast cells attach to and migrate on laminin-, fibronectin-, and matrigel-coated surfaces in response to $Fc_{\epsilon}RI$-mediated signals. Clin Exp Allergy. 1993;23:270-275.
13. Dastych J, Metcalfe DD. Stem cell factor induces mast cell adhesion to fibronectin. J Immunol. 1994;152:213-219.
14. Guo CB, Kagey-Sobotka A, Lichtenstein LM, Bochner BS. Immunophenotyping and functional analysis of purified human uterine mast cells. Blood. 1992;79:708-709.
15. Sperr WR, Ahis H, Czerwenka K, et al. Differential expression of cell surface integrins on human mast cells and human basophils. Ann Hematol. 1992;65:10-16.
16. Columbo M, Bruce SB, Marone G. Human skin mast cells express functional β_1 integrins that mediate adhesion to extracellular matrix proteins. J Immunol. 1995;154:6058-6064.
17. Krüger-Kasagakes S, Grützkau A, Baghramian R, Henz BM. Interactions of immature human mast cells with extracellular matrix: expression of specific adhesion receptors and their role in cell binding to matrix proteins. J Invest Dermatol. 1996;106:538-543.
18. Krüger-Krasagakes S, Grützkau A, Krasagakis K, Hoffmann S, Henz BM. Adhesion of human mast cells to extracellular matrix provides a co-stimulatory signal for cytokine production. Immunology. 1999;98:253-257.
19. Bischoff SC, Schwengberg S, Raab R, Manns MP. Functional properties of human intestinal mast cells cultured in a new culture system: enhancement of IgE receptor-dependent mediator release and response to stem cell factor. J Immunol. 1997;159:5560-5567.
20. Mierke CT, Ballmeier M, Werner U, Manns MP, Welte K, Bischoff SC. Human endothelial cells regulate survival and proliferation of human mast cells. J Exp Med. 2000;192:801-812.
21. Schuppan D, Cantaluppi MC, Becker J, et al. Undulin, an extracellular matrix glycoprotein associated with collagen fibrils. J Biol Chem. 1990;265:8823-8832.
22. Walzog B, Schuppan D, Heimpel C, Hafezi-Moghadam A, Gaehtgens P, Ley K. The leukocyte integrin Mac-1 (CD11b/CD18) contributes to binding of human granulocytes to collagen. Exp Cell Res. 1995;218:28-38.
23. Somasundaram R, Schuppan D. Type I, II, III, IV, V, and VI collagens serve as extracellular ligands for the isoforms of platelet-derived growth factor (AA, BB, and AB). J Biol Chem. 1996;271:26884-26891.
24. Luque A, Gómez M, Puzon W, Takada Y, Sánchez-Madrid F, Cabana C. Activated conformations of very late activation integrins detected by a group of antibodies (HUTS) specific for a novel regulatory region (355-425) of the common β1 chain. J Biol Chem. 1996;271:11067-11075.
25. Qatsha KA, Rudolph C, Marmé D, Schächtele C, May WS. Gö 6976, a selective inhibitor of protein kinase C, is a potent antagonist of human immunodeficiency virus 1 induction from latent/low-level-producing reservoir cells in vitro. Proc Natl Acad Sci U S A. 1993;90:4674-4678.
26. Kuo mL, Yang NC. Reversion of v-H-ras transformed NIH 3T3 cells by apigenin through inhibiting mitogen activated protein kinase and its downstream oncogenes. Biochem Biophys Res Commun. 1995;212:767-775.
27. Yano H, Nakanishi S, Kimura K, et al. Inhibition of histamine secretion by wortmannin through the blockade of phosphatidylinositol 3-kinase in RBL-2H3 cells. J Biol Chem. 1993;268:25846-25856.
28. Furitsu T, Tsujimura T, Tono T, et al. Identification of mutations in the coding sequence of the proto-oncogene c-kit in a human mast cell leukemia cell line causing ligand-independent activation of c-kit product. J Clin Invest. 1993;92:1736-1744.
29. Heinrich MC, Dooley DC, Freed AC, et al. Constitutive expression of steel factor gene by human stromal cells. Blood. 1993;82:771-783.
30. Romagnani S. The role of lymphocytes in allergic disease. J Allergy Clin Immunol. 2000;105:399-408.
31. Brunner T, Heusser CH, Dahinden CA. Human peripheral blood basophils primed by IL-3 produce IL-4 in response to immunoglobulin E receptor stimulation. J Exp Med. 1993;177:605-611.
32. Dvorak AM, Monahan RA, Osage JE, Dickersin GR. Mast cell degranulation in Crohn's disease. Lancet. 1978;1:498.
33. Gelbmann CM, Mestermann S, Gross V, Kollinger M, Scholmerich J, Falk W. Strictures in Crohn's disease are characterized by an accumulation of mast cells colocalized with laminin but not with fibronectin or vitronectin. Gut. 1999;45:210-217.
34. Bischoff SC, Wedemeyer J, Herrmann A, et al. Quantitative assessment of intestinal eosinophils and mast cells in inflammatory bowel disease. Histopathology. 1996;28:1-13.
35. Bischoff SC, Schwengberg S, Wordelmann K, Weimann A, Raab R, Manns MP. Effect of *c-kit* ligand, stem cell factor, on mediator release by human intestinal mast cells isolated from patients with inflammatory bowel disease and controls. Gut. 1996;28:104-114.
36. Lorentz A, Schwengberg S, Mierke C, Manns MP, Bischoff SC. Human intestinal mast cells produce IL-5 in vitro upon IgE receptor cross-linking and in vivo in the course of intestinal inflammatory disease. Eur J Immunol. 1999;29:1496-1503.
37. Levi-Schaffer F. Mast cell/fibroblast interactions in health and disease. Chem Immunol. 1995;61:161-185.
38. Kinashi T, Springer TA. Steel factor and c-kit regulate cell-matrix adhesion. Blood. 1994;83:1033-1038.
39. Kinashi T, Escobedo JA, Williams LT, Takatsu K, Springer TA. Receptor tyrosine kinase stimulates cell-matrix adhesion by phosphatidylinositol 3 kinase and phospholipase C-γ1 pathways. Blood. 1995;86:2086-2090.
40. Kinashi T, Asaoka T, Setoguchi R, Takatsu K. Affinity modulation of very late antigen-5 through phosphatidylinositol 3-kinase in mast cells. J Immunol. 1999;162:2850-2857.
41. Hughes PE, Renshaw MW, Pfaff M, et al. Suppression of integrin activation: a novel function of a ras/raf-initiated MAP kinase pathway. Cell. 1997;88:521-530.