ORIGINAL ARTICLE

Activation of Basophils by Stem Cell Factor: Comparison With Insulin-Like Growth Factor-I

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■ **Abstract**

Background: Basophils are an active participant in the pathogenesis of local inflammation in allergic diseases such as asthma, but it is not fully known how basophil activation is regulated in inflamed tissue.
Objective: In order to clarify the control mechanisms of basophil activation in chronic inflammation and at remodeling sites, we analyzed the effects of fibroblast-derived cytokines, stem cell factor (SCF), and insulinlike growth factor-I (IGF-I) on basophils.
Methods: The effects of SCF and IGF-I on degranulation and surface activation marker expression by basophils were assessed and compared.
Results: SCF enhanced human basophil histamine release elicited by some, but not all, secretagogues; degranulation in response to IgE- or FcεRI-mediated stimulation and 12-*o*-tetradecanoyl-phorbol-13-acetate (TPA) was enhanced by SCF. SCF slightly enhanced ionophore A23187-induced histamine release by basophils from some donors, but it failed to affect the release elicited by monocyte chemoattractant protein-1 (MCP-1), formylmethionyl-leucyl-phenylalanine (FMLP) or C5a. The repertoire of secretagogues responsive to SCF was similar to that of IGF-I. Expression levels of both CD11b and CD69 markers were significantly enhanced by the combination of SCF and IGF-I.
Conclusions: These results suggest that SCF and IGF-I may modify the activation of basophils in a similar and/or synergistic fashion. Interaction of basophils with these cytokines might be involved in the pathogenesis of local inflammation and the remodeling process in asthma.

Key words: Basophils. Insulin-like growth factor. Stem cell factor.

■ **Resumen**

Antecedentes: Los basófilos son un participante activo en la patogenia de la inflamación local en enfermedades alérgicas como el asma, pero no se sabe a ciencia cierta como se regula la activación de los basófilos en tejidos inflamados.
Objetivos: El objetivo fue analizar los efectos de las citocinas derivadas de los fibroblastos, el factor de células madre (FCM) y la somatomedina-I (Sm-I) en los basófilos, con el fin de esclarecer los mecanismos de control de la activación de basófilos en los casos de inflamación crónica y en los sitios donde se desarrollan procesos de remodelación.
Métodos: Se examinaron y compararon los efectos del FCM y de la Sm-I en la degranulación y en la expresión en superficie de los marcadores de activación de los basófilos.
Resultados: El FCM aumentó la liberación de histamina de los basófilos humanos aumentada por el FCM obtenida mediante algunos, pero no todos los secretagogos; la degranulación en respuesta a la IgE o la estimulación mediada por FcεRI y 12-o-tetradecanoil-forbol-13-acetato (TFA). El FCM aumentó levemente la liberación de histamina de los basófilos inducida por el ionóforo A23187 a partir de algunos donantes, pero no afectó ni a la liberación obtenida mediante la proteína 1 quimiotaxina de monocitos (PQM-1), ni al formil-metionil-leucil-fenilalanina (FMLF) o al C5a. El repertorio de secretagogos causantes del FCM fue similar al de la Sm-I. Los niveles de expresión tanto de CD11b como de CD69 se vieron aumentados por la combinación de FCM y de Sm-I.
Conclusiones: Estos resultados sugieren que el FCM y la Sm-I pueden modificar la activación de los basófilos de un modo similar y/o sinérgico. La interacción de los basófilos con estas citocinas puede estar implicada en la patogenia de la inflamación local y los procesos de remodelación en el asma.

Palabras clave: Basófilos. Somatomedina-I. Factor de las células madre.

Introduction

Basophils are the least common component of peripheral blood leukocytes, comprising only 0.5% to 1% of total blood cells. However, basophils contain abundant biogenic amines such as histamine in their cytoplasmic granules. When basophils are exposed to an antigen, immunoglobulin (Ig) E on their surface becomes cross-linked, leading to rapid release of their granule contents and newly synthesized lipid mediators. Thus, basophils are recognized as active participants in the pathogenesis of IgE-mediated immediate hypersensitivity reactions [1].

Accumulation of basophils at inflamed tissue sites is a hallmark of antigen-induced late-phase inflammatory reactions and chronic allergic diseases such as asthma. Recent reports, based on the findings of functional and activation marker studies, have indicated that locally migrated basophils are in an activated state [2,3]. In addition, analyses of local mediator profiles have shown that mediators secreted locally during late-phase reactions in the airways may derive from basophils rather than mast cells. A recent study using IgE-transgenic mice has further demonstrated that tissue basophils can be a key conductor cell type during very-late-phase, antigen-induced inflammation, although they constitute a minor proportion of the infiltrating leukocytes [4]. These results suggest that the regulatory mechanisms controlling activation of locally infiltrated basophils may be particularly important in helping us to understand the precise pathogenesis of allergic diseases.

Several types of bioactive molecules may be involved in the regulatory process of local basophil activation, and cytokines are probably one of the most important. Accumulated evidence indicates that, among such proteins, hematopoietic growth factors most potently affect mature granulocytes, including basophils, inducing alteration of several of their functions and biological indices [5-8]. However, the actions of other cytokines, especially those primarily known to affect tissue-resident cells, and the cooperative effects of these cytokines are much less understood. In this study, we analyzed the effects of stem cell factor (SCF) on human basophils and found that SCF and another fibroblast-derived cytokine, insulinlike growth factor-I (IGF-I), showed similar and overlapping actions on basophil functions.

Materials and Methods

Reagents

The following reagents were purchased as indicated: dextran T500 and Percoll (Pharmacia Fine Chemicals, Uppsala, Sweden), phosphate-buffered saline (PBS), fetal calf serum (FCS), and RPMI 1640 (Gibco, Grand Island, New York, USA), piperazine-N,N'-bis-2-ethanesulfonic acid (PIPES), 12-o-tetradecanoyl-phorbol-13-acetate (TPA) and formylmethionyl-leucyl-phenylalanine (FMLP) (Sigma Chemical Co., St Louis, Missouri, USA), human recombinant SCF, human monocyte chemoattractant protein-1 (MCP-1), human recombinant interleukin-3 (IL-3) and IGF-I (PeproTech, London, UK), and ionophore A23187 (Calbiochem-Behring,

La Jolla, California, USA). The antibodies used in this study were purchased as follows: fluorescein isothiocyanate (FITC)-conjugated anti-human CD69 mAb (IgG1, clone FN50, eBioscience, San Diego, California, USA), FITC-conjugated mouse IgG1, PE-conjugated anti-human CD11b mAb (IgG1, clone Bear1), phycoerythrin (PE)-conjugated mouse IgG1 (Coulter Immunotech, Marseille, France), FITC-conjugated goat anti-human IgE (Biosource, Camarillo, California, USA), and goat anti-human IgE Ab (affinity purified, stock concentration 14 mg/mL) (Medical & Biological Laboratories, Nagoya, Japan). A mouse IgG2b anti-human FcεRI α-chain mAb, CRA-1, was also used; this antibody can bind to the FcεRI α-chain regardless of whether or not it is occupied by IgE [9].

Cell Preparation

Throughout this study, each experiment was performed at least in duplicate using basophils obtained from 1 donor. Experiments using basophils from different donors were performed separately, and not simultaneously. Venous blood was drawn from healthy volunteers with no history of atopic disease. For the histamine release studies, basophil preparations were prepared by the dextran sedimentation procedure as previously described [10]. For CD11b surface expression analysis, basophils were semi-purified by density centrifugation using Percoll solutions of different densities (1.080 and 1.070 g/mL). To obtain basophils with high purity for CD69 analysis, Percoll-separated basophils were further purified by negative selection with MACS beads (Basophil Isolation Kit; Miltenyi BioTech, Belgisch-Gladbach, Germany) according to the manufacturer's instructions [2]. After the MACS procedures, the purity of the basophils was more than 95%.

Histamine Release

Basophils prepared by dextran sedimentation were resuspended in PIPES buffer containing 2 mM Ca^{2+}, 0.5 mM Mg^{2+}, and 0.03% human serum albumin (PIPES-ACM) before being preincubated at 37°C for 30 minutes in polystyrene tubes with or without SCF or other cytokines. Mediator release was initiated by the addition of secretagogues. After additional incubation for 45 minutes at 37°C, the supernatants were collected. The released histamine was measured by an automated fluorometric technique [10]. Experiments were performed in duplicate or triplicate. Histamine release was expressed as a percentage of total cellular histamine after subtracting the spontaneous release (usually <7%).

Analysis of Surface Marker Expression

CD69 expression analyses were performed using highly purified basophils as previously described [2,11]. Briefly, basophils (purity >95%) were cultured in RPMI 1640 supplemented with 10% FCS and antibiotics (100 U/mL of penicillin and 100 μg/mL of streptomycin) at 37°C in 5% CO_2 in flat-bottomed, 96-well culture plates (Iwaki, Chiba, Japan). Surface CD69 expression was analyzed using basophils cultured for 24 hours, since induction of CD69 is known to be a slow process [2]. Most basophils (> 85%) were alive and

nonapoptotic after 24 hours of culture. SCF or other cytokines were added throughout the culture period. The cells were then incubated with human IgG (5 mg/mL) for 10 minutes at room temperature and stained with FITC-conjugated anti-human CD69 mAb at 10 µg/mL for 30 minutes. Isotype-matched FITC-conjugated mouse IgG1 was used as a negative control.

CD11b expression on basophils was assessed using Percoll-purified basophils (purity approximately 15%) [6,11]. Following stimulation with SCF and/or other cytokines in PIPES-ACM for 30 minutes at 37°C, cells were preincubated with human IgG (5 mg/mL) for 10 minutes at room temperature to block nonspecific staining. The cells were then stained with PE-conjugated anti-human CD11b mAb at 5 µg/mL and FITC-conjugated goat polyclonal anti-human IgE Ab at 10 µg/mL for 30 minutes. Isotype-matched PE-conjugated mouse IgG1 was used as a negative control instead of PE–antihuman-CD11b mAb.

Flow cytometric analysis for CD69 or CD11b on the surface of basophils were performed using EPICS XL System II (Coulter, Miami, Florida, USA). The median values of fluorescence intensity were converted to molecules of equivalent soluble fluorochrome (MESF) units, as described previously [2]. Semi-quantified levels of surface molecule expression on basophils were calculated using the following formula: $\Delta MESF = $ (MESF of cells stained with anti-CD69 or anti-CD11b mAb) – (MESF of cells stained with control IgG).

Statistics

All data are expressed as the mean (SEM). Statistical analysis was performed as described previously [8,9]. In brief, the differences between values were analyzed by means of parametric ANOVA (Statcel version 2, OMS-publishing, Saitama, Japan) based on the presumed normal distribution of each group assessed. When the ANOVA indicated a significant difference, the Bonferroni/Dunn post-hoc test was used to compare individual groups.

Results

Analysis of Basophil Degranulation: Effect of SCF and Comparison With IGF-I

Degranulation of human basophils was assessed based on the percentage of histamine release. Direct histamine release was not induced when basophils were stimulated with SCF alone at up to 10 nM. However, release of histamine was boosted when basophils were preincubated with SCF before stimulation with a secretagogue such as anti-IgE antibody, confirming the reports by Columbo et al [12]. As shown in Figure 1, basophils pretreated with SCF at 10 nM for 30 minutes released a higher percentage of histamine when stimulated with each of anti-IgE antibody, anti-FcεRI α-chain CRA-1 mAb, and TPA. SCF at 10 nM also boosted basophil histamine release induced by optimal concentration of anti-IgE
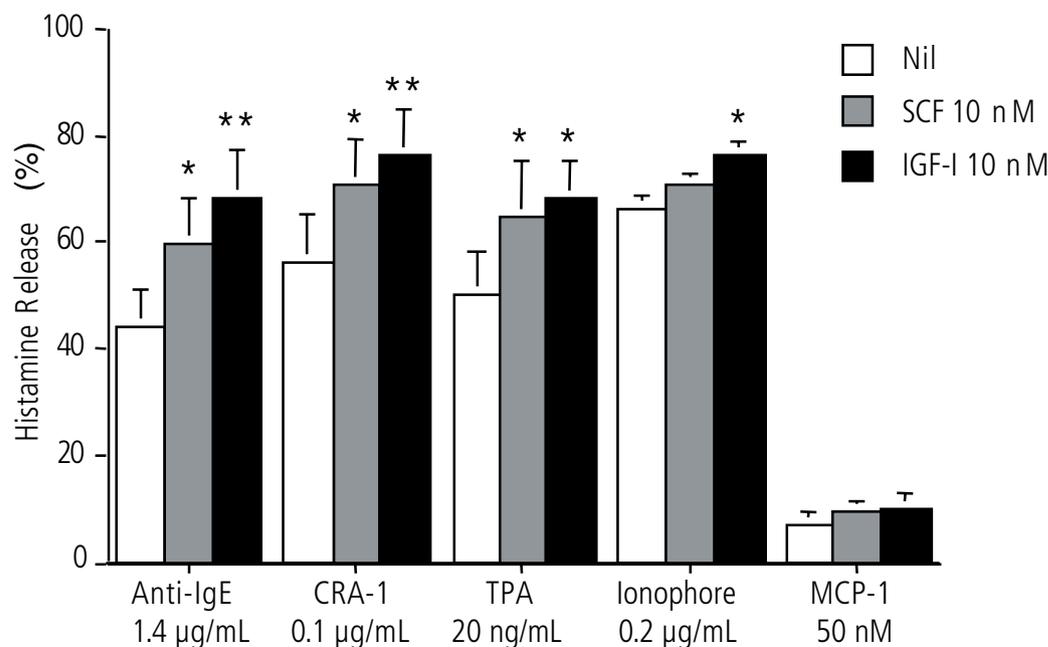

Figure 1. Effects of SCF and IGF-I on basophil histamine release. Basophil preparations were preincubated with or without SCF or IGF-I at 10 nM for 30 minutes at 37°C and then mixed with various secretagogues. Following incubation at 37°C for 45 minutes, the supernatant of each sample was collected for later histamine assay. Data are shown as the mean (SEM) obtained from 5 separate experiments using basophils from 5 different donors. * $P < .05$, ** $P < .01$, versus corresponding values of cells without SCF or IGF-I pretreatment (Nil).
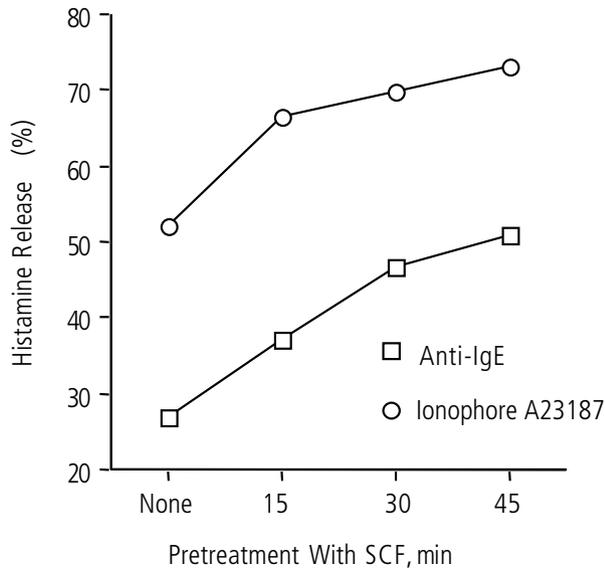
Figure 2. Time-dependent priming effect of SCF on basophil histamine release. Basophils were preincubated with SCF at 3 nM for the indicated time periods, and then an equal volume of anti-IgE Ab (final concentration: 1.4 µg/mL) or Ca ionophore A23187 (final concentration: 0.2 µg/mL) was added. Data shown are representative of 2 separate experiments using basophils from different donors; another experiment generated similar results.
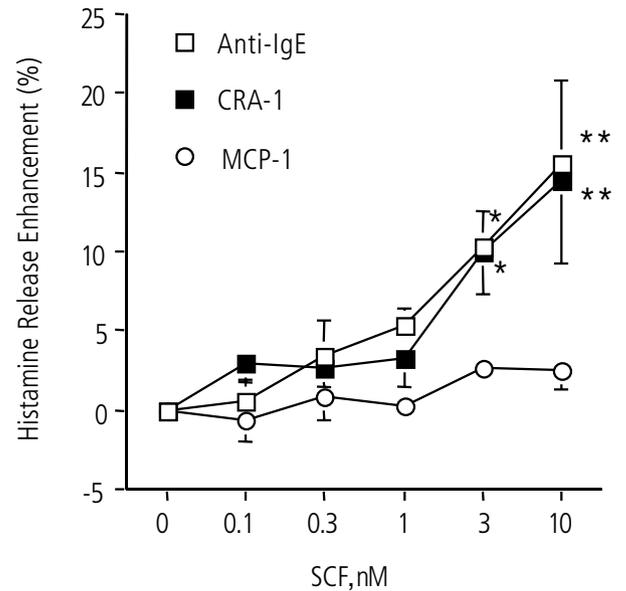

Figure 3. Concentration-dependent effect of SCF on basophil histamine release. Basophils were pretreated with various doses of SCF for 30 minutes at 37°C and then stimulated with anti-IgE (1.4 µg/mL), CRA-1 mAb (0.1 µg/mL), or MCP-1 (50 nM). Data shown are the percentage of histamine release in excess of the corresponding release from non-pretreated cells (44.1% for anti-IgE-stimulated cells, 56.3% for CRA-1 mAb, and 6.90% for MCP-1). Bars represent the SEM (n = 5). * $P < .05$, ** $P < .01$, versus the percent release from non-pretreated cells.

antibody (14 µg/mL). The same pretreatment with SCF slightly enhanced histamine release by basophils from some donors when they were stimulated with Ca ionophore A23187, but the enhancement did not reach statistical significance.

As reported previously, IGF-I is known to enhance histamine release from basophils stimulated with anti-IgE and also with other secretagogues [10]. In the present study, IGF-I actually upregulated the percentages of release induced by anti-IgE, CRA-1 mAb, TPA, and Ca ionophore A23187 with statistical significance. The extent of histamine release enhancement by IGF-I was comparable to or even greater than the enhancement by SCF. However, pretreatment of basophils with SCF or IGF-I failed to show obvious enhancement of basophil histamine release induced by a chemokine, MCP-1 [13] (Figure 1), or other secretagogues such as FMLP or C5a (data not shown). Figure 2 shows the representative time-course results; basophils pretreated with SCF at 3 nM for 15 to 45 minutes showed time-dependent enhancement of histamine release evoked by anti-IgE, and for basophils that showed enhancement of ionophore-induced histamine release by SCF, a similar time course was observed for histamine release by Ca ionophore A23187. Therefore, we chose a 30-minute preincubation time for SCF, and this was also adopted for pretreatment with IGF-I in the rest of our studies [10].

Next, we investigated whether the enhancement of basophil histamine release by SCF was concentration-dependent (Figure 3). SCF at 1 nM or lower did not show obvious enhancement of the release of histamine. However, SCF at 3 nM or 10 nM significantly boosted anti-IgE- and CRA-1 mAb-induced histamine release from basophils. These results suggest that relatively high concentrations of SCF and IGF-I are necessary to induce basophil activation; the findings are in clear contrast to those for the most potent basophil-active cytokine, IL-3, which is effective even at picomolar concentrations. On the other hand, SCF failed to affect MCP-1–induced histamine release at any of the tested concentrations.

Assessment of Surface Marker Expression on Basophils

Based on these results, we thought SCF might be similar to IGF-I in the way it modifies basophil activation. Therefore, we analyzed the effects of SCF and IGF-I on the expression levels of activation-related surface molecules on basophils. SCF at 1 nM or 10 nM slightly enhanced the CD11b level on basophils, but the enhancement did not reach statistical significance (Figure 4A), which is consistent with a previous report [14]. The CD11b-enhancing effect of IGF-I at 10 nM was minimal and insignificant. However, basophils treated with 10 nM of both SCF and IGF-I demonstrated significantly higher levels of CD11b induction on their surface. As basophil
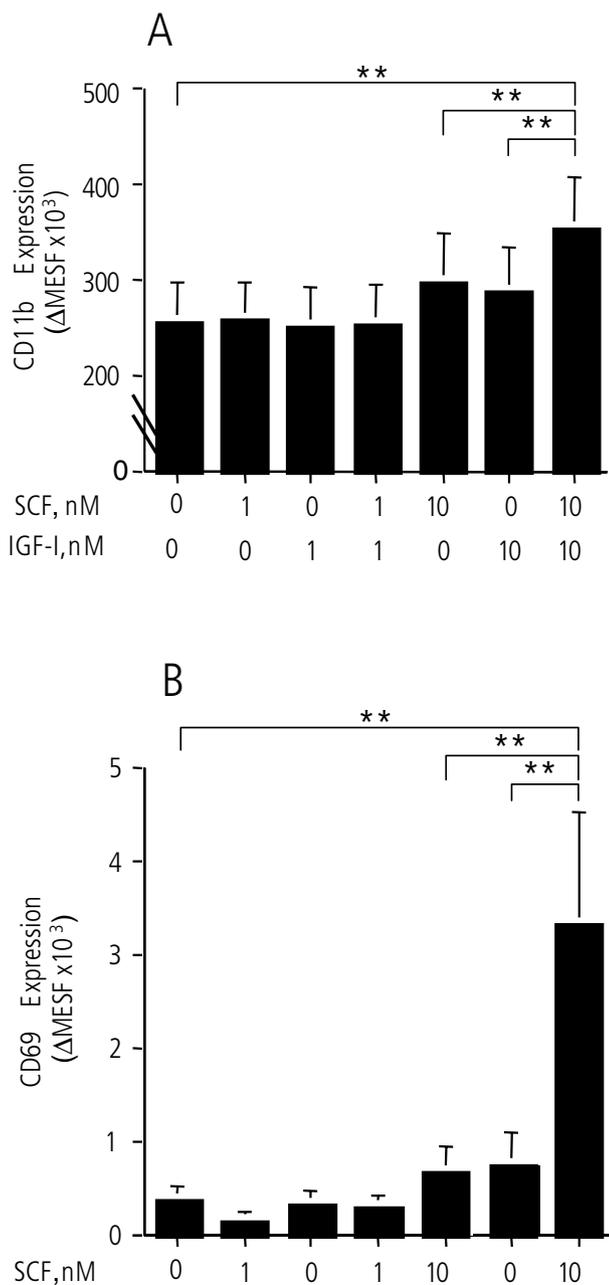
Figure 4. Effects of SCF and IGF-I on A) CD11b and B) CD69 expression on the surface of basophils. A) Percoll-separated basophils were incubated with or without SCF and/or IGF-I for 30 minutes at 37°C, and then surface CD11b expression was measured. The expression level of surface molecules was converted to molecules of equivalent soluble fluorochrome (MESF) units. Bars represent the SEM (n = 6). B) Highly purified basophils were incubated for 24 hours with SCF and/or IGF-I, and CD69 expression on the cells was assessed. Bars represent the SEM (n = 5). ** *P* < .01.

CD11b expression is known to be enhanced by IL-3 [11], we further tested whether SCF affected IL-3–induced CD11b expression on basophils. However, the effect of SCF was highly variable and inconsistent among donors: SCF enhanced IL-3–induced CD11b expression on basophils from some donors, but the opposite effect was observed on cells from others (data not shown). We then assessed the effects of SCF and IGF-I on CD69 expression on basophils. The combination of SCF and IGF-I at 10 nM each obviously induced CD69 expression on highly purified basophils, whereas each of these cytokines alone showed no effect (Figure 4B). Basophil CD69 expression is known to be strongly induced by IL-3, but SCF at 10 nM showed no effect on induction by IL-3 at various concentrations from 1 pM to 300 pM (data not shown). These results indicate that SCF modulates basophil surface activation marker expression in cooperation with IGF-I, but not with IL-3.

Discussion

In this study, we assessed the effects of SCF on basophils and compared the findings with the effects of IGF-I, another fibroblast-derived cytokine. Previous reports have demonstrated that basophil degranulation by anti-IgE antibody can be enhanced by short (10 minutes) preincubation of basophils with SCF [12], although some authors failed to show the enhancement [15]. In the present study, SCF enhanced degranulation of basophils elicited by TPA- and IgE-mediated stimulation, and slightly enhanced degranulation induced by Ca ionophore A23187. However, SCF failed to affect histamine release from basophils exposed to MCP-1, C5a, or FMLP. Importantly, the SCF-responsive and -unresponsive secretagogue repertoires were similar to those of IGF-I, that is, IGF-I also failed to affect basophil degranulation induced by C5a, FMLP, and MCP-1 [10, present study]. These results indicate that SCF and IL-3 show different profiles of secretagogues related to cytokine-induced enhancement: IL-3 augments basophil degranulation induced by all types of secretagogues [7].

Surface-expressed molecules such as CD11b and CD69 are recognized as activation markers of basophils; these markers can be strongly upregulated by IL-3, a well-known basophil-active cytokine. In our study, SCF by itself failed to induce CD11b or CD69 expression in basophils; lack of SCF-induced CD11b expression was consistent with the findings of Heinemann et al [14]. IGF-I also failed to significantly upregulate the surface levels of CD11b or CD69 on basophils. Although basophil CD11b expression is reported to be enhanced by IGF-I [16], we felt that the levels of enhancement were minimal and variable among donors in our experiments. However, SCF and IGF-I cooperatively enhanced the expression of each of CD11b and CD69 on the surface of basophils. From these surface-marker studies, SCF does not seem to act on basophils cooperatively with IL-3, since SCF failed to show consistent augmentation of IL-3-induced expression of CD11b and CD69 on basophils.

Basophils constitute only a small percentage of locally migrated inflammatory cells during late-phase allergic

reactions and chronic allergic diseases, but this cell type may be an important source of mediators such as histamine [17-19]. In healthy conditions, basophils reside in peripheral blood. In allergic states, basophils extravasate from the vasculature, penetrate the basement membrane, and migrate into the tissues. The microenvironment of allergic tissue can include numerous types of mediators and cytokines, and these factors might affect the exacerbation of local inflammation and/or the remodeling process. Thus, it is important to understand how such microenvironmental factors regulate the actions of basophils.

SCF was first cloned more than 15 years ago [20]. Prior to that, loss of a putative c-Kit receptor or its ligand (later named SCF) had been thought to account for the mast cell-deficient phenotype of W/W^v and Sl/Sl^d mice. Today, it is well known that SCF is a key cytokine, acting not only on stem cells and melanocytes, but also on mast cells, all of which express c-Kit, a receptor for SCF. SCF is well known to regulate the proliferation and maturation of immature rodent mast cells. This is the most important cytokine for the induction of human mast cells, and it is capable of critically affecting their development, maturation, chemotaxis, activation, and apoptosis inhibition [20-23].

Cumulative evidence has shown that SCF may be involved in the pathogenesis of allergic diseases. Analyses of bronchoalveolar lavage fluid obtained from pollen-sensitive subjects and nasal lavage fluid from allergic rhinitis patients have demonstrated mast cell chemotactic activity to which SCF may contribute significantly [24,25]. In addition, it was recently reported that neutralization of SCF decreased peribronchial fibrotic remodeling in an antigen-induced chronic asthma model in mice [26]. Presumably, SCF secreted from fibroblasts may act on cells with c-Kit expression, such as mast cells, and, in turn, mediators released from activated mast cells may stimulate fibroblasts or other cells. The results of the previous studies [12,14] and the present findings suggest that human basophils may also be involved in these exacerbation mechanisms; that is, SCF activates human basophils, and the mediators released, such as histamine, may worsen tissue inflammation.

Our results suggest that SCF and IGF-I represent similar regulatory modes of basophil activation. Although SCF and IGF-I are generally considered to be separate cytokines acting on different subsets of cells, our results indicate that basophils may functionally be a target cell type for both. Indeed, both of the receptors for these cytokines—c-Kit for SCF and IGF-IR—belong to the tyrosine kinase receptor family, and their intracellular signals consist of multiple pathways including rapid components (such as activation of Src or Src-related kinases) and late events involving alteration of gene transcription (through mitogen-activated protein kinase activation) [27,28]. Some of these pathways may account for the overlapping and synergistic aspects of the SCF and IGF-I basophil activation profiles. The effects of SCF on basophils appeared less obvious than those on mast cells, in which strong functional induction is observed [12]. We surmise that the strength of the effects may derive from differences in receptor expression levels. We confirmed that, in contrast to mast cells, basophils only possess low levels of surface c-Kit expression, as reported by others [12,14].

Although basophils may be an important cellular source of proinflammatory mediators, the exact role of basophils in the tissue remodeling process in chronic asthma is not yet clear. A recent study by Hartnell [16] has clearly indicated that human nasal polyps contain IGFs, which account for basophil-selective chemotactic activity. However, it is not known whether basophils in the lung are major target cells of locally secreted SCF and/or IGF-I. In this context, further analyses aimed at identifying the specific roles of basophils and tissue-derived cytokines such as SCF and IGF-I in chronic asthma will be important [29]. The findings of the present study, combined with those of future studies, will clarify the potential of basophils as an important target in the treatment of chronic asthma associated with tissue-remodeling mechanisms.

Acknowledgments

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