The Early Growth Response Factor-1 Is Involved in Stem Cell Factor (SCF)-induced Interleukin 13 Production by Mast Cells, but Is Dispensable for SCF-dependent Mast Cell Growth

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The stem cell factor (SCF) plays a central role in the regulation of mast cell function and growth. However, roles of transcription factors involved in these processes remain incompletely defined. The early growth response factor-1 (Egr-1) is a member of the zinc finger transcription factor family. A role for Egr-1 in SCF-induced mast cell activation and growth was investigated in mouse bone marrow-derived mast cells (BMMC). The stimulation of BMMC with SCF induced a strong expression of Egr-1 mRNA. SCF-induced Egr-1 nuclear translocation and DNA binding were demonstrated by electrophoretic mobility shift assay (EMSA) and immunofluorescence assay. SCF-induced IL-13 expression was significantly reduced at both mRNA and protein levels in Egr-1-deficient BMMC. Interestingly, Egr-1 deficiency had little effect on SCF-induced mast cell growth. SCF-induced Egr activation likely requires tyrosine phosphorylation because a tyrosine kinase inhibitor PP2 blocked SCF-induced nuclear protein binding to Egr probe as determined by EMSA. Thus, Egr-1 is required for SCF-induced IL-13 expression, but not mast cell growth. Egr-1 represents a novel mechanism for SCF-induced mast cell activation.

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Mast cells play a central role in allergic reactions. They originate from pluripotential progenitor cells in bone marrow and acquire mature phenotype in tissues through migration and differentiation (1). Mast cells are characterized by expression of high affinity IgE receptor (FcεRI) and c-Kit (CD117), the stem cell factor (SCF) receptor (1). The c-Kit ligand, SCF, is critical for the development, survival, and proliferation of mast cells (reviewed in Ref. 2). SCF initiates its effects by binding to c-Kit, which possesses intrinsic tyrosine kinase activity (2). Binding of SCF to c-Kit results in receptor dimerization and activation of protein kinase activity. Activation of c-Kit initiates multiple signaling pathways as a result of interaction with several enzymes and adaptor proteins (3, 4). These include Src kinase, phosphotyrosine nucleotide-3 (PI 3)-kinase, phospholipase C (PLC)-γ, mitogen-activated protein (MAP) kinase pathways, and others (3, 4). SCF-induced activation of the Src family of tyrosine kinases has been implicated in gene transcription (5, 6) and mast cell chemotaxis (3, 4, 7). SCF-induced PI 3-kinase is essential for mast cell development (3, 4, 8). Thus, each SCF-induced signaling pathway is associated with specific biological functions.

Upon activation, mast cells secrete three major categories of mediators including preformed mediators such as histamine, lipid mediators such as leukotrienes and various cytokines and chemokines, such as IL-13 (1, 9). Although mast cells are able to produce all three categories of mediators, the kinetic, amount, and type of particular mediators secreted are dependent upon the nature of individual stimuli (10). Studies of the regulation of release of these mediators from mast cells have largely focused on FcεRI-mediated signaling pathways. However, FcεRI functions physiologically in the context of c-Kit. FcεRI and c-Kit serve distinct as well as overlapping functions in mast cells (11). This is because FcεRI and c-Kit mediate unique and convergent signals for release of inflammatory mediators from mast cells (11). Recently we demonstrated that a transcription factor, early growth response-1 (Egr-1) is required for the FcεRI-induced cytokine TNF and IL-13 production by mast cells (12). It is unclear whether Egr-1 regulates SCF-induced mast cell cytokine production or SCF-dependent mast cell growth.

Egr-1 is an 80–82-kDa zinc finger transcription factor. It is the prototype of the Egr family that includes Egr-1, Egr-2, Egr-3, and Egr-4, and the Wilm’s tumor product (13, 14). Members of the Egr family have been associated in a large number of biological effects including cell growth and gene expression (13, 14). Importantly, the biological effects of Egr-1 appear to be cell type-specific (13–16). Through interaction with other trans-
scription factors or tissue-specific factors, Egr-1 acts to activate or inhibit gene expression or cell differentiation (13–16).

In this study, we examined SCF-induced expression and function of Egr-1 in mast cells. SCF stimulated a rapid expression of Egr-1 mRNA and protein. By using Egr-1-deficient mast cells, we showed that SCF-induced IL-13 production requires Egr-1. Inhibition of Src family kinases, but not PI 3-kinase, PKC, or MAP kinase blocked SCF-induced Egr-1 activation, suggesting a role for Src kinases in SCF-induced Egr-1 activation. Surprisingly, SCF induced mast cell growth was not affected in the absence of Egr-1. Taken together, Egr-1 represents a novel mechanism in SCF-induced cytokine IL-13 production by mast cells.

MATERIALS AND METHODS

Animals—Egr-1-deficient mice and control C57BL/6NTac mice were purchased from Taconic Farms (Germantown, NY). The protocols were approved by the University Committee on Laboratory Animals, Dalhousie University, in accordance with the guidelines of the Canadian Council on Animal Care.

Antibodies and Reagents—Antibodies to Egr-1 (sc-189 for immunofluorescence study and sc-189X for the blockade of DNA-protein complex formation), Egr-2 (sc-20690), Egr-3 (sc-22801X), and Egr-4 (sc-19868X) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Alexa 594-conjugated goat anti-rabbit IgG (Fab')2 fragment was purchased from Molecular Probes (Eugene, OR). FITC anti-mouse CD117 mAb (CL8936F), FITC rat IgG2a (CLCR2A01) were purchased from Cedarlane Laboratories Limited (Ontario, Canada). Recombinant murine stem cell factor (SCF, catalog no. 250-03) was purchased from PeproTech Incorporation (Rocky Hill, NJ).

Mast Cell Culture and Stimulation—Murine primary cultured bone marrow-derived mast cells (BMMC) were cultured as previously described (12). Following 4–5 weeks of culture, mast cell purity of greater than 98% was achieved as assessed by toluidine blue staining (pH 1.0) of fixed cytocentrifuged preparations. BMMC (1 × 10^6 cells/ml) were activated by addition of various concentrations of SCF (0.5–100 ng/ml) for various times (5 min to 24 h). In the case of IgE+antigen stimulation, BMMC were sensitized with IgE directed against trinitrophenyl (TNP) as previously described (12). TNP-bovine serum albumin (BSA; Biosearch Technologies, Inc., Novato, CA) was used as an antigen.

Nuclear Extract Preparation and Electrophoretic Mobility Shift Assay (EMSA)—Nuclear protein extracts were obtained using a nuclear extract kit (catalog no. 40010; Active Motif, Carlsbad, CA), according to the manufacturer’s protocol. All preparation procedures were carried out at 4 °C. Total protein concentration was determined using the Bio-Rad protein assay (catalog no. 500-0006; Bio-Rad Laboratories). EMSA was carried out as previously described (12). The following double-stranded oligonucleotides were used: wild-type Egr-1 probe, 5′-ATG CTG GCC AGG GGC GAG CGG GTA CGA ACG-3′ (catalog no. 1200011; Geneka Biotechnology, Montreal, QC, Canada). Note, this probe recognizes all 4 Egr family members, although it is indicated as an Egr-1 probe by the manufacturer); mutant Egr-1 probe, 5′-GGA GCC AGG GTA GAG CGG GTA CGA ACG-3′ (catalog no. 1400011; Geneka Biotechnology).
alog no. sc-189; Santa Cruz Biotechnology) for 3 h at 4 °C. Cells were then stained for 2 h with Alexa Fluor-594-conjugated goat anti-rabbit IgG (F(ab)2) (Molecular Probes). Fluorescence-labeled mast cells were cytocentrifuged (Cytospin 3, Shandon, United Kingdom) onto slides at 4.5 × 10^3 g (200 rpm) for 5 min. To visualize cell nuclei, slides were mounted with DAPI, a fluorescent groove-binding probe for DNA, before coverslip attachment. Cells were examined using a fluorescence microscope (Nikon Eclipse E600; Nikon, Tokyo, Japan).

### Fluorescence-activated Cell Sorting (FACS) Analysis

For analysis of c-Kit expression, BMMCs were stained with a FITC-conjugated rat anti-mouse CD117 (c-kit) monoclonal Ab (mAb) (IgG2a) for 30 min at 4 °C. FITC-rat IgG2a was used as an isotypic control. Cells were analyzed by a FACScaliber flow cytometer (BD Biosciences). Fluorescence-labeled mast cells also were cytocentrifuged (Cytospin 3, Shandon, UK) onto slides at 4.5g (200 rpm) for 5 min. To visualize cell nuclei, slides were mounted with DAPI, a fluorescent groove-binding probe for DNA, before coverslip attachment. Cells were examined using a fluorescence microscope (Nikon Eclipse E600; Nikon, Tokyo, Japan). For analysis of FcεRI expression, BMMCs were sensitized with IgE and then stained with FITC-conjugated rat anti-mouse IgE mAb (BD Biosciences) for 1 h at 4 °C. FITC-rat IgG1 was used as a control.

For cell proliferation assay, BMMCs (1 × 10^6) were incubated with 1 μM 5 (6)-carboxyfluorescein diacetate N-succinimidyl ester (CFSE, catalog no. 21888, Sigma-Aldrich) in phosphate buffer for 8 min, and were then washed with 2% FBS RPMI 1640 medium for three times. CFSE-labeled BMMCs were cultured at 37 °C, 5% CO₂ incubator for 5 days with 0, 0.5, 5, 25, 50, or 100 ng/ml of SCF. Cells were counted using hemocytometer and also analyzed by flow cytometer.

### Cytokine Assays

The levels of IL-13, IL-4, IL-5, IL-10, MCP-1 (CCL-2), and RANTES (CCL-5) were measured by enzyme-linked immunosorbent assay (ELISA) using Quantikine Mouse IL-13 Immunoassay (for IL-13) and Duoset antibody pairs (for all other cytokines and chemokines) from R&D Systems (Minneapolis, MN) according to the manufacturer’s protocol.

### RESULTS

**SCF-induced Expression of Egr Products in Mouse BMMC**

To determine if SCF stimulation induces expression of Egr family members in mast cells, mouse BMMC were treated with SCF at the concentration of 50 ng/ml for 15, 30, 60, or 180 min. Expression of Egr products were examined by quantitative real-time PCR. Egr levels were normalized to GAPDH in each sample. An average value of Egr products after GAPDH normalization at the time point of 15 min (the highest Egr expression level) was used as a calibrator to determine the relative levels of Egr expression at various conditions (Fig. 1A). SCF treatment induced a rapid and transient expression of Egr-1 (Fig. 1, A and
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SCF-induced expression of Egr-1 protein was determined by immunofluorescence assay. BMMC after treatment with SCF (100 ng/ml) for 2 h were fixed, permeabilized and stained with anti-Egr-1 Ab. Alexa-594-conjugated secondary Ab was used to visualize Egr-1 expression. Nuclei were stained by DAPI. SCF-induced Egr-1 protein localized in the nucleus of the BMMC (Fig. 2).

**SCF-induced Egr Activation in Mouse BMMC**—To determine if SCF stimulation induces Egr activation, nucleus proteins from SCF-treated BMMC were examined by EMSA. An Egr DNA probe, which can bind Egr products (Egr-1, -2, -3, and -4) was used. Significant Egr DNA binding can be seen after treatment with SCF (50 ng/ml) for 40, 60, and 120 min. Egr DNA binding activity declined after 3–6 h (Fig. 3A). Dose-dependent experiment showed that mast cells responded to SCF even at the very low concentration (0.5 ng/ml). A strong Egr binding was observed when BMMC were stimulated with 5 ng/ml of SCF (Fig. 3B), a concentration close to the physiological SCF level of 3.3 ng/ml in human serum (17). Significant Egr binding was found when BMMC were stimulated with SCF at the concentrations of 25–100 ng/ml (Fig. 3B).

Egr DNA binding specificity was confirmed by competitive binding with unlabeled Egr probe, but not mutant Egr probe or Sp-1 probe (Fig. 3C). Because Sp-1 has been shown to be closely associated with Egr-1 function because of the similarity in their DNA binding sequences, we examined whether SCF induced Sp-1 DNA binding. No Sp-1 activation can be observed in SCF-stimulated mast cells (Fig. 3C). As a control, the specific binding of Sp-1 was shown using nuclear extracts from Hela cells (Fig. 3D).

To further confirm Egr binding specificity, antibodies to Egr-1, -2, -3, and -4 were used in an EMSA supershift assay. Anti-Egr-1 or anti-Egr-2 antibodies diminished Egr DNA binding activity, whereas anti-Egr-3 or anti-Egr-4 antibodies had little effect. These results were consistent with that shown in Fig. 1 that Egr-1 and -2 but not Egr-3 or -4 were induced by SCF stimulation.

**FIGURE 3. Stimulation of mast cells by SCF induces Egr-1 but not Sp-1 activation.** A, mouse BMMCs were stimulated with various concentrations of SCF for 2 h. B, mouse BMMCs were stimulated with SCF (50 ng/ml) for various times. Nuclear proteins were isolated and subjected to EMSA analysis (see “Materials and Methods”) using 32P-labeled Egr-1- or Sp-1-specific probes. Blank, no nuclear proteins were added; NT, no treatment, nuclear proteins were isolated from BMMCs without SCF stimulation. C, nuclear proteins from SCF (50 ng/ml, 120 min)-treated BMMCs (SCF) or from untreated BMMCs (NT) were subjected to DNA probe competition experiment using unlabeled probes or a mutant probe to demonstrate specific Egr-1 binding. 50×-concentrated unlabeled Egr-1 oligonucleotide was used to compete with 32P-labeled Egr-1 oligonucleotide, whereas 50× concentrated unlabeled mutant Egr-1 (mEgr-1) or Sp-1 oligonucleotides were used as nonspecific control probes. Nuclear proteins from no treatment group or SCF-treated cells were analyzed for Sp-1 activation by using 32P-labeled Sp-1 probe. No Sp-1 binding was observed in SCF-stimulated BMMCs. D, as a control of Sp-1 binding study, nuclear extracts from HeLa cells (Promega) were subjected to EMSA using 32P-labeled Sp-1 oligonucleotide. 32P-labeled Sp-1 binding was blocked by unlabeled Sp-1 probe, but not by unlabeled Egr-1 or AP-1 probes (50×). E, antibody blockade of the DNA-protein complex formation. Nuclear proteins from SCF (50 ng/ml, 2 h) treated BMMCs or from untreated BMMCs (NT) were incubated with or without specific antibodies to Egr-1, Egr-2, Egr-3, or Egr-4 for 2 h on ice before EMSA experiment using the 32P-labeled Egr-1 oligonucleotide.

B) and Egr-2 (supplemental Fig. S1). We also examined expression of Egr-3, Egr-4, and the Wilm’s tumor product. Little or no Egr-3, Egr-4, or the Wilm’s tumor product can be detected in these samples (data not shown). PCR products were also separated in agarose gels and visualized by ethidium bromide staining. Representative gels were presented in Fig. 1B and supplemental Fig. S1. A rapid and strong Egr-1 and Egr-2 expression can be seen at 15 and 30 min after SCF treatment. Egr-1 and Egr-2 levels in the unstimulated mast cells were undetectable.
**Egr in Stem Cell Factor-dependent Mast Cell Activation**

SCF-induced Mast Cell Growth in the Absence of Egr-1—Because SCF is a major mast cell growth factor, we determined if SCF-induced Egr-1 expression is required for mast cell growth. Bone marrow cells from *Egr-1*−/− mice and wild-type control mice were used to culture for BMMC. We used conventional method to culture BMMC by using WEHI-3B conditioned medium as a source for IL-3. *Egr-1*−/− BMMC showed similar level of c-kit and IgE receptor expression when compared with wild-type BMMC (Fig. 4A and supplemental Fig. S2) (12). *Egr-1*−/− BMMC and wild-type BMMC were labeled with CFSE and treated with various concentrations of SCF (0.5, 5, 25, 50, or 100 ng/ml) for 5 days. Cells were then examined by flow cytometry. Treatment with SCF induced a concentration dependent reduction of CFSE fluorescence intensity, suggesting cell proliferation. *Egr-1* deficiency did not affect SCF-induced decrease of CFSE fluorescence intensity (mast cell proliferation) (Fig. 4B). Moreover, a similar trend of SCF-induced increase of mast cell numbers was observed in both *Egr-1*−/− BMMC and wild-type BMMC (Fig. 4C). In addition, no morphological difference was observed between *Egr-1*−/− and wild-type BMMC by toluidine blue staining or electron microscopic analysis (data not shown). These results suggest that Egr-1 is not required for SCF-induced mast cell growth in vitro.

To examine if Egr-1 is needed for mast cell development in vivo, tissues from the ear and back skin of *Egr-1*−/− and wild-type mice were used for alcian blue staining for mast cells. Consistent with the in vitro results, no difference of mast cell number in these tissues was observed (supplemental Fig. S3, A and B).

To further examine if Egr-1 affects mast cell apoptosis, BMMC were treated with camptothecin (5 μM, 24 h). Cell lysates were used to examine the cleavage of D4-GDI, an endogenous caspase 3 substrate. Similar level of D4-GDI cleavage in *Egr-1*−/− and wild-type BMMC was observed (supplemental Fig. S4B).

**Decreased IL-13 Production in Egr-1-deficient Mast Cells Following SCF Stimulation**—In addition to promotion of mast cell growth, SCF modulates mast cell function by regulating expression of various inflammatory mediators. SCF has been shown to directly induce IL-13 expression by human mast cells (11). To determine if Egr-1 is required for SCF-dependent IL-13 pro-

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**FIGURE 4. No effect of Egr-1 deficiency on c-Kit expression and mast-cell growth.** Bone marrow cells from wild-type mice or *Egr-1*−/− mice were cultured in conditioned media in vitro for 4 weeks and examined by fluorescence microscope or flow cytometry for c-Kit expression and cell growth. A, BMMCs were stained with FITC-conjugated rat anti-mouse c-Kit mAb or FITC-conjugated rat IgG2a isotypic control. No difference in c-Kit expression was observed between *Egr-1*+/− and *Egr-1*−/− BMMCs. B, *Egr-1*+/− and *Egr-1*−/− BMMCs were labeled with carboxyfluorescein diacetate succinimidyl ester (CFSE, 1 μM). Labeled BMMCs were left untreated (NT) or were treated with various concentrations of SCF (0.5, 5, 25, 50, or 100 ng/ml) for 5 days. CFSE fluorescence intensity was examined by flow cytometry. SCF induced decrease of CFSE fluorescence intensity in a dose-dependent manner. No difference of SCF-induced decrease of CFSE fluorescence intensity was observed between *Egr-1*+/− and *Egr-1*−/− mast cells (one representative of two individual experiments was shown). C, mature BMMCs were cultured with various concentrations (0.5, 5, 25, 50, or 100 ng/ml) of SCF for 5 days. Cells were enumerated by counting. Data are expressed as mean ± S.E. using cell numbers on Day 0 as a baseline (n = 3).
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SCF not only directly stimulates mast cell mediator secretion, it also modulates IgE-dependent cytokine production. SCF synergistically potentiates antigen (TNP)/IgE-induced IL-13 (Fig. 6A) and IL-4 (Fig. 6B) production. Comparing to wild-type BMMC, Egr-1-deficient BMMC showed 69% reduction for IL-13 (Fig. 6A) and 53% reduction for IL-4 (Fig. 6B) in response to SCF + TNP/IgE stimulation. This result suggests that Egr-1 is required for the full scale production of IL-13 and IL-4 during SCF and TNP/IgE co-stimulation. Interestingly, Egr-1 deficiency had little or no effects on SCF/IgE-induced MCP-1 production (supplemental Fig. S5). In addition, RANTES, IL-10, and IL-5 were undetectable in any BMMC samples (data not shown).

To examine if SCF and TNP/IgE co-stimulation has synergistic effects on Egr-1 expression and activation, BMMC were treated with various concentrations of TNP-BSA (0.5, 2, 10 ng/ml) alone or in the presence of SCF (50 ng/ml) for 6 h. Cell-free supernatants were collected for detection of IL-13 and IL-4 by ELISA. Data are expressed as mean ± S.E. (n = 3).

FIGURE 6. Effects of Egr-1 deficiency on SCF+IgE-induced IL-13 and IL-4 production and Egr-1 activation. A and B, BMMCs from Egr-1-deficient and wild-type mice were sensitized with anti-TNP-IgE, and then stimulated with TNP-BSA (0.5, 2, 10 ng/ml) alone or TNP-BSA (10 ng/ml) + SCF (50 ng/ml) for 6 h. Cell-free supernatants were collected for detection of IL-13 and IL-4 by ELISA. Data are expressed as mean ± S.E. (n = 3). C and D, BMMC from wild-type mice after IgE sensitization were pretreated with SCF (50 ng/ml) for 1 h. BMMC were then stimulated with various concentrations of TNP-BSA (0.5, 2, 10 ng/ml) for 1 h. Nuclear proteins were isolated and subjected to EMSA analysis using 32P-labeled Egr-1 probe. Densitometry analysis was performed based on three separate experiments (D).

Tyrosine Kinase Activity in SCF-induced Egr Activation—To examine possible mechanisms involved in SCF-induced Egr activation, BMMC were pretreated for 1 h with various inhibitors for different protein kinases and phosphatases. These inhibitors include PP2 (tyrosine kinases), SB 203580 (p38 mitogen-activated protein kinase, p38 MAPK), PD 98059 (extracel-
Egr in Stem Cell Factor-dependent Mast Cell Activation

![FIGURE 7. Effects of protein kinase/phosphatase inhibitors on SCF-induced Egr-1 activation.](image)

A. BMMCs were pretreated with PP2 (10 μM), SB 203580 (10 μM), PD 98059 (50 μM), wortmannin (100 nM), rapamycin (100 nM), okadaic acid (500 nM) for 1 h. Subsequently, cells were stimulated with SCF (50 ng/ml) for 2 h. Nuclear proteins were extracted and subjected to analysis of Egr-1 by EMSA. B. BMMCs were pretreated with various concentrations of PP2 (0.1, 0.5, 5, or 10 μM) for 1 h. Then cells were stimulated with SCF (50 ng/ml) for 2 h. Nuclear proteins were isolated and analyzed by EMSA for Egr-1 binding.

DISCUSSION

Increased mast cell numbers and increased mast cell functional activities are major features of allergic disorders. SCF is a critical factor for both mast cell growth and functional activation (2). Mast cell precursors as well as mature mast cells express the SCF receptor, c-Kit, on their surface (2). In mice, mutations on the c-Kit or the SCF (steel) loci lead to mast cell deficiency (18–21). In human, SCF is critical for in vitro generation of mast cells from CD34+ progenitors (22, 23). Thus, SCF is essential for mast cell development. SCF is also critical for mast cell functional activation. Injection of recombinant SCF into human subjects subcutaneously results in mast cell hyperplasia and mast cell activation, as evidenced by a wheal-and-flare reaction at the injection site, electron microscopic findings of mast cell degranulation, and increased levels of mast cell tryptase and histamine metabolites (24). In vivo, SCF may stimulate release of inflammatory mediators under certain conditions although the literature is inconsistent on this matter. Several reports indicated that SCF stimulates degranulation, synthesis of inflammatory lipids, and transcription of cytokines and chemokines including IL-13 (25–32). Major efforts have been made in an attempt to dissect molecular mechanisms involved in SCF-dependent mast cell development and SCF-mediated functional regulation. Here, we demonstrated that Egr-1 is induced by SCF and is required for SCF-stimulated IL-13 production by mast cells. However, Egr-1 is not required for SCF-dependent mast cell growth although Egr-1 is essential for the development of a range of other cell types (33–35).

We provide evidence that SCF stimulates Egr-1 expression at both mRNA and protein levels. In addition, SCF promotes Egr DNA binding activity in mast cells. At 5 ng/ml, SCF showed a prominent effect on Egr DNA binding activity as determined by EMSA. In the human, the SCF level in serum averages 3.3 ± 1.1 ng/ml (17). It is possible that the SCF level at local tissues where mast cells reside is likely much higher. Thus, SCF stimulates Egr activation at a physiologically relevant concentration.

To examine whether SCF-induced Egr-1 expression and activation is needed for mast cell activation, Egr-1-deficient mast cells were used. SCF-induced IL-13 production was reduced in Egr-1-deficient mast cells suggesting that Egr-1 is required for SCF-induced IL-13 production. IL-13 is a 112 amino acid Th2 cytokine that has been shown to play a crucial role in the pathogenesis of asthma (36). It has been implicated in the induction of airway hyperresponsiveness and mucus hypersecretion (37, 38). SCF may play an essential role in the regulation of IL-13 production because anti-IgE-induced IL-13 production by human mast cells is dependent on SCF (39). In addition, inhibition of SCF induced decreased airway hyper-responsiveness (40). Interestingly, Egr-1 has been shown to play a key role in IL-13-induced biological responses in vivo and in vitro (41, 42). Moreover, Egr-1-deficient mice showed diminished TNF production and reduced airway hyper-responsiveness (43). Thus, the interplays between Egr-1 and IL-13 in the presence of SCF may play a role in the pathogenesis of allergic inflammation.

There are five members of Egr family, including Egr-1, -2, -3, -4, and the Wilm’s tumor product (13, 14). They share a highly homologous DNA-binding domain, which recognizes an identical DNA response element (14). We found that both Egr-1 and Egr-2, but not Egr-3, Egr-4, or Wilm’s tumor product were induced in mast cells by SCF stimulation. Supershift assay confirmed the DNA binding activity of Egr-1 and Egr-2 induced by SCF. Although Egr family members possess structural similarities and share DNA binding sequence, their biological functions are likely distinct. This notion is supported by the fact that the phenotypes of Egr-1-deficient and Egr-2-deficient mice are vastly different. Homozygous deletion of Egr-1 does not lead to any abnormality in embryonic stem cell growth or differentiation. Mice carrying a germ-line knock-out of Egr-1 mature to adulthood and do not show any morphological abnormalities (44). In contrast, Egr-2 deficiency leads to impaired brain development and death during the first 2 weeks after birth (45). We found that the levels and time course pattern of Egr-2 expression following SCF stimulation were similar between Egr-1-deficient and wild-type mast cells (data not shown). Thus, it is possible that there is no functional redundancy between Egr-1 and Egr-2 in SCF-induced mast cell activation. The role for
Egr-1 has been shown to be important for the development of macrophages (15), lymphocytes (35), and neuronal cells (14). We tested whether Egr-1 is needed for the development of mast cells. The lack of effect of Egr-1 in SCF-induced mast cell growth is intriguing considering that SCF plays an essential role in mast cell development. These results further support the concept that the effect of Egr-1 is cell type-specific.

Upstream signaling pathways in SCF-induced Egr-1 activation are unclear. Stimulation of c-Kit by its ligand SCF leads to activation of its intrinsic tyrosine kinase activity and phosphorylation of key tyrosine residues within the receptor. This phosphorylated c-Kit provides docking sites for a number of Src-homology 2 (SH2)-containing molecules leading to activation of multiple signaling pathways, including the Src kinase pathway, PI 3-kinase pathway, MAP kinase pathway, phospholipase Cγ pathway, and JAK/STAT pathway (3, 4). Specific signaling pathway induced by SCF appears to be associated with its specific biological effects. For example, PI 3-kinase pathway is essential for SCF-induced bone marrow-derived mast cell proliferation and development (8, 46). PI 3-kinase inhibitor wortmannin had no effect on SCF-induced Egr-1 activation, suggesting that PI 3-kinase may not be involved in SCF-induced Egr-1 activation. This appears to be consistent with the lack of effect of Egr-1 on SCF-induced mast cell growth. Similarly, the p38 MAP kinase inhibitor (SB 203580), Erk kinase inhibitor (PD 98059), PKC inhibitor (Ro 318220), or mTOR inhibitor (rapamycin) had no effect on SCF-induced Egr-1 DNA binding, suggesting that these kinases may not be the upstream molecules responsible for SCF-induced Egr-1 activation. In contrast, we found that a chemical inhibitor for Src kinase PP2 (47) blocked SCF-induced Egr-1 activation in a concentration-dependent manner, suggesting that the Src pathway is likely responsible for SCF-induced Egr-1 activation in mast cells. SCF-induced activation of members of the Src family kinases is involved in SCF-dependent gene transcription (5, 6). Accordingly, it is likely that activation of the SCF-Src-Egr-1 pathway leads to IL-13 production by mast cells.

Mast cells could serve as a potent source for IL-13 (11, 48, 49). During allergic inflammation, mast cells are likely produce mediators in the context of SCF and antigen co-stimulation. We observed a potent synergistic effect of SCF and antigen (TNP) on IL-13 production. Interestingly, a significant reduction of IL-13 production induced by SCF and antigen/IgE co-stimulation was observed in Egr-1-deficient mast cells, suggesting an important role for Egr-1 in the regulation of IL-13 production in allergic inflammation.

In summary, we provide evidence that SCF induces Egr-1 expression and DNA binding in mast cells. Egr-1 deficiency leads to decreased IL-13 production by mast cells. Surprisingly, Egr-1 is not required for SCF-induced mast cell growth. Src kinase inhibitor blocked SCF-induced Egr-1 activation. Thus, c-Kit-Src-Egr-1 pathway represents a novel mechanism in the regulation of IL-13 production by mast cells. This finding has implications for the regulation of allergic inflammation.

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REFERENCES

1. Metcalfe, D. D., Baram, D., and Mekori, Y. A. (1997) *Physiol. Rev.* 77, 1033–1079
2. Galli, S. J., Zsebo, K. M., and Geissler, E. N. (1994) *Adv. Immunol.* 55, 1–96
3. Ronnstrand, L. (2004) *Cell. Mol. Life Sci.* 61, 2535–2548
4. Roskoski, R., Jr. (2005) *Biochem. Biophys. Res. Commun.* 337, 1–13
5. Lennartsson, J., Blume-Jensen, P., Hermanson, M., Ponten, E., Carlberg, M., and Ronnstrand, L. (1999) *Oncogene* 18, 5546–5553
6. Bondzi, C., Litz, J., Dent, P., and Krystal, G. W. (2000) *Cell Growth Differ.* 11, 305–314
7. O’Laughlin-Bunner, B., Radoosevic, N., Taylor, M. L., Shivakrupa, DeBerry, C., Metcalfe, D. D., Zhou, M., Lowell, C., and Linnekin, D. (2001) *Blood* 98, 345–350
8. Fukao, T., Yamada, T., Tanabe, M., Terauchi, Y., Ota, T., Takayama, T., Asano, T., Takeuchi, T., Kadokawa, T., Hata Ji, J., and Koyasu, S. (2002) *Nat. Immunol.* 3, 295–304
9. Kinet, J. P. (1999) *Annu. Rev. Immunol.* 17, 931–972
10. Galli, S. J., Kalesnikoff, J., Grimaldston, M. A., Piliponsky, A. M., Williams, C. M., and Tsai, M. (2005) *Annu. Rev. Immunol.* 23, 749–786
11. Hundley, T. R., Gilfillan, A. M., Tkaczyk, C., Andrade, M. V., Metcalfe, D. D., and Beaven, M. A. (2004) *Blood* 104, 2410–2417
12. Li, B., Power, M. R., and Lin, T. J. (2006) *Blood* 107, 2814–2820
13. Gashler, A., and Sukhatme, V. P. (1995) *Prog. Nucleic Acids Res. Mol. Biol.* 50, 191–224
14. Beckmann, A. M., and Wilce, P. A. (1997) *Neurochem Int.* 31, 477–510; discussion 517–476
15. Nguyen, H. G., Hoffman-Liebermann, B., and Liebermann, D. A. (1993) *Cell* 72, 197–209
16. Faour, W. H., Alaeddine, N., Mancini, A., He, Q. W., Jovanovic, D., and Di Battista, J. A. (2005) *J. Biol. Chem.* 280, 9536–9546
17. Langley, K. E., Bennett, L. G., Wypych, J., Yancik, S. A., Liu, X. D., Westcott, K. R., Chang, D. G., Smith, K. A., and Zsebo, K. M. (1993) *Blood* 81, 656–660
18. Geissler, E. N., Ryan, M. A., and Housman, D. E. (1988) *Cell* 55, 185–192
19. Zsebo, K. M., Williams, D. A., Geissler, E. N., Broudy, V. C., Martin, F. H., Atkins, H. L., Hsu, R. Y., Birkett, N. C., Okino, K. H., Murdock, D. C., Jacobsen, F. W., Langley, K. E., Smith, K. A., Takeishi, T., Cattanach, B. M., Galli, S., and Suggs, S. V. (1990) *Cell* 63, 213–224
20. Huang, E., Nocka, K., Beier, D. R., Chu, T. Y., Buck, J., Lahm, H. W., Wellner, D., Leder, P., and Besmer, P. (1990) *Cell* 63, 225–233
21. Kitamura, Y., and Go, S. (1979) *Blood* 53, 492–497
22. Valnet, P., Spanbolch, E., Sperr, W. R., Sillaber, C., Zsebo, K. M., Agis, H., Strobl, H., Geissler, K., Bettelheim, P., and Lechner, K. (1992) *Blood* 80, 2237–2245
23. Kirshenbaum, A. S., Goff, J. P., Kessler, S. W., Micaj, J. M., Zsebo, K. M., and Metcalfe, D. D. (1992) *J. Immunol.* 148, 772–777
24. Costa, J. A., Demetri, G. D., Harrist, T. J., Dvorak, A. M., Hayes, D. F., Merica, E. A., Menchaca, D. M., Gringeri, A. J., Schwartz, L. B., and Galli, S. J. (1996) *J. Exp. Med.* 183, 2681–2686
25. Gagari, E., Tsai, M., Lantz, C. S., Fox, L. G., and Galli, S. J. (1997) *Blood* 89, 2654–2663
26. Colombo, M., Horowitz, E. M., Botana, L. M., MacGlashan, D. W., Jr., Bochner, B. S., Gillis, S., Zsebo, K. M., Galli, S. J., and Lichtenstein, L. M. (1992) *J. Immunol.* 149, 599–608
27. Coleman, J. W., Holliday, M. R., Kimber, I., Zsebo, K. M., and Galli, S. J. (1993) *J. Immunol.* 150, 556–562
28. Takaiishi, T., Morita, Y., Hirai, K., Yamaguchi, M., Ohta, K., Noda, E., Morita, T., Ito, K., and Miyamoto, T. (1994) *Allergy* 49, 837–842
29. Gibb, B. F., Arm, J. P., Gibson, K., Lee, T. H., and Pearce, F. L. (1997) *Eur. J. Pharmacol.* 327, 73–78
30. Taylor, A. M., Galli, S. J., and Coleman, J. W. (1995) *Immunology* 86, 427–433
31. Garrington, T. P., Ishizuka, T., Papst, P. J., Chayama, K., Webb, S., Yujiri, T., Sun, W., Sather, S., Russell, D. M., Gibson, S. B., Keller, G., Gelfand, E. W., and Johnson, G. L. (2000) *EMBO J.* 19, 5387–5395
32. Kobayashi, H., Okayama, Y., Ishizuka, T., Pawankar, R., Ra, C., and Mori, M. (1998) *Clin. Exp. Allergy* 28, 1219–1227
33. Krishnaraju, K., Hoffman, B., and Liebermann, D. A. (2001) *Blood* 97, 1298–1305
34. Krishnaraju, K., Nguyen, H. Q., Liebermann, D. A., and Hoffman, B. (1995) *Mol. Cell. Biol.* 15, 5499–5507
35. Shao, H., Kono, D. H., Chen, L. Y., Rubin, E. M., and Kaye, I. (1997) *J. Exp. Med.* 185, 731–744
36. Willis-Karp, M. (1999) *Annu. Rev. Immunol.* 17, 255–281
37. Grunig, G., Warnock, M., Wakil, A. E., Venkayya, R., Brombacher, F., Rennick, D. M., Sheppard, D., Mohrs, M., Donaldson, D. D., Locksley, R. M., and Corry, D. B. (1998) *Science* 282, 2261–2263
38. Kuperman, D. A., Huang, X., Koth, L. L., Chang, G. H., Dolganov, G. M., Zhu, Z., Elias, J. A., Sheppard, D., and Erle, D. J. (2002) *Nat. Med.* 8, 885–889
39. Kanbe, N., Kurosawa, M., Yamashita, T., Kurimoto, F., Yanagihara, Y., and Miyachi, Y. (1999) *Int. Arch. Allergy Immunol.* 119, 138–142
40. Berlin, A. A., Hogaboam, C. M., and Lukacs, N. W. (2006) *Lab. Invest* 86, 557–565
41. Ingram, J. L., Antao-Menezes, A., Mangum, J. B., Lyght, O., Lee, P. J., Elias, J. A., and Bonner, J. C. (2006) *J. Immunol.* 177, 4141–4148
42. Cho, S. J., Kang, M. J., Homer, R. J., Kang, H. R., Zhang, X., Lee, P. J., Elias, J. A., and Lee, C. G. (2006) *J. Biol. Chem.* 281, 8161–8168
43. Silverman, E. S., De Sanctis, G. T., Boyce, J., Maclean, J. A., Jiao, A., Green, F. H., Grasemann, H., Faunce, D., Fitzmaurice, G., Shi, G. P., Stein-Streilein, J., Milbrandt, J., Collins, T., and Drazen, J. M. (2001) *Am. J. Respir. Crit. Care Med.* 163, 778–785
44. Lee, S. L., Tourtellotte, L. C., Wesselschmidt, R. L., and Milbrandt, J. (1995) *J. Biol. Chem.* 270, 9971–9977
45. Schneider-Maunoury, S., Topilko, P., Seitandou, T., Levi, G., Cohen-Tannoudji, M., Pourrin, S., Babinet, C., and Charnay, P. (1993) *Cell* 75, 1199–1214
46. Timokhina, I., Kissel, H., Stella, G., and Besmer, P. (1998) *EMBO J.* 17, 6250–6262
47. Tatton, L., Morley, G. M., Chopra, R., and Khwaja, A. (2003) *J. Biol. Chem.* 278, 4847–4853
48. Burd, P. R., Thompson, W. C., Max, E. E., and Mills, F. C. (1995) *J. Exp. Med.* 181, 1373–1380
49. Jaffe, J. S., Raible, D. G., Post, T. J., Wang, Y., Glauem, M. C., Butterfield, J. H., and Schulman, E. S. (1996) *Am. J. Respir. Cell Mol. Biol.* 15, 473–481