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Original article

Molecular mechanisms for the release of chemokines from human leukemic mast cell line (HMC)-1 cells activated by SCF and TNF-α: roles of ERK, p38 MAPK, and NF-κB

Background: Mast cells play pivotal roles in IgE-mediated airway inflammation and other mast cell-mediated inflammation by activation and chemoattraction of inflammatory cells.
Objective: We investigated the intracellular signaling mechanisms regulating chemokine release from human mast cell line-1 (HMC-1) cells activated by stem cell factor (SCF) or tumor necrosis factor (TNF)-α.
Methods: Chemokine gene expressions were assessed by reverse transcription-polymerase chain reaction, while the releases of chemokines were determined by flow cytometry or enzyme-linked immunosorbent assay (ELISA). To elucidate the intracellular signal transduction regulating the chemokine expression, phosphorylated-extracellular signal-regulated kinase (ERK), phosphorylated-p38 mitogen-activated protein kinase (MAPK) and nuclear translocated nuclear factor (NF)-κB-DNA binding were quantitatively assessed by ELISA.
Results: Either SCF or TNF-α could induce release from HMC-1 cells of interleukin (IL)-8, monocyte chemoattractant protein (MCP)-1, regulated upon activation normal T-cell expressed and secreted (RANTES), and I-309, while SCF and TNF-α induced release of macrophage inflammatory protein (MIP)-1β and interferon-γ-inducible protein-10 (IP-10), respectively. Using various selective inhibitors for signaling molecules, we found that the inductions of IL-8, MCP-1, and I-309 were mediated by either SCF-activated ERK or TNF-α-activated p38 MAPK, while the induction of IP-10 by TNF-α was mediated by both activated p38 MAPK and NF-κB. The induction of RANTES by SCF or TNF-α was mediated by ERK and NF-κB, respectively, and SCF induced MIP-1β release was mediated by ERK.
Conclusion: The above results therefore elucidated the different intracellular signaling pathways regulating the release of different chemokines from SCF and TNF-α-activated mast cells, thereby shedding light for the immunopathological mechanisms of mast cell-mediated diseases.

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Key words: basic mechanisms; chemokines; mast cells.

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Mast cells are central effector cells that cause immediate-type hypersensitivity. Upon allergen provocation, cross linkage of IgE bound on mast cells, via the high-affinity receptors triggers the release of an array of inflammatory mediators including histamine, neutral proteases and heparin sulphate, prostaglandins, and cysteinyl leukotrienes, as well as various cytokines and chemokines that are involved in recruitment and activation of mast cells and other leukocytes such as eosinophils (1, 2). Mast cells are also involved in both natural and acquired immunity (3) and T helper (Th)-mediated inflammation such as inflammatory bowel disease (4), and rheumatoid arthritis (5).

All the mast cell-mediated inflammatory reactions are characterized by an accumulation of mast cells in the inflammatory sites (6). For example, the selective micro-localization of mast cells within specific airway structures, such as the airway smooth muscle and submucosal glands, is important in the pathophysiology of inflammatory lung disease (7). Apart from the release of inflammatory mediators, mast cells also play their multiple immunopathological roles in many inflammatory disorders by releasing multiple chemokines to recruit different subtypes of leukocytes (1, 8).

In inflammatory cascades, human mast cells can response to stem cell factor (SCF) secreted by fibroblasts and epithelial cells, resulting in enhanced mast cell survival, delayed apoptosis, differentiation, migration and priming for mediator and chemokine release via SCF receptor (c-kit) (2, 8–10). It is known that pro-inflammatory cytokine tumor necrosis factor (TNF)-α is released from mast cells, macrophages and T cells via

IgE-dependent mechanisms in allergic responses (11, 12). Tumor necrosis factor-α can stimulate TNF-αR1 receptors on the cell surface of mast cells for mediator release (2).

Previous studies have suggested that the transcription factor nuclear factor (NF)-κB is involved in the expression of many inflammatory cytokines and adhesion molecules of mast cells (13). Extracellular signal-regulated kinase (ERK) and p38 mitogen-activated protein kinase (MAPK) were found to play a role of adenosine A_{2B} receptor-mediated interleukin (IL)-8 production and IgE-mediated IL-6 production in mast cells (14, 15). However, the intracellular mechanisms coordinated by MAPK and NF-κB for the release of various chemokines of mast cells in inflammatory response have not been well studied. In an attempt to further elucidate the immunological roles of mast cells in inflammation, the intracellular mechanisms for the release of a panel of chemokine IL-8, monocyte chemoattractant protein-1 (MCP-1), regulated upon activation normal T-cell expressed and secreted (RANTES), I-309, macrophage inflammatory protein-1 β (MIP-1β), and interferon-γ-inducible protein-10 (IP-10) from SCF and TNF-α-activated mast cells were investigated.

Materials and methods

Reagents and antibodies

Human recombinant SCF and TNF-α were obtained from Pepro-Tec Inc., NJ. Selective ERK inhibitor PD98059, p38 MAPK inhibitor SB203580 and IκB-α phosphorylation inhibitor BAY117082 were purchased from Calbiochem Corp, CA.

Cell culture

Human mast cell line-1 (HMC-1) cells were a generous gift from Dr. J. H. Butterfield of the Mayo Clinic, MN (16). These cells were maintained in suspension culture at a density between 3 and 7×10^5 cells/ml in Iscove's medium supplemented with 10% (v/v) fetal bovine serum (Gibco Laboratories, NY), and 1.2 mM α-thioglycerol (Sigma Chemical Co, MO). They were kept under a humidified atmosphere with 5% CO_2 at 37 °C.

Endotoxin-free solution

Cell culture medium, which was free of detectable lipopolysaccharide (LPS) (< 0.1 EU/ml) was purchased from Gibco. All other solutions were prepared using pyrogen-free water and sterile polypropylene plasticware. No solution contained detectable LPS, as determined by the Limulus amoebocyte lyase assay (sensitivity limit 12 pg/ml; Associate of Cape Cod, Wooks Hole, MA).

Quantitative analysis of IL-8, MCP-1, RANTES, IP-10, MIP-1β, and I-309

Interleukin-8, MCP-1, RANTES, and IP-10 concentrations in culture supernatant were determined using chemokine cytometric bead array (CBA) kit (BD Pharmingen, CA) with a FACSCalibur flow cytometer (Becton Dickinson Corp, CA) (17). In CBA, bead populations with distinct fluorescence intensities had been coated with capturing antibodies specific for different chemokines. These bead populations could be resolved in the fluorescence channels of the flow cytometer. After the beads had been incubated with 50 μl of culture supernatant, different chemokines in the sample were captured by their corresponding beads. The chemokine captured beads were then mixed with phycoerythrin-conjugated detection antibodies to form immune complexes. Following incubation, washing and acquisition of fluorescence data, the concentration results were generated in graphical format using the BD CBA software. Concentrations of I-309 and MIP-1β were quantitated by enzyme-linked immunosorbent assay (ELISA) kit from R&D systems Inc., MN.

Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was extracted from HMC-1 cells using Tri-Reagent (Molecular Research Center Inc., OH).

Extracted RNA was reverse transcribed into first-strand complementary DNA using First-Strand cDNA Synthesis Kit (Amersham Biosciences Corp, NJ). Polymerase chain reaction was performed in a reaction mixture containing 3 mM $MgCl_2$, 200 μM dNTPs, 1 unit of AmpliTaq Gold DNA polymerase (Perkin Elmer Corp, CA), 50 pmol of 5′ and 3′ primers (Invitrogen Inc., CA) in PCR reaction buffer (1 min each at 94, 56, and 72 °C) for 30 cycles for β-actin, IL-8, IP-10, MCP-1, RANTES, I-309, and MIP-1β after an initial 12 min of denaturation at 94 °C. All RT-PCR were performed in the linear range of the PCR reaction according to the preliminary experiments. Polymerase chain reaction primers were as the following: IL-8 sense, 5′-CTGTGTGAAGGTG CAGTTTTGCC-3′ and antisense, 5′-CTCAGCCCTCTTCAA AAACTTCTCC-3′, yielding a 237-bp product (18); IP-10 sense, 5′-CCTGCTTCAAATATTTCCCT-3′ and antisense, 5′-CCTTC CTGTATGTGTTTGGA-3′, yielding a 229-bp product (18); MCP-1 sense, 5′-AATGCCCCAGTCACCTGCTGTTAT-3′ and antisense, 5′-GCAATTTCCCCAAGTCTCTGTATC-3′, yielding a 427-bp product (18); RANTES sense, 5′-ATATTCCTCGGACAC CACAC-3′ and antisense, 5′-CACGTCCAGCCTGGGGAAGG-3′, yielding a 370-bp product (18); MIP-1β sense, 5′-TACCATG AAGCTCTGCGTGACT-3′ and antisense 5′-TTAAGAGAAG GGACAGGAACT-3′, yielding a 397-bp product (19); I-309 sense, 5′-GCCCAAGCCAGACCAGAAGACA-3′ and antisense 5′-AAGCAGGGCAGAAGGAATGGTG, yielding a 403-bp product (19); β-actin sense, 5′-AGCGGGAAATCGTGCGTG-3′ and antisense, 5′-CAGGGTACATGGTGGTGCC-3′, yielding a 300-bp product (18). After the amplification reaction using PTC-200 DNA Engine™ (MJ Research Inc., MA), PCR products were electrophoresed on 2% agarose gel in TAE buffer (pH 8.0) and stained with ethidium bromide. The electrophorectic bands were documented with Gene Genius Gel Documentation System (Syngene Inc., Cambridge, UK).

Quantitative measurement of phosphorylated-ERK and phosphorylated-p38 MAPK concentration and detection of p38 MAPK activity

The concentrations of phosphorylated-ERK and phosphorylated-p38 MAPK in cell protein lysate of HMC-1 cells were quantitated by ELISA using the reagent kits of Assay Designs Inc., MI. p38 MAPK activity was assessed by the detection of phosphorylated ATF-2 using p38 MAP kinase activity assay kit (Cell Signaling Technology Inc., MA).

Quantitative assay of NF-κB activity

Nuclear proteins of HMC-1 cells were extracted with NE-PER™ nuclear and cytoplasmic extraction reagents (Pierce Chemical Co, IL) for the quantitative determination of NF-κB activity. Nuclear extracts were subjected to a test for NF-κB protein/NF-κB oligonucleotide binding using Mercury™ TransFactor NF-κB p50 kit (BD Biosciences Clontech Corp, CA).

Statistical analysis

Data in figures were presented as histograms plus SD. Differences between groups were assessed by the nonparametric Mann–Whitney rank sum test. A probability $P < 0.05$ was considered significantly different. All analyses were performed using the Statistical software GraphPad Prism (GraphPad Prism for Windows, Version 3.00, GraphPad Software, Inc., CA).

Results

Effect of SCF and TNF-α on the induction of chemokines of HMC-1 cells

Figure 1 shows that HMC-1 cells alone at 6, 12, and 24 h expressed relatively less or undetectable mRNA gene expression of chemokine IL-8, IP-10, MCP-1, I-309, MIP-1β, and RANTES. However, either SCF (50 ng/ml) or TNF-α (20 ng/ml) could up-regulate the mRNA gene expression of IL-8, RANTES, and I-309, while SCF and TNF-α could up-regulate the mRNA gene expression of MIP-1β and IP-10, respectively, at 6 and 12 h but not 24 h treatment of HMC-1 cells. mRNA gene expression of MCP-1 was found to be significantly up-regulated in HMC-1 cells by 12 h but not 6 and 24 h treatment with SCF and TNF-α.β-actin was used as positive control and it remained constant in all treatments. Similar to the results of mRNA expression, SCF (50 ng/ml) or TNF-α (20 ng/ml) could significantly induce IL-8, MCP-1, RANTES, and I-309 at 24 h incubation from HMC-1 cells (all $P < 0.05$, Fig. 2). Besides, TNF-α could significantly induce IP-10 release, and SCF could induce MIP-1β release ($P < 0.001$, Fig. 2). The dose and incubation time of SCF (50 ng/ml) and TNF-α (20 ng/ml) have been optimized according to our previous publication using HMC-1 cells (20).

Effects of SCF, TNF-α on the induction of ERK, p38 MAPK, and NF-κB activities of HMC-1 cells

Total cellular protein extracted from HMC-1 cells after cytokine stimulation was used for the determination of phosphorylated p38 MAPK and phosphorylated ERK. Figure 3A,B shows that SCF could activate ERK but not p38 MAPK pathway that peaked at 30 min and declined afterwards. It was also observed that TNF-α did not cause any phosphorylation of the ERK but caused activation of p38 MAPK reaching peak level at 15 min and declined afterwards.

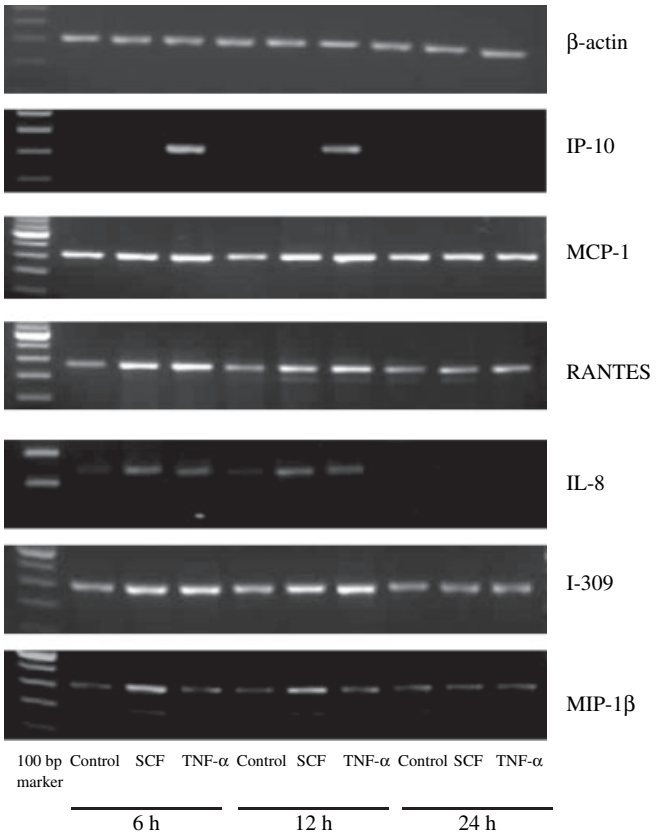

Figure 1. Effects of stem cell factor (SCF) and tumor necrosis factor (TNF)-α on mRNA expression of β-actin, interferon-γ-inducible protein (IP)-10, monocyte chemoattractant protein (MCP)-1, regulated upon activation normal T-cell expressed and secreted (RANTES), interleukin (IL)-8, I-309, and macrophage inflammatory protein (MIP)-1β in human mast cell line-1 (HMC-1) cells. Total RNA was extracted from HMC-1 (1×10^6/well) after treatment with or without SCF (50 ng/ml) or TNF-α (20 ng/ml) for 6, 12, and 24 h, and then reverse transcribed into cDNA and analyzed by polymerase chain reaction (PCR). The β-actin housekeeping gene was used as the control.

Enzyme-linked immunosorbent assay of NF-κB protein/NF-κB oligonucleotide binding (Fig. 3C) showed that the peak level of nuclear translocated NF-κB protein occurred at 7 h after treatment of TNF-α but not SCF. Subsequently, the level of TNF-α induced NF-κB protein binding declined at 12 h.

Effect of inhibitors on TNF-α and SCF induced phosphorylated p38 MAPK, ERK, and NF-κB activity in HMC-1 cells

Pretreatment of ERK inhibitor PD98059 (50 μM) and p38 MAPK inhibitor SB 203580 (20 μM) could suppress the SCF induced phosphorylation of ERK and TNF-α induced p38 MAPK catalyzed phosphorylation of ATF-2 in HMC-1 cells, respectively (Fig. 4A,B). As shown in Fig. 3C, pretreatment of NF-κB inhibitor

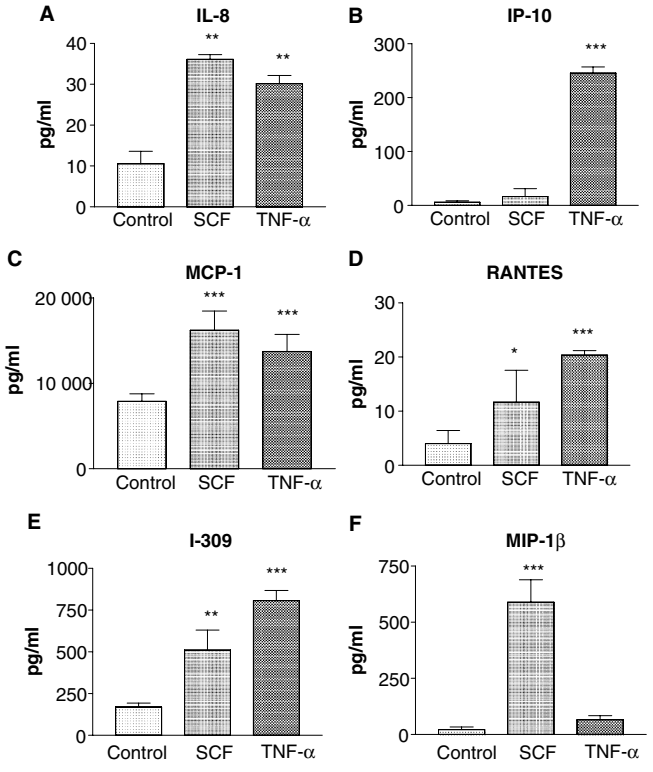

Figure 2. Effects of stem cell factor (SCF) and tumor necrosis factor (TNF)-α on the release of (A) interleukin (IL)-8, (B) interferon-γ-inducible protein (IP)-10, (C) monocyte chemoattractant protein (MCP)-1, (D) regulated upon activation normal T-cell expressed and secreted (RANTES), (E) I-309, and (F) macrophage inflammatory protein (MIP)-1β from human mast cell line-1 (HMC-1) cells. Human mast cell line-1 cells (1×10^6/well) were cultured with or without SCF (50 ng/ml) or TNF-α (20 ng/ml) for 24 h in a 24-well plate. Interleukin-8, IP-10, MCP-1, RANTES and I-309 and MIP-1β released into the culture supernatant were determined by human chemokine cytometric bead array (CBA) kit using flow cytometry and enzyme-linked immunosorbent assay (ELISA), respectively. Results are expressed as the mean plus SD from five independent experiments. $*P < 0.05$, $**P < 0.01$, and $***P < 0.005$ when compared with the control.

BAY117082 (70 μM) could suppress TNF-α induced nuclear translocation of NF-κB and the subsequent NF-κB protein/NF-κB oligonucleotide binding in HMC-1 cells.

Effects of PD98059, SB203580, and BAY117082 on the SCF and TNF-α induced chemokine release from HMC-1 cells

As shown in Fig. 5, PD98059 (20, 50, and 100 μM) could significantly suppress SCF induced release of IL-8, MCP-1, RANTES, I-309, and MIP-1β from HMC-1 cells (all $P < 0.05$). In Fig. 6, SB203580 (10, 20, and 50 μM) could significantly suppress TNF-α induced release of IL-8, IP-10, MCP-1, and I-309. BAY117082

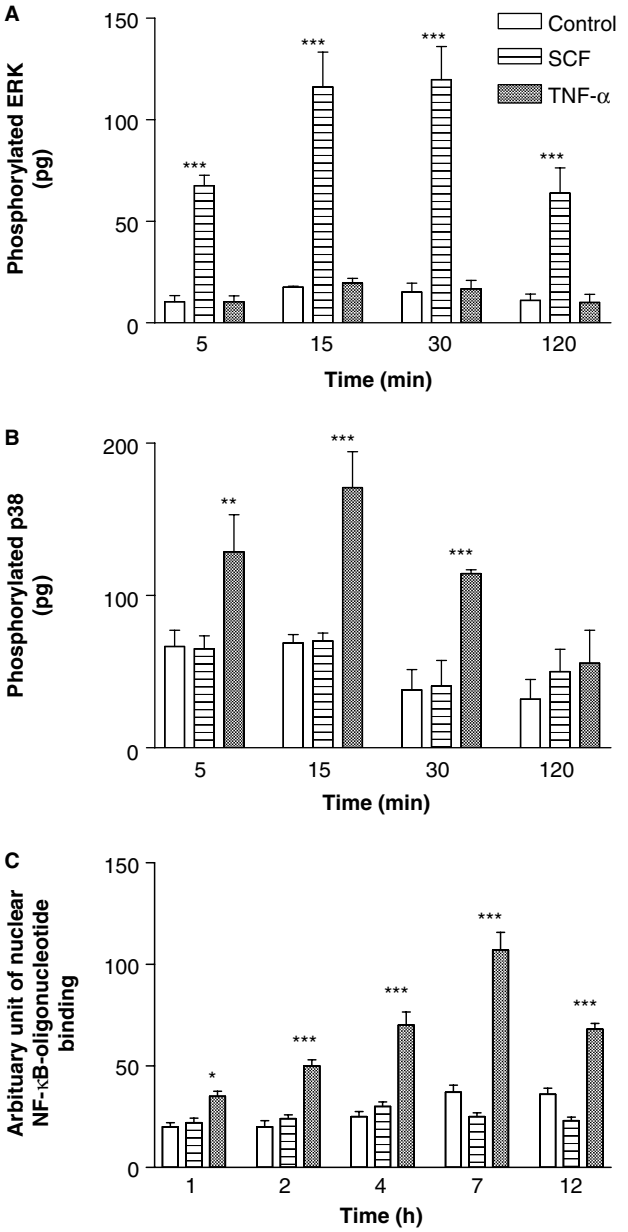

Figure 3. Effects of stem cell factor (SCF) and tumor necrosis factor (TNF)-α on the activation of extracellular signal-regulated kinase (ERK), p38 mitogen-activated protein kinase (MAPK), and NF-κB activity of human mast cell line-1 (HMC-1) cells. Human mast cell line-1 cells (1×10^6 cells/ml) were stimulated for various time periods with SCF (50 ng/ml) or TNF-α (20 ng/ml). Extracted total cellular protein (10 ng) from treated and untreated (control) HMC-1 cells after 5, 15, 30, and 120 min were used for the enzyme-linked immunosorbent assay (ELISA) of (A) phosphorylated-ERK and (B) phosphorylated-p38 MAPK. (C) Extracted nuclear protein (30 μg) after treatment for 1, 2, 4, 7, and 12 h was used for the assay of NF-κB protein/NF-κB oligonucleotide binding using Mercury™ TransFactor NF-κB p50 kit. Results are expressed as mean plus SD of six independent experiments. $*P < 0.05$, $**P < 0.01$, and $***P < 0.005$ when compared with the control.

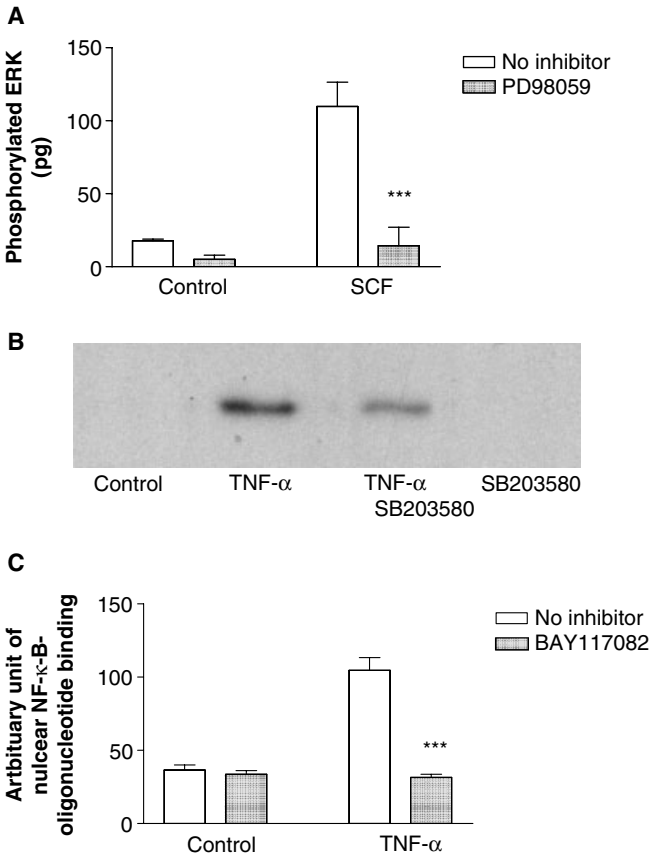

Figure 4. Effects of inhibitors on tumor necrosis factor (TNF)-α and stem cell factor (SCF) induced phosphorylated extracellular signal-regulated kinase (ERK), p38 mitogen-activated protein kinase (MAPK), and NF-κB activities in human mast cell line-1 (HMC-1) cells. Human mast cell line-1 cells (1×10^6 cells/ml) were stimulated by SCF (50 ng/ml) or TNF-α (20 ng/ml) for different time with or without 1 h pretreatment of PD098059 (50 μM), SB203580 (20 μM), or BAY117082 (70 μM). Total cellular protein was extracted from HMC-1 cells for the measurement of (A) phosphorylated ERK using enzyme-linked immunosorbent assay (ELISA) at 30 min, (B) p38 MAPK activity by the detection of phosphorylated ATF-2 using p38 MAPK activity assay kit at 15 min, and nuclear protein was extracted for (C) NF-κB protein/NF-κB oligonucleotide binding using Mercury™ TransFactor NF-κB p50 kit at 7 h. Results in (A, C) are expressed as the mean plus SD from five independent experiments. ***$P < 0.005$ when compared to no inhibitor control. For (B), experiments were performed in three independent replicates with essential identical results and representative results are shown.

(30, 70, and 100 μM) could suppress IP-10 and RANTES release from TNF-α-activated HMC-1 cells (all $P < 0.01$).

Discussion

The HMC-1 is a well established and widely used cell line exhibiting many characteristics of mast cells (16). As shown from other and our previous study, these cells can be activated by phorbol esters, calcium ionophore, SCF, and TNF-α. Human mast cell line-1 cells can therefore serve as a useful mast cell system for the study of the expression of chemokines during inflammation (1, 20). We showed in the present study that upon SCF and TNF-α stimulation, HMC-1 cells could induce the release a panel of chemokines including IL-8 for neutrophils, MCP-1 for Th2 cells, eosinophils and macrophages, RANTES for activated T cells and eosinophils, IP-10 for Th1 cells, MIP-1α for monocytes and natural killer (NK) cells, and I-309 for Th2 cells. Since we observed that SCF and TNF-α could not exhibit any significant effect on the proliferation of HMC-1 cells (data not shown), the induction of release of chemokines was not due to the increase of cell number.

Mast cells were found to be the abundant and major source of MIP-1β in the lymph nodes during hypersensitivity (21), and MIP-1β could profoundly recruit T cells from the circulation to lymph nodes during the initiation of the primary immune response (22). Previous studies have also shown that I-309, which is constitutively over expressed by mast cells, could markedly be elevated after stimulation via high-affinity Fcε receptor I (FcεRI) on mast cells for Th2 chemoattraction to trigger allergic inflammation (23, 24). Monocyte chemoattractant protein-1, which is also constitutively over expressed in HMC-1 cells, acts as the crucial chemokine in attracting eosinophils and macrophages in several inflammatory diseases including asthma and parasitic infestation (2). Interferon-γ-inducible protein-10 was shown to be induced by interferon (IFN)-α in mast cells during innate immune response against bacterial infection (25). Allergic dermatitis and other chronic inflammatory skin diseases have also been shown to be mediated by the Th1 cells recruited by IP-10, which is mainly derived from mast cells (26). Since TNF-α is a Th1 related pro-inflammatory cytokine, it is reasonable that TNF-α but not SCF could induce Th1 chemokine IP-10 release in mast cells. Interleukin-8 is responsible for recruitment and degranulation of neutrophil and granulocytopenia during inflammation (27, 28). Stem cell factor has been shown to induce airway hypersensitivity by the release of RANTES for the recruitment of activated T cells (29). Together, the above six chemokines are chemotactic factors of Th1 cells for cell-mediated immunity; Th2 cells for humoral immunity, macrophage, neutrophils and natural killer cells for innate immune response, and eosinophils for allergic inflammation. In conjunction with other mast cell-derived cytokines such as IL-1, IL-3, IL-4, IL-5, IL-6, IL-13, and TNF-α (2), our results therefore confirmed that mast cells can orchestrate differential immunological roles by the activation and chemoattraction of different leukocytes and immune effector cells in various mast cell-mediated inflammations.

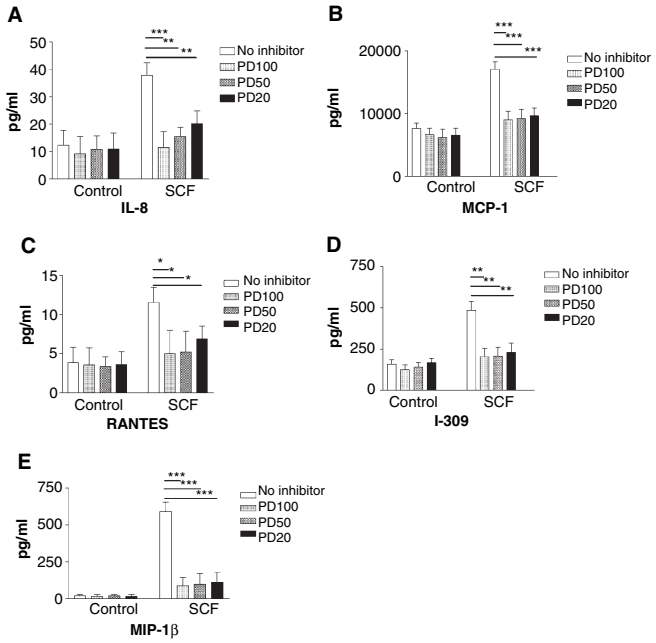

Figure 5. Effects of PD98059 on the stem cell factor (SCF) induced release of (A) interleukin (IL)-8, (B) monocyte chemoattractant protein (MCP)-1, (C) regulated upon activation normal T-cell expressed and secreted (RANTES), (D) I-309, and (E) macrophage inflammatory protein (MIP)-1β from human mast cell line-1 (HMC-1) cells. Human mast cell line-1 cells (1×10^6 cells/ml) were treated without or with PD98059 (20, 50, 100 μM) for 1 h followed by stimulation with SCF (50 ng/ml) for 24 h. Flow cytometry-based cytometric bead array (CBA) was used to detect IL-8, MCP-1 and RANTES, and enzyme-linked immunosorbent assay (ELISA) was used to detect I-309 and MIP-1β in culture supernatant. Results are expressed as mean plus SD of five experiments. $^*P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.005$ when compared with no inhibitor control.

Regarding the molecular regulatory mechanisms in HMC-1 cells, we applied several widely used specific inhibitors PD98059, SB203580, and BAY117082 to elucidate the intracellular signaling mechanisms regulating the induction of different chemokines. Following our previous publication for the toxicity test of different inhibitors on HMC-1 cells (20), we adopted the optimal concentrations of BAY117082 (30–100 μM), PD98059 (20–100 μM), and SB203580 (10–50 μM) with the highest inhibitory effect for chemokine release without any cell toxicity. Results indicated that SCF could induce ERK activity while TNF-α could induce both p38 MAPK and NF-κB activity (Figs 3 and 4). Moreover, we also observed that the selective inhibitors of c-Jun N terminal kinase (JNK) and Janus kinase (JAK) pathway, i.e., SP600125 and AG-490, respectively, had no effect in either SCF or TNF-α induced chemokine expression (data not shown), thereby indicating that there was no involvement of JNK- and JAK-signal transduction and activator of transcription pathways in chemokine expression in SCF- or TNF-α-activated HMC-1 cells. As shown in Figs 5 and 6, inductions of IL-8, MCP-1, and I-309 were mediated by either SCF-activated ERK or TNF-α-activated p38 MAPK, while the induction of IP-10 by TNF-α was mediated by both activated p38 MAPK and NF-κB. The induction of RANTES by SCF or TNF-α was mediated by ERK and NF-κB respectively, and SCF induced MIP-1β release was mediated by ERK. The above discrepancies of the intracellular signaling mechanisms of SCF and TNF-α induced expression of different chemokines may be due to the discrete activation of intracellular signaling pathways (i.e., SCF induced ERK and TNF-α induced p38 MAPK and NF-κB) and differential regulation of various transcription factors for the production of different chemokines. There are increasing evidences showing that MAPK, e.g., p38 MAPK, is required for NF-κB-dependent gene expression (30) and cross talk between discrete intracellular signaling pathways (31). Further experiments are required to confirm this type of regulatory mechanisms for the discrete responses upon different cytokine treatment for the release of various chemokines from mast cells.

It has been documented that HMC-1 cell has a mutated c-kit (SCF receptor) that is constitutively phosphorylated on tyrosine residues (32). We have performed additional experiments using selective c-kit inhibitors ST1571 (imatinib mesilate) and PP1, which are selective inhibitors of c-kit receptor tyrosine kinase and c-kit src tryrosine kinase, respectively (20). Results showed that both optimal effective concentration of STI571 (0.1 μM) and PP1 (5 μM) could not significantly inhibit the basal level of the release of all studied chemokines from HMC-1 cells (data not shown). These results therefore indicated that the constitutive phosphorylation on tyrosine residues of c-kit in HMC-1 cells cannot contribute to the high-basal level of protein expression of chemokines.

Apart from the studied signaling molecules, we speculate that there may be other signaling pathways such as phosphoinositide-3 kinase (PI3K)/Akt and various transcription factors contributing to the cytokine and chemokine gene expression and synthesis in HMC-1 cells. Protein kinase C (PKC) has recently been found to regulate the NF-κB-dependent transcription (33), therefore, the role of PKC in chemokine release also required further investigation. Regarding the protein release, there may be some other unidentified intracellular mechanisms that are responsible for the basal secretion and release of preformed chemokines via the export by the Golgi apparatus. Therefore, even the gene expression and protein synthesis are suppressed, the basal release of chemokines may not be affected in HMC-1 cells.

In view of the increasing prevalence of allergic diseases such as asthma, allergic rhinitis, and eczema worldwide (34), there is a need for novel and safe treatments of the underlying inflammation of these mast cell-mediated diseases (35). Apart from histamine release by

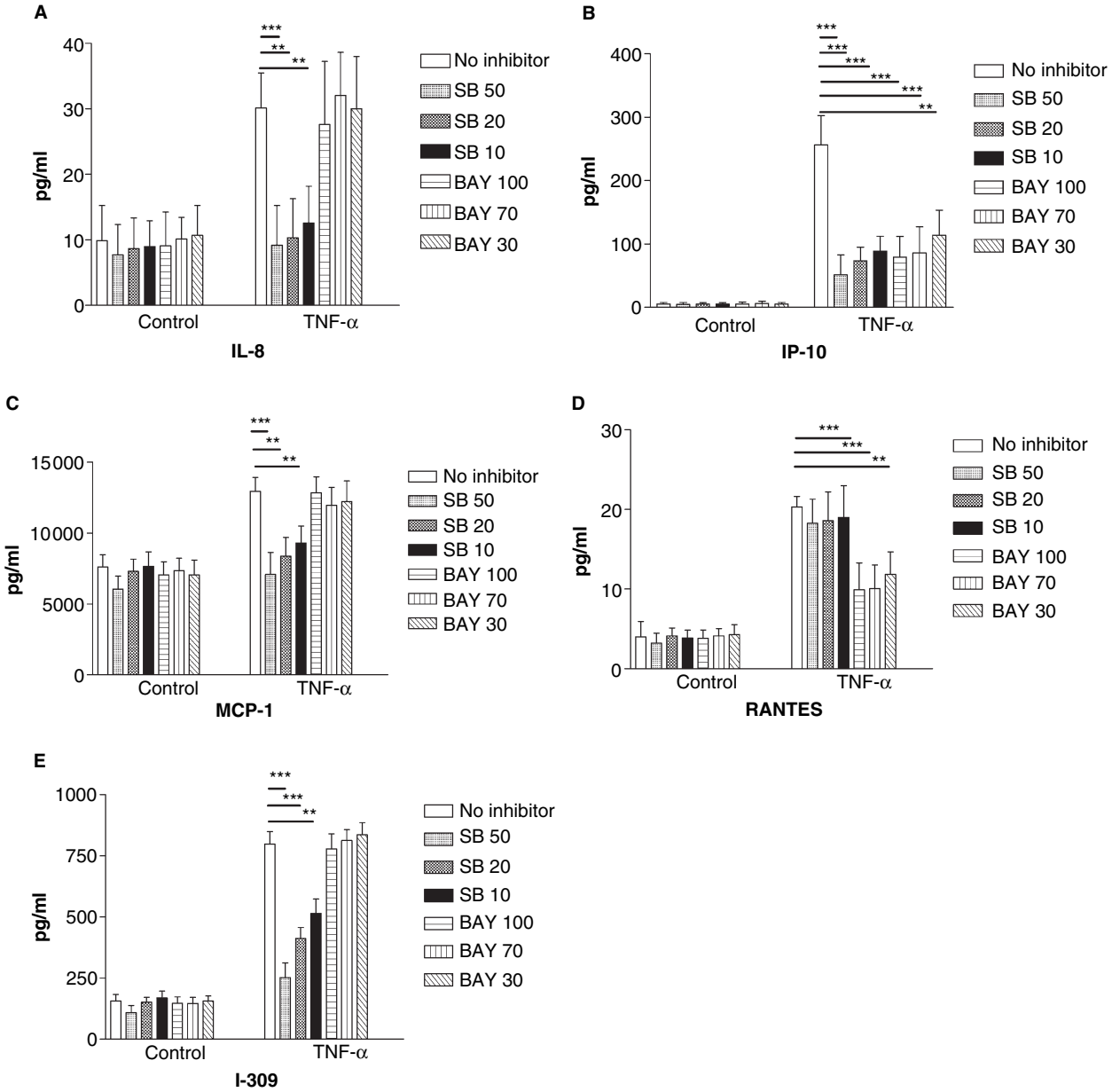

Figure 6. Effects of SB203580 and BAY117082 on the tumor necrosis factor (TNF)-α induced release of (A) interleukin (IL)-8, (B) interferon-γ-inducible protein (IP)-10, (C) monocyte chemoattractant protein (MCP)-1, (D) regulated upon activation normal T-cell expressed and secreted (RANTES), and (E) I-309 from human mast cell line-1 (HMC-1) cells. Human mast cell line-1 cells (1×10^6 cells/ml) were treated without or with SB203580 (10, 20, 50 μM) or BAY117082 (30, 70, 100 μM) for 1 h followed by stimulation with TNF-α (20 ng/ml) for 24 h. Flow cytometry-based cytometric bead array (CBA) was used to detect IL-8, IP-10, MCP-1, and RANTES, and enzyme-linked immunosorbent assay (ELISA) was used to detect I-309 in culture supernatant. Results are expressed as mean plus SD of triplicate experiments. $^{**}P < 0.01$ and $^{***}P < 0.005$ when compared with no inhibitor control.

degranulation, mast cells play differential roles in the inflammation by initiating and orchestrating immune responses by the release of various chemokines and cytokines via differential intracellular signal transduction pathways. In conjunction with our previous study regarding the cross-talk between different signaling pathways for the fine control of adhesion molecule expression on HMC-1 cells (20), our present results therefore provide further new insight that the activation of HMC-1 mast cell are under fine, diversified and complicated intracellular regulation. Because of recent advances in the application of p38 MAPK and NF-κB inhibitors as potential anti-inflammatory agents (36, 37), our study of molecular mechanisms of the release of HMC-1 cells

derived chemokines may represent potential targets for pharmacologic intervention using bio-therapeutic inhibitors for treating diseases varying from mast cell-relate diseases to allergic asthma.

Acknowledgments

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