

Suppression of CXCR4 expression in mast cells upon IgE-mediated antigen stimulation

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Abstract

Objective Recent studies have demonstrated that a variety of chemokine receptors are expressed in mast cells. We investigated the changes in mRNA expression of CXCRs in murine IL-3-dependent bone marrow-derived mast cells (BMMCs) to clarify how the CXCR expression is regulated in mast cells.

Methods Expression of CXCR mRNA was measured by RNase protection assay. Functional expression of CXCRs was confirmed by monitoring intracellular Ca^{2+} mobilization.

Results CXCR4 mRNA expression was transiently induced in BMMCs in serum-dependent fashion and was completely suppressed upon IgE-mediated antigen stimulation. In contrast, CXCR5 mRNA expression was induced upon IgE-mediated antigen stimulation. Changes in the intracellular Ca^{2+} mobilization induced by CXCL12 strongly indicated the functional expression of CXCR4. The decrease in CXCR4 and the increase in CXCR5 mRNA expression was also observed in BMMCs

stimulated with thapsigargin, a phorbol ester, and stem cell factor.

Conclusion The mRNA expression of CXCR4 is differentially regulated in BMMCs upon various stimuli including IgE-mediated antigen stimulation.

Keywords Mast cell · Chemokine · CXCR4 · CXCR5 · SCF

Introduction

Recent studies have suggested that the chemokine system plays a critical role in migration and tissue distribution of leukocytes including mast cells [1, 2]. Expression of chemokine receptors in mast cells and basophils has been explored using various culture models [2, 3]. Mast cells are known to originate from hematopoietic stem cells in the bone marrow and migrate into connective and mucosal tissues, in which their maturation and terminal differentiation occur, although it remains to be clarified as to how mast cell progenitors are recruited from the circulation to these specific tissues [4, 5]. Furthermore, Wang et al. reported that activated mast cells can migrate from the skin to the draining lymph nodes in the sensitization phase of a dinitrofluorobenzene-induced contact hypersensitivity model [6], although the exact signaling molecules regulating the migration are unknown.

Chemokine-mediated leukocyte migration is regulated not only by chemokine production but also by chemokine receptor expression, especially in the case of 'homeostatic' chemokines, which regulate lymphocyte differentiation and lymphoid tissue development in addition to leukocyte traffic [1]. One of the 'homeostatic' chemokine receptors, CXCR4, has been regarded as a possible candidate for the

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critical chemokine receptors that regulate the recruitment of mast cell progenitors from peripheral blood into tissues [7, 8]. On the other hand, Byrne et al. have recently demonstrated that UV radiation-induced mast cells migration to draining lymph nodes was suppressed by a CXCR4 antagonist [9], raising the possibility that CXCR4 is involved in stimulated migration of mast cells. In this study, we investigated the changes in mRNA expression of CXCRs in IL-3-dependent bone marrow-derived mast cells (BMMCs) activated by various stimuli including IgE-mediated antigen stimulation.

Materials and methods

Preparation of BMMC

Specific pathogen-free, 8-week-old female Balb/c mice were obtained from Japan SLC (Hamamatsu, Japan). All the experiments were performed according to the Guideline for Animal Experiments of Kyoto University and the Guidelines for Animal Experiments of Mukogawa Women's University. BMMCs were prepared as described previously [10]. After 4 weeks of culture, more than 95% of the viable cells demonstrated metachromasy, as assessed by staining with acidic Toluidine blue, confirming that these were immature mast cells.

IgE-dependent antigen stimulation

BMMCs were found to respond to IL-3 immediately after changing the medium, which is, for example, characterized by a transient increase in histamine synthesis. Therefore, BMMCs cultured for 24 h in the same complete RPMI medium were designed as the control. Control BMMCs were then sensitized with 1 µg/ml of an anti-DNP IgE (SPE-7; Sigma, St. Louis, MO) for 12 h in the same medium under standard culture conditions. The cells were twice washed with the complete medium to remove the unbound IgE and then incubated in the fresh complete medium in the presence or absence of 30 ng/ml of DNP-conjugated HSA (Sigma) for the periods indicated.

RNase protection assay

RNase protection assay (RPA) was performed using a multiprobe template set [RiboQuant, Pharmingen, San Diego, CA: mCR-6: CXCR2, CXCR4, CXCR5 (BLR-1) and two housekeeping gene, L32 and GAPDH] as previously described [11]. The intensity of each protected band was presented as the relative intensity normalized with that protected by the GAPDH probe.

Measurement of cytosolic Ca^{2+} concentrations

Cytosolic Ca^{2+} concentrations were measured as previously described [10]. Fluorescent intensities were measured, at an excitation wavelength of 340 or 380 nm and an emission wavelength of 510 nm, with a fluorescence spectrometer (CAF-100; Jasco, Tokyo, Japan).

Results

Changes in CXCR4 and CXCR5 mRNA expression in BMMCs upon IgE-mediated antigen stimulation

Previous reports have indicated the expression of CXCR4 in mast cells and basophils [7, 8, 12–15], although it remains unknown whether its expression levels change upon IgE-mediated antigen stimulation. We quantified the mRNA expression levels of CXCR2, 4, and 5 in BMMCs by RPA (Fig. 1a, b). Expression of CXCR2 was not observed in mouse BMMCs under any conditions tested. Basal mRNA expression of CXCR4 was quite low in control BMMCs, and the amount of its mRNA did not change significantly during 12 h of incubation with an anti-DNP IgE. CXCR4 expression was drastically induced by incubation in IgE-free fresh medium, and the induction was abrogated in the presence of the antigen. We then investigated the time course of mRNA expression of CXCR4 in IgE-sensitized BMMCs incubated in the fresh IgE-free medium in the presence or absence of antigen (Fig. 1c). In the absence of antigen, a significant increase in the CXCR4 mRNA expression was observed 30 min after the replacement of the medium. The transcriptional induction of CXCR4 was transient with its peak at 6 h after replacement of the medium. Induction of CXCR4 mRNA expression was also observed in the case of the fresh medium without IL-3 (data not shown).

In addition to the changes of CXCR4 expression, a drastic increase in mRNA expression of CXCR5 was observed (Fig. 1a, b). The transcriptional induction of CXCR5 was transient with its peak at 6 h after the antigen stimulation (Fig. 1d).

Changes in mRNA expression of CXCR4 and CXCR5 in BMMCs stimulated with thapsigargin, A23187, a phorbol ester, and SCF

Our results suggested that antigen stimulation suppresses the transcriptional induction of CXCR4 in BMMCs. Intracellular Ca^{2+} mobilization is one of the prominent responses induced by IgE-mediated antigen stimulation. A Ca^{2+} ionophore, A23187, and an inhibitor of sarco/endoplasmic Ca^{2+} -ATPase, thapsigargin, both of which are

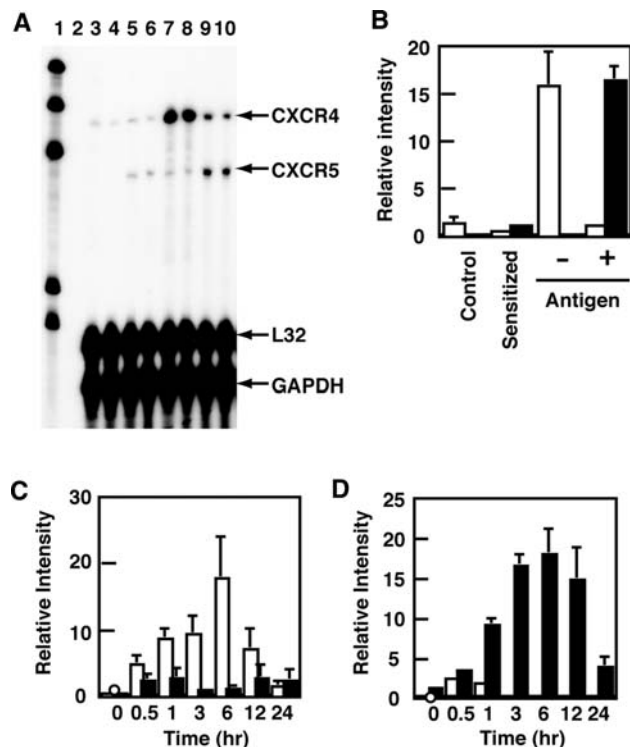


Fig. 1 Expression of CXC chemokine receptors, CXCR4 and CXCR5, in BMMCs upon antigen stimulation. BMMCs were sensitized with an anti-DNP IgE (1 μ g/ml) for 12 h, twice washed, and further incubated for 6 h in the presence or absence of DNP-conjugated HSA (30 ng/ml). **a** Total RNA (10 μ g) was extracted from cells at each time point [lanes 3 and 4; control BMMC (no sensitization), lanes 5 and 6; IgE-sensitized BMMC, lanes 7 and 8; sensitized BMMC incubated for 6 h without antigen, lane 9 and 10; sensitized BMMC incubated for 6 h with antigen] and then subjected to RPA using the [^{32} P]labeled riboprobes (lane 1). Lane 2 presents a negative control (yeast tRNA). Protected bands are indicated by the arrows. **b** The intensity of each protected band (open columns CXCR4, closed columns CXCR5) was normalized according to that of GAPDH. The relative intensities are presented as the means $\pm$ SEM ($n = 3-5$). **c,d** Sensitized BMMC were incubated in the presence (closed columns) or absence (open columns) of DNP-conjugated HSA for the periods indicated. The intensity of each protected band (**c** CXCR4 and **d** CXCR5) was normalized according to that of GAPDH. The intensities of the bands in the control (open circles) are also presented. The relative intensities are presented as the means $\pm$ SEM ($n = 3-5$)

known to augment cytosolic Ca^{2+} concentrations, were found to significantly suppress the CXCR4 mRNA expression induced upon replacement of the medium (Fig. 2). Treatment of the cells with phorbol 12-myristate 13-acetate (PMA) also suppressed the CXCR4 mRNA expression. Furthermore, SCF, which was also found to augment cytosolic Ca^{2+} concentrations in BMMCs, drastically suppressed the CXCR4 induction. These reagents and SCF did not affect the basal levels of mRNA expression of CXCR4 in BMMCs (data not shown).

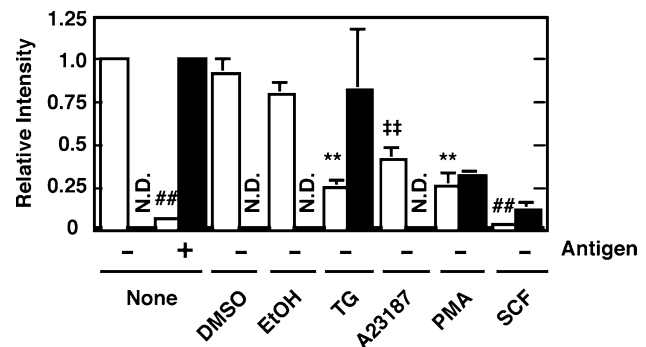


Fig. 2 Differential expression of CXCR4 and CXCR5 in mast cells in the presence of various mast cell activators. BMMC sensitized with an anti-DNP IgE (1.0 μ g/ml) for 12 h were twice washed and incubated for 6 h in the presence of indicated stimulants [30 ng/ml DNP-conjugated HSA (+Antigen), 10 nM thapsigargin (TG), 0.3 μ M A23187, 10 nM PMA, 100 ng/ml SCF]. The relative intensities (CXCR4; open columns, CXCR5; closed columns) are presented as the means $\pm$ SEM ($n = 3-5$). ##Significant at $P < 0.01$ (Student's t test: vs None). **Significant at $P < 0.01$ (Student's t test: vs DMSO). **Significant at $P < 0.01$ (Student's t test: vs EtOH). ND Not detected

Induction of CXCR5 was detected in the presence of thapsigargin, PMA, and SCF (Fig. 1d). However, treatment of the cells with A23187 failed to induce the CXCR5 mRNA expression in various concentrations tested (Fig. 2; and data not shown).

Serum-dependent induction of CXCR4 mRNA expression

Spontaneous induction of CXCR4 under culture conditions has also been reported in freshly isolated human basophils [13] and peripheral blood neutrophils [16], although it remains to be clarified which kinds of factors are involved in such response. Since BMMCs are IL-3-dependent cultured cells, it is possible that freshly added IL-3 induces the CXCR4 expression in our system. However, addition of IL-3 in the conditioned medium did not augment the mRNA levels of CXCR4 in BMMCs up to 100 ng/ml of IL-3 (data not shown). We therefore investigated the effects of FBS on CXCR4 mRNA expression in BMMCs (Fig. 3a). CXCR4 mRNA expression was induced concentration-dependently by FBS. Dialyzed FBS (molecular weight cutoff 10-kDa) was also found to induce the expression of CXCR4, although this induction was weaker than that by FBS, especially in higher concentrations. Functional expression of CXCR4 was investigated by measuring the intracellular Ca^{2+} mobilization induced by CXCL12. A transient increase in the cytosolic Ca^{2+} concentration induced by CXCL12 was detected in BMMCs 6 h after the replacement of the medium, but not in the control BMMCs or in BMMCs obtained 6 h after antigen stimulation (Fig. 3b). Homologous desensitization of the

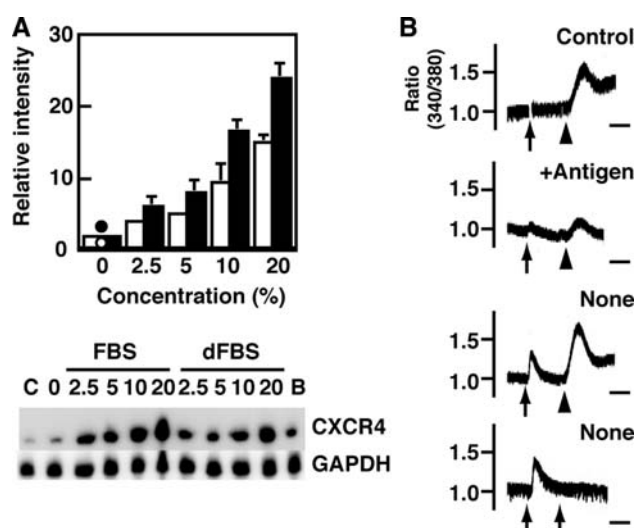


Fig. 3 Serum-dependent induction of CXCR4 and Ca^{2+} mobilization induced by CXCL12. **a** BMMC were sensitized with an anti-DNP IgE (1 $\mu\text{g}/\text{ml}$) for 12 h in the presence of 10% FBS, twice washed, and incubated for 6 h in the fresh medium containing the indicated concentrations of FBS (FBS, closed columns) or dialyzed FBS (dFBS, open columns). RPA was performed as described in the legend to Fig. 1. The intensity of each protected band corresponding to CXCR4 mRNA was normalized according to that of GAPDH. The intensity of the band in the control cells (open circles) and that in the cells incubated in the presence of 1% BSA (closed circles) are also presented. The relative intensities are presented as the means $\pm$ SEM ($n = 3-5$). **b** BMMC were sensitized with an anti-DNP IgE (1 $\mu\text{g}/\text{ml}$) for 12 h (Control), twice washed, and incubated for 6 h in the fresh medium with (+Antigen) or without (None) DNP-conjugated HSA (30 ng/ml). The cells were then sequentially stimulated with CXCL12 (arrows, 100 ng/ml) and SCF (arrowheads, 100 ng/ml). Bars 2 min. This is a representative figure of three independent experiments showing similar results

CXCL12-induced intracellular Ca^{2+} mobilization was confirmed. The intracellular Ca^{2+} mobilization induced by SCF was observed in BMMCs under these conditions, which was not affected by pretreatment with CXCL12. The increase in the cytosolic Ca^{2+} concentration induced by SCF was smaller in BMMCs upon IgE/Ag stimulation, which may be due to the decreased surface expression of c-kit under this condition (data not shown).

Discussion

Spontaneous induction of CXCR4 in freshly isolated human leukocytes under in vitro culture conditions has been demonstrated using flow cytometry [13, 16], although it remains unknown how the surface expression of CXCR4 is induced and whether the induction is transcriptionally regulated. We found that serum constituents are involved in the induction of CXCR4 mRNA expression in BMMCs. Since the levels of induction was moderately decreased upon dialysis of the serum, it is possible that low molecular

weight factors are also involved in the induction. Our results suggest that expression of CXCR4 in tissue mast cells may be up-regulated upon plasma extravasation under inflammatory conditions. Such up-regulation of CXCR4 may enhance the migration of activated mast cells toward lymph nodes. Messenger RNA expression of CXCR4 in BMMCs was found to be suppressed by antigen stimulation. Although a recent study demonstrated that the progesterone-mediated reduction of CXCL12-induced migration of a human mast cell line [17], it remains unknown whether IgE-mediated antigen stimulation affects the expression of CXCR4. The suppression of CXCR4 expression was also observed upon treatment with thapsigargin, A23187, PMA, and SCF. These results suggested that intracellular Ca^{2+} mobilization and activation of PKC may play important roles in the transcriptional suppression of CXCR4. Peacock et al. demonstrated that activation of T cell receptor leads to the decreased surface expression of CXCR4 in human T cell lines [18]. Since Ca^{2+} influx is known to be mediated by the same Ca^{2+} channel, Ca^{2+} -release activated Ca^{2+} channel, both in T cell receptor activation and Fc ϵ RI activation [19], it is possible that the common signaling pathway is utilized to suppress the CXCR4 expression in activated T cells and BMMCs. Down-regulation of CXCR4 was also reported in isolated human basophils, in which the surface expression of CXCR4 was significantly decreased by treatment with IL-3, IL-4, IL-5, or GM-CSF for 24 h [13]. It is possible that these cytokines produced by BMMCs upon antigen stimulation are involved in the suppression of CXCR4 mRNA expression in an autocrine/paracrine manner. A previous study demonstrated that the expression of CXCR4 was detectable only in mast cell progenitors derived from human cord blood cells (4-week-old culture) and not in prolonged in vitro culture (9-week-old culture) [14]. These results and our findings indicate that the expression of CXCR4 in mast cells may be strictly regulated according to the differentiation stages and activation states. SCF-mediated suppression of CXCR4 expression may contribute to infiltration and settlement of mast cell progenitors from the circulation into the connective tissues, since SCF is abundantly expressed and regulates the terminal differentiation of mast cells.

The induction of CXCR5 upon antigen stimulation in BMMCs was unexpected, since CXCR5 expression in mast cells has not been reported and the absence of CXCR5 mRNA was demonstrated in human cord blood-derived cultured mast cells and in HMC-1 cells [8]. The only known ligand for CXCR5, CXCL13, is known to be expressed exclusively in the follicles of the secondary lymphoid organs, such as Payer's patches, spleen, and lymph node [20]. The inducible mRNA expression of CXCR5 in BMMCs upon antigen stimulation raises the

possibility that antigen-stimulated mast cells can migrate into the secondary lymphoid organs. Expression of CXCR4 and CXCR5 is regulated in a coordinated manner in B cells [21, 22]. On the contrary, a previous study demonstrated that plasma cells are sensitive to CXCL12, but down-regulate CXCR5 and CCR7, and suggested that contrasting expression of CXCR4 and CXCR5 plays critical roles in regulation of plasma cell distribution [23]. The opposite expression profile of CXCR4 and CXCR5 may contribute to selective recruitment of activated mast cells into lymph nodes; CXCL13 drives migration of antigen-stimulated mast cells whereas CXCR4 is involved in mast cell migration under inflammatory conditions. However, surface expression of CXCR5 and intracellular Ca^{2+} mobilization induced by CXCL13 could not be observed in BMMCs upon antigen stimulation (data not shown). Further analyses are required to determine the conditions where functional CXCR5 is expressed in mast cells.

In summary, we demonstrated here that CXCR4 expression is induced by serum but suppressed upon IgE-mediated antigen stimulation in BMMCs. The opposite mRNA expression profiles between CXCR4 and CXCR5 were found in BMMCs stimulated with thapsigargin, a phorbol ester, and stem cell factor. These results raise the possibility that mast cell migration and distribution is regulated at least in part by CXCR4 and CXCR5.

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