Mast Cells Produce Interleukin-25 upon FcεRI-mediated Activation

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ABSTRACT

Interleukin (IL)-25 is a recently described T helper 2 (Th2) cell-derived cytokine that belongs to IL-17 family and induces the production of IL-4, IL-5, and IL-13 from an unidentified non-T cell population. Here, we show that mast cells are also potent IL-25-producing cells. When bone marrow-derived mast cells (BMMCs) were stimulated by IgE cross-linking, IL-25 mRNA was induced within 30 min in a calcineurin-dependent manner and the levels of IL-25 mRNA were comparable to that of activated Th2 cells. Production of IL-25 by mast cells was also detected at protein levels by immunoblotting. These results suggest that mast cells may enhance Th2-type immune response by producing IL-25.

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INTRODUCTION

Recently, cytokines homologous to IL-17 have been identified by database searching. Five new family members have been identified, namely IL-17B, IL-17C, IL-17D, IL-17E/IL-25, and IL-17F, in which these molecules possess 20-30% homology to IL-17 (1, 2). Among IL-17 family cytokines, it has been shown that the in vivo and in vitro biological activities of IL-25 are markedly different from those described for IL-17 and other IL-17 family cytokines (2-7). The expression of IL-25 results in the expansion of eosinophils through the production of IL-5 from an unidentified non-T cell population (2-4), whereas other IL-17 family cytokines induce the expansion of neutrophils (5-7). In addition, IL-25 induces elevated gene expression of IL-4 and IL-13 in multiple tissues and the resultant Th2-type immune responses, including increased serum IgE levels and pathological changes in the lungs and digestive tract with eosinophilic infiltrates, increased mucus production, and epithelial cell hyperplasia (2-4), indicating that IL-25 is capable of amplifying allergic inflammation.

Although it has been shown that IL-25 mRNA is exclusively expressed in polarized Th2 cells (4), quantitative RT-PCR analysis shows that IL-25 mRNA is detected in multiple tissues, including colon, uterus, stomach, small intestine, kidney, and lung (2-4), suggesting that, in addition to Th2 cells, other cell types may produce IL-25. We show here that primary bone marrow-derived mast cells (BMMCs) produce IL-25 upon IgE cross-linking, suggesting that, in addition to Th2 cells, mast cells are potent IL-25 producers and mast cell-derived IL-25 may be involved in the augmentation of Th2-type immune response.
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METHODS

Cell culture

BMMCs were prepared and cultured as described previously (8). Over 98% of cells obtained after 4 weeks of culture were positive for c-kit expression. CFTL-15 cells (9) were cultured in RPMI 1640 medium containing IL-3. For stimulation of BMMCs via Fcε receptors, BMMCs were first incubated with mouse anti-DNP IgE (1 µg/ml, YAMASA, Tokyo) at 37°C for 2 h, washed twice with RPMI1640 medium, and then incubated with DNP-HSA (human serum albumin) (50 ng/ml, Sigma, St. Louis, MO) at 37°C for 1 h. In some experiments, BMMCs were stimulated with IL-3 (10 ng/ml, R&D Systems Inc., Minneapolis, MN), stem cell factor (SCF; 10 ng/ml, a gift from Kirin Brewery Co., Takasaki, Gunma), LPS (1 µg/ml, Sigma), A23187 (500 ng/ml, Sigma), and PMA (100 ng/ml, Sigma) at 37°C for 1 h. Th0 cells and Th2 cells were prepared from DO11.10 TCR transgenic mice and stimulated as described previously (10). Splenic B cells and M12.4.5 cells were stimulated with LPS as described elsewhere (11).

RT-PCR assay

Total cellular RNA was prepared and RT-PCR analysis was performed as described previously (11). The following primer pairs were used for PCR: IL-25 (ATGTACCAGGCTGTTGCATTCTTG and CTAAGCCATGACCCGGGGCC), IL-17 (AGGCCCTCAGACTACCTCAACC and GCCTCTGAA TCCACA TTCCTTG), IL-17B (CTGACTTGGTGGGATGGACTG and ATTCACGCAACCCAAACATAGG), and TNF-α (ATGAGCAGAAGCATGATCC and GAAGACTCCTCCCAGGTATATG). Primer pairs for IL-17C, IL-17D, and IL-17F were described elsewhere (2). RT-PCR for β-actin was performed as a control. All PCR amplifications were performed at least three times with multiple sets of experimental RNAs.
**Taqman PCR analysis**

Expression of IL-25 mRNA was determined by real-time Taqman PCR using standard protocol on ABI PRISM 7000 instrument (Applied Biosystems, Foster City, CA). The following PCR primers and a fluorogenic probe were used: sense primer CACACTGCCTAGCCTACAGA, antisense primer TGTGGTAAAGTGGGACGGAGTT, and probe FAM CTCCCACATGGACCCGCTGGG TAMARA. Taqman PCR for TNF-α was performed as described previously (3). The levels of IL-25 or TNF-α mRNA were normalized to the levels of GADPH mRNA (Applied Biosystems).

**Immunoblotting**

After BMMCs were stimulated with A23187 + PMA at 37°C for 3 h, culture supernatant was collected by centrifugation, concentrated with Microcon (Millipore), and separated on 12% SDS gel. Rabbit antisera to murine IL-25 were produced using synthetic peptide (CPSKEQEPPEEW) as an antigen according to the standard protocol (12). The antigenic peptide did not exhibit any significant similarity to other IL-17 family members. Immunoblotting was performed as described previously (13).
RESULTS and DISCUSSION

To determine whether mast cells produce IL-25 upon activation, we first examined the expression of IL-25 mRNA in BMMCs by RT-PCR analysis. When BMMCs were stimulated with IgE cross-linking by anti-DNP IgE + DNP-HSA, BMMCs produced IL-25 mRNA at the comparable levels to that of activated Th2 cells (Fig. 1A). We also obtained similar results using sorted c-kit positive BMMCs (data not shown). CFTL-15 cells, a mast cell line, also expressed IL-25 mRNA upon activation with A23187 + PMA (Fig. 1A). In addition, we confirmed the expression of IL-25 mRNA by sequencing. By contrast, neither splenic B cells nor a B cell line (M12 cells) expressed IL-25 mRNA upon activation with LPS (Fig. 1A). Among various conditions we tested, the best stimulation for IL-25 mRNA expression in BMMCs was IgE cross-linking (anti-DNP IgE + DNP-HSA) or A23187 + PMA (Fig. 1B). A23187 alone or PMA alone induced IL-25 mRNA expression at lower levels (Fig. 1B). LPS also weakly induced IL-25 mRNA expression (Fig. 1B). On the other hand, neither IL-3 nor SCF induced IL-25 mRNA expression in BMMCs (Fig. 1B). Interestingly, cyclosporin A inhibited IL-25 mRNA expression induced by IgE cross-linking (Fig. 1B). Real-time PCR analysis revealed that cyclosporin A significantly decreased the IL-25 mRNA expression by 88% (n = 4, p<0.01) (Fig. 1C). These results suggest that a calcineurin-dependent pathway is essential for IL-25 mRNA expression in IgE-stimulated mast cells.

We next examined the kinetics of IL-25 mRNA expression in mast cells. As shown in Fig. 1D, IL-25 mRNA expression reached a peak within 1 h after stimulation and decreased to the baseline levels at 3 h. The peak of IL-25 production was faster than that of TNF-α production (Fig. 1D). In addition, analogous kinetics of IL-25 as well as TNF-α production was observed by real-time PCR analysis (Fig. 1E). Moreover, we found that 513 bp fragment of 5' region of IL-25 gene exhibited the responsiveness to A23187 + PMA stimulation in CFTL-15 cells (data not shown), suggesting that IL-25 mRNA is induced by the direct activation of the IL-25 promoter.
To examine IL-25 production at protein levels, we generated antisera to murine IL-25. The antisera could detect recombinant murine IL-25 but did not react with IL-17 (Fig. 1F). When BMMCs were stimulated with A23187 + PMA for 3 h and culture supernatant was subjected to immunoblotting, a specific band was detected at approximately 17kDa with antisera to murine IL-25 but not with pre-immune serum (Fig. 1G). These results suggest that BMMCs release IL-25 protein upon activation.

Finally, to determine whether mast cells selectively produce IL-25, we performed RT-PCR analysis for other IL-17 family cytokines (IL-17, IL-17B, IL-17C, IL-17D, or IL-17F). Interestingly, in addition to IL-25 mRNA, BMMCs expressed IL-17F mRNA upon IgE cross-linking (Fig. 2). In contrast, BMMCs did not express mRNA for IL-17, IL-17B, IL-17C, or IL-17D (Fig. 2 and data not shown). On the other hand, activated Th2 cells expressed mRNA not only for IL-25 and IL-17F but also for IL-17 (Fig. 2), suggesting that the expression of IL-17 family cytokines is differently regulated between mast cells and Th2 cells.

In summary, we show that, upon IgE cross-linking, mast cells produce IL-25. Because IL-25 induces IgE production and eosinophilic inflammation in multiple tissues through the expression of IL-4, IL-5, and IL-13, our findings suggest that mast cell-derived IL-25 may play a pivotal role in IgE-dependent atopic diseases.
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REFERENCES


FIGURE LEGENDS

Figure 1. Mast cells produce IL-25 upon activation via IgE receptors.

(A) Mast cells express IL-25 mRNA upon activation. Th0 cells and Th2 cells were stimulated with plate-bound anti-CD3 antibody at 37 °C for 1 h. Splenic B cells and M12.4.5 cells were stimulated with LPS (1 µg/ml) for 1 h. BMMCs were incubated with anti-DNP IgE for 2 h and then surface IgE was cross-linked with DNP-HSA for 1 h. CFTL-15 cells were stimulated with A23187 (500 ng/ml) + PMA (100 ng/ml) for 1 h. Total cellular RNA was prepared from these cells and RT-PCR for IL-25 and β-actin (as a control) mRNA was performed. Preliminary experiments revealed that the peak of IL-25 mRNA expression in anti-CD3-stimulated Th2 cells was 1 h after stimulation. Shown are representative data from 5 independent experiments. (B) Cyclosporin A inhibits IL-25 mRNA induction in mast cells. BMMCs were stimulated with the following stimuli for 1 h; IL-3 (10 ng/ml), SCF (10 ng/ml), LPS (1 µg/ml), anti-DNP IgE, anti-DNP IgE + DNP-HSA, A23187 (500 ng/ml), PMA (100 ng/ml), and A23187 + PMA. Where indicated, cyclosporin A (CyA) was added at 1 µg/ml. RT-PCR analysis for IL-25 and β-actin mRNA was performed as described in the Methods. Shown are representative data from 4 independent experiments. (C) Real-time PCR analysis for the effect of cyclosporin A. BMMCs were incubated with anti-DNP IgE alone or anti-DNP IgE + DNP HSA for 1 h in the presence or absence of cyclosporin A (1 µg/ml). Real-time PCR analysis for IL-25 as well as GADPH (as a control) mRNA was performed and the levels of IL-25 mRNA were normalized to the levels of GADPH mRNA. Data are means ± SD from four experiments. *significantly different from the mean value of control responses (without cyclosporin A), p<0.01. (D) Kinetics of IL-25 mRNA expression upon activation. BMMCs were incubated with anti DNP IgE and then surface IgE was cross-linked with DNP-HSA. At indicated times after IgE cross-linking, total cellular RNA was prepared and RT-PCR for IL-25, TNF-α, and β-actin mRNA was performed. Shown are representative data from 4 independent experiments. (E) Real-time PCR analysis of IL-25 and TNF-α mRNA expression. Similar
to D, BMMCs were stimulated with anti-DNP IgE + DNP-HSA and total cellular RNA was prepared at indicated times after stimulation. Real-time PCR analysis for IL-25 and TNF-α as well as GADPH (as a control) mRNA was performed. The levels of IL-25 or TNF-α mRNA were normalized to the levels of GADPH mRNA. Data are means ± SD from four experiments. (F) Detection of IL-25 at protein levels. COS7 cells were transiently transfected with IL-25 expression vector (pME18S-IL-25) or control vector (pME18S) and these cell lysates as well as recombinant IL-17 (0.5 µg, Endogen, Woburn, MA) were subjected to immunoblotting with rabbit antisera to murine IL-25 or pre-immune rabbit serum. (G) Mast cells release IL-25 upon activation. BMMCs were stimulated with A23187 + PMA for 3 h. Culture supernatant was collected by centrifugation, concentrated with Microcon, separated on 12% SDS gel, and blotted with rabbit antisera to IL-25 or with pre-immune rabbit serum. Shown is a representative blot from 4 independent experiments.

Figure 2. Mast cells produce IL-25 and IL-17F but not IL-17.

BMMCs were stimulated with anti-DNP IgE + DNP-HSA and Th2 cells were stimulated with anti CD3 antibody as described in Fig. 1A. RT-PCR analysis for IL-25, IL-17, and IL-17F mRNA was performed. Shown are representative data from 4 independent experiments.
Fig. 1

A

B

C

D

E

F

G
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