

Brief report

Mast cells produce interleukin-25 upon FcεRI-mediated activation

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Interleukin-25 (IL-25) is a recently described T helper 2 (T_H2) cell–derived cytokine that belongs to the 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 were stimulated by immunoglobulin E cross-linking, IL-25 mRNA was induced within 30 minutes in a calcineurin-dependent manner, and the levels of IL-25 mRNA were comparable with those of activated T_H2 cells. Production of IL-25 by mast

cells was also detected at protein levels by immunoblotting. These results suggest that mast cells may enhance T_H2-type immune response by producing IL-25. (Blood. 2003;101:3594-3596)

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Introduction

Recently, cytokines homologous to interleukin (IL)–17 have been identified by database searching. There have been 5 new family members identified, namely IL-17B, IL-17C, IL-17D, IL-17E/IL-25, and IL-17F, which possess 20% to 30% homology to IL-17.^{1,2} Among IL-17 family cytokines, it has been shown that the *in vivo* and *in vitro* biologic 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 T helper 2 (T_H2)–type immune responses (increased serum immunoglobulin E [IgE] levels and pathologic 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 T_H2 cells,⁴ quantitative reverse transcriptase–polymerase chain reaction (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 T_H2 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 T_H2 cells, mast cells are potent IL-25 producers and mast cell–derived IL-25 may be involved in the augmentation of T_H2-type immune response.

CFTL-15 cells⁹ were cultured in RPMI 1640 medium containing IL-3. For stimulation of BMMCs via Fcε receptors, BMMCs were first incubated with mouse anti-dinitrophenol (DNP) IgE (1 μg/mL, YAMASA, Tokyo, Japan) at 37°C for 2 hours, washed twice with RPMI 1640 medium, and then incubated with DNP-HSA (human serum albumin; 50 ng/mL, Sigma, St Louis, MO) at 37°C for 1 hour. In some experiments, BMMCs were stimulated with IL-3 (10 ng/mL, R&D Systems, Minneapolis, MN), stem cell factor (SCF; 10 ng/mL, a gift from Kirin Brewery, Takasaki, Gunma, Japan), lipopolysaccharide (LPS, 1 μg/mL; Sigma), A23187 (500 ng/mL, Sigma), and phorbol myristate acetate (PMA; 100 ng/mL, Sigma) at 37°C for 1 hour. T_H0 cells and T_H2 cells were prepared from DO11.10 T-cell receptor 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 GCCTCTGAATCCACATTCCTTG), IL-17B (CTGACTTGGTGGGATGGACTG and ATTCACGCAACCCAAACATAGG), and tumor necrosis factor α (TNF-α) (ATGAGCACAGAAAGCATGATCC and GAAGACTCCTCCAGGTATATG). 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 3 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 CACACTGCGTCAGCCTACAGA, antisense primer TGTGGTAAAGTGGGACGGAGTT, and probe FAM CTCCCA-CATGGACCCGCTGGG 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 glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA (Applied Biosystems).

Study design

Cell culture

BMMCs were prepared and cultured as described previously.⁸ More than 98% of cells obtained after 4 weeks of culture were positive for c-kit

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Immunoblotting

After BMMCs were stimulated with A23187 + PMA at 37°C for 3 hours, culture supernatant was collected by centrifugation, concentrated with Microcon (Millipore, Billerica, MA), and separated on 12% sodium dodecyl sulfate (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 levels comparable with those of activated T_H2 cells (Figure 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 (Figure 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 (Figure 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 (Figure 1B). A23187 alone or PMA alone induced IL-25 mRNA expression at lower levels (Figure 1B). LPS also weakly induced IL-25 mRNA expression (Figure 1B). On the other hand, neither IL-3 nor SCF induced IL-25 mRNA expression in BMMCs (Figure 1B). Interestingly, cyclosporin A inhibited IL-25 mRNA expression induced by IgE cross-linking (Figure 1B). Real-time PCR analysis revealed that cyclosporin A significantly decreased the IL-25 mRNA expression by 88% ($n = 4$, $P < .01$) (Figure 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 Figure 1D, IL-25 mRNA expression reached a peak within 1 hour after stimulation and decreased to the baseline levels at 3 hours. The peak of IL-25 production was faster than that of TNF- α production (Figure 1D). In addition, analogous kinetics of IL-25 as well as TNF- α production was observed by real-time PCR analysis (Figure 1E). Moreover, we found that a 513-base pair (bp) fragment of the 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.

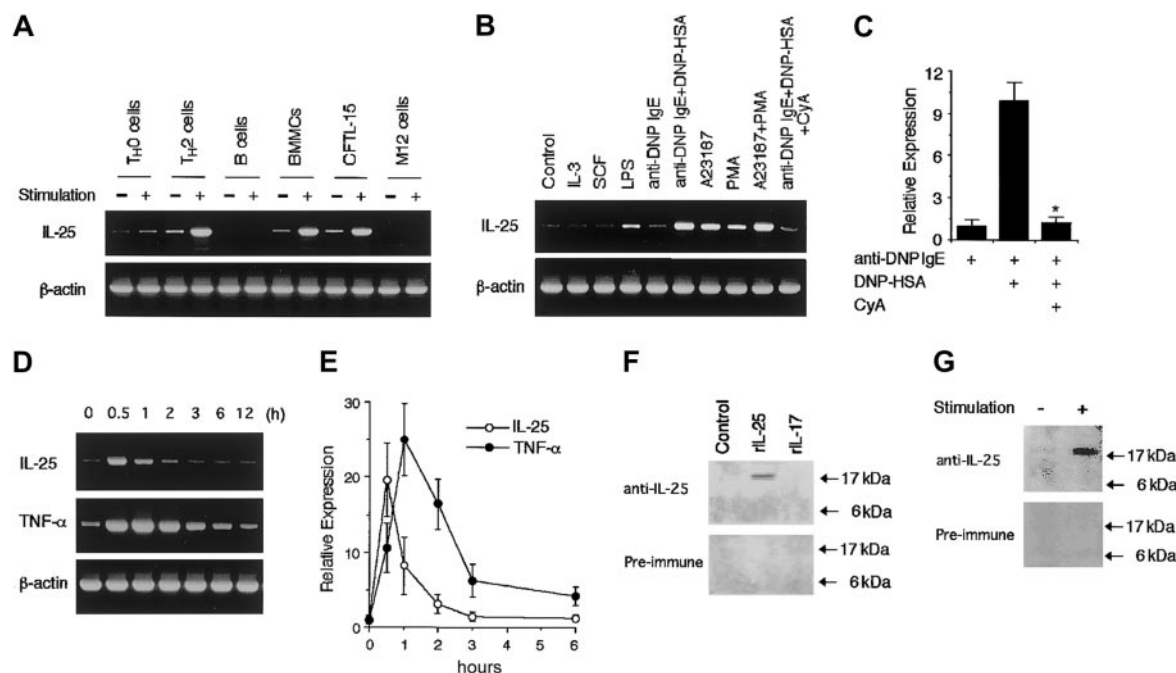


Figure 1. Mast cells produce IL-25 upon activation via IgE receptors. (A) Mast cells express IL-25 mRNA upon activation. T_H0 cells and T_H2 cells were stimulated with plate-bound anti-CD3 antibody at 37°C for one hour. Splenic B cells and M12.4.5 cells were stimulated with LPS (1 μ g/mL) for one hour. BMMCs were incubated with anti-DNP IgE for 2 hours and then surface IgE was cross-linked with DNP-HSA for 1 hour. CFTL-15 cells were stimulated with A23187 (500 ng/mL) + PMA (100 ng/mL) for one hour. 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 T_H2 cells was one hour 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 one hour: IL-3 (10 ng/mL), SCF (10 ng/mL), LPS (1 μ g/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 μ g/mL. RT-PCR analysis for IL-25 and β -actin mRNA was performed as described in "Study design." 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 one hour in the presence or absence of cyclosporin A (1 μ g/mL). Real-time PCR analysis for IL-25 as well as GAPDH (as a control) mRNA was performed, and the levels of IL-25 mRNA were normalized to the levels of GAPDH mRNA. Data are means $\pm$ SD from 4 experiments. *Significantly different from the mean value of control responses (without cyclosporin A); $P < .01$. (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 panel 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 ($\circ$) and TNF- α ($\bullet$) as well as for GAPDH (as a control) mRNA was performed. The levels of IL-25 or TNF- α mRNA were normalized to the levels of GAPDH mRNA. Data are means $\pm$ SD from 4 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 μ g, Endogen, Woburn, MA) were subjected to immunoblotting with rabbit antisera to murine IL-25 or preimmune rabbit serum. (G) Mast cells release IL-25 upon activation. BMMCs were stimulated with and without A23187 + PMA for 3 hours. Culture supernatant was collected by centrifugation, concentrated with Microcon, separated on 12% SDS gel, and blotted with rabbit antisera to IL-25 or with preimmune rabbit serum. Shown is a representative blot from 4 independent experiments.

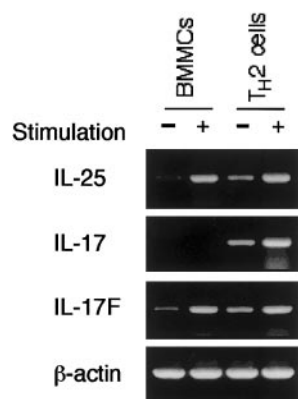


Figure 2. Mast cells produce IL-25 and IL-17F but not IL-17. BMMCs were stimulated with anti-DNP IgE + DNP-HSA and T_H2 cells were stimulated with anti-CD3 antibody as described in Figure 1A. RT-PCR analysis for IL-25, IL-17, and IL-17F mRNA was performed. Shown are representative data from 4 independent experiments.

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 (Figure 1F). When BMMCs were stimulated with A23187 + PMA for 3 hours and culture supernatant was subjected to immunoblotting, a specific

band was detected at approximately 17 kDa with antisera to murine IL-25 but not with preimmune serum (Figure 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 (Figure 2). In contrast, BMMCs did not express mRNA for IL-17, IL-17B, IL-17C, or IL-17D (Figure 2 and data not shown). On the other hand, activated T_H2 cells expressed mRNA not only for IL-25 and IL-17F but also for IL-17 (Figure 2), suggesting that the expression of IL-17 family cytokines is differently regulated between mast cells and T_H2 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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