

Original article

IL-25 regulates the expression of adhesion molecules on eosinophils: mechanism of eosinophilia in allergic inflammation

Background: Interleukin-25 (IL-25) is a novel T-helper-2 (Th2) cytokine of the IL-17 family that plays a key role in allergic inflammation. Recent studies reported that over-expression of IL-25 in mouse induces eosinophilia. We investigated the effect of IL-25 on the expression of several adhesion molecules on human eosinophils and the underlying intracellular mechanisms.

Methods: Viability of eosinophils was measured by annexin V-fluorescein isothiocyanate (FITC) assay. Gene expression and surface expression of intercellular adhesion molecule (ICAM)-1 (CD54), ICAM-3 (CD50), L-selectin (CD62L), leukocyte function-associated antigen (LFA-1) (CD11a/CD18) and very late antigen-4 (VLA-4, CD49d/CD29) on eosinophils were measured by reverse transcriptase-polymerase chain reaction (RT-PCR) and flow cytometry, respectively. Adhesion of eosinophils to fibronectin was assessed using the fibronectin-coated insert system.

Results: Viability of eosinophils was significantly enhanced by IL-25 from 41% to 76% dose-dependently. IL-25 could significantly upregulate the surface expression of ICAM-1, but suppress those of ICAM-3 and L-selectin on eosinophils in a dose-dependent manner. Adhesion of eosinophils to fibronectin was also significantly enhanced by IL-25. Besides, pre-incubation with p38 mitogen-activated protein kinases (MAPK) inhibitor SB203580, C-Jun NH₂-terminal protein kinases (JNK) inhibitor SP600125 and proteasome inhibitor MG-132 could significantly restrain the effects of IL-25 on surface expression of L-selectin, ICAM-1 and ICAM-3, respectively, and also on the adhesion of eosinophils onto fibronectin (all $P < 0.05$).

Conclusions: Our findings suggest an essential role of IL-25 in enhancing survival and regulating surface expression of ICAM-1, ICAM-3 and L-selectin on human eosinophils through the activation of p38 MAPK, JNK and nuclear factor (NF)- κ B pathways, thereby shedding light on the molecular mechanisms of IL-25-induced eosinophilia in allergic inflammation.

P. F. Y. Cheung, C. K. Wong, W. K. Ip, C. W. K. Lam

Department of Chemical Pathology, The Chinese University of Hong Kong, Prince of Wales Hospital, N.T., Hong Kong

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Prof. C. W. K. Lam
Department of Chemical Pathology
The Chinese University of Hong Kong
Prince of Wales Hospital
Shatin
Hong Kong

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There has been increasing interest in the cytokine interleukin-17 (IL-17) because of its essential roles in inflammation and numerous diseases (1). We have previously found that plasma concentration of IL-17 was elevated in patients with allergic asthma (2) and systemic lupus erythematosus (3). Five members of the IL-17 family have been identified: IL-17B, IL-17C, IL-17D, IL-17E/IL-25 and IL-17F (4–8). IL-25 seems to have biological activities different from the other family members (9).

Interleukin-25 is expressed in T-helper-2 (Th2)-polarized T lymphocytes (9) and bone marrow-derived mast cells (10). Several studies have shown that intranasal administration of IL-25-expressing adenovirus vector resulted in the production of Th2 cytokines IL-4, IL-5, IL-13 and specific eosinophil chemokine eotaxin in lung tissue and bronchoalveolar lavage fluid (9, 11). In addition, epithelial cell hyperplasia, increased mucus

secretion, and airway hyperreactivity were found to have been developed in mice upon intraperitoneal administration of recombinant IL-25 (11). The above findings suggest the role of IL-25 in amplifying allergic inflammation.

Interestingly, IL-25 has also been shown to induce eosinophilia in murine models (12, 13). Eosinophilia is a hallmark of allergic diseases such as allergic asthma (14, 15). Once they migrate into the site of allergic inflammation, activated eosinophils cause tissue damage and manifestation of allergic diseases by releasing granular cytotoxic protein including major basic protein (MBP), eosinophilic cationic protein (ECP), lipid mediators, chemokines and cytokines. Levels of these proteins have been extensively correlated with clinical symptoms of asthma (16). The recruitment and migration of eosinophils through the vascular endothelial cells therefore play a crucial role in the pathogenesis of allergic inflammation.

The mechanisms controlling eosinophil trafficking involve the regulation of chemoattractant-dependent interaction with vascular endothelium (17). Such an interaction requires a profile of adhesion molecules expressed on the cell membrane of eosinophils including: (i) integrin family: very late antigen-4 (VLA-4, CD49d/CD29) and leukocyte function-associated antigen (LFA-1) (CD11a/CD18); (ii) selectin family: L-selectin (CD62L); and (iii) immunoglobulin (Ig) family: intercellular adhesion molecule (ICAM)-1 (CD54) and ICAM-3 (CD50) (18). In view of the relevance of these adhesion molecules to eosinophilia, the investigation of the effect of IL-25 on their expression on eosinophils might provide clues for modulating allergic responses.

In order to explore the intracellular mechanisms underlying the functional effects of IL-25, we examined the activation of several intracellular signaling molecules including mitogen-activated protein kinases (MAPKs) and nuclear factor (NF)- κ B. MAPKs and NF- κ B have been demonstrated to control the majority of inflammatory responses in allergic diseases, and a number of inhibitors have been developed as potential therapeutic drugs to suppress the activation of these signaling pathways (19). In the present study, two competitive inhibitors, SB203580 and SP600125, which bind to the ATP-binding site of p38 MAPK and JNK, respectively, were used to suppress the corresponding downstream kinase activities. For NF- κ B pathway, we used a proteasome inhibitor MG-132 to prevent the degradation of the phosphorylated inhibitor κ B, thereby preventing the release of activated NF- κ B to translocate into nucleus for DNA binding and the subsequent gene transcription (19).

Materials and methods

Reagents and antibodies

Recombinant human IL-25 was obtained from R&D Systems Inc. (Minneapolis, MN, USA). Fluorescein isothiocyanate (FITC)-conjugated mouse anti-human ICAM-1, ICAM-3, CD18, CD49d, L-selectin monoclonal antibody, and fluorescein-conjugated mouse IgG₁ and IgG_{2b} isotype were purchased from BD Pharmingen (San Diego, CA, USA). Proteasome inhibitor N-cbz-Leu-Leu-Leucinal (MG-132), JNK inhibitor SP600125, and p38 MAPK inhibitor SB203580 were purchased from Calbiochem Corp (San Diego, CA, USA). SB203580 was dissolved in water, while MG-132 and SP600125 were dissolved in dimethyl sulfoxide (DMSO). In all studies, the final concentration of DMSO was $\leq 0.1\%$ (vol/vol).

Isolation of human blood eosinophils from buffy coat and eosinophil culture

Fresh human buffy coat obtained from the Hong Kong Red Cross Blood Transfusion Service was diluted 1:2 with phosphate-buffered saline (PBS) at 4°C and centrifuged using an isotonic Percoll solution (density 1.082 g/ml; Amersham Biosciences Corp, Piscataway, NJ, USA) for 30 min at 1000 g. The eosinophil-rich granulocyte

fraction was collected and washed twice with cold PBS containing 2% fetal calf serum. The cells were then incubated with anti-CD16 magnetic beads (Miltenyi Biotec, Bergisch Gladbach, Germany) at 4°C for 45 min and CD16-positive neutrophils were depleted by passing through a LS+ column (Miltenyi) within a magnetic field. With this preparation, the drop-through fraction contained eosinophils with a purity of at least 98% as assessed by Hemacolor rapid blood smear stain (E Merck Diagnostica, Darmstadt, Germany). The isolated eosinophils were cultured in RPMI1640 medium supplemented with 10% fetal bovine serum (FBS) and 20 mM Hepes (Gibco Co, Eggenstein, Germany).

Endotoxin-free solutions

Cell culture medium was purchased from Gibco, free of detectable lipopolysaccharide (LPS) (< 0.1 EU/mL). 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; Biowhittaker Inc, Walkersville, MD, USA).

Reverse transcription-polymerase chain reaction

Total RNA from eosinophils was extracted using Tri-Reagent (Molecular Research Center Inc, Cincinnati, OH, USA). Equal amount of extracted RNA was reverse transcribed into first-strand complementary DNA using First-Strand cDNA Synthesis Kit (Amersham). Polymerase chain reaction (PCR) was performed in a reaction mixture containing 3 mM MgCl₂, 200 μ M dNTPs, 1 unit of AmpliTaq Gold DNA polymerase (Applied Biosystems Corp, Foster City, CA, USA), 50 pmol of 5' and 3' primers (Invitrogen Corp, Foster City, CA, USA) in PCR reaction buffer (1 min each at 94, 60 and 72°C) for 28 cycles for β -actin, ICAM-1, ICAM-3, and L-selectin after an initial 12 min of denaturation at 94°C. All reverse transcription (RT)-PCR analyses were performed in the linear range of the PCR reaction according to the preliminary experiments. PCR primers were the following: β -actin sense, 5'-AGCGGGAAATCGTGCGTG-3' and antisense, 5'-CAGGGTACATGGTGGTGCC-3', yielding a 300-bp product (20); ICAM-1 sense, 5'-CGTGCCGCACTGAAGTGGAC-3' and anti-sense, 5'-CCTCACAC TTCCTGTCTACCT-3', yielding a 447-bp product (20); ICAM-3 sense, 5'-AGTTCTTGACAGGAACAGTAGC-3', and anti-sense, 5'-TGAAGACGTACATTAAGGCCAGT-3', yielding a 380-bp product; L-selectin sense, 5'-TTCTCAATGATTAAAGGAGGGTGA-3' and anti-sense, 5'-CCTCAGAAAAGACAAAAAGC TGA-3', yielding a 395-bp product. After the amplification reaction using PTC-200 DNA Engine (MJ Research Inc, Waltham, MA, USA), PCR products were electrophoresed on 2% agarose gel in tris-acetate-EDTA (TAE) buffer (pH 8.0) and stained with ethidium bromide. The electrophoretic bands were documented with Gene Genius Gel Documentation System (Syngene Inc, Cambridge, UK).

Apoptosis assay

Apoptosis of eosinophils was assessed by the TACSTM Annexin V-FITC assay (Trevigen Inc., Gaithersburg, MD, USA) using flow cytometry (FACSCalibur, BD Biosciences Corp, San Jose, CA, USA) on eosinophils gated on the basis of their forward and side light scatter with any cell debris excluded from analysis. The population of viable cells was characterized by low mean fluorescence of both annexin V-FITC and propidium iodide (PI)-phycoerythrin (PE).

Immunofluorescence staining and flow cytometry

Eosinophils (5×10^5 cells/0.5 ml), after the preceding treatments, were washed and resuspended with cold PBS supplemented with 0.5% bovine serum albumin. After blocking with 2% human pooled serum for 20 min at 4°C and washing with PBS supplemented with 0.5% bovine serum albumin, cells were incubated either with FITC-conjugated mouse anti-human adhesion molecule monoclonal antibody or fluorescein-conjugated mouse IgG₁ and IgG_{2b} isotype for 30 min at 4°C in the dark. After washing, the cells were finally resuspended in 1% paraformaldehyde in 1x PBS as fixative. Expression of surface adhesion molecule on 10 000 viable cells was then analyzed by flow cytometry (FACSCalibur) as mean fluorescence intensity (MFI), which includes both the changes of adhesion molecule expression on individual cell and the percentage of cells expressing the adhesion molecules.

Eosinophil cell adhesion assay

The adhesion assay was performed in a 24-well fibronectin-coated insert system with pore size 3 µm in diameter (BD Biosciences). Pre-warmed culture media (1400 µl) was added to the 24-well plate. The fibronectin-coated insert was carefully put in place, and then 500 µl of treated or untreated eosinophils at 1×10^6 cells/ml were added to each insert. The system was incubated at 37°C, in a 5% CO₂, humidified atmosphere and cells were allowed to adhere onto the insert for 4 h. After incubation, inserts were carefully taken out and eosinophil adherence was assessed by counting the number of eosinophils adhered to the fibronectin-coated insert in four high-power fields (magnification: 400×) per well under inverted microscope.

Statistical analysis

All data in the figures are expressed as mean $\pm$ SEM. Differences between groups were assessed by the parametric unpaired Student's *t*-test. A probability $P < 0.05$ was considered significantly different. All analyses were performed using the statistical software GraphPad Prism for Windows (Version 3.0; GraphPad Software, San Diego, CA, USA).

Results

Effect of IL-25 on enhancing survival of eosinophils

Figure 1A–E shows that significant differences in survival were observed between the control and IL-25-treated eosinophils. After incubation with IL-25 (10–100 ng/ml) for 24 h, the percentage of viable cells increased from 41% to 76% in a dose-dependent manner (all $P < 0.05$; Fig. 1F).

Effect of IL-25 on the expression of ICAM-1, ICAM-3, L-selectin, CD18 and CD49d on human eosinophils

To generate a profile of adhesion molecules that may be regulated by IL-25, the effect of IL-25 (50 ng/ml) on ICAM-1, ICAM-3, L-selectin, CD18, and CD49d was examined (Figs 2 and 3). Figure 2 shows the kinetics and dose response of IL-25-inducing effects on the surface expression of ICAM-1, ICAM-3, and L-selectin. We

found that IL-25 (10–100 ng/ml) could significantly upregulate the surface expression of ICAM-1 (Figs 2A and 3A) while downregulating those of ICAM-3 (Figs 2B and 3B) and L-selectin (Figs 2C and 3C) in a dose-dependent manner. In addition, the maximal responses occurred at 16 h of IL-25 stimulation for all of the three adhesion molecules, while the effective dose of IL-25 was 50 ng/ml. However, IL-25 (50 ng/ml) did not have any significant effect on the surface expression of CD18 (Fig. 3D) and CD49d (Fig. 3E).

In view of such regulation of ICAM-1, -3, and L-selectin by IL-25, we evaluated the effect of IL-25 on the mRNA expression levels of ICAM-1, -3 and L-selectin. RT-PCR results showed that IL-25 (50 ng/ml) could induce the mRNA expression of ICAM-1 (Fig. 4B), but suppress those of ICAM-3 (Fig. 4C) and L-selectin (Fig. 4D).

Effect of MG-132, SP600125 and SB203580 on IL-25-regulated surface expression of ICAM-1, ICAM-3 and L-selectin

Based on the results of viability test of different inhibitors, we chose MG-132 (5 µM), SP600125 (3 µM) and SB203580 (7.5 µM) for the subsequent inhibition experiments. Figure 5 shows that pre-treatment of eosinophils with proteasome inhibitor MG-132, JNK inhibitor SP600125 and p38 MAPK inhibitor SB203580 for 1 h could significantly suppress the IL-25-induced upregulation of ICAM-1, and downregulation of ICAM-3 and L-selectin (all $P < 0.05$).

Effect of IL-25 on adhesion of eosinophils to fibronectin

Figure 6 shows that IL-25 could significantly increase the number of eosinophils adhered to fibronectin-coated insert. Pre-treatment of eosinophils with MG-132, SB203580 or SP600125 for 1 h prior to stimulation could significantly restrain the enhancing effect of IL-25 on the adherence of eosinophils to fibronectin (all $P < 0.05$; Fig. 6).

Discussion

While the physiological effects of IL-25 have been well illustrated in animal models (9, 11, 12), the responsible cell populations expressing the IL-25 receptor are still poorly recognized in man. The receptor for IL-25 was first identified as IL-17B receptor (IL-17BR) (4), also called IL-17 receptor homolog 1 (IL-17Rh1) (7), and Evi 27 (21). Our group has, for the first time, examined the protein expression of IL-17BR in human eosinophils, and found that they constitutively express protein of IL-17BR (22). We also found that the median plasma concentration of IL-25 in allergic asthmatic patients was significantly higher than that of normal control subjects. It has been

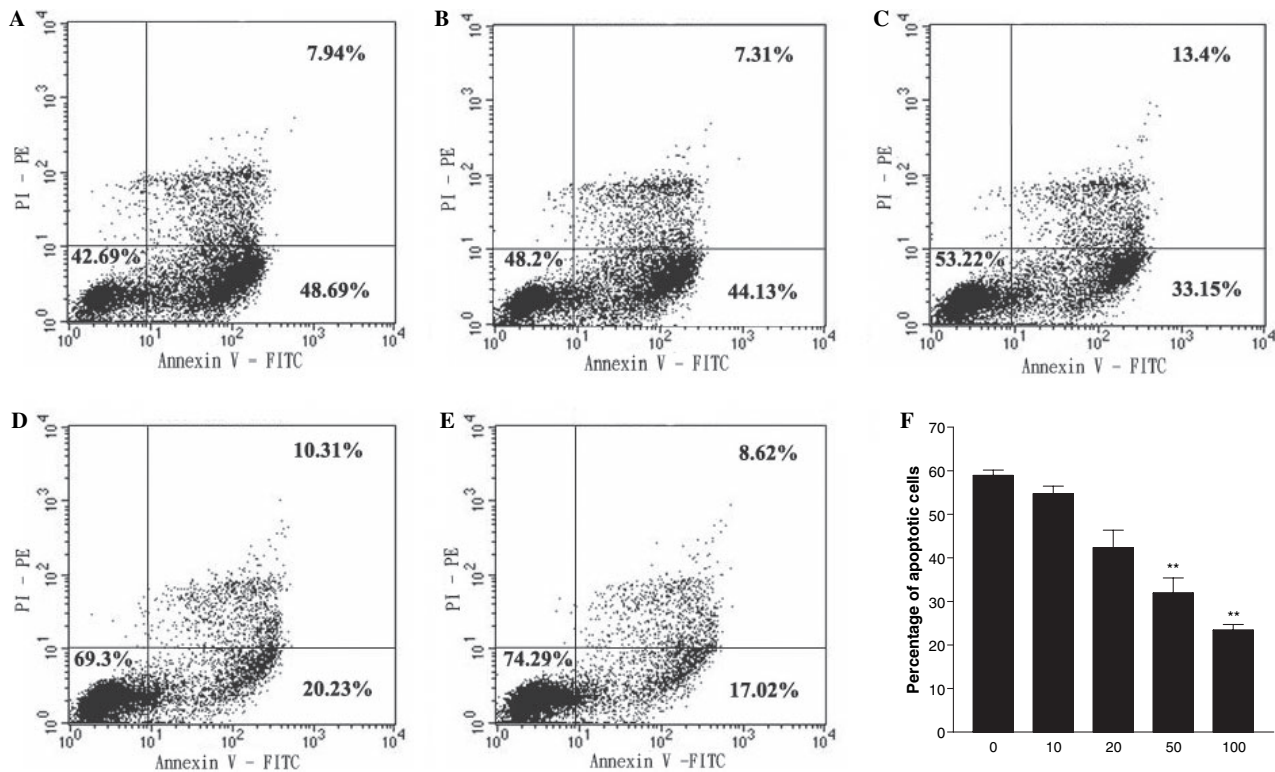


Figure 1. Effect of IL-25 on the viability of eosinophils. Eosinophils (5×10^5 cells/ml) were untreated (A), or treated with IL-25 at (B) 10 ng/ml, (C) 20 ng/ml, (D) 50 ng/ml or (E) 100 ng/ml for 24 h. Viability of eosinophils was determined by flow cytometry. Flow analysis was performed on eosinophils gated on the basis of their forward and side light scatter with any cell debris excluded from analysis. The population of viable cells was characterized by low mean fluorescence of both annexin V-FITC and propidium iodide (PI)-PE (lower left quadrant), while that of apoptotic cells was shown by high mean fluorescence of Annexin V-FITC but low PI-PE (lower right quadrant), and dead cells represented by high mean fluorescence of both Annexin V-FITC and PI-PE (upper right quadrant). These figures are representatives from three independent experiments with similar results. (F) Apoptotic cells were presented as percentage of gated cells and are expressed as the arithmetic mean plus SEM of three independent experiments. ** $P < 0.01$ when compared with medium control.

reported that IL-25 could induce eosinophilia in mouse (12, 13). In this study, we found that the viability of IL-25-treated eosinophils was greatly enhanced when compared with untreated ones (Fig. 1), indicating that IL-25 could delay eosinophil apoptosis and therefore promote eosinophilia in allergic inflammation. The above results therefore prompted us to study the effect of IL-25 on the expression of adhesion molecules in human eosinophils.

In the present study, we found that IL-25 could significantly upregulate the cell surface and mRNA expression of ICAM-1, but suppress those of ICAM-3 and L-selectin dose-dependently (Figs 2 and 4). However, this cytokine did not induce any significant change in surface expression of CD18 and CD 49d (Fig. 3), which may be due to the differential regulation of intracellular signal transduction pathways responsible for different adhesion molecules. In addition, another clinical report indicated that VLA4 on eosinophils did not change in atopic asthmatic children (23). ICAM-1 has been well demonstrated to potentiate inflammatory process in childhood asthma (24). Although our previous publica-

tion (25) showed that IL-25 did not induce any effect on the surface expression of ICAM-1 on human mast cell line-1 (HMC-1), it could significantly upregulate that of eosinophils in a dose-dependent manner (Fig. 2a). This may be due to the different intracellular signal transduction pathways in eosinophils and mast cells. The interaction of ICAM-1 and integrins has been shown to be essential for the recruitment and transendothelial migration of eosinophils (26). Therefore, upregulation of ICAM-1 expression on eosinophils might promote allergic inflammation. As ICAM-1 can bind to fibronectin (27), a broad-range cell adhesion molecule, we investigated the effect of IL-25 on the adhesion of eosinophils to fibronectin. Our result showed that IL-25 (50 ng/ml) could significantly enhance the adhesion of eosinophils to fibronectin (Fig. 6), thereby shedding light on the role of upregulated level of surface ICAM-1 expression on the cells.

Unlike ICAM-1, ICAM-3 is highly expressed on resting eosinophil surface. However, the significance of this high expression level remains unclear. A previous

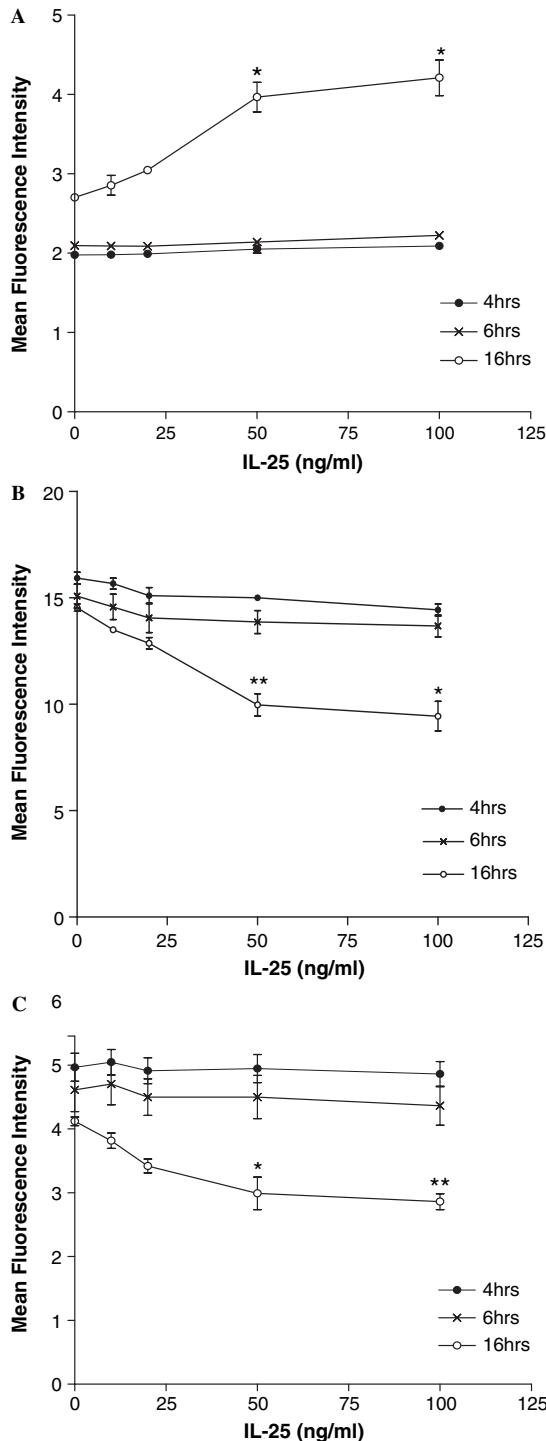


Figure 2. Effect of IL-25 on cell surface expression of: (A) ICAM-1, (B) ICAM-3, and (C) L-selectin on eosinophils. Eosinophils (5×10^5 /well) were cultured with or without IL-25 (10–100 ng/ml) for 4, 8 and 16 h in a 24-well plate. Surface expression of adhesion molecules of 10 000 cells was analyzed by flow cytometry as mean fluorescence intensity. Results have been normalized by subtracting appropriate isotypic control and are expressed as the arithmetic mean $\pm$ SEM of five independent experiments. * $P < 0.05$, ** $P < 0.01$ when compared with medium control.

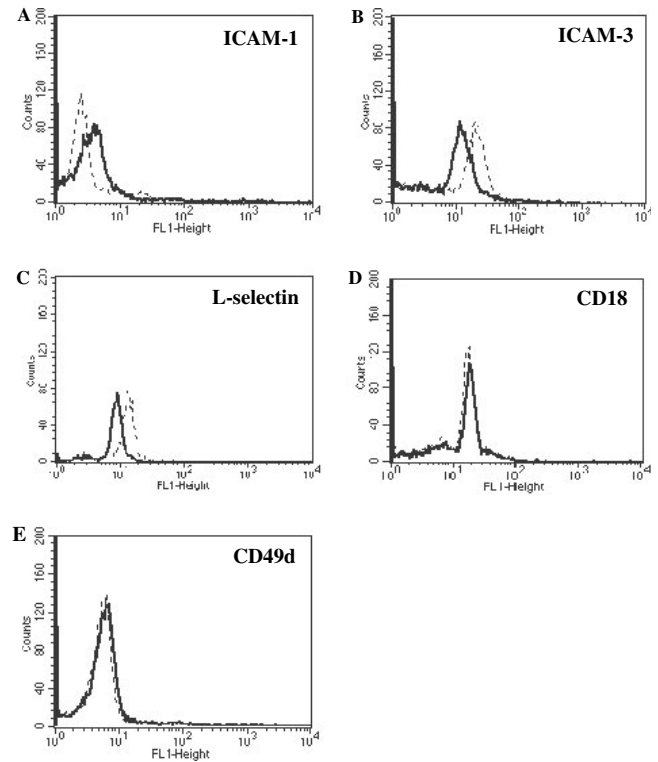


Figure 3. Effects of IL-25 on cell surface expression of: (A) ICAM-1, (B) ICAM-3, (C) L-selectin, (D) CD18 and (E) CD49d on eosinophils. Eosinophils (1×10^6 cells/ml) were treated with (solid line) or without (dotted line) IL-25 (50 ng/ml) for 16 h. Surface expression of the adhesion molecules on eosinophils was determined by flow cytometry. Results are expressed as histograms of relative cells counts with mean fluorescence intensity. These figures are representatives from three independent experiments with similar results.

study has shown that the expression level of ICAM-3 on eosinophils was significantly reduced after exposure to the combination of granulocyte macrophage-colony stimulating factor (GM-CSF) and TNF- α (28). Moreover, ligation of ICAM-3 on eosinophils could suppress GM-CSF synthesis, suggesting that ICAM-3 might possess anti-inflammatory function (28). From our results, eosinophils exposed to IL-25 appeared to switch to a 'suppressed ICAM-3 and enhanced ICAM-1' status, favoring the proinflammatory responses induced by upregulation of ICAM-1.

In addition to ICAM-3, we found that L-selectin expression was also significantly suppressed by IL-25. Our finding concurs with several other studies showing that activated eosinophils could downregulate L-selectin (29–31). Unlike most of the other adhesion molecules, the function of the selectin family is uniquely restricted to the interaction of leukocyte with vascular endothelium. L-selectin mediates the initial attachment of eosinophils to endothelial cells before their firm adhesion and diapedesis at sites of inflammation (32). As eosinophils pass through

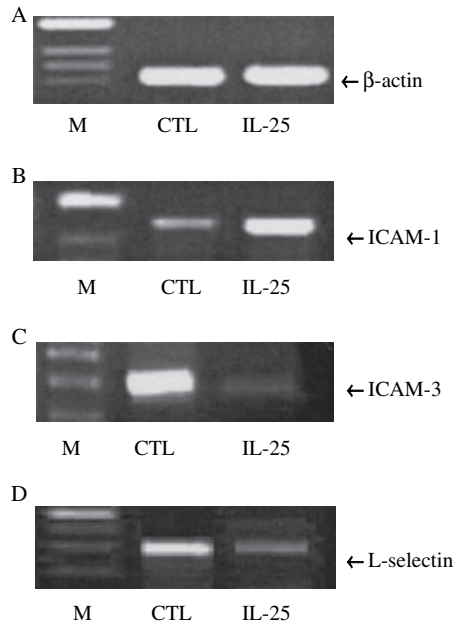


Figure 4. Representative RT-PCR analysis of β -actin, ICAM-1, ICAM-3 and L-selectin mRNA expression in eosinophils. Total RNA was extracted from eosinophils (1×10^7 per treatment) after treated with or without IL-25 (50 ng/ml) for 4 h, followed by reverse transcription and PCR analysis. The β -actin house-keeping gene was used as the control. M: 100 base-pair molecular size marker; CTL: control.

the diverse beds of vascular endothelium, a differential utilization of various adhesion molecules is observed (33). Based on our observation, we postulate that IL-25 may play a role in eosinophil flattening and transmigration by lowering the expression of L-selectin. However, further studies with appropriate functional assays for L-selectin are needed to support this hypothesis. In summary, we suggest that IL-25 may induce eosinophilia in asthmatic lung by enhancing the adhesion of eosinophils onto bronchial epithelial cells and the subsequent transmigration into the inflammatory sites.

Apart from the regulation of adhesion molecule expression, our results revealed that IL-25 could significantly induce Th2 cytokine IL-6, chemokines IL-8, MCP-1 and MIP-1 α synthesis and release from eosinophils in a dose-dependent manner (22). Together with the upregulation of ICAM-1 and suppression of ICAM-3 and L-selectin, our *in vitro* studies support that IL-25 plays a key role in several immunopathological characteristics during allergic asthma, including eosinophilia, Th2 responses, and infiltration of inflammatory cells into sites of inflammation.

To elucidate the mechanism underlying the regulatory effects of IL-25 on ICAM-1, ICAM-3 and L-selectin on eosinophils, we investigate the intracellular signal transduction pathway(s) involved. It was found that the regulatory effects of IL-25 on surface expres-

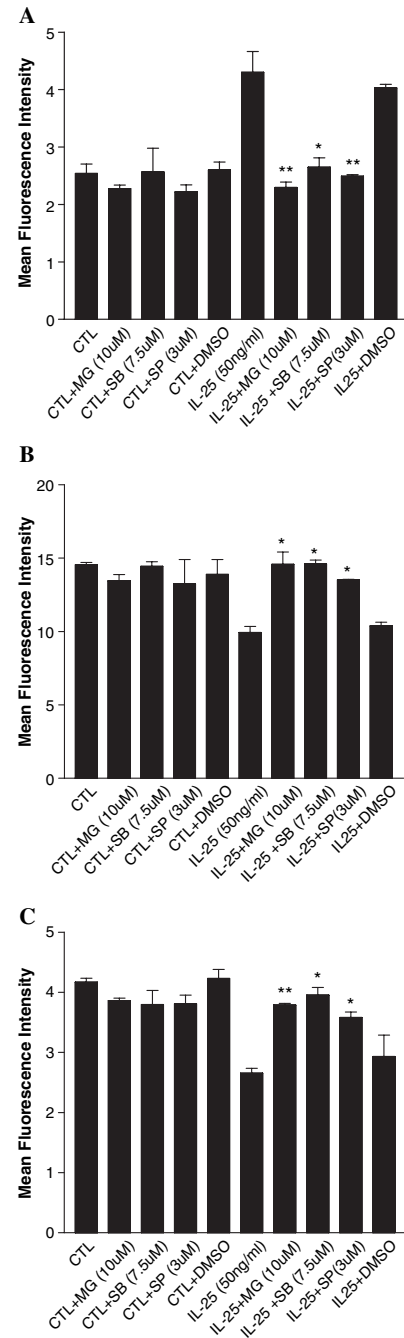


Figure 5. Effect of MG-132, SB203580 and SP600125 on the IL-25-induced surface expression of (A) ICAM-1, (B) ICAM-3 and (C) L-selectin on eosinophils. Eosinophils (5×10^5 /well) were pretreated with MG-132 (5 μ M), SB203580 (7.5 μ M), or SP600125 (3 μ M) for 1 h followed by incubation with or without IL-25 (50 ng/ml) for further 16 h. Surface expression of the adhesion molecules of 10 000 cells was assessed by flow cytometry as mean fluorescence intensity. Results are expressed as the arithmetic mean plus SEM from five independent experiments. DMSO (0.1%) was used as the DMSO control. * $P < 0.05$, ** $P < 0.01$ when compared with the IL-25 control. CTL: medium control; MG: MG-132; SB: SB203580; SP: SP600125.

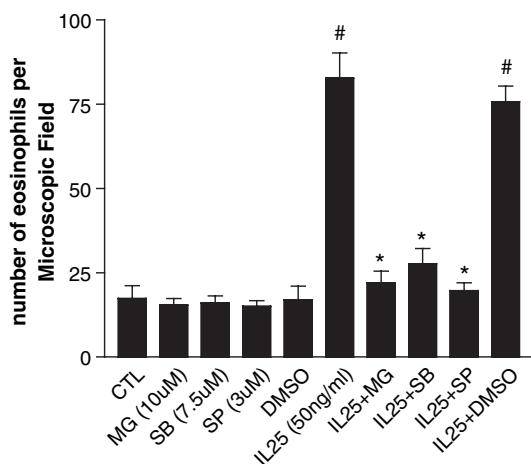


Figure 6. Effect of MG-132, SB203580, and SP600125 on IL-25-induced adhesion of eosinophils onto fibronectin. Purified eosinophils (1×10^6 cells/ml) were treated with or without MG-132 (10 μ M), SB203580 (7.5 μ M) or SP600125 (3 μ M) for 1 h prior to stimulation with IL-25 (50 ng/ml) for further 16 h. Results are expressed as the arithmetic mean numbers of cells adhered to fibronectin-coated insert in four random high power field ($\times 400$) plus SEM of quadruplicate experiments. DMSO (0.1%) was used as the DMSO control. # $P < 0.05$ compared with medium control; * $P < 0.05$ compared with IL-25 control. CTL: medium control; MG: MG-132; SB: SB203580; SP: SP600125.

sion of ICAM-1, ICAM-3 and L-selectin on eosinophils was greatly abrogated by proteasome inhibitor MG-132, JNK inhibitor SP600125, and p38 MAPK inhibitor SB203580. Although MG-132 (10 μ M), SP600125 (3 μ M) and SB203580 (7.5 μ M) exhibited $< 10\%$ toxicity on eosinophils according to our previous

publication (22), it did not have any effect on the adhesion molecule results that were presented with MFI per 10 000 viable eosinophils. Besides, the effect of IL-25 on the adhesion of eosinophils to fibronectin was also significantly restrained by these inhibitors. Moreover, our group has previously found that IL-25 could induce the activation of NF- κ B, p38 MAPK and JNK pathways (22). Altogether, these findings suggested the important role of NF- κ B, JNK and p38 MAPK pathways in mediating the effect of IL-25 on surface expression of ICAM-1, ICAM-3 and L-selectin on eosinophils.

To our knowledge, this is the first report on the regulation of the expression of adhesion molecules on eosinophils by the novel Th2 cytokine, IL-25. We have also shown that the IL-25 induced effects on human eosinophils are mediated through intracellular NF- κ B, JNK and p38 MAPK pathways. Our results underline the important role of IL-25 in eosinophilia, as well as in the overall allergic reaction. In addition to allergic inflammation, mounting evidence suggests that eosinophils can contribute to other pathological processes such as neoplastic, vasculitis granulomatous, interstitial pulmonary and parasitic diseases (34, 35). However, more information is needed to fully understand the mechanism of eosinophil involvement in different diseases. A better understanding of eosinophils may, therefore, provide a biochemical basis for the development of novel clinical strategies.

Acknowledgments

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