

p38 MAP kinase regulates TNF α -, IL-1 α - and PAF-induced RANTES and GM-CSF production by human bronchial epithelial cells

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Summary

Background RANTES and granulocyte macrophage-colony stimulating factor (GM-CSF) play an important role in the production of allergic inflammation of the airway through their chemotactic activity for eosinophils. Recent studies have indicated that p38 mitogen-activated protein (MAP) kinase regulates cytokine expression in various cells; however, the role of p38 MAP kinase in RANTES and GM-CSF production in human bronchial epithelial cells (BECs) has not yet been determined.

Objective In the present study, we examined serine phosphorylation of MKK3 and MKK6 which is the upstream regulator of p38 MAP kinase and p38 MAP kinase activation in tumour necrosis factor (TNF)- α , interleukin (IL)-1 α and platelet-activating factor (PAF)-stimulated BECs and the effect of SB 203580 as the specific inhibitor for p38 MAP kinase activity on RANTES and GM-CSF expression in order to clarify the intracellular signal regulating RANTES and GM-CSF production by human BECs.

Results The results showed that TNF α , IL-1 α and PAF induced serine phosphorylation of MKK3 and MKK6, and p38 MAP kinase activation in BECs. SB 203580 inhibited p38 MAP kinase activity and RANTES and GM-CSF production by TNF α -, IL-1 α - or PAF-stimulated human BECs.

Conclusions These results indicate that p38 MAP kinase plays an important role in TNF α -, IL-1 α - or PAF-activated signalling pathway which regulates RANTES and GM-CSF production by BECs and that the specific inhibitor for p38 MAP kinase activity might be useful for the treatment of allergic inflammation of the airway.

Keywords: bronchial epithelial cells, GM-CSF, p38 MAP kinase, RANTES

Clinical and Experimental Allergy, Vol. 30, pp. 48–55. Submitted 29 August 1998; revised 12 November 1998; accepted 8 March 1999.

Introduction

Bronchial asthma is a disease that is characterized by episodic reversible airway obstruction, airway hyper-responsiveness, and allergic inflammation in the airway [1]. The pathogenesis of allergic inflammation is complex and involves multiple inflammatory cells, cytokines and mediators [2,3]. Of these inflammatory cells, eosinophils

play a pivotal role in the production of allergic inflammation, and increased infiltration of eosinophils into the asthmatic airway is a characteristic feature of allergic inflammation [3,4]. Various cytokines which promote the infiltration of eosinophils into the airway have been demonstrated. Of these cytokines, RANTES and GM-CSF which are known to be produced by bronchial epithelial cells (BECs) [5,6], have been shown to be involved in the production of allergic inflammation through their chemotactic activity for eosinophils [7,8].

The mitogen-activated protein (MAP) kinases are

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important mediators of signal transduction from the cell membrane to the nucleus. Super-family of mammalian MAP kinases have been molecularly characterized: extra-cellular signal-regulated kinase (ERK), c-Jun NH₂-terminal kinase (JNK) and p38 MAP kinase [9]. p38 MAP kinase is activated in various cells by pro-inflammatory cytokines, environmental stresses, DNA-damaging agents, and haematopoietic growth factor [9–18]. We have previously demonstrated that p38 MAP kinase regulates IL-8 expression in tumour necrosis factor (TNF)- α , interleukin (IL)-1 α and platelet-activating factor (PAF)-stimulated BECs [19]. However, the role of p38 MAP kinase in the expression of RANTES and GM-CSF which play an important role in the recruitment of eosinophils into the airway, has not been determined yet. In the present study, we made the role of p38 MAP kinase in cytokine expression in BECs and the production of allergic inflammation of the airway more precise by examining the role of p38 MAP kinase pathway in RANTES and GM-CSF production by BECs. To this end, we attempted to examine serine phosphorylation of MKK3 and MKK6 which is the upstream regulator of p38 MAP kinase [20–24] and p38 MAP kinase activation in TNF α -, IL-1 α - and PAF-stimulated BECs and the effect of SB 203580 as the specific inhibitor for p38 MAP kinase activity on RANTES and GM-CSF production in order to clarify the intracellular signal regulating RANTES and GM-CSF production by BECs.

Materials and methods

Reagents

Human recombinant IL-1 α and TNF α were kindly provided by Ohtsuka Pharmaceutical Co. Ltd. (Tokushima, Japan) and Dainippon Pharmaceutical Co. Ltd. (Osaka, Japan), respectively. PAF was obtained from Sigma Chemicals Co. (St. Louis, MO, USA). The pyridinylimidazole SB 203580, the specific inhibitor for p38 MAP kinase activity [25], which was kindly provided by Smithkline Beecham (King of Prussia, PA, USA) and was dissolved in dimethyl sulfoxide.

Cell culture

Transformed human bronchial epithelial cell line (BET-1A) was kindly provided by Dr Hajime Nakamura (Yamagata University School of Medicine). The cells (1×10^4 cells/mL) were placed onto collagen-coated 24-well flat-bottom tissue culture plate (Iwaki, Tokyo, Japan) for determination of cytokine production and collagen-coated tissue culture dish (Iwaki) for analysis of serine phosphorylation of MKK3/MKK6 and p38 MAP kinase activity using Ham's F12 medium containing 1% penicillin-streptomycin, 5 μ g/mL of insulin (GIBCO, Grand Island, NY, USA), 5 μ g/mL

of transferrin (GIBCO), 25 ng/mL of epidermal growth factor (EGF, Collaborative Research, Lexington, MA, USA), 15 μ g/mL of endothelial cell growth supplement (ECGS, Collaborative Research), 2×10^{-10} mol/L triiodothyronine (GIBCO), and 10^{-7} mol/L hydrocortisone (GIBCO). The cells were grown until they were sub-confluent and the medium was changed. In order to examine the effect of TNF α , IL-1 α and PAF on serine phosphorylation of MKK3 and MKK6, and p38 MAP kinase activation in BECs, the cells that had been pre-incubated with Ham's F12 medium without insulin, transferrin, EGF, ECGS, triiodothyronine and hydrocortisone (growth factor free medium) for 16 h were stimulated with TNF α , IL-1 α or PAF and cultured in growth factor free medium for the desired times. To examine TNF α -, IL-1 α - and PAF-induced RANTES and GM-CSF production by BECs, and the effect of SB 203580 as the p38 specific inhibitor on p38 MAP kinase activity and on RANTES and GM-CSF production, the cells that had been pre-incubated with growth factor free medium for 16 h were pre-incubated with or without SB 203580 for 1 h, and then the cells were stimulated either with TNF α , IL-1 α or PAF and cultured in growth factor free medium for the desired times at 37°C in humidified 5% CO₂ atmosphere. At the end of culture periods, the culture supernatants for determination of RANTES and GM-CSF protein were harvested, centrifuged and the supernatants were collected, filtered through a millipore filter and stored at -80°C until assay.

Measurement of RANTES and GM-CSF

The concentrations of RANTES and GM-CSF in the culture supernatants from BECs were measured by commercially available ELISA kits (Amersham International, Amersham, UK). ELISA was performed according to the manufacturer's instruction. All samples were assayed in duplicate.

Western blot analysis of MKK3 and MKK6

The serine phosphorylation of MKK3 and MKK6 kinase was analysed by commercially available kits (Phospho-Plus™ MKK3/MKK6 [Ser189/207] Antibody Kit, New England Biolabs Inc., Beverly, MA, USA). Phosphospecific MKK3/MKK6 antibody supplied in this kit selectively detects Ser189- and Ser207-phosphorylated MKK3 and MKK6, respectively. As the activation sites of both proteins are closely related, both phosphoproteins are detected by this antibody. Polyclonal antibodies were produced by immunizing rabbits with a synthetic phospho-Ser189 peptide (KLH coupled) corresponding with residues 185–194 of human MKK3. Analysis of serine phosphorylation of MKK3 and MKK6 was performed according to the manufacturer's instruction. Briefly, the cells that had been

washed with cold TRIS-buffered saline were lysed in SDS buffer (62.5 mmol/L TRIS-HCl, pH 6.8, 2% w/v SDS, 10% glycerol, 50 mmol/L DTT, 0.1% w/v bromphenol blue) for 15 min on ice and sonicated for 2 s to shear DNA. The samples were heated in a boiling water bath for 5 min to fully denature the proteins prior to electrophoresis and then centrifuged at 12 000 *g* for 5 min to remove insoluble debris. After separating proteins from cell lysate by 15% SDS-PAGE, proteins were electrophoretically transferred to nitrocellulose membrane and the membrane was washed with 0.1% Tween-20 supplemented with TRIS-buffered saline (washing buffer). To block non-specific protein binding, the membrane was incubated with 0.1% Tween-20 supplemented with TRIS-buffered saline containing 5% w/v non-fat dried skimmed milk for 3 h at room temperature. It was then incubated with phosphospecific MKK3/MKK6 antibody (affinity purified rabbit polyclonal IgG) at 1:1000 dilution in 0.1% Tween-20 supplemented with TRIS-buffered saline containing 5% BSA at 4°C overnight with gentle shaking. After washing with washing buffer three times, it was incubated for 1 h with gentle shaking at room temperature with the horseradish peroxidase-conjugated antirabbit antibody (1:2000) and horseradish peroxidase-conjugated antibiotin antibody (1:1000) to detect biotinylated protein markers, and then washed three times with washing buffer. Blots were incubated with enhanced chemiluminescence solution (LumiGLO) for 1 min at room temperature and exposed on Kodak XAR film. To show the amounts of MKK3 precipitated, blots were stripped and reprobed using phosphorylation state-independent MKK3-specific antibody to determine total MKK3 levels. Phosphorylation state-independent MKK3-specific antibodies (affinity purified rabbit polyclonal IgG, New England BioLabs Inc.) are produced by immunizing rabbits with a synthetic peptide (KLH coupled) corresponding with residues 296–309 of human MKK3. This antibody dose not cross-react with MKK6, MKK4, MEK1 and MEK2.

p38 MAP kinase assay

The activity of p38 MAP kinase was analysed by commercially available kits (p38 MAP Kinase Assay Kit, New England Biolabs Inc.). The kit employs two different antibodies, anti-p38 MAP kinase antibody which is specific for p38 MAP kinase and dose not cross-react with ERK1/2 or JNK, and antiphosphospecific ATF-2 antibody to detect p38 MAP kinase-induced phosphorylation of ATF-2. Analysis of activity of p38 MAP kinase was performed according to the manufacturer's instruction. Briefly, BECs that had been washed with ice-cold PBS were lysed with 1.0 mL of ice-cold lysis buffer (20 mmol/L TRIS pH 7.4, 150 mmol/L NaCl, 1 mmol/L EDTA, 1 mmol/L EGTA, 1% Triton,

2.5 mmol/L sodium pyrophosphate, 1 mM β -glycerolphosphate, 1 mM Na_3VO_4 , 1 $\mu\text{g}/\text{mL}$ Leupeptin) plus 1 mmol/L PMSF in the six well plate on ice for 5 min. After sonication, the cell lysate was microcentrifuged for 10 min at 4°C. 200 μL of cell lysate was incubated with anti-p38 MAP kinase antibody (1:100 dilution) to selectively immunoprecipitate p38 MAP kinase from cell lysates at 4°C overnight with gentle shaking. The resulting immunoprecipitate was then mixed with protein A sepharose beads. After microcentrifugation, the pellet was washed twice with lysis buffer and then washed twice with kinase buffer (25 mmol/L TRIS pH 7.5, 5 mmol/L β -glycerolphosphate, 2 mmol/L DTT, 0.1 mmol/L Na_3VO_4 , 10 mmol/L MgCl_2). The pellet was suspended in 50 μL of kinase buffer with 100 $\mu\text{mol}/\text{L}$ ATP and 2 μg of ATF-2 fusion protein, and then incubated for 30 min at 30°C. The pellet was mixed with the sample buffer consisting of 62.5 mmol/L TRIS base, 10% glycerol, 50 mmol/L DTT and 2% SDS, and then heated in a boiling water bath for 5 min to fully denature the protein prior to electrophoresis. Equal amounts of protein (5 $\mu\text{g}/\text{lane}$) were separated in a 15% SDS-PAGE, and transferred to a polyvinylidene difluoride sheet (Millipore, Bedford, MA, USA) by electroelution with a constant current of 200 mA for 90 min at room temperature. After blocking with 0.1% Tween 20 supplemented with PBS (T-PBS) containing dried skimmed milk overnight, the sheet was incubated with antiphosphospecific ATF-2 antibody (1:1000 dilution) at 4°C overnight. The sheet was then washed three times with T-PBS, and incubated with horseradish peroxidase (HRP)-conjugated antirabbit antibody (1:2000) and HRP-conjugated antibiotin antibody (1:2000) for 1 h at room temperature. After washing with T-PBS three times, the sheet was incubated with 10 mL of the ECL (enhanced chemiluminescence) solution for 1 min according to manufacturer's instructions, and exposed on Fuji medical X-ray film (AIF new RX) for 1 min.

Statistical analysis

Statistical significance was analysed using analysis of variance (ANOVA). *P*-values less than 0.05 were considered significant.

Results

TNF α , IL-1 α and PAF induce RANTES and GM-CSF production. Firstly, we examined a dose-dependent induction of RANTES and GM-CSF production by BECs. To this end, the culture supernatants from BECs stimulated with various concentrations of TNF α , IL-1 α or PAF were harvested at 24 h after cultivation (Fig. 1). The concentrations of RANTES and GM-CSF in the culture supernatants from

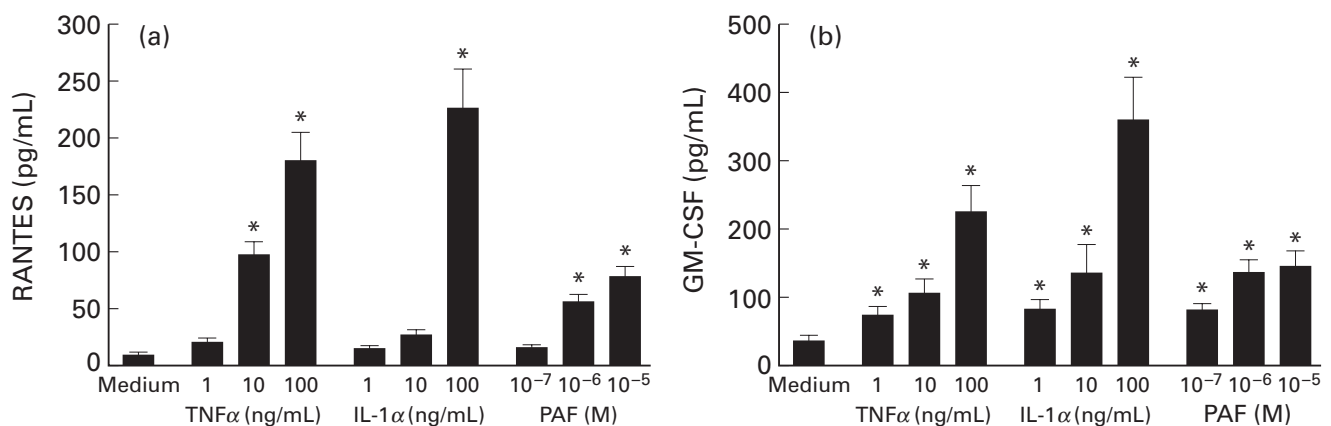


Fig. 1. TNF α , IL-1 α and PAF induce RANTES and GM-CSF production by BEC. BEC were cultured either with medium, various concentrations of TNF α , IL-1 α or PAF, and the concentrations of RANTES (a) and GM-CSF (b) in the culture supernatants were determined at 24 hours after cultivation as described in Materials and methods. The results are expressed as the mean $\pm$ SD in three different experiments. *, $P < 0.01$ compared with RANTES or GM-CSF concentration in BEC cultured with medium.

TNF α -stimulated culture increased in a dose-dependent manner. Similarly, the concentrations of RANTES and GM-CSF in the culture supernatants from IL-1 α - or PAF-stimulated culture increased in a dose-dependent manner.

TNF α , IL-1 α and PAF cause serine phosphorylation of MKK3 and MKK6. To determine whether TNF α , IL-1 α and PAF induce serine phosphorylation of MKK3 and MKK6, BECs were stimulated and immunoblot study was performed. The immunoblot study of lysate of TNF α -stimulated BECs with a specific antibody which was produced by immunizing rabbits with human MKK3 detected phosphorylated serine MKK3 and MKK6 showing that amounts of phosphorylated serine of MKK3 and MKK6 protein increased. Amounts of phosphorylated serine of MKK3 and MKK6 increased in IL-1 α - and PAF-stimulated BECs (Fig. 2a, upper panel). Blots shown in the upper panel of Fig. 2(a) were stripped and reprobed using a MKK3-specific control antibody. Lower panel of Fig. 2(a) showed that equal amounts of MKK3 protein were immunoblotted with MKK3-specific control antibody regardless of the stimulation with TNF α , IL-1 α and PAF, indicating that TNF α , IL-1 α or PAF stimulation-induced increases in serine phosphorylation of MKK3 and MKK6 occurred in the absence of changes in MKK3 protein levels. When BECs were cultured with growth factor-free medium instead of stimulation with TNF α , IL-1 α and PAF, an increase in amounts of serine phosphorylation of MKK3 and MKK6 was not observed (data not shown).

TNF α , IL-1 α and PAF cause p38 MAP kinase activation, and SB 203580 inhibits p38 MAP kinase activity. We next examined whether TNF α , IL-1 α and PAF could induce p38 MAP kinase activation. p38 MAP kinase activity was analysed by a specific immunoprecipitation

with anti-p38 MAP kinase antibody followed by an *in vitro* kinase assay of its substrate, ATF-2. As shown in Fig. 3, TNF α , IL-1 α and PAF activated p38 MAP kinase as the demonstrated by the increased phosphorylation of ATF-2. Pretreatment of the cells with SB 203580 as the specific inhibitor for p38 MAP kinase activity abolished TNF α -, IL-1 α - and PAF-induced increases in p38 MAP kinase activity.

SB 203580 inhibits TNF α -, IL-1 α - and PAF-induced RANTES and GM-CSF production

TNF α , IL-1 α and PAF induced the production of RANTES and GM-CSF and the activation of p38 MAP kinase pathway. In addition, SB 203580 inhibited this stimuli-induced p38 MAP kinase activity. These results suggested that TNF α -, IL-1 α - or PAF-induced RANTES and GM-CSF production might be mediated through p38 MAP kinase-dependent pathway. To test this possibility, BECs that had been pre-incubated with or without various concentrations of SB 203580 were cultured either with TNF α , IL-1 α or PAF, and the concentrations of RANTES and GM-CSF in the culture supernatants were determined at 24 h after cultivation. As shown in Fig. 4, SB 203580 inhibited RANTES production by TNF α -, IL-1 α - or PAF-stimulated BECs in a concentration-dependent manner. Similarly, SB 203580 inhibited GM-CSF production by TNF α -, IL-1 α - or PAF-stimulated BECs in a concentration-dependent manner.

The total number of cells and cell viability at the end of culture period of each experiment determined by trypan blue exclusion assay did not differ with culture conditions, suggesting that TNF α -, IL-1 α - or PAF-induced RANTES and GM-CSF production and the inhibition by p38 MAP

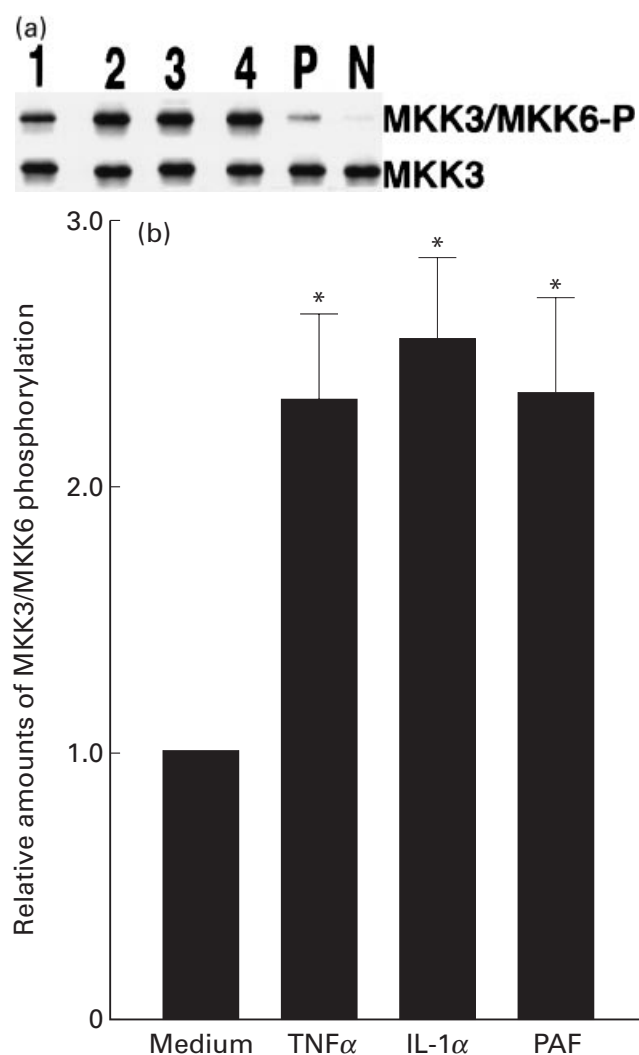


Fig. 2. TNF α , IL-1 α and PAF cause serine phosphorylation of MKK3 and MKK6 in BEC. BEC were stimulated with medium (lane 1), 100 ng/mL of TNF α (lane 2), 100 ng/mL of IL-1 α (lane 3), 10^{-5} mol/L of PAF (lane 4) for 10 minutes. The lysates from BEC were separated by a 10% SDS-polyacrylamide gel, transferred to membranes, and blotted with a specific antibody to phosphorylated serine of MKK3 and MKK6 (MKK3/MKK6-P; upper panel of a). Blots shown in the upper panel were stripped and reprobed using a MKK3-specific control antibody to show the amounts of MKK3 blotted (MKK3; lower panel of a). Lane P, positive protein prepared from NIH 3T3 cells stimulated with UV treatment for phosphorylated serine of MKK3 and MKK6; Lane N, negative protein prepared from NIH 3T3 cells without UV treatment. Three identical experiments independently performed gave similar results. The amounts of serine phosphorylation of MKK3 and MKK6 were quantitated by NIH image analyzer and are presented as the amounts of serine phosphorylation of MKK3 and MKK6 relative to control cells treated without agonist (1.0) (b). The results are expressed as the mean $\pm$ SD in three different experiments. * P < 0.01 compared with control cells treated without agonist.

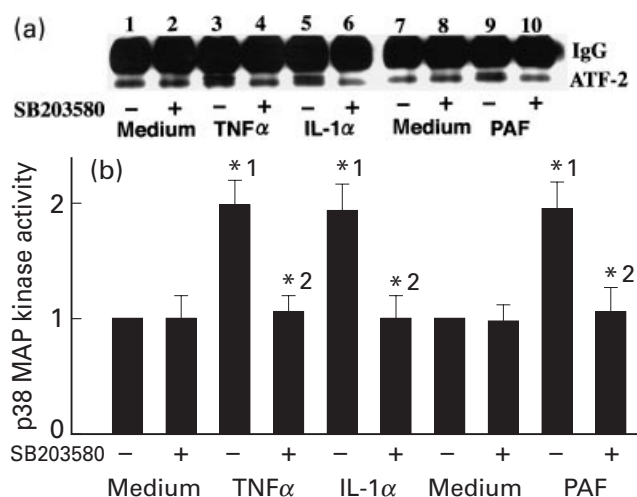


Fig. 3. TNF α , IL-1 α and PAF activate p38 MAP kinase in BEC and SB 203580 inhibits p38 MAP kinase activity. BEC that had been cultured either with or without SB 203580 (10 μ mol/L) for 60 minutes were stimulated with TNF α (100 ng/mL), IL-1 α (100 ng/mL) or PAF (10^{-5} mol/L) for 15 minutes. At the end of cultivation, the cell lysates from the cells were incubated with anti-p38 MAP kinase antibody to selectively immunoprecipitate p38 MAP kinase from the cells lysates and the immunoprecipitates were incubated with ATF-2 fusion protein in the presence of ATP, which allowed immunoprecipitated-active p38 MAP kinase to phosphorylate ATF-2. The samples were separated by a 15% SDS-PAGE, transferred to membranes, and blotted with anti-phosphospecific ATF-2 antibody. The cells were cultured with medium (lane 1), SB 203580 (lane 2), TNF α (lane 3), TNF α and SB 203580 (lane 4), IL-1 α (lane 5), IL-1 α and SB 203580 (lane 6), medium (lane 7), SB 203580 (lane 8), PAF (lane 9) or PAF and SB 203580 (lane 10) (a). SB 203580 was dissolved in dimethyl sulfoxide in this study and the concentrations of dimethyl sulfoxide were 0.01%, which had no effect. Three identical experiments independently performed gave similar results. p38 MAP kinase activity as demonstrated by the increased phosphorylation of ATF-2 was quantitated by NIH image analyser and are presented as p38 MAP kinase activity relative to control cells treated without agonist (1.0) (b). The results are expressed as the mean $\pm$ SD in three different experiments. *1; * P < 0.01 compared with control cells pretreated with medium instead of SB 203580 followed by the cultivation with medium. *2; * P < 0.01 compared with cells pretreated with medium instead of SB 203580 followed by the stimulation with TNF α , IL-1 α or PAF.

kinase inhibitor of RANTES and GM-CSF production did not result from cytotoxicity.

Discussion

In the present study, we examined the signal transduction pathway in TNF α -, IL-1 α - and PAF-induced RANTES and GM-CSF production by human BECs. The results

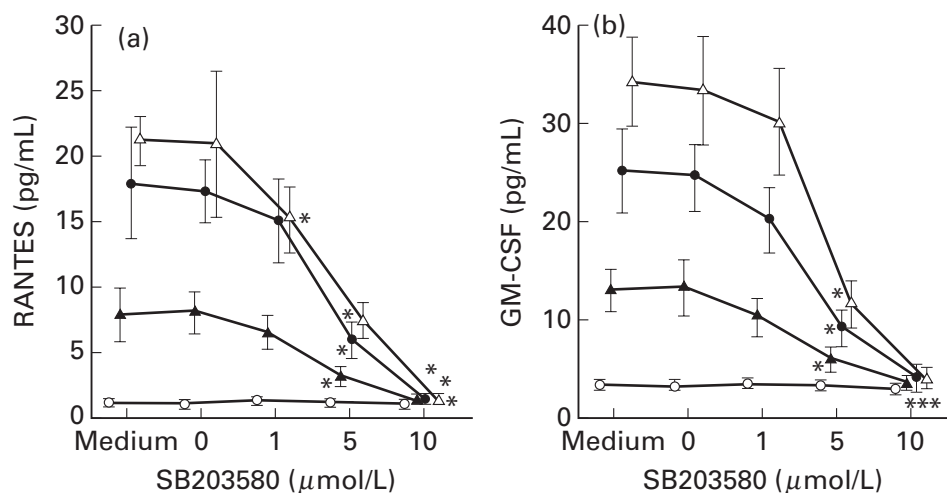


Fig. 4. SB 203580 inhibits TNF α -, IL-1 α - and PAF-induced RANTES and GM-CSF production by BEC. BE that had been preincubated with or without various concentrations of SB 203580 for 1 h were cultured either with medium, 100 ng/mL of TNF α , 100 ng/mL of IL-1 α or 10^{-5} mol/L of PAF and the concentrations of RANTES (a) and GM-CSF (b) in the culture supernatants were determined 24 h after cultivation as described in Materials and methods. SB 203580 was dissolved in dimethyl sulfoxide in this study. 0.5 μ mol/L, 1 μ mol/L, 5 μ mol/L and 10 μ mol/L of SB 203580 contained 0.0005%, 0.001%, 0.005% and 0.01% of dimethyl sulfoxide, respectively. These concentrations of dimethyl sulfoxide had no effect on TNF α -, IL-1 α - and PAF-induced RANTES and GM-CSF production. The results are expressed as the mean $\pm$ sd in five different experiments. *, $P < 0.01$ compared with RANTES or GM-CSF concentrations in BEC cultured without SB 203580.

showed that TNF α , IL-1 α and PAF induced RANTES and GM-CSF production and serine phosphorylation of MKK3 and MKK6 and p38 MAP kinase activation in human BECs. SB 203580 as the specific inhibitor for p38 MAP kinase activity inhibited TNF α -, IL-1 α - and PAF-induced p38 MAP kinase activity and RANTES and GM-CSF production. These results indicate that p38 MAP kinase pathway play an important role in TNF α -, IL-1 α - and PAF-activated signalling pathway which regulates RANTES and GM-CSF production by human BECs.

In the present study, we examined the role of p38 MAP kinase pathway in TNF α -, IL-1 α - and PAF-induced RANTES and GM-CSF production by BECs. TNF α , IL-1 α and PAF were used as inducer for RANTES and GM-CSF production by BECs, since TNF α , IL-1 α and PAF have been shown to be involved in the production of allergic inflammation [4,26–31] and can stimulate BECs to induce cytokine expression in BECs [5,6].

The p38 MAP kinase pathway has been characterized. Activation of p38 MAP kinase is mediated by dual phosphorylation of threonine and tyrosine by MKK3 and MKK6 [20–24]. p38 MAP kinase activation culminates in the phosphorylation of downstream cytosolic and nuclear factors that regulate various cellular responses [12,32,33]. In the present study, we firstly examined whether TNF α , IL-1 α and PAF could induce serine phosphorylation of MKK3 and MKK6 which are the upstream regulators

of p38 MAP kinase [19–23]. Second, p38 MAP kinase activation was analysed. The results showed that TNF α , IL-1 α and PAF induced serine phosphorylation of MKK3/MKK6 as well as p38 MAP kinase activation. In addition, SB 203580 inhibited TNF α -, IL-1 α - and PAF-induced p38 MAP kinase activity. These results indicated that TNF α , IL-1 α and PAF actually activated p38 MAP kinase pathway. Finally, we determined the role of p38 MAP kinase in TNF α -, IL-1 α - and PAF-induced RANTES and GM-CSF production by BECs using SB 203580 as the specific inhibitor for p38 MAP kinase activity. Up to 10 μ mol/L of SB 203580 was used, since the previous study with analysis of the role of p38 MAP kinase in cytokine expression showed that 10 μ mol/L of SB 203580 almost completely inhibited the expression of cytokines [14,18,19]. The results showed that 10 μ mol/L of SB 203580 almost completely inhibited TNF α -, IL-1 α - and PAF-induced RANTES and GM-CSF production by BECs. Since, very recently, 10 μ mol/L of SB 203580 has been shown to also inhibit JNK activity [34], we cannot conclude that p38 MAP kinase alone regulates TNF α -, IL-1 α - and PAF-induced RANTES and GM-CSF production. However, the results with serine phosphorylation of MKK3 and MKK6, p38 MAP kinase activation, and the inhibition of p38 MAP kinase activity and RANTES and GM-CSF production by SB 203580 indicate that p38 MAP kinase, at least, plays an important role in TNF α -, IL-1 α - and PAF-activated signalling

pathway which regulates RANTES and GM-CSF production by BECs. We analysed p38 MAP kinase activity by a specific immunoprecipitation with anti-p38 MAP kinase antibody followed by *in vitro* kinase assay of its substrate, ATF-2. p38 MAP kinase activity was demonstrated by the increased phosphorylation of ATF-2. Although the concentrations of RANTES and GM-CSF in the culture supernatants from PAF-stimulated BECs were lower than those in TNF α - or IL-1 α -stimulated BECs, amounts of phosphorylated ATF-2 in PAF-stimulated BECs were comparable with those in TNF α - or IL-1 α -stimulated BECs. Time-course of RANTES and GM-CSF production by PAF-stimulated BECs was similar to that by TNF α - or IL-1 α -stimulated BECs (data not shown). Discrepancy between p38 MAP kinase activity as demonstrated by amounts of phosphorylated ATF-2 and the concentrations of cytokines might result from the sensitivity to detect p38 MAP kinase activity. In addition, other signalling pathway(s) might be involved in regulating RANTES and GM-CSF production.

Bronchial asthma is regarded as an allergic inflammation of the airways [1–4]. Although the pathogenesis of allergic inflammation is complex, RANTES and GM-CSF which exhibit a chemotactic activity for eosinophils [7,8] have been shown to play a pivotal role of the production of allergic inflammation through the recruitment of eosinophils into the site of allergic inflammation. Therefore, the inhibition of RANTES and GM-CSF production by BECs is an important strategy for the treatment of bronchial asthma. The specific inhibitor for p38 MAP kinase activity, SB 203580, has been thought to be useful in the treatment of inflammatory diseases [25]. In the present study, we showed the inhibition of RANTES and GM-CSF production by SB 203580. Therefore, the specific inhibitor for p38 MAP kinase activity, SB 203580, may have a potential effect on controlling allergic inflammation through inhibiting RANTES and GM-CSF production.

From the data presented here, we conclude that TNF α -, IL-1 α - and PAF-induced RANTES and GM-CSF production by BECs is, at least, regulated by p38 MAP kinase. The inhibition of p38 MAP kinase pathway by the specific inhibitor for p38 MAP kinase activity is thought to be an important strategy for the treatment of bronchial asthma.

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