

Proinflammatory cytokine-induced and chemical mediator-induced IL-8 expression in human bronchial epithelial cells through p38 mitogen-activated protein kinase-dependent pathway

Ken Matsumoto, MD, Shu Hashimoto, MD, PhD, Yasuhiro Gon, MD, PhD,
Tomoko Nakayama, MD, PhD, and Takashi Horie, MD, PhD Tokyo, Japan

The p38 mitogen-activated protein (MAP) kinase is activated in various cells by proinflammatory cytokines and environmental stresses. However, little is known about the role of p38 MAP kinase in proinflammatory cytokine- and chemical mediator-induced cytokine expression in human bronchial epithelial cells (BECs). In this study we examined the role of p38 MAP kinase in IL-8 expression in BECs to clarify the signal transduction pathway regulating IL-8 expression in BECs stimulated with tumor necrosis factor- α (TNF- α), IL-1 α , and platelet-activating factor (PAF). We used TNF- α , IL-1 α , and PAF as inducers for the analysis of the signal transduction pathway and determined IL-8 expression in BECs because TNF- α , IL-1 α , and PAF are known to induce cytokine expression in BECs, and these proinflammatory cytokines and PAF are described to have a role in the production of allergic inflammation. The results showed that TNF- α , IL-1 α , and PAF induced tyrosine phosphorylation of p38 MAP kinase in a dose- and time-dependent manner. The specific p38 MAP kinase inhibitor, SB 203580, completely inhibited TNF- α , IL-1 α , or PAF-induced IL-8 protein and mRNA expression in BECs. These results indicated that p38 MAP kinase plays an important role in TNF- α , IL-1 α , or PAF-activated signaling pathway, which regulates IL-8 expression in BECs. In addition, these results provide new evidence on a strategy for treatment of airway inflammation with the specific p38 MAP kinase inhibitor. (J Allergy Clin Immunol 1998;101:825-31.)

Key words: Tumor necrosis factor- α , IL-1 α , platelet-activating factor, p38 mitogen-activated protein kinase, bronchial epithelial cells

Bronchial asthma is a disease that is characterized by episodic reversible airway obstruction, airway hyperresponsiveness, and allergic inflammation in the airway.¹ The pathogenesis of allergic inflammation is complex and involves multiple inflammatory cells and mediators.^{2,3} The involvement of macrophage-derived cytokines such as tumor necrosis factor (TNF)- α and IL-1 in the production of allergic inflammation has been described,⁴⁻⁸ and TNF- α and IL-1 have been described to

Abbreviations used

BEC:	Bronchial epithelial cell
MAP:	Mitogen-activated protein
PAF:	Platelet-activating factor
SDS:	Sodium dodecylsulfate
TNF- α :	Tumor necrosis factor- α

stimulate airway epithelial cells to produce a variety of cytokines that initiate and/or amplify allergic inflammation.⁹⁻¹³ Of these cytokines, IL-8, which is well known to be produced by bronchial epithelial cells (BECs),¹¹⁻¹³ has been suggested to have a role in the pathogenesis of allergic inflammation.^{4, 14-17}

The mitogen-activated protein (MAP) kinases are important mediators of signal transduction from the cell membrane to the nucleus. Several subgroups of mammalian MAP kinases have been molecularly characterized, including extracellular signal-regulated kinase, c-Jun NH₂-terminal kinase, and p38 MAP kinase.¹⁸ The p38 MAP kinase, which is a mammalian homologue of the HOG-1 of *Saccharomyces cerevisiae*,¹⁸⁻²⁰ is activated in various cells by proinflammatory cytokines, environmental stresses, DNA-damaging agents, and hematopoietic growth factor.¹⁸⁻²⁷ However, little is known about the role of p38 MAP kinase in proinflammatory cytokine- and chemical mediator-induced cytokine expression in human BECs. As mentioned, IL-8 produced in BECs play an important role in the production of airway inflammation. Therefore clarification of the signal transduction pathway in IL-8 expression and regulation of IL-8 expression in BECs are thought to be important strategies for the treatment of airway inflammatory diseases.

In this study we examined the role of p38 MAP kinase in the induction of IL-8 expression in human BECs to clarify the signal transduction pathway in the IL-8 expression in BECs stimulated with proinflammatory cytokines such as TNF- α and IL-1 α and chemical mediators such as platelet-activating factor (PAF).

METHODS

Reagents

Human recombinant IL-1 α and TNF- α were kindly provided by Ohtsuka Pharmaceutical Co. Ltd. (Tokushima, Japan) and

From the First Department of Internal Medicine, Nihon University School of Medicine, Tokyo.

Received for publication Dec. 4, 1997; revised Feb. 2, 1998; accepted for publication Feb. 17, 1998.

Reprint requests: Shu Hashimoto, MD, PhD, First Department of Internal Medicine, Nihon University School of Medicine, 30-1 Otaguchikamimachi, Itabashi-Ku, Tokyo 173, Japan.

Copyright © 1998 by Mosby, Inc.

0091-6749/98 \$5.00 + 0 1/1/89693

Dainippon Pharmaceutical Co. Ltd. (Osaka, Japan), respectively. PAF was obtained from Sigma Chemicals Co. (St. Louis, Mo.). The pyridinyl imidazole SB 203580, the specific p38 MAP kinase inhibitor,²⁸ was kindly provided by SmithKline Beecham and was dissolved in dimethyl sulfoxide.

Cell culture

Transformed human BEC 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 plates (Iwaki, Tokyo, Japan) for determination of cytokine production and collagen-coated tissue-culture dishes (Iwaki) for western blot and northern blot analysis with Ham's F12 medium. The medium contained 1% penicillin-streptomycin, 5 μ g/ml insulin (GIBCO, Grand Island, N.Y.), 5 μ g/ml transferrin (GIBCO), 25 ng/ml epidermal growth factor (Collaborative Research, Lexington, Mass.), 15 μ g/ml endothelial cell growth supplement (Collaborative Research), 2×10^{-10} mol/L triiodothyronine (GIBCO), and 10^{-7} mol/L hydrocortisone (GIBCO). The cells were grown until they were subconfluent and the medium was changed. To examine the effect of proinflammatory cytokines and chemical mediators on tyrosine phosphorylation of p38 MAP kinase in BECs, the cells that had been pretreated with Ham's F12 medium without insulin, transferrin, epithelial growth factor, endothelial cell growth supplement, triiodothyronine, and hydrocortisone (growth factor-free medium) for 1 hour were stimulated with various concentrations of IL-1 α , TNF- α , or PAF and incubated for the desired times. To examine proinflammatory cytokine- and chemical mediator-induced IL-8 expression in BECs and the effect of SB 203580 as the p38-specific inhibitor, the cells that had been preincubated with growth factor-free medium (with or without SB 203580) for 1 hour were stimulated with TNF- α , IL-1 α , or PAF and cultured for the desired times at 37° C in humidified 5% CO₂ atmosphere. At the end of the culture periods, the culture supernatants for determination of IL-8 protein were harvested and centrifuged, and the supernatants were collected, filtrated with a millipore filter, and stored at -80° C until assayed. The cells for analysis of IL-8 mRNA expression were collected and stored at -80° C until analysis of mRNA expression.

Measurement of IL-8

The concentration of IL-8 in the culture supernatants from BECs were measured by commercially available ELISA kits (Amersham International, Aylesbury, U.K.). ELISA was performed according to the manufacturer's instruction. All samples were assayed in duplicate.

Western blot analysis of p38 MAP kinase

The tyrosine phosphorylation of p38 MAP kinase was analyzed by commercially available kits (PhosphoPlus p38 MAPK Antibody Kit; New England Biolabs, Inc., Beverly, Mass.). Analysis of tyrosine phosphorylation of p38 MAP kinase was performed according to the manufacturer's instruction. Briefly, the cells that had been washed with cold Tris-buffered saline were lysed in sodium dodecylsulfate (SDS) buffer (62.5 mmol/L Tris-HCl [pH 6.8], 2% wt/vol SDS, 10% glycerol, 50 mmol/L dithiothreitol, and 0.1% wt/vol bromophenol blue) for 15 minutes on ice and sonicated for 2 seconds to shear DNA. The samples were heated in a boiling water bath for 5 minutes to fully denature the proteins before electrophoresis and then centrifuged at 12,000 g for 5 minutes to remove insoluble debris. After separating proteins from cell lysate by 15% sodium

dodecylsulfate-polyacrylamide gel electrophoresis, proteins were electrophoretically transferred to a nitrocellulose membrane, and the membrane was washed with 0.1% Tween-20 supplemented with Tris-buffered saline (washing buffer). To block nonspecific protein binding, the membrane was incubated with 0.1% Tween-20 supplemented with Tris-buffered saline containing 5% wt/vol nonfat dry milk for 3 hours at room temperature. It was then incubated with specific antibody to phosphorylated tyrosine of p38 MAP kinase (affinity-purified rabbit polyclonal IgG) at 1:1000 dilution in 0.1% Tween-20 supplemented with Tris-buffered saline containing 5% bovine serum albumin at 4° C overnight with gentle shaking. After washing with washing buffer three times, it was incubated for 1 hour with gentle shaking at room temperature with the horseradish peroxidase-conjugated anti-rabbit antibody (1:2000) and horseradish peroxidase-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers. It was then washed three times with washing buffer. Blots were incubated with enhanced chemiluminescence solution (LumiGLO) for 1 minute at room temperature and exposed on KODAK XAR film. To show the amounts of p38 MAP kinase precipitated, blots were stripped and reprobed with phosphorylation state-independent p38 MAP kinase-specific antibody to determine total p38 MAP kinase levels (affinity-purified rabbit polyclonal IgG).

Northern blot analysis

Total RNA was prepared with an RNA extraction kit (RNAzol B.; Cinna Scientific, Friedswald, Tex.) by using the acid guanidine thiocyanate-phenol-chloroform extraction methods. Total RNA (10 μ g) was denatured in a solution containing formaldehyde and formamide and electrophoresed in a 1% agarose gel containing formaldehyde.²⁹ Then it was capillary transferred onto a nylon membrane (Hybond N, Amersham). The membrane was prehybridized with rapid hybridization buffer (Amersham) and then hybridized with ³²P-labeled probes for 2 hours at 65° C. The probes used in this study were the PstI-PstI fragments of cDNA for β -actin and the full length of cDNA for IL-8,³⁰ which was kindly provided by Dr. Koji Matsushima (Tokyo University School of Medicine, Department of Hygiene). After hybridization, the membrane was washed with standard saline citrate containing SDS and then autoradiographed with Kodak XAR film at -70° C.

Statistical analysis

Statistical significance was analyzed by using analysis of variance. A probability (*p*) value less than 0.05 was considered significant.

RESULTS

TNF- α , IL-1 α , and PAF induce IL-8 production

First, we examined the dose-dependent induction of IL-8 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 hours after cultivation (Fig. 1). The concentrations of IL-8 in the culture supernatants from TNF- α -stimulated culture increased in a dose-dependent manner. Similarly, the concentrations of IL-8 in the culture supernatants from IL-1 α - or PAF-stimulated culture increased in a dose-dependent manner.

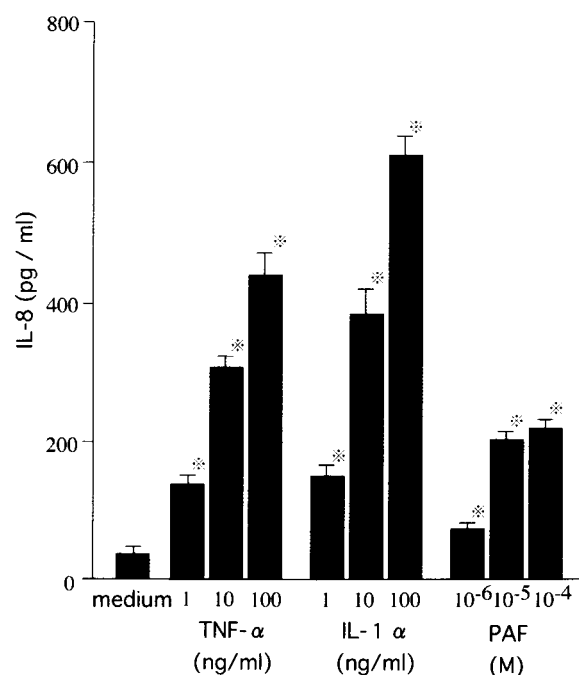


FIG. 1. TNF- α , IL-1 α , and PAF induce IL-8 production by BECs. BECs were cultured either with medium or various concentrations of TNF- α , IL-1 α , or PAF, and concentrations of IL-8 in culture supernatants were determined at 24 hours after cultivation as described in Methods section. Results are expressed as means $\pm$ SD in three different experiments. * $p < 0.01$ compared with IL-8 concentration in BECs cultured with medium.

TNF- α , IL-1 α , and PAF cause tyrosine phosphorylation of p38 MAP kinase

To determine whether TNF- α , IL-1 α , and PAF induce tyrosine phosphorylation of p38 MAP kinase, BECs were stimulated for the desired times and p38 MAP kinase was immunoblotted. An immunoblot study of lysate of BECs with a specific antibody to phosphorylated tyrosine of p38 MAP kinase antibody showed that stimulation of the cells with TNF- α caused an increase in tyrosine phosphorylation of p38 MAP kinase in a dose-dependent manner (Fig. 2, *A*, upper panel). To determine the time course of tyrosine phosphorylation of p38 MAP kinase, BECs were stimulated with 100 ng/ml of TNF- α for the desired times as indicated. Amounts of phosphorylated tyrosine of p38 MAP kinase increased at 5 minutes and returned to near basal levels at 15 minutes, indicating that tyrosine phosphorylation of p38 MAP kinase was transient (Fig. 2, *B*, upper panel). IL-1 α - or PAF-induced tyrosine phosphorylation of p38 MAP kinase was in a dose-dependent manner (Fig. 3, *A*, upper panel and Fig. 4, *A*, upper panel). Amounts of IL-1 α -induced tyrosine phosphorylation of p38 MAP kinase increased at 5 minutes and returned to near basal levels at 30 minutes (Fig. 3, *B*, upper panel). Amounts of PAF-induced tyrosine phosphorylation of p38 MAP kinase increased at 10 minutes and returned to near basal levels at 60 minutes (Fig. 4, *B*, upper panel). Equal amounts of p38 MAP kinase protein were immunoblot-

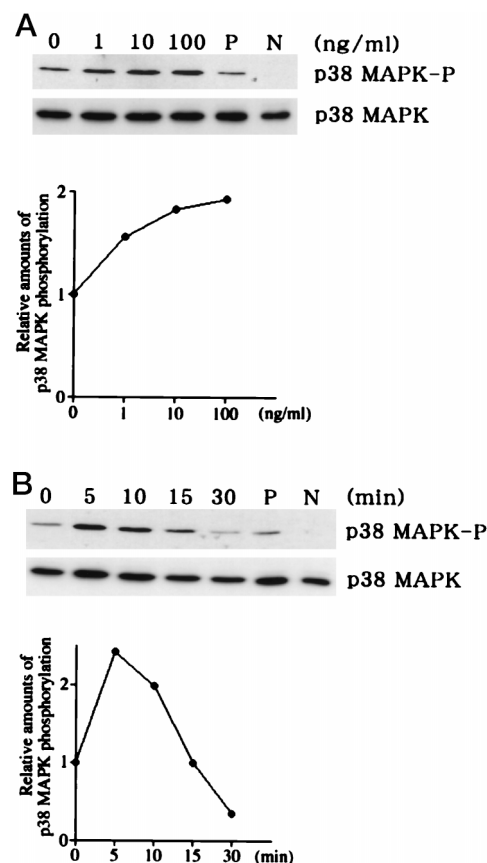


FIG. 2. TNF- α causes tyrosine phosphorylation of p38 MAP kinase in BECs. BECs were stimulated with various concentrations of TNF- α for 5 minutes (*A*) and were stimulated with TNF- α (100 ng/ml) for desired times as indicated (*B*). Lysates from BECs were separated by 10% SDS-polyacrylamide gel, transferred to membranes, and blotted with specific antibody to phosphorylated tyrosine of p38 MAP kinase (p38 MAPK-P; upper panels). Blots shown in upper panels were stripped and reprobed with p38 MAP kinase-specific antibody to show amounts of p38 MAP kinase blotted (p38 MAPK; lower panels). Lane P represents positive protein prepared from C-6 glioma cells stimulated with anisomycin for phosphorylated tyrosine of p38 MAP kinase, and lane N represents negative protein prepared from C-6 glioma cells unstimulated with anisomycin. Amounts of p38 MAP kinase phosphorylation were quantitated by NIH image analyzer and are presented as amounts of p38 MAP kinase phosphorylation relative to control cells treated without agonist (1.0). Three identical experiments independently performed showed similar results.

ted with p38 MAP kinase-specific antibody regardless of dose of TNF- α , IL-1 α , and PAF and time of culture periods, indicating that TNF- α , IL-1 α , or PAF stimulation-induced increases in tyrosine phosphorylation of p38 MAP kinase occurred in the absence of changes in p38 MAP kinase protein levels (Figs. 2, *A*, 2, *B*, 3, *A*, 3, *B*, 4, *A*, and 4, *B*, lower panels). When BECs were cultured with growth factor-free F12 medium in the absence of TNF- α , IL-1 α , and PAF, an increase in amounts of tyrosine phosphorylation of p38 MAP kinase was not observed at any point during the culture periods (data not shown).

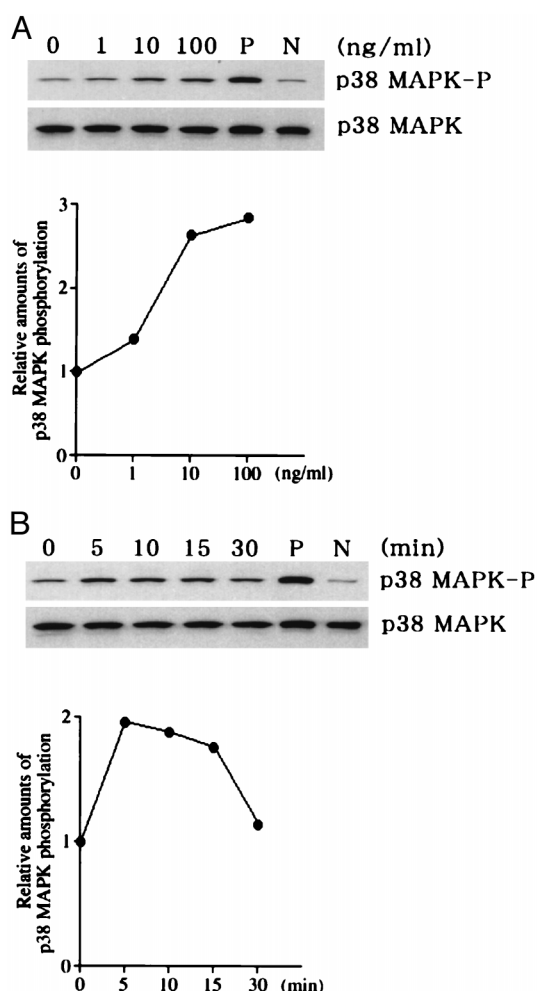


FIG. 3. IL-1 α causes tyrosine phosphorylation of p38 MAP kinase in BECs. BECs were stimulated with various concentrations of IL-1 α for 5 minutes (**A**) and were stimulated with IL-1 α (100 ng/ml) for desired times as indicated (**B**). Lysates from BECs were separated by 10% SDS-polyacrylamide gel, transferred to membranes, and blotted with specific antibody to phosphorylated tyrosine of p38 MAP kinase (p38 MAPK-P; *upper panels*). Blots shown in *upper panels* were stripped and reprobed with p38 MAP kinase-specific antibody to show amounts of p38 MAP kinase blotted (p38 MAPK; *lower panel*). Lane P represents positive protein for phosphorylated tyrosine of p38 MAP kinase, and lane N represents negative protein. Amounts of p38 MAP kinase phosphorylation were quantitated by NIH image analyzer and are presented as amounts of p38 MAP kinase phosphorylation relative to control cells treated without agonist (1.0). Three identical experiments independently performed showed similar results.

SB 203580 as the specific inhibitor of p38 MAP kinase inhibits TNF- α -, IL-1 α -, and PAF-induced IL-8 production

TNF- α , IL-1 α , and PAF induced IL-8 production and tyrosine phosphorylation of p38 MAP kinase in BECs. These results suggested that TNF- α , IL-1 α , or PAF stimulation-induced IL-8 production might be mediated through p38 MAP kinase-dependent pathway. To test this possibility, BECs that had been preincubated with or

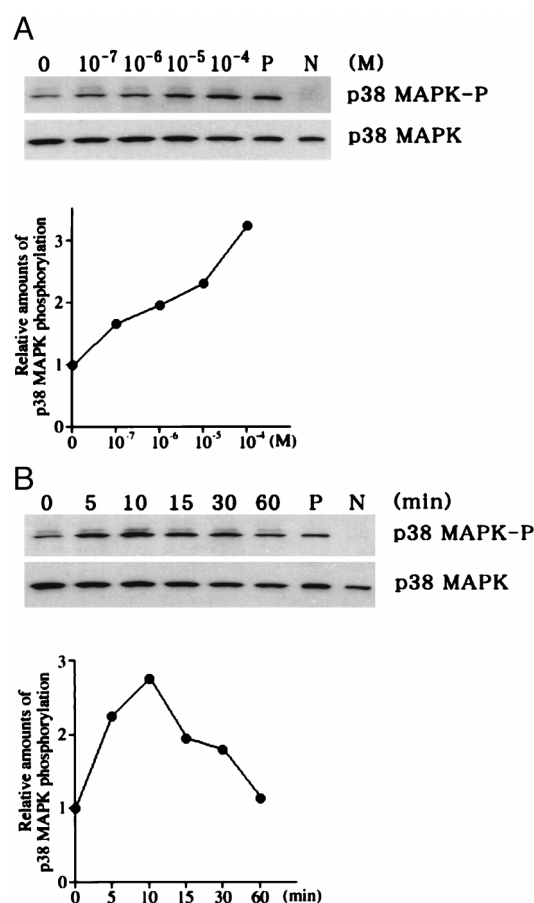


FIG. 4. PAF causes tyrosine phosphorylation of p38 MAP kinase in BECs. BECs were stimulated with various concentrations of PAF for 5 minutes (**A**) and were stimulated with PAF (10⁻⁵ mol/L) for desired times as indicated (**B**). Lysates from BECs were separated by 10% SDS-polyacrylamide gel, transferred to membranes, and blotted with specific antibody to phosphorylated tyrosine of p38 MAP kinase (p38 MAPK-P; *upper panels*). Blots shown in *upper panels* were stripped and reprobed with p38 MAP kinase-specific antibody to show amounts of p38 MAP kinase blotted (p38 MAPK; *lower panels*). Lane P represents positive protein for phosphorylated tyrosine of p38 MAP kinase, and lane N represents negative protein. Amounts of p38 MAP kinase phosphorylation were quantitated by NIH image analyzer and are presented as amounts of p38 MAP kinase phosphorylation relative to control cells treated without agonist (1.0). Three identical experiments independently performed showed similar results.

without SB 203580 were cultured either with TNF- α , IL-1 α , or PAF, and the concentrations of IL-8 in the culture supernatants were determined at 24 hours after cultivation. As shown in Fig. 5, the concentrations of IL-8 in the culture supernatants from the cells cultured with TNF- α in the presence of SB 203580 were lower than those from the cells cultured with TNF- α in the absence of SB 203580, indicating that SB 203580 inhibited TNF- α -induced IL-8 production. Similarly, SB 203580 inhibited IL-1 α - or PAF-induced IL-8 production.

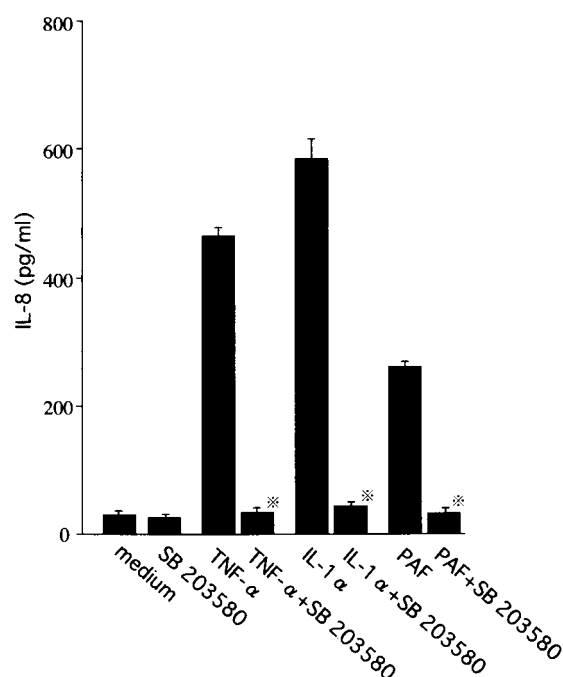


FIG. 5. Specific MAP kinase inhibitor SB 203580 inhibits TNF- α -, IL-1 α -, and PAF-induced IL-8 production by BECs. BECs that had been preincubated with or without SB 203580 (10 μ mol/L) for 1 hour were cultured either with medium, TNF- α (100 ng/ml), IL-1 α (100 ng/ml), or PAF (10⁻⁵ mol/L), and concentrations of IL-8 in culture supernatants were determined at 24 hours after cultivation as described in Methods section. Concentration of dimethyl sulfoxide used in this study was 0.01%, which had no effect. Results are expressed as means $\pm$ SD in five different experiments. * p < 0.01 compared with IL-8 concentration in BECs cultured without SB 203580.

SB 203580 as the specific inhibitor of p38 MAP kinase inhibits TNF- α -, IL-1 α -, and PAF-induced IL-8 mRNA expression

An inhibitory effect of the specific p38 MAP kinase inhibitor on TNF- α -, IL-1 α -, or PAF-induced IL-8 protein production by BECs suggested that this action may have resulted from an inhibitory effect of this inhibitor on IL-8 gene expression. To test this possibility, BECs that had been preincubated with or without SB 203580 were cultured either with TNF- α , IL-1 α , or PAF, and IL-8 mRNA expression was analyzed at 6 hours after cultivation. As shown in Fig. 6, IL-8 mRNA expression was upregulated when BECs were stimulated with TNF- α (lane 3), IL-1 α (lane 5), or PAF (lane 7), whereas SB 203580 inhibited TNF- α -, IL-1 α -, or PAF-induced upregulation of IL-8 mRNA expression (lane 4, TNF- α plus SB 203580; lane 6, IL-1 α plus SB 203580; and lane 8, PAF plus SB 203580). The levels of β -actin mRNA expression did not vary significantly with culture conditions.

The total number of cells and cell viability at the end of the culture period of each experiment determined by trypan blue exclusion assay did not differ with culture

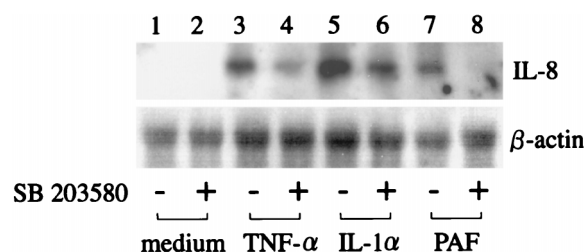


FIG. 6. Specific MAP kinase inhibitor SB 203580 inhibits TNF- α -, IL-1 α -, and PAF-induced IL-8 mRNA expression in BECs. BECs that had been preincubated with or without SB 203580 (10 μ mol/L) for 1 hour were cultured either with medium, TNF- α (100 ng/ml), IL-1 α (100 ng/ml), or PAF (10⁻⁵ mol/L) for 6 hours, and IL-8 mRNA expression and β -actin mRNA expression were analyzed by northern blotting analysis as described in Methods section. Lanes 1, 3, 5, and 7 represent cells cultured with medium, TNF- α (100 ng/ml), IL-1 α (100 ng/ml), and PAF (10⁻⁵ mol/L), respectively. Lanes 2, 4, 6, and 8 represent cells cultured with SB 203580, TNF- α plus SB 203580, IL-1 α plus SB 203580, and PAF plus SB 203580, respectively. Concentration of dimethyl sulfoxide used in this study was 0.01%, which had no effect. Three identical experiments independently performed showed similar results.

conditions. Figs. 1, 5, and 6 suggest that TNF- α , IL-1 α , or PAF induced IL-8 expression, and the inhibition by p38 MAP kinase inhibitor of IL-8 expression did not result from cell cytotoxicity.

DISCUSSION

In this study we examined the signal transduction pathway in TNF- α -, IL-1 α -, and PAF-induced IL-8 expression in human BECs. The results showed that TNF- α , IL-1 α , and PAF induced IL-8 expression and tyrosine phosphorylation of p38 MAP kinase in BECs. The specific p38 MAP kinase inhibitor SB 203580 completely inhibited TNF- α -, IL-1 α -, and PAF-induced IL-8 expression in BECs, indicating that p38 MAP kinase plays an important role in TNF- α -, IL-1 α -, and PAF-activated signaling pathway, which regulates IL-8 expression in BECs.

In this study we used TNF- α , IL-1 α , and PAF as inducers for examination of signal transduction pathway in cytokine expression in BECs because TNF- α , IL-1 α , and PAF are known to induce cytokine expression in BECs.⁹⁻¹³ Furthermore, the involvement of TNF- α and IL-1 in the production of allergic inflammation and the development of late asthmatic responses have been shown.⁴⁻⁸ PAF is known to contribute to producing allergic inflammation through activation of eosinophils and inducing airway smooth muscle contraction.^{4, 31, 32}

TNF- α and IL-1 α have been shown to activate p38 MAP kinase in various cells. However, little is known about the effect of these cytokines on p38 MAP kinase activation in BECs. Furthermore, it has not been determined whether PAF activates p38 MAP kinase. In this study we showed that PAF, as well as TNF- α and IL-1 α , induced tyrosine phosphorylation of p38 MAP kinase in BECs. Other subgroups of MAP kinase, extracellular signal-regulated kinase and c-Jun NH₂-terminal kinase,

are also activated in response to TNF- α and IL-1 α .^{21, 34} Therefore we used SB 203580 as the specific p38 MAP kinase inhibitor^{28, 35} to determine the signal transduction pathway regulating IL-8 expression in TNF- α -, IL-1 α -, or PAF-stimulated BECs. As a result, SB 203580 completely inhibited IL-8 expression in TNF- α -, IL-1 α -, or PAF-stimulated BECs, indicating that p38 MAP kinase plays an important role in TNF- α -, IL-1 α -, and PAF-activated signaling pathway, which regulates IL-8 expression in BECs.

To determine the level at which SB 203580 inhibited IL-8 protein production, we performed northern blot analysis. SB 203580 inhibited TNF- α -induced upregulation of IL-8 mRNA expression in BECs. A similar inhibitory effect by SB 203580 was also evident in IL-1 α - and PAF-induced IL-8 mRNA expression. These results indicated that SB 203580 might inhibit TNF- α -, IL-1 α -, and PAF-induced IL-8 expression at the level of transcription.

The promoter of the gene encoding IL-8 contains sequences for binding several nuclear transcription factors, including NF- κ B and AP-1.^{36, 37} These transcription factors participate to various extents in the inducible expression of the genes encoding IL-8. The p38 MAP kinase has been implicated in the activation of multiple transcription factors, including NF- κ B, CHOP, and ATF-2.^{21, 38, 39} In this study the transcription factor that involves IL-8 expression has not been investigated. Therefore further study should be done to clarify the links between p38 MAP kinase activation and IL-8 gene expression. Alternatively, it has been shown that p38 MAP kinase may regulate VCAM expression at the posttranscriptional level.²⁰ SB 203580 inhibited IL-8 mRNA expression and protein production in this study, suggesting that p38 MAP kinase might not involve the posttranscriptional regulation.

IL-8 exhibits a chemotactic activity for neutrophils and T lymphocytes.¹⁴ In addition, a chemotactic activity of IL-8 for eosinophils has been documented.⁴¹ These cells have been described to play an important role in the production of allergic inflammation.²⁻⁴ Therefore IL-8 produced by BECs stimulated with TNF- α , IL-1 α , and PAF contribute to the production of allergic inflammation through recruitment of these cells into the site of inflammation. On the basis of the role of IL-8 in the production of allergic inflammation, the inhibition of IL-8 production is an important strategy for the treatment of bronchial asthma. The specific p38 MAP kinase inhibitor SB 203580 was originally discovered as an inhibitor of lipopolysaccharide-induced production of IL-1 and TNF- α in the human monocytic cell line, THP-1.²⁸ This drug has been thought to be useful in the treatment of inflammatory diseases.²⁸ In this study we showed the inhibition of IL-8 production by SB 203580. Therefore the specific p38 MAP kinase inhibitor SB 203580 may have a potential effect on controlling allergic inflammation through inhibiting IL-8 production.

From the data presented here, we concluded that TNF- α , IL-1 α , and PAF induce IL-8 expression in BECs

through p38 MAP kinase-dependent pathway. These results provide new evidence on the mechanism in TNF- α -, IL-1 α -, and PAF-induced IL-8 expression in BECs and a strategy for treatment of airway inflammation with the specific p38 MAP kinase inhibitor.

We thank Ms. Ikuko Takeshita for her technical assistance.

REFERENCES

1. American Thoracic Society. Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. *Am Rev Respir Dis* 1987;136:225-44.
2. Djukanovic R, Roche W, Wilson JW, Beasley CRW, Twentyman OP, Howarth PH, et al. Mucosal inflammation in asthma. *Am Rev Respir Dis* 1990;142:434-57.
3. Corrigan CJ, Kay AB. T cells and eosinophils in the pathogenesis of asthma. *Immunol Today* 1992;12:501-7.
4. Kay AB. Asthma and inflammation. *J Allergy Clin Immunol* 1991; 87:893-910.
5. Shah A, Church MK, Holgate ST. Tumor necrosis factor alpha: a potential mediator of asthma. *Clin Exp Allergy* 1995;25:1038-44.
6. Kips JC, Tavernier J, Pauwels PA. Tumor necrosis factor causes bronchial hyperresponsiveness in rats. *Am Rev Respir Dis* 1992;145: 332-6.
7. Herandez A, Omini C, Daffonchio L. Interleukin-1: a possible mediator of lung inflammation and airway hyperreactivity. *Pharmacol Res* 1991;24:385-93.
8. Watson ML, Smith D, Bourne AD, Thompson RC, Westwick J. Cytokines contribute to airway dysfunction in antigen-challenged guinea pigs: inhibition of airway hyperreactivity, pulmonary eosinophil accumulation, and tumor necrosis factor generation by pretreatment with an interleukin-1 receptor antagonist. *Am J Respir Cell Mol Biol* 1993;8:365-9.
9. Marini M, Soloperto M, Mezzetti M, Fasoli A, Mattoli S. Interleukin-1 binds to specific receptors on human bronchial epithelial cells and upregulates granulocyte/macrophage colony-stimulating factor synthesis and release. *Am J Respir Cell Mol Biol* 1991;4:519-24.
10. Takizawa H, Ohtoshi T, Ohta K, Shunsei S, Yamaguchi M, Suzuki N, et al. Interleukin-6/B cell stimulating factor-II is expressed and released by normal and transformed human bronchial epithelial cells. *Biochem Biophys Res Commun* 1992;187:596-602.
11. Kown OJ, Au BT, Collins PD, Baraniuk JN, Adcock IM, Chung KF, et al. Inhibition of interleukin-8 expression by dexamethasone in human cultured airway epithelial cells. *Immunology* 1994;81:389-94.
12. Takizawa H, Ohtoshi T, Kikutani T, Okazaki H, Akiyama N, Sato M, et al. Histamine activates bronchial epithelial cells to release inflammatory cytokines in vitro. *Int Arch Allergy Immunol* 1995;108:260-7.
13. Levine SJ. Bronchial epithelial cell-cytokine interactions in airway inflammation. *J Invest Med* 1995;43:241-9.
14. Baggiolini M, Dewald B, Moser B. Interleukin-8 and related chemotactic cytokines—CXC and CC chemokines. *Adv Immunol* 1994; 55:97-179.
15. Marini M, Vittori E, Hollemborg J, Mattoli S. Expression of the potent inflammatory cytokines, granulocyte-macrophage-colony-stimulating factor and interleukin-6 and interleukin-8, in bronchial epithelial cells of patients with asthma. *J Allergy Clin Immunol* 1992;89:1257-67.
16. Bellini AH, Yoshimura E, Vittori M, Marini M, Mattoli S. Bronchial epithelial cells of patients with asthma release chemoattractant factors for T lymphocytes. *J Allergy Clin Immunol* 1993;92:412-24.
17. Hallsworth MP, Soh CPC, Lane SJ, Arm JP, Lee TH. Selective enhancement of GM-CSF, TNF- α , IL-1 β and IL-8 production by monocytes and macrophages of asthmatic subjects. *Eur Respir J* 1994;7:1096-102.
18. Davis RJ. MAPKs: new JNK expand the group. *Trends Biochem Sci* 1994;19:470-3.
19. Han J, Lee JD, Bibbs L, Ulevitch RJ. A Map kinase targeted by endotoxin and hyperosmolarity in mammalian cells. *Science* 1994; 265:808-11.
20. Rouse J, Cohen P, Trigon S, Morahge M, Alonso-Liamazares A,

- Zamanillo D, et al. A novel kinase cascade triggered by stress and heat shock that stimulates MAPKAP kinase-2 and phosphorylation of the small heat shock proteins. *Cell* 1994;78:1027-34.
21. Raingeaud J, Gupta S, Rogers JS, Dickens M, Han J, Ulevitch R, et al. Pro-inflammatory cytokines and environmental stress cause p38 mitogen-activated protein kinase activation by dual phosphorylation on tyrosine and threonine. *J Biol Chem* 1995;270:7420-6.
22. Freshney N, Rawlinson L, Guesdon F, Jones E, Cowley S, Hsuan J, et al. Interleukin-1 activates a novel protein kinase cascade that results in the phosphorylation of Hsp 27. *Cell* 1994;78:1039-49.
23. Beyaert R, Cuenda A, Berghe WV, Plaisance S, Lee JC, Haegeman G, et al. The p38/JNK mitogen-activated protein kinase pathway regulates interleukin-6 synthesis in response to tumor necrosis factor. *EMBO J* 1996;15:1914-23.
24. Pandey P, Raingeaud J, Kaneki M, Weichselbaum R, Davis RJ, Kufe D, et al. Activation of p38 mitogen-activated protein kinase by c-abl-dependent and -independent mechanism. *J Biol Chem* 1996;271:23775-9.
25. Morihuchi T, Toyoshima F, Gotoh Y, Iwamatsu A, Irie K, Mori E, et al. Purification and identification of a major activator for p38 from osmotically shocked cells. Activation of mitogen-activated protein kinase kinase 6 by osmotic shock, tumor necrosis factor- α , and H2O₂. *J Biol Chem* 1996;272:26981-8.
26. Nick JA, Avdi NJ, Gerwins P, Johnson GL, Worthen GS. Activation of a p38 mitogen-activated protein kinase in human neutrophils by lipopolysaccharide. *J Immunol* 1996;156:4867-75.
27. Foltz IN, Lee JC, Young PR, Schrader JW. Hemopoietic growth factors with the exception of interleukin-4 activate the p38 mitogen-activated protein kinase pathway. *J Biol Chem* 1997;272:3296-301.
28. Lee JC, Laydon JT, McDonnell PC, Gallagher TF, Kummer S, Green D, et al. A protein kinase involved in the regulation of inflammatory cytokine biosynthesis. *Nature* 1994;372:739-46.
29. Thomas PS. Hybridization of denatured RNA transferred or dotted to nitrocellulose paper. *Methods Enzymol* 1983;100:255-66.
30. Mukaida M, Shiroo M, Matsushima K. Genomic structure of the human monocyte-derived neutrophil chemotactic factor IL-8. *J Immunol* 1989;143:1366-71.
31. Borish L, Joseph BZ. Inflammation and the allergic response. *Clin Allergy* 1992;76:765-87.
32. Arm JP, Lee TK. The pathobiology of bronchial asthma. *Adv Immunol* 1992;51:323-82.
33. Walters EH, Ward C, Li X. Bronchial lavage in asthma research. *Respirology* 1996;1:233-45.
34. Sluss HK, Barrett T, Derijard B, Davis RJ. Signal transduction by tumor necrosis factor mediated by JNK protein kinases. *Mol Cell Biol* 1994;14:8376-84.
35. Cuenda A, Rouse J, Doza YN, Meier R, Cohen P, Gallagher TF, et al. SB 203580 is a specific inhibitor of a MAP kinase homologue which is stimulated by cellular stress and interleukin-1. *FEBS Lett* 1995;364:229-33.
36. Baeuerle PA, Henkel T. Function and activation of NF- κ B in the immune system. *Annu Rev Immunol* 1994;12:141-79.
37. Hobbie S, Chen LM, Davis RJ, Galán JE. Involvement of mitogen-activated protein kinase pathways in the nuclear responses and cytokine production induced by *Salmonella typhimurium* in cultured intestinal epithelial cells. *J Immunol* 1997;159:5550-9.
38. Wane XZ, Ron D. Stress-induced phosphorylation and activation of the transcription factor CHOP (GADD153) by p38 MAP kinase. *Science* 1996;272:1347-9.
39. Wesselborg S, Bauer MKA, Vogt M, Schmitz ML, Schulze-Osthoff K. Activation of transcription factor NF- κ B and p38 mitogen-activated protein kinase is mediated by distinct and separate stress effector pathway. *J Biol Chem* 1997;272:12422-9.
40. Pieterse A, Tilly BC, Gaestel M, de Jone N, Lee JC, Koster JF, et al. P38 mitogen-activated protein kinase regulates endothelial VCAM-1 expression at the post-transcriptional level. *Biochem Biophys Res Commun* 1997;230:44-8.
41. Collins PD, Weg VB, Faccioli LH, Watson ML, Moqbel R, Williams TJ. Eosinophil accumulation induced by human interleukin-8 in the guinea-pig in vivo. *Immunology* 1993;79:312-8.