

Intracellular glutathione regulates tumour necrosis factor- α -induced p38 MAP kinase activation and RANTES production by human bronchial epithelial cells

S. HASHIMOTO, Y. GON, K. MATSUMOTO, I. TAKESHITA, T. MACHINO and T. HORIE

First Department of Internal Medicine, Nihon University School of Medicine

Summary

Background RANTES plays an important role in the production of allergic inflammation of the airway through its chemotactic activity for eosinophils. The cellular reduction and oxidation (redox) changes are involved in the activation of p38 mitogen-activated protein (MAP) kinase and the induction of cytokine expression. It has previously been shown that tumour necrosis factor (TNF)- α activates p38 mitogen-activated protein (MAP) kinase to produce cytokine, including RANTES, that N-acetylcysteine (NAC) attenuates cytokine production by human bronchial epithelial cells (BECs), and that sensitivity to TNF α is inversely correlated with cellular redox state. However, a role of cellular redox regulated by intracellular glutathione (GSH) in TNF α -induced p38 MAP kinase activation and p38 MAP kinase-mediated RANTES production by human BECs has not been determined.

Objective Human BECs were exposed to NAC or buthionine sulfoximine (BSO). TNF α -induced p38 MAP kinase activation and p38 MAP kinase-mediated RANTES production by human BECs were then examined in order to clarify these issues.

Results The results showed that: NAC attenuated TNF α -induced p38 MAP kinase activation and RANTES production; SB 203580 as the specific inhibitor of p38 MAP kinase activity attenuated TNF α -induced RANTES production; BSO facilitated TNF α -induced p38 MAP kinase activation and RANTES production; SB 203580 attenuated BSO-mediated facilitation of TNF α -induced RANTES production; and the intracellular GSH increased in NAC-treated cells, whereas the intracellular GSH was reduced in BSO-treated cells.

Conclusions These results indicate that cellular redox regulated by GSH is critical for TNF α -induced p38 MAP kinase activation and p38 MAP kinase-mediated RANTES production by human BECs.

Keywords: glutathione, p38 MAP kinase, RANTES, bronchial epithelial cells

Clinical and Experimental Allergy, Vol. 31, pp. 144–151. Submitted 19 August 1999; revised 31 March 2000; accepted 9 May 2000.

Introduction

Bronchial asthma is a disease that is characterized by episodic reversible airway obstruction, airway hyperresponsiveness, and allergic inflammation in the airway [1]. Multiple inflammatory cells, cytokines and mediators participate in the pathogenesis of bronchial asthma [2–3]. Of these

inflammatory cells, eosinophils play a pivotal role in the production of allergic inflammation, and the accumulation of eosinophils in the asthmatic airway is a characteristic feature of allergic inflammation [3,4]. Various cytokines which display chemotactic activity for eosinophils have been identified. Of these cytokines, RANTES, which is known to be produced by bronchial epithelial cells (BECs) [5,6], has been shown to be involved in the production of allergic inflammation [7,8]. Therefore, it is important to clarify the mechanism of RANTES production by BECs.

Correspondence: S. Hashimoto, First Department of Internal Medicine, Nihon University School of Medicine, 30–1 Oyaguchikamimachi, Itabashi-ku, Tokyo 173–8610, Japan.

The mitogen-activated protein (MAP) kinases are important mediators of signal transduction from the cell membrane to the nucleus [9]. The activation of p38 MAP kinase, which belongs to MAP kinase superfamily, elicits a variety of biological responses including cytokine expression. p38 MAP kinase is activated by a variety of extracellular stresses [10–16]. Among these, oxidative stresses can activate p38 MAP kinase cascade, and it has previously been shown that (i) tumour necrosis factor- α (TNF α) activates p38 MAP kinase to produce RANTES, granulocyte-macrophage stimulating factor (GM-CSF) and interleukin-8 (IL-8) by human BECs [17], (ii) N-acetylcysteine attenuates IL-8 production [18], and (iii) a redox control protein, thioredoxin (TRX), negatively regulates p38 MAP kinase activation and p38 MAP kinase-mediated IL-6 production [19]. These observations indicate that there may be a link between the cellular reduction/oxidation (redox) state and p38 MAP kinase-mediated cytokine expression. However, a role for cellular redox regulated by intracellular glutathione (GSH) in TNF α -induced p38 MAP kinase activation and p38 MAP kinase-mediated RANTES production by human BECs has not been determined. In the present study, BECs were exposed to NAC which acts as a precursor of GSH synthesis, thus scavenging reactive oxygen species (ROS) [20]. BECs were also exposed to BSO which selectively inhibits γ -glutamylcysteine synthetase, thus inhibiting GSH synthesis [21]. Thereafter, the cells were stimulated with TNF α , and the threonine and tyrosine phosphorylation of p38 MAP kinase and p38 MAP kinase-mediated RANTES production were examined in order to clarify these issues.

Materials and methods

Reagents

NAC and BSO were obtained from Sigma Chemical Co. (St Louis, MO, USA). The pyridinyl imidazole SB 203580, the specific inhibitor for p38 MAP kinase activity [22], was kindly provided by Smithkline Beecham (King of Prussia, PA, USA) and was dissolved in dimethyl sulfoxide. Human recombinant TNF α was kindly provided by Dainippon Pharmaceutical Co. Ltd (Osaka, Japan).

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 a collagen-coated tissue culture dish (Iwaki, Tokyo, Japan) for western blot, or a 24-well flat bottom tissue culture plate (Iwaki) for determination of cytokine production using Ham's F12 medium containing

1% penicillin-streptomycin, 5 μ g/mL insulin (Gibco, Grand Island, NY, USA), 5 μ g/mL transferrin (Gibco), 25 ng/mL epidermal growth factor (EGF, Collaborative Research, Lexington, MA, USA), 15 μ g/mL 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 subconfluent and the medium was changed. In order to examine the effect of NAC and BSO on TNF α -induced threonine, and tyrosine phosphorylation of p38 MAP kinase and RANTES production, growth factor-starved BECs that had been pre-incubated with NAC or BSO were stimulated with TNF α , and cultured for the desired times as indicated. In order to examine the effect of SB 203580 on RANTES production, growth factor-starved BECs that had been pre-incubated with or without SB 203580 were stimulated with TNF α , and cultured for 24 h at 37 °C, in humidified 5% CO₂ atmosphere. At the end of the culture periods, the culture supernatant fluids were harvested for determination of RANTES protein, centrifuged, and the supernatant fluids collected, filtered with a Millipore filter and stored at – 80 °C until assay.

Western blot analysis of threonine and tyrosine phosphorylation of p38 MAP kinase

The threonine and tyrosine phosphorylation of 38 MAP kinase was analysed by commercially available kits (PhosphoPlus™ p38 MAPK Antibody Kit, New England Biolabs, Inc., Beverly, MA, USA). Analysis of threonine and 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 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 bromophenol 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, the cell lysate containing 10 μ g of protein was 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 dry milk, for 3 h at room temperature. It was then incubated with specific antibody to phosphorylated threonine and 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% 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 horseradish peroxidase-conjugated anti-rabbit antibody (1:2000) and horseradish peroxidase-conjugated antibiotin 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 min at room temperature and exposed on KODAK XAR film. Blots were stripped and reprobed using phosphorylation-state independent p38 MAP kinase-specific antibody to determine total p38 MAP kinase levels (affinity purified rabbit polyclonal IgG).

Measurement of RANTES

The concentrations of RANTES in the culture supernatant fluids from BECs were measured by a commercially available ELISA kit (Amersham International, Aylesbury, UK). ELISA was performed according to the manufacturer's instruction. All samples were assayed in duplicate.

Measurement of intracellular GSH levels

The intracellular GSH levels were measured using methods described previously [23]. Briefly, the cells were washed with ice-cold PBS. After washing, the cells were collected and suspended in ice-cold PBS, and then 10% trichloroacetic acid was added to the cell suspension. The mixture was centrifuged at 4 °C, after which the supernatant fluid was extracted with diethylether. The resulting extract was incubated with 200 µM NADPH (Sigma) and 1 unit/mL of GSH reductase (Sigma) for 10 min at 37 °C, and then 1 mM of 5,5-dithiobis(2-nitro-benzonic acid) (DTNB, Sigma) was added and the reaction measured at 415 nm absorbance.

Statistical analysis

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

Results

TNFα activates p38 MAP kinase and NAC attenuates TNFα-induced p38 MAP kinase activation

Activation of p38 MAP kinase is mediated by dual phosphorylation of the threonine and tyrosine residues of p38 MAP kinase [11]. Increases in threonine and tyrosine phosphorylation of p38 MAP kinase reflect activation state of p38 MAP kinase. Consequently, the threonine and tyrosine phosphorylation of p38 MAP kinase was examined. BECs were stimulated

with TNFα (10 ng/mL) for the desired times and p38 MAP kinase was immunoblotted. Amounts of phosphorylated threonine and tyrosine of p38 MAP kinase increased at 5 min, these levels being sustained between 10 and 15 min; thereafter, they returned to near-basal levels at 60 min, indicating that threonine and tyrosine phosphorylation of p38 MAP kinase was transient (Fig. 1, upper panel). In order to examine the effect of NAC on TNFα-induced p38 MAP kinase activation, the cells that had been pre-incubated with NAC (10 mmol/L) for 1 h were stimulated with TNFα, and amounts of phosphorylated threonine and tyrosine of p38 MAP kinase were analysed 5 min after stimulation. Amounts of phosphorylated threonine and tyrosine of p38 MAP kinase were lower in NAC-pre-treated cells than those in NAC-untreated cells, indicating that the pre-treatment of NAC attenuated TNFα-induced p38 MAP kinase activation (Fig. 2, upper panel). The lower panels of Fig. 1 and Fig. 2 show that equal amounts of p38 MAP kinase protein were immunoblotted with phosphorylation state-independent p38MAP kinase-specific antibody, regardless of culture conditions.

NAC attenuates TNFα-induced IL-8 production

NAC attenuated TNFα-induced p38 MAP kinase activation (Fig. 2). It has previously been shown that p38 MAP kinase regulates TNFα-induced RANTES production by BECs [17]. From this evidence, the attenuation of p38 MAP kinase activation by NAC might result in reducing TNFα-induced RANTES production. To test this possibility, the effect of NAC on TNFα-induced RANTES production was examined. Simultaneously, the effect of SB 203580 (10 µmol/L) on TNFα-induced RANTES production was also examined to confirm the role of p38 MAP kinase in RANTES production. As shown in Fig. 3, the pre-treatment of the cells with NAC attenuated TNFα-induced RANTES production. SB 203580 attenuated TNFα-induced RANTES production, indicating and confirming that p38 MAP kinase plays an important role in the TNFα-activated signalling pathway which regulates RANTES production by BECs. These results indicate that the cellular GSH increasing agent, NAC, negatively regulated TNFα-induced p38 MAP kinase activation and p38 MAP kinase-mediated RANTES production.

BSO facilitates TNFα-induced p38 MAP kinase activation

The effect of the GSH depleting agent, BSO, on TNFα-induced p38 MAP kinase activation and RANTES production by BECs was investigated. To this end, the cells that had been pre-incubated with BSO (100 µmol/L) for 16 h were stimulated with TNFα, and amounts of phosphorylated threonine and tyrosine of p38 MAP kinase were analysed

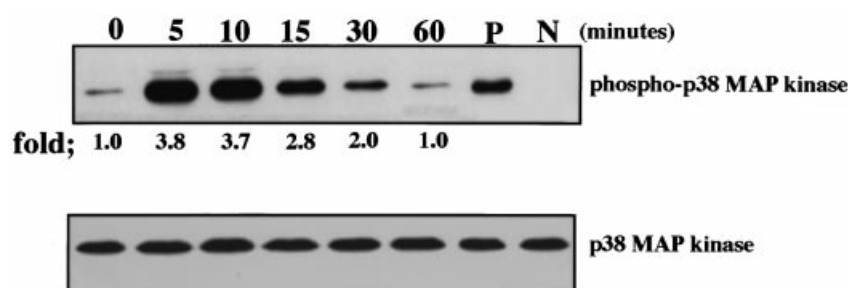


Fig. 1. TNF α activates p38 MAP kinase. BECs were stimulated with TNF α (10 ng/mL) for the desired times as indicated. The lysates from BECs were separated by a 15% SDS-PAGE, transferred to membranes, and blotted with a specific antibody to phosphorylated threonine and tyrosine of p38 MAP kinase (phospho-p38 MAP kinase; upper panel). Blots shown in the upper panels were stripped and reprobed, using a phosphorylation state-independent p38 MAP kinase-specific antibody, to show the amounts of p38 MAP kinase blotted (p38MAP kinase; lower panel). Lane P, positive protein prepared from C-6 glioma cells stimulated with anisomycin for phosphorylated threonine and tyrosine of p38 MAP kinase; Lane N, negative protein prepared from C-6 glioma cells unstimulated with anisomycin. The amounts of p38 MAP kinase phosphorylation relative to control cells treated without agonist (1.0). Fold increase in amounts of phosphorylated p38 MAP kinase proteins is indicated below. Three identical experiments independently performed gave similar results.

5 min after stimulation. Amounts of phosphorylated threonine and tyrosine of p38 MAP kinase were higher in BSO-pre-treated cells than in BSO-untreated cells, indicating that the pre-treatment with BSO facilitated TNF α -induced p38 MAP kinase activation (Fig. 4). The lower panel of Fig. 4 shows that equal amounts of p38 MAP kinase protein were immunoblotted with phosphorylation state-independent p38 MAP kinase-specific antibody, regardless of culture conditions.

BSO facilitates TNF α -induced RANTES production

Since activated p38 MAP kinase is an important signal which regulates RANTES production by BECs, the facilitation of TNF α -induced p38 MAP kinase activation

by BSO might result in the facilitation of RANTES production. To test this possibility, the cells that had been pre-treated with BSO were stimulated with TNF α and RANTES concentrations were determined. Simultaneously, the effect of SB 203580 on RANTES production was examined. As shown in Fig. 5, the concentrations of RANTES from the culture supernatant fluids of TNF α -stimulated cells were higher in BSO-pre-treated cells than in BSO-untreated cells, indicating that the pre-treatment of BSO facilitated TNF α -induced RANTES production. The pre-treatment of the cells with SB 203580 attenuated RANTES production by the BSO-pre-treated cells with TNF α stimulation. These results indicate that p38 MAP kinase is an important signal leading to RANTES production by BSO-pre-treated cells. The total number of

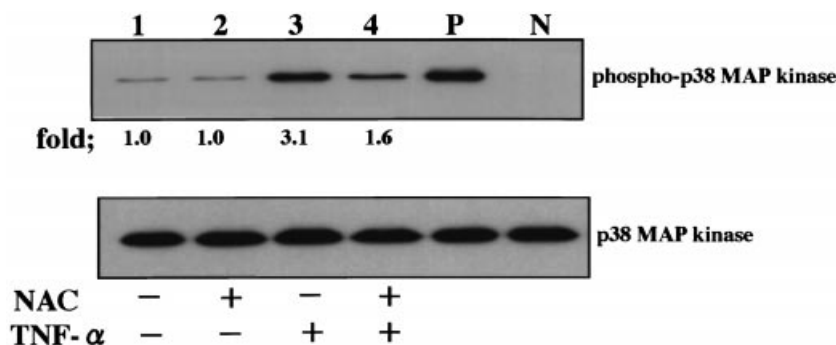


Fig. 2. NAC attenuates TNF α -induced p38 MAP kinase activation. BECs that had been pre-treated either with medium or NAC (10 mmol/L) for 1 h were stimulated with TNF α (10 ng/mL) for 5 min. The lysates from BECs were separated by a 15% SDS-PAGE, transferred to membranes, and blotted with a specific antibody to phosphorylated threonine and tyrosine of p38 MAP kinase (phospho-p38 MAP kinase; upper panel). Blots shown in the upper panel were stripped and reprobed using a phosphorylation state-independent p38 MAP kinase-specific antibody to show the amounts of p38 MAP kinase blotted (p38MAP kinase; lower panels). The cells were cultured with medium (lane 1), NAC (lane 2), TNF α (lane 3) and TNF α and NAC (lane 4). Lane P, lane N and fold were as described in Fig. 1 legend. Three identical experiments independently performed gave similar results.

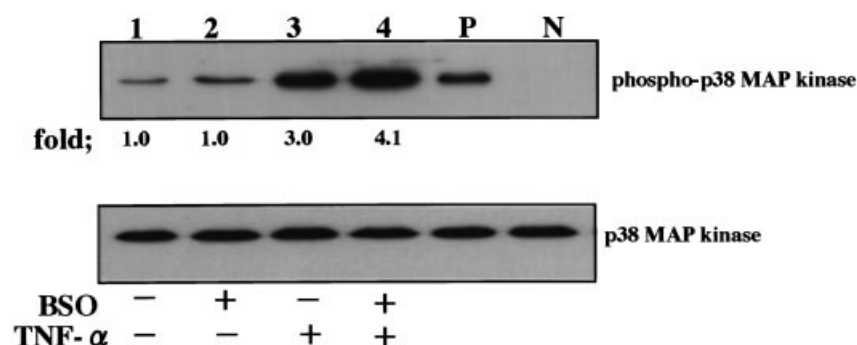


Fig. 3. BSO facilitates TNF α -induced p38 MAP kinase activation. BECs that had been pre-treated either with medium or BSO (100 μ mol/L) for 16 h were stimulated with TNF α (10 ng/mL) for 5 min. The lysates from BECs were separated by a 15% SDS-PAGE, transferred to membranes, and blotted with a specific antibody to phosphorylated threonine and tyrosine of p38 MAP kinase (phospho-p38MAP kinase; upper panel). Blots shown in the upper panels were stripped and reprobed using a phosphorylation state-independent p38 MAP kinase-specific antibody to show the amounts of p38 MAP kinase blotted (p38MAP kinase; lower panel). The cells were cultured with medium (lane 1), BSO (lane 2), TNF α (lane 3) and TNF α and BSO (lane 4). Lane P, lane N and fold were as described in Fig. 1 legend. Three identical experiments independently performed gave similar results.

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 conditions. Figure 3 and Fig. 5 suggest that TNF α -induced RANTES production, the attenuation by NAC and SB 203580 of RANTES production, and BSO-mediated facilitation of RANTES production, did not result from cell cytotoxicity.

Cellular redox state

It is known that NAC acts as a precursor of GSH synthesis [20] and BSO inhibits GSH synthesis [21]. The intracellular

GSH and H₂O₂ levels were measured in order to confirm the intracellular GSH and H₂O₂ levels in this study. When the cells were incubated with NAC for 1 h, the intracellular GSH increased (Fig. 6a). By contrast, when the cells were incubated with BSO for 16 h, the intracellular GSH was reduced (Fig. 6b).

Discussion

In the present study, the role of cellular redox regulated by intracellular GSH in TNF α -induced p38 MAP kinase activation and p38 MAP kinase-mediated RANTES

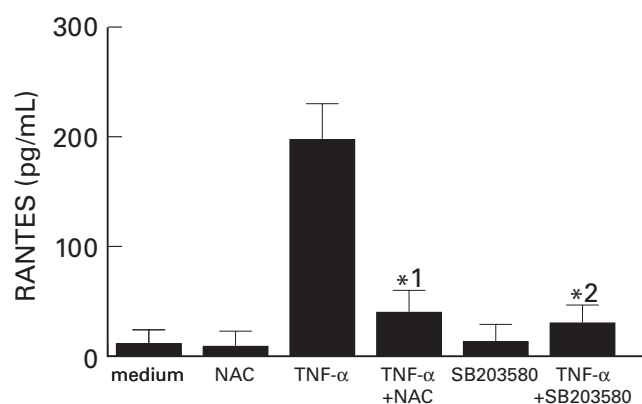


Fig. 4. NAC attenuates TNF α -induced RANTES production. BECs that had been pre-treated either with medium or NAC (10 mmol/L) for 1 h were cultured with medium or TNF α (10 ng/mL). Simultaneously, the cells that had been pre-treated with SB 203580 (10 μ mol/L) were stimulated with TNF α (10 ng/mL) to examine the effect of SB 203580 on TNF α -induced RANTES production. The concentrations of RANTES in the culture supernatant fluids were determined at 24 h after stimulation with TNF α . 10 μ mol/L SB 203580 contained 0.01% dimethyl sulfoxide, which had no effect on RANTES production. The results are expressed as mean $\pm$ SD in five different experiments. *1; $P < 0.01$ compared with RANTES concentrations in the culture supernatant fluids from TNF α -stimulated BECs cultured without NAC. *2; $P < 0.01$ compared with RANTES concentrations in the culture supernatant fluids from TNF α -stimulated BECs cultured without SB 203580.

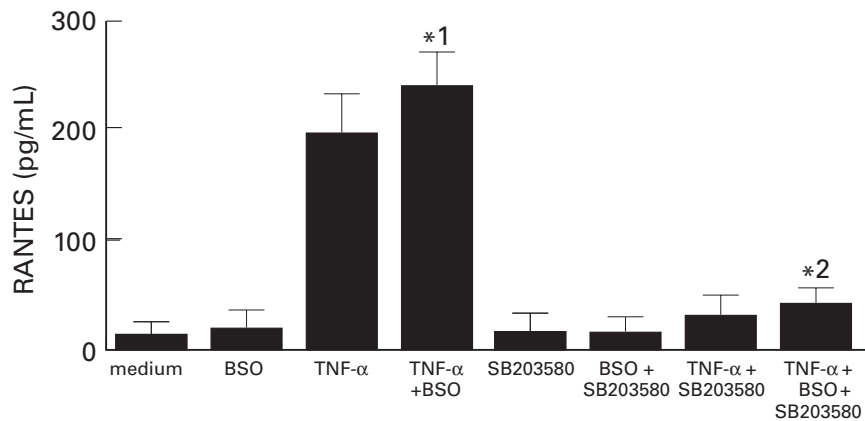


Fig. 5. BSO facilitates TNF α -induced RANTES production and SB 203580 attenuates RANTES production. BECs that had been pre-treated with medium or BSO (100 μ mol/L) for 16 h followed by the treatment with medium or SB 203580 (10 μ mol/L) for 1 h were cultured with medium or TNF α 10 ng/mL. The concentrations of RANTES in the culture supernatant fluids were determined at 24 h after stimulation with TNF α . 10 μ mol/L SB 203580 contained 0.01% dimethyl sulfoxide, which had no effect on RANTES production. The results are expressed as mean $\pm$ SD in five different experiments. *1; $P < 0.05$ compared with RANTES concentrations in the culture supernatant fluids from TNF α -stimulated BECs cultured without BSO and SB 203580. *2; $P < 0.01$ compared with RANTES concentrations in the culture supernatant fluids from BSO-pre-treated BECs followed by stimulation with TNF α .

production by human BECs was examined. The results showed that (i) NAC attenuated TNF α -induced p38 MAP kinase activation and RANTES production; (ii) SB 203580 attenuated TNF α -induced RANTES production; (iii) BSO facilitated TNF α -induced p38 MAP kinase activation and RANTES production; (iv) SB 203580 attenuated BSO-mediated facilitation of TNF α -induced RANTES production; and (v) the intracellular GSH increased in NAC-treated cells, whereas the intracellular GSH was reduced in BSO-treated cells. These results indicated that TNF α -induced p38 MAP kinase activation and p38 MAP kinase-mediated RANTES production by human BECs are at least regulated by intracellular GSH levels.

The cellular redox changes are involved in the activation of MAP kinase superfamily [15,16, 27,28] and the induction of cytokine expression [24,25]. Reactive oxygen species (ROS) generated by TNF-TH stimulation have been described as acting as signalling intermediates for TNF α -induced cytokine expression, since ROS are generated by TNF α stimulation [24–26] and antioxidants inhibit TNF α -induced cytokine expression [18,29,30]. In addition, ROS *per se* can activate p38 MAP kinase [15,16]. It was previously shown that p38 MAP kinase regulates TNF α -induced RANTES production by human BECs [17]. Redox control proteins, including GSH and TRX, have been shown to modulate c-Jun-NH₂-terminal kinase [31] and p38 MAP kinase activation [19]. However, a role for cellular redox regulated by intracellular GSH in TNF α -induced p38 MAP kinase activation and p38 MAP kinase-mediated RANTES production by BECs has not been determined. To clarify these issues, NAC and BSO were used. In

the present study, the intracellular GSH increased in NAC-treated cells, whereas the intracellular GSH was reduced in BSO-treated cells. The results showed that NAC attenuated TNF α -induced p38 MAP kinase activation and p38 MAP kinase-mediated RANTES production, indicating that TNF α -induced p38 MAP kinase activation and p38 MAP kinase-mediated RANTES production are negatively regulated by increasing intracellular GSH levels. By contrast, BSO facilitated TNF α -induced p38 MAP kinase activation and p38 MAP kinase-mediated RANTES

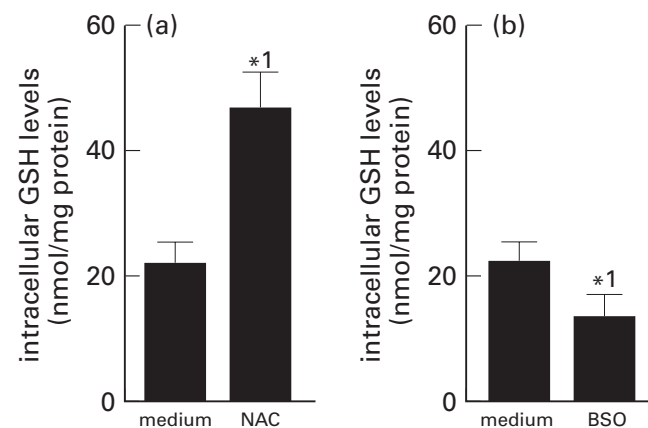


Fig. 6. Cellular redox state. The intracellular GSH levels in BECs were measured at 1 h after incubation with 10 mmol/L NAC (a), and at 16 h after incubation with 100 μ mol/L BSO (b). The results are expressed as mean $\pm$ SD in three different experiments. *1; $P < 0.05$ compared with the intracellular GSH levels in the cells cultured without NAC or BSO.

production, indicating that TNF α -induced p38 MAP kinase activation and p38 MAP kinase-mediated RANTES production are superinduced by a depleting intracellular GSH pool. Collectively, these results indicate that p38 MAP kinase activation and p38 MAP kinase-mediated RANTES production by BECs with TNF α stimulation are inversely regulated with intracellular GSH levels. ROS have been proposed to act as signalling intermediates for various TNF α -induced cellular responses, including cytokine expression [18,29,30]. It was proposed that ROS might be involved in TNF α -induced p38 MAP kinase activation in BECs based on the following evidence: (i) scavenging ROS by intracellular GSH increasing agent, NAC, attenuates TNF α -induced p38 MAP kinase activation; and (ii) disability of scavenging ROS by a depleting intracellular GSH pool superinduces TNF α -induced p38 MAP kinase activation. These results support the hypothesis that ROS might be involved in p38 MAP kinase activation in TNF α -stimulated BECs.

There are several possible mechanisms by which TNF α induces p38 MAP kinase activation and cellular redox, regulated by intracellular GSH, modulates TNF α -induced p38 MAP kinase activation in BECs. Recently, apoptosis signal-regulating kinase (ASK1) was identified as a MAP kinase which activates p38 MAP kinase cascades [32,33]. TNF α -induced ASK1 activation is regulated by the cellular redox state. TRX which binds directly to the N-terminal portion of ASK1 inhibits ASK1 activity [34]. TRX-mediated inhibition of ASK1 activity depends on their interaction, and a reduced form of TRX is critical for the direct inhibition of ASK1 activity [33]. Conversely, when the cells are exposed to oxidative stress, the oxidation of TRX may disrupt their interaction and consequently, activates ASK1. Alternatively, TNF α causes ASK1 activation through ROS-mediated dimerization of ASK1 [34]. In either way, the cellular redox state is critical for ASK1 activation. Although the up-stream regulator of p38 MAP kinase activation in BECs with TNF α stimulation was not examined, ASK1 activation might be involved in consequent p38 MAP kinase activation, and intracellular GSH could modulate p38 MAP kinase activation by modulating ASK1 activity. However, further study should be undertaken to clarify these issues.

Although the pathogenesis of allergic inflammation is complex, BECs play an important role in the production of allergic inflammation by producing a variety of cytokines [5]. Therefore, it is important to attenuate the production of cytokines. The present results showed that NAC attenuated TNF α -induced RANTES production by BECs. Antioxidant, including NAC, therapy has been reported to be useful in the treatment of acute lung injury [35,36]. It is not known, at this time, whether NAC is capable of producing a beneficial effect on controlling the production of allergic inflammation of bronchial asthma. However, an

understanding of the redox control mechanism of TNF α -induced RANTES production by BECs indicates a strategy for the therapy controlling allergic inflammation of bronchial asthma.

From the data presented here, it is concluded that cellular redox regulated by intracellular GSH is critical for TNF α -induced p38 MAP kinase activation and p38 MAP kinase-mediated RANTES production by human BECs. The attenuation of p38 MAP kinase activation and RANTES production by NAC is thought to be an important strategy for the treatment of bronchial asthma.

Acknowledgements

This work was financially supported, in part, by Grant-in-Aid for High-Tech Research Center from the Japanese Ministry of Education, Science, Sports and Culture to Nihon University

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 et al. Mucosal inflammation in asthma. *Am Rev Respir Dis* 1990; **142**:434–57.
- 3 Corrigan CJ, Kay ABT. 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 Takizawa H. Airway epithelial cells as regulator of airway inflammation (Review). *Int J Mol Med* 1998; **1**:367–78.
- 6 Berman N, Robichaud A, Krishnan VL et al. Expression of RANTES in human airway epithelial cells: effects of corticosteroids and interleukin-4 and -13. *Immunol* 1996; **87**:599–603.
- 7 Humbert M. Pro-eosinophilic cytokines in asthma. *Clin Exp Allergy* 1996; **26**:123–7.
- 8 Baggiolini M, Dahinden CA. CC chemokines in allergic inflammation. *Immunol Today* 1994; **15**:127–33.
- 9 Davis RJ. MAPKs: new JNK expand the group. *Trends Biochem Sci* 1994; **19**:470–3.
- 10 Han J, Lee JD, Bibbs L, Ulevitch RJA. Map kinase targeted by endotoxin and hyperosmolarity in mammalian cells. *Science* 1994; **265**:808–11.
- 11 Raingeaud J, Gupta S, Rogers JS 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.
- 12 Hashimoto S, Matsumoto K, Gon K et al. Hyperosmolarity-induced IL-8 expression in human bronchial epithelial cells through p38 MAP kinase. *Am J Respir Crit Care Med* 1999; **159**:634–40.
- 13 Gon Y, Hashimoto S, Matsumoto K et al. Cooling and rewarming-induced IL-8 expression in human bronchial

- epithelial cells through p38 MAP kinase-dependent pathway. *Biochem Biophys Res Commun* 1998; **249**:156–60.
- 14 Matsumoto K, Hashimoto S, Gon Y et al. Proinflammatory cytokine- and chemical mediator-induced IL-8 expression in human bronchial epithelial cells through p38 MAP kinase-dependent pathway. *J Allergy Clin Immunol* 1998; **101**:825–31.
- 15 Moriguchi T, Toyoshima F, Gotoh Y 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.
- 16 Clerk A, Fuller SJ, Michael A, Sugden PH. Stimulation of 'stress-regulated' mitogen-activated protein kinases (stress-activated protein kinases/c-jun N-terminal kinases and p38-mitogen-activated protein kinase) in perfused rat hearts by oxidative and other stresses. *J Biol Chem* 1998; **273**:7228–34.
- 17 Hashimoto S, Matsumoto K, Gon Y et al. p38 mitogen-activated protein kinase regulate TNF- α , IL-1 α , and PAF-induced RANTES GM-CSF production by human bronchial epithelial cells. *Clin Exp Allergy* 2000; **30**:48–55.
- 18 Matsumoto K, Hashimoto S, Gon Y et al. N-acetylcysteine inhibits IL-1 α -induced IL-8 secretion by human bronchial epithelial cells. *Respir Med* 1998; **92**:512–5.
- 19 Hashimoto S, Matsumoto K, Gon Y et al. Thioredoxin negatively regulates p38 MAP kinase activation and IL-6 production by tumor necrosis factor- α . *Biochem Biophys Res Commun* 1999; **258**:443–7.
- 20 Cotgreave I, Moldeus P, Schuppe I. The metabolism of N-acetylcysteine by human endothelial cells. *Biochem Pharmacol* 1991; **42**:13–6.
- 21 Wu X, Bishopric NH, Discher DJ et al. Physical and functional sensitivity of zinc finger transcription factors to redox changes. *Mol Cell Biol* 1996; **16**:1035–46.
- 22 Lee JC, Laydon JT, McDonnell PC et al. A protein kinase involved in the regulation of inflammatory cytokine biosynthesis. *Nature* 1994; **372**:739–46.
- 23 Anderson ME. Determination of glutathione and glutathione disulfide in biological samples. *Meth Enzymol* 1985; **113**:548–55.
- 24 Schreck R, Baeuerle PA. A role of oxygen radicals as second messengers. *Trend Cell Biol* 1991; **1**:39–42.
- 25 Schreck R, Albermann K, Baeuerle PA. Nuclear factor κ B: an oxidative stress-responsive transcriptional factor of eukaryotic cells (a review). *Free Rad Res Commun* 1992; **17**:221–37.
- 26 Meier B, Radeke HH, Selle S et al. Human fibroblasts release reactive oxygen species in response to interleukin-1 and tumor necrosis factor- α . *Biochem J* 1989; **263**:539–45.
- 27 Guyton K, Yusen L, Gorospe M et al. Activation of mitogen-activated protein kinase by H₂O₂. Role of cell survival following oxidant injury. *J Biol Chem* 1996; **271**:4438–42.
- 28 Laderoute KR, Webster KA. Hypoxia/reoxygenation stimulates Jun kinase activity through redox signaling in cardiac myocytes. *Cir Res* 1997; **80**:336–44.
- 29 Talley AK, Dewhurst S, Perry SW et al. Tumor necrosis factor alpha-induced apoptosis in human neural cells: protection by the antioxidant N-acetylcysteine and the genes bcl-2 and crmA. *Mol Cell Biol* 1995; **15**:2359–466.
- 30 Droge W, Eck H-P, Mihm S. HIV-induced cysteine deficiency and T-cell dysfunction — a rationale for treatment with N-acetylcysteine. *Immunol Today* 1992; **13**:211–4.
- 31 Wilhelm D, Bender K, Knebel A, Angel P. The levels of intracellular glutathione is a key regulator for the induction of stress-activated signal transduction pathways including Jun N-terminal protein kinases and p38 kinase by alkylating agents. *Mol Cell Biol* 1997; **17**:4792–800.
- 32 Ichijo H, Nishida E, Irie K et al. Induction of apoptosis by ASK1, a mammalian MAPKKK that activates SAPK/JNK and p38 signaling pathway. *Science* 1997; **275**:90–4.
- 33 Saitoh M, Nishitoh H, Fujii M et al. Mammalian thioredoxin is a direct inhibitor of apoptosis signal regulating kinase (ASK) 1. *EMBO J* 1998; **9**:2596–606.
- 34 Gotoh Y, Cooper JA. Reactive oxygen species- and dimerization-induced activation of apoptosis signal-regulating kinase 1 in tumor necrosis factor- α signal transduction. *J Biol Chem* 1998; **273**:17447–82.
- 35 Bernard GR. Potential of N-acetylcysteine as treatment for the adult respiratory distress syndrome. *Eur Respir J* 1990; **3** (Suppl):486S–498S.
- 36 Suter PM, Domenighetti G, Shaller MD et al. N-acetylcysteine enhances recovery from acute lung injury in man. A randomized, double blind, placebo-controlled clinical study. *Chest* 1996; **109**:163–637.