

Role of leukotriene B₄ in bronchial hyperresponsiveness induced by interleukin-8

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ABSTRACT: Repeated intranasal administration of interleukin 8 (IL-8) induces bronchial hyperresponsiveness (BHR) accompanied by lower airway neutrophil accumulation (ANA) in guinea-pigs. Leukotriene B₄ (LTB₄) is a chemotactic factor for neutrophils.

To elucidate whether LTB₄ and neutrophil elastase are involved in the IL-8-induced BHR and ANA, the effects of a LTB₄ antagonist (ONO-4057) and a neutrophil elastase inhibitor (ONO-5046) on the responses were examined. IL-8 (5 µg·kg⁻¹) was administered intranasally to guinea-pigs twice weekly for 3 weeks. One day after the last administration, animals were anaesthetized and artificially ventilated through tracheal cannulae, and lateral pressure at the tracheal cannula (*P*_{ao}) was measured as an overall index of airway responses to inhaled histamine. ONO-4057 (2 or 20 mg·kg⁻¹) or ONO-5046 (30 or 300 mg·kg⁻¹) was administered intraperitoneally 24 and 1 h before anaesthesia.

ONO-4057, but not ONO-5046, significantly inhibited the IL-8-induced BHR and ANA, assessed by bronchoalveolar lavage, in a dose-dependent manner.

These findings suggest that interleukin 8 causes bronchial hyperresponsiveness and airway neutrophil accumulation in guinea-pigs *in vivo*. In part this appears to be due to release of leukotriene B₄, whereas it may not be mediated by neutrophil elastase.

Eur Respir J 1998; 11: 306–311.

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Keywords: Airway neutrophil influx, bronchial hyperresponsiveness, interleukin 8, leukotriene B₄, neutrophil elastase

Received: July 30 1996
Accepted after revision September 29 1997

This study was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture (07670662) of the Japanese Government.

Interleukin 8 (IL-8) was purified from lipopolysaccharide (LPS)-stimulated human peripheral blood mononuclear cell culture supernatants [1]. IL-8 is known as a potent chemoattractant and activating factor for neutrophils and as a chemoattractant for T-lymphocytes [2, 3]. However, recent studies have shown that IL-8 also triggers histamine and leukotriene release from basophils [4], induces contraction of airway smooth muscle [5] and causes eosinophil infiltration *in vivo* [6]. In addition, it has been demonstrated that bronchial epithelial cells and eosinophils stimulated with calcium ionophores can produce IL-8 *in vitro* [7, 8], suggesting an important role of IL-8 in the genesis and persistence of bronchial inflammation in asthma. We recently showed the elevated level of IL-8 in sputum obtained from asthmatic patients, especially during asthma attacks [9].

We recently reported that repeated intranasal administration of IL-8 (twice a week for 3 weeks) induced bronchial hyperresponsiveness (BHR) and neutrophil accumulation in the lower airways in guinea-pigs *in vivo* [10]. Leukotriene B₄ (LTB₄) is an arachidonate 5-lipoxygenase product and a chemotactic factor for neutrophils. The present study was conducted to examine whether LTB₄ and neutrophil elastase are involved in the IL-8-induced BHR and lower airway neutrophil influx. We assessed the effect of an LTB₄ antagonist (ONO-4057) [11] and a human neutrophil elastase inhibitor (ONO-

5046) [12] on the BHR to inhaled histamine and on bronchoalveolar lavage cells in the IL-8-treated guinea-pigs.

Materials and methods

Animals

Male, albino, Hartley strain guinea-pigs weighing 350–400 g were obtained from Sankyo Laboratory Service (Toyama, Japan). After arrival at the Institute of Animal Experiments in our university, the animals were kept in conventional housing facilities for 1 week before the start of treatment with IL-8. They were allowed to drink and feed *ad libitum*. This animal experiment was performed according to Principles of Laboratory Animal Care formulated by the Institute of Animal Experiments in Kanazawa University.

Experimental protocols

Thirty nine guinea-pigs received intranasal administration of 5 µg·kg⁻¹ of IL-8 and six received phosphate-buffered saline (PBS) twice a week for 3 weeks. Bronchial responsiveness was assessed 24 h after the last administration of IL-8 and then bronchoalveolar lavage (BAL)

was performed. ONO-4057 or ONO-5046 was given intraperitoneally 24 and 1 h before anaesthesia of animals. ONO-4057 and ONO-5046 were dissolved in physiological saline to make the studied concentrations. In the ONO-4057 study, animals treated with IL-8 were divided into the following three groups: 1) treated with 2 mg·kg⁻¹ of ONO-4057 (n=6); 20 mg·kg⁻¹ of ONO-4057 (n=6); and saline (n=6). As a normal control group six animals were treated with saline after intranasal administration of PBS for 3 weeks. In the ONO-5046 study, IL-8 treated animals were given 30 (n=7) or 300 mg·kg⁻¹ of ONO-5046 (n=7) or saline (n=7).

To assess nonspecific bronchodilator and/or broncho-protective action of ONO-4057 and ONO-5046, the effects of these compounds on bronchial responsiveness to inhaled histamine were examined in normal guinea-pigs. ONO-4057 at a dose of 2 (n=6) or 20 mg·kg⁻¹ (n=6) or saline (n=6) was administered intraperitoneally 1 h before anaesthesia. In the same manner, 30 (n=6) or 300 mg·kg⁻¹ of ONO-5046 (n=6) or saline (n=6) was given.

Treatment with IL-8

IL-8 was dissolved in PBS at a concentration of 5 µg·mL⁻¹. One millilitre per kilogram of the IL-8 solution was administered intranasally twice weekly for 3 weeks. Endotoxin content of the IL-8 solution was not detectable, being less than 50 pg·mg protein⁻¹ (Phrodick Limulus Test kit, Seikagaku Kogyo, Tokyo) [13].

Assessment of bronchial responsiveness

One day after the last administration of IL-8, guinea-pigs were anaesthetized with sodium pentobarbital (75 mg·kg⁻¹ *i.p.*). The animals were placed in the supine position and the trachea was cannulated with a polyethylene tube (outside diameter 2.5 mm, inside diameter 2.1 mm).

After surgery the guinea-pigs were artificially ventilated by a small animal respiratory pump (Model 1680, Harvard Apparatus Co. Inc., South Natick, MA, USA) adjusted to a tidal volume (*V*_T) of 10 mL·kg⁻¹ at a rate of 60 strokes·min⁻¹. The change in lung resistance to inflation, the lateral pressure of the tracheal tube (pressure at the airway opening; *P*_{ao} in cmH₂O), was measured using a pressure transducer (Model TP-603T; Nihon Koden Kogyo Co. Ltd., Tokyo, Japan). Since the change in *P*_{ao} following inhalation of leukotriene C₄ (LTC₄) represented the average of the changes in pulmonary resistance (*R*_L) and reciprocal dynamic lung compliance (1/*C*_{dyn}) [14], we used *P*_{ao} as an overall index of bronchial response to bronchoactive agents.

When all procedures were completed, the animals received twice the *V*_T for two breaths by clamping the outlet port of the respirator to unify the volume history of the lung [14].

After *P*_{ao} had stabilized, increasing doses of histamine (25, 50, 100 and 200 µg·mL⁻¹) were inhaled at 5 min intervals under continuous ventilation. The aerosol was generated during a 20 s period by an ultrasonic nebulizer developed for small animals at our institution [15]. The amount of aerosol was 15.2 µL·min⁻¹, and 46.4% of the aerosol was deposited in the lung as measured by radio-

aerosol technique [15]. The median aerodynamic diameter of the particles of normal saline was 3.59±1.96 µm (mean±SD). All of histamine inhalation challenges were completed within 60 min.

Analysis of bronchoalveolar lavage cells

BAL was performed immediately after the determination of bronchial responsiveness. The lower airways were lavaged *via* the tracheal cannula with 10 mL sterile saline at 37°C. The fluid was recovered by gentle aspiration with a disposable syringe. The BAL fluid was immediately centrifuged at 1,500 revolutions per minute (rpm) for 10 min. After discarding the supernatant, the cells were washed twice in Hank's solution and resuspended in 1 mL of Hank's solution. They were then counted manually in a Burkert Chamber. Cyto-centrifuged preparations (Cytospin 2, Shandon Southern Products Ltd., UK) were stained with May Giemsa and a differential cell count was performed on 300 cells according to standard morphological criteria.

Statistical analysis

Dose-response curves for histamine-induced bronchoconstriction in the three treatment groups in each study were compared by repeated measure analysis of variance (ANOVA). Bronchoconstrictor responses to each concentration of histamine were compared among the three treatment groups using factorial ANOVA. Cellular composition of BAL fluid was compared between each pair of groups using the Mann-Whitney U-test. A *p*-value of 0.05 or less was considered significant. All results were expressed as mean±SEM.

Chemicals

The following chemicals were used: sodium pentobarbital (Abbot Laboratories, North Chicago, IL, USA); histamine (Wako Pure Chemical Ind., Osaka, Japan); ONO-4057 (5-(2-(2-carboxyethyl)-3-(6-(4-methoxyphenyl)-5E-hexenyl)oxyphenoxy)valeric acid) and ONO-5046 (N-(2-(4(2,2-dimethylpropionyloxy)phenylsulfonylamino)aminoacetic acid) (Ono Pharmaceutical Co. Ltd., Osaka, Japan). Human recombinant IL-8 was prepared as reported previously [13, 16].

Results

Effects of ONO-4057

IL-8-induced bronchial hyperresponsiveness (fig. 1). Pre-histamine inhalation values for *P*_{ao} were 10.8±0.4 cmH₂O in PBS-administered animals treated with saline, 9.7±0.4 cmH₂O in IL-8 administered animals treated with saline, and 10.0±0.3 and 9.5±0.4 cmH₂O in the IL-8-administered animals treated with 2 and 20 mg·kg⁻¹ of ONO-4057, respectively; these values were not significantly different. The dose-response curve for percentage increases in *P*_{ao}

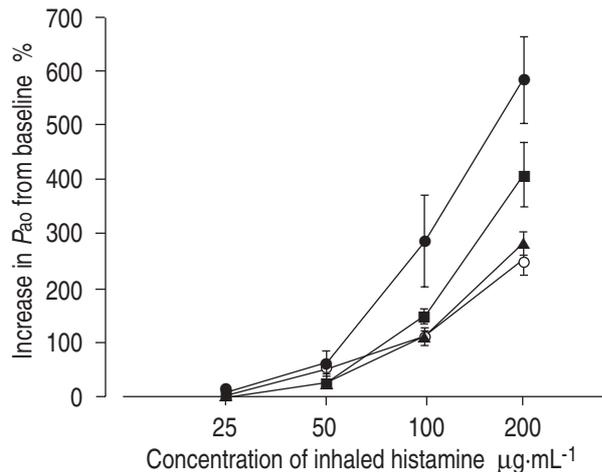


Fig. 1. – Effects of a selective leukotriene B₄ (LTB₄) antagonist (ONO-4057), in guinea-pigs, on bronchial hyperresponsiveness to inhaled histamine induced by intranasal administration of 5 µg·kg⁻¹ of interleukin-8 (IL-8) twice weekly for 3 weeks. ONO-4057 was administered intraperitoneally at a dose of 2 or 20 mg·kg⁻¹ 24 and 1 h before anaesthesia of animals. Animals received either repeated intranasal administration of phosphate-buffered saline and treatment with saline (negative control; ○), repeated intranasal administration of IL-8 and treatment with saline (positive control; ●), or repeated intranasal administration of IL-8 and treatment with 2 mg·kg⁻¹ (■) or 20 mg·kg⁻¹ (▲) ONO-4057. The histamine dose-response curve was significantly ($p < 0.05$) different between positive and negative control animals using repeated measure analysis of variance (ANOVA). The dose-response curves of the IL-8-administered animals treated with 2 and 20 mg·kg⁻¹ of ONO-4057 differed significantly ($p < 0.02$, repeated measure ANOVA) from the positive control animals. P_{ao} : pressure at the airway opening.

from the baseline value caused by increasing doses of inhaled histamine was significantly ($p < 0.05$) different between the PBS-administered animals treated with saline and the IL-8-administered animals treated with saline, indicating IL-8-induced BHR. The histamine dose-response curve was significantly ($p < 0.02$) different among the IL-8-administered animals treated with saline and 2 and 20 mg·kg⁻¹ of ONO-4057, showing a dose-dependent inhibition of IL-8-induced bronchial hyperresponsiveness by ONO-4057.

Naive bronchial responsiveness (fig. 2). The P_{ao} values before histamine inhalation were 10.2 ± 0.4 , 10.5 ± 0.4 and 9.9 ± 0.4 cmH₂O in animals treated with saline and 2 and 20 mg·kg⁻¹ of ONO-4057, respectively, and these values were not significantly different. The dose-response curves for percentage increases in P_{ao} from the baseline value caused by ascending doses of inhaled histamine were not different among the three groups, showing no nonspecific bronchodilator or bronchoprotective effect of ONO-4057.

IL-8-induced airway neutrophil influx (fig. 3). Total cells recovered from BAL fluid were 2.10 ± 0.28 cells·mL⁻¹ in PBS-administered animals treated with saline, 2.1 ± 0.2 cells·mL⁻¹ in IL-8 administered animals treated with saline, and 2.1 ± 0.3 and $2.7 \pm 0.2 \times 10^5$ cells·mL⁻¹ in IL-8-administered animals treated with 2 and 20 mg·kg⁻¹ of ONO-4057, respectively; these values were not significantly different. The percentage of neutrophils in BAL fluid was significantly greater in IL-8-administered and saline-treated animals ($21.8 \pm 1.6\%$) compared with PBS-administered animals ($2.6 \pm 0.4\%$) ($p < 0.01$) (fig. 3). The value was significantly ($p < 0.01$) decreased by the

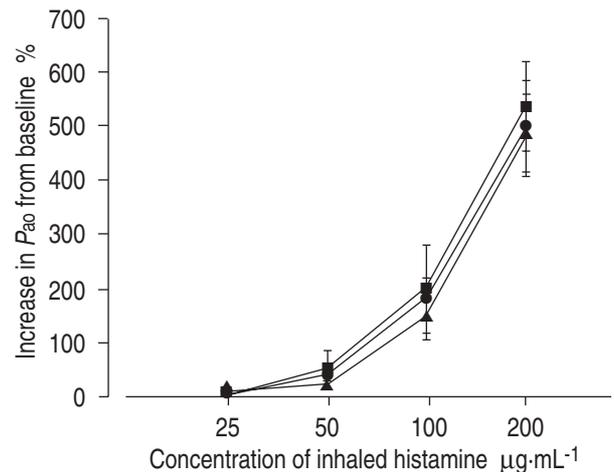


Fig. 2. – Effects of a selective leukotriene B₄ (LTB₄) antagonist (ONO-4057) on bronchial responsiveness to inhaled histamine in naive guinea-pigs. ONO-4057 was administered intraperitoneally at a dose of 2 or 20 mg·kg⁻¹ h before anaesthesia of animals. The histamine dose-response curve was not significantly different among animals treated with 2 and 20 mg·kg⁻¹ of ONO-4057 and saline by repeated measure analysis of variance (ANOVA). ●: saline (n=6); ■: 2 mg·kg⁻¹ ONO-4057; ▲: 20 mg·kg⁻¹ ONO-4057. For definitions, see legend to figure 1.

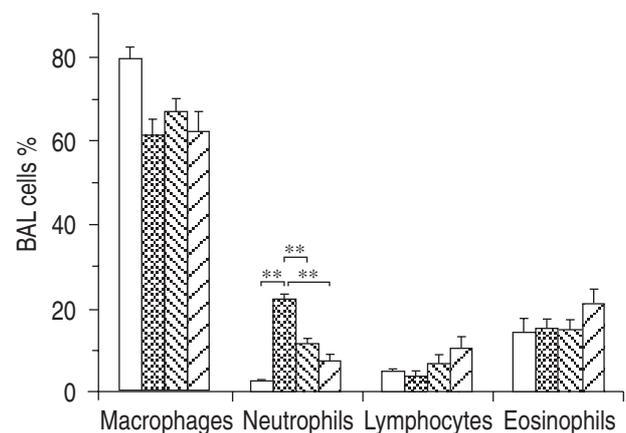


Fig. 3. – Effect of a selective leukotriene B₄ (LTB₄) antagonist (ONO-4057) on cellular composition of bronchoalveolar lavage (BAL) fluid induced by 3 weeks of treatment with interleukin-8 (IL-8) in guinea-pigs. Animals received either repeated intranasal administration of PBS and treatment with saline (negative control; □), repeated intranasal administration of IL-8 and treatment with saline (positive control; ▨), or repeated intranasal administration of IL-8 and treatment with 2 mg·kg⁻¹ (▩) or 20 mg·kg⁻¹ (▧) ONO-4057. **: $p < 0.01$ (factorial analysis of variance (ANOVA)). For definitions, see legend to figure 1.

ONO-4057 treatment ($11.5 \pm 1.5\%$ at 2 mg·kg⁻¹ and $7.3 \pm 1.6\%$ at 20 mg·kg⁻¹) compared with the saline treatment (fig. 3).

Effects of ONO-5046

IL-8-induced bronchial hyperresponsiveness (fig. 4). The P_{ao} values before histamine provocation were 10.0 ± 0.2 , 10.9 ± 0.1 and 10.9 ± 0.3 cmH₂O in the IL-8-administered animals treated with saline and 30 and 300 mg·kg⁻¹ of ONO-5046, respectively. The dose-response curves for percentage increase in P_{ao} from the baseline value caused by increasing doses of inhaled histamine were not significantly different among these three treatment groups,

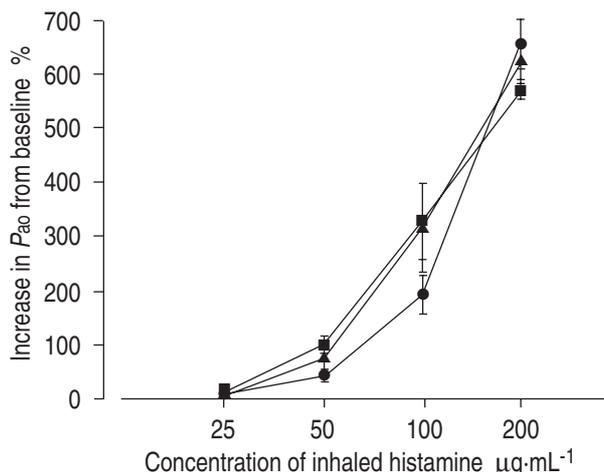


Fig. 4. – Effects of a selective neutrophil elastase inhibitor (ONO-5046) in guinea-pigs, on bronchial hyperresponsiveness to inhaled histamine induced by intranasal administration of 5 µg·kg⁻¹ of interleukin-8 (IL-8) twice weekly for 3 weeks. ONO-5046 was administered intraperitoneally at a dose of 30 or 300 mg·kg⁻¹ 24 and 1 h before anaesthesia of animals. Animals received repeated intranasal administration of IL-8 and treatment with either saline (●: control, n=7), or 30 mg·kg⁻¹ (■) or 300 mg·kg⁻¹ ONO-5046 (▲). For definitions see legend to figure 1.

showing no inhibitory effect of ONO-5046 on IL-8-induced BHR.

Naive bronchial responsiveness (fig. 5). The P_{ao} values before histamine inhalation were 9.1±0.3, 9.3±0.4 and 9.5±0.3 cmH₂O in animals treated with saline and 30 and 300 mg·kg⁻¹ of ONO-5046, respectively; these values were not significantly different. The dose-response curves for percentage increases in P_{ao} from the baseline value caused by increasing doses of inhaled histamine was not different among the three groups.

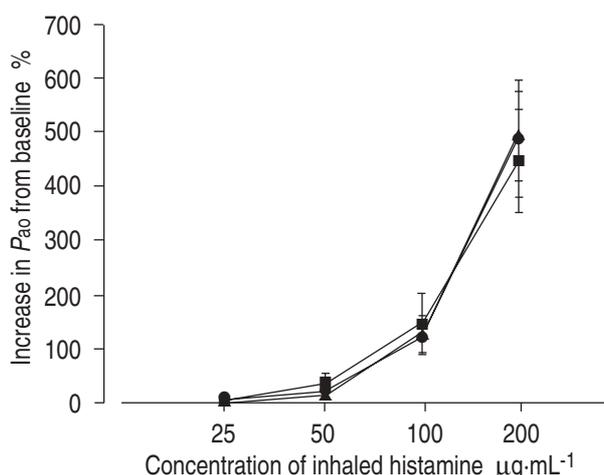


Fig. 5. – Effects of a selective neutrophil elastase inhibitor (ONO-5046) on bronchial responsiveness to inhaled histamine in naive guinea-pigs. ONO-5046 was administered intraperitoneally at a dose of 30 or 300 mg·kg⁻¹ 1 h before anaesthesia of animals. The histamine dose-response curves were not significantly different among animals treated with 30 and 300 mg·kg⁻¹ of ONO-5046 and saline (repeated measure analysis of variance (ANOVA)). ●: repeated intranasal administration of interleukin-8 (IL-8) and treatment with saline (control, n=6); ■: repeated intranasal administration of IL-8 and treatment with 30 mg·kg⁻¹ ONO-5046 (n=6); ▲: repeated intranasal administration of IL-8 and treatment with 300 mg·kg⁻¹ ONO-5046. For definitions see legend to figure 1.

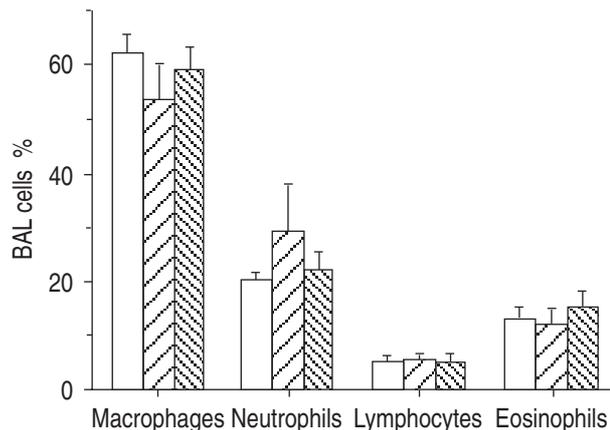


Fig. 6. – Effect of a selective neutrophil elastase inhibitor (ONO-5046) on cellular composition of bronchoalveolar lavage (BAL) fluid induced by 3 weeks of treatment with interleukin-8 (IL-8) in guinea-pigs. □: repeated intra-nasal administration of IL-8 and treatment with saline (control); ▨: repeated intranasal administration of IL-8 and treatment with 30 mg·kg⁻¹ ONO-5046; ▩: repeated intranasal administration of IL-8 and treatment with 300 mg·kg⁻¹ ONO-5046. For further definitions see legend to figure 1.

IL-8-induced airway neutrophil influx (fig. 6). Total cells recovered from BAL fluid were 1.87±0.23, 5.81±0.10 and 4.64±0.71 ×10⁵ cells·mL⁻¹ in animals treated with saline and 30 and 300 mg·kg⁻¹ of ONO-5046, respectively. Although the value was significantly greater with 30 mg·kg⁻¹ (p<0.01) and 300 mg·kg⁻¹ of ONO-5046 treatments (p<0.01) compared with saline treatment, the percentage of neutrophils in BAL fluid was not significantly different among the three groups (fig. 6).

Discussion

Three week intranasal administration of IL-8 in guinea-pigs enhanced bronchial responsiveness to inhaled histamine, which was accompanied by lower airway neutrophil accumulation. An LTB₄ antagonist (ONO-4057) significantly inhibited the IL-8-induced BHR and reduced airway neutrophil influx in a dose dependent fashion, while a neutrophil elastase inhibitor ONO-5046 did not.

The P_{ao} measurement used in this study does not clearly distinguish between airway oedema, airway smooth muscle contraction and lung elasticity. Since P_{ao} increased immediately after antigen and histamine inhalation and the bronchodilator procaterol almost completely inhibited the increases in P_{ao} [17], it is likely that the increase in P_{ao} is indicative of bronchoconstriction in our experimental system.

Although we did not perform histological examination to assess lower airway inflammatory cells in this study, we have previously showed that the IL-8-induced BHR was accompanied by increased neutrophils, but not eosinophils, in both BAL fluid and bronchial tissues [10]. As the neutrophil counts in BAL fluid were significantly correlated with those in bronchial tissues, we evaluated only BAL cell population in this study. A significant correlation between neutrophil inflammation and BHR has been shown in animal studies [18–20], while SMH *et al.* [21] have reported that administration of IL-8 (10 µg) to guinea-pigs by the intraperitoneal route induced an increase

in T-lymphocytes (maximal at 4 h) and eosinophils (maximal at 24 h) in BAL fluid. They have also reported that a single inhalation of IL-8 (50 µg) caused an increase in the numbers of eosinophils, but not lymphocytes, in BAL fluid as compared with the control vehicle (0.25% bovine serum albumin (BSA)). Neither single intraperitoneal nor aerosolized administration of IL-8 induced BHR to intravenously administered histamine. Accordingly, there has been controversy over whether IL-8 causes accumulation of neutrophils into the airways and produces BHR. We do not know why bronchial responsiveness was enhanced by IL-8 in our study but not in that of SMITH *et al.* [21]. Single administration of IL-8 in the study of SMITH *et al.* [21] and repeated administration in the present study may account for the discrepancy. We administered IL-8 repeatedly because we hypothesized that repeated and/or continuous production of IL-8 in the lower airways might contribute to the BHR seen in stable asthmatics.

To elucidate the role of LTB₄ in the BHR and lower airway neutrophil influx caused by repeated administration of IL-8 the effects of a selective LTB₄ antagonist ONO-4057 [11] on these responses were examined. ONO-4057 displaces the binding of ³H-labelled LTB₄ to the LTB₄ receptor in human neutrophils (equilibrium inhibition constant (K_i) = 3.7±0.9 nM) and inhibits the LTB₄-induced rise in cytosolic free calcium with an inhibitory concentration of 50% (IC₅₀) value of 0.7±0.3 µM and human neutrophil aggregation, chemotaxis and degranulation induced by LTB₄ with IC₅₀ values of 3.0±0.1, 0.9±0.1 and 1.6±0.1 µM, respectively, without showing any agonist activity at concentration up to 30 µM [11]. Orally administered ONO-4057 prevents LTB₄-induced transient neutropenia and intradermal neutrophil migration in guinea-pigs with IC₅₀ values of 25.6 and 5.3 mg·kg⁻¹ [11]. The present results on ONO-4057 indicate the importance of LTB₄ in the increased bronchial responsiveness and lower airway neutrophil accumulation induced by IL-8.

ONO-5046 is a competitive inhibitor of human neutrophil elastase [12]. The effects of ONO-5046 in the present experimental model were studied to examine whether the BHR is mediated by release of neutrophil elastase from accumulating neutrophils induced by repeated intranasal administration of IL-8. ONO-5046 inhibits human neutrophil elastase (IC₅₀=0.044 µM, K_i=0.2 µM) and leucocyte elastase obtained from rabbits, rats, hamsters and mice [12]. ONO-5046 does not inhibit trypsin, thrombin, plasmin, plasma kallikrein, chymotrypsin or cathepsin G even at 100 µM [13]. ONO-5046 suppresses lung haemorrhage in hamsters by intratracheal administration (ID₅₀ = 82 µg·kg⁻¹) and increase of skin capillary permeability in guinea-pigs by intravenous administration (inhibitory dose of 50% (ID₅₀)=9.6 mg·kg⁻¹), both of which are induced by human neutrophil elastase [12]. It has been shown that infusion of ONO-5046 (1 or 10 mg·kg⁻¹·h⁻¹) dose-dependently inhibits LTB₄-induced polymorphonuclear leucocyte-mediated increase in pulmonary microvascular permeability [22]. SAKAWA *et al.* [23] recently reported that ONO-5046 showed concentration-dependent inhibition of guinea-pig neutrophil elastase activity (IC₅₀ value of 23.2±1.2 nM), and that the inhibition was competitive, with a K_i value of 7.65±0.62 nM by Dixon analysis. They also showed that 150 mg of ONO-5046 given intravenously significantly inhibited increase in the lung wet/dry ratio induced by lipopolysaccharide in guinea-

pigs. In the present study, intraperitoneal administration of ONO-5046 did not reduce the BHR induced by IL-8 at doses of 30 or 300 mg·kg⁻¹, the latter dose being considered sufficient to inhibit action of neutrophil elastase. ONO-5046 did not alter BAL cell distribution, while it increased total cell number in BAL fluid. FUMIO *et al.* [24] have shown that continuous infusion of ONO-5046 (10 mg·kg⁻¹·h⁻¹) in allergic sheep reduces antigen-induced early and late bronchoconstriction, neutrophil recruitment, increase in LTB₄ in BAL fluid and BHR. Although we do not know the reason for the discrepancy between the present results and those of FUMIO *et al.* [24], it is possible that the administration route of this compound may be responsible.

In conclusion, the present study suggests that repeated intranasal administration of interleukin 8 induces bronchial hyperresponsiveness and lower airway neutrophil accumulation in part through release of leukotriene B₄, in which neutrophil elastase may not be involved.

Acknowledgement: The authors wish to thank Ono Pharmaceutical Co., Ltd., Osaka, Japan for kindly supplying ONO-4057 and ONO-5046.

References

1. Mukaida N, Harada A, Yasumoto K, Matsushima K. Properties of pro-inflammatory cell type-specific leukocyte chemotactic cytokine, interleukin (IL-8) and monocyte chemotactic and activating factor (MCAF). *Microbiol Immunol* 1992; 36: 773–789.
2. Baggolini M, Walze A, Kunkel SL. Neutrophil-activating peptide-1/interleukin 8, a novel cytokine that activates neutrophils. *J Clin Invest* 1989; 84: 1045–1049.
3. Leonard EJ, Yoshimura T. Neutrophil attractant/activation protein-1 (NAP-1) (interleukin-8). *Am J Respir Cell Biol* 1990; 2: 479–486.
4. Krieger M, Brunner T, Bischoff SC, *et al.* Activation of human basophils through the IL-8 receptor. *J Immunol* 1992; 149: 2662–2667.
5. Burrows LJ, Piper PJ. Human recombinant neutrophil-activating factor/interleukin-8 is a spasmogen of airway smooth muscle (abstract). *Cytokine* 1989; 1: 100.
6. 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: 312–318.
7. Marini M, Vittori E, Hollemborg J, Mattoli SM. 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: 1001–1009.
8. Braun RK, Franchini M, Erard F, *et al.* Human peripheral blood eosinophils produce and release interleukin-8 on stimulation with calcium ionophore. *Eur J Immunol* 1993; 23: 956–960.
9. Kurashima K, Mukaida N, Fujimura M, Schröder JM, Matsuda T, Matsushima K. Increase of chemokine levels in sputum precedes exacerbation of acute asthma attacks. *J Leukocyte Biol* 1996; 59: 1–4.
10. Xiu Q, Fujimura M, Nomura M, *et al.* Involvement of thromboxane A₂ in bronchial hyperresponsiveness induc-

- ed by interleukin-8 in guinea pigs. *Clin Exp Allergy* 1995; 25: 51–59.
11. Kishikawa K, Tateishi N, Maruyama T, Seo R, Toda M, Miyamoto T. ONO-4057, a novel, orally active leukotriene B₄ antagonist: effects on LTB₄-induced neutrophil functions. *Prostaglandins* 1992; 44: 261–275.
 12. Kawabata K, Suzuki M, Sugitani M, Imaki K, Toda M, Miyamoto T. ONO-5046, a novel inhibitor of human neutrophil elastase. *Biochem Biophys Res Commun* 1991; 177: 814–820.
 13. Furuta R, Yamagishi J, Kotani H, *et al.* Production and characterization of recombinant human neutrophil chemo-tactic factor. *J Biochem* 1989; 106: 436–441.
 14. Fujimura M. Inhibitory effect of steroids on slow reacting substance of anaphylaxis (SRS-A) mediated bronchoconstriction in guinea pig. *Jpn J Allergol* 1983; 32: 365–375.
 15. Minami S, Okafuji K, Saga T, *et al.* A new quantitative inhalation apparatus for small animals. *Jpn J Chest Dis* 1983; 21: 252–258.
 16. Matsushima K, Morishita K, Yoshimura T, *et al.* Molecular cloning of a human monocyte-derived neutrophil chemo-tactic factor (MDNCF) and the induction of MDNCF mRNA by interleukin 1 and tumor necrosis factor. *J Exp Med* 1988; 167: 1883–1893.
 17. Fujimura M, Sakamoto S, Nishi K, Saito M, Miyake Y, Matsuda T. Inhibitory effect of inhaled procaterol on anaphylactic bronchoconstriction and thromboxane production in guinea pigs. *Clin Exp Allergy* 1991; 21: 189–194.
 18. Holtzman MJ, Fabbri LM, O'Byrne PM, *et al.* Importance of airway inflammation for hyperresponsiveness induced by ozone. *Am Rev Respir Dis* 1983; 127: 686–690.
 19. O'Byrne PM, Leikauf GD, Aizawa H, *et al.* Leukotriene B₄ induces airway hyperresponsiveness in dogs. *J Appl Physiol* 1985; 59: 1941–1946.
 20. Pauwels RA, Kips JC, Peleman RA, Van Der Straeten ME. The effect of endotoxin inhalation on airway responsiveness and cellular influx in rats. *Am Rev Respir Dis* 1990; 141: 540–545.
 21. Smith D, Burrows L, Westwick J. Interleukin-8 - a mediator of inflammatory lung disease? In: Westwick J, ed. *Chemotactic Cytokines*. New York, Plenum Press, 1991; pp. 119–126.
 22. Yoshimura K, Nakagawa S, Koyama S, Kobayashi T, Homma T. Roles of neutrophil elastase and superoxide anion in leukotriene B₄-induced lung injury in rabbit. *J Appl Physiol* 1994; 76: 91–96.
 23. Sakamaki F, Ishizaka A, Urano T, *et al.* Effect of a specific neutrophil elastase inhibitor, ONO-5046, on endotoxin-induced acute lung injury. *Am J Respir Crit Care Med* 1996; 153: 391–397.
 24. Fujimoto K, Kubo K, Shinozaki S, Okada K, Matsuzawa Y, Kobayashi T. Neutrophil elastase inhibitor reduces asthmatic responses in allergic sheep. *Respir Physiol* 1995; 100: 91–100.