

In vivo airway eosinophil accumulation induced by polymyxin-B reduces bronchial responsiveness in guinea pigs

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Summary

Background Chronic desquamative eosinophilic bronchitis and bronchial hyper-responsiveness have been considered essential for bronchial asthma. However, it has not been studied whether airway eosinophils enhance or inhibit bronchial responsiveness *in vivo*.

Objective This study was conducted to elucidate the influence of airway eosinophil accumulation on bronchial responsiveness *in vivo*.

Materials and methods Guinea pigs were transnasally treated with 75 µg/kg of polymyxin-B or vehicle twice a week for a total of 3 weeks. Guinea pigs were surgically cannulated and artificially ventilated 24 h after the last administration of polymyxin-B or vehicle. Ten minutes after the installation of artificial ventilation, ascending doses of methacholine, acetylcholine or histamine were inhaled for 20 s at intervals of 5 min. Subsequent study was conducted 20 min after treatment of 60 mg/kg of indomethacin in the same manner. Final study was conducted in naive guinea pigs after single inhalation of 75 µg/mL of polymyxin B.

Results The proportion of eosinophils in bronchoalveolar lavage fluid significantly increased in guinea pigs treated with polymyxin-B compared with vehicle. Bronchial responsiveness to inhaled methacholine, acetylcholine and histamine was significantly decreased by the polymyxin-B treatment. This protective effect induced by polymyxin B was abolished by pretreatment of indomethacin. A significant increase in bronchial responsiveness was observed after a single inhalation of polymyxin B.

Conclusion These results suggest that *in vivo* airway eosinophils may reduce non-specific bronchial responsiveness through inhibitory or bronchoprotective prostanoids.

Keywords: polymyxin-B, airway eosinophil accumulation, bronchial responsiveness

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Introduction

Bronchial asthma is characterized by a reversible airflow limitation, and bronchial hyperresponsiveness to specific and non-specific stimuli which is considered to depend on airway inflammatory cells, mainly eosinophils [1,2]. Several investigators [3–5] have reported the positive

correlation between the bronchial hyperresponsiveness and the percentage of eosinophils in bronchoalveolar lavage fluid (BALF) in asthmatics.

However, recent investigators have failed to demonstrate the relationship between the bronchial hyperresponsiveness and the number of eosinophils in biopsied bronchial tissues [6–10]. We have reported that bronchial responsiveness to methacholine is not heightened in patients with eosinophilic bronchitis with cough receptor hypersensitivity and eosinophilic pneumonia despite the increase of eosinophils

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in bronchial mucosa [11,12]. Gibson *et al.* [13] have also shown that sputum eosinophilia is not associated with bronchial hyperresponsiveness. Therefore, the exact role of eosinophils in the airways *in vivo* remains unknown.

This study was designed to elucidate whether *in vivo* airway eosinophil accumulation potentiates or reduces non-specific bronchial responsiveness.

Materials and methods

A model of eosinophilic bronchitis

Administration of Polymyxin-B. Male, albino, Hartley strain guinea pigs weighing 200 g were obtained from Sankyo Laboratory Service (Toyama, Japan). They were transnasally administered 75 µg/kg of polymyxin-B twice a week for a total of 3 weeks. For each procedure, animals were mildly anaesthetized by diethyl ether. Control animals were administered normal saline as vehicle in the same manner. They had grown 400–450 g when each of the following studies was performed.

Installation of artificial respiration. Guinea pigs were anaesthetized by an i.p. injection of 75 mg/kg sodium pentobarbital and placed in the supine position 24 h after the last administration of polymyxin-B or vehicle. The trachea was cannulated with a polyethylene tube (2.5 mm outside diameter and 2.1 mm inside diameter). The animals were artificially ventilated using a small animal respirator (Model 1680; Harvard Apparatus Co., Inc., South Natick, MA, USA) adjusted to a tidal volume of 10 mL/kg at a rate of 60 strokes/min. The changes in lung resistance to inflation, the lateral pressure of the tracheal tube (pressure at the airway opening (Pao), cmH₂O) were measured using a pressure transducer (Model TP-603T; Nihon Koden Kogyo Co., Ltd, Tokyo, Japan). Since we [14,15] demonstrated that the changes in Pao after an antigen challenge in passively sensitized guinea pigs treated with and without antihistamines represented the averages of the changes in pulmonary resistance and reciprocal dynamic lung compliance, we used change in the Pao as an overall index of the bronchial response to bronchoactive agents. By clamping the outlet port of the respirator, we overinflated the animals by two times tidal volume for two breaths [15].

All the animal procedures in this study complied with the standard set out in the guidelines for the care and use of laboratory animals in Takara-machi campus of Kanazawa University.

Experimental protocols

Each of the following studies was carried out separately.

Influence of polymyxin-B-induced airway eosinophil accumulation on bronchial responsiveness. The guinea pigs were assigned into two groups: polymyxin-B nasally administered group and control group ($n = 8$ in each group). Ten minutes after the installation of artificial ventilation, when the Pao had stabilized, ascending doses of methacholine (12.5, 25, 50, 100 and 200 µg/mL) in study 1, acetylcholine (25, 50, 100 and 200 µg/mL) in study 2, or histamine (12.5, 25, 50 and 100 µg/mL) in study 3 were inhaled for 20 s by the nebulizer at intervals of 5 min. The nebulizer generated the aerosol at a rate of 15.2 µg/min and 46.2% of the aerosol generated was deposited in the lungs as measured by the radioaerosol technique [16]. The median aerodynamic diameter of the particles of normal saline was 3.59 ± 1.96 µm (mean $\pm$ S.D.) [17].

The cell composition of the BALF from guinea pigs prepared as each experimental protocol was compared with that from control ones. Sterile saline (10 mL) was instilled, at room temperature, through a polyethylene cannula introduced into the trachea, and recovered immediately. Following this manner, the same procedure was repeated; a total of 20 mL of sterile saline was used for BAL. After filtrating the fluid through a double layer of Dacron nets, cells were centrifuged at 1000 rev/min for 12 min and resuspended in 3 mL of RPMI 1640 (GIBCO, Grand Island, NY, USA). Following dilution with an equal volume of Türk solution, the number of cells was counted in a Bürker chamber. The fluid was then resuspended with RPMI 1640 to make a cell suspension containing 2×10^5 cells/mL. Smears for differential cell counts were prepared by cytocentrifugation (Cytospin 2; Shandon Southern Products Ltd, UK) at 800 rev/min for 8 min. After staining with May–Grünwald–Giemsa stain (Diff-Quick; American Scientific Products, McGraw Park, IL, USA), 300 cells were counted.

Influence of indomethacin on polymyxin-B-induced decrease in non-specific bronchial responsiveness. Guinea pigs prepared in the same manner according to studies 1–3 were assigned into two groups: 60 mg/kg for the indomethacin-administered group and the saline administered group as control ($n = 8$ in each group). The guinea pigs were anaesthetized and cannulated 20 min after an i.p. administration of indomethacin or its vehicle. Ten minutes after the installation of artificial ventilation ascending doses of methacholine (12.5, 25, 50, 100 and 200 µg/mL) in study 4, acetylcholine (25, 50, 100 and 200 µg/mL) in study 5, or histamine (12.5, 25, 50 and 100 µg/mL) in study 6 were inhaled for 20 s by the nebulizer at intervals of 5 min.

Immediate influence of polymyxin-B inhalation on non-specific bronchial responsiveness. The guinea pigs weighing 400 g were assigned into two groups ($n = 8$ in

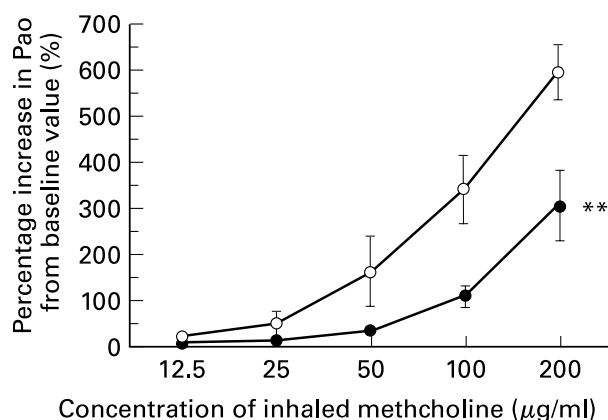


Fig. 1. Effect of polmyxin-B-induced airway eosinophil accumulation on bronchial responsiveness to inhaled methacholine in guinea pigs. Treatment with 75 µg/kg of polmyxin-B ($n = 8$) (●) and vehicle ($n = 8$) (○) twice a week for 3 weeks is shown. Error bars indicate mean $\pm$ S.E.M. ** $P < 0.02$ compared with the vehicle group. Pao, Pressure at the airway opening.

each group) as study 7. Ten minutes after the installation of artificial ventilation, the control group was given aerosolized saline and the polmyxin-B group was administered aerosolized 75 µg/mL of polmyxin-B, the same concentration nasally administered in studies 1–6, for 20 s by the nebulizer. Ten minutes after this procedure, ascending doses of methacholine (12.5, 25, 50 and 100 µg/mL) were inhaled for 20 s by the nebulizer at intervals of 5 min.

The cell composition of the BALF from guinea pigs was compared with that from control ones in the same manner described above.

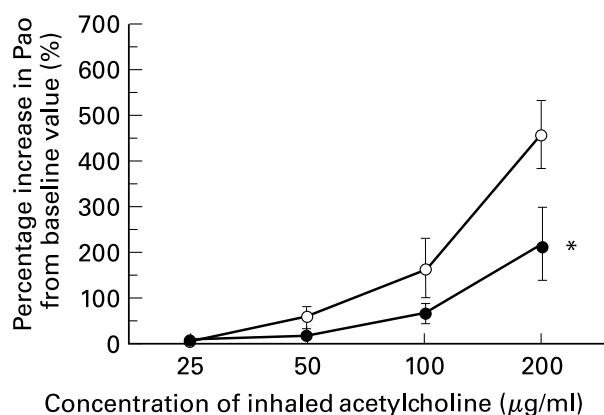


Fig. 2. Effect of polmyxin-B-induced airway eosinophil accumulation on bronchial responsiveness to inhaled acetylcholine in guinea pigs. Treatment with 75 µg/kg of polmyxin-B ($n = 8$) (●) and vehicle ($n = 8$) (○) twice a week for 3 weeks is shown. Error bars indicate mean $\pm$ S.E.M. * $P < 0.02$ compared with the vehicle group. Pao, Pressure at the airway opening.

Chemicals

The following chemicals were used: sodium pentobarbital (Abbot Laboratories, North Chicago, IL, USA), methacholine (Wako Pure Chemical Ind., Osaka, Japan), acetylcholine (Wako Pure Chemicals), histamine (Wako Pure Chemicals), indomethacin (Wako Pure Chemicals), polmyxin-B sulphate (Pfizer Taito Co. Ltd, Tokyo, Japan), and diethyl ether (Wako Pure Chemicals).

Statistical analysis

One-way analysis of variance (ANOVA) was used for differences in baseline Pao value or cell component of BALF between groups. The degree of bronchial responsiveness was evaluated using dose–response curve for percent increase in the Pao from the baseline induced by each bronchoconstrictor, and was analysed by two-way ANOVA. These analyses were performed using the software StatView 4.02 (Abacus Concepts, Berkeley, CA, USA). A P -value of < 0.05 was considered statistically significant, and values were presented as the mean $\pm$ S.E.M.

Results

Influence of polmyxin-B-induced airway eosinophil accumulation on bronchial responsiveness

The Pao values before methacholine challenge did not differ significantly between guinea pigs treated with repeated intranasal administration of polmyxin-B (10.4 ± 0.3 cmH₂O) and saline (10.7 ± 0.4 cmH₂O) in study 1. Figure 1 shows the percent increase in Pao after inhalation of increasing concentrations of methacholine. Bronchial responsiveness to inhaled methacholine was significantly reduced in animals given polmyxin-B compared with saline.

The baseline Pao values before acetylcholine challenge did not differ significantly between treatment with polmyxin-B (10.5 ± 0.2 cmH₂O) and saline (11.2 ± 0.6 cmH₂O) in study 2. Figure 2 shows the percent increase in Pao after inhalation of increasing concentrations of acetylcholine. Repeated intranasal administration of polmyxin-B significantly reduced bronchial responsiveness to inhaled acetylcholine compared with saline administration.

Values of the Pao before histamine challenge did not differ significantly between treatment with polmyxin-B (10.1 ± 0.4 cmH₂O) and saline (11.1 ± 0.5 cmH₂O) in study 3. Figure 3 shows the percent increase in Pao after inhalation of increasing concentrations of histamine. Polmyxin-B treatment significantly reduced bronchial responsiveness to inhaled histamine.

The number of total cells recovered per millilitre lavage

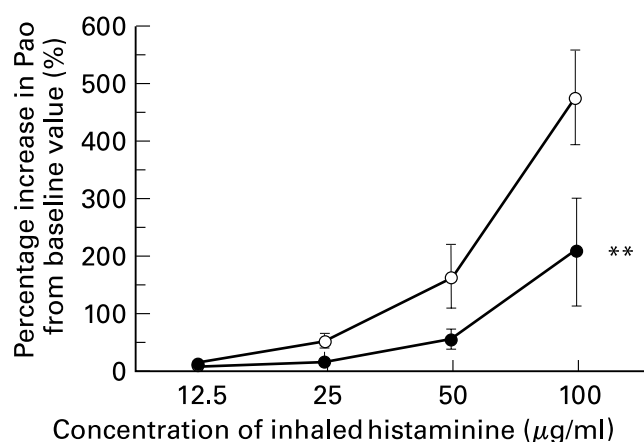


Fig. 3. Effect of polmyxin-B-induced airway eosinophil accumulation on bronchial responsiveness to inhaled histamine in guinea pigs. Treatment with 75 μg/kg of polmyxin-B ($n = 8$) (●) and vehicle ($n = 8$) (○) twice a week for 3 weeks is shown. Error bars indicate mean $\pm$ S.E.M. ** $P < 0.05$ compared with the vehicle group. Pao, Pressure at the airway opening.

fluid did not differ significantly between transnasal treatment with polmyxin-B ($13.1 \pm 2.6 \times 10^5$ cells/mL) and saline ($10.5 \pm 1.8 \times 10^5$ cells/mL). BAL cell findings are shown in Fig. 4. The proportion of eosinophils in BALF significantly increased in guinea pigs treated with polmyxin-B compared with the control animals. There were no significant differences between these groups in the proportion of neutrophils and lymphocytes. The mean number of nuclear segmentations in eosinophils in BALF after treatment with polmyxin B are shown in Fig. 5. The numbers of nuclear segmentations of eosinophils were significantly higher than those in animals after treatment with polmyxin B.

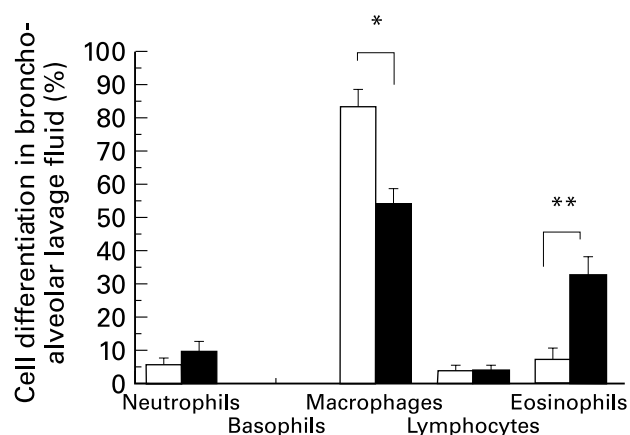


Fig. 4. Bronchoalveolar cell finding after repeated transnasal administration of polmyxin-B. The groups treated with polmyxin-B ($n = 8$) (■) and vehicle ($n = 8$) (□) are shown. Error bars indicate mean $\pm$ S.E.M. * $P < 0.05$; ** $P < 0.01$ compared with the vehicle group.

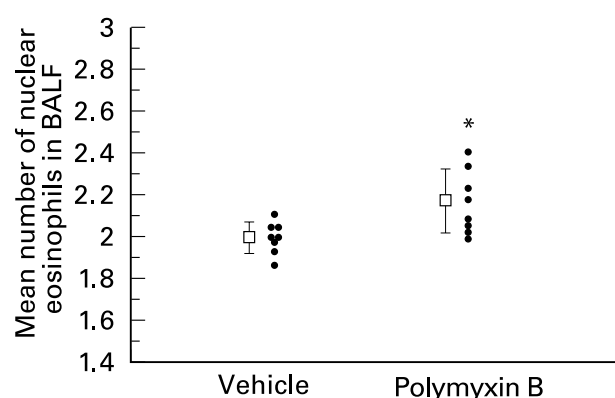


Fig. 5. Mean number of nuclear segmentations of eosinophils in bronchoalveolar lavage fluid (BALF). Each open square with vertical bar represents mean $\pm$ S.D. * $P \leq 0.05$ compared with the vehicle group.

Influence of indomethacin on polmyxin-B-induced decrease in non-specific bronchial responsiveness

Mean value ($\pm$ S.E.M.) of the baseline Pao immediately before methacholine inhalation was 10.2 ± 0.4 , 10.2 ± 0.3 , 9.8 ± 0.3 and 10.0 ± 0.2 cmH₂O in animals given repeated intranasal polmyxin-B with and without indomethacin, saline with and without indomethacin, respectively, in study 4. There were no significant differences among them. Figure 6 shows the influence of indomethacin on methacholine-induced bronchoconstriction. The dose-response curve of the methacholine-induced increase in Pao was significantly inhibited by repeated intranasal treatment with polmyxin-B, whereas this reduction of bronchial responsiveness was abolished by indomethacin.

The baseline Pao values immediately before acetylcholine inhalation were 9.8 ± 0.2 , 9.7 ± 0.3 , 9.6 ± 0.3 and 10.0 ± 0.3 cmH₂O with repeated intranasal treatment with polmyxin-B with and without indomethacin, and intranasal treatment of saline with and without indomethacin, respectively, in study 5. There were no significant differences among them. Figure 7 shows the influence of indomethacin on bronchoconstriction caused by inhalation of acetylcholine. The dose-response curve of the acetylcholine-induced increase in Pao was significantly inhibited by repeated intranasal treatment with polmyxin-B, whereas this reduction in bronchial responsiveness was abolished by the indomethacin treatment.

The values of baseline Pao immediately before histamine inhalation were 10.4 ± 0.3 , 10.2 ± 0.3 , 9.9 ± 0.2 and 10.1 ± 0.3 cmH₂O with treatment with polmyxin-B with and without indomethacin, and treatment with saline with and without indomethacin, respectively, in study 6. There were no significant differences among them. Figure 8 shows the influence of indomethacin treatment on bronchoconstriction

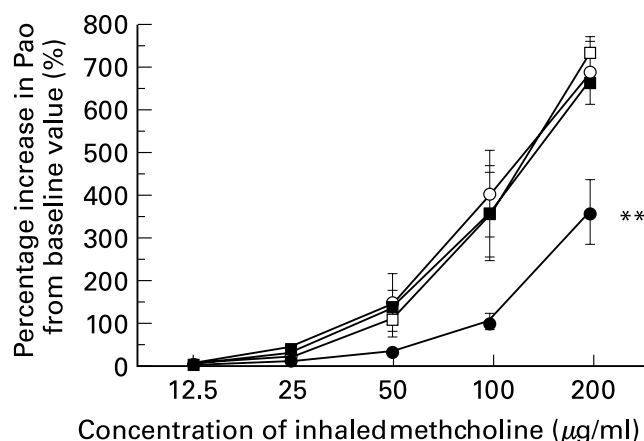


Fig. 6. Influence of i.p. administration of 60 mg/kg of indomethacin on methacholine-induced bronchoconstriction. The groups administered indomethacin after 3-week treatment with polmyxin-B ($n = 8$) (■), administered vehicle after 3-week treatment with polmyxin-B ($n = 8$) (●), administered indomethacin after 3-week treatment with vehicle ($n = 8$) (□) and administered vehicle after 3-week treatment with vehicle as control ($n = 8$) (○). Error bars indicate mean $\pm$ S.E.M. ** $P < 0.02$ compared with the control group. Pao, Pressure at the airway opening.

induced by inhalation of histamine. The dose–response curve of the histamine-induced increase in Pao was significantly inhibited by the repeated intranasal treatment with polmyxin-B, whereas this reduction of bronchial responsiveness was abolished by treatment with indomethacin.

Immediate influence of polmyxin-B inhalation on non-specific bronchial responsiveness

The Pao values before methacholine challenge did not differ significantly between a single inhalation of polmyxin-B (9.2 ± 0.5 cmH₂O) and saline (9.6 ± 0.5 cmH₂O) in study 7. Figure 9 shows the percent increase in Pao after inhalation of increasing concentrations of methacholine. Bronchial responsiveness to methacholine was significantly increased 10 min after the single inhalation of polmyxin-B.

The number of total cells recovered per millilitre lavage fluid did not differ significantly between single inhalation of polmyxin B ($8.4 \pm 1.8 \times 10^5$ cells/mL) and saline ($9.2 \pm 2.1 \times 10^5$ cells/mL). BAL cell findings were not significantly different between guinea pigs given the single inhalation of polmyxin-B and saline as shown in Fig. 10.

Discussion

In this study, airway eosinophil accumulation, which was induced by repeated transnasal administration of

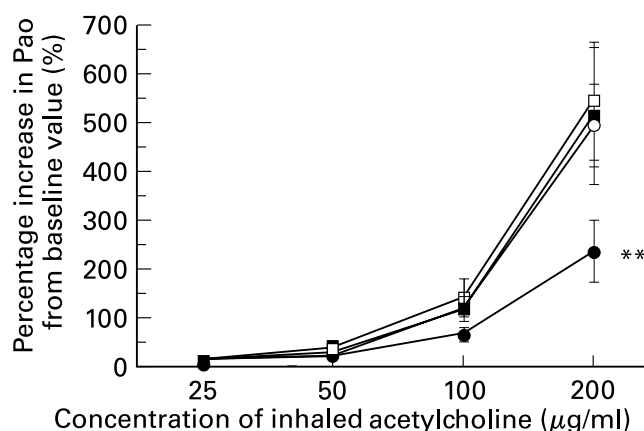


Fig. 7. Influence of i.p. administration of 60 mg/kg of indomethacin on acetylcholine-induced bronchoconstriction. The groups administered indomethacin after 3-week treatment with polmyxin-B ($n = 8$) (■), administered vehicle after 3-week treatment with polmyxin-B ($n = 8$) (●), administered indomethacin after 3-week treatment with vehicle ($n = 8$) (□) and administered vehicle after 3-week treatment with vehicle as control ($n = 8$) (○). Error bars indicate mean $\pm$ S.E.M. ** $P < 0.02$ compared with the control group. Pao, Pressure at the airway opening.

polmyxin-B, reduced bronchial responsiveness to inhaled methacholine, acetylcholine and histamine.

This reduction of bronchial responsiveness was abolished by treatment with indomethacin, while bronchial

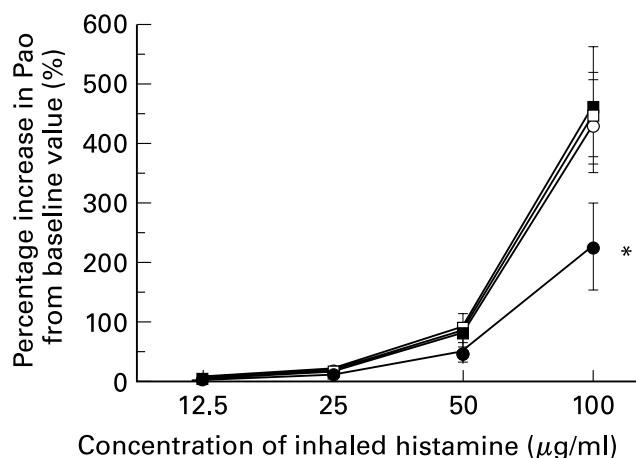


Fig. 8. Influence of i.p. administration of 60 mg/kg of indomethacin on histamine-induced bronchoconstriction. The groups administered indomethacin after 3-week treatment with polmyxin-B ($n = 8$) (■), administered vehicle after 3-week treatment with polmyxin-B ($n = 8$) (●), administered indomethacin after 3-week treatment with vehicle ($n = 8$) (□) and administered vehicle after 3-week treatment with vehicle as control ($n = 8$) (○). Error bars indicate mean $\pm$ S.E.M. * $P < 0.05$ compared with the control group. Pao, Pressure at the airway opening.

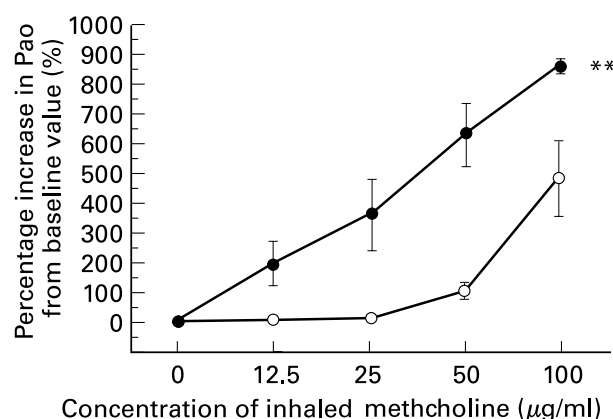


Fig. 9 Direct effect of polymyxin-B on bronchial responsiveness to inhaled methacholine. Treatment with polymyxin-B ($n = 8$) (●) and vehicle ($n = 8$) (○) 10 min before methacholine challenge is shown. Error bars indicate mean $\pm$ S.E.M. ** $P < 0.02$ compared with the vehicle group. Pao, Pressure at the airway opening.

responsiveness to methacholine was increased 10 min after a single inhalation of polymyxin-B. These results suggest that chronic eosinophil accumulation may inhibit non-specific bronchial responsiveness via the defence mechanism such as inhibitory or bronchoprotective prostanoids *in vivo*.

Recently, chronic desquamative eosinophilic bronchitis has been considered to be a fundamental feature of bronchial asthma [18]. The association between increased number of eosinophils in the airways and bronchial asthma has been well recognized [19] and the significant correlation between the degree of bronchial hyperresponsiveness and airway eosinophil accumulation in stable asthmatics has been demonstrated [3–5]. The role of eosinophil-derived mediators in the development of bronchial hyperresponsiveness has been postulated because of the significant correlation between the concentration of major basic protein (MBP) and the number of eosinophils in BALF in asthmatics and the protection by an antibody of MBP against antigen-induced bronchial hyperresponsiveness [3].

However, Sanjar and colleagues [8,9] have reported that there is no significant increase in the bronchial responsiveness after platelet-activating factor (PAF) challenge or antigen challenge by aerosol in guinea pigs despite the airway eosinophil accumulation. Sasaki and colleagues [20] have reported that IL-5, which is known as a chemotactic factor for eosinophils, does not increase bronchial responsiveness in guinea pigs, and others have failed to demonstrate the relationship between the bronchial hyperresponsiveness and the number of eosinophils in biopsied bronchial mucosa in asthmatics [6,7,10].

Gibson and colleagues [13] have also shown that sputum eosinophilia is not associated with bronchial hyperresponsiveness. Moreover, we have shown that bronchial responsiveness to methacholine is not increased in patients with bronchodilator-resistant cough with sputum eosinophilia and cough hypersensitivity [11] and eosinophilic pneumonia [12], despite the increase of eosinophils in bronchial mucosa. These findings suggest that eosinophilic inflammation may not be essential for the development of bronchial asthma and bronchial hyperresponsiveness.

We have reported an animal model of eosinophilic bronchitis which is made by repeated transnasal administration of polymyxin-B. The increase in the number of eosinophils in the mucosa and the injury to airway epithelium in trachea, large bronchi and medium bronchi, but not in the alveolar region or the lung structure, have been proven in the animal model [21]. So we conducted this study to elucidate the influence of airway eosinophil accumulation on bronchial responsiveness *in vivo*, and clearly revealed that polymyxin-B-induced airway eosinophil accumulation reduced non-specific bronchial responsiveness to methacholine, acetylcholine and histamine. We also showed that the direct effect of polymyxin-B was not related to this defence mechanism, because a single administration of polymyxin-B increased bronchial responsiveness to methacholine. The attenuation of bronchial responsiveness by the airway eosinophil accumulation was first reported by Lefort and colleagues using acetylcholine in sensitized guinea pigs after an inhalation of antigen, but they did not address its mechanism [22]. Our findings confirm this attenuating effect of airway eosinophil accumulation on bronchial responsiveness not only to acetylcholine but also to methacholine and histamine.

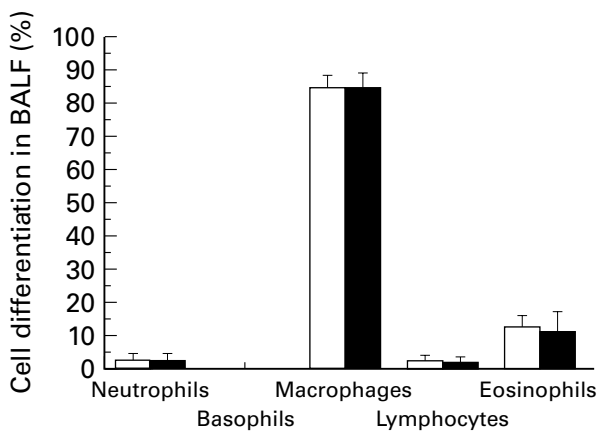


Fig. 10 Bronchoalveolar cell finding after single administration of polymyxin-B. The groups treated with polymyxin-B ($n = 8$) (■) and vehicle ($n = 8$) (□) are shown. Error bars indicate mean $\pm$ S.E.M. BALF, Bronchoalveolar lavage fluid.

Acetylcholine and methacholine are direct bronchoconstrictors. Histamine causes bronchoconstriction directly and in part indirectly via its vagal nerve stimulation. As high doses of barbiturates are known to suppress vagal nerve reflexes, it is considered that histamine induces bronchoconstriction in large part by its direct effect on airway smooth muscle. Accordingly, it is suggested that airway eosinophil accumulation attenuates non-specific bronchial responsiveness. We did not measure eosinophilic cationic protein levels in this study. However, activation of eosinophils recruited in this study was demonstrated through nuclear hypersegmentation. This finding is compatible with the previous *in vivo* study by Davis and colleagues [23] showing eosinophil activation induced by polymyxin-B.

The decreased response to repeated inhalation of bronchial stimuli has been recognized as tachyphylaxis [24–26]. Manning *et al.* have shown that bronchial responsiveness to both acetylcholine and histamine is decreased after histamine-induced bronchoconstriction in asthmatics [24]. They have also indicated its defence mechanism as the release of inhibitory prostaglandins that protect the airway against further bronchoconstriction [25]. Other investigators have reported that histamine-induced contraction of guinea pig trachea causes release of prostaglandin E [26]. Thus we conducted the present study and showed that this defence mechanism was abolished by treatment with the cyclooxygenase inhibitor indomethacin. Our previous study showed that repeated administration of polymyxin-B accelerate the releasability of histamine [27]. Taking all the data together, it is suggested that the attenuating effect of airway eosinophil accumulation on non-specific bronchial responsiveness may result from the release of inhibitory mediators such as prostaglandin E₂ and prostaglandin I₂, but the cell source is unknown.

In conclusion, the present study clearly demonstrates that *in vivo* airway eosinophil accumulation induced by repeated administration of polymyxin-B causes reduced response to non-specific bronchial stimuli through the defence mechanism associated with inhibitory prostaglandins. Further *in vivo* studies may be required to elucidate the role of airway eosinophil accumulation in bronchial responsiveness using other compounds accumulating and activating eosinophils.

References

- 1 Boushey HA, Holtzman MJ, Sheller JR, Nadel JA. Bronchial hyperreactivity. *Am Rev Respir Dis* 1980; 121:389–413.
- 2 Pretolani N, Lefort J, Vargaftig BB. Active immunization induces lung hyperresponsiveness in the guinea-pig pharmacological modulation and triggering role of the booster injection. *Am Rev Respir Dis* 1988; 138:1572–8.
- 3 Wardlaw AJ, Dunnette S, Gleich GJ, Collins JV, Kay AB. Eosinophils and mast cells in bronchoalveolar lavage in subjects with mild asthma. *Am Rev Respir Dis* 1988; 137:62–9.
- 4 Ferguson AC, Wong FWM. Bronchial hyperresponsiveness in asthmatic children: correlation with macrophages and eosinophils in bronchoalveolar fluid. *Chest* 1989; 96:988–91.
- 5 Kirby JC, Hargreave FE, Gleich GJ, O'Byrne PM. Bronchoalveolar cell profiles of asthmatic and nonasthmatic subjects. *Am Rev Respir Dis* 1987; 136:379–83.
- 6 Djukanovic R, Wilson JW, Britten KR, Wilson SJ, Walls AF, Roche WR, Howarth PH, Holgate ST. Quantitation of mast cells and eosinophils in the bronchial mucosa of symptomatic atopic asthmatics and healthy control subjects using immunohistochemistry. *Am Rev Respir Dis* 1990; 142:863–71.
- 7 Sanjar S, Aoki S, Boubekur K, Chapman ID, Smith D, Kings MA, Morley J. Eosinophil accumulation in pulmonary airways of guinea-pigs. *Br J Pharmacol* 1990; 99:267–72.
- 8 Sanjar S, Aoki S, Kristersson A, Smith D, Morley J. Antigen challenge induces pulmonary airway hyperreactivity in sensitized guinea-pigs: the effect of anti-asthma drugs. *Br J Pharmacol* 1990; 99:679–86.
- 9 Smith D, Burrows L, Westwick J. Interleukin-8 a mediator of inflammatory lung disease? In: Westwick J, ed. *Chemotactic cytokines*. New York: Plenum Press, 1991:119–26.
- 10 Azzawi M, Bradley B, Jeffery PK et al. Identification of activated T lymphocytes and eosinophils in stable atopic asthma. *Am Rev Respir Dis* 1990; 142:1407–13.
- 11 Nishi K, Myou S, Ohya T, Fujimura M, Matsuda T. Bronchial biopsy findings in patients with chronic nonproductive cough resistant to bronchodilator therapy (abstract in Japanese). *Jap J Allergol* 1992; 41:302.
- 12 Fujimura M, Matsuda T. Airway inflammation and bronchial responsiveness (Japanese). In: Itoh K, Tomioka H, eds. *Airway inflammation: pathogenesis of bronchial asthma*. Tokyo: Axel Springer Japan Publishing Inc., 1995:46–60.
- 13 Gibson PG, Dolovich J, Denburg J, Ramsdale EH, Hargreave EF. Chronic cough: eosinophilic bronchitis without asthma. *Lancet* 1989; i:1346–8.
- 14 Fujimura M. Inhibitory effect of steroids on slow reacting substance of anaphylaxis (SRS-A) mediated bronchoconstriction in guinea-pigs. *Jpn J Allergol* 1983; 32:365–75.
- 15 Minami S. Studies on effect of histamine and slow reacting substance of anaphylaxis (SRS-A) on bronchospasm produced by antigen inhalation in passively sensitized guinea pigs. *Juzen Igakkai Zasshi* 1983; 92:811–21(in Japanese).
- 16 Minami S, Okafuji K, Saga T, Fujimura M, Kanamori K, Miyabo S, Hattori K, Kawai K. A new quantitative inhalation apparatus for small animals. *Jap J Chest Dis* 1983; 21:252–8.
- 17 Kurashima K, Fujimura M, Tsujiura M, Matsuda T. Effect of surfactant inhalation on allergic bronchoconstriction in guinea pigs. *Clin Exp Allergy* 1997; 27:337–42.
- 18 Bousquet J, Chanez P, Lacoste JY et al. Eosinophilic inflammation in asthma. *N Engl J Med* 1990; 323:1033–9.
- 19 Ellis AG. The pathological anatomy of bronchial asthma. *Am J Med Sci* 1908; 136:407–29.

- 20 Sasaki Y, Ichinose M, Miura M et al. A model for chronic eosinophilic inflammation of airways induced by repeated inhalation of mouse recombinant IL-5 (mrIL-5) in guinea-pigs (Abstract). *Jpn J Chest Dis* 1993; 31:403.
- 21 Ogawa H, Fujimura M, Saito M, Matsuda M, Akao N, Kondo K. The effect of neurokinin antagonist FK-224 on the cough response to inhaled capsaicin in a new model of guinea-pig eosinophilic bronchitis induced by intranasal polymyxin B. *Clin Auton Res* 1994; 4:19–27.
- 22 Lefort JL, Nahori MA, Ruffié C, Vargaftig BB, Pretolani M. In vivo neutralization of eosinophil-derived major basic protein inhibits antigen-induced bronchial hyperreactivity in sensitized guinea pigs. *J Clin Invest* 1996; 97:1117–21.
- 23 Davis WB, Husney RM, Mohammed BS, Pawluk LJ, Ackerman GA, Sagone AL. Activation and degeneration of guinea pig peritoneal eosinophils during chronic polymyxin B stimulation. *J Leuk Biol* 1989; 45:147–54.
- 24 Manning PJ, O'Byrne PM. Histamine bronchoconstriction reduced bronchoconstriction reduced airway responsiveness in asthmatic subjects. *Am Rev Respir Dis* 1988; 137:1323–5.
- 25 Manning PJ, Jones GL, O'Byrne PM. Tachyphylaxis to inhaled histamine in asthmatic subjects. *J Appl Physiol* 1987; 63:1572–7.
- 26 Grodzinska L, Panczenko B, Gryglewski RJ. Generation of prostaglandin E-like material by the guinea-pig trachea contracted by histamine. *J Pharm Pharmacol* 1975; 27:88–91.
- 27 Ishiura Y, Fujimura M, Myou S, Amemiya T, Sasaki S, Matsuda T. Airway eosinophil accumulation potentiates immediate asthmatic response in guinea pigs. *Allergy Clin Immunol Int* 1997; 4:277(Abstract).