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Interleukin-8 inhalation directly provokes bronchoconstriction in guinea pigs

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Background: Although it has been reported that the concentration of interleukin (IL)-8 in nasal lavage fluid and sputum and its production in bronchial epithelium were increased in asthmatic subjects, the direct effects of IL-8 on the airways *in vivo* is unclear.

Methods: We examined bronchoconstriction in response to IL-8 inhalation through an endotracheal cannula in anesthetized, artificially ventilated guinea pigs.

Results: Inhalation of IL-8 at concentrations of 1 and 10 µg/ml caused significant bronchoconstriction, as revealed by the elevation of pressure at the airway opening. Moreover, the bronchoconstriction induced by IL-8 was significantly inhibited by the antihistamines diphenhydramine and terfenadine, suggesting the involvement of histamine release in the IL-8-induced bronchoconstriction. No significant leukocyte infiltration was observed in the bronchoalveolar lavage fluid or histologic findings 25 min after the first IL-8 inhalation.

Conclusions: IL-8 provokes bronchoconstriction without leukocyte accumulation in the airways, mediated in part by histamine release, in guinea pigs.

Chronic airway inflammation is a main feature of bronchial asthma. Several lines of evidence indicate that eosinophils and neutrophils accumulate in the airways after challenge with antigens, as evidenced by increase of eosinophils and neutrophils in bronchoalveolar lavage fluid (BALF) (1, 2). Hence, both eosinophils and neutrophils are probably involved in the pathophysiology of asthma.

Various cytokines, produced by tissue resident cells as well as infiltrating leukocytes, are presumed to be involved

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in the signaling between cells and to contribute to the pathophysiology of bronchial asthma (3, 4). Interleukin-8 (IL-8), a novel chemotactic and activating cytokine for neutrophils, was purified initially from lipopolysaccharide (LPS)-stimulated human peripheral blood mononuclear cell culture supernatants (5), and could be produced by a number of cells present in lung tissue, such as bronchial epithelial cells (6), macrophages (7), mast cells (8), endothelial cells (9), and fibroblasts (10). Several lines of evidence indicate that IL-8 may also act as a potent eosinophil chemoattractant (11, 12).

These observations prompted us to speculate that IL-8 might contribute to the pathogenesis of asthma. Indeed, it has been demonstrated that the concentration of IL-8 in sputum (13) and its production in bronchial epithelium (6) were increased in asthmatic subjects compared with nonasthmatics. Recently, we also demonstrated that repeated exposure of IL-8 caused bronchial hyperresponsiveness (BHR) and airway neutrophil infiltration, and that thromboxane A₂ was important in the development of BHR induced by IL-8 (14). However, IL-8 can induce the release of several chemical mediators (15–17), suggesting that IL-8 may have effects on the airway by inducing release of chemical mediator(s) *in vivo*. We examined here the effects of inhaled IL-8 on bronchomotor tone without reference to leukocyte infiltration into the airway.

Material and methods

Chemicals

The following chemicals were used: sodium pentobarbital (Abbot Laboratories, North Chicago, IL, USA), methacholine (Wako Pure Chemical Ind., Osaka, Japan), and diphenhydramine (Wako Pure Chemical Ind., Osaka, Japan). Human recombinant IL-8 and terfenadine were the kind gift of Dainippon Pharmaceutical Co. (Osaka, Japan) and Hoechst Marion Roussel, Inc. (Tokyo, Japan), respectively.

Installation of artificial respiration

Male, albino, Hartley strain guinea pigs weighing 300–350 g were obtained from Sankyou Laboratory Service (Toyama, Japan). They were quarantined in the Animal Research Center of Kanazawa University for 1 week before the study.

Guinea pigs were anesthetized by an intraperitoneal injection of 75 mg/kg of sodium pentobarbital and were

placed in a supine position. After the trachea was cannulated with a polyethylene tube (outside diameter, 2.5 mm; inside diameter, 2.1 mm), the animals were artificially ventilated by 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; *P*_{ao}, cmH₂O), were measured with a pressure transducer (TP-603T, Nihon Koden Kogyo Co., Tokyo, Japan), according to the modified method of Konzett & Roessler (18), as described by Jones et al. (19). Since we confirmed that the change in *P*_{ao} after inhalation of leukotriene C₄ and histamine represented the average of the changes in pulmonary resistance (RL) and reciprocal dynamic lung compliance (1/C_{dyn}) (20), *P*_{ao} was used as an overall index of bronchial response to bronchoactive agents (21). The animals were overinflated two times the tidal volume for two breaths by closing the outlet port of the respirator to unify the volume history of the lung.

All the animal procedures in this study complied with the standards set out in the Guidelines for the Care and Use of Laboratory Animals at Takara-machi Campus of Kanazawa University.

Bronchoconstrictor effect of IL-8

Fifteen minutes after the installation of artificial ventilation, *P*_{ao} had stabilized, and increasing doses of IL-8 (1, 10, 100, 1000, and 10 000 ng/ml), dissolved in phosphate-buffered saline (PBS), were administered intrabronchially to the guinea pigs in the positive control group. Each inhalation was done in 20 s at intervals of 5 min under continuous ventilation with an ultrasonic nebulizer (22). The nebulizer generated the aerosol at the rate of 15.2 µl/min during the 20-s period, and 46.4% of the generated aerosol was deposited in the lung, as measured by the radioaerosol technique (22). The animals in the negative control group were given an aerosol consisting of only PBS with the same time schedule as those in the positive control group. For examination of the involvement of histamine, the antihistamine diphenhydramine (20 mg/kg, dissolved in saline, intraperitoneally 30 min before the anesthesia; *n*=7 in each group), a selective histamine H₁ antagonist terfenadine (0.2 or 2.0 mg/kg, dissolved in carboxymethyl cellulose orally 1 h before the anesthesia; *n*=7 in each group), or each vehicle was administered, and the animals inhaled ascending doses of IL-8.

Analysis of bronchoalveolar lavage cells

After the examination of the effect of diphenhydramine on IL-8-induced bronchoconstriction, 10 ml of sterile saline at 37°C was infused twice through the tracheal cannula. The fluid was recovered manually, through gentle aspiration with a disposable syringe after each infusion. The recovered BALF was immediately centrifuged by 800 rpm for 10 min. After the supernatant was discarded, the cell pellets were resuspended in 10 ml of RPMI-1640 (Grand Island Biological Co., Grand Island, NY, USA) for determination of number of the cells with Türk's solution as well as differential counts of the cells by cytocentrifugation (Cytospin 2, Shadon, UK). For differential counting, 300 cells were counted after staining with May-Grünwald-Giemsa stain.

Histologic examination

After the examination of the effect of terfenadine on IL-8-induced bronchoconstriction, the heart and lungs were removed and inflated with 10% formalin solution. Tissues were taken from adjacent sites at three places along the axial airway, running from the right main bronchus to distal right lower lobe, the first block containing extrapulmonary bronchus at the hilus and the second intrapulmonary bronchus (150–300 µm in a diameter). Tissues were stained with hematoxylin-eosin.

Quantitative light-microscopic techniques were used to identify the location and severity of inflammatory cell infiltration. All slides were examined in a blinded fashion. The first score, the number of inflammatory cells in the epithelium or the subepithelium (the layer of connective tissue immediately beneath the epithelium and superficial to a dense layer of elastin), was determined at each location. We averaged the numbers of inflammatory cells infiltrated in 150 µm of length at three different sites of each airway. The second score, for estimating the damage to the bronchial epithelium, we termed the "injury score"; the total points of 10 locations. Each point was defined by the degree of desquamation in a 150-µm length of airway epithelium; 0 point for intact airway epithelium, 1 point for less than 50% desquamation, 2 points for greater than 50% desquamation, and 3 points for complete desquamation.

Statistical analysis

Bronchoconstriction to each concentration of IL-8 was determined as percent increase in *Pao* compared with the

value before the first inhalation of IL-8. Variation among three or more groups was statistically analyzed by analysis of variance (ANOVA) followed by Fisher's protected least significant difference. Variation between two groups was tested by Student's *t*-test. Significance was based on a 95% confidence level ($P<0.05$), and all measurements were expressed as mean $\pm$ standard error of the mean (SEM).

Results

There were no significant differences between the baseline *Pao* values before inhalation of IL-8 and PBS. Aerosolized administration of IL-8 caused transient increases in *Pao*, which reached a maximal level 63.6 ± 5.3 s after exposure to IL-8. Pretreatment with diphenhydramine (data not shown) or terfenadine (Fig. 1) significantly lessened the increase in *Pao* induced by IL-8, indicating that release of histamine might be involved in IL-8-induced bronchoconstriction.

No significant differences in the recovery rate of BALF were observed among groups. Inhalation of IL-8 failed to

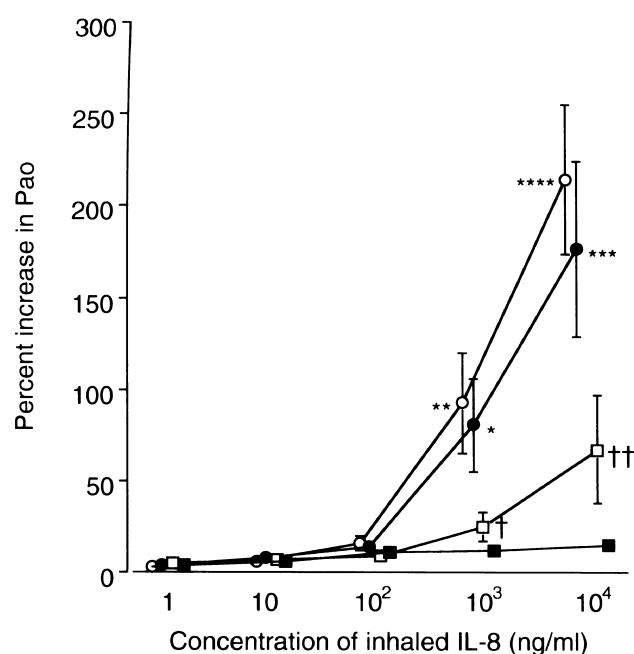


Figure 1. Bronchoconstrictor response to inhaled IL-8 and effect of terfenadine. Bronchoconstriction was expressed as percent increase in pressure at airway opening (*Pao*) in positive control group (open circles), 2.0 mg/kg of terfenadine group (open squares), 0.2 mg/kg of terfenadine group (closed circles), and negative control group (closed squares), as described in Material and methods section ($n=7$ in each group). Error bars indicate mean $\pm$ SEM. Statistical significance is indicated as follows; * $P<0.02$, ** $P<0.01$, *** $P<0.005$, and **** $P<0.001$ compared with negative control group; † $P<0.02$ and †† $P<0.01$ compared with positive control group.

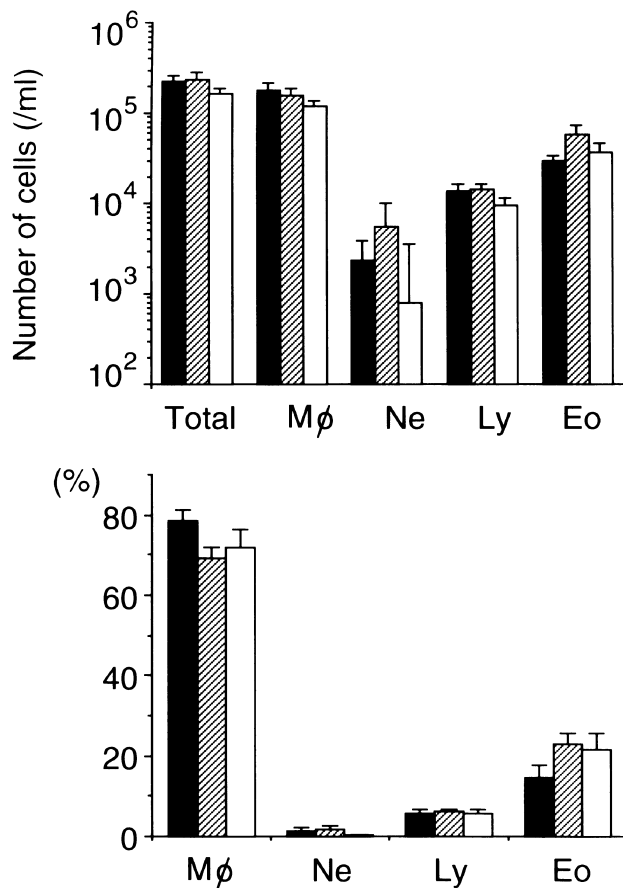


Figure 2. Inhaled IL-8-induced change in BAL cell composition and effect of diphenhydramine. Absolute number (top panel) and percentage (bottom panel) of total cells, macrophages (Mφ), neutrophils (Ne), lymphocytes (Ly), and eosinophils (Eo) were determined on BALF collected from either negative control (closed columns), positive control (shaded columns), or diphenhydramine group (open columns) after examination of effect of diphenhydramine on IL-8-induced bronchoconstriction ($n=7$ in each group). Error bars indicate mean $\pm$ SEM. No significant difference was observed between three groups.

increase numbers of total cells, neutrophils, and eosinophils in BALF (Fig. 2). No increase in infiltration of neutrophils or eosinophils in bronchial tissues, or epithelial damage was histologically observed in the animals that inhaled IL-8 (Table 1).

Discussion

The present study shows that the inhalation of IL-8 induces bronchoconstriction in guinea pigs through induction of histamine release. The number of leukocytes, particularly neutrophils and eosinophils, in BALF or airway subepithelium did not change significantly. These results suggest that IL-8 causes bronchoconstriction in guinea pigs without inducing leukocyte infiltration.

Table 1. Histologic findings in guinea pigs given inhalation of interleukin-8 or PBS after examination of effect of diphenhydramine on interleukin-8-induced bronchoconstriction (mean $\pm$ ESM)

	PBS ($n=7$)	IL-8 ($n=7$)
Neutrophil infiltration		
Extrapulmonary bronchus		
Epithelium (cells)*	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
Subepithelium (cells)	0.11 $\pm$ 0.05	0.14 $\pm$ 0.07
Intrapulmonary bronchus		
Epithelium (cells)	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
Subepithelium (cells)	0.21 $\pm$ 0.10	0.18 $\pm$ 0.07
Eosinophil infiltration		
Extrapulmonary bronchus		
Epithelium (cells)	0.07 $\pm$ 0.05	0.11 $\pm$ 0.11
Subepithelium (cells)	1.39 $\pm$ 0.28	1.43 $\pm$ 0.36
Intrapulmonary bronchus		
Epithelium (cells)	0.11 $\pm$ 0.07	0.14 $\pm$ 0.09
Subepithelium (cells)	1.86 $\pm$ 0.39	1.54 $\pm$ 0.38
Injury score		
Extrapulmonary bronchus	2.28 $\pm$ 0.47	1.71 $\pm$ 0.52
Intrapulmonary bronchus	2.14 $\pm$ 0.77	1.86 $\pm$ 0.94

*Cells means average of cell numbers counted in 150- μ m length at three different sites. No significant difference was observed between two groups.

Konzett & Roessler's method does not clearly distinguish between airway edema and airway smooth-muscle contraction. It is likely that the increase in *Pao* indicates bronchoconstriction, since our results demonstrated that *Pao* increased transiently and immediately after inhalation of IL-8 and that there was no edema in the bronchial wall sections. However, since the histologic fixation/dehydration procedures would modulate/reduce any airway wall edema present, further research, such as measuring airway microvascular leakage, is required to draw a conclusion.

Burrows et al. demonstrated previously that IL-8 is a spasmogen for airway smooth muscle *in vitro* (23). Moreover, several independent groups have reported that IL-8 causes concentration-dependent histamine release from human basophils *in vitro* (16), and that IL-8 induces histamine release in IL-3-pretreated human blood basophils (17). IL-8 at 10^{-8} M and 10^{-7} M activates human basophils by a receptor-mediated mechanism similar to that operating in neutrophils (17). However, at higher concentration (10^{-6} M) of IL-8, histamine release is presumed to be induced by cationic interactions without involvement of the IL-8 receptor (17). Since the molecular mass of IL-8 is about

8 kDa, the highest concentration of inhaled IL-8 in our study is about 1.25×10^{-9} M. Thus, it is likely that receptor-mediated mechanisms rather than cationic interactions were responsible for the histamine release in our experiments. Furthermore, the activity of IL-8 on the main histamine-containing cells of the guinea pig airway, mainly the mast cells, is unknown. Thus, it is tempting to speculate that IL-8 might induce histamine release from mast cells, in turn leading to bronchoconstriction.

Collins et al. demonstrated that the intradermal injection of IL-8 induces local neutrophil infiltration in guinea pigs *in vivo* and that eosinophil infiltration also occurred 1 and 2 h after injection of IL-8 (11). In addition, Smith et al. reported that the absolute number and the percentage of eosinophils in BALF were elevated only at 24 and 48 h after aerosolized administration of IL-8 in guinea pigs (12). In the present study, eosinophils in BALF or bronchial tissues did not increase, but it is possible that a significant increase in eosinophils might be observed even in our model if examined at 24 h.

Since BHR did not develop 4 h after aerosolized administration of approximately 5 ng (0.01% of 50 µg reaches the airway) of IL-8 in guinea pigs (12), IL-8-induced BHR may be unable to last for several hours. We recently demonstrated that BHR was induced by intranasal administration of 5 µg/kg/day of IL-8 twice a week for 3 weeks accompanied by an

increase of neutrophils both in BALF and bronchial tissues in guinea pigs (14). Medhurst et al. (24) also reported that BHR to histamine is induced by intraperitoneal injection of IL-8 at 2 µg/kg at 24 and 48 h in guinea-pig perfused lungs. As these two reports did not describe the airway response immediately after administration of high doses of IL-8, it may be possible that ongoing bronchoconstriction might contribute to the apparent BHR. In future studies to investigate the effect of IL-8 on the airway, we should also pay attention to the direct effect of IL-8 itself.

In conclusion, IL-8 has bronchoconstrictive action partly through releasing histamine in guinea pigs without cellular infiltration into the airway. Although our results obtained in control, unsensitized guinea pigs may not be applicable to the inflammatory asthmatic response in sensitized human subjects, we believe that this study may lead to better design in future human work that will clarify the effect of IL-8 on bronchomotor tone in asthmatic subjects.

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