

Effect of interleukin-1 β on airway hyperresponsiveness and inflammation in sensitized and nonsensitized Brown-Norway rats

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Airway responsiveness (AR) to inhaled acetylcholine and bradykinin and inflammatory cell recruitment in bronchoalveolar lavage fluid (BALF) were studied in inbred male Brown-Norway rats actively sensitized to ovalbumin and later given 500 U interleukin-1 β (IL-1 β) intratracheally. We examined animals 14 to 21 days after initial sensitization at 18 to 24 hours after the intratracheal administration of IL-1 β . We evaluated AR to acetylcholine as $-\log PC_{200}$, which is $-\log_{10}$ transformation of provocative concentration of acetylcholine producing 200% increase in lung resistance, and to bradykinin as percent increase in lung resistance. BALF was examined as an index of inflammatory changes within the lung. Although there was no significant difference in baseline lung resistance, nonsensitized and sensitized animals that were given IL-1 β demonstrated a significant increase of AR to bradykinin at 18 to 24 hours and a significant increase of neutrophil counts in BALF, which was already observed by 4 to 6 hours. There was a significant correlation between AR to bradykinin and neutrophil counts in BALF in all animals ($r = 0.644$; $p < 0.0005$). We conclude that intratracheal administration of IL-1 β induces the inflammatory changes, which are characterized by an increase in neutrophil counts in BALF, and increased AR to bradykinin, and that active sensitization per se does not potentiate the effect of IL-1 β on AR to acetylcholine or bradykinin or on airway inflammation. (J ALLERGY CLIN IMMUNOL 1994;93:464-9.)

Key words: Active sensitization, ovalbumin, Brown-Norway rats, interleukin-1 β , airway responsiveness, acetylcholine, bradykinin, bronchoalveolar lavage fluid

Reversible airflow obstruction and airway hyperresponsiveness (AHR) to a wide variety of bronchoconstrictor stimuli are characteristic features of asthma.^{1, 2} Although the precise mechanisms of AHR have not been elucidated, airway inflammation is an important determinant of AHR.^{3, 4} There is increasing interest in the possible role of cytokines in the amplification and modulation of both allergic and nonallergic inflammation. The sources of these cytokines, as

Abbreviations used

AHR:	Airway hyperresponsiveness
BALF:	Bronchoalveolar lavage fluid
IL-1 β :	Interleukin-1 β
R _L :	Lung resistance
PC ₂₀₀ :	Provocative concentration producing 200% increase in lung resistance

well as the target cells for their actions, are pleiomorphic and include inflammatory cells, which are central to asthmatic process. The release of one of these cytokines, interleukin-1 (IL-1), by alveolar macrophages in patients with asthma is enhanced⁵ and the level of IL-1 β in bronchoalveolar lavage fluid (BALF) of patients with symptomatic asthma is higher than that of normal subjects or patients with asymptomatic asthma.^{6, 7} IL-1 induces the release of histamine from human basophils in the presence of deuterium oxide.⁸ In addition, IL-1 β induces the expression of intercellular adhesion molecule-1 on

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TABLE I. Characteristics of four experimental groups and procedures performed for each group

Group	n	Body weight (gm)	Intraperitoneal injection ($\times 3$)	Intratracheal injection	Baseline R_L before ACh (cm H ₂ O/ml/sec)	Baseline R_L before BK (cm H ₂ O/ml/sec)
NS	7	322 $\pm$ 6	—	Saline	0.38 $\pm$ 0.06	0.46 $\pm$ 0.09
NI	6	322 $\pm$ 11	—	IL-1 β	0.29 $\pm$ 0.05	0.35 $\pm$ 0.05
SS	8	309 $\pm$ 11	OA + Al (OH) ₃	Saline	0.32 $\pm$ 0.03	0.42 $\pm$ 0.03
SI	7	281 $\pm$ 8	OA + Al (OH) ₃	IL-1 β	0.30 $\pm$ 0.04	0.46 $\pm$ 0.09

ACh, Acetylcholine; BK, bradykinin; OA, ovalbumin.

human airway epithelial cells⁹ and endothelium.¹⁰ Recently, an IL-1 receptor antagonist has been shown to inhibit allergen-induced AHR in sensitized guinea pigs, suggesting a role for IL-1 in this model.¹¹

In view of the relationship between airway inflammation and bronchial hyperresponsiveness, we examined whether IL-1 β could affect the airway response to acetylcholine and bradykinin. We therefore compared the effects of IL-1 β on the increase in pulmonary resistance caused directly (by acetylcholine) or indirectly (by bradykinin). Because active sensitization could prime airway responses,¹² we also examined the effects of IL-1 β in actively sensitized Brown-Norway rats.

METHODS

Sensitization to ovalbumin and intratracheal injection of IL-1 β

We studied four separate groups of inbred male pathogen-free Brown-Norway rats, which weighed 250 to 350 gm. Two of the four groups were given intraperitoneal injections of 1 ml of a 1 mg ovalbumin/100 mg Al(OH)₃ in 0.9% (wt/vol) saline suspension on days 1, 2, and 3. Intratracheal injections of saline solution or IL-1 β were performed with a 26-gauge needle after blunt dissection of the soft tissues of the neck was performed to expose the trachea while animals were anesthetized with an intraperitoneal injection of 2 mg/kg midazolam and an intramuscular injection of 0.4 ml/kg Hypnorm (0.315 mg/ml of fentanyl citrate and 10 mg/ml of fluanisone).

Rats were divided into groups as follows (Table I). Group NS consisted of nonsensitized animals that were given 100 μ l saline solution intratracheally. Group NI consisted of nonsensitized animals that were given 500 U recombinant human IL-1 β in 100 μ l saline solution intratracheally. Group SS consisted of sensitized animals 14 to 21 days after initial sensitization to ovalbumin that were given 100 μ l saline solution intratracheally. Group SI consisted of sensitized animals 14 to 21 days after initial sensitization to ovalbumin that were given 500 U recombinant human IL-1 β in 100 μ l saline solution.

Because recombinant human IL-1 β has been re-

ported to displace antibody binding to recombinant rat IL-1 β ¹³ in addition to the considerable homology of 78% between human and rat IL-1 β ,¹⁴ recombinant human IL-1 β was used in this study.

In preliminary experiments, 500 U IL-1 β diluted in saline solution alone induced an increase in the number of neutrophils in BALF to the same extent as in a previous report.¹⁵ We therefore used the same amount of IL-1 β throughout this study.

Measurement of lung function and airway responsiveness to acetylcholine and bradykinin

Airway responsiveness was measured 18 to 24 hours after intratracheal administration of IL-1 β or saline solution. Rats were anesthetized with an initial dose of 60 to 80 mg/kg pentobarbital sodium injected intraperitoneally. Additional pentobarbital was administered as required to maintain adequate anesthesia. A tracheal cannula (10 mm length and 1.3 mm internal diameter) was inserted into the lumen of the cervical trachea through a tracheostomy and tied snugly with suture material. A polyethylene catheter was inserted into the left carotid artery to monitor blood pressure and heart rate with a pressure transducer. The right external jugular vein was cannulated for administration of intravenous drugs and fluids. Animals were then connected to a small animal respirator (Harvard Apparatus, Ltd., Edenbridge, Kent, U.K.) and ventilated with 10 ml/kg air at a rate of 90 strokes/min. Transpulmonary pressure was measured with a pressure transducer (model FCO 40; $\pm$ 1000 mm of H₂O, Furness Controls, Ltd., Bexhill, Sussex, U.K.) with one side attached to an air-filled catheter inserted into the right pleural cavity and the other side attached to a catheter connected to a side port of the intratracheal cannula. The ventilatory circuit had a total volume of 20 ml. Airflow was measured with a pneumotachograph (model F1L; Mercury Electronics, Ltd., Glasgow, Scotland) connected to a transducer (model FCO 40; $\pm$ 20 mm of H₂O, Furness Controls, Ltd.). The signals from the transducers were digitized with a 12-bit analog-digital board (NB-MIO-16, National Instruments, Austin, Texas) connected to a Macintosh II computer (Apple Computer Inc., Cupertino, Calif.) and analyzed with software (LabView, National Instruments, Austin, Tex-

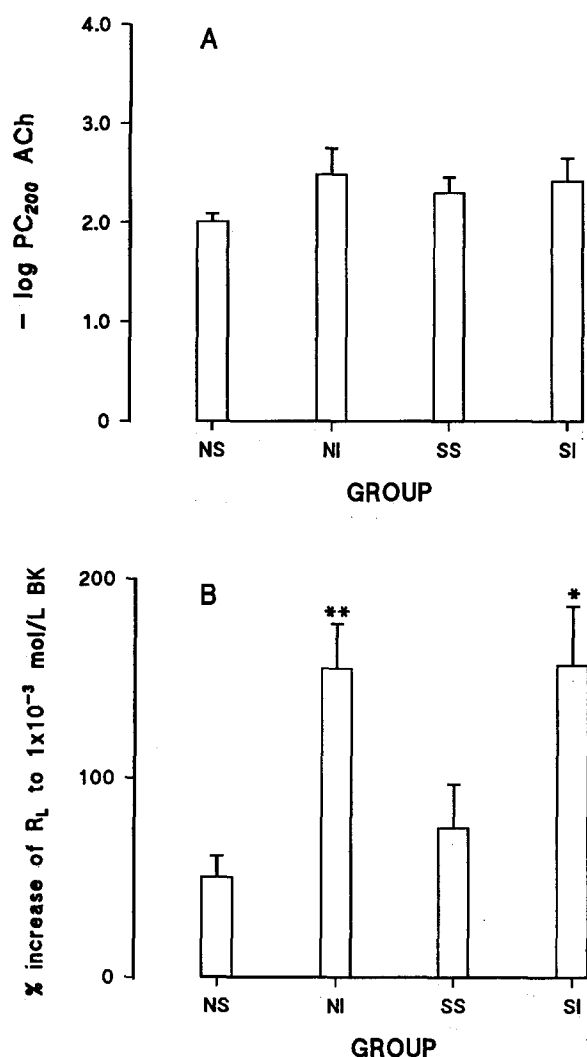


FIG. 1. $-\log PC_{200}$ to acetylcholine (mean $\pm$ SEM) and percent increase of R_L to 1×10^{-3} mol/L bradykinin (mean $\pm$ SEM) for four groups are shown in A and B (see Table I for individual groups). ** $p < 0.005$ compared with NS group; * $p < 0.05$ compared with SS group. ACh, Acetylcholine; BK, bradykinin.

as), which was programmed to instantaneously calculate lung resistance (R_L) according to the method of von Neergard and Wirz.¹⁶ Transpulmonary pressure and mean blood pressure were also monitored throughout the experiments. Aerosols were generated with an ultrasonic nebulizer (model 2511; PulmoSonic, DeVilbiss Health Care Inc., Somerset, Pa.) and were administered to the airways through a separate ventilatory system, which bypassed the pneumotachograph. The volume of this circuit was 50 ml. The mean mass diameter of the aerosol was $3.8 \mu\text{m}$, with a geometric standard deviation of 1.3, measured with a laser droplet and particle analyzer (model 2600C, Malvern Instruments, Derbyshire, U.K.).

Animals were initially injected with propranolol (1 mg/kg intravenously) to inhibit adrenergic effects. A

dose of inhaled saline solution was administered for 45 breaths, and the subsequent R_L value was used as baseline to acetylcholine. Starting 3 minutes after saline exposure, increasing half-log₁₀ concentrations of acetylcholine were administered by inhalation (45 breaths) with the initial concentration set at 10^{-4} mol/L. Increasing half-log concentrations were administered at 5- to 7-minute intervals with one hyperinflation of twice the tidal volume applied between each acetylcholine concentration, performed by manually blocking the outflow of the ventilator. The challenge was stopped when an increase in R_L that exceeded the initial baseline by 200% was obtained. Animals were allowed to recover to baseline R_L . Then a dose of inhaled saline solution was administered for 45 breaths, and the subsequent R_L value was used as baseline to bradykinin. Because of the development of tachyphylaxis to repeated doses of bradykinin, we examined the response to only one dose of bradykinin at 10^{-3} mol/L, administered by inhalation for 45 breaths. The $-\log PC_{200}$, which is $-\log_{10}$ transformation of provocative concentration of acetylcholine producing a 200% increase in R_L , was calculated by the log-linear interpolation of concentration-response curves, and the percent increase of R_L to bradykinin was measured in individual animals.

Bronchoalveolar lavage and cell counting

After measurement of lung function parameters, rats were administered an overdose of pentobarbital (100 mg/kg intravenously), and lungs were lavaged with 2 ml aliquots of saline solution 10 times through a polyethylene tube introduced through the tracheostomy. Lavage fluid was centrifuged (500 g for 10 minutes at 4°C), and the cell pellet was resuspended in 0.5 ml of Hanks' balanced salt solution. Total cell counts were performed by adding 10 μl of the cell suspension to 90 μl of Kimura stain, and cells were counted in a Neubauer chamber (American Optical Corp., Southbridge, Mass.) under a light microscope. Differential cell counts were determined from cytopun preparations, which were made by centrifuging at 300 rpm for 5 minutes and stained by May-Grünwald stain. Cells were identified as macrophages, neutrophils, eosinophils, lymphocytes, basophils, and epithelial cells by standard morphology; 500 cells were counted under $400\times$ magnification and the percentage and absolute number of each cell type were calculated.

Histologic examination

After bronchoalveolar lavage, lungs were removed, inflated with 10% (vol/vol) formalin to a pressure of 25 cm H_2O , and fixed in formalin. Paraffin-embedded sections (8 μm thick) of the airways and lungs were stained with hematoxylin-eosin.

Time course of IL-1 β -induced effects

To determine whether IL-1 β -induced airway effects could occur earlier than the 18- to 24-hour time point, we studied nonsensitized Brown-Norway rats 4 to 6

TABLE II. Total cell counts and differentiated cell counts ($\times 10^6$) in BALF in each group at 18 to 24 hours

Group	n	Total cell count	Macrophage count	Neutrophil count	Lymphocyte count	Eosinophil count
NS	7	5.69 $\pm$ 0.59	5.49 $\pm$ 0.61	0.11 $\pm$ 0.05	0.07 $\pm$ 0.02	0.04 $\pm$ 0.02
NI	6	23.92 $\pm$ 1.96*	7.07 $\pm$ 0.65	16.77 $\pm$ 2.16*	0.06 $\pm$ 0.03	0.01 $\pm$ 0.01
SS	8	5.82 $\pm$ 0.80	5.55 $\pm$ 0.77	0.10 $\pm$ 0.05	0.05 $\pm$ 0.01	0.09 $\pm$ 0.04
SI	7	30.81 $\pm$ 6.31†	9.28 $\pm$ 2.42	21.42 $\pm$ 5.02†	0.07 $\pm$ 0.01	0.01 $\pm$ 0.01

* $p < 0.0001$ compared with group NS.† $p < 0.0001$ compared with group SS.

hours after IL-1 β administration. Airway responsiveness to acetylcholine and bradykinin was measured, followed by bronchoalveolar lavage as described above.

Drugs

We used the following agents and drugs: ovalbumin (grade V, salt-free), acetylcholine, and bradykinin (Sigma Chemical Co., Poole, Dorset, U.K.), Al(OH)₃ (BDH Chemicals Ltd., Dorset, Poole, U.K.), propranolol (Inderal, ICI, plc, Macclesfield, Cheshire, U.K.), pentobarbital sodium (Sagatal, May & Baker, Ltd., Dagenham, U.K.), midazolam (Hypnovel, Roche Products Ltd., Herts, U.K.), and fentanyl citrate and flunisolone (Hypnorm, Jannssen Pharmaceuticals Ltd., Oxon, U.K.). Recombinant human IL-1 β was a gift of Glaxo Laboratories Ltd., Greenford, U.K.

Data analysis

All values are expressed as means $\pm$ SEM. Statistical significance of differences in variance between independent groups was assessed with the F test. Unpaired Student's *t* test (two-tailed) was used to test the significance of differences between means of the two independent groups if equal variance was found between the two groups; otherwise the Mann-Whitney U test (two-tailed) was used. Probability (*p*) values of less than 0.05 were considered to be significant.

RESULTS

Administration of IL-1 β

Animals that were given either IL-1 β or saline solution intratracheally demonstrated momentary respiratory distress characterized by labored breathing followed by tachypnea, but recovered spontaneously.

Effect of IL-1 β at 18 to 24 hours

Airway responsiveness to acetylcholine. There was no significant difference in baseline R_L measured before acetylcholine challenge in the four groups. Inhaled acetylcholine caused a concentration-dependent increase in R_L . After inhalation of each concentration of acetylcholine, peak R_L was reached within 45 seconds and recovered within 2 to 5 minutes. Mean $-\log PC_{200}$

to acetylcholine values for all the groups are shown in Fig. 1, A.

Airway responsiveness to acetylcholine of groups NI and SI, which were given IL-1 β intratracheally, demonstrated a small but nonsignificant change compared with groups NS and SS, which were given saline solution intratracheally. The $-\log PC_{200}$ s to acetylcholine in groups NS, NI, SS, and SI were 2.01 ± 0.08 , 2.49 ± 0.26 , 2.30 ± 0.16 , and 2.42 ± 0.23 , respectively.

Airway responsiveness to bradykinin. There was no significant difference in R_L measured before bradykinin challenge in the four groups.

Groups NI and SI groups demonstrated significantly increased airway responsiveness to bradykinin compared with groups NS and SS ($p < 0.005$, $p < 0.05$, respectively). The mean percent increases in R_L to bradykinin for the NS, NI, SS, and SI groups were $49.9\% \pm 10.7\%$, $154.8\% \pm 22.3\%$, $74.6\% \pm 22.3\%$, and $156.4\% \pm 29.6\%$, respectively (Fig. 1, B). Sensitized rats showed no further enhanced responses to bradykinin after IL-1 β administration.

Cellular content of BALF. A total volume of 20 ml saline solution was lavaged, and the recovery volume was 18 to 19 ml.

Groups NI and SI demonstrated significant increased total cell numbers, characterized by significant increased neutrophil numbers in BALF 18 to 24 hours after the administration of IL-1 β compared with groups NS and SS ($p < 0.0005$, $p < 0.005$, respectively). There was no significant difference in numbers of macrophages, eosinophils, lymphocytes, basophils, or epithelial cells among the four groups. The values of total cell numbers and differential cell counts are summarized in Table II.

Histologic changes. Groups NI and SI demonstrated migration of neutrophils into the bronchoalveolar space and submucosal infiltration of neutrophils in the intrapulmonary airways without causing pulmonary parenchymal necrosis. These findings were not observed in rats not treated with IL-1 β .

Relationship between airway responsiveness to acetylcholine and bradykinin and cells in BALF.

There was a significant correlation between percent increase of R_L to bradykinin and the degree of neutrophil counts in BALF when the data from all of the

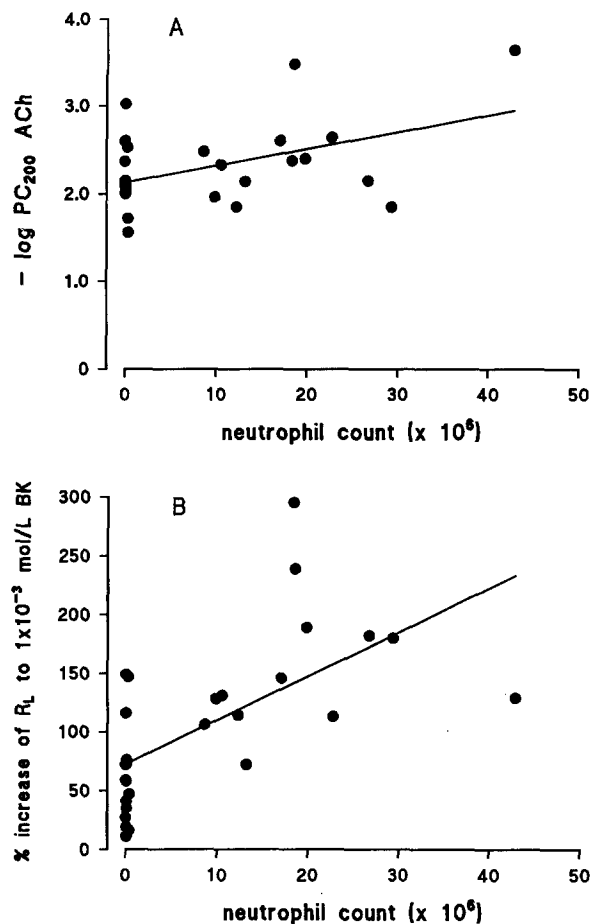


FIG. 2. Relationship between $-\log PC_{200}$ and acetylcholine or percent increase of R_L to 1×10^{-3} mol/L bradykinin and the degree of neutrophil counts in BALF in all animals. There is a significant correlation (for panel B, Spearman's correlation coefficient $r = 0.644$, $p < 0.0005$). Ach, Acetylcholine; BK, bradykinin.

different experimental groups were analyzed together (Spearman's correlation coefficient (r) = 0.644; $p < 0.0005$), but not between $-\log PC_{200}$ acetylcholine and the degree of neutrophil counts in BALF (Fig. 2, A and B).

Effect of IL-1 β at 4 to 6 hours

IL-1 β induced a significant increase in neutrophil counts in BALF at 4 to 6 hours but had no significant effect on airway responsiveness to acetylcholine or bradykinin (Table III).

DISCUSSION

In this study, we have demonstrated that airway responsiveness to inhaled bradykinin was significantly increased 18 to 24 hours after the intratracheal administration of 500 U recombinant human IL-1 β in anesthetized rats, whereas there was

no significant increase in airway responsiveness to acetylcholine. We have also demonstrated that IL-1 β -induced inflammatory changes within the lungs were characterized by the accumulation of large numbers of neutrophils in lungs within 4 to 6 hours of administration, which is consistent with previous reports.^{15, 17} In addition, the number of neutrophils in BALF correlated significantly with airway responsiveness to bradykinin. Active sensitization caused no enhancement of IL-1 β -induced airway responses and inflammation.

We have shown that an increase in airway responses to bradykinin, but not to acetylcholine, can be demonstrated. This suggests that the response of the airways to a bronchoconstrictor acting directly on the airway smooth muscle (such as acetylcholine) is not altered by IL-1 β . However, bradykinin-induced airway effects may be mediated via the release of tachykinin,¹⁸ and its contribution to the increase in pulmonary resistance may also involve airway microvascular leakage resulting in edema of the airway wall.¹⁸ Thus the enhanced responses to bradykinin after IL-1 β administration may represent "indirect" effects such as an enhanced release of tachykinins or enhanced propensity of bradykinin for increased airway microvascular leakage. Another potential explanation for the enhanced responses to bradykinin may relate to the loss of neutral metalloendopeptidase (enkephalinase), which degrades tachykinins and bradykinin; and inhibition of this activity, which is localized predominantly to airway epithelium has been shown to enhance bradykinin-induced bronchoconstriction and airway edema.¹⁹ Because the airway epithelium appears to be intact after IL-1 β administration, mechanisms other than epithelial damage may be involved in the explanation of any potential inhibition of neutral metalloendopeptidase activity.

The strong correlation between neutrophil numbers in BALF and the bronchoconstrictor responses to bradykinin suggests that neutrophils may modulate bradykinin-induced increases in R_L . It is possible that oxidant stresses resulting from the influx of neutrophils may lead to inactivation of epithelial neutral endopeptidase activity. IL-1 β has been shown to have the capacity to stimulate the release of superoxide anions from neutrophils,²⁰ and HOCl, a product of neutrophil myeloperoxidase-catalyzed oxidation of chloride, has been reported to induce AHR to substance P but not to acetylcholine in vitro through inactivation of airway mucosal neutral endopeptidase.²¹

Active sensitization to ovalbumin may increase

TABLE III. Effect of IL-1 β on airway responses at 4 to 6 hours

Group	n	Weight (gm)	-log PC ₂₀₀ ACh	% increase of R _L to BK*	Cell count in BALF (1 $\times$ 10 ⁶)	
					total	Neutrophil
Saline	6	232 $\pm$ 5	2.02 $\pm$ 0.13	48.7 $\pm$ 12.2	4.38 $\pm$ 0.37	0.06 $\pm$ 0.01
IL-1 β	6	235 $\pm$ 5	2.26 $\pm$ 0.12	67.5 $\pm$ 13.4	26.75 $\pm$ 9.02†	20.64 $\pm$ 8.54†

Results are expressed as means $\pm$ SEM.

ACh, Acetylcholine; BK, bradykinin.

*1 $\times$ 10⁻³ mol/L bradykinin.

†p < 0.005 compared with saline-treated group.

mediator-induced responses of lung tissue and release of mediators in guinea pigs.^{12, 22} We therefore also examined the effects of IL-1 β in actively sensitized Brown-Norway rats. Active sensitization per se, however, did not potentiate the effect of IL-1 β on airway responses to acetylcholine or bradykinin or on airway inflammation.

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