

Potential role of interleukin-1 in allergen-induced late asthmatic reactions in guinea pigs: Suppressive effect of interleukin-1 receptor antagonist on late asthmatic reaction

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Interleukin (IL)-1 is a pluripotential proinflammatory cytokine and is thought to be involved in the pathogenesis of bronchial asthma and late asthmatic reactions (LARs). To determine whether IL-1 plays a role in LAR, guinea pigs sensitized with Ascaris antigen were used. We evaluated IL-1 production by immunostaining with anti-IL-1 β antibody and elucidated the action of IL-1 in LAR with recombinant IL-1 receptor antagonist. Immunostaining revealed that IL-1 β -like immunoreactivity-positive cells increased in the airway walls and in bronchoalveolar lavage fluid after the antigen challenge. IL-1 receptor antagonist protein pretreatment reduced the generation of LAR in terms of pulmonary resistance. IL-1 receptor antagonist protein pretreatment did not change cellular components but reduced the percentage of hypodense eosinophils in bronchoalveolar lavage fluid. We also studied the direct effect of recombinant human IL-1 β on pulmonary resistance and eosinophil activity measured as released eosinophil peroxidase activity. Recombinant human IL-1 β did not change pulmonary resistance but primed eosinophils to release eosinophil peroxidase activity in response to platelet activating factor. Therefore these results suggest that IL-1 was produced in sensitized pulmonary tissue of guinea pigs by allergen exposure and played a role in the generation of LAR, at least partially by modulating the activation of eosinophils. (J ALLERGY CLIN IMMUNOL 1995;95:1236-45.)

Key words: Eosinophils, bronchial asthma, pulmonary resistance

Late asthmatic reaction (LAR) is an inflammatory reaction of airways occurring 4 or 5 hours after allergen exposure and persisting for 24 hours or more in some patients with bronchial asthma, and it is characterized by the infiltration of inflammatory cells, predominantly eosinophils. Studying the pathogenesis of LAR might be beneficial to the understanding of bronchial asthma, because airway inflammation is now recognized as an important factor in its pathogenesis. Various animal models

Abbreviations used

BALF:	Bronchoalveolar lavage fluid
EPO:	Eosinophil peroxidase
IL:	Interleukin
IRAP:	Interleukin-1 receptor antagonist protein
LAR:	Late asthmatic reaction
PAF:	Platelet activating factor
PBS:	Phosphate-buffered saline
PIPES:	Piperazine-N,N'-bis[2-ethanesulfonic acid]
R1:	Pulmonary resistance

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have been developed to clarify the pathogenesis of LAR.^{1,2} We have also reported the development of LAR in actively sensitized guinea pigs, which had histologic changes such as eosinophil infiltra-

tion, airway wall edema, and plugging in the lumen of airways, similar to those of bronchial asthma.³ On the other hand, recent studies have demonstrated that pluripotential proinflammatory cytokines such as interleukin(IL)-1 and tumor necrosis factor- α are related to various forms of inflammatory disease.⁴⁻⁶ Among them, IL-1 has been reported to be increased in the bronchoalveolar lavage fluid (BALF) of patients with symptomatic asthma.⁷

Thus the aim of this study was to clarify the involvement of IL-1 in LAR with the guinea pig model of LAR. First, we attempted to determine whether IL-1 was produced after the antigen challenge in this guinea pig model by means of immunostaining with anti-IL-1 β antibody. Next, using IL-1 receptor antagonist protein (IRAP), which is a member of the IL-1 family and binds to identical receptors of IL-1 without inducing agonistic effect,^{8,9} we attempted to determine whether IL-1 participated in the development of LAR and in the changes of cellular components and eosinophil density distribution in the BALF of the late phase.

METHODS

Animals

Male, Hartley strain guinea pigs, weighing 200 to 250 gm, were obtained commercially (Charles River, Yokohama, Japan). Animal care and use were in accordance with the principles stated in the "Guide for the Care and Use of Laboratory Animals" (Prime Minister's Office of Japan, Publication no. 6, 1980). Protocols described below were approved by the Animal Care and Use Committee, Tohoku University.

Antigens

Extracts from *Ascaris suum* were prepared according to a previously reported method.¹⁰ We obtained frozen *Ascaris* nematodes from a local slaughterhouse. They were washed several times with physiologic saline solution and were homogenized in a Waring blender (Waring Products, New Hartford, Conn.). After centrifugation at 10,000 rpm for 30 minutes, the supernatant was collected and dialyzed against several changes of phosphate-buffered saline (PBS). By further centrifugation at 10,000 rpm for 30 minutes, we obtained a clear supernatant, which was stored at -20°C .

Sensitization procedure

Guinea pigs were injected intraperitoneally twice at an interval of 2 weeks with a mixture of 20 μg of *Ascaris* extract and 20 mg of silica (S 5130; Sigma Chemical Co., St. Louis, Mo.) dissolved in 1 ml of physiologic saline solution. One week after the second intraperitoneal injection, animals inhaled aerosolized *Ascaris* extract (2.5 mg/ml in physiologic saline solution) for 30 seconds

through a mask and an ultrasonic nebulizer (NE-U06; Omron, Kyoto, Japan).

Detection of IL-1 β by immunohistochemistry

One week after the last sensitization procedure, guinea pigs inhaled aerosolized *Ascaris* antigen (2.5 mg/ml) for 30 seconds. Before and 30 minutes, 1, 2, 4, 8, and 24 hours after the inhalation, guinea pigs were killed with an overdose of sodium pentobarbital (60 mg/kg) injected intraperitoneally. The lungs were removed from the guinea pigs and placed in periodate-lysine-paraformaldehyde, embedded in paraffin.

Immunostaining was performed on 5 μm sections with goat polyclonal anti-murine IL-1 β (AB-401-NA; R & D Systems, Minneapolis, Minn.) and the streptavidin-biotin complex and alkaline phosphatase method (K391; Dako, Glostrup, Denmark). During the same time course lung specimens taken from nonsensitized and *Ascaris* antigen-challenged guinea pigs were also prepared. The cells in the BALF from antigen-challenged guinea pigs with or without sensitization were studied in the same way. BALF was collected before and 1, 2, and 6 hours after the antigen challenge. As a negative control, specimens were treated with normal goat serum instead of anti-IL-1 β antibody. We stained the cells in the BALF from normal guinea pigs, which were incubated 2 hours with or without lipopolysaccharides (10 $\mu\text{g}/\text{ml}$) with anti-IL-1 β antibody as controls. We confirmed the cross-reactivity and the selectivity of anti-IL-1 β antibody (AB-401-NA, R & D Systems) to guinea pig IL-1 β by performing an absorption test and immunostaining with an indifferent antibody made from the same species (anti-granulocyte-macrophage colony-stimulating factor antibody; AB-415-NA, R & D Systems). An absorption test was performed for each specimen with the supernatant of the antibody solution (10 $\mu\text{g}/\text{ml}$) incubated with 100 ng of recombinant murine IL-1 β (401-ML, R & D Systems) and centrifuged at 10,000 g for 1 hour. IL-1 β -like immunoreactivity-positive specimens did not show positive staining with this supernatant. Immunostaining with indifferent antibody showed a different pattern. Thus the antibody for IL-1 β used in this study was specific for IL-1 β of guinea pigs. Each group or each time point contained three guinea pigs.

Measurement of pulmonary resistance

Pulmonary resistance (R1) was measured during spontaneous tidal breathing. Guinea pigs were anesthetized with an intraperitoneal injection of urethane, 2 gm/kg. Tracheostomy was performed, and an endotracheal tube (inner diameter = 2.1 mm, length = 2.5 cm) was inserted through the tracheostoma. A pneumotachograph (no. 8300; Hans Rudolph, Kansas City, Mo.) coupled to a differential pressure transducer (model LCVR, 0 to 2 cm H_2O ; Celesco, Canoga Park, Calif.) was connected to the endotracheal tube to measure airflow. Volume was obtained by electrical integration of

the flow signal with an integrator (EI-601G; Nihon-koden, Tokyo, Japan). Esophageal pressure was measured with a 9 cm length of water-filled polyethylene tube (inner diameter = 0.75 mm) and a pressure transducer (TP-200T, Nihon-koden). Airway opening pressure was measured by a pressure transducer (model LCVR, 0 to 5 cm H₂O; Celesco) connected between the endotracheal tube and the pneumotachograph. Transpulmonary pressure was obtained by subtraction of airway opening pressure from esophageal pressure. R1 was calculated by the subtraction method of Mead and Whittenberger.¹¹

Effect of IRAP on LAR

We injected IRAP (1 mg/kg; Upjohn, Kalamazoo, Mich.) (group I, $n = 6$) or PBS (group II, $n = 6$ and group III, $n = 6$) intravenously to actively sensitized guinea pigs 30 minutes before antigen exposure. Guinea pigs in groups I and II inhaled aerosolized *Ascaris* extract (2.5 mg/ml in physiologic saline solution) carried by a constant airflow (600 ml/min) twice within a 5-minute period (total = 1 minute) through an endotracheal tube. Guinea pigs in group III (half of the guinea pigs that were given PBS intravenously) inhaled saline solution as a control. R1 was measured before the injection of IRAP or PBS; before and immediately after the antigen challenge; 5, 15, and 30 minutes; and then from 1 hour to 6 hours at 1-hour intervals after the antigen challenge. Secretions were removed with paper strings before each R1 measurement procedure. BALF was collected immediately after the completion of R1 measurement 6 hours after the antigen challenge. After animals were killed with an intraperitoneal injection of sodium pentobarbital and exsanguination, the airways were lavaged through the endotracheal tube with physiologic saline solution (3 times, total 30 ml). BALF was centrifuged at 1200 rpm for 5 minutes. Pellets were resuspended in 4 ml of physiologic saline solution. Total cell number was counted with a hemocytometer (improved Neubauer counting chamber), and a 100 μ l aliquot was cytocentrifuged with Cytospin2 (Shandon, Inc., Pittsburgh, Pa.) and stained with Wright-Giemsa stain. The cells were differentiated as macrophages, lymphocytes, eosinophils, or neutrophils.

Comparison of eosinophil density distribution

Distribution of eosinophil density in BALF was compared between the IRAP-pretreated group and the nontreated group. Guinea pigs were immunized and challenged in the same way as those in group I ($n = 5$) and group II ($n = 5$). BALF was collected from all guinea pigs 5 hours after the antigen challenge and centrifuged at 1200 rpm for 5 minutes. Pellets were resuspended in 4 ml of 24 nmol/L piperazine-N,N'-bis[2-ethanesulfonic acid] (PIPES) solution (pH 7.3, containing 125 mmol/L NaCl, 5 mmol/L KCl, and 0.03% bovine serum albumin), and 200 μ l aliquots were used for total

and differential cell counts. The remainder was centrifuged again, and cells were resuspended in 2 ml of PIPES solution containing 1.065 gm/ml Percoll (Pharmacia, Uppsala, Sweden). Percoll discontinuous gradients were prepared by a modification of Gartner's method.¹² Serial discontinuous densities of Percoll (1.112, 1.100, 1.090, 1.085, 1.080, and 1.070 gm/ml) in PIPES solution were layered into a 25 ml conical polycarbonate tube (Beckman, Fullerton, Calif.). Cell suspension was mounted gently onto the Percoll discontinuous gradient and centrifuged at 2800 rpm, 4° C, for 20 minutes. Then each layer was separated, and total cell count and eosinophil percentage were measured after a wash with PIPES solution. Data are expressed as the percentage of eosinophils of each density per total number of eosinophils. As previously reported, we defined eosinophils less than 1.085 gm/ml as hypodense, 1.085 to 1.090 gm/ml as normodense, and 1.100 gm/ml or greater as hyperdense.¹³ Percentage of each component was compared between two groups.

Effect of recombinant human IL-1 β on R1

With the use of eight guinea pigs, the direct effect of IL-1 β on R1 was examined. Guinea pigs were anesthetized and tracheostomized as described above. In four guinea pigs, 1 μ g/kg of rhIL-1 β (Otsuka, Tokushima, Japan) was instilled through the endotracheal tube. In the other guinea pigs, the same concentration of bovine serum albumin was instilled. Before and 1, 3, 5, 15, 30, 60, 120, and 240 minutes after the instillation, R1 was measured.

Effect of rhIL-1 β on eosinophils

With guinea pig eosinophils, the direct effect of rhIL-1 β was studied. Eosinophils were harvested from the peritoneal cavity of horse serum-sensitized and stimulated guinea pigs by washing with PBS. They were washed several times with PIPES solution and resuspended in 1.068 gm/ml Percoll PIPES solution. The cell suspension was mounted onto the Percoll discontinuous gradient (1.112, 1.086, and 1.081 gm/ml in PIPES solution) and centrifuged at 2800 rpm, 4° C for 20 minutes. Cells on the 1.112 gm/ml Percoll solution were washed several times with PIPES solution. After cell number and eosinophil percentage were determined, cells were separated into groups of 1×10^7 cells for eosinophil peroxidase (EPO) activity measurement.

EPO activity was measured as previously described.¹⁴ Purified guinea pig eosinophils, prepared as described above (purity > 97%, 1×10^7 cells), were incubated for 120 minutes at 37° C with rhIL-1 β (1 ng/ml or 100 ng/ml) or without rhIL-1 β (total volume = 1 ml) and then incubated 30 minutes with or without platelet activating factor (PAF) (10^{-6} mol/L). After cooling and centrifugation at 1200 rpm for 5 minutes, the supernatant (150 μ l) was added to the substrate solution (0.1 mmol/L *o*-phenylenediamine dihydrochloride in a 0.05 mol/L Tris buffer containing 0.1% Triton X-100 and

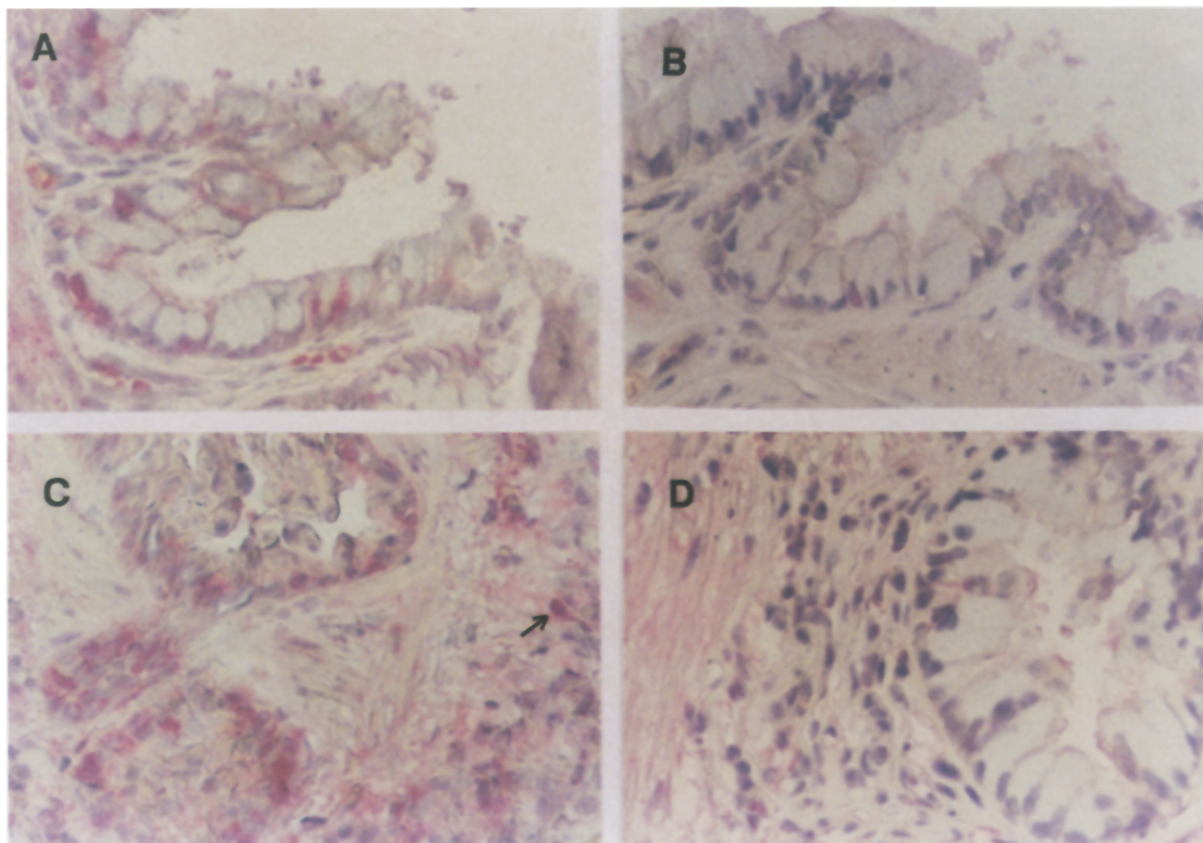


FIG. 1. Immunostaining of the airways taken from guinea pigs sensitized and exposed to *Ascaris* antigen. **A**, The airway 2 hours after the antigen exposure was immunostained with anti-IL-1 β antibody. Cells in the bronchial epithelium and a few cells in the submucosal layer were stained in red with streptavidin-biotin/alkaline phosphatase and Fast Red substrates (K699, Dako). **B**, The same airway section as shown in **A** was immunostained with anti-IL-1 β -antibody-absorbed solution. No cells were stained in red. **C**, The airway 8 hours after antigen exposure. Cells in the epithelium, and a few cells in the peribronchiolar layer (arrow) demonstrated positive staining for IL-1 β . **D**, The airway 24 hours after antigen exposure. No cells showed positive staining for IL-1 β , although cell infiltrations to the submucosal layer were observed. Counterstaining was performed with hematoxylin.

1 mmol/L hydrogen peroxide) and incubated at 37° C for 30 minutes. After addition of 200 μ l of 4 mol/L sulfuric acid, the absorbance at 492 nm was measured with a spectrophotometer (220A; Hitachi, Tokyo, Japan). With a standard curve made by horseradish peroxidase, the EPO concentration was determined.

Statistical analysis

We considered up to 1 hour after the challenge as the immediate phase, and from 2 hours to 6 hours as the late phase. We compared change in R1 versus time area under the curve of each phase after the challenge with the Mann-Whitney U test between the groups. If a difference was observed, we compared the increase in R1 at each time point with the Mann-Whitney U test. The Wilcoxon test was used to compare the changes in R1 from baseline. R1 changes after the rhIL-1 β instillation were evaluated in the same way. Cell number in BALF and percentage of each density of eosinophils were compared by using the

Mann-Whitney U test. EPO activity was compared by using Student's *t* test. Probability (*p*) values of less than 0.05 were considered to be significant.

RESULTS

Immunohistochemistry

Immunostaining with the anti-IL-1 β antibody revealed the appearance of IL-1 β -positive cells after the antigen challenge. No IL-1 β -positive cells were present at any time after the antigen challenge without the sensitization or before the antigen challenge with the sensitization. IL-1 β -positive cells were observed among the epithelial cells and in the submucosal layer of bronchi from 2 hours to 8 hours after the challenge (Fig. 1, *A* and *C*). Few IL-1 β -positive cells were observed in the alveolar space at any time. At 24 hours after the antigen challenge, IL-1 β -

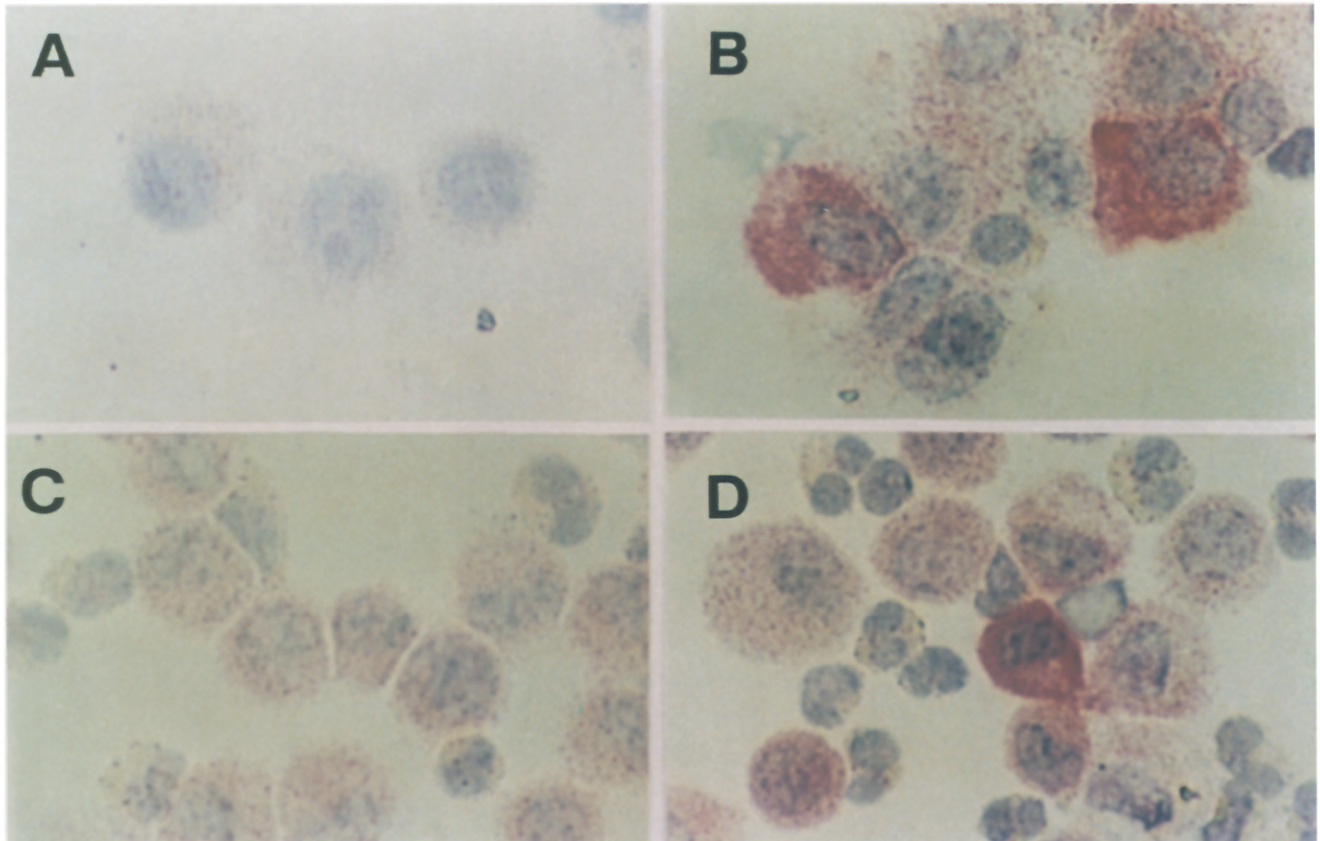


FIG. 2. Cells in BALF. **A**, Cells from nonsensitized and nonchallenged guinea pigs demonstrated very weak staining for IL-1 β . **B**, Cells from normal guinea pigs (same as in **A**) were incubated with lipopolysaccharide (10 μ g/ml, 2 hours). Macrophages showed stronger staining than in **A**. **C**, One hour after challenge, macrophages showed weak staining, but the intensity was stronger than in **A**. **D**, Six hours after antigen challenge, macrophages showed staining as strong as that in **B**.

positive cells disappeared from the airways, although cells were still infiltrating in the submucosal layer (Fig. 1, *D*). The specimens used for the absorption test (Fig. 1, *B*) or incubated with normal goat serum were not stained.

In the BALF, macrophages demonstrated weak staining for IL-1 β even in the state of nonsensitization without challenge. The intensity was not changed after the antigen challenge in the nonsensitized guinea pigs, nor until 1 hour after the antigen challenge in the sensitized guinea pigs. In the sensitized guinea pigs, macrophages demonstrated stronger staining for IL-1 β from 2 hours after the antigen challenge (Fig. 2). Other types of cells did not show positive staining.

Effect of IRAP on R1 changes after antigen challenge

R1 values before the intravenous injections of IRAP and PBS were 84.3 ± 4.5 and 87.5 ± 5.5 (mean $\pm$ SEM, $\times 10^{-3}$ cm H₂O/ml/sec), and those

at 30 minutes after the injection were 91.2 ± 5.7 and 90.3 ± 4.9 , respectively. No significant differences in R1 values between the two groups and two time points were observed. Fig. 3 shows the time course of changes in R1 after the challenge in the three groups; IRAP-pretreated and antigen-challenged (group I), PBS-pretreated and antigen-challenged (group II), and PBS-pretreated and saline-challenged (group III). In the saline-challenged animals (group III), no significant change in R1 was observed until 6 hours after the inhalation of saline solution. In the antigen-challenged guinea pigs, with or without IRAP injection, a biphasic increase in R1 was observed after the antigen challenge and was significantly different from that of the saline-challenged guinea pigs in both the immediate and the late phase ($p < 0.05$). In the IRAP-pretreated guinea pigs (group I), the increase in R1 was reduced in the late phase after the antigen challenge compared with that of the PBS-pretreated guinea pigs (group II) ($p < 0.05$).

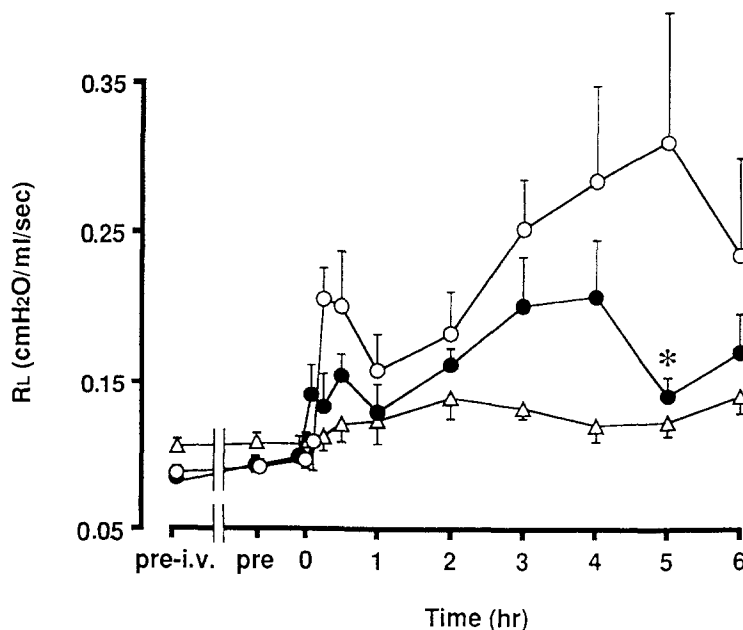


FIG. 3. Time course of changes in R1 after the challenge in the three groups; antigen challenged with IRAP pretreatment (group I, ●, $n = 6$), without pretreatment (group II, ○, $n = 6$), and saline-challenged (IRAP pretreatment [–], group III, △, $n = 6$) groups. Significant increases in R1 in both the immediate and late phases were observed in antigen-challenged guinea pigs (○), compared with saline-challenged guinea pigs (△) ($p < 0.05$). Increase in R1 was significantly reduced in the late phase (2 to 6 hours) after antigen challenge in group I (●) compared with group II (○) (detected with Mann-Whitney U test for change in R1 versus time area under the curve). Further, significance was observed at the 5-hour time point. * $p < 0.05$. R_L , Pulmonary resistance.

The increase in R1 5 hours after the antigen challenge in group I was significantly less than that in group II ($p < 0.05$). The increase in R1 tended to be smaller also in the immediate phase compared with that in group II, but the difference in R1 between the two groups was not significant. A possible correlation between the magnitude of the immediate-phase response and that of the LAR calculated as the change in R1 versus time area under the curve or the maximal increase in R1 was investigated, but none was observed ($r = 0.14$ and 0.23 for each parameter, respectively).

Effect of IRAP on changes in cell differential in BALF after antigen challenge

Total cell counts in the BALF at 6 hours after the challenge were 230.5 ± 35.4 , 219.8 ± 32.5 , and 88.7 ± 14.2 (mean $\pm$ SEM, $\times 10^5$ cells) for groups I, II, and III, respectively. Significant increases in cell numbers were observed in the antigen-challenged groups (groups I and II). Among them, eosinophils markedly increased in the antigen-challenged guinea pigs (Fig. 4). Lymphocytes also increased in the antigen-challenged guinea pigs.

On the other hand, significant differences were not observed in the numbers of macrophages and neutrophils between the antigen-challenged and the saline-challenged guinea pigs. Pretreatment of guinea pigs with IRAP did not affect the number of cells. No significant difference in cell number was observed between groups I and II.

To examine a more detailed cell differential between the IRAP-pretreated and the PBS-pretreated groups at the time point that the significant difference in R1 was observed, BALF was collected at 5 hours after the antigen challenge in the other guinea pigs. At this time point also, we could not find any difference in the numbers of each type of cell between the guinea pigs with and without IRAP (Table I). Then we compared eosinophil density distribution of the two groups. Eosinophil density distribution was significantly different in the two groups ($p < 0.05$, Fig. 5). Compared with the nontreated group, the percentage of eosinophils under 1.080 gm/ml was significantly suppressed ($p < 0.05$), and that of eosinophils over 1.100 gm/ml was significantly increased ($p < 0.05$) in the IRAP-pretreated group.

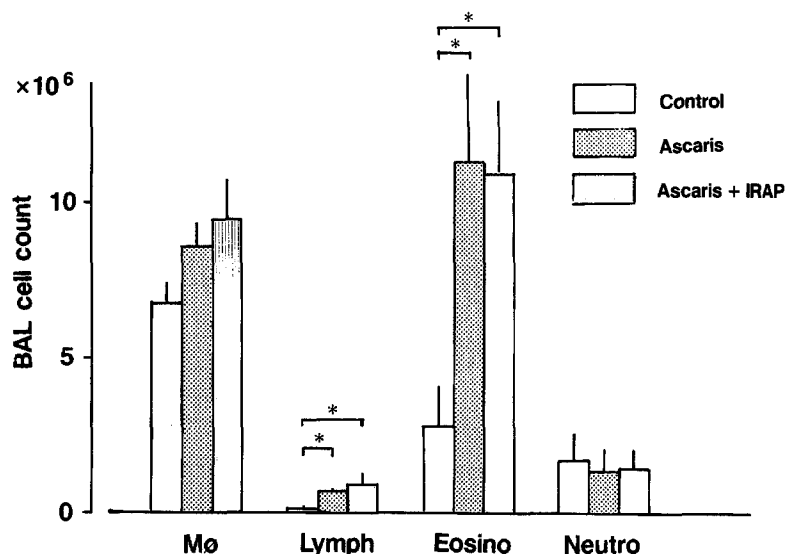


FIG. 4. Cellular components in BALF 6 hours after challenge. Significant increases in eosinophil and lymphocyte cell counts were observed after antigen challenge ($p < 0.05$). However, no differences in cell counts were observed between IRAP-pretreated and nontreated groups. No difference in macrophage or neutrophil cell count was observed among three groups. BAL, Bronchoalveolar lavage; Mø, macrophages.

TABLE I. Cell count in BALF 5 hours after the challenge

	IRAP (-)	IRAP (+)
Eosinophils ($\times 10^5$)	166.1 $\pm$ 14.5	177.2 $\pm$ 13.4
Hypodense (%) (<1.085 gm/ml)	41.8 $\pm$ 9.6	17.1 $\pm$ 4.7*
Normodense (%) (1.085 to 1.090)	46.7 $\pm$ 8.0	49.4 $\pm$ 4.7
Hyperdense (%) (≥ 1.100)	11.5 $\pm$ 4.1	33.5 $\pm$ 8.5*
Macrophages ($\times 10^5$)	106.7 $\pm$ 13.2	127.2 $\pm$ 25.4
Lymphocytes ($\times 10^5$)	7.2 $\pm$ 2.9	6.6 $\pm$ 2.4
Neutrophils ($\times 10^5$)	6.3 $\pm$ 2.7	1.6 $\pm$ 0.9

Values are expressed as means $\pm$ SEM.

* $p < 0.05$ from IRAP (-) group.

Effect of rhIL-1 β instillation on R1

During the period of measurement, no significant increase in R1 was observed in the rhIL-1 β -instilled guinea pigs compared with the baseline R1. No significant difference in percent change in R1 was observed between the rhIL-1 β -instilled and the bovine serum albumin-instilled guinea pigs (Fig. 6).

Effect of rhIL-1 β on eosinophils

Incubation of eosinophils with rhIL-1 β did not increase the release of EPO activity at either the high (100 ng/ml) or low dose (1 ng/ml) but augmented the

PAF-induced release of EPO activity (Table II). Further, we tested the inhibitory effect of IL-1ra on IL-1-induced eosinophil priming. Incubation of eosinophils with IRAP (100 μ g/ml) together with high-dose human IL-1 β (100 ng/ml) reduced the PAF-induced release of EPO to 0.38 ± 0.04 compared with that with only human IL-1 β (100 ng/ml) (0.49 ± 0.04 mU, $p < 0.05$), whereas incubation with IL-1ra did not change the baseline release of EPO.

DISCUSSION

By means of immunostaining with anti-IL-1 β antibody, we observed the generation of IL-1 β in the pulmonary tissue after the antigen challenge in the guinea pig model of LAR. In this study the cells in the bronchial epithelium and macrophages showed positive staining for IL-1 β . It is possible that IL-1 β was produced in the bronchial epithelial cells, because Mattoli et al.¹⁵ have reported that IL-1 β -like immunoreactivity was released from bronchial epithelial cells in response to toluene 2,4-diisocyanate. Otherwise, IL-1 β that was produced in other cells, such as macrophages, was trapped in the airway epithelium. Macrophages in the BALF showed very weak staining for IL-1 β , even in the state of nonstimulation, and demonstrated stronger staining after the antigen challenge. Thus IL-1 β was constitutively produced in macrophages and was augmented by antigenic stimuli. It has already been reported that IL-1 β is

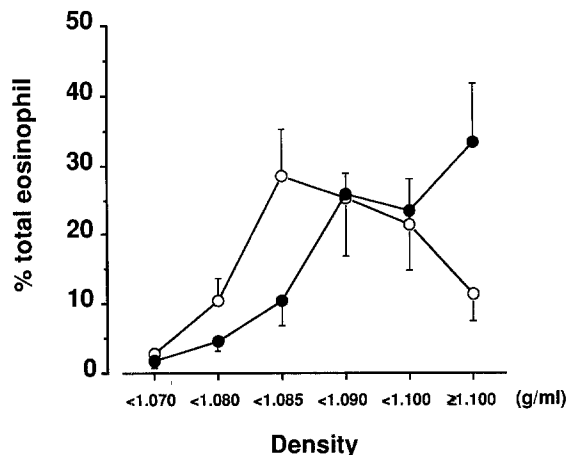


FIG. 5. Eosinophil density distribution in the IRAP-pretreated guinea pigs (●, $n = 5$) and in the IRAP pretreatment (–) guinea pigs (○, $n = 5$). IRAP pretreatment shifted eosinophil density from hypodense to hyperdense.

increased in the BALF of patients with symptomatic asthma,⁷ and allergen challenge to the skin of atopic subjects induces the release of IL-1 β at the site of the challenge.¹⁶ Thus it is speculated that, in the case of LAR also, IL-1 β is produced at the site of allergic reaction. However, to our knowledge, there has been no evidence of IL-1 β production in LAR in human or animal models. Gosset et al.¹⁷ reported that IL-1 β did not increase in the culture supernatant of alveolar macrophages collected 18 to 20 hours after allergen exposure in patients with bronchial asthma with LAR, whereas tumor necrosis factor- α and IL-6 did increase. In this study we observed the appearance of IL-1 β -positive cells from 2 hours to 8 hours after the antigen challenge in the lung specimens and no IL-1 β -positive cells at 24 hours. Augmented IL-1 β production might be limited within hours after antigen challenge.

Next, we studied the effect of IL-1 in the LAR by using IRAP. Pretreatment of guinea pigs with IRAP reduced the increase in R1 in the late phase after the antigen challenge. IL-1 is a potent proinflammatory cytokine, which may induce airway inflammation and an increase in R1. In a model of pulmonary inflammation, intratracheal instillation of IL-1 β induced the accumulation of neutrophils in the BALF.¹⁸ Thus to confirm whether IL-1 induced by antigen exposure directly increased R1, we instilled a high dose of rhIL-1 β (about 100 times the previously reported dose¹⁸) directly into the tracheas of guinea pigs. But instillation of rhIL-1 β did not increase R1 during the period of measurement. The effect of IL-1 on LAR might be

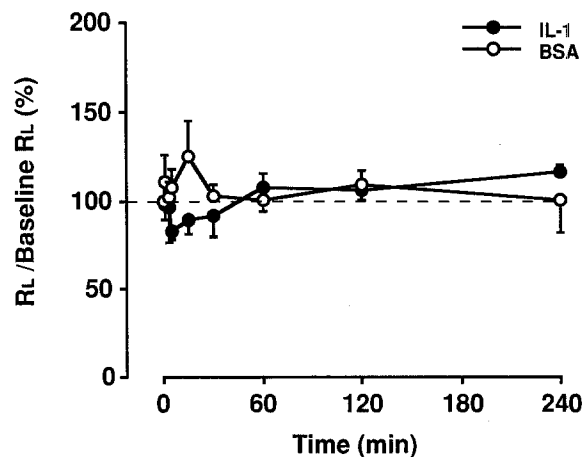


FIG. 6. Time course of percent changes in R1 after instillation of rhIL-1 β (1 μ g/kg, ●, $n = 4$) or the same concentration of bovine serum albumin (○, $n = 4$). No significant change in R1 was observed after the instillation of rhIL-1 β . No significant difference in percent change in R1 was observed between the two groups. BSA, Bovine serum albumin; R_L , Pulmonary resistance.

TABLE II. EPO activity (mU/10⁷ cells)

	PAF (–)	PAF (+)
Control	0.19 $\pm$ 0.05	0.39 $\pm$ 0.01
IL-1 1 ng/ml	0.19 $\pm$ 0.06	0.44 $\pm$ 0.03
IL-1 100 ng/ml	0.16 $\pm$ 0.04	0.49 $\pm$ 0.04*

Values are expressed as means $\pm$ SEM.

* $p < 0.01$ from PAF (+) control eosinophils.

mediated by the activation of cells that are related to LAR, such as eosinophils, macrophages, and T lymphocytes.

We observed a significant increase in eosinophil cell count in the BALF at the late phase after the antigen challenge. Eosinophils release various mediators that can induce changes in lung mechanics, such as leukotriene C₄,¹⁹ major basic protein, and EPO.²⁰ Thus eosinophils might have participated in the increase in R1 after the challenge. Further, in this study we found that pretreatment of guinea pigs with IRAP shifted the eosinophil density distribution from hypodense to hyperdense, although the significant increase of eosinophil infiltration to the bronchoalveolar space in the late phase after the antigen challenge was not affected by IRAP. Many studies on eosinophils have indicated that hypodense is the activated state of eosinophils, which show increased oxygen consumption,²¹ leukotriene C₄ production,²² and cytotoxicity against cellular targets.²³ We speculated

that IRAP reduced the increase in R1 in LAR by inhibiting the activation of eosinophils in the late phase after the challenge and that not only accumulation but also activation of eosinophils is necessary for the induction and augmentation of LAR.

In this study we observed that IL-1 was involved in the activation of eosinophils in LAR in this animal model. Then we selected a means of activity measurement (EPO assay) more direct than density distribution to determine whether IL-1 directly functioned on guinea pig eosinophils. IL-1 did not directly induce the release of a chemical mediator from eosinophils but primed eosinophils. Baskar and Pincus²⁴ have also reported that IL-1 augmented the IgE-dependent activation of eosinophils. IL-1 might have participated in the priming of eosinophils in the augmentation of LAR. These results also indicate the binding ability of human IL-1 β and human IRAP to the IL-1 receptor on guinea pig eosinophils.

Several other indirect pathways between IL-1 and eosinophils are also possible. IL-1 stimulates macrophages and T-lymphocytes to induce various cytokines, such as granulocyte macrophage-colony stimulating factor, IL-3, IL-4 and IL-5. Granulocyte-macrophage colony-stimulating factor, IL-3, and IL-5 are responsible for the activation of eosinophils. IL-4 induces the expression of Fc receptor for IgE on eosinophils. Moreover, IL-1 augments the expression of various adhesion molecules on endothelial cells including intercellular adhesion molecule-1, one of the counter-receptors of the eosinophil integrin, macrophage-1.²⁵ Wegner et al.²⁶ demonstrated that inhibition of macrophage-1 by monoclonal antibody in antigen-challenged primates reduced the eosinophil activation measured by EPO activity in BALF without affecting eosinophil infiltration into the bronchoalveolar space. To our knowledge, this is the only report describing the discrepancy between the influx and the activation of eosinophils in an in vivo model, although various adhesion molecules are involved and very late antigen-4-vascular cell adhesion molecule interaction is thought to be the major component in the accumulation and perhaps the activation of eosinophils. It is possible that in this study, IRAP has inhibited only the interaction between intercellular adhesion molecule-1 and macrophage-1 to prevent the activation but not the influx of eosinophils.

Various cells and their mediators are thought to participate in the changes of respiratory function in the late phase after antigen challenge. IL-1 can modulate many of them, such as the activation of

macrophages, lymphocytes, endothelial cells, eosinophils, and others. Thus the effect of IL-1 on eosinophils, which we report here, may be only one of the pathways that can cause an increase in R1 in the late phase after antigen challenge. The interaction of IL-1 with these cells (i.e., macrophages, lymphocytes, neutrophils, endothelial cells, and others) in the LAR requires further study.

In this study the immediate-phase increase in R1 appeared to be reduced by IRAP. Immediate asthmatic reaction is mainly mediated by histamine. It is known that IL-1 augments the release of histamine.²⁷ Our immunohistologic study revealed that IL-1 β -positive cells are already present before the antigen challenge. We speculated that IL-1 β might participate to some extent in the production of the immediate-phase reaction.

In summary, we found that IL-1 β production occurred in the cells in airway walls and in BALF after the antigen challenge; and by using IRAP, we found that IL-1 modulated the increase of R1 and the activation of eosinophils in the late phase after the antigen challenge. Because the rhIL-1 β did not directly increase R1, the effect of IL-1 on LAR was thought to be mediated by other mediators. A priming effect on eosinophils by rhIL-1 β was observed in this study. It is speculated that IL-1 induced by allergen exposure augmented LAR, partially by modulating the activation of eosinophils.

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REFERENCES

1. Abraham WM, Delehunt JC, Yerger L, Marchette B. Characterization of a late phase pulmonary response following antigen challenge in allergic sheep. *Am Rev Respir Dis* 1983;128:839-44.
2. Sasaki H, Yanai M, Shimura S, et al. Late asthmatic response to *Ascaris* antigen challenge in dogs treated with metirapone. *Am Rev Respir Dis* 1987;136:1459-65.
3. Iijima H, Ishii M, Yamauchi K, et al. Bronchoalveolar lavage and histologic characterization of late asthmatic response in guinea pigs. *Am Rev Respir Dis* 1987;136:922-9.
4. Wooley PH, Whalen JD, Chapman DL, et al. The effect of an interleukin-1 receptor antagonist protein on type II collagen and antigen-induced arthritis in mice. *Arthritis Rheum* 1993;36:1305-14.
5. Ohlsson K, Bjork P, Bergenfeldt M, Hageman R, Thompson RC. Interleukin-1 receptor antagonist reduces mortality from endotoxin shock. *Nature* 1991;348:550-2.

6. Hervé P, Flesch M, Tiberghien P, et al. Phase I-II trial of a monoclonal anti-tumor necrosis factor α antibody for the treatment of refractory severe acute graft-versus-host disease. *Blood* 1992;79:3362-8.
7. Broide DH, Lotz M, Cuomo AJ, Coburn DA, Federman EC, Wasserman SI. Cytokines in symptomatic asthma airways. *J ALLERGY CLIN IMMUNOL* 1992;89:958-67.
8. Arend WP. Interleukin 1 receptor antagonist. A new member of the interleukin 1 family. *J Clin Invest* 1991;88:1445-51.
9. Carter DB, Deibel MR Jr, Dunn CJ, et al. Purification, cloning, expression and biological characterization of an interleukin-1 receptor antagonist protein. *Nature* 1990;344:633-7.
10. Strejan G, Campbell DH. Hypersensitivity to *Ascaris* antigens. I. Skin-sensitizing activity of serum fractions from guinea pigs sensitized to crude extracts. *J Immunol* 1967;98:893-900.
11. Mead J, Whittenberger JL. Physical properties of human lungs measured during spontaneous respiration. *J Appl Physiol* 1953;5:779-96.
12. Gartner I. Separation of human eosinophils in density gradients of polyvinylpyrrolidone-coated silica gel (Percoll). *Immunology* 1980;40:133-6.
13. Aizawa T, Sekizawa K, Aikawa T, et al. Eosinophil supernatant causes hyperresponsiveness of airway smooth muscle in guinea pig trachea. *Am Rev Respir Dis* 1990;142:133-7.
14. Kroegel C, Yukawa T, Dent G, Venge P, Chung KF, Barnes PJ. Stimulation of degranulation from human eosinophils by platelet-activating factor. *J Immunol* 1989;142:3518-26.
15. Mattoli S, Mianze S, Calabro F, Mezzetti M, Fasoli A, Allegra L. Bronchial epithelial cells exposed to isocyanates potentiate activation and proliferation of T-cells. *Am J Physiol* 1990;259:L320-7.
16. Bochner BS, Charlesworth EN, Lichtenstein LM, et al. Interleukin-1 is released at sites of human cutaneous allergic reactions. *J ALLERGY CLIN IMMUNOL* 1990;86:830-9.
17. Gosset P, Tsicopoulos A, Wallaert B, et al. Increased secretion of tumor necrosis factor and interleukin-6 by alveolar macrophages consecutive to the development of the late asthmatic reaction. *J ALLERGY CLIN IMMUNOL* 1991;88:561-71.
18. Ulich TR, Yin S, Guo K, del Castillo J, Eisenberg SP, Thompson RC. The intratracheal administration of endotoxin and cytokines. III. The interleukin-1 (IL-1) receptor antagonist inhibits endotoxin- and IL-1-induced acute inflammation. *Am J Pathol* 1991;138:521-4.
19. Dahlen S, Hedqvist P, Hammerstrom S, Samuelsson B. Leukotrienes are potent constrictors of human bronchi. *Nature* 1980;288:484-6.
20. Gundel RH, Letts LG, Gleich GJ. Human eosinophil major basic protein induces airway constriction and airway hyperresponsiveness in primates. *J Clin Invest* 1991;87:1470-3.
21. Winqvist I, Olofsson T, Olsson I, Persson A-M, Hallberg J. Altered density, metabolism and surface receptors of eosinophils in eosinophilia. *Immunology* 1982;47:531-9.
22. Shaw RJ, Walsh GM, Cromwell J, Moqbel R, Spry CJF, Kay AB. Activated human eosinophils generate SRSA leukotrienes after physiological (IgG-dependent) stimulation. *Nature* 1985;316:120-2.
23. Capron M, Spiegelberg HL, Prin L, et al. Role of IgE receptors in effective function of human eosinophils. *J Immunol* 1984;132:462-8.
24. Baskar P, Pincus SH. Selective regulation of eosinophil degranulation by interleukin 1. *Proc Soc Exp Biol Med* 1992;199:249-54.
25. Wegner CD, Gundel RH, Reilly P, Haynes N, Letts LG, Rothlein R. Intercellular adhesion molecule-1 (ICAM-1) in the pathogenesis of asthma. *Science* 1990;247:456-9.
26. Wegner CD, Gundel RH, Churchill L, Souza DJ, Stearns CD, Letts LG. Mac-1 (CD11b/CD18) mediates antigen-induced eosinophil activation and airway hyperresponsiveness in monkeys [Abstract]. *Am Rev Respir Dis* 1992;145:A461.
27. Falus A, Meretey K. Histamine: an early messenger in inflammatory and immune reactions. *Immunol Today* 1992;13:154-6.