

In vitro responses to antigen stimulation: comparison between human lung parenchyma resected from asthmatic patients and non-asthmatic patients

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Background: The airway of asthmatic patients is hyperresponsive to various stimuli in vivo. There are, however, only a few reports that compared the in vivo responsiveness of asthmatic patients and non-asthmatic subjects to those of lung parenchyma in vitro.

Objectives: To compare the contractile response, release of various chemical mediators, and responsiveness to drugs in samples of lung parenchyma excised from asthma patients with those of non-asthmatic subjects.

Methods: Human lung parenchymal strips were subjected to passive sensitization with sera of 5+ RAST titer to mites. The strip was suspended in a magnus bath containing a buffer solution. Parenchymal contraction was induced by $\text{PGF}_2\alpha$. After washing, the baseline concentrations of thromboxane B_2 (TXB_2), leukotriene (LT), and histamine were measured in each bath and then contraction was induced by the addition of a mite antigen. The concentrations of TXB_2 , LT, and histamine were measured after contraction. The inhibitory effects of TXA_2 synthetase inhibitor (DP-1904) and TXA_2 receptor antagonist (AA-2414) were also evaluated in both tissue samples.

Results: There were no significant differences between lung parenchymal tissues of asthmatic and non-asthmatic patients with regard to $\text{PGF}_2\alpha$ -induced contraction, antigen-induced contraction, release of chemical mediators, and the response to drugs.

Conclusion: Unlike the response in vivo, there are no differences in the response to stimuli in vitro between lung parenchymal tissues of asthmatic and non-asthmatic patients.

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INTRODUCTION

Several studies have identified differences in airway responsiveness between asthmatics and normal subjects in vivo.^{1–4} Few studies, however, have

shown a lack of differences in vitro between in lung tissues of asthmatics and normal subjects.^{5,6} On the other hand, Rubinfeld and coworkers⁷ demonstrated in vitro differences in lungs of animals models of asthma. In the present study, we used tissue samples of human lung parenchyma to examine in vitro differences in contractility, release of chemical mediators, and responsiveness to certain drugs between asthmatic and non-asthmatic subjects.

MATERIAL AND METHODS

Tissue specimens were obtained from surgically removed lungs of five pa-

tients with lung cancer and bronchial asthma and five patients with lung cancer only. Each patient with bronchial asthma satisfied the definition of asthma by the NIH-NHL&BI Global Strategy for Asthma Management and Prevention.⁸ The clinical characteristics of the patients are shown in Table 1. All the patients were checked for airway responsiveness to methacholine by an astograph. An astograph is the method developed by Takishima et al⁹ where the respiratory resistance (R_{rs}) is measured during inhalation of methacholine. Airway hyperresponsiveness is evaluated as the minimum cumulative dose (D_{min}) of methacholine that induced an increase in R_{rs} . All patients with asthma had the airway hyperresponsiveness and all without asthma had no airway hyperresponsiveness. All the asthmatic patients had histories of dyspnea accompanied by wheezing, and had 15% or greater than 15% reversibility of FEV_1 with bronchodilator. The mean age of patients with asthma (68.0 ± 5.5 years) was not different from that of non-asthmatics (68.6 ± 5.0 years). The mean forced expiratory volume in one second/forced expiratory volume (FEV_1/FVC) ratio of asthmatics before surgery was $65.6 \pm 9.5\%$ while that of non-asthmatics was $69.8 \pm 3.7\%$. All patients were smokers, and all patients with asthma were non-atopic. Their asthma medication included 400 μg /day of inhaled steroid and inhaled β stimulants as needed. No patient had been treated with radiotherapy or anti-cancer therapy before the operation.

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Table 1. Characteristics of Patients

No.	Sex	Age (y)	FEV ₁ (%pred)	Smoking	Positive RAST	Asthma	Dmin* (Unit)
1	M	75	49.7	+	0	+	0.42
2	M	66	83.8	+	0	+	0.82
3	F	66	67.7	+	0	+	1.43
4	M	72	71.4	+	0	+	0.53
5	M	61	65.4	+	0	+	1.52
6	F	62	68.2	+	0	—	>50.0
7	M	66	76.3	+	0	—	>50.0
8	M	68	68.6	+	0	—	>50.0
9	M	74	68.5	+	0	—	>50.0
10	M	73	67.4	+	0	—	>50.0

* Dmin: the minimum cumulative dose of methacholine that induced an increase in respiratory resistance. One unit of Dmin equals 1 minute of inhalation of metacholine aerosol solution at 1.0 mg/mL.

The tissue samples were obtained within about two hours of the start of surgery. The study was approved by the Ethics Committee of Nagasaki University School of Medicine and informed consent was obtained.

Preparation of Samples of Human Lung Parenchyma

The method of Creese and Temple¹⁰ was used to prepare lung parenchymal specimens as described in our report.¹¹ Following removal of the lung tissue, parenchymal strips measuring approximately 4 x 4 x 20 mm and weighing 200 to 300 mg (dry weight) were cut from macroscopically normal lung sections. The strips were passively sensitized for 24 hours at 4°C with a 1:5 dilution of sera from patients with asthma with a 5+ RAST titer to mites. The tissue strip was suspended in a magnus bath containing 20 mL Krebs-Henseleit solution (in mM: 117 NaCl, 4 KCl, 25 NaHCO₃, 2.4 MgSO₄, 1.2 NaH₂PO₄, 2.5 CaCl₂ and 11 dextrose), bubbled with 5% CO₂ 95% O₂ and maintained at 37°C, pH 7.4. Muscle tension was measured by a pressure transducer (TB-601T, Nihon Koden Kogyo, Tokyo) and recorded on a polygraph recorder (Polygraph System, Nihon Koden Kogyo). The initial smooth muscle tension was 1.0 g in each lung parenchymal strip.

PGF₂ α -Induced Contraction

To confirm the contractility of each strip, contraction was initially induced by the addition of 10⁻⁴ M of PGF₂ α to each bath, and a contraction curve

(tension versus time) for each strip was recorded. We selected PGF₂ α rather than methacholine because the former could induce stronger contraction. After recording the maximum contraction induced by PGF₂ α , the strip was washed several times with Krebs-Henseleit solution. Antigen-induced contraction was expressed as a percentage of the maximum PGF₂ α -induced contraction (see below).

Mite Antigen-Induced Contraction and Inhibitory Effects of Drugs

Following determination of the maximum contraction induced by PGF₂ α and repeated washing, the tissue strip was allowed to recover to baseline tension. When complete recovery was established, the buffer volume was adjusted to 20 mL to which 10⁻⁵ M of DP-1904, a TXA₂ synthetase inhibitor (Dai-ichi Pharmaceuticals Inc., Tokyo, Japan) or 10⁻⁶ M of AA-2414, a TXA₂ receptor antagonist (Takeda Chemical Industries, Osaka, Japan) was added. Parenchymal contraction was induced 15 minutes later by adding 0.5 μ g/mL of mite antigen¹⁰ to the buffer. As a control, the strip was placed in the buffer solution alone. The drug concentration used in the present experiment was determined based on its effect in intact animals.^{12,13} Antigen-induced contraction reached a maximum level in 10 to 30 minutes. By examining the maximum contraction level, contractility and the responsiveness to each drug were compared between asthmatic and non-asthmatic samples of lung parenchyma.

TXB₂ Release and Inhibitory Effects of Drugs

We also measured the concentration of TXB₂ since TXA₂ is metabolized quickly to TXB₂. For this purpose, media were collected before and 30 minutes after the addition of the antigen. The level of TXB₂ was measured by radioimmunoassay, and the values were expressed in pg/100 mg tissue. The concentration of TXB₂ at 30 minutes after the addition of antigen in asthmatic lung parenchyma was compared with that of non-asthmatic lung parenchyma. We also compared the effect of DP-1904 and AA-2414 on the release of TXB₂ between lung parenchyma of asthmatics and non-asthmatics. Since the antigen-induced contraction generally reached a peak value within 10 to 20 minutes, or by 30 minutes at the latest, the amount of TXB₂ released in the test was examined during the initial 30-minute period.

Measurement of Leukotrienes

The concentration of LT within 30 minutes of addition of the mite antigen was compared in samples of asthmatic and non-asthmatic lung parenchyma. Leukotriene was regarded as a peptide LT, thus grouping LT-C₄, LT-D₄, and LT-E₄ together. Leukotriene was measured by radioimmunoassay, similar to the method used for the measurement of TXB₂. The values were expressed in pg/100 mg tissue.

Measurement of Histamine

The concentration of histamine within 30 minutes of addition of antigen was

compared in samples of asthmatic and non-asthmatic lung parenchyma. Histamine was measured by radioimmunoassay and the values were expressed in nMol/mg tissue.

Statistical Methods

Data were expressed as mean $\pm$ SD. Differences in PGF₂ α -induced contraction, mite antigen-induced contraction, release of TXB₂, LT, and histamine and the response to various drugs were compared in asthmatic and non-asthmatic lung parenchymal tissue using the paired *t* test. A *P* value $< .05$ was considered significant.

RESULTS

PGF₂ α -Induced Contraction

The maximum level of PGF₂ α -induced contraction in asthmatic lung parenchyma (2.4 ± 1.6 g/g tissue) was not significantly different from that measured in non-asthmatic lung parenchyma (2.3 ± 0.75 g/g tissue, Fig 1).

Mite Antigen-Induced Contraction and Inhibitory Effects of Drugs

The maximum mite antigen-induced contraction of asthmatic lung parenchyma ($49.4 \pm 7.7\%$ of the maximum PGF₂ α -induced contraction) was not different from that of non-asthmatic ($46.68 \pm 18.33\%$, Fig 2). Neither DP-1904 nor AA-2414 produced a signif-

icant change in peak contraction level recorded using asthmatic and non-asthmatic lung parenchymal tissues. When the peak control level was considered as 100%, the inhibition rates of DP-1904 and AA-2414 were about 7% and 20%, respectively.

TXB₂ Release and Inhibitory Effects of Drugs

There was no significant difference between asthmatic and non-asthmatic lung parenchymal tissue samples with regard to the concentration of TXB₂ measured within 30 minutes of adding the antigen (Fig 3A). More than 90% of the released TXB₂ was inhibited by DP-1904. The inhibition rate of AA-2414 was about 25%. The effects of these drugs on tissue samples from asthmatic and non-asthmatic were not different.

Measurement of Leukotrienes

The concentrations of LT measured within 30 minutes of the addition of mite antigen were not significantly different between asthmatic and non-asthmatic lung parenchymal tissues (Fig 3B).

Measurement of Histamine

Finally, the concentrations of histamine measured at 30 minutes after the addition of antigen were not significantly different between asthmatic and

non-asthmatic lung parenchyma (Fig 3C).

DISCUSSION

Passively sensitized resected lung tissues of animals are often used when we do experiments to examine bronchial asthma in vitro. In most cases, when human lung tissue is used, samples are obtained from non-asthmatic patients. Are there any differences in in vitro responsiveness which are otherwise present in vivo, between lungs with bronchial asthma and those without asthma? With regard to human asthmatics in vivo, there are many reports that have confirmed the presence of airway hyperresponsiveness to histamine,¹ methacholine,² prostaglandin,³ and leukotriene E₄.⁴ On the other hand there are only a few in vitro studies that used lung tissue samples from asthmatics, and most were published in the 1980s. Vincenc et al⁵ compared lung tissue samples resected from 5 patients who had airway hyperresponsiveness to inhalation of histamine ($PD_{20} < 4.0$) and those resected from nine patients who had no airway hyperresponsiveness. They reported a lack of difference in the response to histamine between the two lung tissues in vitro. Similarly, Armour et al⁶ reported a lack of correlation between the provocative concentration of histamine resulting in a 20% fall in FEV₁ (PC_{20}) in vivo and the histamine-induced contraction of bronchi in vitro. Based on these results, both reports concluded that the mechanism of airway hyperresponsiveness in vivo may not include the characteristic features of airway smooth muscles. In both reports mentioned above, the patients did not have clinical symptoms of asthma although they had airway hyperresponsiveness to histamine.

In the present study, we targeted patients who were clinically diagnosed with bronchial asthma. They had also checked their airway hyperresponsiveness to methacholine. First of all we evaluated the PGF₂ α -induced contraction, but the results showed no significant differences in the contraction in vitro between lung parenchymal tissue

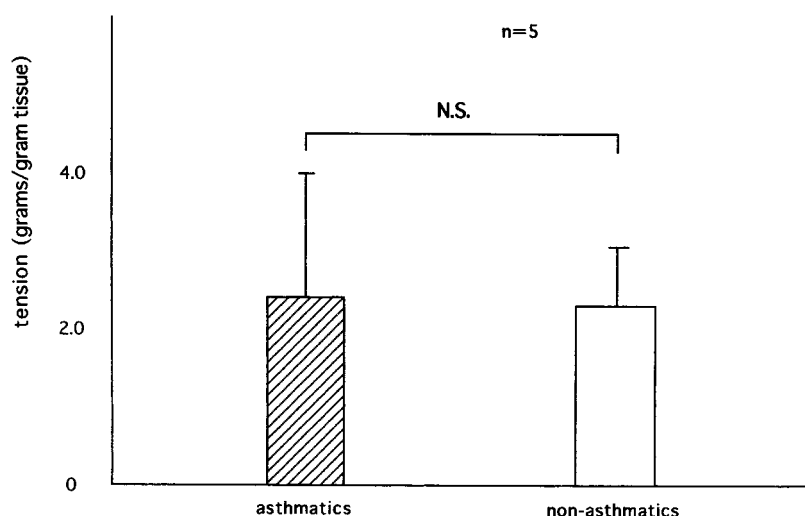


Figure 1. The maximum contraction of asthmatic lung parenchyma and non-asthmatic parenchyma induced by PGF₂ α . Data represent the mean $\pm$ SD of five specimens in each group.

of asthmatics and non-asthmatics. This result is in agreement with the reports of Vincenc et al⁵ and Armour et al.⁶ The latter group, however, used the bronchus in their experiments, while the former group used both parenchymal and bronchial tissues. Interestingly, in that study no difference between these two components of the lung were detected with regard to maximal contraction but the values of EC₅₀ (50% of the maximal response) were significantly different with the bronchus being 28 times more sensitive than the parenchyma.⁵ They also reported the presence of a wide variability in the response of the parenchymal strips. It is possible that these differences are due to the presence of several cell types and contractile components (eg, vascular smooth muscles) in the parenchyma. For example, the presence of LT results in contraction of both airway and vascular smooth muscles.¹⁴ Vascular smooth muscle in turn influences the contraction of parenchymal tissue, thus explaining the difference between the contraction of central airways and that of parenchymal tissue. In this regard, previous studies have described differences between the central airways and peripheral tissues in their responsiveness to cholinergic agonists,¹⁵ and LTC₄, D₄,^{16,17} or the existence of mast cells.¹⁸⁻²¹ Further experiments are necessary to compare bronchial and parenchymal tissue contractile characteristics using lung tissues of asthmatic patients. Anyway, concerning human lung parenchymal tissue, our result showed that there were no significant differences in the PGF₂α-induced contraction in vitro between asthmatics and non-asthmatics.

We also evaluated the contractile response to the mite antigen. There was no significant difference between asthmatic lung parenchyma and non-asthmatic lung parenchyma. Rubinfeld et al⁷ used dogs to evaluate the antigen-induced contraction. They prepared canine trachealis muscles that were resected from two groups of dogs. In the first group, the animals were exposed to *Ascaris* aerosol antigen which resulted in increased pulmonary resis-

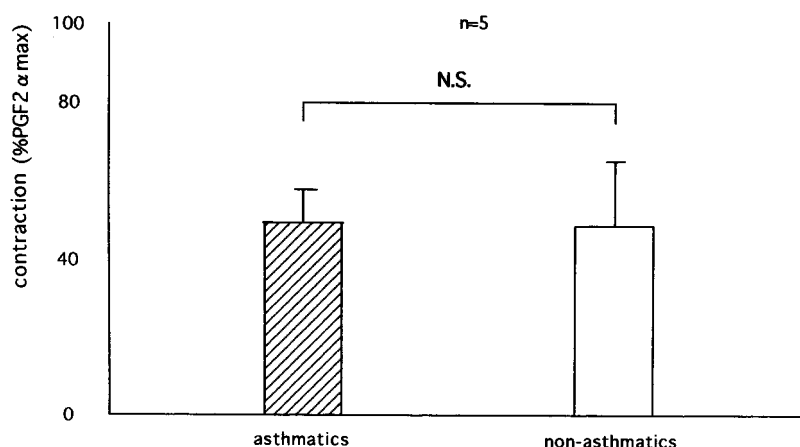


Figure 2. The maximum contraction of asthmatic and non-asthmatic parenchymal tissues induced by mite antigen. Data represent the mean $\pm$ SD of five specimens in each group.

tance. The second group consisted of non-reactive dogs. They studied the contraction of these muscles in vitro following the addition of *Ascaris* antigen. Their results showed that while the contraction of tissues from *Ascaris*-reactive dogs were higher than those of non-reactive ones, the differences was not statistically significant. When the response to low concentrations of methacholine was examined, trachealis

muscles from reactive dogs contracted more strongly than those of non-reactive dogs. It is possible that tissues from humans may react in a manner different from that of other animals. It should be noted that our asthmatic patients were all non-atopic and tissues of all subjects were passively sensitized in vitro, while the dogs used by Rubinfeld and coworkers⁷ were naturally sensitized to *Ascaris*. A careful

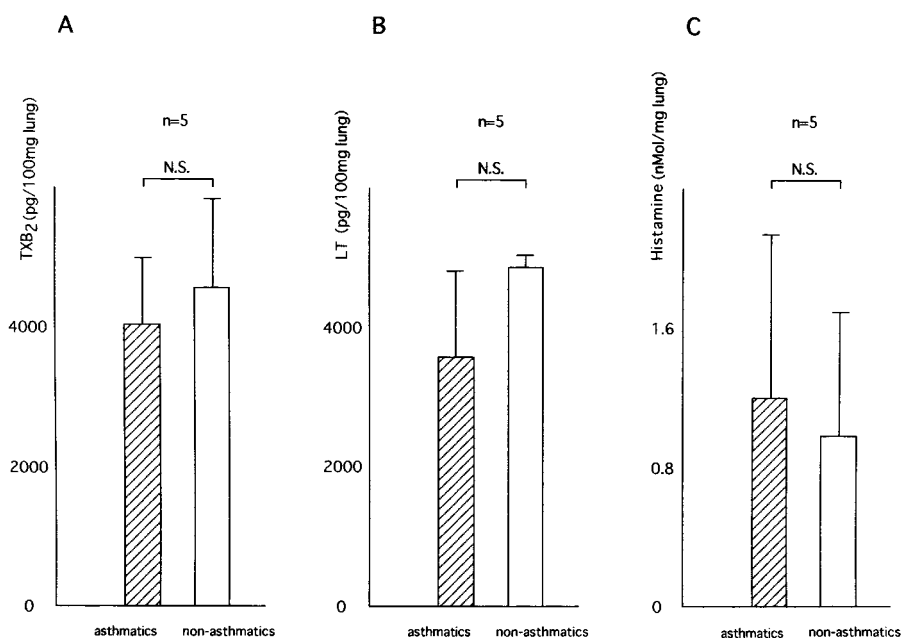


Figure 3. Release of TXB₂ (A), LT (B), and histamine (C) from asthmatic and non-asthmatic parenchymal tissues after mite antigen stimulation. Data represent the mean $\pm$ SD released during 30 minutes in five different specimens. n = 5 in A, B, and C.

search of the literature showed no studies that have previously compared mite antigen-induced changes in lung tissues of asthmatics and non-asthmatics in vitro. Interestingly, four of the patients studied by Vincenc et al⁵ who were not responsive to histamine were atopic, but other patients showed no differences in responsiveness to histamine. Unfortunately, the response to mite antigen was not examined in that study.

We also measured the concentrations of TXB₂, LT and histamine released from the lung parenchyma following antigen stimulation. Our results showed no differences between lung parenchyma of asthmatics and non-asthmatics. Previous studies have identified two types of mast cells, connective tissue mast cells (CTMC) and mucosal mast cells (MMC). The histamine content of CTMC is 12-times higher than that of MMC.²² Based on our results, it is possible that the number and ability to store, produce and release chemical mediators by mast cells of the lung parenchyma of asthmatic patients are similar to those of non-asthmatic patients.

Finally, we evaluated the response to drugs. Two types of agents were used in the present study: TXA₂ synthetase inhibitor and TXA₂ receptor antagonist, which react to the enzyme and receptor, respectively. As we previously reported, LT inhibitor had the significant inhibitory effect to the contraction of the lung resected from non-asthmatic patient using the same experiment system as this time. Then we could expect the same result on the lung from asthmatic patients. Actually we did only one preliminary experiment with LT inhibitor and could see the inhibitory effect of the contraction. To the contrary, the inhibitory effect of TXA₂ inhibitor and antagonist on the contraction of the lung from non-asthmatic patients were not significant even though these drugs had already been used for the therapy of bronchial asthma in Japan. Then we had the interest whether the effect of these drugs was different between the lungs from asthmatic patients and non-asthmatic

patients. We selected TXA₂ inhibitor and antagonist. Our results using these agents suggest a lack of specific features of these enzymes and receptors in asthmatic lungs relative to non-asthmatic subjects.

In conclusion, our results showed that unlike the reaction in vivo, no differences in PGF₂α-induced contraction, antigen-induced contraction, release of chemical mediators, and responsiveness to drugs were noted in vitro in lungs obtained from asthmatic patients and non-asthmatic patients. Before starting these experiments, we predicted that the responses of the parenchyma resected from asthmatics would be greater than that of non-asthmatics in vitro. Our results suggest that there are no differences in ability of contraction of smooth muscle or the amount of the chemical mediators that are released by stimuli. We think both asthmatics and non-asthmatics showed a response which was not consistent with airway hyperresponsiveness, in which the in vitro response did not reflect the in vivo response. Our experimental design did not allow examination of differences due to central airways, nervous system, and peripheral blood cells (eg, lymphocytes, eosinophils) and other factors. It is possible that one or more of these components may cause airway hyperresponsiveness.

REFERENCES

- Cockcroft DW, Killian DN, Mellon JJA, Hargreave FE. Bronchial reactivity to inhaled histamine: a method and clinical survey. *Clin Allergy* 1977;7:235-243.
- Juniper EF, Frith PA, Hargreave FE. Airway responsiveness to histamine and methacholine: relationship to minimum treatment to control symptoms of asthma. *Thorax* 1981;36:575-579.
- Hardy CC, Robinson C, Tattersfield AE, Holgate ST. The bronchoconstrictor effect of inhaled prostaglandin D₂ in normal and asthmatic men. *N Engl J Med* 1984;311:209-213.
- O'Hickey SP, Arm JP, Rees PJ, et al. The relative responsiveness to inhaled leukotriene E₄, methacholine and histamine in normal and asthmatic subjects. *Eur Respir J* 1988;1:913-917.
- Vincenc SK, Black LJ, Yan K, et al. Comparison of in vivo and in vitro responses to histamine in human airways. *Am Rev Respir Dis* 1983;128:875-879.
- Armour LC, Lazar MN, Schellenberg RR, et al. A comparison of in vivo and in vitro human airway reactivity to histamine. *Am Rev Respir Dis* 1984;129:907-910.
- Rubinfeld RA, Rinard AG, Mayer ES. Responsiveness of isolated tracheal smooth muscle in a canine model of asthma. *Lung* 1982;160:99-107.
- Global strategy for asthma management and prevention. NHLBI/WHO work shop report. 1995;1-8.
- Takishima T, Hida W, Sasaki H, et al. Direct-writing recorder of the dose-response curves of the airway to methacholine. *Chest* 1981;80:600-606.
- Creese BR, Temple DM. The mediators of allergic contraction of human airway smooth muscle: a comparison of bronchial and lung parenchymal strip preparations. *Clin Exp Pharmacol Physiol* 1986;13:103-119.
- Fukushima C, Shimoda T, Matsuse H, et al. Inhibitory effect of thromboxane A₂ synthetase inhibitor, DP-1904, on antigen-induced contraction of human lung parenchyma. *Ann Allergy* 1996;77:483-487.
- Takami M, Matsumoto K, Takata Y, et al. Possible role of thromboxane A₂ in hyperresponsiveness of isolated rat lung tissue in a Sephadex-induced eosinophilia model. *Int Arch Allergy Immunol* 1995;106:401-409.
- Ashida Y, Matsumoto T, Kuriki H, et al. A novel anti-asthmatic quinone derivative, AA-2414 with a potent antagonistic activity against a variety of spasmogenic prostanoids. *Prostaglandins* 1989;38:91-112.
- Hanna CJ, Bach KM, Pare DP, Schellenberg. Slow-reacting substances (LT) contract human airway and pulmonary vascular smooth muscle in vitro. *Nature* 1981;290:343-344.
- Vornanen M, Tirfi R. Cholinergic responses in different section of rat airways. *Acta Physiol Scand* 1981;113:177-182.
- Szarek JL, Evans NJ. Pharmacologic responsiveness of rat parenchymal strips, bronchi, and bronchioles. *Exp Lung Res* 1988;14:575-585.
- Drazen JM, Austen FK, Lewis AR, et al. Comparative airway and vascular

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- activities of leukotrienes C-1 and D in vivo and in vitro. *Proc Natl Acad Sci USA* 1980;77:4354–4358.
18. Kaliner M. Asthma and mast cell activation. *J Allergy Clin Immunol* 1989; 83:510–520.
19. Holgate ST, Benyon CR, Howarth HP, et al. Relationship between mediator release from human lung mast cells in vitro and in vivo. *Int Arch Allergy Appl Immunol* 1985;77:47–56.
20. Du T, Spapienza S, Eidelmam HD, et al. Morphometry of the airways during late responses to antigen challenge in the rat. *Am Rev Respir Dis* 1991;143: 132–137.
21. Bachelet CM, Bernandin FJ, Fleury-Feith J. Distribution and histochemical characterization of pulmonary mast cells in the rat and guinea pig. *Int Arch Allergy Appl Immunol* 1988;87: 225–229.
22. Katz RH, Kaye ER, Austen FK. Mast cell biochemical and functional heterogeneity. *Transplant Proc* 1991;23: 2900–2904.
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