BENEFITS OF INDUCED SPUTUM FOR THE EVALUATION OF THERAPEUTIC EFFICACY IN PATIENTS WITH BRONCHIAL ASTHMA

Ohnishi, Hisashi
Maeda, Hitoshi
Nishimura, Yoshihiro
Yokoyama, Mitsuhiro

(Citation)
The Kobe journal of the medical sciences,44(4):149-161

(Issue Date)
1998-08

(Resource Type)
departmental bulletin paper

(Version)
Version of Record

(URL)
https://hdl.handle.net/20.500.14094/E0001008

Kobe J. Med. Sci. 44, 149/161, August 1998

BENEFITS OF INDUCED SPUTUM FOR THE EVALUATION OF THERAPEUTIC EFFICACY IN PATIENTS WITH BRONCHIAL ASTHMA

Hisashi OHNISHI, Hitoshi MAEDA, Yoshihiro NISHIMURA, and Mitsuhiro YOKOYAMA

First Division, Department of Internal Medicine
Kobe University School of Medicine

INDEXING WORDS

hypertonic saline; beclomethason dipropionate (BDP); bronchial hyperresponsiveness; eosinophil

SYNOPSIS

Airway inflammation is a major factor in the pathogenesis of asthma. Inducing sputum by hypertonic saline is a noninvasive method of assessment of the airway inflammation in asthmatic patients. To investigate sputum induction as a method for assessing airway inflammation and to evaluate the effect of inhaled beclomethasone dipropionate (BDP) in asthmatic patients, we examined the bronchial hyperresponsibility (BHR), pulmonary function and differential cell counts in induced sputum of the patients before and after BDP therapy. In asthmatic patients, the percentage of eosinophils in induced sputum was significantly higher than that in non-asthmatic subjects. Ten patients with atopic asthma (four men and six women; mean [± SD] age, 30.5 ± 12.4 years) participated. Their mean percentage of eosinophils (%eosinophils) in induced sputum fell from 22.9 ± 7.2 % to 13.9 ± 8.3 % ($p<0.05$) by 3 months of BDP treatment. The percentage of eosinophils in induced sputum before BDP treatment was significantly correlated with the ratio of the forced expiratory

Received for publication: April 19, 1998

Authors' names in Japanese: 大西 尚、前田 均、西村善博
横山光宏

volume in one second to the forced vital capacity (FEV1%) at baseline ($r = -0.75$, $p < 0.05$), but not with log Dmin at baseline ($p = 0.18$). The change in FEV1% between at baseline and post-treatment correlated significantly with the change in the sputum eosinophil percentage ($r = -0.79$, $p < 0.01$). In addition, there was a significant correlation between the change of log Dmin and the change of the sputum eosinophil percentage ($r = -0.64$, $p < 0.05$).

In conclusion, analysis of induced sputum is a safe, noninvasive, repeatable and useful method to assess the clinical condition of bronchial inflammation in patients with bronchial asthma.

Introduction

Airway inflammation is a major factor in the pathogenesis of asthma. Although bronchial biopsy and bronchial lavage studies have contributed to elucidation of eosinophilic airway inflammation,[2] the assessment of bronchial inflammation in patients with asthma by bronchoscopy is potentially hazardous. The existence of eosinophils in the sputum of asthmatic patients has been known for a long time.[3,18] Though during exacerbations asthmatic patients often expectorate sputum, they have little phlegm during stable periods. Sputum induction by inhaled hypertonic saline was recently introduced as a noninvasive method for the assessment of airway inflammation in asthmatic patients.[4,7,9,14,15] We confirmed in a preliminary study that the method of sputum induction by inhaled hypertonic saline was safe. In the present study, to further investigate sputum induction as a method for assessing the airway inflammation and to assess the effect of inhaled beclomethasone dipropionate (BDP) in asthmatic patients, we examined the bronchial hyperresponsiveness (BHR), pulmonary function and the percentage of eosinophils in induced sputum of patients before and after BDP therapy.

Materials and Methods

Subjects

Ten subjects with mild to moderate asthma (four men and six women; mean [± SD] age, 30.5 ± 12.4 years)(Nos.1-5; mild asthma, Nos.6-10; moderate asthma) and eight normal healthy subjects (five

men and three women; mean [± SD] age, 26.5 ± 2.3 years) participated in this study. No healthy subjects had chest symptoms and history of atopic diseases. All asthmatics had specific IgE antibody to more than one positive antigen judged by radioallergosorbent test. None had had spontaneous expectorated sputum or respiratory tract infection for at least four weeks. Two subjects were current smokers, others were non-smokers. Patients were excluded from the study if they had taken systemic corticosteroids in any form during the previous two months. On study days, all patients withheld their usual medications for 24 hours except for inhaled procaterol as needed.

Informed consent was obtained from each patient for our protocol.

Study Design

Analysis of the percentage of eosinophils in complete blood and IgE, spirometry, and a methacholine inhalation test were performed before starting the BDP therapy. Sputum induction followed the methacholine provocation test. Eight patients had not taken medication of inhaled corticosteroid, and the other two patients (Nos.1 and 10) had used inhaled corticosteroid of 400 μg/day for 10 weeks before this study. Beginning on the day after their first visit, all patients started inhaled corticosteroid (BDP; 400-800 μg/day) medication in proportion to the severity of their disease state. At their second visit, 3 months later, the same examinations were repeated for all patients. The number of inhalations of procaterol taken as required had been recorded for study period. No patient dropped out of the study during inhalation therapy.

Induction of sputum

Induction of sputum was performed by the method of Pin et al.[14] After the methacholine inhalation test, each subject inhaled salbutamol (2.5mg/ml, 2 min). After the respiratory resistance (Rrs) recovered to the baseline value, the subjects inhaled 3% hypertonic saline using an ultrasonic nebulizer for about 10 min, and then coughed up and expectorated sputum into a container. In our preliminary study, breathing deeply and rinsing the mouth were necessary to gather adequate sputum. After the sputum was

expectorated or if troublesome symptoms occurred, the inhalation of hypertonic saline was stopped.

Sputum processing

The sputum was transferred to a Petri dish, and the macroscopic characteristics of the sputum were assessed. After an equal volume of 2% N-acetylcysteine (NAC) was added to the induced sputum sample, it was mixed gently by vortex mixer. The homogenized sputum was centrifuged at 1500 rpm for 10 min. The cell pellets were mounted on slides and fixed by dry air. The smears were stained with Diff-Quik (International Reagents Co., Kobe, Japan). Differential cell counts were performed by two investigators who did not know the clinical details of the subjects. The investigators counted at least 500 nucleated cells, and the percentages of each cell count of the two investigators were averaged. When the cell counts varied widely between the investigators, we discarded the samples as inadequate and inhomogenated sputum.

Methacholine inhalation test

The methacholine inhalation test was performed by the method of Takishima et al. with an Astograph (TCK-6000M, Chest Co., Tokyo, Japan),[16] which can write dose-response curves of respiratory resistance (Rrs) with tidal breathing during continuous inhalation. The methacholine was administered in twofold incremental concentrations from 0.049 to 25 mg/ml every minute. Dmin, the minimum cumulative dose required to start to decrease respiratory conductance from the baseline, was evaluated as an indicator for the bronchial sensitivity. Bronchodilators, xanthine derivatives, and inhaled corticosteroid were withheld from the subjects for 24 hours before this test.

Statistical analysis

Data are presented as mean values ± SD. The analysis of Dmin values was performed on logarithmically transformed data, because it showed logarithmic normal distribution. The evaluation of differences in FEV1%, Dmin and %eosinophils in induced sputum between before and after BDP treatment was performed by Student's t-test for paired data. Correlations were examined by least square linear regression analysis. A p value less than 0.05 was considered to be significant.

Table 1. Characteristics of study subjects.

No.	Age (y.o)	Sex	Smoking	FEV1% (%)	Eosinophil # (/μl)	Allergen
1	50	M	(-)	82.8	3954	M, HD
2	29	M	(-)	70	731	M, HD
3	26	M	(-)	53.2	490	M, HD
4	28	F	(-)	77.9	281	M, HD
5	23	F	(-)	78.7	1015	M, HD
6	29	F	(-)	86.6	775	M, HD
7	56	F	(-)	81.2	519	M, HD
8	24	F	(+)	66.5	249	M, HD
9	19	F	(-)	83.7	504	M, HD
10	21	M	(+)	80.2	608	M, HD
mean±SD	30.5±12.4	M/F:4/6		76.1±10.1	912±1092	

FEV1% : the ratio of the forced expiratory volume in one second to the forced vital capacity

cell counts of eosinophils in peripheral blood

M : mite, HD : house dust

Table 2. Changes of PEF and doses for period of treatment with BDP.

No.	Period (Weeks)	Min. PEF (L/min)	Max. PEF (L/min)	BDP (μg)	Other drugs
1	12	240	560	400	
2	17	580	800	400	
3	18	400	600	400	
4	16	250	450	400	
5	12	360	480	400	
6	24	320	430	400	
7	34	310	430	400	T, A
8	42	300	500	400→800	T
9	35	280	400	400	
10	22	450	700	400→800	T, β_2
mean±SD	23.2±10.4				

period from baseline examination to examination after BDP treatment

T : theophylline, A : azelastin, BDP : beclomethasone dipropionate

β2 : oral administration of β2-adrenergic stimulant

Min. PEF : minimum peak expiratory flow for study period

Max. PEF : maximum peak expiratory flow for study period

Table 3. Changes of procaterol use, eosinophils, FEV1%, and Dmin after treatment with Beclomethasone Dipropionate.

No.	Procaterol use (puffs/week)		Eosinophil (%) *		FEV1% (%)		Dmin (Units)	
	before	after	before	after	before	after	before	after
1	8	0	21	3.8	82.8	89.3	0.473	14.952
2	28	1	24.2	5.7	70	94.5	0.447	2.036
3	28	0	34	14.4	53.2	73.1	0.16	0.252
4	48	2	28.8	9.1	77.9	89.7	0.088	0.186
5	7	0	23.3	11.2	78.7	81.3	0.786	1.723
6	28	1	6.9	4.6	86.6	79	1.463	0.786
7	28	2	20.1	21.6	81.2	83.7	0.046	0.039
8	7	2	25.8	26.2	66.5	61.7	0.317	0.252
9	21	3	18.8	18.9	83.7	86.3	9.119	11.619
10	14	2	26.5	23.8	80.2	83.8	1.411	2.244
mean±SD	21.7±13.0	1.3±1.1	22.9±7.2	13.9±8.3	76.1±10.1	82.2±9.4	1.43±2.75	3.41±5.33

* differential cell counts of eosinophil in induced sputum

FEV1% : the ratio of the forced expiratory volume in one second to the forced vital capacity

Dmin : minimum dose of methacholine for bronchial hyperreactivity

Result

Clinical characteristics of subjects

The clinical characteristics of the subjects are shown in Table I. Of all subjects, two showed low values of FEV1%, but examinations of spirometry, methacholine inhalation test and sputum induction by hypertonic saline were safely done. All 10 subjects were atopic (positive reaction to mites and house dust). Two subjects (Nos. 8 and 10) were smokers. Inhalation doses of BDP were 400 μg/day in eight patients, but for the other two subjects, the dose was increased from 400 μg to 800 μg/day because their symptoms were not improving enough (Table II). Nos. 8 patient had had inhaled corticosteroid of 400 μg/day for 38 weeks and 800 μg/day for 4 weeks, Nos. 10 patient had inhaled corticosteroid of 400 μg/day for 16 weeks and 800 μg/day for 6 weeks.

Safety and success of the method of induced sputum

All patients had a mild cough and a feeling of salty taste after the induced sputum procedure, but their symptoms were self-controllable. Spirometry was measured both pre- and post-inhalation of the hypertonic saline, and no decline in FEV1% was observed, perhaps because the patients were clinically in the relatively stable condition of asthma and used inhaled salbutamol before the inhalation of hypertonic saline. The induction of sputum was successfully performed in all patients. The instructions to inhale the hypertonic saline deeply and to cough up the sputum were helpful to the subjects. Sputum from asthmatics contained a significantly greater percentage of eosinophils compared with those from normal subjects (22.9 ± 7.2 % vs. 1.43 ± 0.65 %, $p< 0.05$, respectively)(Fig.1).

Effect of inhaled BDP

By the end of the BDP treatment period, the all subjects' symptoms had notably improved. The number of procaterol use had remarkably reduced at all subjects after BDP treatment (21.7 ± 13.0 puffs/week vs. 1.3 ± 1.1 puffs/week, $p< 0.001$) (Table III). Their peak expiratory flow (PEF) were more than 80% of their personal best values. After the treatment, the FEV1% and Dmin values were significantly improved (76.1 ± 10.1 % vs. 82.2 ± 9.4 %, $p< 0.05$; 1.43 ± 2.75 log U vs. 3.41 ± 5.33 log U, $p< 0.05$, respectively)

(Table III). The percentage of eosinophils in the induced sputum was significantly decreased after BDP treatment (22.9 ± 7.2 % vs. 13.9 ± 8.3 %, p< 0.05, respectively) (Table III).

Correlation between cell count in induced sputum and physiological examination

The percentage of eosinophils in induced sputum at baseline correlated significantly with FEV1% at baseline (r = -0.75, p < 0.05) (Fig.2A), but not with log Dmin at baseline (p = 0.18) (Fig.2B). The change of FEV1% from before to after treatment correlated significantly with the change of %eosinophils in the induced sputum between before and after treatment (r = -0.79, p < 0.01) (Fig.3A). In addition, there was a significant correlation between the change of log Dmin and the change of the percentage of eosinophils in induced sputum (r = -0.64, p < 0.05) (Fig.3B).

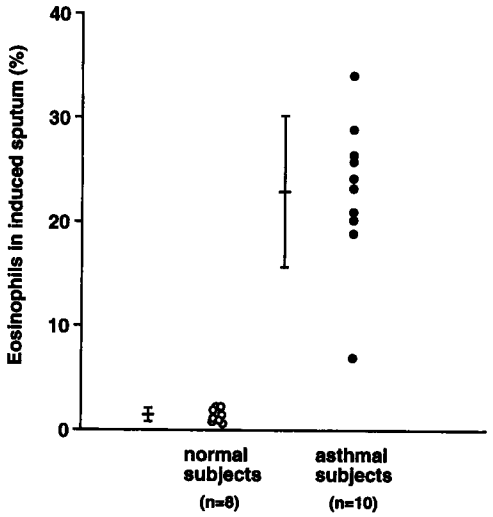

Figure 1. Individual data of the percentage of eosinophils in induced sputum from normal subjects and asthmatics. Open circles represent normal subjects, closed circles patients with asthma.

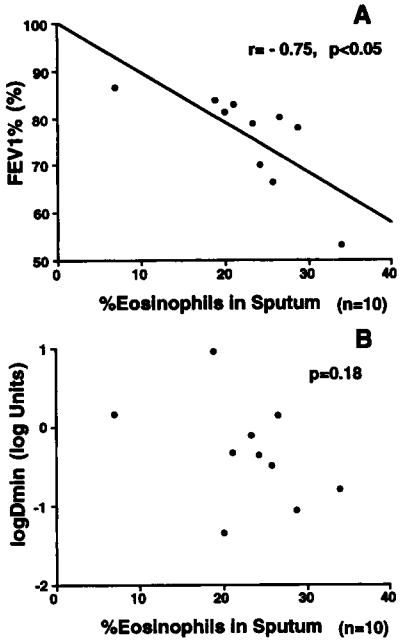

Figure 2. Relationship between the ratio of eosinophils in induced sputum and the parameters of a pulmonary function test. There was a significant correlation between the percentage of eosinophils in induced sputum and FEV1% (A), but no significant correlation between the percentage of eosinophils in induced sputum and log Dmin (B). FEV1%, the percentage of forced expiratory volume at one second; Dmin, minimum dose of methacholine for bronchial hyperreactivity.

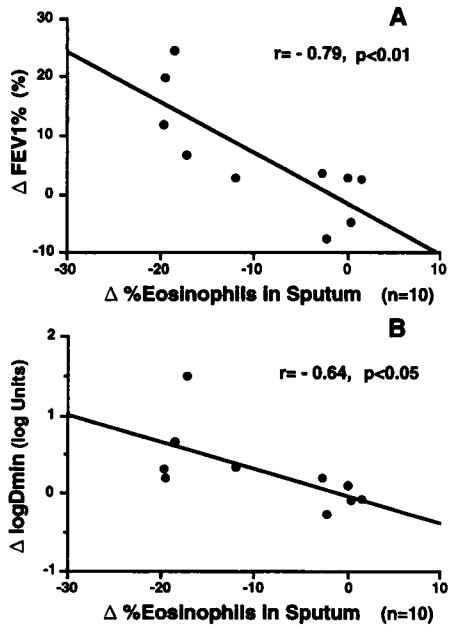

Figure 3. Relationship between the change of eosinophils in induced sputum and the change of parameters in the pulmonary function test before and after beclomethasone dopropionate treatment. (A) A significant correlation between the change in eosinophils in induced sputum and the change in FEV1% was shown. (B) A moderate correlation was shown between the change in eosinophils in induced sputum and the change in log Dmin. FEV1%, the percentage of forced expiratory volume at one second; Dmin, the minimum cumulative dose required to start to decrease respiratory conductance from the baseline.

Discussion

In the present study, to evaluate the clinical and cytological improvement in the condition of patients with bronchial asthma, we examined the results of a pulmonary function test, bronchial hyperresponsiveness, and the percentage of eosinophils in induced sputum before and after BDP treatment in asthmatic subjects. Sputum induction was safely and successfully performed in these asthmatic subjects. In our preliminary study, however, only one of eighty asthmatics, who showed low FEV1% on the examination day, developed a mild bronchoconstriction during the inhalation of hypertonic saline in spite of inhalation of salbutamol as premedication, and she showed the decrease of PEF after about six hours. Though the safety of the inhalation of hypertonic saline has been reported,[14,15] the late asthmatic response should be monitored, especially when the patient has a low FEV1% and an attack during the inhalation. The success rate of gathering the sputum after the inhalation of hypertonic saline was 79.2%[14] and 96%[9] in earlier studies. In the present study, all patients expectorated adequate sputum following inhalation of 3% saline, but we emphasize that to achieve an adequate quality and quantity of sputum, the patients made efforts to deeply inhale the hypertonic saline. In our preliminary study, we sometimes could not gather adequate samples without deep inhalation by the subjects.

Inhaled corticosteroid therapy has been established as one of the most effective treatments for chronic asthma. Inhaled corticosteroids have also been shown to reduce the number and activation of inflammatory cells in the airway in biopsy studies.[5,10,13] Laitinen et al. reported that there was a reduction in the numbers of eosinophils, macrophages, mast cells, and lymphocytes after 3 months of inhaled corticosteroid therapy.[13] Similar results have been reported in bronchoalveolar lavage of asthmatic patients, with a reduction in eosinophil number after the treatment of inhaled budesonide.[1] The eosinophil percentage in induced sputum correlated significantly with those in bronchoalveolar lavage[7], so sputum induction is a non-invasive, useful method to evaluate the change of the bronchial eosinophilic inflammation with the treatment of BDP.

Continuous treatment with inhaled steroids reduces airway

responsiveness to inhaled histamine and methacholine in asthmatic patients. Several studies have shown an improvement of FEV1% and a reduction in bronchial hyperresponsiveness after steroid inhalation for 2 or 3 months.[6,11,21] In our present study, to elucidate the effect of BDP on FEV1% and hyperresponsiveness, the subjects inhaled BDP at least for 3 months. Though their symptoms was improved and the number of procaterol use was respectively decreased, the percentage of eosinophils in the induced sputum, FEV1% and Dmin valeus were not changed after BDP treatment at some patients (Nos. 6-10). Nos. 6-10 subjects were moderate asthmatic patients and there may heva been a difference of severity of disease between Nos.1-5 subjects and Nos.6-10 subjects. We suspected that the possible reasons why there were no remarkable changes of the percentage of eosinophils in induced sputum, FEV1% and Dmin in Nos.6-10 subjects were that the subjects probably had undertreatment of BDP, shorter periods of BDP treatment. The limitation of this study was the difficulty to assessment for the seasonal variation of airway inflammation in atopic asthmal patients. Although all subjects participated in November and December in our study, periods of BDP treatment were not fixed because of the variation of their symptom's improvement.

The changes in the percentage of eosinophils in the bronchoalveolar lavage were also found to be significantly correlated with bronchial hyperresponsiveness, in addition to FEV1%.[8,12,17,19,20] Moreover, in induced sputum studies, the percentage of eosinophils showed a significant correlation both FEV1% and PC20-histamine.[4,14] However, Iredale et al. reported that they found no correlation between PD20-hypertonic saline (HS) and %eosinophils on differential cell counts in induced sputum samples.[9] It is still controversial as to whether the relationship between the percentage of eosinophils in induced sputum samples and bronchial hyperresponsiveness is significant. In our study, there was a significant correlation between the percentage of eosinophils in induced sputum and FEV1%, but we found no correlation between the percentage of eosinophils and Dmin. We showed that the change of %eosinophils in the differential cell counts of induced sputum between before and after treatment had a significant correlation with the changes of FEV1% and Dmin. We suspect that the %eosinophils in differential cell counts of induced sputum samples does not always reflect the severity of bronchial

hyperresponsiveness. However, we suggest that this parameter (%eosinophils) is useful to repeatably evaluate the clinical condition of asthmatic patients during treatment.

In conclusion, the results of this study showed that there was no correlation between the percentage of eosinophils in induced sputum and Dmin, but that the change in %eosinophils in differential cell counts in induced sputum before and after treatment had significant correlations with the changes in FEV1% and Dmin before and after treatment. It is very important to examine the efficacy of therapy over a long term in asthmatic treatment. The gathering of induced sputum is safe, noninvasive and repeatable, and the analysis of differential cell counts is very useful to evaluate the clinical condition of bronchial inflammation in asthmatic patients who are treated with anti-inflammatory medications.

References

1. Ädelroth, E., Rosenhall, L., Johansson, S-A., Linden, M., and Venge, P. : Am. Rev. Respir. Dis. 1990. 142. 91/99. Inflammatory cells and eosinophilic activity in asthmatics investigated by bronchoalveolar lavage.
2. Bradley, B.L., Azzawi, M., Jacobson, M., Assoufi, B., Collins, J.V., Irani, A.M., Schwartz, L.B., Durham, S.R., Jeffery, P.K., and Kay, A.B. : J. Allergy Clin. Immunol. 1991. 88. 661/674. Eosinophils, T-lymphocytes, mast cells, neutrophils, and macrophages in bronchial biopsy specimens from atopic subjects with asthma: Comparison with biopsy specimens from atopic subjects without asthma and normal control subjects and relationship to bronchial hyperresponsiveness.
3. Brown, HM. : Lancet. 1958. ii. 245/247. Treatment of chronic asthma with predonisolone. Significance of eosinophilia in the sputum.
4. Claman, D.M., Boushey, H.A., Liu, J., Wong, H., and Fahy, J.V. : J. Allergy Clin. Immunol. 1994. 94. 861/869. Analysis of induced sputum to examine the effects of predonisone on airway inflammation in asthmatic subjects.
5. Djukanovic, R., Wilson, J.W., Britten, Y.M., Wison, S.J., Walls, A.F., Roche, W.R., Howarth, P.H., and Holgate, S.T. : Am. Rev.

Respir. Dis. 1992. 145. 669/674. Effect of an inhaled corticosteroid on airway inflammation and symptoms of asthma.

6. Dutoit, J.I., Salome, C.M., and Woolcock, A.J. : Am. Rev. Respir. Dis. 1987. 136. 1174/1178. Inhaled corticosteroids reduced the severity of bronchial hyperresponsiveness in asthma, but oral theophylline does not.
7. Fahy, J.V., Wong, H., Liu, J., and Boushey, H.A. : Am. Rev. Respir. Dis. 1995. 152. 53/58. Comparison of samples collected by sputum induction and bronchoscopy from asthmatic and healthy subjects.
8. Ferguson, A.C., Whitelaw, M., and Brown, H. : J. Allergy Clin. Immunol. 1992. 90. 609/-613. Correlation of bronchial eosinophil and mast cell activation with bronchial hyperresponsiveness in children with asthma.
9. Iredale, M.J., Wanklyn, S.A.R., Phillips, I.P., Kransz, T., and Ind. P.W. : Clin. Exp. Allergy. 1994. 24. 940/945. Non-invasive assessment of bronchial inflammation in asthma: no correlation between eosinophilia of induced sputum and bronchial responsiveness to inhaled hypertonic saline.
10. Jeffery, P.K., Godfrey, R.W., Ädelroth, E., Nelson, F., Rogers, A., and Johansson, S-A. : Am. Rev. Respir. Dis. 1992. 145. 890/899. Effect of treatment on airway inflammation and thickening of basement membrane reticular collagen in asthma.
11. Juniper, E.F., Kline, P.A., Vanzieleghem, M.A., Ramsdale, E.H., O'Byrne, P.M., and Hargreave, F.E. : Am. Rev. Respir. Dis. 1990. 142. 832/836. Effect of long-term treatment with an inhaled corticosteroid (Budesonide) on airway hyperresponsiveness and clinical asthma in nonsteroid-dependent asthmatics.
12. Kirby, J.G., Hargreave, F.E., Gleich, G.J., and O'Byrne, P.M. : Am. Rev. Respir. Dis. 1987. 136. 379/383. Bronchoalveolar cell profiles of asthmatic and nonasthmatic subjects.
13. Laitinen, L.A., Laitinen, A., and Haahtela, T. : J. Allergy Clin. Immunol. 1992. 90. 32/42. A comparative study of the effects of an inhaled corticosteroid, budesonide, and of a β-agonist, terbutaline, on airway inflammation in newly diagnosed asthma.
14. Pin, I., Gibson, P.G., Kolendowicz, R., Girgis-Gabardo, A., Denburg, J.A., and Hargreave, J. : Thorax. 1992. 47. 25/29. Use of induced sputum cell counts to investigate airway inflammation in asthma.

15. Pin, I., Freitag, A.P., O'Byrne, P.M., Girgis-Gabardo, A., Watson, R.M., Dolovich, J., Denburg, J.A., and Hargreave, F.E.. : Am. Rev. Respir. Dis. 1992. 145. 1265/1269. Changes in the cellular profile of induced sputum after allergen induced asthmatic responses.
16. Takishima, T., Hida, W., Sasaki, H., Suzuki, S., and Sasaki, T. : Chest. 1981. 80. 600/606. Direct-writing recorder of the dose-response curves of the airway to Methacholine.
17. Vathenen, A.S., Knox, A.J., Winsniewski, A., and Tattersfield, A.E. : Am. Rev. Respir. Dis. 1991. 143. 1317/1321. Time course of change in bronchial reactivity with an inhaled corticosteroid in asthma.
18. Vieira, V.G. and Prolla, J.C. : J. Clin. Pathol. 1979. 32. 1054/1057. Clinical evaluation of eosinophils in the sputum.
19. Walker, C., Kaegi, M.K., Broun, P., and Blaser, K. : J. Allergy Clin. Immunol. 1991. 88. 935/942. Activated T cells and eosinophilia in bronchoalveolar lavages from subjects with asthma correlate with disease severity.
20. Wardlaw, A.J., Dunnette, S., Gleich, G.J., Collins, J.V., and Kay, A.B. : Am. Rev. Respir. Dis. 1988. 137. 62/69. Eosinophils and mast cells in bronchoalveolar lavage in subjects with mild asthma; relationship to bronchial reactivity.
21. Woolcock, A.J., Yan, K., and Salome, C.M. : Clin. Allergy. 1988. 18. 165/176. Effect of therapy on bronchial hyperresponsiveness in the long-term management of asthma.