

Original Article

Bronchial and nasal responsiveness in atopic asthma and allergic rhinitis patients: Relationship of local responsiveness to cytokine production by peripheral blood mononuclear cells

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ABSTRACT

To investigate the relationship between local responsiveness and allergic symptoms, bronchial and nasal responsiveness were measured in the following four groups of subjects: (i) bronchial asthma patients with serum house dust mite (HDM)-specific IgE antibody; (ii) allergic rhinitis patients with serum HDM-specific IgE antibody; (iii) normal control subjects with HDM-specific IgE antibody; and (iv) normal control subjects without IgE antibody specific for 10 common aero-allergens. Bronchial hyperresponsiveness was detected in all subjects with asthma (group 1) and in some subjects from groups 2 and 3, but not in subjects from group 4. Nasal hyperresponsiveness was found in all subjects with allergic rhinitis (group 2) and in some subjects from groups 1 and 3, but not in subjects from group 4. These findings indicate that local hyperresponsiveness of the non-diseased organ is influenced by an individual's atopic status. Interleukin (IL)-4 and IL-5 production by peripheral blood mononuclear cells (PBMC) was measured after stimulation with HDM in groups 1, 2 and 3 and was found to be similar in all three groups. A

correlation between bronchial hyperresponsiveness and *in vitro* cytokine production was noted in asthma patients. These results suggest that the capacity of IL-4 or IL-5 production by PBMC may reflect local hyperresponsiveness in case of asthma.

Key words: allergic rhinitis, atopy, bronchial asthma, bronchial responsiveness, interleukin-4, interleukin-5, nasal responsiveness

INTRODUCTION

Bronchial asthma (BA) is a disease characterized by increased bronchial reactivity.¹ Patients with perennial rhinitis have a hyperreactive nasal mucosa (nasal hyperreactivity).^{2,3} It has been suggested that allergic rhinitis patients who have bronchial hyperresponsiveness are more likely to develop asthma.^{4,5} In contrast, it has not been clearly determined whether BA patients have nasal hyperreactivity, despite the strong epidemiologic associations between atopic asthma and allergic rhinitis.⁶ Although increased airway responsiveness has been reported in patients with allergic rhinitis who show no asthma symptoms,^{4,7} it is not clear whether the association is predominantly with rhinitis itself or with the atopic status of the rhinitis patients.

Of all potential allergens in the environment associated with asthma, allergy to house dust mite (HDM) is the

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most common independent risk factor for the development of the disease.⁸ It is also known that HDM is a significant perennial indoor allergen that can precipitate allergic rhinitis.⁹ In the present study we used serum IgE antibody titer to HDM to evaluate atopic status.

To investigate the association between local responsiveness and allergic symptoms among individuals with serum HDM-specific IgE antibody, we studied four groups of patients and normal subjects. Our purpose was to determine how local responsiveness (bronchial or nasal) and cytokine production from peripheral blood mononuclear cells (PBMC) influenced the occurrence of BA and allergic rhinitis.

METHODS

Subjects

Subjects with the following characteristics were chosen randomly from our clinics (Internal Medicine and Otolaryngology, Osaka Teishin Hospital and Osaka University Medical School, Osaka, Japan) and volunteers: (i) patients with a clinical diagnosis of BA who had no history of allergic rhinitis and had elevated serum levels of HDM-specific IgE antibody ($n = 13$); (ii) patients with a clinical diagnosis of allergic rhinitis who had no history of asthma and had elevated serum levels of HDM-specific IgE antibody ($n = 11$); (iii) volunteers working at our hospital with no history of asthma or allergic rhinitis who had elevated serum

levels of HDM-specific IgE antibody ($n = 10$); and (iv) volunteers working at our hospital with no specific IgE antibody for the following 10 aero-allergens ($n = 9$): *Dermatophagoides pteronyssinus* (Der p), *Dermatophagoides farinae* (Der f), house dust 1, house dust 2, ragweed pollen, orchard grass, *Aspergillus fumigatus*, *Candida albicans*, *Alternaria alternata* and Japanese cedar pollen. The clinical characteristics of the subjects are shown in Table 1. Specific IgE antibodies were measured with a Pharmacia CAP System (Pharmacia, Uppsala, Sweden) by external commercial laboratories. A CAP-RAST class of 2 or more was considered positive (class 0, < 0.34 UA/mL; class 1, 0.35–0.69 UA/mL; class 2, 0.7–3.4 UA/mL; class 3, 3.5–17.4 UA/mL; class 4, 17.5–49.9 UA/mL; class 5, 50.0–99.9 UA/mL; class 6, > 100 UA/mL). House dust mite was Der f in both the CAP-RAST assay and in the *in vitro* studies. Forced vital capacity and forced expiratory volume in 1 s were measured using a spirometer (Autospiro AS-500; MINATO Ltd, Osaka, Japan).

Bronchial and nasal responsiveness

Bronchial responsiveness was measured with an Astograph (Chest Corp., Tokyo, Japan, a direct-writing recorder for respiratory resistance (Rrs) dose–response curves, during continuous inhalation of methacholine at stepwise incremental concentrations.¹⁰ Respiratory resistance was measured by the forced oscillation method. It showed a curvilinear increase with an increase

Table 1. Clinical characteristics of subjects

	Group 1	Group 2	Group 3	Group 4
No. subjects	13	11	10	9
Gender (M/F)	6/7	3/8	6/4	5/4
Smoker/non-smoker	2/11	2/9	2/8	3/6
Age (years)*	44.7 (17.9)	34.9 (14.6)	37.0 (15.5)	34.9 (13.5)
CAP-RAST score for HDM (Der f)*	3.31 (1.11)	3.54 (0.93)	3.50 (1.08)	0
Serum IgE level (IU/mL)**	399 (26–1200)	308 (24–710)	889 (32–4400)	68 (7–200)
Eosinophil count in peripheral blood (/mm ³)**	513 (200–1500)	314 (0–640)	295 (65–670)	121 (61–240)
% FVC*	89.6 (14.0)	95.4 (13.0)	95.6 (7.2)	91.8 (16.1)
FEV _{1.0} %*	81.5 (13.1)	92.0 (6.3)	91.8 (7.7)	92.5 (5.3)

*Data are the mean with SD given in parentheses; **data are the mean with the range given in parentheses.

% FVC, % of predicted forced vital capacity; FEV_{1.0}%, % of predicted forced expiratory volume in 1 s; HDM, house dust mite.

Group 1, bronchial asthma patients with serum HDM-specific IgE antibody; group 2, allergic rhinitis patients with serum HDM-specific IgE antibody; group 3, control subjects with no symptoms of allergic diseases who have serum HDM-specific IgE antibody; group 4, control subjects with no symptoms of allergic diseases and no specific serum IgE antibody for a panel of 10 aero-allergens, including HDM.

in the methacholine concentration. The minimum dose of methacholine ($D_{\min}$) was used as an indicator of bronchial sensitivity and was defined as the cumulative dose at the inflection point where the reciprocal of Rrs (Grs) decreased linearly; $D_{\min}$ was expressed in units equal to a 1.0 mg/mL aerosol inhalation of methacholine for 1 min. The existence of bronchial hyperresponsiveness was determined by a $D_{\min}$ value < 50 .¹⁰ Drugs were discontinued at least 12 h before the study. Nasal responsiveness was measured by placing a piece of round paper (Toyo-Roshi No. 5, Tokyo, Japan) containing increasing doses of histamine chloride over the surface of the inferior turbinate bilaterally.³ The logarithm of the histamine chloride concentration that induced sneezing and nasal secretion was defined as nasal responsiveness. Histamine chloride was prepared at concentrations ranging from 10^5 to $10 \mu\text{g/mL}$ by serial 10-fold dilution. Bronchial responsiveness and nasal responsiveness were measured on separate days (1–2 weeks apart). All subjects were free from wheezing, cough, nasal discharge, sneezing and respiratory tract infection for at least 3 weeks before the test. Fully informed consent was obtained from all subjects participating before entry into the study.

Reagents and cells

RPMI 1640 (Gibco, Grand Island, NY, USA), supplemented with 10% fetal bovine serum (FBS; Gibco), 2-mercaptoethanol (0.05 mmol/L), penicillin (100 U/mL) and streptomycin (0.1 mg/mL), was used as the culture medium. House dust mite (*Der f*) extract was provided by Torii Co. Ltd (Tokyo, Japan).

Measurement of interleukin-4 and -5 production

Peripheral blood mononuclear cells were isolated from heparinized venous blood by density gradient centrifugation on Ficoll-Hypaque (Pharmacia, Uppsala, Sweden).

Isolated mononuclear cells ($2 \times 10^6/\text{mL}$) were added to each well of a 24-well culture plate in 0.5 mL culture medium with mite extract ($10 \mu\text{g/mL}$), which was the optimal concentration for interleukin (IL)-4 synthesis.¹¹ After 48 h, the supernatant was collected and the amount of IL-4 and IL-5 was measured by ELISA (Compact Interleukin 4 ELISA kit; CLB, Amsterdam, The Netherlands; ELISA for human Interleukin 5; Immunotech International, Marseilles, France, respectively). Using

these assays, 0.1–1000 pg/mL IL-4 or 2–1000 pg/mL IL-5 was measured in the supernatants and was compared with different concentrations of recombinant IL-4 or IL-5. Interleukin-4 and -5 production was measured within 6 weeks after the assessment of bronchial and nasal responsiveness.

Statistical analysis

The Student's *t*-test and the Mann–Whitney *U*-test were used for comparisons between groups. Correlations were investigated using Spearman's rank correlation test.

RESULTS

Bronchial responsiveness

All 13 asthma patients (group 1) showed bronchial hyperresponsiveness ($D_{\min} < 50$; Fig. 1). Three of 11

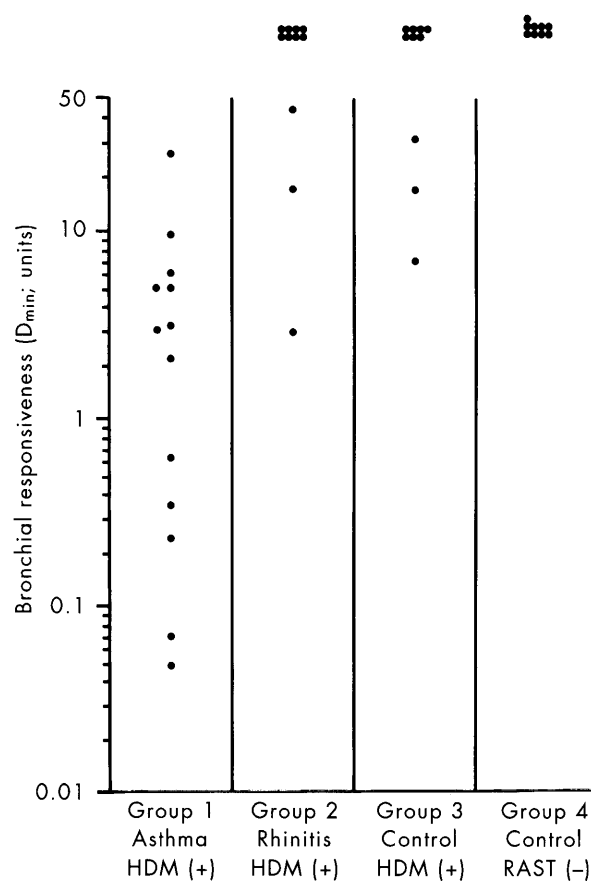


Fig. 1 Individual values for the minimum dose of methacholine ($D_{\min}$) in the four groups, where $D_{\min}$ is an indicator of bronchial responsiveness and $D_{\min} > 50$ is normal.

rhinitis patients (group 2) and three of 10 HDM-specific IgE positive normal control subjects (group 3) also showed bronchial hyperresponsiveness. Overall, 55.9% of subjects with elevated levels of HDM-specific IgE showed bronchial hyperresponsiveness. In contrast, none of the nine normal control subjects without specific IgE (group 4) showed bronchial hyperresponsiveness.

Nasal responsiveness

In a preliminary study, normal individuals without a history of rhinitis showed a nasal responsiveness of 4 or 5. Most normal control subjects without specific IgE (7/9; group 4) also showed a nasal responsiveness of 4 or 5 (Fig. 2). Seven of 13 BA patients (group 1) and eight of 10 HDM-specific IgE positive normal control subjects (group 3) showed nasal hyperresponsiveness (score ≤ 3). All 11 rhinitis patients (group 2) showed nasal hyperresponsiveness and it was more prominent than in the other three groups. Overall, 76.4% of subjects with elevated levels of HDM-specific IgE showed nasal hyperresponsiveness.

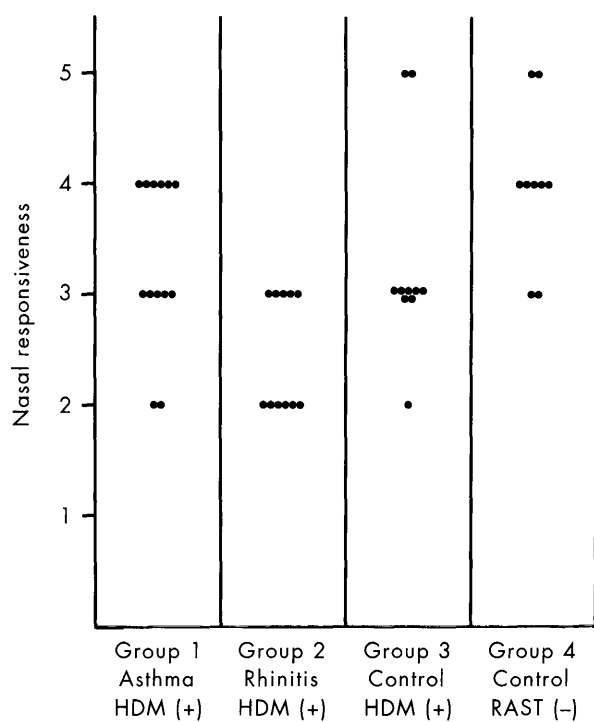


Fig. 2 Individual nasal responsiveness to histamine chloride in the four groups. A nasal responsiveness of 4 or 5 is normal.

Interleukin-4 and -5 production from PBMC

Peripheral blood mononuclear cells from subjects in groups 1–3 were stimulated with HDM extract *in vitro* and the IL-4 and -5 levels in culture supernatants were

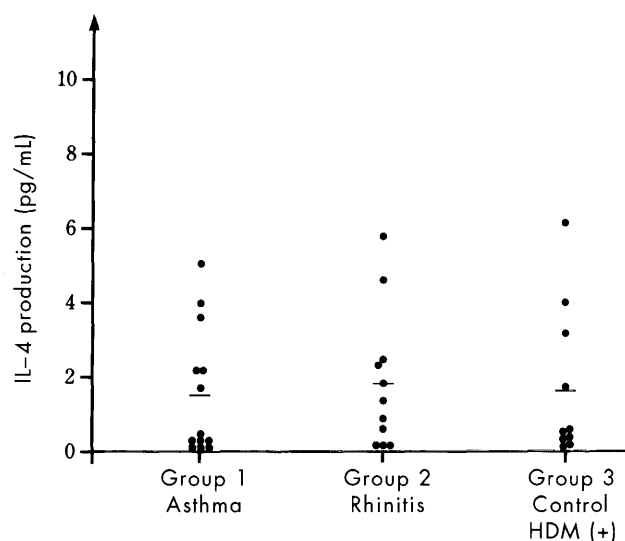


Fig. 3 Interleukin (IL)-4 production by peripheral blood cells stimulated with house dust mite. Mean values, expressed as the geometric mean, are represented by horizontal bars.

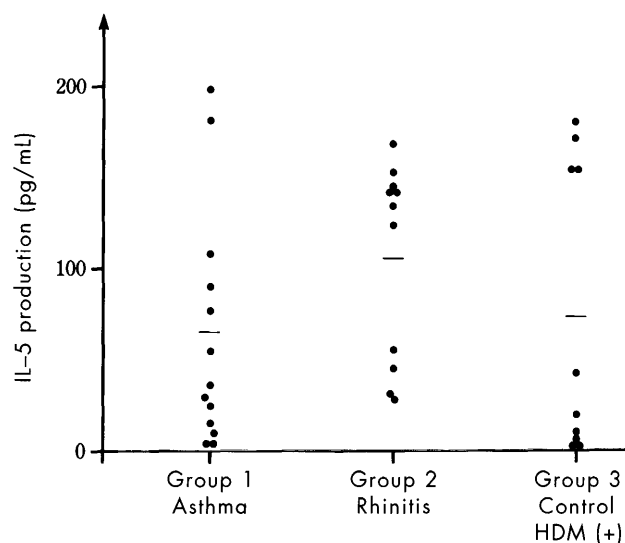


Fig. 4 Interleukin (IL)-5 production by peripheral blood mononuclear cells stimulated with house dust mite. Geometric mean values are represented by horizontal bars.

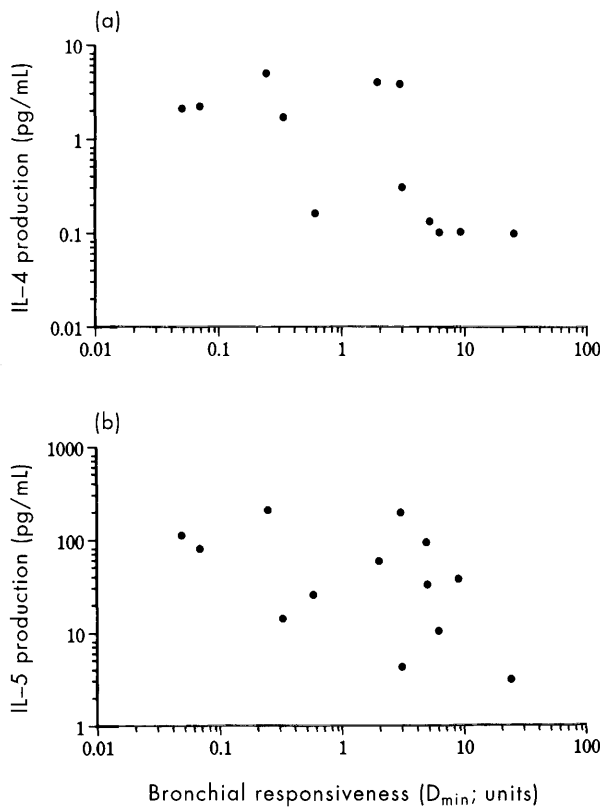


Fig. 5 (a) Relationship between interleukin (IL)-4 and the minimum dose of methacholine (D_{min}) in bronchial asthma (group 1). There is a significant negative correlation ($r = -0.79$; $P < 0.01$). (b) Relationship between IL-5 and D_{min} in bronchial asthma patients (group 1). There is a weak negative correlation ($r = 0.51$; $P = 0.07$).

measured (Figs 3,4). The mean IL-4 levels were 1.5 ± 1.8 , 1.8 ± 1.9 and 1.7 ± 2.1 pg/mL in groups 1, 2 and 3, respectively, while the respective IL-5 levels were found to be 64 ± 65 , 106 ± 54 and 75 ± 81 pg/mL. There was no significant difference in the production of either cytokine among the different groups with HDM-specific IgE positivity.

Local responsiveness and cytokine production

To test whether local responsiveness (bronchial and nasal responsiveness) may be influenced by cytokine production in peripheral blood, the relationship between local responsiveness and IL-4 or -5 production was investigated. There was no correlation between nasal responsiveness and IL-4 or -5 production among the

subjects in each of the groups 1–3 or among all subjects in these three groups combined. However, the relationship between D_{min} (bronchial responsiveness) and IL-4 production in asthma patients (group 1) showed a strong negative correlation (Fig. 5a; $r = -0.79$; $P < 0.01$), although no such correlation was found among all subjects who had bronchial hyperresponsiveness ($D_{min} < 50$) in groups 1–3. The relationship between D_{min} and IL-5 production in asthma patients showed a weaker negative correlation (Fig. 5b; $r = -0.51$; $P = 0.07$), while IL-5 and bronchial responsiveness showed no relationship among all subjects with bronchial hyperresponsiveness ($D_{min} < 50$) in groups 1–3. The relationship between D_{min} and cytokine production could not be assessed separately in groups 2 or 3 because only three subjects showed bronchial hyperresponsiveness in each group. There was a strong positive correlation between IL-4 and -5 production in BA patients ($r = 0.66$; $P < 0.03$; data not shown).

DISCUSSION

The results of the present study suggest an association between atopy and local hyperresponsiveness. Of the subjects with an elevated serum level of HDM-specific IgE, all asthma patients (group 1) had bronchial hyperresponsiveness ($D_{min} < 50$). In addition, approximately 30% of both allergic rhinitis patients (group 2) and subjects with no allergic symptoms (group 3) had bronchial hyperresponsiveness. In contrast, the normal control subjects with no aero-allergen-specific IgE (group 4) did not have bronchial hyperresponsiveness. Similar results were obtained in the case of nasal responsiveness. Of the subjects with elevated serum HDM-specific IgE, all rhinitis patients (group 2) had nasal hyperresponsiveness and more than half of both groups 1 and 3 also had moderate nasal hyperresponsiveness. Most normal control subjects with no aero-allergen-specific IgE had normal nasal responsiveness. Witt *et al.* have reported that the prevalence of bronchial histamine responsiveness in adults increased from 5.8% in subjects who did not respond to allergen prick tests to 22.2% in subjects who responded to all five allergen groups.¹² Woolcock *et al.* reported that 7.3% of non-atopic subjects showed bronchial hyperresponsiveness compared with 16.5% of those with severe atopy, and stated that there was a significant association between bronchial responsiveness and atopy in random adult populations.¹³ Our results are compatible with these

findings and extend the association with atopy to nasal hyperresponsiveness.

It is well known that allergic rhinitis patients often have bronchial hyperresponsiveness,⁴⁻⁷ but whether BA patients have nasal hyperresponsiveness has been unclear. In accordance with our clinical experience, more than half the asthma patients with serum HDM-specific IgE (group 1) showed nasal hyperresponsiveness in the present study. This may explain why asthma patients often complain of nasal symptoms. Although bronchial responsiveness showed almost no overlap between asthma patients and non-atopic normal control subjects, nasal responsiveness did not completely separate rhinitis patients from non-atopic normal control subjects. This may be because nasal responsiveness can be acquired readily after nasal infection and rhinitis is more common than asthma. In any event, local responsiveness of the non-diseased organ was increased to some extent among serum HDM-specific IgE positive subjects (groups 2 and 3 for bronchial responsiveness and groups 1 and 3 for nasal responsiveness), suggesting that local responsiveness of the non-diseased organ may be affected by an individual's atopic status.

Interleukin-4 is known to be a mast cell growth factor¹⁴ and it also promotes B cell isotype switching to IgE.¹⁵ Allergen-induced IgE-dependent mast cell degranulation may be responsible for acute bronchoconstrictor responses in atopic asthma.¹⁶ Interleukin-5 promotes the differentiation,¹⁷ vascular adhesion¹⁸ and *in vitro* survival of eosinophils¹⁹ and eosinophil infiltration and activation is known to contribute to chronic bronchial hyper-reactivity.²⁰ It has also been shown that atopic asthma is associated with increased expression of mRNA for IL-3, IL-4, IL-5 and granulocyte-macrophage colony stimulating factor in the bronchi.²¹ It has also been reported that cells expressing mRNA for IL-4 and -5 increase during the allergen-induced nasal response in allergic rhinitis.²² Although increases in IL-4 and IL-5 activity has been shown in diseased organs (bronchial mucosa or recovered cells from bronchoalveolar lavage for BA and nasal mucosa for allergic rhinitis), *in vitro* cytokine production by PBMC stimulated with antigen has not been well characterized in patients with allergic diseases. Secrist *et al.* reported that allergen immunotherapy decreased IL-4 production by CD4⁺ T cells from allergic individuals.²³ Ochi *et al.* reported that both cultured lymphocytes and basophils from the peripheral blood of allergic individuals produced IL-4 in response to allergen.¹¹ In the present study, we evaluated the *in vitro*

production of IL-4 and -5 by PBMC stimulated with HDM and found that the levels of these cytokines were similar among serum HDM-specific IgE-positive subjects (groups 1-3). This finding implies that the amount of these cytokines produced *in vitro* is not related to either the presence or the type of allergic disease.

Next we evaluated the correlation between local responsiveness and various clinical parameters. No correlation was found between nasal responsiveness and the total serum IgE level, the eosinophil count in the peripheral blood and IL-4 or -5 production by PBMC stimulated with HDM. Bronchial responsiveness (D_{min}) was negatively correlated with IL-4 production by PBMC among asthma patients (group 1). Although there was a negative correlation between bronchial responsiveness and IL-5 production by PBMC among asthma patients, it was not statistically significant. Bronchial responsiveness was also not correlated with various clinical parameters, such as the total serum IgE level or the eosinophil count in the peripheral blood. We used methacholine for the assay of bronchial responsiveness and should point out, however, that the possibility exists that the use of histamine for bronchial responsiveness may provide a more significant correlation between bronchial responsiveness and cytokine production by PBMC. Robinson *et al.* reported a negative correlation between airway methacholine responsiveness and the number of bronchoalveolar lavage cells expressing IL-5 mRNA, but not IL-4 mRNA.²⁴ Ackerman *et al.* reported that the level of airway hyperresponsiveness in atopic asthma patients was negatively correlated with the IL-5 immunoreactivity of bronchial biopsies, but not with IL-4 immunoreactivity.²⁵ Our results appear to be different from those of Robinson *et al.*²⁴ and Ackerman *et al.*²⁵ One reason may be that we assayed *in vitro* cytokine production by PBMC stimulated with antigen, whereas they assayed either bronchoalveolar lavage cells or bronchial mucosa. However, the significant negative correlation between bronchial hyperresponsiveness and IL-4 production, as well as the weaker negative correlation between bronchial hyperresponsiveness and IL-5 production in our asthma patients, supports the notion that the capacity of cytokine production by PBMC may reflect bronchial responsiveness among asthmatic patients.

Finally, there were two non-asthmatic subjects who had bronchial hyperresponsiveness in the asthmatic range, atopic status (an HDM CAP-RAST score ≥ 3) and an above average production of both IL-4 and -5 by peripheral blood (one in the allergic rhinitis group and

one with no allergic symptoms). The reason why these subjects did not develop asthma symptoms is unclear and the additional factors necessary to provoke overt symptoms of asthma need to be defined in the future.

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