

Relation between bronchial responsiveness to methacholine and levels of IgE antibody against *Dermatophagoides farinae* and serum IgE in asthmatic children

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Summary

The relation between non-specific bronchial responsiveness and allergic sensitivity was evaluated in children with asthma. Bronchial responsiveness was determined by methacholine inhalation challenge test, and was expressed as a provocative concentration of methacholine causing a 20% fall in FEV₁ (PC₂₀). RAST titre for *Dermatophagoides farinae* and serum IgE level were evaluated as parameters of allergic sensitivity. When the PC₂₀ values, RAST titres for *D. farinae*, and serum IgE levels of 47 asthmatic children and 16 normal controls were compared, the asthmatic children had significantly lower PC₂₀ values and higher *D. farinae*-RAST titres and serum IgE levels than the normal controls. The correlation analyses in 47 asthmatic children have shown that there is no significant correlation between PC₂₀ values and RAST titres for *D. farinae* ($r=0.04$, $P>0.1$) or between PC₂₀ values and serum IgE levels ($r=0.03$, $P>0.1$). These results suggest that although both bronchial hyperresponsiveness and allergic sensitization may be in some way related to one another in children with asthma, the magnitude of allergic sensitization does not influence the degree of the non-specific bronchial responsiveness.

Clinical and Experimental Allergy, Vol. 23, pp. 450–454. Submitted 6 December 1991; revised 11 November 1992; accepted 30 November 1992.

Introduction

Asthma is a complex disorder often associated with bronchial hyperresponsiveness (BHR) and involving heterogeneous factors [1]. The allergic reaction is believed in many cases to play a major role in the development of asthma in children, because high levels of specific IgE antibodies against common inhaled allergens show significant correlations not only with a clinical history of asthma, but also with the results of provocation tests for the corresponding allergens [2–5]. However, the relation between allergy and BHR remains unclear.

House dust mite is thought to be one of the potent allergens triggering asthmatic attacks. The mite sensitivity is shown to be a much greater risk factor for

development of asthma than other aero-allergens such as pollens [6,7]. Furthermore, it has been suggested that sensitivity to the house dust mite may increase BHR, as prolonged avoidance of exposure to mites led to a reduction in BHR [8,9]. In this study, we examined the bronchial responsiveness to methacholine, the levels of IgE antibody against *Dermatophagoides farinae* by RAST and serum IgE in 47 asthmatic children, and evaluated the relation between bronchial responsiveness and allergic sensitivity.

Materials and methods

Subjects

A total of 47 asthmatic patients (32 boys and 15 girls), 7 to 15 years of age (mean 10.1), were selected from those attending the out-patient clinic at the University Hospital of Tsukuba. Asthma was defined according to the criteria of the American Thoracic Society [10], and only patients

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who had had four or more episodes of wheezing with shortness of breath during the past 12 months were included in this study. Patients with cystic fibrosis, humoral immunodeficiency and Kartagener's syndrome were excluded from this study, because these illnesses were almost always associated with chronic bronchitis. Patients were also excluded if during the previous month they had had an acute asthma attack requiring theophylline infusion therapy or a respiratory tract infection, or if they had taken corticosteroids in any form for 2 weeks or more, sodium cromoglycate, ketotifen, azelastin hydrochloride and theophylline for 24 hr, or any β -agonist for 8 hr. Sixteen healthy volunteers (10 boys and 6 girls) aged 8 to 13 years (mean 10.5) were also studied as a control group. They had never had asthma or any allergic disease. None of them had experienced respiratory tract infection during the month preceding the test. Their pulmonary function was within the normal range. Parents gave written consent for their children to be included in this study.

Methacholine inhalation tests

The methacholine challenge test was performed according to the standardized procedure recommended by Japanese Society of Allergology [11]. The challenge test was done if the subject's FEV₁/FVC value was more than 70% in the initial pulmonary function test. Subjects were administered aerosols of methacholine from a hand-held DeVilbiss No. 646 nebulizer (DeVilbiss Co., Somerset, PA, U.S.A.) by tidal breathing for 2 min. The operating airflow rate was 5 l/min. The isotonic saline was inhaled first as a control. This was followed by doubling concentrations of methacholine from 0.049 to 25 mg/ml. The FEV₁ was measured after each inhalation with a Minato A 300 Autospirometer (Minato, Inc., Tokyo, Japan). The percentage FEV₁ fall was calculated from the post saline-FEV₁. The test was continued until the FEV₁ had fallen > 20% or until the maximal concentration of methacholine had been administered. The bronchial sensitivity was expressed as the concentration of methacholine that would provoke a fall in FEV₁ of 20% (PC₂₀). At the end of the test, any fall in FEV₁ was reversed with an inhalation of orciprenaline.

Measurement of RAST titre

The Sepharose RAST method was described in detail in a previous article [12]. Briefly, 3 mg of freeze-dried antigen of *D. farinae*, supplied by Torii Co. Ltd, Tokyo, was coupled to 300 mg (dry weight) of CNBr-activated Sepharose 4B (Pharmacia, Uppsala, Sweden), according to the method described by Wide *et al.* [13]. The allergen-

coupled particles (ACP) were suspended in RAST buffer obtained from Phadebas RAST kits (Pharmacia) at a concentration of 10 mg/ml. Fifty microlitres of ACP suspension was incubated for 2 hr at room temperature with 50 μ l of ¹²⁵I-labelled anti-IgE obtained from Phadebas RAST kits, and then added to the tube and further incubated for 16 hr at room temperature. Excess of labelled anti-IgE was washed away with 0.15 mol/l of NaCl solution containing 1% Tween 20. The radioactivity bound to the ACP was measured in a gamma counter. RAST determinations were done in duplicate, and the mean percentage of binding was calculated by dividing the counts bound to ACP by total counts times 100. The cut-off point between RAST positivity and negativity was defined as 350% of the background binding produced by 2% HSA. The results were regarded as positive when the percentage binding of sample serum was higher than the cutoff value. Serial twofold dilutions of sera were prepared and then subjected to RAST. The maximum dilution of serum producing RAST positivity was expressed as a RAST titre.

Determination of total IgE levels

Serum IgE levels were determined by RIST kits (Pharmacia). The mean value of duplicate determinations was expressed as the total serum IgE level.

Statistics

Data were analysed by the computer program of the Statistical Analysis System (SAS Institute, U.S.A.) after first normalizing methacholine PC₂₀ values, RAST titres, and IgE concentrations by logarithmic transformations, using the Chi-square test and Pearson's correlation analysis: *P* values < 0.05 were considered significant.

Results

Bronchial sensitivity (PC₂₀), and *D. farinae* specific and serum IgE levels for 47 asthmatics and 16 normal controls were compared (Fig. 1). Since some 70% of our asthmatic children showed a maximal RAST value for *D. farinae* in the ordinary Phadebas RAST assay, here, *D. farinae*-specific IgE levels were determined by examining the RAST end-point titre, using serially twofold diluted sera. The average PC₂₀ value was 0.8 $\pm$ 1.4 mg/ml for asthmatic children and 20.0 $\pm$ 8.1 mg/ml for normal controls; the mean RAST titres were 420 $\pm$ 513 and 15 $\pm$ 34, and the average IgE level 845 $\pm$ 719 and 151 $\pm$ 230 IU/ml, respectively. Thus, asthmatic children had significantly lower PC₂₀ values and higher RAST titres and serum IgE levels than normal controls (*P* < 0.01).

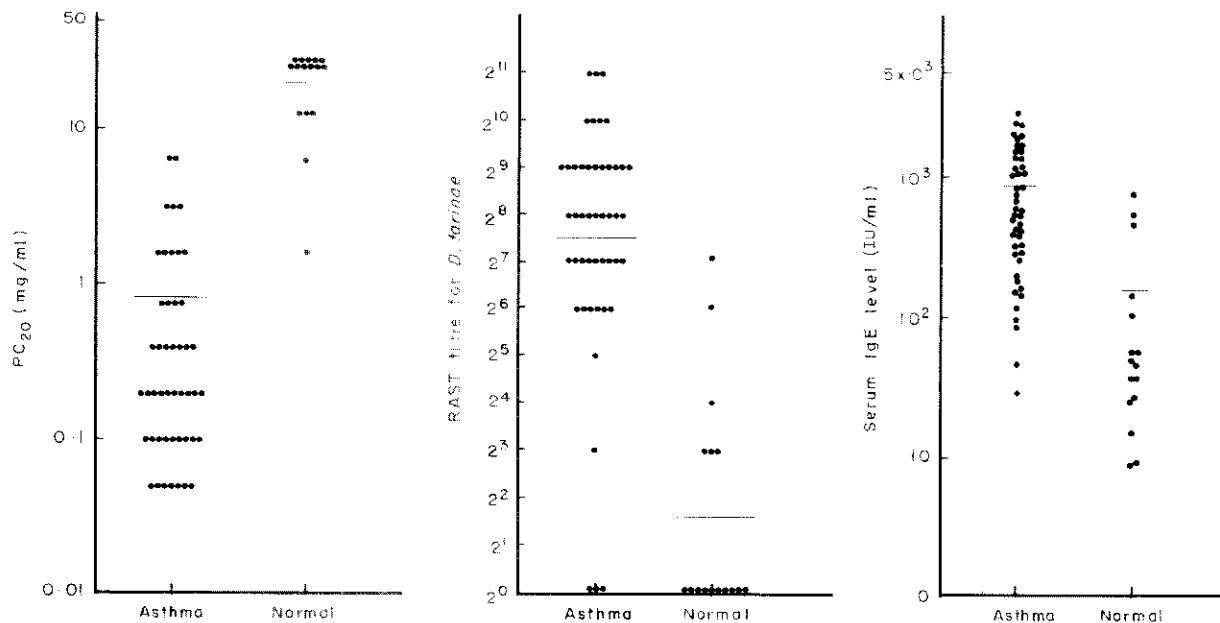


Fig. 1. Bronchial sensitivity to methacholine (PC_{20}), RAST titre for *D. fariniae*, and serum IgE level in 47 asthmatic and 16 normal children. Horizontal bars in the graph indicate average values for each group.

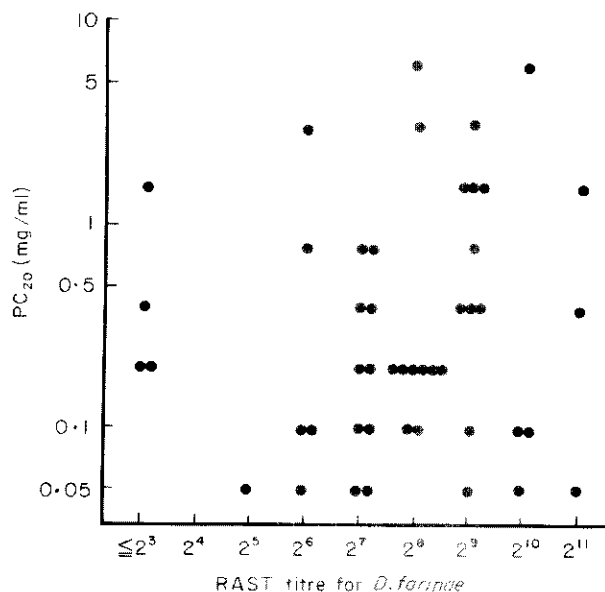


Fig. 2. Relation between PC_{20} value and IgE antibody level against *D. fariniae* ($r=0.04$; $P>0.1$). In the calculation of correlation coefficient, PC_{20} values and RAST titres were logarithmically transformed.

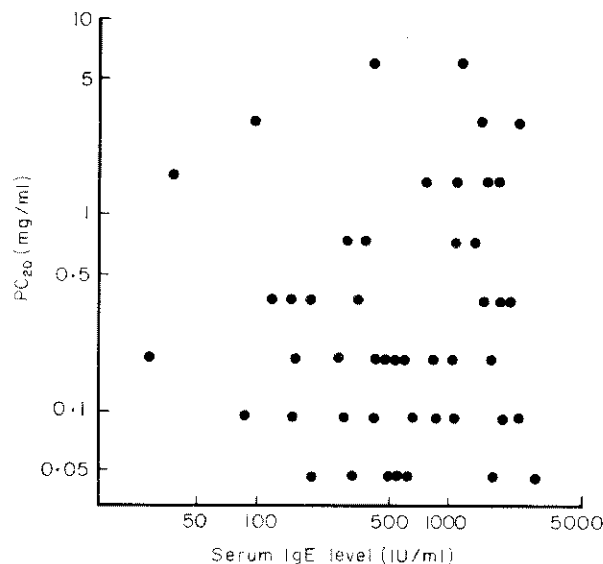


Fig. 3. Relation between PC_{20} value and total serum IgE level ($r=0.03$; $P>0.1$). In the calculation of correlation coefficient, PC_{20} and IgE levels were logarithmically transformed.

The PC_{20} values of the 47 asthmatic children were compared with their RAST titres for *D. fariniae*. As shown in Fig. 2, there was no significant correlation between the two ($r=0.04$; $P>0.1$). When the PC_{20} values were similarly compared with *D. fariniae*-RAST titres in the 43

children with high RAST titre for *D. fariniae* (≥ 32), no significant correlation was found ($r=0.19$; $P>0.1$).

As shown in Fig. 3, when the PC_{20} values of the 47 asthmatic children were compared with their total serum IgE levels, no significant correlation was found ($r=0.03$; $P>0.1$).

On the other hand, there was an intimate correlation between the RAST titres for *D. farinae* and the total serum IgE levels in the 47 asthmatic children ($r=0.76$; $P<0.01$).

Discussion

The present results have shown that there is no significant correlation between the degree of the bronchial sensitivity to methacholine and *D. farinae*-specific and total serum IgE levels in asthmatic children. Regarding the relation of allergy to non-specific bronchial responsiveness, conflicting results have been reported. Cockcroft *et al.* [14] observed a significant positive association between bronchial responsiveness to histamine and skin-test reactivity to common inhaled allergens in adult atopic asthmatic subjects. Peat *et al.* [15] have reported that the severity of bronchial reactivity to histamine in children is related to their severity of atopic status, as measured by the number and size of the skin reactions to common allergens. Bryant and Burns [16] observed no significant difference in bronchial responsiveness to methacholine between adult subjects with atopic asthma and asthmatic subjects without atopy. Neijens *et al.* [17] have shown that there was no significant correlation between the threshold dose of histamine and basophil histamine release with allergen. Our results are in agreement with the latter observations, and suggest that, even in children whose asthma is almost always atopic, the allergic sensitivity does not directly influence the degree of non-specific bronchial responsiveness.

In our given subjects, serum IgE levels were significantly correlated with RAST titres for *D. farinae*. Since *D. farinae* is a dominant environmental allergen in this country [18], it is likely that *D. farinae*-specific IgE levels contribute much to the elevation of total serum IgE levels.

It has been reported that experimental exposure to allergen increases the non-specific bronchial reactivity to inhaled histamine or methacholine in allergic subjects with asthma [8,19]. Atopic asthmatics have been observed to display increased non-specific bronchial responsiveness during periods of seasonal pollen exposure [20,21]. Conversely, prolonged avoidance of allergen exposure can reduce non-specific bronchial responsiveness in allergic asthmatics [9]. From these observations, it can be supposed that continuing exposure to mite allergen may increase the non-specific bronchial reactivity that predisposes mite-reactive children to the development of asthma. However, the present results do not agree with this notion, suggesting rather that the non-allergic factors are still important in the pathogenesis of BHR in atopic asthmatics.

However, the lack of a demonstrable correlation between bronchial reactivity and *D. farinae*-specific IgE levels does not exclude the possibility that there is some association between bronchial reactivity and allergic sensitivity. Our results have also shown that both the BHR and sensitization to *D. farinae* frequently occur together in asthmatic children. Further experimentation will be required to determine whether it is possible that the difference in the number of mast cells in the bronchi [22], the concentration of IgE fixed to these cells, their ability to release chemical mediators [23], and the sensitivity of bronchial smooth muscle to mediators [24] in *D. farinae*-reactive asthmatic children may influence the magnitude of bronchial reactivity. The inflammation of airway including eosinophilic infiltration may alter the contractility of the smooth muscle [25,26].

Allergy and BHR could also be genetically linked, or associated on the basis of a shared underlying cause, such as an abnormality of the bronchial mucosa. More remains to be learned about the biological basis of the relation between allergy and non-specific airway responsiveness.

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