

Short communication

Relationship between cutaneous allergen response and airway allergen-induced eosinophilia

Background: Determinants of changes in airway caliber after allergen challenge include nonallergic airway responsiveness, immune response and dose of allergen given. However, determinants of the airway inflammatory response to allergens remain to be determined.

Aim: To assess the relationship between skin reactivity to airborne allergens and lower airway eosinophilic response to allergen exposure in asthma and allergic rhinitis.

Methods: Forty-two subjects with mild allergic asthma (mean age 24 years) and 14 nonasthmatic subjects with allergic rhinitis (mean age 25 years) had allergen skin prick tests and titration with the allergen chosen for subsequent challenge. On a second visit, 31 asthmatic subjects had a conventional challenge while 11 asthmatic subjects and all rhinitic subjects had a low-dose allergen challenge over four subsequent days. Induced sputum samples were obtained at 6 and 24 h after the conventional challenge and at days 2 and 4 of the low-dose challenge.

Results: In the asthmatic group, there was a weak correlation between wheal diameter induced by the concentration used for challenge and increase in eosinophils 6 h postconventional challenge ($r = 0.372$, $P = 0.05$), but no correlation was observed following the low-dose challenge. Rhinitic subjects showed a correlation between wheal diameter with the allergen dose used for bronchoprovocation and increase in eosinophils at day 2 of low dose ($r = 0.608$, $P = 0.02$).

Conclusion: This study suggests that immediate immune responsiveness to allergen, assessed by the magnitude of the skin response, is a significant determinant of allergen-induced airway eosinophilia and can help to predict the airway inflammatory response.

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Asthma is defined as an airway disease characterized by reversible airflow limitation and airway hyperresponsiveness (AHR), associated with an airway inflammatory process and restructuring (1, 2). One of the main features of asthmatic airways is an increase in number and activation of inflammatory cells, particularly eosinophils (3–5), which produce and release substances such as cationic proteins, and a large variety of cytokines and other mediators.

Allergen exposure may lead to the development or worsening of asthma in sensitized subjects (6, 7). Inhalation of allergens by allergic asthmatic subjects may induce different types of allergic responses, such as the early response (EAR), occurring in the first hour after exposure, and the late response (LAR), observed 2–8 h thereafter (8). The latter is accompanied by airway inflammation, as shown by an increase in the number of eosinophils and lymphocytes in the airway wall and lumen (9, 10). The cellular and humoral inflammatory responses to allergen have been extensively studied not

only from bronchial biopsies and bronchoalveolar lavage, but also induced sputum analysis. Pizzichini et al. (11) showed an increased number of eosinophils and CD4+ cells, and increased amounts of eosinophil cationic protein and tryptase in the sputum of mild asthmatic subjects compared with normal controls. Pin et al. (12) looked at airway inflammation using induced sputum analysis before and 32 h after asthmatic responses to allergen in asthmatic subjects and observed a significant increase in eosinophils and metachromatic cells.

Airway inflammation has also been observed following repeated low-dose allergen exposure in asthmatic subjects. This usually happens without clinical manifestations or fall in expiratory flows, but is associated with an increase in airway responsiveness (13). Allergen-induced airway inflammation has also been reported in nonasthmatic subjects after natural (14) or laboratory (15) exposure, although the influence of low-dose allergen challenge remains to be documented in these subjects.

Analyses of the relationships between allergen skin and lower airway responses expressed as a fall in expiratory flows showed that skin titration could help to estimate inhaled-allergen sensitivity in subjects with mild to moderate cutaneous allergen sensitivity, although not accurately (16). This may be explained by the fact that airway asthmatic responses to allergen in sensitized subjects are dependent on the nonallergic airway responsiveness, the dose of allergen provided, and the immune response to allergen, as determined by skin sensitivity or specific IgE measures. These relationships have led to the development of a formula to predict the allergen provocative dose that gives a 20% fall in forced expiratory volume in 1 s (FEV_1) (PD_{20}) (17, 18). For a similar degree of nonallergic airway responsiveness, a smaller dose of allergen is therefore required to induce a 20% fall in FEV_1 in a subject with a high degree of immunologic response compared with a subject with a low degree of immune response.

We do not know, however, what the relationship is between lower airway inflammatory response and allergic skin responses in asthmatic or nonasthmatic atopic subjects. As airway inflammatory response to allergens is frequently used to study the mechanisms implicated in asthma development, it would be of great value to determine this relationship. Moreover, it could help us to predict the magnitude of the airway allergen-induced response from skin test titration. In this study, we correlated the skin reactivity to aeroallergens with airway eosinophilic response to allergen challenge in mild allergic asthmatic subjects and nonasthmatic subjects with allergic rhinitis.

Methods

Subjects

Allergic asthmatic and allergic rhinitic subjects were recruited from the Laval Hospital Asthma and Allergy Clinic. Asthmatic subjects had mild asthma as shown by a need for only β_2 -agonists to control asthma symptoms and normal or near-normal expiratory flows. None of the allergic rhinitis subjects had experienced asthma symptoms or taken any asthma medication in the past.

All subjects had a positive skin response to one or more aeroallergens and were able to produce adequate sputum samples. They were nonsmokers or had not smoked for at least 6 months. None had been exposed to an allergen to which they were sensitized (apart from house dust mite) for at least 1 month, and none had had any respiratory tract infection for at least that same time period. Subjects sensitized to animals did not have pets at home. The study was approved by the local Ethics Committee and all subjects signed an informed consent form.

Study design

This study is an analysis of the relationship between cutaneous reactivity and lower airway eosinophilic responses in subjects with mild allergic asthma who came to the laboratory for either

conventional or low-dose allergen challenges, and in nonasthmatic subjects with allergic rhinitis after low-dose allergen challenges.

All subjects presented to the laboratory for a baseline visit. Allergy skin prick tests and titration were performed, followed by spirometry and methacholine bronchoprovocation. During that visit, induced sputum was also obtained. One to 7 days later, subjects came back in the morning to undergo either a conventional allergen challenge ($n = 32$ asthmatic subjects) or a low-dose allergen challenge ($n = 14$ rhinitic subjects and 11 asthmatic subjects). For the conventional challenge, induced sputum was obtained during the LAR and again 24 h following the challenge. For the low doses, a sputum sample was obtained in the afternoons of the second and fourth challenges, and again 1 week later.

Allergen skin prick tests and titration

Atopy was determined using skin prick tests to 26 common allergens. Normal saline and histamine were used as negative and positive controls, respectively. Skin wheal diameter was determined at 10 min using two perpendicular diameter measurements. A positive reaction was defined as a skin wheal diameter of 3 mm or more to at least one allergen. Skin titration was done with the allergen to which the subject was the most sensitized (as assessed by prick tests). Increasing concentrations were applied on the forearm and the diameter was measured at 15 min as for the skin prick tests. The smallest concentration still giving 2 mm in wheal diameter was used to determine the concentration for allergen challenge.

Spirometry and methacholine challenge

Baseline FEV_1 and forced vital capacity (FVC) were measured according to American Thoracic Society (ATS) specifications (19). Baseline FEV_1 was defined as the best of three reproducible curves. The predicted values for expiratory flows were obtained from Knudson et al. (20). Methacholine tests were done according to the method described by Juniper et al. (21).

Allergen challenges

Conventional allergen challenges were performed as described by Cockcroft et al. (18). To determine which dose of allergen would give a 20% fall in FEV_1 (PD_{20}), we used the equation described by Cockcroft et al. (18). For conventional challenge, the starting dose of allergen given for inhalation corresponded to four doubling doses below the calculated PD_{20} . Subjects inhaled allergen for 2 min, and FEV_1 was recorded 10 min later. Increasing doses were administered until the 10-min FEV_1 had fallen by 15% or more from the highest baseline FEV_1 . After allergen inhalation, FEV_1 was recorded at 10, 20, 30, 45, 60, 90 and 120 min, and then hourly until 7 h after allergen inhalation. The EAR corresponded to a fall of $\geq 15\%$ in FEV_1 during the first hour following allergen inhalation. The LAR was calculated similarly as a $\geq 10\%$ decrease in FEV_1 between 3 and 7 h after allergen challenge.

Low-dose allergen challenges were done as described in a recent publication (22). Briefly, allergen doses corresponding to nine, eight, and seven doubling doses below the calculated PD_{20} allergen, were administered on four consecutive mornings for 2 min. FEV_1 was measured 10 min later, and if the fall in FEV_1 was lower than 5% from the highest FEV_1 at baseline, the next concentration was administered for a maximum of three doses. After the last concentration, FEV_1 was monitored at 10-min intervals for 30 min.

Induced sputum

Sputum induction was performed according to the method described by Pin et al. (12) and modified by Pizzichini et al. (23). Briefly, after pretreatment with inhaled salbutamol, subjects inhaled hypertonic saline (3, 4 and 5%) for 7 min each via a Medix electronic nebulizer (Medix, Catthorp, UK) without a valve or nose clip. They were then instructed to rinse their mouth and blow their nose before trying to expectorate into a sterile dish.

Sputum was processed within 2 h following the induction according to the method currently used in our laboratory (22). Briefly, mucous plugs were selected, weighted and mixed with four times their volume of dithiothreitol (DTT) (Sputolysin; Calbiochem Corp., La Jolla, CA) and the reaction was stopped with an equal volume of Dulbecco's phosphate buffer saline (D-PBS) 1X. After filtration, cells were counted and suspension was adjusted to 0.5–1 million cells/ml. Two slides were prepared using a Shandon III cytocentrifuge (Shandon Scientific Lim., Astmoor, UK) and stained with Diff-Quik (Dade Diagnostics of P.R. Inc., Aguada, Puerto Rico) for differential cell count. Four hundred cells were counted by a technician blind to the clinical details.

Data analysis

In order to assess whether there were any relationships between the magnitude of lower airways inflammation and the immune response, we correlated the variation of sputum eosinophils (Δ eosinophils) following allergen challenge (whether conventional or low-dose) to the mean wheal diameter of the skin reaction. Sputum eosinophilia measured on day 2 of low-dose allergen challenges was used to correlate with skin responses, as this had been previously shown to be the peak inflammatory response to allergen using this method in rhinitic subjects, while it was obtained at 6 h after conventional challenge for a similar reason. The skin diameter was the one corresponding to the last dose of allergen inhaled during the challenge.

Results are expressed as median values (25th and 75th percentiles). Relationships between parameters are expressed with Pearson's correlation coefficients. The results were considered significant if P -values were ≤ 0.05 . Data were analyzed using the statistical package SAS (SAS Institute Inc., Cary, NC).

Results

Subjects characteristics

The baseline clinical characteristics of subjects are shown in Table 1. Analysis included data from 42 allergic asthmatic subjects aged 18–45 years and 14 nonasthmatic subjects with allergic rhinitis, aged 18–45 years. All subjects completed the study.

Table 1. Subjects characteristics

Type of allergen challenge	Subject	<i>n</i>	Age (years)	Sex (F/M)	FEV ₁ (% pred.)	PC ₂₀ (mg/ml)	Allergen (AU/ml)
Conventional	A	31	25 $\pm$ 4	19/12	93 $\pm$ 2	3 $\pm$ 1	394 $\pm$ 35
Low dose	A	11	23 $\pm$ 2	9/2	98 $\pm$ 1	4 $\pm$ 1	2 $\pm$ 1
Low dose	R	14	25 $\pm$ 2	9/5	104 $\pm$ 4	65 $\pm$ 15	33 $\pm$ 12

Values are given as mean $\pm$ SEM. FEV₁, forced expiratory volume in 1 s; PC₂₀, provocative concentration of methacholine that gives a 20% fall in FEV₁; A, asthmatic subjects; R, rhinitic nonasthmatic subjects.

Allergen-induced fall in FEV₁

Mean EAR and LAR falls in FEV₁ following conventional allergen challenge in asthmatic subjects, were 27.2 and 23.1%, respectively. Two asthmatic subjects presented only an isolated EAR. There was no fall in FEV₁ $>$ 5% in rhinitic subjects nor in asthmatic subjects following any low-dose allergen challenge and none of these subjects experienced any allergic or asthmatic symptoms throughout the challenge days.

Lower airway inflammatory response to allergen

Following conventional allergen challenge, median (25th and 75th percentiles) increase in percentage of eosinophils between baseline and 6 h was 6.0% (2.5, 15.0); between baseline and 24 h it was 4.0% (1.6, 20.1). Following low-dose challenge, median increase in percentage of eosinophils between baseline and day 2 was 1.25% (0, 8.4) in rhinitic subjects and 0.8% (0, 3.5) in asthmatic subjects; between baseline and day 4 it was 0.8% (0, 3.3) in rhinitic subjects and 1.0% (0, 2.8) in asthmatic subjects (22).

Correlations between skin and airway inflammatory responses

We observed a weak correlation between skin wheal diameter and bronchial eosinophilia in asthmatic subjects at the LAR ($r = 0.372$, $P = 0.05$) (Fig. 1). Following day 2 of low-dose challenge, there was a significant correlation between skin reactivity and percentage of eosinophils in rhinitic subjects ($r = 0.608$, $P = 0.02$) (Fig. 2), but not in asthmatic subjects. There was no significant correlation following 4 days of low dose or 1 week later.

Discussion

Airway inflammatory responses may be influenced by many factors, including the immunologic status of the subject, particularly the level of IgE present on airway inflammatory cells. Although this level may not necessarily correspond to the number of IgE antibodies attached to skin inflammatory cells (e.g., mast cells), previous studies have suggested a correlation between cutaneous immediate allergic responses and airway responses to methacholine in asthmatic subjects. Consequently, both immunologic status and AHR were used to

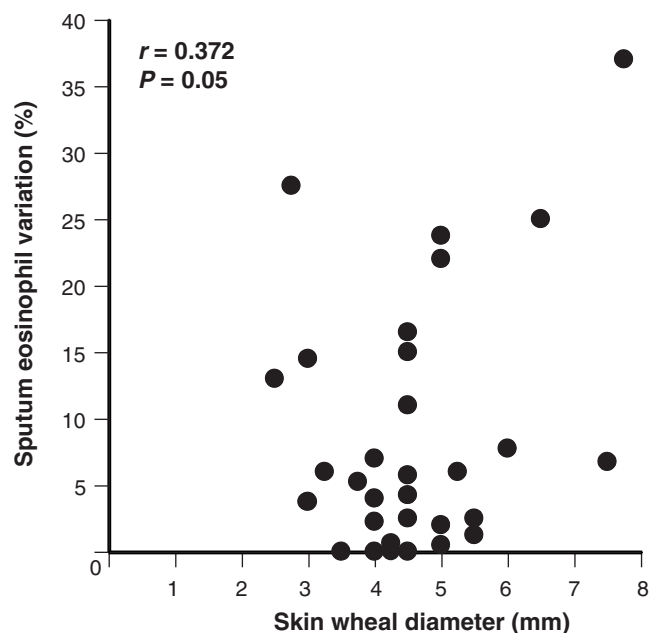


Figure 1. Correlation between the mean skin wheal diameter at titration for the inhaled concentration of allergen and the variation of sputum eosinophils at the LAR after conventional allergen challenge in asthmatic subjects compared with baseline.

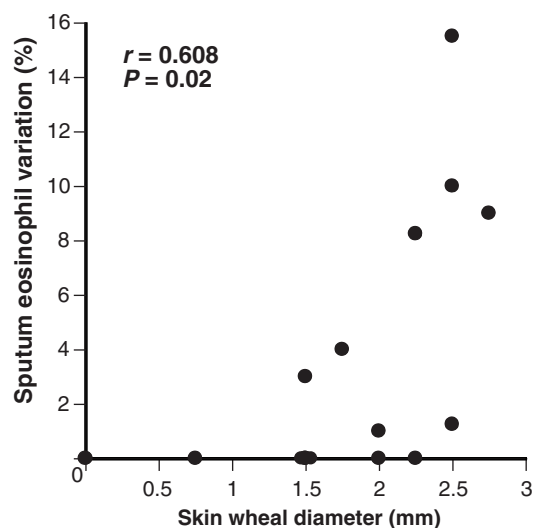


Figure 2. Correlation between the mean skin wheal diameter at titration for the inhaled concentration of allergen and the variation of sputum eosinophils following 2 days of low-dose allergen challenge in rhinitic subjects compared with baseline.

develop an equation to predict airway responsiveness depending on allergen concentration (18). It was therefore of interest to determine whether there was a correlation between cutaneous and inflammatory airway

responses. This study suggests that immune response to allergens, as assessed by the magnitude of the immediate skin response, is a significant determinant of allergen-induced airway eosinophilia. To our knowledge, such analysis of the correlation between skin and airway inflammatory responses to allergens has not been previously reported.

We observed a positive correlation between skin immune reactivity and airway inflammation in both rhinitic (following low-dose challenge) and asthmatic subjects (following conventional challenge), although it was more marked in the former group. This difference may be explained by factors such as the presence of airway obstruction in asthmatic subjects, which could contribute to reduced penetration of the antigen into the bronchial tree. The fact that the doses of allergen given during the low-dose challenge were much lower for asthmatic subjects than for rhinitic subjects could explain the lack of correlation in this group following low-dose allergen challenge. Therefore, a higher dose of allergen seems to be needed to induce more change in sputum eosinophils in asthmatic subjects. This could be due to the airway inflammation already present in the airways of asthmatic subjects as compared with rhinitic subjects. Indeed, the magnitude of the change in sputum eosinophils was smaller in asthmatic subjects than in rhinitic subjects. Nonallergic airway response could have influenced the dose of allergen given, but this should not affect the correlation between skin and airway inflammatory responses, as we compared responses for doses of allergen determined using the same equation. It seems, however, that the low doses of allergen induce significantly fewer changes in airway inflammation in asthmatic subjects with already some baseline airway inflammation than in rhinitic subjects, making the correlation between the two types of responses more difficult to observe with this type of challenge.

The magnitude of the skin response to allergens could help to assess the potential for a given allergen exposure to induce airway inflammation. This may provide an indirect, noninvasive method for predicting airway inflammatory responses, not only in asthmatic patients but also in nonasthmatic atopic subjects. It would be of interest to determine whether those with more marked skin responses are at increased risk of developing asthma in the next few years and what impact the type of allergen and current allergen exposure have on that risk. This could lead to earlier preventative measures in possible 'at risk' subjects.

In conclusion, this study demonstrates that there is a relationship between allergic immune response and lower airway inflammatory response in asthmatic and rhinitic atopic subjects as assessed by induced sputum analysis. Further studies are required to identify the determinants of allergen-induced airway inflammatory responses and the possibility to determine the risk of developing asthma in the future through assessment of cutaneous responses.

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