Skin tests, T cell responses and self-reported symptoms in children with allergic rhinitis and asthma due to house dust mite allergy

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Working title:

Correlation between allergen-induced inflammation and symptom severity in children with allergic rhinitis

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Summary

Background: In allergic responses a distinction is made between an early phase response, several minutes after allergen exposure, and a late phase response after several hours. During the late phase, eosinophils and T cells infiltrate in the mucosa and play an important role in inflammation.

Objective: To examine the relationship between allergen-induced late phase skin responses and *in vitro* T cell reactivity. In addition, the relationship between allergen-induced skin or T cell responses and the severity of self-reported symptoms was studied in children with house dust mite allergy.

Methods: A total of 59 house-dust-mite allergic children (6-18 years) were recruited in general practice. These children or their parents rated their nasal and asthma symptoms on diary cards during one month. Allergen skin tests were performed and read after 15 minutes (early phase) and 6 hours (late phase). Allergen-specific T cell proliferation was determined and Th_2 cytokine (IL-5 and IL-13) secretion was analyzed.

Results: The size of the late phase skin response correlated with *in vitro* T cell proliferation (r_s=0.38, p=0.003) but not with Th_2 cytokine secretion (r_s=0.16, p=0.2 both for IL-5 and IL-13). Moreover, the late phase skin response and T cell proliferation correlated with asthma symptoms (r_s=0.30, p=0.02 for skin response and r_s=0.28, p=0.03 for T cell proliferation) but not with nasal symptoms (r_s=0.19, p=0.15 for skin response and r_s=0.09, p=0.52 for T cell proliferation). The early phase skin response correlated with the nasal symptom score (r_s=0.34, p=0.01) but not with asthma symptom scores (r_s<0.005,p=0.97).

Conclusion: In this study the late phase skin test response correlated with *in vitro* T cell proliferation but not with Th_2 cytokine secretion. We found weak or absent correlations between late phase skin responses and symptoms of asthma or rhinitis in children with house dust mite allergy. This suggests that late phase skin responses reflect certain T cell properties but are of limited value for the evaluation of airway symptoms in atopic children.

Key words:

Allergic rhinitis, asthma, children, house dust mite, late phase response, symptom severity

Introduction

Allergic rhinitis (AR) is an inflammatory condition in which symptoms are not confined to an early phase response within minutes after allergen exposure, but may be present several hours after allergen contact (late phase response). Symptoms of the early phase response are sneezing, itching and rhinorrhea. During the late phase response congestion, fatigue, malaise, and irritability become more prominent. During this late phase, inflammatory cells such as eosinophils and T cells infiltrate the mucosa. Especially Th_2 cytokines, such as IL-4, IL-5 and IL-13, play a central role in the late phase allergic response. [1-3]

As in AR, early and late inflammatory changes in the bronchi of asthmatic patients, with influx of activated eosinophils and T cells, can be demonstrated.[4-6] Both nasal and bronchial mucosa are elements of a "united airway" and therefore AR and asthma are both manifestations of the same allergic disease.[4] As a consequence, asthma and rhinitis often coexist in the same patients. It appears that at least 80% of asthmatic patients suffer from rhinitis and around 20-30% of patients with AR also have asthma.[2,7]

In vivo skin testing or detection of *in vitro* allergen-specific responses can be helpful to test whether a patient with a relevant clinical history is allergic to a specific allergen. After intradermal injection of allergen in the skin of a sensitive subject a type-I reaction develops rapidly, peaks after 10-20 min, and subsides within a few hours. After several hours a late diffuse edematous response may appear at the allergen injection site.[8] *In vitro*, allergen-specific T cell responses and Th_2 cytokine production can be assessed.

In this study we hypothesized that the extent of the late phase skin response in house dust mite (HDM)-allergic children is associated with *in vitro* T cell activity to HDM, since these are both manifestations of the late phase inflammatory response. To test this hypothesis, we investigated skin responses after intradermal injection of HDM and determined allergen-induced T cell proliferation and Th_2 cytokine production. In addition, we examined the relationship between *in vivo* skin testing or *in vitro* T cell response and the severity of self-reported nasal or bronchial symptoms in children with a proven HDM allergy.

Methods and subjects

Patient selection

Fifty-nine children (aged 6 to 18 years) with allergic rhinitis and established HDM allergy were selected from electronic medical records in general practice. The inclusion criteria were: presence of specific IgE antibodies to HDM in serum (≥ 0.7 kU/l), a history of allergic rhinitis during at least 1 year, and a nasal symptom score of at least 4 out of 12 (see below). Before scoring symptoms, nasal corticosteroids were withheld for 4 weeks before the study period. The presence of asthma was assessed using the International Study of Asthma and Allergies in Childhood (ISAAC) core questionnaire.[9]

The study was approved by the ethical review board of Erasmus MC. All patients and / or their parents gave informed consent for the study.

Measurement of nasal or asthma symptoms

All participants or their parents scored their nasal symptoms on diary cards during a period of 1 month in October or in November. Nasal symptoms (sneezing, itching nose, watery running nose and nasal blockage) were scored on a 0-3 scale (0=none, 1=mild, 2=moderate, 3=severe). In total, a maximal daily cumulative nasal symptom score of 12 could thus be obtained. Children or parents scored their asthma symptoms (wheeze/breathless and dry cough during night) daily during 1 month on diary cards. In total, a maximum daily asthma symptom score of 6 (0-3 for wheeze/breathless and 0-3 for dry cough during night) could be obtained.

A mean symptom score was determined by calculating the mean daily score over the entire month. Only diaries with at least 15 out of 30 completely filled out pages were included in the analyses.

Skin testing

Allergy skin testing was performed by intracutaneous injection of 0.02 ml *Dermatophagoides pteronyssinus* in the forearm (concentration 30 U/ml, manufactured by ALK-Abelló, Nieuwegein, the Netherlands). We chose to perform an intracutaneous skin test rather than the usual skin prick test because intracutaneous injection of the allergen is the most feasible and convenient way to induce a late phase response after the early phase skin response.[8] As a positive control, histamine (concentration 0.01 mg/ml) was injected, negative control was dilution buffer. Reactions were read after 15 min (early response) and after 6 h (late response). The area of the skin response in mm^2 was measured by a specially developed scanning program. The early phase response was considered positive when its size was at least half the size of the histamine response (expressed as a Histamine-Equivalent-Intra-Cutaneous index or HEIC index). This cut-off value was determined by applying a modified algorithm proposed by Niemijer et al.[10] Late phase skin responses with a surface of more than 100 mm^2 were regarded positive, a surface of 50-100 mm^2 was considered borderline. Children were not allowed to take antihistamines within 24 h prior to skin testing.

HDM-specific T cell proliferation

Blood was drawn before skin testing. Peripheral blood mononuclear cells (PBMC) were isolated from heparinized blood by density centrifugation on Ficoll-Paque Plus (GE Healthcare, Uppsala, Sweden). PBMCs were used in a lymphocyte proliferation test (LPT). Cells were resuspended in complete medium (RPMI+HEPES+glutamax supplemented with gentamicin (Gibco, Gibco BRL, Life Technologies, Rockville, USA) and 5% heat inactivated human serum (Sanquin, Rotterdam, the Netherlands) and stimulated by culturing in the presence or absence of 2 IR/ml *D. pteronyssinus* (Stallergènes, France). All cell cultures were performed in quadruplicate in a final volume of 200 ml with a cell concentration of $2x10^6$ cells/ml in 96-well round bottomed microtitre plates. To determine HDM-specific proliferative responses, cells were cultured for 5 days at 37°C, 5% CO_2, 95% humidity. During the last 16 h, cells were pulsed with 0.5 µCi/well ^{3}H thymidine (Pharmacia, UK). Radioactivity was measured with a β-platereader and the proliferative capacity was assessed by the stimulation index (SI), calculated as the ratio of mean ^{3}H thymidine uptake in stimulated to non-stimulated cultures. The SI was considered positive when it exceeded 2.0.

HDM-specific cytokine production

To determine HDM-specific cytokine production, 1 ml of PBMCs with a concentration of $2x10^6$ cells/ml were cultured in duplicate in a 24-well plate with or without 2 IR/ml *D. pteronyssinus* (Stallergènes, France). After 5 days of culture, supernatants were harvested and stored at –20 °C until testing. IL-5, IL-

13, and IFN-γ cytokine production was measured following the manufacturer's instructions (for IL-5 and IFN-γ eBioscience, San Diego, USA; for IL-13 R&D Systems, Abingdon, UK). The lower detection limits for these cytokines are: 7.8 pg/ml for IL-5; 3.1 pg/ml for IL-13 and 12.5 pg/ml for IFN-γ. HDM-induced cytokine production was assessed by subtracting the cytokine concentration of non-stimulated from stimulated culture supernatants.

Measurement of exhaled NO

Exhaled nitric oxide (NO) was measured with a handheld FENO device (NIOX MINO, Aerocrine Solna, Sweden) in accordance with the manufacturers guidelines and literature references[11]. Exhalation duration was 10 seconds for all children.

Statistical analysis

Associations between variables were assessed using Spearman's correlation coefficient (r_s), since data were not normally distributed. A p-value of 0.05 (2-sided) was considered to be the limit of significance in all analyses. SPSS 11.0 for Windows was used for all analyses.

Results

Clinical characteristics of the study population

Baseline characteristics of the 59 children are summarized in Table 1. All children had elevated serum concentrations of HDM-specific IgE antibodies (mean 43.2 kU/l, range 0.7 to >100 kU/l). The mean daily nasal symptom score was 3.4 (range 0.3 to 8.8). Of the 59 children, 27 (46%) reported an earlier physician's diagnosis of asthma. However, 70% of children reported wheeze. The mean asthma symptom score was 0.9 (range 0 to 5.4) for the total group. Patients diagnosed as having asthma had an asthma symptom score of 1.4 whereas children without asthma had a score of 0.4 (p=0.002).

The early phase skin response to HDM was determined in 52 patients only. A positive response was detected in 51/52 patients (98%). Of the 59 children, 48 (81%) had a positive late phase skin response and 7/59 (12%) had a borderline positive late response.

Correlation between in vivo skin tests and in vitro T cell responses

Positive HDM-specific T cell proliferation responses were found in 51/59 (86%) and Th_2 cytokine production was found in 44/59 (75%) of children. The size of the early phase skin response showed a borderline significant correlation with T cell proliferation (r_s=0.25, p=0.07) and a significant positive correlation with cytokine secretion (r_s=0.3, p= 0.03 for both cytokines). The size of the late phase skin response correlated significantly with *in vitro* T cell proliferation (r_s=0.38, p=0.003, fig 1a) but not with cytokine secretion (r_s=0.16, p=0.2 both for IL-13 and IL-5, fig 1b).

The size of early phase and late phase skin responses demonstrated a clear positive correlation within subjects (r_s=0.51, p<0.001). *In vitro* T cell proliferation and cytokine production also showed a positive association (r_s=0.34, p=0.01 for IL-5 and r_s=0.36, p=0.01 for IL-13).

Correlation between skin responses and nasal or asthma symptoms

The early phase skin response correlated with the nasal symptom score (r_s=0.34, p=0.01; fig 2a) but not with asthma symptom scores (r_s<0.005, p=0.97, fig 2b), whereas the late response correlated with asthma symptoms (r_s=0.30, p=0.02, fig 2d) but not with nasal symptoms (r_s=0.19, p=0.15, fig 2c).

Correlation between T cell responses and nasal or asthma symptoms

Allergen-specific T cell proliferation was associated with asthma symptoms (r_s=0.28, p=0.03) but not with nasal symptoms (r_s=0.09, p=0.52). Cytokine production (both IL-5 and IL-13) did not correlate with nasal symptoms (r_s=0.14 for IL-5 and r_s=0.02 for IL-13) or bronchial symptoms (r_s=0.14 for IL-5 and r_s=0.15 for IL-13). Next to allergen-specific T cell responses and asthma symptoms, exhaled NO was determined as a measure for asthma disease severity. There was no correlation between the level of exhaled NO and disease severity (i.e. asthma symptom score, R_s=0.134, p=0.32). However, exhaled NO correlated with *in vitro* T cell proliferation (R_s=0.363, p=0.005) and Th_2 cytokine production (R_s=0.427, p=0.001 for IL-5 and R_s=0.350, p=0.007 for IL-13).

Discussion

The results of the present study show that in HDM-allergic children *in vivo* late phase skin responses correlate with *in vitro* T cell proliferation but not with Th_2 cytokine secretion. Correlations between skin tests and T cell responses on the one hand and atopic airway symptoms on the other were weak and inconsistent.

Allergen-specific T cells play an important role in the development of a late phase allergen response. A subset of Th_2 lymphocytes upon activation produce cytokines such as IL-13, IL-5 and IL-4.[12] Especially IL-5 is responsible for the cascade of eosinophil activation, whereas IL-13 and IL-4 are crucial in IgE production.[13] Whereas an association emerged between the extent of a late phase skin response and the level of the *in vitro* T cell proliferation, no correlation was found between the extent of the late phase skin reaction and production of Th_2 cytokines. So, whereas there is an involvement of allergen-specific T cells in the late phase response, we were not able to attribute this to Th_2 cells in particular.

Other studies report a lack of correlation between absolute amounts of IL-5 or IL-13 secretion and proliferative responses.[14,15] Perhaps a combination of qualitative (i.e. cytokine profiles) and quantitative responses (i.e. proliferation rate) serve to characterize the allergic responder phenotype.

It is possible that cytokines were derived from basophils rather than T cells. Recent evidence suggests that basophils are an important source of cytokines such as interleukin-4, perhaps more important than T cells. This would make

any correlation involving cytokine production and T cell activity difficult to interpret.

A correlation between late asthmatic response (LAR) and inflammation (i.e. allergen-specific proliferative response of T cells) has been reported.[16] However, no contribution of the late cutaneous response was found in association with LAR or inflammation; the lack of a relationship between allergen-induced late inflammatory response in the skin and airways in the latter study could be explained by variations in measurement.

Very few studies have addressed the association between clinical symptoms and the outcome of *in vivo* and *in vitro* tests. In two studies the severity of asthma appeared to be associated with the number of positive skin tests and the magnitude of the immediate skin reaction[17] or with total and specific IgE.[18] In seasonal allergic rhinitis skin tests do not predict symptom severity in the pollen season.[19]

More research has focused on the relationship between *in vivo* or *in vitro* tests and allergen-specific bronchial[16,20-22] or nasal[22] airway reactivity.

In line with previous challenge studies[16,21] our results suggest that asthmatic complaints are related to late inflammatory changes, i.e. the level of allergen-specific proliferative response of T cells *in vitro* and the late phase cutaneous response *in vivo*. In our study, the early skin response is not correlated with asthmatic complaints, but with nasal symptoms, whereas the late phase cutaneous reaction was not related with rhinitis severity. Also, the nasal allergic reaction is better predicted by immediate skin test than by late skin reactions.[23] However, all challenge studies demonstrate that non-specific airway reactivity is

more important in predicting airway reactivity to allergens than indices of allergic sensitization. This might, in part, explain the weak correlations found in the present study.

Whereas in both AR and asthma early and late phase responses play an important role,[24] it seems feasible that sneezing and itching are features of an early phase response, and that during the late phase response asthma and nasal congestion are more prominent. During the early phase response, histamine and leukotrienes are important mediators that are generated from resident airway cells. Therefore, antihistamines and anti-leukotrienes are effective against direct complaints of the nose and the early skin response.[5]

Limitations of the study

Correlations in our study were significant but not strong. This might be explained by the fact that the complaints of patients are determined by many factors, such as degree of sensitization, allergen exposure, non-specific airway hyperresponsiveness, experience, use of specific medication, and symptom perception. Since the interaction between all these variables influences the outcome, we would not expect a high correlation coefficient.

Another explanation might be that children were allowed to take symptomatic asthma or allergy medication in case of complaints. This might result in a decrease of self-reported symptoms, and a weaker correlation between T cell responses and symptoms.

In conclusion, we have shown a correlation between late phase allergen-induced skin responses and T cell proliferation, but not Th_2 cytokine secretion. Elevated levels of Th_2 cytokines did not correlate with the late phase response or with clinical symptoms. We speculate that both late phase skin tests and in vitro T cell tests reflect immunological abnormalities in atopic children, but that other mechanisms determine the severity of reported atopic symptoms.

Acknowledgments

The authors thank all the children and their parents for their participation; Ellen Duijster-Kunst (Dept. of Allergology, Erasmus MC) for taking blood samples and performing skin tests; Margaretha Lambers and Danielle Hijdra (Dept. of Lung Diseases, Erasmus MC) for conducting the lymphocyte proliferation tests and cytokine analysis, and the Dept. of Clinical Chemistry (Erasmus MC) for conducting the RAST tests.

Funding and conflict of interest

The study was sponsored by Artu-Biologicals, Lelystad, The Netherlands. The Authors declare that they have no conflict of interest.

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Table 1: Characteristics of the study population

	n = 59
Age - years (Mean, SD)	11.6 (2.9)
Gender - Male (n, %)	28 (48%)
Weight - kg (Mean, SD)	47 (14)
Height - cm (Mean, SD)	153 (16)
FeNO - ppb (Mean - Range)	38 (4-173)
Allergy / Asthma symptoms	
Nasal symptom score (Mean, Range)	3.4 (0.3 - 8.8)
Asthma symptom score (Mean, Range)	0.9 (0.0 - 5.4)
In vivo HDM specific skin test	
Early skin test - HEIC (Mean, Range)	1.7 (0.4 - 3.3)
Late skin test - mm^2 (Mean, Range)	871 (0 - 2728)
In vitro HDM specific allergy test	
specific IgE - kU/L (Mean, Range)	43.2 (0.7 - >100)
Proliferation - SI (Mean, Range)	6.8 (0.8 - 36.6)
IL-5 - pg/mL (Mean, Range)	893 (0 - 11278)
IL-13 - pg/mL (Mean, Range)	849 (0 - 5433)

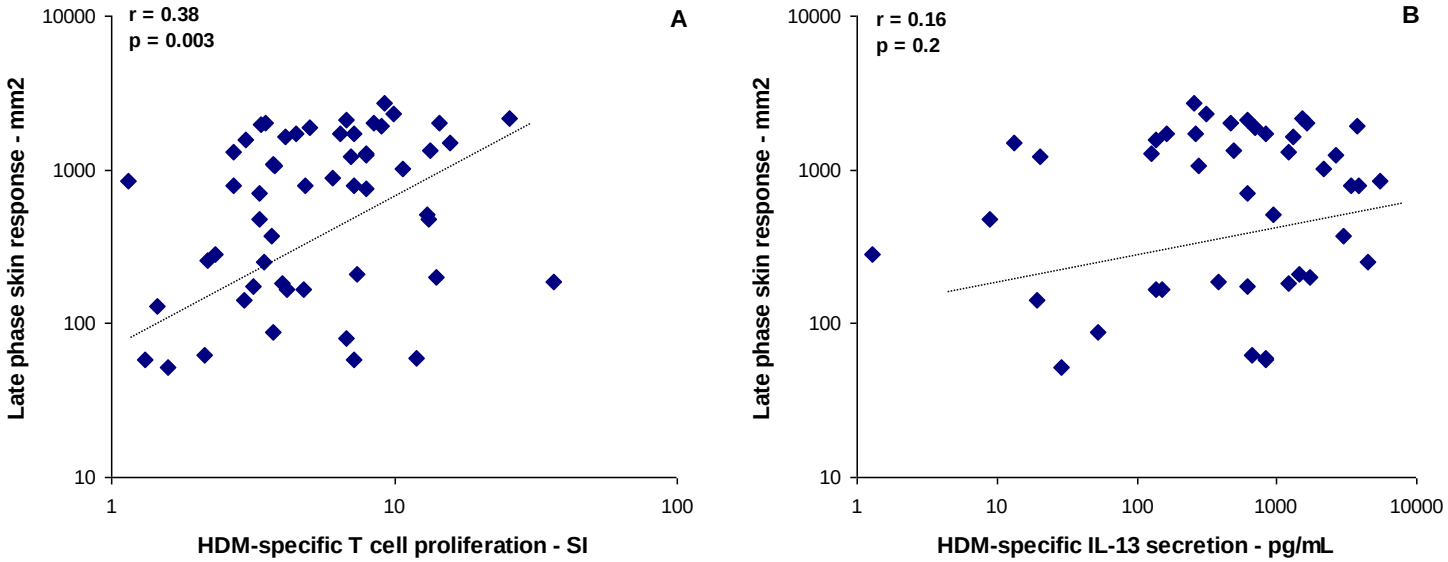

Figure 1: Correlation between *in vivo* late phase skin response and *in vitro* T cell proliferation (A) and IL-13 secretion (B).

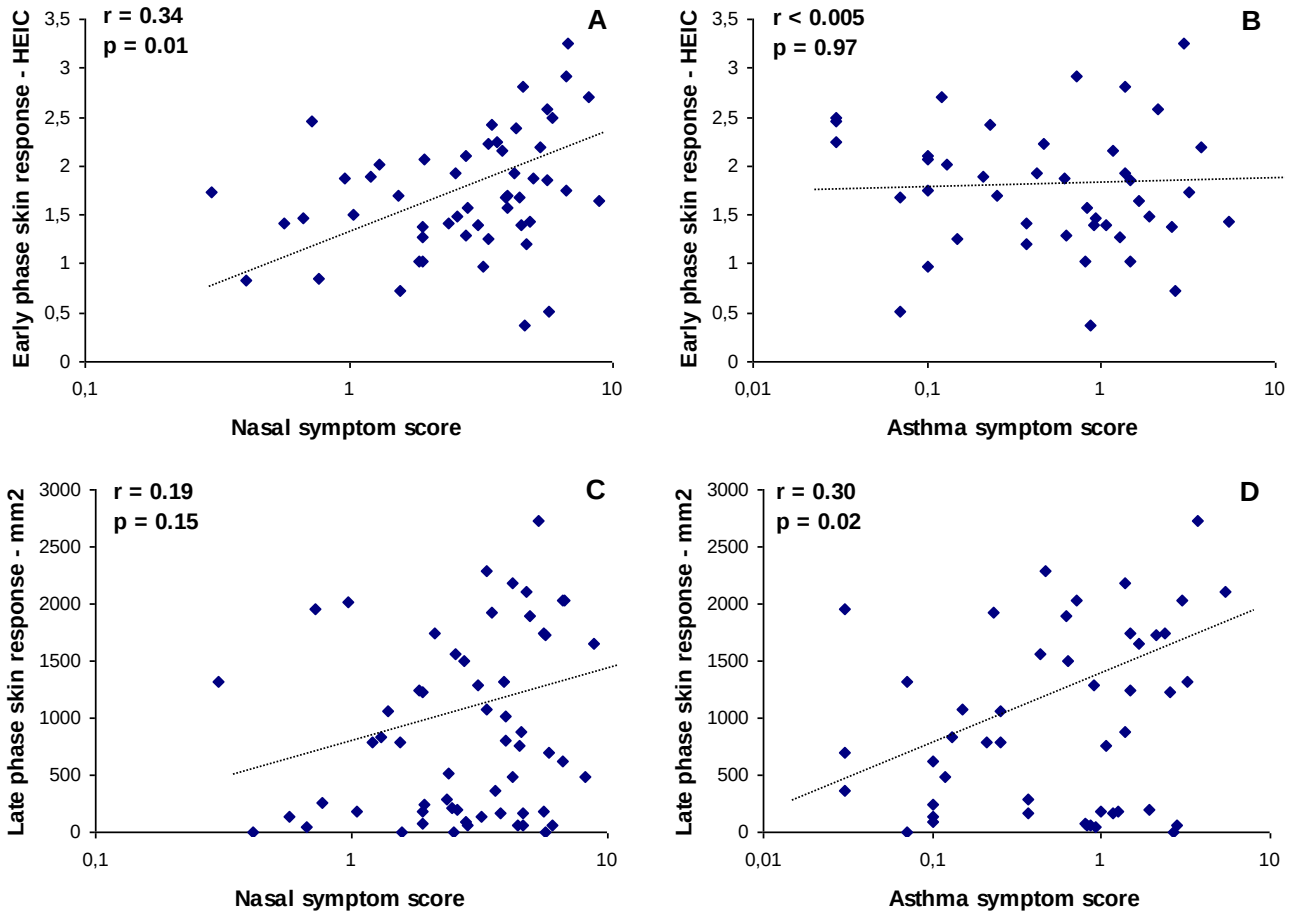

Figure 2: Correlation between nasal / asthmatic symptoms and *in vivo* early / late phase skin response.