

## Comparison of Allergen-Induced Late Inflammatory Reactions in the Nose and in the Skin in House Dust Mite-Allergic Patients with or without Asthma

Christa E. Lopuhaä<sup>a</sup> Mariëlle J. de Riemer<sup>b</sup> Theo A. Out<sup>b</sup>  
Deman H.S. Sjamsoedin<sup>c</sup> Rob C. Aalberse<sup>d</sup> Henk M. Jansen<sup>a</sup>  
Jaring S. van der Zee<sup>a</sup>

Departments of <sup>a</sup>Pulmonology, <sup>b</sup>Experimental Immunology and <sup>c</sup>Otorhinolaryngology, Academic Medical Center, and <sup>d</sup>CLB Sanquin Blood Supply Foundation, Amsterdam, The Netherlands

### Key Words

Asthma · Rhinitis · Nasal allergen challenge ·  
House dust mite · Late-phase inflammation

### Abstract

**Background:** It remains to be established which factors contribute to the occurrence of asthma in allergic individuals. We hypothesized that differences in the late allergic inflammatory reaction to allergen between asthmatic and non-asthmatic house dust mite-allergic individuals might contribute to the difference in the clinical presentation of allergy. **Aim:** To compare allergen-induced changes in parameters for cellular inflammation during the phase of the late allergic reaction in the skin and nose, in house dust mite-allergic individuals with or without asthma. **Material and Methods:** Nasal and dermal allergen challenges with house dust mite (*Dermatophagoides pteronyssinus*) extract were performed in 52 house dust mite-allergic individuals, of whom 26 had mild to moderate persistent asthma and 26 had perennial rhinitis without current or past asthmatic symptoms. Serial nasal lavage samples were analyzed for the pres-

ence of inflammatory cells (eosinophils and neutrophils) and soluble markers associated with cellular inflammation [interleukin-5 (IL-5), interleukin-8 (IL-8), eosinophil cationic protein (ECP) and myeloperoxidase (MPO)]. Macroscopic late phase skin reactions were studied after intracutaneous skin tests with house dust mite extract.

**Results:** Fixed dose nasal allergen provocation elicited a similar degree of immediate allergic reaction as judged by plasma protein exudation and histamine concentrations in asthma and non-asthmatic rhinitis. Subsequently, no differences between groups were found during the phase of the late allergic reaction (4–24 h) in inflammatory cell influx, plasma protein leakage, ECP or MPO. Likewise, there were no differences in levels of chemotactic cytokines IL-5 and IL-8. In agreement with the results of nasal challenge, the late skin reaction after dermal challenge with a fixed allergen dose and after an allergen dose 10,000 times above the skin threshold for an early skin reaction did not differ between the groups. **Conclusion:** House dust mite-allergic patients with or without asthma have very similar late allergic inflammatory reactions in the skin and in the nose after allergen challenge. Hence, it is unlikely that the occurrence of pulmonary

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Correspondence to: Dr. J.S. van der Zee  
Department of Pulmonology, F4-208  
Academic Medical Center, PO Box 22700  
NL–1100 DE Amsterdam (The Netherlands)  
Tel. +31 20 566 4356, Fax +31 20 691 7584, E-Mail j.s.vanderzee@amc.uva.nl

symptoms in asthma is explained by a general tendency of asthmatics to have an enhanced late allergic cellular inflammatory response. Nasal and dermal allergen provocations are adequate models to study allergen-induced inflammation but probably lack the pivotal link which is essential for the development of asthma.

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## Introduction

Inhalant allergy plays an important causal role in the development of asthma and rhinitis. Although allergic sensitization is demonstrable in 30–40% of the population in the western industrialized countries, only 5–10% suffer from allergic asthma and 10–20% suffer from allergic rhinitis. The vast majority of allergic asthmatic patients also experience symptoms of allergic rhinitis [1, 2]. By contrast, allergic rhinoconjunctivitis is not necessarily accompanied by asthmatic symptoms although this group may have an increased chance of developing symptoms of the lower airways. Increased non-specific bronchial hyperresponsiveness is reported for the group of allergic rhinitis, but this is less pronounced than in asthma [3, 4]. Likewise, inflammatory changes in the bronchi similar to the bronchial inflammation found in asthmatics are demonstrable in patients with allergic rhinitis without asthma [5]. Results of studies on quantitative differences in bronchial inflammation between allergic asthma and non-asthmatic rhinitis remain inconclusive [6–8]. Bronchial allergen challenge is able to induce early bronchoconstriction in rhinitics as well as in asthmatics [9, 10]. However, it is generally found that a late asthmatic reaction, which is associated with an increase in cellular inflammation in the bronchi, occurs more frequently and is more pronounced in asthma. It was our study hypothesis that differences between asthmatic and non-asthmatic allergy might be associated with differences in the late cellular inflammatory response to allergen. To test this hypothesis we performed a prospective experimental study to investigate allergen-induced inflammation in the bronchi, in the nose and in the skin in house dust mite (HDM)-allergic patients with or without asthma. Here, we describe the results of the nasal and dermal allergen provocation tests. The results of the bronchial allergen provocations are published elsewhere [11]. Intracutaneous skin provocation was done with a fixed allergen dose as well as with an allergen dose titrated to a standardized early skin reaction. Primary outcome of the skin provocation test was the magnitude of the dermal infiltration after 7 h.

Nasal provocation was performed with a fixed dose of HDM extract for all individuals and the reaction was monitored by measuring changes in repeated nasal lavages. Primary outcomes were levels of interleukin-5 (IL-5), IL-8, eosinophil cationic protein (ECP), myeloperoxidase (MPO) and cell differentials obtained during the phase of the late allergic reaction (4–24 h). Early protein leakage ( $\alpha_2$ -macroglobulin, A2M) and histamine levels were monitored as an indicator for early mast cell degranulation in the nose [12–15]. The study was designed to detect a 25% difference in late skin reactivity and a 30% difference in log-normalized levels of soluble markers with a power of 0.80 and a 95% confidence level.

## Materials and Methods

### Patients

Patients with asthma, diagnosed according to the American Thoracic Society (ATS) criteria, were recruited from the Outpatient Department of Pulmonology, and had a documented history of recurrent episodes of wheezing, chest tightness and dyspnoea and a normal lung function between asthmatic attacks [16]. Patients with perennial rhinitis were recruited from the Outpatient Department of Otorhinolaryngology and were characterized by episodes of sneezing, watery rhinorrhoea, pruritus in the nose, eyes and palate and nasal obstruction [17]. Patients with non-asthmatic perennial rhinitis were only included if they did not have a history of current or past dyspnoea or wheezing, use of bronchodilators or a doctor's diagnosis of asthma.

The following inclusion criteria applied to all participants: (1) FEV1  $\geq$  70% of predicted value; (2) specific IgE level to HDM  $>0.5$  IU/ml; (3) able to stop short-acting  $\beta_2$ -adrenoceptor agonists for at least 8 h, oral antihistamines for 2 weeks and inhaled corticosteroids for 6 weeks prior to the start of the study; (4) no significant change in environmental allergen exposure (e.g. not moved) during the past 12 months, and (5) whenever co-sensitization against pollen was present, patients were studied outside the relevant season.

Exclusion criteria for all were (1) history of immunotherapy, (2) respiratory tract infection 6 weeks prior to the study, (3) immunosuppressive medication, and (4) smoking. The AMC Medical Ethics Committee approved the study, and all subjects gave written informed consent.

### Study Design

Prior to the inclusion all subjects were screened by the radio-allergosorbent test (RAST) and the skin prick test with a standard panel of aero-allergens and by spirometry.

Corticosteroids were withheld for 6 weeks and antihistamines for 2 weeks before the study period. On the first day of the study intracutaneous tests were performed and blood samples were obtained. The next day nasal challenge with diluent was followed by allergen challenge 1 h later. Changes induced by diluent challenge were monitored by three nasal lavages during the 1st hour. Similarly, allergen challenge was followed by three lavages in the 1st hour and subsequently by lavages every hour for 8 h. Twenty-four hours after allergen challenge nasal lavage and venepuncture were repeated.

### Allergen Extract

A standardized freeze-dried *Dermatophagoides pteronyssinus* extract (ALK Diephuis, Houten, The Netherlands) was dissolved in phosphate-buffered saline, pH 7.4 (PBS), 0.03% human serum albumin (HSA), 0.5% phenol (ALK Diephuis) at 1 mg/ml [equivalent to 10<sup>6</sup> SQ (ALK Standard Quality) units/ml and 50,000 BU (biological units)/ml] and was kept at -20°C in small aliquots. This allergen batch was used for all assays, and contained 85 µg Der p 1 and 9.7 µg Der p 2/mg of protein. Allergen dilutions for tests in vivo and in vitro were made freshly from stock. For nasal allergen challenge it was diluted in PBS, 0.03% (w/v) HSA, 0.0125% (w/v) benzalkonium chloride (ALK Benelux, Houten, The Netherlands).

### Dermal Allergen Challenge

Tenfold dilutions of the extract and 30 BU/ml were administered intracutaneously into the forearm as described before [18]. In addition, diluent control and histamine dihydrochloride at 0.1 mg/ml were tested. After 15 min, wheal and flare reactions were determined by marking both reactions and copying the marks on a form via an adhesive tape. Results were summarized by the sum of the largest diameter and its orthogonal diameter. At 7 h the late skin induration was quantified by the sum of the largest diameter and the orthogonal diameter. Late phase reactions were evaluated for 30 BU/ml and for the allergen concentration 10,000 times above the threshold concentration for eliciting an early skin reaction.

### Nasal Allergen Challenge

Nasal allergen challenge and repeated nasal lavages were performed according to the method described by Naclerio et al. [19]. For lavage, both nostrils were instilled with 5 ml of 0.154 M NaCl at 37°C with the patient sitting with the head in 45° anteflexion. After 10 s dwell time the fluid was collected in a polypropylene tube and immediately put on ice. The challenge protocol started with 4 baseline nasal washings and subsequent application of oxymetazoline 0.05%, 0.0125% benzalkonium chloride dissolved in NaCl 0.9% to limit mucosal swelling. Subsequently, diluent fluid (0.15 ml) was sprayed into both nostrils as a control challenge and nasal lavages were performed after 20, 30 and 40 min. Then, allergen challenge was performed with 0.15 ml of 10,000 BU/ml in each nostril. Nasal lavage was repeated 20, 30 and 40 min after application of allergen. Thereafter, nasal washings were done hourly from 3 to 8 h and again 24 h after challenge. During the procedure the subjects were allowed to wipe their noses but were asked not to blow them.

### Sample Processing

The lavage fluids were centrifuged for 5 min at 700 g. The supernatant was separated from the cells and stored in aliquots at -20°C. The cell pellet was resuspended in PBS and an equal volume of dithiothreitol (DTT) solution (10 mM DTT in 135 mM Tris buffer, pH 8.0) was added followed by mixing the sample in a shaking water bath for 15 min at 37°C (DTT; Sigma Chemical Company, St. Louis, Mo., USA). After centrifugation for 5 min at 700 g, the cell pellet was resuspended in 1 ml PBS containing 0.5% HSA and cells were counted in a Bürker counting chamber. Cells were then cytocentrifuged at 550 rpm for 2 min in a Shandon Cytocentrifuge and stained with Romanovsky (Diff-Quick) and Jenner Giemsa. All differential cell counts were performed by one investigator blinded for patient identity and sample time point. Epithelial cells (squamous and non-squamous), neutrophils, eosinophils, lymphocytes and macrophages were identified. A total of 500–1,000 cells were counted, depending

on the percentage of eosinophils. Due to low cell numbers in repeated lavages data from samples 4–24 h after challenge were pooled.

### Protein Assays

Levels of ECP were determined in the nasal lavage supernatants with an ELISA described previously [20]. The detection limit was 15 pg/ml. Assay reagents (rabbit-anti-human ECP antiserum and anti-ECP antibody-biotin) were kindly donated by Dr. A. Zuurbier (CLB Sanquin, Amsterdam, The Netherlands). A standard dilution curve of ECP was derived from Pharmacia & Upjohn (Uppsala, Sweden). MPO [21], IL-5 [22], IL-8 [23] and A2M [24] were measured by ELISA as described earlier. Histamine was measured with an automated fluorometric analysis as described by Siraganian [25]. Total serum IgE was quantified by a binding and an inhibition assay, as described before [26]. Levels of specific IgE against HDM (*D. pteronyssinus*) were measured in duplicate with the RAST [26]. The results were expressed in international units per millilitre using an in-house standard, calibrated against the World Health Organization standard [27].

### Statistical Analysis

SPSS, version 10.0 Statistics UK (Chicago, Ill., USA), was used for statistical analyses. The analysis of the ECP, MPO, IL-5 and IL-8 during the late allergic reaction was done with the average of the values measured at 4, 6 and 8 h after challenge in addition to the results on separate time points. When applicable data were log-transformed to obtain normal distribution of data. Data of primary end points are reported by the mean and standard error of the mean (SE). Within-group changes were evaluated with paired t test or Wilcoxon test (IL-5). Between-group comparisons were done with t test and analysis of variance controlling for the level of specific IgE to HDM. The Mann-Whitney test was done in the absence of a (log)-normal distribution of data (IL-5 and skin reactions). Comparison of groups with respect to allergen-induced changes in parameters was done with the analysis of covariance with postchallenge values as dependent variable, the patient group as fixed factor and baseline values as a covariate. Associations between parameters were determined with the Spearman rank test. All reported p values are two-tailed and p levels of <0.05 are considered significant.

## Results

A summary of demographic and clinical data of both patient groups is presented in table 1. There was no difference in mean age and total IgE between the groups. The amount of IgE directed against HDM was slightly lower in the non-asthmatic group. Adjustment for this parameter was added to the statistical analysis of differences in the primary outcomes between groups. As expected, there was a significant difference in baseline FEV1 and histamine thresholds between asthmatics and non-asthmatic patients.

### Nasal Challenge

Mean recovery of the 10 ml lavage fluid was similar in asthmatics and non-asthmatics [7.7 ml (SD 0.9) vs. 7.6 ml

(SD 0.9),  $p > 0.2$ ]. The baseline eosinophil and neutrophil counts and levels of A2M, ECP, MPO, IL-5 and IL-8 were similar in the nasal washings of asthma and rhinitis without asthma (table 2).

The repeated nasal lavage procedure caused some wash-out effect of soluble markers and cells. This is shown by a gradual decrease in the concentrations of the soluble markers during the initial baseline lavages and after diluent challenge. It generally resulted in levels at 40 min after the diluent challenge of about 10–15% of the initial baseline lavage. Extended control procedures during 24 h after diluent challenge in a limited number of patients indicated a slight further decrease in soluble markers up to 40 min after the sham challenge. This was followed by a gradual return to values immediately before sham challenge at 6 h and to baseline values at 24 h (fig. 1). Therefore, 24-hour lavages, with no preceding lavage for 16 h, were compared with the initial baseline lavages and these results are summarized in table 2. For lavages up to 8 h after allergen challenge the lavage 40 min after the diluent

**Table 1.** Patient characteristics

|                                       | Rhinitis without asthma | Asthma        |
|---------------------------------------|-------------------------|---------------|
| Gender (male/female)                  | 6/20                    | 6/20          |
| Age (mean, range), years              | 27 (18–37)              | 27 (18–40)    |
| FEV1 <sup>a</sup> , % predicted       | 103.2 (12.7)            | 94.7 (14.6)** |
| Pc20 histamine <sup>b</sup> , mg/ml   | 9.6 (2.7)               | 1.2 (4.0)**   |
| Perennial rhinitis symptoms, %        | 100                     | 73            |
| Total IgE <sup>b</sup> , IU/ml        | 126.1 (3.8)             | 243.4 (3.8)   |
| HDM-specific IgE <sup>b</sup> , IU/ml | 13.0 (3.4)              | 26.6 (2.8)*   |
| <i>RAST results<sup>c</sup></i>       |                         |               |
| Grass pollen, % pos                   | 50                      | 50            |
| Tree pollen, % pos                    | 46                      | 31            |
| Cat or dog dander, % pos              | 23                      | 50            |
| HDM mono-allergy, % pos               | 31                      | 38            |

\*  $p < 0.05$ ; \*\*  $p < 0.01$ .  
<sup>a</sup> Mean and standard deviation.  
<sup>b</sup> Geometric mean and standard deviation.  
<sup>c</sup> Percentage of patients with positive test results.

**Table 2.** Analysis of changes in nasal lavage from baseline to 24 h postchallenge

|                                               | Rhinitis without asthma (n = 26) |                   |                      | Asthma (n = 26)       |                   |                      | Non-asthmatics vs. asthmatics |                   |
|-----------------------------------------------|----------------------------------|-------------------|----------------------|-----------------------|-------------------|----------------------|-------------------------------|-------------------|
|                                               | baseline                         | 24 h <sup>a</sup> | p value <sup>b</sup> | baseline <sup>a</sup> | 24 h <sup>a</sup> | p value <sup>b</sup> | baseline <sup>c</sup>         | 24 h <sup>d</sup> |
| Eosinophils <sup>e</sup> 10 <sup>4</sup> /l   | 2.2 (1.6)                        | 19.9 (1.4)        | 0.001                | 3.8 (1.7)             | 14.8 (1.5)        | 0.004                | >0.2                          | >0.2 (>0.2)       |
| ECP, ng/ml                                    | 2.20 (1.3)                       | 4.32 (1.4)        | 0.003                | 1.6 (1.3)             | 4.0 (1.4)         | 0.002                | >0.2                          | >0.2 (>0.2)       |
| IL-5, pg/ml                                   | <0.1                             | 1.53 (0.7)        | 0.02                 | <0.1                  | 1.51 (0.5)        | 0.04                 | >0.2                          | NA (>0.2)         |
| Neutrophils <sup>e</sup> , 10 <sup>5</sup> /l | 2.5 (1.5)                        | 8.1 (1.4)         | 0.003                | 1.4 (1.6)             | 7.7 (1.5)         | 0.001                | >0.2                          | >0.2 (>0.2)       |
| IL-8, pg/ml                                   | 136.0 (1.2)                      | 140.0 (1.1)       | >0.2                 | 95.1 (1.2)            | 94.9 (1.2)        | >0.2                 | >0.1                          | >0.2 (>0.1)       |
| MPO, ng/ml                                    | 75.5 (1.2)                       | 72.9 (1.2)        | >0.2                 | 45.9 (1.3)            | 38.2 (1.3)        | >0.2                 | >0.1                          | >0.2 (>0.05)      |
| A2M, ng/ml                                    | 503.6 (1.2)                      | 642.7 (1.3)       | >0.2                 | 404.7 (1.3)           | 490.1 (1.4)       | >0.2                 | >0.2                          | >0.2 (>0.2)       |

Within-group comparison: paired t test (Wilcoxon-signed ranks for IL-5); between-group comparison: unpaired t test (baseline and unadjusted 24 h) and analysis of covariance (24 h adjusted for baseline) signifying differences in changes from baseline (Mann-Whitney test for IL-5). NA = Not available.

<sup>a</sup> Values represent mean with SE in parentheses, geometric mean and SE for ECP, IL-8, MPO, A2M.

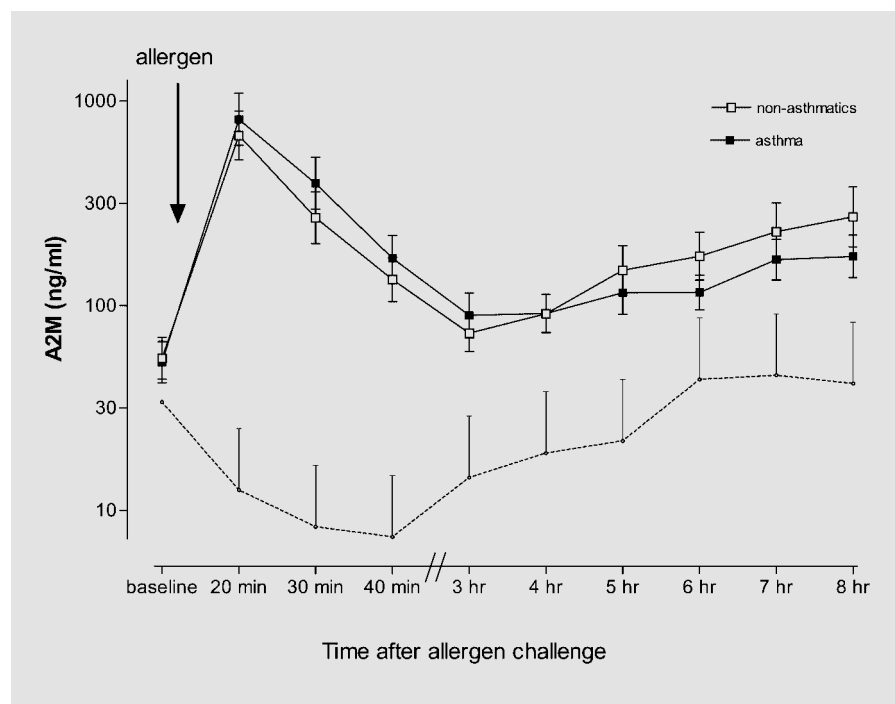
<sup>b</sup> Values represent 24 h versus baseline.

<sup>c</sup> Values represent p values between groups.

<sup>d</sup> Values represent p values adjusted for baseline with unadjusted values in parentheses.

<sup>e</sup> Pooled cell data from baseline lavages and from lavages 4–24 h after challenge.

**Fig. 1.** Level of A2M in nasal lavages after nasal allergen challenge. Baseline was level after diluent challenge. - - - = Geometric mean (SE) of A2M values after diluent sham challenge in 3 patients. There was a significant increase in A2M during the early allergic reaction (20–40 min, both groups  $p < 0.0001$ ) and during the phase of the late allergic reaction (4–8 h) in both groups (both groups  $p < 0.001$ ), relative to baseline as well as relative to controls after sham challenge. There were no significant differences between asthmatic and non-asthmatic patients during early phase and late phase (all  $p$  values  $> 0.1$ ).



challenge and immediately before allergen challenge was considered the baseline and these results are summarized in figures 1 and 2. In none of the patients was plasma protein exudation found during the 1st hour after diluent challenge. During the 1st hour after allergen administration A2M levels increased in all patients and to a similar extent in both groups (fig. 1). In contrast, HDM challenge did not induce an increase in A2M in patients without sensitization to HDM, excluding a significant non-immunological effect of the HDM extract on the nasal mucosa.

A2M expressed as area under the curve (AUC) during the 1st hour after allergen challenge was not significantly different between the two groups (fig. 1). This was not changed by adjustment for specific IgE. Similarly, histamine levels during the early allergic reaction did not differ between asthmatics and non-asthmatics [mean (SE): asthma 0.76 ng/ml (0.10), non-asthmatics 0.81 ng/ml (0.12),  $p > 0.2$ ].

#### *Changes in Markers of Inflammation during the Phase of the Late Allergic Reaction*

Plasma protein exudation as detected by the level of A2M showed a second increase during the phase of the late allergic reaction with a maximum 8 h postchallenge in both groups (fig. 1). The latter increases were statistically significant in the non-asthmatics as well as in the asthma

group (both  $p < 0.001$ ). At 24 h after the challenge levels there was no statistically significant difference with baseline values (table 2). The course of A2M as assessed by the AUC from 2 to 8 h was not different between the two groups. This was unaffected by adjustment for IgE directed against HDM. In addition, there was no difference between the subgroups of asthmatic patients with or without rhinitis symptoms.

There were increases in IL-5 and ECP in nasal washings during the period 4–8 h after allergen challenge which were statistically significant in both groups (fig. 2). At 24 h postchallenge both IL-5 and ECP levels were still elevated compared to baseline values (table 2). The increases at 4–8 and 24 h after challenge were statistically indistinguishable for asthma and rhinitis without asthma.

The levels of IL-8 and MPO also increased significantly in both groups between 4 and 8 h after challenge compared to levels after diluent challenge (fig. 2). In contrast to IL-5 and ECP, IL-8 and MPO levels had returned to baseline levels after 24 h (table 2). Again, no difference was found between the groups at any of the time points studied.

Due to low cell numbers in the repeated nasal washings data for influx of inflammatory cells were pooled for 4–24 h. There was a significant increase in both groups for eosinophil and neutrophil numbers compared to baseline

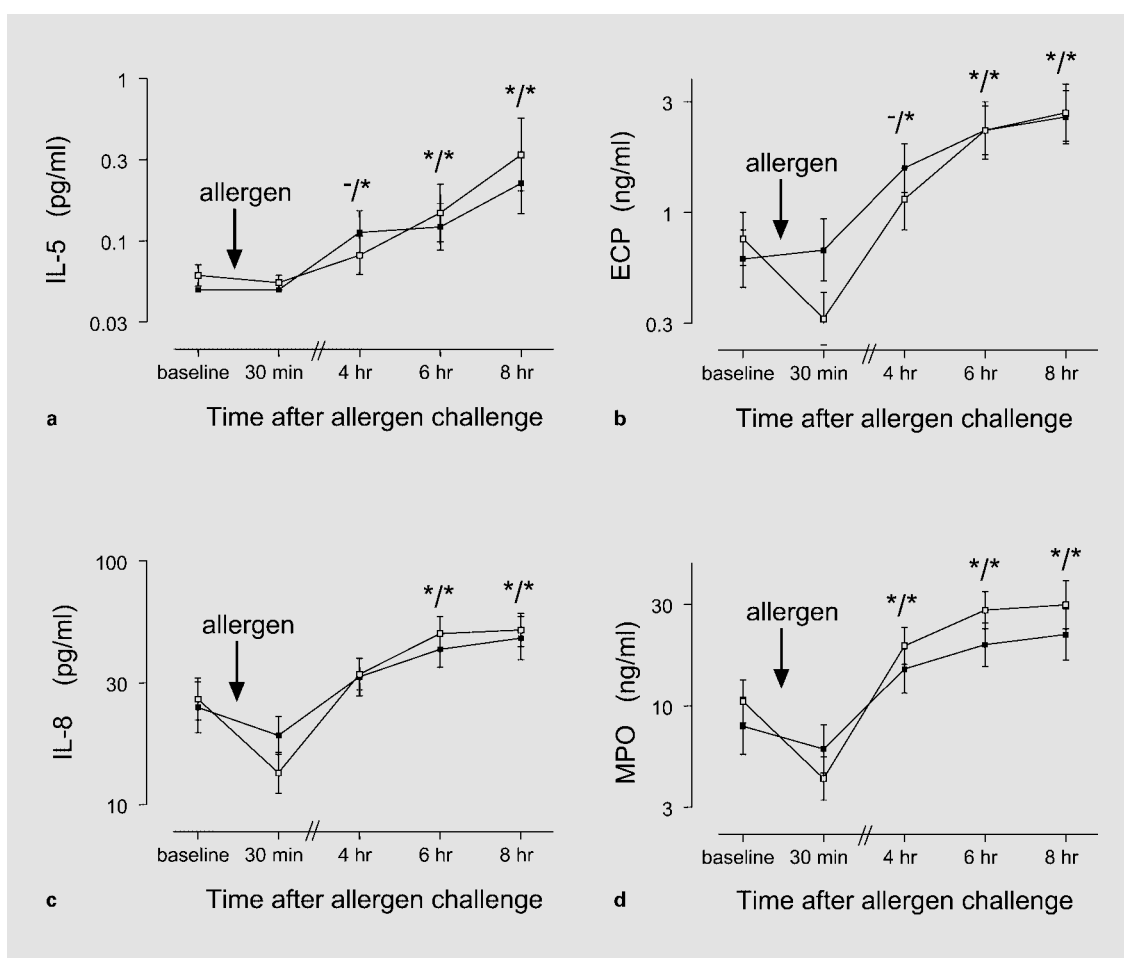


**Fig. 2.** Concentrations of IL-5 (**a**), ECP (**b**), IL-8 (**c**) and MPO (**d**) in nasal washings during the first 8 h after nasal allergen challenge. Baseline was level after diluent challenge. ■ = Asthma; □ = non-asthmatic rhinitis. \* $p < 0.05$  indicates increases compared to baseline levels within the non-asthmatic rhinitis and asthma groups. There were no significant differences in these parameters between both groups at any of the time points studied. Error bars indicate standard error of the mean.

numbers, but no significant difference between groups (table 2). Results were not affected by controlling for the level of specific IgE to HDM.

#### Correlation between Outcomes

IL-5 early during the late phase (4–6 h) was associated with late-phase levels of ECP ( $R_s = 0.46$ ,  $p = 0.001$ ), A2M ( $0.40$ ,  $p = 0.003$ ) and eosinophil numbers ( $R_s = 0.47$ ,  $p = 0.001$ ), but was not associated with neutrophils ( $p = 0.6$ ) and MPO ( $p = 0.16$ ). An early rise in IL-8 (4–6 h) was associated with late-phase levels of MPO ( $R_s = 0.72$ ,  $p < 0.0001$ ), A2M ( $R_s = 0.42$ ,  $p = 0.002$ ), ECP ( $R_s = 0.47$ ,  $p = 0.001$ ) and showed a trend for an association with post-

challenge neutrophil numbers ( $R_s = 0.26$ ,  $p = 0.07$ ), but no association with eosinophil numbers ( $p = 0.9$ ).

#### Dermal Challenge

The allergen threshold concentration for a positive early skin reaction was significantly lower in asthma than in non-asthmatic rhinitis [geometric mean (SE) asthma  $0.09$  BU/ml ( $1.4$ ), non-asthmatics  $0.21$  BU/ml ( $1.3$ ),  $p = 0.04$ ]. However, after correction for the difference in the level of HDM-specific IgE the difference between the groups disappeared ( $p = 0.3$ ). Skin reactions with histamine were very similar in both groups [median (IQR) flare reaction: asthma  $8.0$  cm ( $2.3$ ), non-asthmatics  $7.5$  cm

(1.5); wheal reaction: asthma 2.3 cm (0.5), non-asthmatics 2.0 cm (0.5),  $p > 0.2$ ]. Early skin reaction with HDM showed no differences in size of the wheal and flare reactions between the asthmatics and non-asthmatics, neither after a challenge with 30 BU/ml [median (IQR) flare reaction: asthma 10.3 cm (3.6), non-asthmatics 9.6 cm (2.8); wheal reaction: asthma 2.7 cm (1.1), non-asthmatics 2.6 cm (0.5), both  $p > 0.2$ ] nor at a concentration of 10,000 times above the skin allergen threshold [median (IQR) flare reaction: asthma 10.4 cm (2.0), non-asthmatics 9.9 cm (2.4); wheal reaction: asthma 2.9 cm (1.0), non-asthmatics 2.9 cm (0.7), both  $p > 0.2$ ].

Obvious late skin reactions were observed in both groups after challenge with 30 BU [median (IQR) skin induration: asthma 9.3 cm (3.9), non-asthmatics 8.1 cm (3.7),  $p > 0.2$ ], as well as after challenge with a concentration 10,000 times above the threshold [median (IQR) skin induration: asthma 10.4 cm (2.0), non-asthmatics 11.1 cm (6.0),  $p > 0.2$ ] with no significant difference between groups.

#### *Associations between Nasal and Dermal Late Phase Reactions*

There were significant associations between the late-phase skin reaction with a fixed dose of allergen and late-phase nasal eosinophils ( $R_s = 0.30$ ,  $p = 0.05$ ), ECP ( $R_s = 0.45$ ,  $p = 0.001$ ), IL-5 ( $R_s = 0.38$ ,  $p = 0.006$ ) and A2M ( $R_s = 0.45$ ,  $p = 0.001$ ). Significant associations with late-phase nasal neutrophils, MPO and IL-8 were lacking ( $p > 0.1$ ). In a stepwise multiple linear regression analysis with a late-phase skin reaction as a variable to be explained and nasal eosinophils, ECP, IL-5, A2M and specific IgE to HDM as explaining variables, IgE to HDM turned out to be the only significant independent predictor.

## **Discussion**

In this study of allergen-induced late allergic inflammation in nose and skin we were not able to detect differences between HDM-allergic individuals with or without asthma. Validation of the classification as being asthmatic or non-asthmatic in this study was supported by a significantly lower baseline FEV1 and histamine threshold in asthma. Both parameters had not been used for inclusion into either group.

The majority of the patients with asthma also reported symptoms of perennial allergic rhinitis, which is in keeping with the literature [28, 29]. No significant differences in nasal and dermal outcomes were found between asth-

matics who had perennial nasal symptoms and those who did not. In agreement, comparison of primary outcomes between non-asthmatic rhinitis and the subgroups of asthmatics with or without symptoms of perennial rhinitis did not reveal differences. Moreover, at baseline nasal inflammation was very similar in non-asthmatic rhinitis and asthma. So, it seems unlikely that any difference in allergen-induced inflammation would have been obscured by differences in the occurrence of nasal symptoms or initial nasal mucosal inflammation.

Because challenge and lavage procedures were identical and recovery of lavage fluids was very similar in non-asthmatics and asthmatics comparison of both groups is not hampered by the wash-out phenomenon of repeated nasal washings. However, estimation of the kinetics of the nasal inflammation during the first hours after challenge may be slightly distorted by the phenomenon.

It cannot be excluded that there are small differences in allergen-induced late cellular inflammation between asthmatics and non-asthmatic rhinitis beyond the detection limit of this study. However, the concordance between dermal and nasal challenge results may argue against this.

Nasal challenge with a fixed dose of allergen resulted in a similar degree of early mast cell activation in both groups as inferred from the similar increase in plasma protein exudation and histamine levels. This is in agreement with literature reporting similar early allergic reactions as reflected by histamine release and plasma protein leakage between allergic patients with or without asthmatic symptoms [9, 30].

The lack of a difference in the late-phase inflammation in the nose and the skin in this study seems to be in contrast with the general notion that allergen-induced bronchial inflammation is increased in asthmatics. The evidence for this is mainly indirect and based on the correlation between bronchial inflammation and nonspecific bronchial hyperresponsiveness on the one hand and the finding that allergen-induced late bronchoconstriction occurs more frequently and is more pronounced in asthmatics than in non-asthmatics on the other hand. Baseline non-specific bronchial hyperresponsiveness, which is more increased in asthma, is the main determinant of the late-phase bronchoconstriction [31]. Therefore, it is impossible to conclude that there will be differences in late-phase cellular inflammation at the bronchial level between asthmatics and non-asthmatics just based on differences in allergen-induced late-phase bronchoconstriction. In fact, the few studies in which allergen-induced bronchial inflammation was directly compared between aller-

gic asthmatics and non-asthmatics produced conflicting results [6, 7]. A direct comparison of allergen-induced bronchial inflammation may be hampered by the differences in baseline non-specific bronchial hyperresponsiveness and possibly also by differences in baseline bronchial inflammation. For this purpose the nasal mucosa is probably a more appropriate target because of the similarity in baseline inflammation and in nasal symptoms in both groups. Moreover, in nasal challenge the allergen dose is not limited by the degree of airway narrowing, as it is in bronchial challenge, offering the opportunity to apply fixed allergen dose regimens which facilitate the direct comparison of allergen-induced inflammation.

The increase in nasal IL-5 and IL-8 and the subsequent influx of inflammatory cells accompanied by the increase in the soluble cell markers ECP and MPO as found in this study is in agreement with the results of other studies [12, 14, 23, 32–35]. The association between increases in the chemoattractants IL-5 and IL-8 and the nasal influx of eosinophils and neutrophils, respectively, during the late phase, is in agreement with their reported role for the recruitment of these inflammatory cells.

The late-phase nasal cellular inflammation closely resembles allergen-induced bronchial inflammation. It is generally accepted that allergen-induced bronchial inflammation contributes to asthmatic symptoms particularly in those individuals with a, possibly genetically determined, increased propensity to develop non-specific bronchial hyperresponsiveness. The high prevalence of asthmatic symptoms in occupational allergy and the ability

to induce asthmatic symptoms in non-asthmatic atopy by artificial high-dose bronchial allergen challenge may demonstrate that high levels of exposure to allergen, which is associated with an exaggerated allergic inflammatory response, may overcome the absence of an inherited propensity to develop bronchial hyperresponsiveness. Natural HDM exposure in our study population was very similar for non-asthmatic rhinitis and asthmatics as judged from the Der p 1 and Der f 1 levels measured in mattress dust samples of all participants (results not shown). This finding renders an environmental role for the occurrence of asthmatic symptoms in this study less likely.

In conclusion, we did not find any differences in late-phase cellular inflammation following nasal and dermal allergen challenge between HDM-allergic patients with or without asthma. Our results confirm that nasal challenge is an adequate model for studying allergen-induced mucosal inflammation. However, the similarity between asthmatic and non-asthmatic patients in allergen-induced nasal and dermal inflammation suggests that ultimately local factors in the bronchial tree are decisive for the development of asthma symptoms. Our results indicate that these factors most likely cannot be targeted in allergic models in organs other than the lower airways.

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## References

- Corren J: Allergic rhinitis and asthma: How important is the link? *J Allergy Clin Immunol* 1997;99:S781–S786.
- Rowe-Jones JM: The link between the nose and lung, perennial rhinitis and asthma – Is it the same disease? *Allergy* 1997;52:20–28.
- Ramsdale EH, Morris MM, Roberts RS, Hargreave FE: Asymptomatic bronchial hyperresponsiveness in rhinitis. *J Allergy Clin Immunol* 1985;75:573–577.
- Eggleston PA: Upper airway inflammatory diseases and bronchial hyperresponsiveness. *J Allergy Clin Immunol* 1988;81:1036–1041.
- Djukanovic R, Lai CK, Wilson JW, Britten KM, Wilson SJ, Roche WR, Howarth PH, Holgate ST: Bronchial mucosal manifestations of atopy: A comparison of markers of inflammation between atopic asthmatics, atopic non-asthmatics and healthy controls. *Eur Respir J* 1992;5:538–544.
- Shaver JR, O'Connor JJ, Pollice M, Cho SK, Kane GC, Fish JE, Peters SP: Pulmonary inflammation after segmental ragweed challenge in allergic asthmatic and nonasthmatic subjects. *Am J Respir Crit Care Med* 1995;152:1189–1197.
- Polosa R, Ciamarra I, Mangano G, Prosperini G, Pistorio MP, Vancheri C, Crimi N: Bronchial hyperresponsiveness and airway inflammation markers in nonasthmatics with allergic rhinitis. *Eur Respir J* 2000;15:30–35.
- Alvarez MJ, Olaguibel JM, Garcia BE, Rodriguez A, Tabar AI, Urbiola E: Airway inflammation in asthma and perennial allergic rhinitis. Relationship with nonspecific bronchial responsiveness and maximal airway narrowing. *Allergy* 2000;55:355–362.
- Stevens WJ, van Bever HP: Frequency and intensity of late asthmatic reactions after bronchial allergen challenge in asthma and rhinitis. *Allergy* 1989;44:471–476.
- Muller BA, Leick CA, Smith RM, Suelzer MT, Richerson HB: Comparisons of specific and nonspecific bronchoprovocation in subjects with asthma, rhinitis, and healthy subjects. *J Allergy Clin Immunol* 1993;91:758–772.
- Lopuhaa CE, Out TA, Jansen HM, Aalberse RC, van der Zee JS: Allergen-induced bronchial inflammation in house dust mite-allergic patients with or without asthma. *Clin Exp Allergy* 2002;32:1720–1727.
- Garrelds IM, de Graaf-in 't Veld T, Nahori MA, Vargaftig BB, Gerth van Wijk R, Zijlstra FJ: Interleukin-5 and eosinophil cationic protein in nasal lavages of rhinitis patients. *Eur J Pharmacol* 1995;275:295–300.
- Wang D, Smits J, Derde MP, Clement P: Concentrations of myeloperoxidase in nasal secretions of atopic patients after nasal allergen challenge and during natural allergen exposure. *Int Arch Allergy Immunol* 1996;110:85–90.

- 14 Jacobi HH, Poulsen LK, Reimert CM, Skov PS, Ulfgren AK, Jones I, Elfman LB, Malling HJ, Mygind N: IL-8 and the activation of eosinophils and neutrophils following nasal allergen challenge. *Int Arch Allergy Immunol* 1998;116:53–59.
- 15 Greiff L, Meyer P, Svensson C, Persson CG, Andersson M: The 'nasal pool'-device for challenge and lavage of the nasal mucosa in children: Histamine-induced plasma exudation responses. *Pediatr Allergy Immunol* 1997;8:137–142.
- 16 Anonymous: Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, November 1986. *Am Rev Respir Dis* 1987;136:225–244.
- 17 Anonymous: International Consensus Report on the diagnosis and management of rhinitis. International Rhinitis Management Working Group. *Allergy* 1994;49:1–34.
- 18 Position paper: Allergen standardization and skin tests. The European Academy of Allergology and Clinical Immunology. *Allergy* 1993;48:48–82.
- 19 Naclerio RM, Meier HL, Kagey-Sobotka A, Adkinson NFJ, Meyers DA, Norman PS, Lichtenstein LM: Mediator release after nasal airway challenge with allergen. *Am Rev Respir Dis* 1983;128:597–602.
- 20 Liu L, Zuurbier AE, Mul FP, Verhoeven AJ, Lutter R, Knol EF, Roos D: Triple role of platelet-activating factor in eosinophil migration across monolayers of lung epithelial cells: Eosinophil chemoattractant and priming agent and epithelial cell activator. *J Immunol* 1998;161:3064–3070.
- 21 Bresser P, Out TA, van Alphen L, Jansen HM, Lutter R: Airway inflammation in nonobstructive and obstructive chronic bronchitis with chronic *Haemophilus influenzae* airway infection. Comparison with noninfected patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2000;162:947–952.
- 22 McNamee LA, Fattah DI, Baker TJ, Bains SK, Hissey PH: Production, characterisation and use of monoclonal antibodies to human interleukin-5 in an enzyme-linked immunosorbent assay. *J Immunol Methods* 1991;141:81–88.
- 23 Nocker RE, Schoonbrood DF, Van de Graaf EA, Hack CE, Lutter R, Jansen HM, Out TA: Interleukin-8 in airway inflammation in patients with asthma and chronic obstructive pulmonary disease. *Int Arch Allergy Immunol* 1996;109:183–191.
- 24 Van de Graaf EA, Out TA, Roos CM, Jansen HM: Respiratory membrane permeability and bronchial hyperreactivity in patients with stable asthma. Effects of therapy with inhaled steroids. *Am Rev Respir Dis* 1991;143:362–368.
- 25 Siraganian RP: Refinements in the automated fluorometric histamine analysis system. *J Immunol Methods* 1975;7:283–290.
- 26 Witteman AM, Stapel SO, Perdok GJ, Sjamsoedin DH, Jansen HM, Aalberse RC, van der Zee JS: The relationship between RAST and skin test results in patients with asthma or rhinitis: A quantitative study with purified major allergens. *J Allergy Clin Immunol* 1996;97:16–25.
- 27 Schuurman J, Perdok GJ, Lourens TE, Parren PW, Chapman MD, Aalberse RC: Production of a mouse/human chimeric IgE monoclonal antibody to the house dust mite allergen Der p 2 and its use for the absolute quantification of allergen-specific IgE. *J Allergy Clin Immunol* 1997;99:545–550.
- 28 Greisner WA, Settipane RJ, Settipane GA: Coexistence of asthma and allergic rhinitis: A 23-year follow-up study of college students. *Allergy Asthma Proc* 1998;19:185–188.
- 29 Danielsson J, Jessen M: The natural course of allergic rhinitis during 12 years of follow-up. *Allergy* 1997;52:331–334.
- 30 Fish JE, Ankin MG, Kelly JF, Peterman VI: Comparison of responses to pollen extract in subjects with allergic asthma and nonasthmatic subjects with allergic rhinitis. *J Allergy Clin Immunol* 1980;65:154–161.
- 31 van der Veen MJ, Van Neerven RJ, De Jong EC, Aalberse RC, Jansen HM, van der Zee JS: The late asthmatic response is associated with baseline allergen-specific proliferative responsiveness of peripheral T lymphocytes in vitro and serum interleukin-5. *Clin Exp Allergy* 1999;29/2:217–227.
- 32 Gosset P, Tillie-Leblond I, Malaquin F, Durieu J, Wallaert B, Tonnel AB: Interleukin-8 secretion in patients with allergic rhinitis after an allergen challenge: Interleukin-8 is not the main chemotactic factor present in nasal lavages. *Clin Exp Allergy* 1997;27:379–388.
- 33 de Graaf-in 't Veld C, Garrelds IM, Koenders S, Gerth van Wijk R: Relationship between nasal hyperreactivity, mediators and eosinophils in patients with perennial allergic rhinitis and controls. *Clin Exp Allergy* 1996;26:903–908.
- 34 Meyer P, Persson CGA, Andersson M, Wollmer P, Linden M, Svensson C, Greiff L: Alpha2-macroglobulin and eosinophil cationic protein in the allergic airway mucosa in seasonal allergic rhinitis. *Eur Respir J* 1999;13:633–637.
- 35 Wang JM, Rambaldi A, Biondi A, Chen ZG, Sanderson CJ, Mantovani A: Recombinant human interleukin 5 is a selective eosinophil chemoattractant. *Eur J Immunol* 1989;19:701–705.