Factors in clinical expression of allergic airways disease

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CHAPTER 6

Comparison of allergen-induced late inflammatory reactions in the nose and in the skin in house dust mite allergic patients with or without asthma

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submitted
Summary

Background: It remains to be established which factors contribute to the occurrence of asthma in allergic individuals. We hypothesised 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 clinical presentation of allergy.

Aim of this study: 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 (D. 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 analysed for the presence 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 hours) 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, the occurrence of pulmonary symptoms in asthma is unlikely 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.
**Introduction**

Inhalant allergy plays an important causal role in the development of asthma and rhinitis. Although allergic sensitisation is demonstrable in 30 to 40% of the population in the western industrialised countries, only 5 to 10% suffer from allergic asthma and 10 to 20% suffer from allergic rhinitis. The vast majority of allergic asthmatic patients also experience symptoms of allergic rhinitis\(^1,2\). By contrast, allergic rhino-conjunctivitis 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 allergic patients with or without asthma. Here, we describe the results of the nasal and dermal allergen provocation tests. Intracutaneous skin provocation was done with a fixed allergen dose as well as with an allergen dose titrated to a standardised early skin reaction. Primary outcome of the skin provocation test was the magnitude of the dermal infiltration after 7 hours.

Nasal provocation was performed with a fixed dose of house dust mite extract for all individuals and the reaction was monitored by measuring changes in repeated nasal lavages. Primary outcomes were levels of IL-5, IL-8, Eosinophil Cationic Protein (ECP), Myeloperoxidase (MPO) and cell differentials obtained during the phase of the late allergic reaction (4-24 hours).
Early protein leakage (alfa-2-macroglobulin) and histamine levels were monitored as an indicator for early mast cell degranulation in the nose\textsuperscript{11-14}. The study was designed to detect a 25\% difference in late skin reactivity and a 30\% difference in log normalised 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\textsuperscript{15}. Patients with perennial rhinitis were recruited from the outpatient department of otorhinolaryngology and were characterised by episodes of sneezing, watery rhinorrhoea, pruritus in the nose, eyes and palate and nasal obstruction\textsuperscript{16}. 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 doctors diagnosis of asthma.

The following inclusion criteria applied to all participants: a) $\text{FEV}_1 \geq 70\%$ of predicted value; b) specific IgE level to house dust mite $> 0.5$ IU/ml; c) able to stop short acting $\beta_2$-adrenoceptor agonists for at least 8 hours, oral anti-histamines for 2 weeks and inhaled corticosteroids for 6 weeks prior to the start of the study; d) no significant change in environmental allergen exposure (e.g. not moved) during the past 12 months; e) whenever co-sensitisation against pollen was present, patients were studied outside the relevant season.

Exclusion criteria for all were: a) history of immunotherapy; b) respiratory tract infection 6 weeks prior to the study; c) immunosuppressive medication; d) 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 Radio-Allergo-Sorbent-Test (RAST) and skin prick test (SPT) with a standard panel of aero-allergens and by spirometry.

Corticosteroids were withheld for 6 weeks and anti-histamines 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 one hour later. Changes induced by diluent challenge were monitored by three nasal lavages during the first hour. Similarly, allergen challenge was followed by three lavages in the first hour and subsequently by lavages every hour for 8 hours. Twenty-four hours after allergen challenge nasal lavage and venepuncture were repeated.

Allergen extract
A standardized freeze dried house dust mite 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, Houten, The Netherlands) at 1 mg/ml (equivalent to 10^6 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 p1 and 9.7 μg Der p2 per 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 (ICT) in the forearm as described before. In addition, diluent control and histamine dihydrochloride at 0.1 mg/ml were tested. After 15 minutes, wheal- and flare reactions were determined by marking both reactions and copying the marks on a form via an adhesive tape. Results were summarised by the sum of the largest diameter and its orthogonal diameter. At 7 hours 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.\textsuperscript{18} 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 seconds 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% benzalkoniumchloride 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 minutes. 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 minutes after application of allergen. Thereafter, nasal washings were done hourly from 3 to 8 hours and again 24 hours after challenge. During the procedure the subjects were allowed to wipe their noses but asked not to blow their nose.

Sample processing
The lavage fluids were centrifuged for 5 minutes at 700 g. 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 dithiotreitol 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 minutes at 37°C (DTT; Sigma Chemical Company, St. Louis, Missouri, USA). After centrifugation for 5 minutes 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 than cyto-centrifuged at 550 rpm for 2 minutes 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 500-1000 cells were counted, depending on the percentage of eosinophils.
Due to low cell numbers in repeated lavages data from samples 4-24 hours after challenge were pooled.

Protein assays
Levels of ECP were determined in the lavage supernatants with an ELISA described previously. 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, IL-5, IL-8 and Alpha-2-macroglobulin (A2M) were measured by ELISA as described earlier. Histamine was measured with an automated fluorometric analysis as described by Siraganian. Total serum IgE was quantified by a binding and an inhibition assay, as described before. Levels of specific IgE against house dust mite (Dermatophagoides pteronyssinus) were measured in duplicate with the Radio-Allergosorbant test (RAST). The results were expressed in international units per millilitre using an in house-standard, calibrated against the World Health Organisation standard.

Statistical analysis
SPSS, version 10.0 Statistics U.K. (Chicago, IL, USA) was used for statistical analyses. The analysis of the ECP, MPO, IL-5 and IL-8 during late allergic reaction was done with the average of the values measured at 4,6 and 8 hours after challenge. When applicable data were log-transformed to obtain normal distribution of data. Within group changes were evaluated with paired t-test and Wilcoxon test (IL-5). Between groups comparisons were done with t-test and analysis of variance controlling for the level of specific IgE to HDM. 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 analysis of covariance with post challenge values as dependent variable, 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 house dust mite 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 FEV$_1$ and histamine thresholds between asthmatics and non-asthmatic patients.

<table>
<thead>
<tr>
<th>Table 1 Patient characteristics.</th>
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<tbody>
<tr>
<td></td>
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<tr>
<td>Gender</td>
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<tr>
<td>Age</td>
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<tr>
<td>FEV$_1$</td>
</tr>
<tr>
<td>Pc$_{20}$histamine</td>
</tr>
<tr>
<td>Perennial rhinitis symptoms</td>
</tr>
<tr>
<td>Total IgE</td>
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<tr>
<td>HDM specific IgE</td>
</tr>
<tr>
<td>RAST results:</td>
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<tr>
<td>Gras pollen$^3$</td>
</tr>
<tr>
<td>Tree pollen$^3$</td>
</tr>
<tr>
<td>Cat or dog dander$^3$</td>
</tr>
<tr>
<td>HDM mono allergy$^3$</td>
</tr>
</tbody>
</table>

$^*$ p<0.05, $^{**}$ p<0.01
$^1$, mean and standard deviation; $^2$, geometric mean and standard deviation.$^3$, (% pos).
### Table 2: Analysis of changes in nasal lavage from baseline to 24 hour post-challenge.

<table>
<thead>
<tr>
<th></th>
<th>Non-asthmatics (n = 26)</th>
<th>Asthma (n = 26)</th>
<th>Non-asthmatics vs. Asthmatics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>baseline</td>
<td>24 hrs</td>
<td>p value</td>
</tr>
<tr>
<td>Mean* (SE)</td>
<td>Mean* (SE)</td>
<td>24 hrs versus baseline</td>
<td>Mean* (SE)</td>
</tr>
<tr>
<td>Eosinophils$^\dagger$</td>
<td>2.2 (1.6)</td>
<td>19.9 (1.4)</td>
<td>0.001</td>
</tr>
<tr>
<td>(10$^4$/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ECP (ng/ml)</td>
<td>2.20 (1.3)</td>
<td>4.32 (1.4)</td>
<td>0.003</td>
</tr>
<tr>
<td>IL-5 (pg/ml)</td>
<td>&lt;0.1 (0.7)</td>
<td>1.53 (0.5)</td>
<td>0.02</td>
</tr>
<tr>
<td>Neutrophils$^\dagger$</td>
<td>2.5 (1.5)</td>
<td>8.1 (1.4)</td>
<td>0.003</td>
</tr>
<tr>
<td>(10$^5$/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-8 (pg/ml)</td>
<td>136.0 (1.2)</td>
<td>140.0 (1.1)</td>
<td>&gt;0.2</td>
</tr>
<tr>
<td>MPO (ng/ml)</td>
<td>75.5 (1.2)</td>
<td>72.9 (1.2)</td>
<td>&gt;0.2</td>
</tr>
<tr>
<td>A2M (ng/ml)</td>
<td>503.6 (1.2)</td>
<td>642.7 (1.3)</td>
<td>&gt;0.2</td>
</tr>
</tbody>
</table>

*, geometric mean and standard error for ECP, IL-8, MPO, A2M.

$, pool cell data from baseline lavages and from lavages 4–24 hr after challenge.

NA, not available;

Within group comparison: paired t-test (Wilcoxon signed ranks for IL-5); between groups comparison unpaired t-test (baseline and unadjusted 24 hrs) and analysis of covariance (24 hrs adjusted for baseline) signifying differences in changes from baseline (Mann-Whitney test for IL-5).
Nasal challenge
Mean recovery of the 10 ml lavage fluid was similar in asthmatics and non-asthmatics (7.7 [SD 0.9] vs. 7.6 [SD 0.9] ml, p>0.2).

The baseline eosinophil and neutrophil counts and levels of alpha-2-macroglobulin (A2M), ECP, MPO, IL-5 and IL-8 were similar in the nasal washings of asthma and non-asthmatic rhinitis (table 2).

The repeated nasal lavage procedure caused some washout 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 minutes after the diluent challenge of about 10-15% of the initial baseline lavage. Extended control procedures during 24 hours after diluent challenge in a limited number of patients indicated a slight further decrease in soluble markers up to 40 minutes after sham challenge, followed by a gradual return to pre-challenge values at 6 hours and to baseline values at 24 hours (fig. 1). Therefore, 24 hours lavage results, with no preceding lavage for 16 hours, was compared with baseline values, and lavages after 4 to 8 hours were compared with post diluent lavages. In none of the patients plasma protein exudation was found during the first hour after diluent challenge. During the first hour after allergen administration A2M levels increased in all patients and to a similar extent in both groups (fig. 1). In contrast, house dust mite challenge did not induce an increase in A2M in patients without sensitisation to house dust mite, excluding a significant non-immunological effect of the house dust mite extract on the nasal mucosa.

A2M expressed as area under the curve (AUC) during the first 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[0.10], non-asthmatics 0.81[0.12] ng/ml, 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 hours post-challenge 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 hours after the challenge levels had returned to baseline values. The course of A2M as assessed by the AUC from 2 to 8 hours, was not different between the two groups. This was unaffected by adjustment for IgE directed against house dust mite. In addition, there was no difference between the subgroups of asthmatic patients with or without rhinitis symptoms.

There was an increase in ECP in nasal washings during the late phase, which was statistically significant in both groups (fig. 2). At 24 hours post-challenge levels were still elevated compared to baseline values (table 2). Again, increases at 4 to 24 hours were statistically indistinguishable for asthma and non-asthmatic rhinitis.
Figure 1 Level of α-2-macroglobulin (A2M) in nasal lavages after nasal allergen challenge. Baseline was level after diluent challenge. The broken line indicates geometric mean (SE) of A2M values after diluent challenge in 3 patients.

There was a significant increase in A2M during the early allergic reaction (20-40 minutes, both groups p<0.0001) and during the phase of the late allergic reaction (4-8 hours) in both group (both groups p<0.001), relative to baseline as well as to controls after sham challenge. There were no significant differences between asthmatics and non-asthmatics during early phase (p = 0.3) and late phase (p = 0.5).
Figure 2 Concentrations of eosinophil cationic protein (ECP) in nasal washings after nasal allergen challenge. Baseline was level after diluent challenge. There was a significant increase in both groups at 4-8 hours (p<0.0001) but there was no significant difference between asthmatics and non-asthmatics (p = 0.8).
Figure 3 Levels of IL-5 in nasal washings following nasal allergen challenge. Baseline was level after diluent challenge. The number of samples below detection level are indicated at each time point. There was a significant increase in IL-5 in both groups at 4-8 hours (Wilcoxon p < 0.05). There were no significant differences between asthmatics and non-asthmatics (Mann-Whitney p = 0.7).
There was also a significant rise in MPO levels at 4 to 8 hours compared to levels after control challenge in both groups (geometric mean\[SE\] asthma 7.9 [1.4] to 20.9 [1.3] ng/ml and non-asthmatics: 10.5 [1.3] to 28.8 [1.3] ng/ml, both p<0.0001). In contrast to ECP, MPO levels returned to baseline levels after 24 hours (table 2).

IL-5 levels significantly increased in both groups during the late allergic reaction and were still elevated after 24 hours (both p<0.05, Wilcoxon) (fig. 3). Again, no difference was found between the groups at any of the time points studied.

IL-8 showed a similar but modest increase in both groups after 4 to 8 hours compared to levels after diluent challenge in the asthma as well as in the non-asthmatic group (geometric mean \[SE\] asthma 24.0 [1.3] to 45.6 [1.2] ng/ml, non-asthmatics 28.1 [1.2] to 45.9 [1.2] ng/ml, p<0.0001 and p = 0.005, respectively). After 24 hours levels were similar to baseline levels (table 2).

Due to low cell numbers in the repeated nasal washings data for influx of inflammatory cells were pooled for 4-24 hours. There was a significant increase in both groups for eosinophil and neutrophil numbers compared to baseline 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 hours) was associated with late phase levels of ECP (Rs = 0.46, p = 0.001), A2M (0.40, p = 0.003) and eosinophil numbers (Rs = 0.47, p = 0.001), but was not associated with neutrophils (p = 0.6) and MPO (p = 0.16). Early rise in Il-8 (4-6 hours) was associated with late phase levels of MPO (Rs = 0.72, p<0.0001), A2M (Rs = 0.42, p = 0.002), ECP (Rs = 0.47, p = 0.001) and showed a trend for an association with post challenge neutrophil numbers (Rs = 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[1.4] BU/ml, non-asthmatics 0.21[1.3] BU/ml, p = 0.04). However, after correction for the difference in the level of HDM specific IgE the difference between groups disappeared (p = 0.3). Skin reactions with histamine were very similar in both groups (median[IQR] flare reaction, asthma: 8.0[2.3] cm, non-asthmatics: 7.5[1.5]cm; wheal reaction, asthma: 2.3[0.5]cm, non-asthmatics: 2.0[0.5]cm, 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[3.6]cm, non-asthmatics: 9.6[2.8]cm; wheal reaction, asthma: 2.7[1.1]cm, non-asthmatics: 2.6[0.5]cm, both p>0.2) nor at a concentration of 10,000 times above the skin allergen threshold (median[IQR] flare reaction, asthma 10.4[2.0]cm, non-asthmatics 9.9[2.4]cm; wheal reaction, asthma: 2.9[1.0]cm, non-asthmatics: 2.9[0.7]cm, 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[3.9]cm, non-asthmatics 8.1[3.7]cm, p>0.2), as well as after challenge with a concentration 10,000 times above the threshold (median[IQR] skin induration, asthma 10.4[2.0]cm, non-asthmatics 11.1[6.0]cm, 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 fixed dose of allergen and late phase nasal eosinophils (Rs = 0.30, p = 0.05), ECP (Rs = 0.45, p = 0.001), IL-5 (Rs = 0.38, p = 0.006) and A2M (Rs = 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 late phase skin reaction as 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 house dust mite 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 literature²⁷,²⁸. No significant differences in nasal and dermal outcomes were found between asthmatics who indicated to have perennial nasal symptoms and those who did not. In concordance, comparison of primary outcomes between non-asthmatic rhinitis and the subgroups of asthmatics with and 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 washout 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 rhinitics 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\textsuperscript{9,29}.

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 non-specific 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\textsuperscript{30}. 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 allergic asthmatics and non-asthmatics produced conflicting results\textsuperscript{6,7}. 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 direct comparison of allergen, induced inflammation.

The increase in nasal IL-5 and IL-8, 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\textsuperscript{11,13,22,31-34}. The association between increases in the chemo-attractants IL-5 and IL-8 and the nasal influx of eosinophils and neutrophils, respectively, during the late phase, are in agreement with their reported role for 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 atopics 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 rhinitics and asthmatics as judged from the Der p 1 and Der f 1 levels measured in mattress dust samples in 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 house dust mite 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 other organs other than the lower airways.

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