

Lower airway inflammatory responses to repeated very-low-dose allergen challenge in allergic rhinitis and asthma

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Summary

Background Low-dose allergen challenge (LDAC) may be a useful tool for studying the capacity of allergens to induce airway inflammation in atopic subjects.

Objective To evaluate lower airway inflammatory changes following repeated inhalation of very low doses of allergen (VLDAC) in non-asthmatic subjects with allergic rhinitis (NAAR) compared with mild allergic asthmatic subjects (AA).

Methods Fourteen NAAR and 11 AA were seen out of the pollen season and had skin prick tests with common aeroallergens. Baseline spirometry (S) and methacholine challenge (MC) were done and blood and induced sputum (IS) differential cell counts were obtained. Each subject underwent VLDAC on four consecutive mornings with a relevant allergen. S, MC, and blood and IS samplings were repeated 6 h after the second and fourth VLDAC and one week later.

Results Although there were, as expected, no changes in FEV₁ or PC₂₀ in either group, mean percentage eosinophils on IS were significantly increased in NAAR on day 2 of VLDAC and decreased in all but one subject on day 4, with a tendency to return to baseline levels one week later. In AA, there was a non-significant trend for sputum eosinophils to increase on day 2; four subjects showed a decrease of eosinophils on day 4 of VLDAC. There was a correlation between eosinophil cationic protein (ECP) levels and eosinophil counts in NAAR throughout the study. There were no variations in other sputum cells or blood inflammatory cells.

Conclusion VLDAC can increase the percentage of eosinophils in IS of NAAR subjects without associated respiratory symptoms nor physiological modifications. A reduction in eosinophilic response despite repeated exposure, more common in NAAR subjects, suggests an adaptation process that needs to be further evaluated.

Keywords allergic rhinitis, asthma, low-dose allergen challenge, inflammation, sputum

Submitted 6 December 2001; revised 19 April 2002; accepted 11 July 2002

Introduction

The prevalence of asthma has been increasing in most countries in the past 20 years [1, 2]. This disease may develop following exposure to environmental substances known to induce an airway inflammatory process, such as allergens in sensitized subjects [3]. Atopy is considered a predisposing factor in the development of asthma, but its clinical manifestations will vary from one subject to another and only about 25% of sensitized atopic subjects will develop asthma [4]. Why some atopic subjects develop asthma and others do not is still unknown, although genetic and environmental influences are considered important.

Airway inflammation [5] and changes in airway structure, also called 'remodeling' [6], may be present in non-asthmatic subjects with allergic rhinitis (NAAR), although in a less intense form than in asthmatic patients [7]. Airway inflammation has

been associated with the increase in airway responsiveness observed during natural or laboratory exposure to allergens in atopic subjects without asthma [8–13]. Lower airway inflammation and remodeling may occur in NAAR following segmental bronchial provocation with increased pulmonary symptoms and decreased pulmonary function [14].

Because asthma is generally already permanent at the time of diagnosis, it would be of interest to identify subjects at increased risk of developing the disease in order to try to prevent its occurrence. There is a possibility that repeated epithelial damage and airway inflammation in allergic rhinitis lead to long-lasting changes in lower airway structure and function and contribute to the development of asthma in atopic subjects [15].

Allergen challenges have been used for a few decades to study the physiopathology of asthma. It has been shown that the magnitude of allergen-induced airway responses may increase with repeated allergen exposure over a few days [16]. A few years ago, low-dose allergen challenge (LDAC) has been introduced as a tool to study airway responses to aeroallergens in asthmatic subjects. This method could be particularly useful in studying the influence of doses of allergen closer to natural

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exposure than usual provocation doses, on lower airway inflammation of non-asthmatic subjects.

In regard to assessment of airway inflammation, bronchial biopsies and bronchoalveolar lavage (BAL) have been used extensively to study lower airway inflammation [17], but bronchoscopy is an invasive technique and cannot be easily repeated over a short period of time. In the past few years, induced sputum (IS) analysis has been shown to be a direct, valid and reproducible method [18–20]. It is non-invasive and can be repeated frequently, making it possible to study the dynamics of airway inflammatory responses.

In this study, we aimed to evaluate lower airway inflammatory changes following very-low-dose allergen challenge (VLDAC) in NAAR using induced sputum analysis, and to compare these changes with those observed in allergic asthmatic patients (AA).

Methods

Subjects

Twenty-five subjects aged 18–45 years were recruited from advertisements in newspapers and from the outpatient clinic's database. Fourteen of them were non-asthmatic subjects with allergic rhinitis (NAAR). Their PC₂₀, the provocative concentration of methacholine giving a 20% fall in forced expiratory volume in one second (FEV₁), had to be > 16 mg/mL but < 128 mg/mL, in order to obtain a measurable value. None of them, however, had experienced asthma symptoms nor had taken any asthma medication. Eleven mild AA were also studied; their PC₂₀ was ≤ 16 mg/mL but their respiratory symptoms were mild, requiring only an inhaled short-acting β₂-agonist on demand.

All subjects had a positive skin response to one or more allergens and were able to produce adequate sputum samples. They were non-smokers or had not smoked for at least six months. None had been exposed to an allergen to which they were sensitized (apart from house dust mite) for at least one month, nor had any respiratory tract infection for at least that same period of time. Subjects sensitized to animals did not have pets at home. The study was approved by the Hospital Ethics Committee and all subjects signed an informed consent form.

Study design

The study included eight visits and was done out of the pollen season. Each subject initially presented to the clinic in the afternoon. During this two-hour baseline visit, they completed a respiratory questionnaire and had skin prick tests, a spirometry and a methacholine challenge. A blood sample and induced-sputum specimen were also obtained. Subjects then came back one to seven days later to undergo very-low-dose allergen challenge (VLDAC) for four consecutive mornings with a relevant allergen. Spirometry, methacholine challenge, blood and induced sputum samplings were repeated six hours after the second and fourth VLDAC, then one week later. Peak expiratory flows (PEF) were recorded twice daily throughout the study.

Questionnaire

Information on past and current medical history was recorded, using the European Community Survey questionnaire [21]. Any

past or present respiratory symptoms, family or personal history of asthma or other diseases were documented.

Skin prick tests and titration

Atopy was determined by skin prick tests with 26 common aeroallergens including birch pollen, ragweed and mixed grasses, cat and dog danders and two house dust mite (HDM) species (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*). Normal saline and histamine were used as negative and positive controls, respectively. The weal size was recorded at 10 min as the mean of two perpendicular measures. A positive response was defined as a skin weal diameter of 3 mm or more.

Skin titration was performed with the allergen showing the largest skin test weal diameter. Dilutions were applied on the forearms and the weal size was recorded at 15 min as for the skin prick tests. The highest dilution of allergen that gave a 2-mm weal was used to determine the concentration for VLDAC.

Spirometry and methacholine challenge

Spirometry was performed according to standardized methods and FEV₁ was measured according to the American Thoracic Society (ATS) specifications [22]. Baseline FEV₁ was defined as the best of three reproducible values. The predicted values for expiratory flows were obtained from Knudson [23]. Spirometry was performed with an ATS-approved spirometer (Vitalograph Medical Instrumentation, Lenexa, KS, USA).

Airway responsiveness to methacholine was measured using the method described by Juniper [24]. Briefly, after a 2-min inhalation of 0.9% saline, increasing concentrations of methacholine were inhaled for 2 min at 5-min intervals or more, until a 20% fall in FEV₁ was observed or until the last concentration of methacholine (128 mg/mL) was given. After each inhalation, FEV₁ was recorded at 30 and 90 s or until FEV₁ had increased. The responses were expressed as the PC₂₀, obtained from the log dose–response curve.

Very-low-dose allergen challenge

As mentioned previously, for bronchoprovocation, using the allergen inducing the greater skin response, we determined the concentrations to be administered using a modification of the method described by Cockcroft [25]. This method, which was similarly used for both study groups, takes into account the PC₂₀ and the results from the titration. For safety purposes, and to explore the minimal dose that could induce an eosinophilia, we used even lower doses than those used in previously published LDAC studies [8, 26, 27]. A pilot study was therefore done before the beginning of this study, to determine the minimum allergen dose that could induce lower airway inflammation (two subjects inhaled 10, 9 and 8 doubling doses below the calculated PD₂₀, which provided a small airway inflammatory response). For both groups, we determined that the dose of allergen to be given on each morning would correspond to 9, 8 and 7 doubling doses lower than the calculated allergen PD₂₀, the dose that would have given a 20% fall in FEV₁ from allergen inhalation. The method described by Cockcroft was used for allergen inhalation [25]. Briefly, after spirometry, subjects inhaled the first allergen dose for 2 min FEV₁ was recorded 10 min later. If the fall in FEV₁ was lower than 5% from the highest FEV₁ at baseline, the next concentration was administered.

A maximum of three doubling doses of allergen were administered. After the last dose, FEV₁ was monitored at 10-min intervals for 30 min.

Sputum induction and processing

Sputum was induced and processed according to the method described by Pin et al. [28] and modified by Pizzichini et al. [20]. Briefly, subjects were pretreated with 200 µg of salbutamol before inhaling increasing concentrations of hypertonic saline (3, 4 and 5%) for 7 min each (for a maximum of 21 min) with a Medix electronic nebulizer (Medix, Catthorp, UK) without a valve or nose clip. After each inhalation, subjects were instructed to blow their nose and to rinse their mouth and swallow the water to minimize postnasal drip and squamous epithelial cell contamination, respectively. FEV₁ was recorded after each inhalation.

Sputum was processed within the two hours following the induction. Using forceps, mucous plugs were separated from saliva, mixed with 4 times their volume of 0.1% dithiothreitol (DTT) (Sputolysin; Calbiochem Corp., La Jolla, CA, USA) and rocked for 15 min. To stop the reaction, an equal volume of Dulbecco's phosphate buffer saline 1X (D-PBS) (Life Technologies (Gibco BRL), Burlington, Ontario, Canada) was added and the suspension was rocked for 5 more minutes. After filtration of the cell suspension through a 48-µm nylon gauze (SEFAR, Scarborough, Ontario, Canada) to remove debris, the resulting suspension was centrifuged at 450 *g* for 5 min. The supernatant was aspirated and aliquots were stored at -70 °C until further analysis. Total cell count was obtained with a Neubauer haemocytometer chamber (Reichert, Buffalo, NY, USA) and expressed as million cells per gram of selected sputum. Cell viability was determined by the trypan blue exclusion method and the procedure was stopped if the viability was 40% or less. Cell suspension was adjusted to 0.5–1 million cells per mL and 2 prewet slides were prepared with a Shandon III cytocentrifuge (Shandon Scientific Lim., Astmoor, Runcorn, Cheshire, UK) using 50 µL of D-PBS 1X and run at 10 *g* for 3 min. Then, 100 µL of cell suspension was added and run at 10 *g* for 5 min. The two slides were stained with Diff-Quik (Dade Diagnostics of P.R. Inc., Newark, USA) for differential cell count and 400 cells were counted. All sputum counts and measurements were performed on coded slides, with the observer blind to the clinical details or study group to which the subject belonged.

ECP measurements in sputum

Levels of eosinophil cationic protein (ECP, µg/L) were measured in diluted (1 : 2) sputum supernatants using radioimmunoassay (RIA) (Pharmacia & UpJohn Diagnostics AB, Uppsala, Sweden) according to the manufacturer's instructions. The sensitivity of the assay was < 2 µg/L. The results were adjusted for the dilution.

Blood sample analysis

On the first visit, blood was collected for complete blood count and total IgE analysis.

Statistical analysis

Results are expressed as mean ± SEM for PC₂₀ and ECP data and as median (25%, 75% interquartile) for sputum cell counts.

To compare subjects at baseline, day 2, day 4 and 1 week post, repeated ANOVA measurements were applied to the data. The normality assumption was verified with the Shapiro-Wilk test and the Brown-Forsythe test was used to verify the homogeneity of variances. After transformation of values, the univariate approach, using randomized block designs, was performed, as it is more powerful than its multivariate counterpart. The normality and variance assumptions were fulfilled. Relationships between parameters were expressed with Pearson's correlation coefficients.

A second analysis was performed to compare NAAR with AA and also to compare different groups on the basis of allergen challenge and house dust mite reactivity. A two-way ANOVA with repeated measurements on one factor was performed to analyse data. The results were considered significant if *P*-values were ≤ 0.05. For all data, comparisons were performed with Tukey's method. All analyses were conducted using the statistical package SAS (SAS Institute Inc, Cary, NC, USA).

Results

Characteristics of subjects

Baseline characteristics of subjects are shown in Table 1. All subjects completed the study and none of them experienced any allergic or asthmatic symptoms through the challenge days.

Expiratory flows and methacholine responsiveness

Peak expiratory flows or baseline FEV₁ did not vary significantly over the study period (less than 5% between visits in both groups). As expected, mean baseline PC₂₀ was significantly lower in AA than in NAAR (3 ± 1 mg/mL and 48 ± 1 mg/mL, respectively, *P* < 0.0001), and did not significantly change throughout the study (Day 2: 3 ± 1 mg/mL and 32 ± 1 mg/mL, respectively, *P* < 0.0001; Day 4: 3 ± 1 mg/mL and 40 ± 1 mg/mL, respectively, *P* < 0.0001; 1 week post: 3 ± 1 mg/mL and 30 ± 1 mg/mL, respectively, *P* < 0.0001).

Sputum and blood sample analysis

The results from sputum differential cell counts are shown in Table 2. The cell viability did not differ statistically throughout the visits and the percentage of squamous epithelial cells remained less than 20% throughout the study. On baseline, AA had a higher median percentage of eosinophils in induced sputum than did rhinitic subjects (1.5% and 0.25%, respectively, *P* = 0.01) (Fig. 1). In AA, there was no statistically significant change in IS, although eosinophils tended to increase on day 2 of VLDAC and decrease slightly on day 4. Rhinitic subjects, on the other hand, demonstrated a significant increase of sputum median percentage eosinophils on day 2 (*P* = 0.0005) that reached the levels observed in AA, followed on day 4 by a more pronounced decrease than that found in AA, although it was not statistically significant (Fig. 1a). In the AA group, seven out of 11 subjects increased their percentage of sputum eosinophils on day 4, but that phenomenon was observable in only one NAAR subject, most (13) showing a reduction in the eosinophil numbers on day 4 compared with day 2 (Figs 2 and 3). The only NAAR subject demonstrating an inflammatory pattern similar to the AA had no particular clinical characteristics that could

Table 1. Characteristics of subjects

Group	Subject	Sex	Age	FEV ₁ (% pred.)	PC ₂₀ (mg/mL)	Allergen inhaled	Starting allergen dose
NAAR	S1	F	23	115	37	Birch	1:32 768
	S2	F	21	105	223	Birch	1:16 384
	S3	M	26	102	17	Mixed grasses	1:131 072
	S4	F	23	127	22	Birch	1:8192
	S5	M	26	104	55	<i>D. pteronyssinus</i>	1:32 768
	S6	M	27	115	19	Mixed grasses	1:524 288
	S7	F	23	111	35	<i>D. pteronyssinus</i>	1:16 384
	S8	F	22	94	64	Mixed grasses	1:16 384
	S9	M	22	105	82	<i>D. farinae</i>	1:2048
	S10	M	28	87	97	Cat	1:128
	S11	F	21	124	34	Birch	1:4096
	S12	F	43	90	32	<i>D. farinae</i>	1:4096
	S13	F	22	92	145	Mixed grasses	1:4096
	S14	F	23	84	44	Mixed grasses	1:16 384
Mean $\pm$ SEM			25 $\pm$ 2	104 $\pm$ 4	48 $\pm$ 1		1:2048
AA	S15	F	20	103	4	<i>D. pteronyssinus</i>	1:16 384
	S16	F	23	93	2	Mixed ragweed	1:131 072
	S17	F	21	104	1	<i>D. pteronyssinus</i>	1:65 536
	S18	M	20	101	4	Mixed grasses	1:131 072
	S19	F	24	97	7	Cat	1:65 536
	S20	F	23	94	8	Mixed grasses	1:32 768
	S21	F	19	96	0,3	Cat	1:262 144
	S22	F	21	93	3	<i>D. pteronyssinus</i>	1:32 768
	S23	F	18	91	3	Birch	1:65 536
	S24	F	23	94	5	<i>D. pteronyssinus</i>	1:65 536
	S25	M	40	101	4	Mixed grasses	1:131 072
Mean $\pm$ SEM			23 $\pm$ 2	98 $\pm$ 1	3 $\pm$ 1		1:4096

Table 2. Cell analysis of sputum samples*

	NAAR Baseline Post-LDAC	Day 2 Post-LDAC	Day 4	1 Week	AA Baseline	Day 2	Day 4	1 Week
Total cell counts (million cells/g mucus)	0.8 (0.6, 1.8)	1.0 (0.6, 1.3)	1.3 (0.8, 1.9)	1.0 (0.7, 1.5)	0.6 (0.3, 1.0)	0.6 (0.2, 1.6)	1.3 (0.4, 1.7)	0.8 (0.3, 2.3)
Differential cell counts (%)								
Lymphocytes	0.8 (0, 2.0)	0.9 (0.2, 3.2)	1.0 (0.5, 3.2)	0.8 (0.2, 2.2)	1.0 (0.3, 2.2)	1.8 (1.0, 2.9)	1.5 (0.5, 2.4)	1.8 (1.5, 2.0)
Macrophages	73.2 (44.0, 85.2)	66.2 (27.2, 77.0)	58.8 (34.0, 77.0)	72.2 (55.3, 81.9)	72.5 (34.1, 79.1)	68.5 (52.2, 69.4)	66.5 (42.1, 69.4)	73.0 (57.8, 80.0)
Neutrophils	17.8 (9.2, 27.5)	27.2 (21.2, 55.5)	33.1 (18.8, 59.0)	24.9 (9.2, 38.0)	20.2 (16.6, 60.0)	22.2 (9.8, 25.9)	30.2 (23.2, 41.2)	22.5 (10.8, 28.5)
Eosinophils	0.2 (0, 1.0)	2.9 (0.5, 8.2)	1.2 (0.2, 3.0)	1.1 (0.8, 1.8)	1.5 (0.8, 4.5)	2.0 (0.8, 4.2)	1.8 (1.0, 8.8)	3.1 (0.2, 8)

*Data are expressed as median (25%, 75% percentiles).

have helped predict his response pattern. He did not have asthma in his family, his PC₂₀ was not lower than the other NAARs (128 mg/mL), he did not have increased IgE levels and prick test for HDM was negative. There was no significant change in the other cells on induced sputum following allergen challenges in either group.

There were no significant variations in sputum or blood eosinophil counts between visits in house dust mite positive (HDM⁺) compared with house dust mite-negative (HDM⁻) subjects or between subjects challenged with HDM compared with the ones challenged with another allergen. Moreover, the increase in sputum eosinophils was not dependent on the allergen given for inhalation. White blood cells and blood differential cell counts were not statistically different at any time-point during the study.

Levels of ECP in sputum

Mean ECP levels were significantly higher in AA than in NAAR at baseline, on day 4 and 1 week post-VLDAC (Fig. 4). On day 2, ECP concentrations tended to increase in NAAR to almost reach ECP concentrations observed in AA, but this increase was not statistically significant. In NAAR, although changes were not significant, ECP levels followed the same pattern as eosinophils: they increased on day 2 and decreased on day 4 to tend to return to baseline levels one week following VLDAC. Consequently, we found a significant correlation between ECP levels and percentage eosinophils in NAAR throughout the study ($r = 0.457$, $P = 0.001$). For the NAAR subject showing an inflammatory pattern similar to that of AA, ECP levels continued to increase on day 4 and then returned to baseline levels one

Fig. 1. Variations in sputum percentage eosinophils between baseline, day 2, day 4 and 1 week following VLDAC in (a) NAAR and in (b) AA. Bars represent medians with 25 and 75 interquartiles. Squares represent means. * $P = 0.01$ vs. baseline NAAR; † $P = 0.0005$ vs. baseline NAAR.

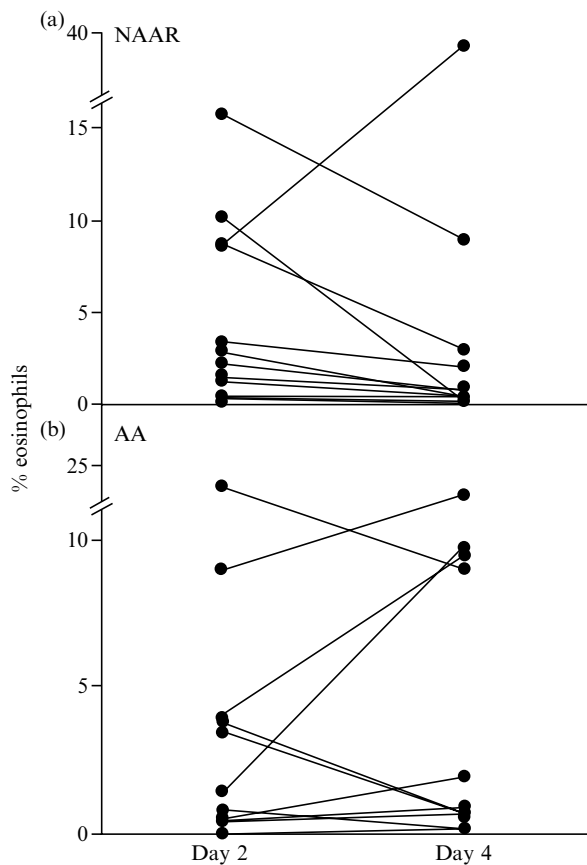
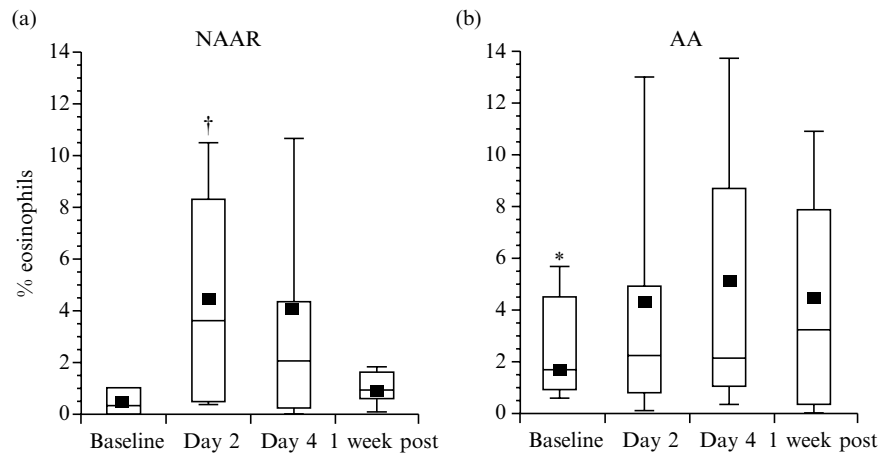


Fig. 2. Percentage of sputum eosinophils in (a) NAAR and in (b) AA between day 2 and day 4 of VLDAC.

week postchallenge. In AA, there were no significant variations of ECP levels over the study visits.

Discussion

Low-dose allergen challenge has been a useful means for studying the inflammatory process observed in mild allergic asthma [8, 29, 30]. This method has, among other advantages, the

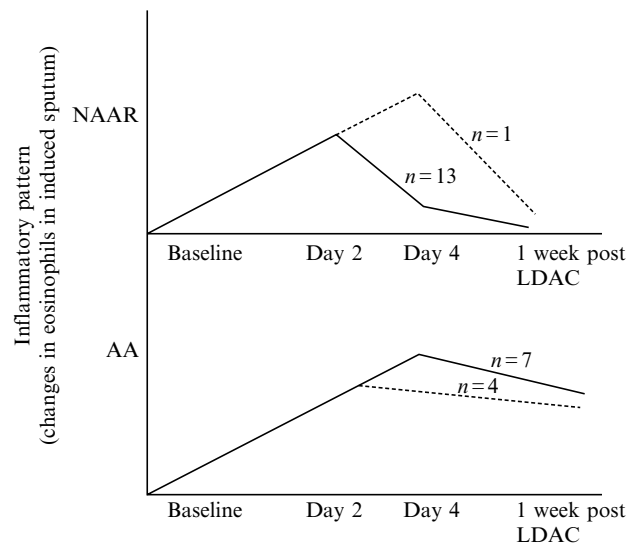


Fig. 3. Patterns of airway inflammation following VLDAC in NAAR and in AA.

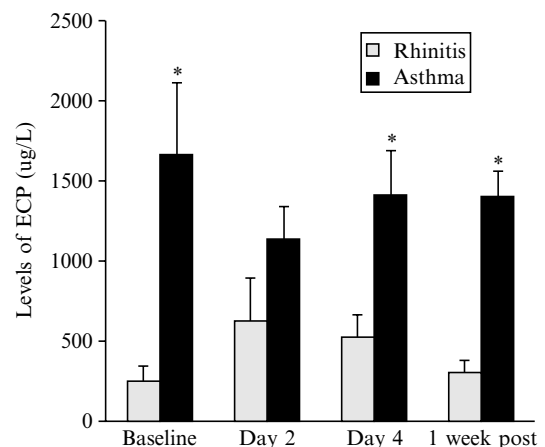


Fig. 4. Mean levels of sputum ECP at baseline, day 2, day 4 and 1 week post-VLDAC in NAAR and in AA. * $P < 0.05$ vs. NAAR.

ability to better mimic natural exposure to aeroallergens by using concentrations that are lower than the ones used in a conventional allergen challenge, and by repeating the exposure over a few days. To our knowledge, although standard or 'high'-doses of allergens have been used to study airway responses in non-asthmatic allergic subjects, VLDAC has never been used before in this population. We thought this could be a most useful way to look at the determinants and mechanisms by which asthma may develop in atopic subjects. In this regard, this study brings new information about the inflammatory response of atopic non-asthmatic compared with those with asthma and suggests that rhinitic subjects show a different pattern of response to allergens that may perhaps explain why some will develop asthma and others will not.

It is recognized that allergic rhinitis is a factor predisposing to the development of asthma [27–29]. Inflammation can be present in the lower airways of non-asthmatic rhinitic subjects during natural allergen exposure, even when there are no clinical symptoms [13, 31] nor any changes in airway responsiveness [32]. Using segmental allergen provocation, Alvarez et al. showed that the allergen-induced increase in sputum eosinophils was similar in NAAR to that found in AA following provocation, but that NAAR subjects had no decrease in methacholine PD₂₀, unlike AA, who had significantly lower PD₂₀ [9]. Moreover, Calhoun et al. compared airway inflammation and airway responsiveness induced by segmental- vs. whole-lung antigen challenge in NAAR [33]. They observed a significant increase in BAL eosinophils 48 h after both allergen challenges and this was associated with a significant fall in FEV₁ 15 min following both challenges and 48 h following whole-lung challenge. However, the doses used to induce airway changes in rhinitic subjects in these two studies were higher than in our study.

Despite these observations, we do not know why some subjects with allergic rhinitis will develop asthma and others will not. By demonstrating that some NAAR may show a reduction in airway inflammation, even when allergen exposure is continuing, we may conclude that some rhinitic subjects have protective or dampening mechanisms against the allergen-induced airway inflammatory process, possibly an 'immunological tolerance' to the inhaled allergen [34].

LDAC has also been shown to induce an inflammatory process in AA, but this was also associated with an increase in airway responsiveness or in clinical symptoms [35]. This study therefore shows that inhalation of very small doses of allergen, even lower than those used in other LDAC studies aimed at giving an allergen dose that would provoke a 5% fall in FEV₁, can induce airway inflammation in NAAR as well as in AA. As a comparison, VLDAC doses are about 3–5 doubling doses lower than for a 'classical' LDAC. Although the variations in sputum eosinophils were not statistically significant in AA, there was a trend toward an increase on day 2 of allergen exposure. The fact that we did use doses of allergens lower than what has been used for inhalation in the past may explain why we failed to observe the significant increases in AA that others found [27, 35]. We think, however, that it was necessary to use the same dose calculations for both groups, in order to minimize the difference in allergen load provided. As we did not want to raise ethical issues by giving doses that would have induced asthmatic reactions in rhinitic subjects, we chose allergen doses that induced only an inflammatory response, without inducing clinical symptoms. However, a next step could be to

use slightly higher doses, to better characterize the variations between the 2 groups and the mechanisms underlying these airway inflammatory responses. In subjects with NAAR, the increase in eosinophils we observed on day 2 was significant; the maximal increase in eosinophils was similar in the two groups, but the magnitude of the change was smaller in AA, their baseline percentage eosinophils being higher than those of rhinitics. Although these observations suggest a potentially important mechanism of protection against allergen-induced lower airway inflammation, they should be confirmed in further studies. We also need more data to explain the differences in allergen responses between allergic rhinitic subjects and allergic asthmatic subjects. These observations may provide some clues in regard to the identification of subjects at risk of developing asthma in the near future.

To our knowledge, this is the first time that two different inflammatory patterns are described in NAAR and in AA following allergen provocation. As the doses of allergen given for inhalation were determined according to the same method for both groups (mean starting allergen dose difference was only of one doubling dilution between the two groups (Table 1)), taking into consideration the estimated PD₂₀ allergen, although it cannot be excluded, it is unlikely that the differences observed in the pattern of allergen response could be attributable to the doses of allergen given.

We observed no statistically significant variations of inflammatory cells in the blood following allergen provocation. Our observations suggest that IS seems a more accurate medium than blood to monitor variations in inflammatory cells in mild AA and that the very limited local inflammatory airway changes are insufficient to be reflected in the blood. In this regard, Roquet et al. failed to observe significant changes in the proportions or activation of blood inflammatory cells following LDAC in AA [36]. On the other hand, it is also likely that the information provided by IS, since it directly explores the airways, is more accurate than data obtained from peripheral blood to reflect airway processes [37].

In conclusion, this study has shown that repeated VLDAC can increase airway inflammation without increasing airway responsiveness, causing airflow obstruction or inducing asthmatic symptoms in subjects with NAAR as well as in allergic AA. This supports the idea that lower airway inflammation can be present before the onset of clinical symptoms or airway hyper-responsiveness. In addition, two types of inflammatory response may be observed, suggesting an adaptation mechanism that seems to be absent in many AA. Although more studies are needed to confirm these observations, they provide new information about the factors involved in the potentially development of asthma.

Acknowledgements

We thank all the subjects for their participation in the study. We thank Serge Simard for the statistical analyses.

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