

Influence of Natural Exposure to Pollens and Domestic Animals on Airway Responsiveness and Inflammation in Sensitized Non-Asthmatic Subjects

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Key Words

Airway inflammation · Airway responsiveness · Allergens · Allergic rhinitis · Sputum, induced

Abstract

Background: Atopy may be a risk factor in the development of asthma. Indoor allergens are considered to be more potent asthma inducers than outdoor ones such as pollens. Lower airway inflammation may be present in non-asthmatic subjects during natural exposure to relevant allergens and may eventually lead to the development of asthma. **Aims:** To document seasonal variation in lower airway responsiveness and inflammation in sensitized non-asthmatic subjects, during natural exposure to allergens, and to determine whether it is more marked in those exposed to animals to which they are sensitized. **Methods:** Twenty-two atopic subjects were seen during and out of the pollen season. All (but the controls) were sensitized to domestic animals, and to trees, grasses or ragweed. Eleven were not exposed to animals at home and 8 were exposed. They were compared with 3 normal controls. A respiratory questionnaire was administered, allergy skin prick tests, spirometry, methacholine challenge, blood and induced sputum with differential cell counts were obtained during the pollen season for all subjects. These tests were repeated

out of the pollen season. **Results:** Throughout the study, none of the subjects had asthma symptoms. Mean PC₂₀ was significantly lower in subjects exposed to animals compared with unexposed subjects or controls, both during and out of the pollen season. In season, subjects exposed to animals had significantly higher sputum eosinophil numbers than unexposed or normal control subjects. **Conclusions:** Non-asthmatic atopic subjects show variable degrees of airway responsiveness and inflammation. However, subjects exposed to animals show higher airway eosinophilia, which may suggest they are at increased risk of developing airway hyperresponsiveness and asthma.

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Introduction

Allergic rhinitis is one of the main manifestations of atopy. Allergen sensitization is associated with an increased risk for developing asthma [1], and subjects with allergic rhinitis may be particularly at risk of developing it [2, 3]. As for asthma, the incidence of allergic rhinitis has increased considerably over the past 20 years [4]. About 80% of asthmatic subjects have rhinitis symptoms and 5–15% of subjects with perennial rhinitis have asthma [5].

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The type and intensity of allergen exposure may be a determinant of clinical manifestations of atopy [6]. Allergens can be divided into two distinct categories according to their indoor or outdoor source. In most countries, house dust mites (HDM) are the major source of indoor allergens followed by animal danders [7]. Indoor allergens may not only cause perennial rhinitis, they are also more frequently associated with asthma [8], while outdoor ones (which cause seasonal rhinitis) are more closely related to allergic rhinitis [6, 9].

Airway hyperresponsiveness (AHR) is one of the main physiological characteristics of asthma. It is also an important determinant in the development of asthma [2]. Studies have demonstrated that subjects with allergic rhinitis can present an increase in airway responsiveness following natural or laboratory exposure to relevant allergens [10–12]. Airway inflammation and remodeling may also be present in non-asthmatic rhinitic subjects, although it is less intense than in asthma [13, 14] and can increase during natural allergen exposure [15, 16].

Changes in airway responsiveness and airway inflammation have been documented in subjects with allergic rhinitis, both during and out of the pollen season. Nevertheless, we do not know if the pattern and intensity of inflammatory changes in subjects with allergic rhinitis induced by natural exposure to indoor allergens are similar to those induced by outdoor ones.

In this study, we aimed to assess changes in airway responsiveness and lower airway inflammation in subjects with allergic rhinitis during and out of the pollen season, and to compare the subjects exposed at home to animals to which they are sensitized with both unexposed subjects and normal controls.

Methods

Subjects

Thirty consecutive subjects aged 18–45 years, 24 with allergic rhinitis and 6 normal controls, were recruited from advertisements in newspapers. Exclusion criteria were a past or present history of asthma, previous asthma symptoms or asthma medication intake, current smoking and/or respiratory tract infection in the month preceding the study. Subjects using nasal corticosteroids were asked to stop taking their medication 1 month prior to their baseline visit.

All subjects but the controls were sensitized to tree, grasses and/or ragweed pollens. Subjects were either exposed ($n = 8$) or not exposed ($n = 16$) to domestic animals. They were compared with 6 non-atopic non-asthmatic normal control subjects. Subjects in the exposed group had daily contact with an indoor animal to which they were sensitized (cat or dog). The study was approved by the Institutional Ethics Committee, and each subject signed an informed consent form.

Study Design

Subjects were asked to attend the laboratory on two occasions. All visits were done in the afternoon in order to minimize circadian variations. For all subjects, the first visit was held during the pollen season (May to September). A respiratory questionnaire was administered, followed by skin prick tests with 26 common aeroallergens, baseline spirometry, methacholine challenge, blood sampling and finally, sputum induction. In the fall/winter (November to February), subjects came back for a second visit, at which time the same procedures, except for the skin prick tests, were performed.

Questionnaire

Each subject filled out a validated respiratory questionnaire based on the European Community Survey Questionnaire [17]. This provided information on past or present respiratory symptoms and family and personal history of asthma and other diseases.

Allergen Skin Prick Test

Atopic status was confirmed by prick tests with 26 common airborne allergens including pollens from trees and grasses, animal danders, molds and HDM. Normal saline and histamine were used as negative and positive controls, respectively. The wheal size was recorded at 10 min as the mean of two perpendicular measures. A positive response was defined as a skin wheal diameter of 3 mm or more.

Spirometry and Methacholine Challenge

Baseline expiratory flows and methacholine challenge were done with an American Thoracic Society (ATS)-approved spirometer (Vitalograph Medical Instrumentation, Lenexa, Kans., USA) and forced expiratory volume in 1 s (FEV₁) was measured according to ATS specifications [18]. The predicted values for expiratory flows were obtained from Knudson et al. [19]. The baseline FEV₁ was calculated from the best of three reproducible curves.

Airway responsiveness to methacholine was measured using the method described by Juniper et al. [20]. Briefly, doubling concentrations of methacholine up to 128 mg/ml were administered for 2 min at 5-min intervals through a Wright nebulizer at an output of 0.13 ml/min. Expiratory flows were recorded 30 and 90 s after each inhalation. The test was interrupted when the subject showed a 20% fall in FEV₁ or when the last concentration had been administered. The response was expressed as the provocative concentration of methacholine that caused a 20% fall in FEV₁ (PC₂₀) obtained from the log concentration-response curve.

Sputum Induction and Processing

Sputum was obtained and processed using the method described by Pin et al. [21] and modified by Pizzichini et al. [22]. After pretreatment with 100 µg of inhaled salbutamol, subjects inhaled increasing concentrations of saline (3, 4 and 5%) for 7 min each, for a maximum of 21 min, using a Medix electronic nebulizer (Medix, Catthorp, UK) without a valve or nose clip. After each inhalation, subjects were instructed to blow their nose and rinse their mouth with water in order to minimize postnasal drip and squamous epithelial cell contamination. After each concentration, FEV₁ was recorded for safety.

The sputum sample was processed as soon as possible within 2 h following the induction. The sample was transferred into a Petri dish and forceps were used to select plugs as free as possible of saliva (non-squamous cells); these were placed in a 15-ml polystyrene tube and weighed. A volume of 0.1% dithiothreitol (Sputolysin; Calbiochem,

Table 1. Characteristics of subjects on entry into the study

Subjects	Sex	Age years	FEV ₁ % pred.	FVC % pred.	PC ₂₀ mg/ml	Allergen sensitization
<i>Unexposed</i>						
S1	F	24	107	102	15	TP, GP, C, D, HDM
S2	F	22	102	99	>128	TP, GP
S3	F	21	92	88	30	TP, C, D, HDM
S4	M	21	105	91	>128	GP
S5	M	26	90	98	>128	GP, HDM
S6	M	27	100	92	41	TP, GP, HDM
S7	M	23	91	94	34	GP, C
S8	F	19	93	91	6	TP, GP, C
S9	F	23	116	117	18	TP, C, D, HDM
S10	F	23	127	117	>128	GP, C, D, HDM
S11	F	22	84	85	24	TP, GP, C, HDM
Mean		23	101	98	40	
<i>Exposed</i>						
S12	M	23	101	108	27	TP, GP, C, HDM
S13	F	23	100	115	9	GP, C, D, HDM
S14	F	21	104	109	2	TP, GP, HDM
S15	F	23	93	87	2	GP, C, D, HDM
S16	M	21	92	115	1	GP, C, D, HDM
S17	F	21	106	92	11	TP, GP, C, D, HDM
S18	F	21	99	97	6	TP, C, D
S19	M	19	109	94	>128	GP, C, D, HDM
Mean		22	101	102	10	
<i>Controls</i>						
S20	M	22	83	92	102	
S21	M	30	105	114	64	
S22	F	23	105	101	128	
Mean		25	98	102	94	

FVC = Forced vital capacity; TP = tree pollens; GP = grass pollens; C = cat; D = dog.

La Jolla, Calif., USA) freshly diluted with distilled water equal to 4 times the weight of the cell plugs was added and mixed gently to ensure homogenization, and the tube was rocked for 15 min. To stop the reaction, an equal volume of Dulbecco's phosphate buffer saline (D-PBS) 1 × [Life Technologies (Gibco BRL), Burlington, Ont., Canada] was added and the tube was again rocked for 5 min. The cell suspension was filtered through a 48-μm nylon gauze (SEFAR, Scarborough, Canada) to remove debris and mucus. The resulting suspension was centrifuged at 450 *g* for 5 min. The supernatant was aspirated and 200-μl aliquots were stored at -80°C until further analysis. Total cell count was obtained with the use of a Neubauer hemacytometer chamber (Reichert, Buffalo, N.Y., USA) and expressed as million cells per gram of selected sputum. Cell viability was determined by the trypan blue exclusion method, and if the sample contained less than 40% cell viability, the procedure was discontinued. Cell suspension was adjusted to 0.5–1 million cells/ml. Two prewet slides (50 μl of D-PBS 1 ×, 300 rpm for 3 min) were prepared with a Shandon III cytocentrifuge (Shandon Scientific, Astmoor, UK) and 100 μl of suspension was added to each slide and run at 300 rpm for 5 min. The slides were used for differential cell count and stained with Diff-Quik (Dade Diagnostics, Aguada, P.R., USA), and

400 cells were counted. All sputum counts and measurements were performed on coded slides by someone blind to the clinical details. Results were expressed as a percentage of total non-squamous cells.

Blood Sample Analysis

Blood was collected for complete blood count and total serum IgE analysis.

Statistical Analysis

Results are expressed as mean ± SEM. Split-plot-in-time analyses of variance (ANOVAs) were used to analyze the data from allergic subjects to account for variation among (1) types of allergy, (2) subjects within type of allergy, (3) times of visit for all types of allergy and for each type, and (4) experimental error. The main plot of the ANOVAs expressed the variation between subjects, while the subplot expressed the variation within subjects. Tests of hypotheses were conducted to ensure that residues were normally distributed (Shapiro-Wilk test) and their variances homogeneous (visual examination of the plot). For PC₂₀ and sputum total cells, we used the logarithm transformation in order to stabilize the variance among treatments. When a factor was significant, Bonferroni's multiple comparisons

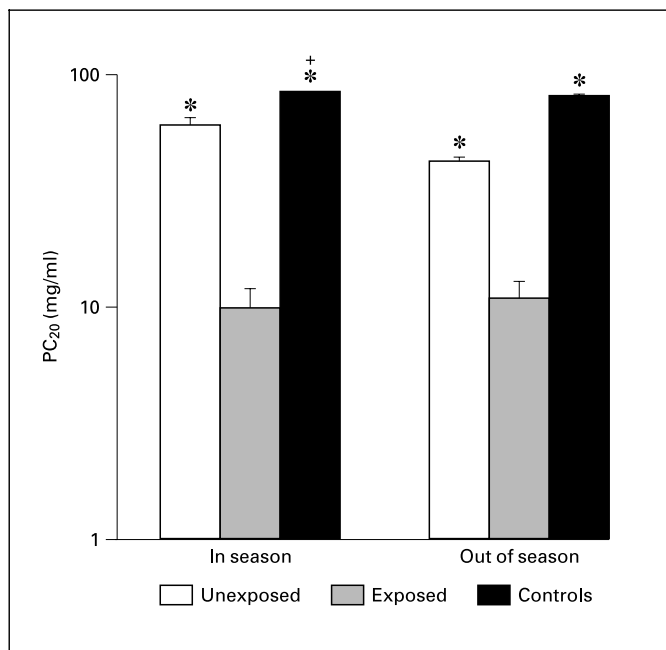


Fig. 1. Variations of mean PC₂₀ between unexposed, exposed and normal control subjects during and out of the pollen season. * $p < 0.0001$ vs. exposed; + $p < 0.0001$ vs. unexposed in season.

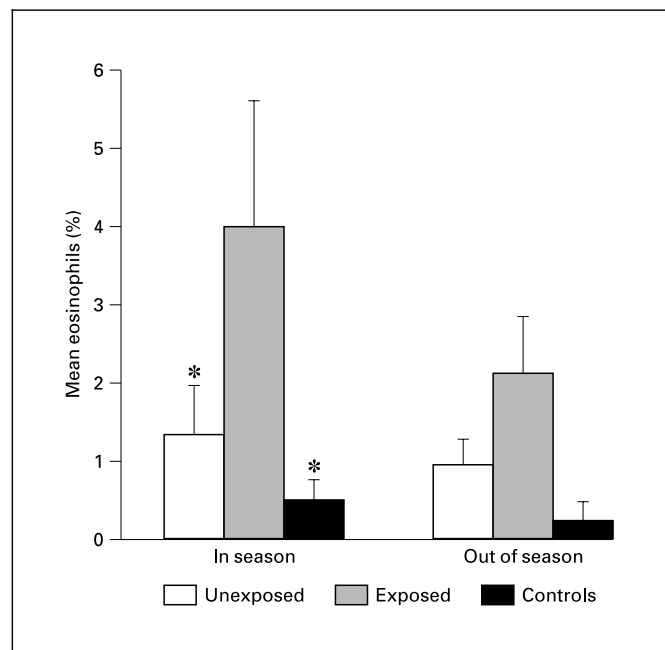


Fig. 2. Variations of mean sputum eosinophils between unexposed, exposed and normal control subjects during and out of the pollen season. Percent eosinophils correspond to the ones in the subgroup analysis. * $p = 0.02$ vs. exposed in season.

were carried out to determine which levels of the factor differed from the others. The results were considered significant if p values were ≤ 0.05 . Analyses were carried out with the SAS software (GLM procedure, SAS Institute, Cary, N.C., USA).

Results

Characteristics of Subjects

For the pollen season visit, we recruited 16 subjects in the unexposed group, 8 in the exposed group and 6 normal subjects (table 1). For the second visit, out of pollen season, 22 subjects came back. Eleven were from the unexposed group, 8 from the exposed group and 3 from the normal control group. Only these latter subjects were included in the statistical analyses.

Airway Responsiveness

All subjects (but controls) had seasonal symptoms of rhinoconjunctivitis. During the pollen season, mean PC₂₀ methacholine was 40 ± 1 mg/ml in the unexposed group, 10 ± 2 mg/ml in the exposed group and 94 ± 1 mg/ml in the control group (fig. 1). Out of pollen season, mean PC₂₀ was 42 ± 2 mg/ml in the unexposed group, 11 ± 2 mg/ml

in the exposed group and 81 ± 1 mg/ml in the controls. Both in and out of pollen season, controls had significantly higher mean PC₂₀ than exposed subjects ($p < 0.0001$ and < 0.0001 , respectively), and unexposed subjects had a significantly higher mean PC₂₀ than exposed ones ($p < 0.0001$ and < 0.0001 , respectively). During the pollen season, PC₂₀ was significantly higher in normal than in unexposed subjects ($p < 0.0001$), but out of pollen season, PC₂₀ between these groups was not statistically different. During the pollen season, only 2 out of 11 (18%) unexposed subjects presented AHR as shown by a reduction of the PC₂₀ under 16 mg/ml compared with 5 out of 8 (62%) exposed subjects. Out of the pollen season, airway responsiveness normalized in all unexposed subjects, but only in 3 exposed subjects. None of the control subjects presented AHR on either visit.

Induced-Sputum Inflammatory Cell Analysis

The results for sputum differential cell counts are shown in table 2. Total cell counts were not significantly different between groups, whether in or out of the pollen season. Although sputum eosinophils were lower out compared to during the pollen season (1.3 ± 0.6 vs. $0.9 \pm 0.3\%$ in unexposed and 4.0 ± 1.6 vs. $2.1 \pm 0.7\%$ in

Table 2. Cell analysis of sputum samples

	In season			Out of season		
	U	E	C	U	E	C
Total cell counts, million cells/g	2.4±0.9	1.0±0.3	3.5±0.9	2.3±0.3	1.4±0.2	2.5±0.5
Differential cell counts, %						
Lymphocytes	1.8±0.3	1.4±0.3	3.0±0.6	2.0±0.5	2.0±0.5	2.6±1.5
Macrophages	66.5±6.7	72.2±9.4	61.9±10.6	59.2±9.2	58.8±10.2	71.7±18.2
Neutrophils	28.9±6.7	21.2±9.8	34.4±10.3	36.5±9.5	36.1±11.0	24.4±15.7
Eosinophils	1.3±0.6*	4.0±1.6	0.5±0.2	0.9±0.3	2.1±0.7	0.2±0.2

Values represent mean ± SEM. * p = 0.02 unexposed subjects vs. exposed ones and controls.
U = Unexposed subjects; E = exposed subjects; C = control subjects.

exposed subjects), this difference did not reach statistical significance whatever the group. There was no significant difference for the other inflammatory cell counts between the two visits. However, during the pollen season, relative numbers of eosinophils were significantly higher in exposed subjects than in unexposed ones (4.0 ± 1.6 vs. $1.3 \pm 0.6\%$, $p = 0.02$) or controls (4.0 ± 1.6 vs. $0.5 \pm 0.2\%$, $p = 0.02$) (fig. 2). Out of pollen season, there was a non-significant trend for eosinophils to be higher in exposed subjects than in controls (2.1 ± 0.7 vs. $0.2 \pm 0.2\%$, $p > 0.05$). There was no significant difference in eosinophil percentages between exposed and unexposed subjects out of pollen season (2.1 ± 0.7 vs. $0.9 \pm 0.3\%$, $p > 0.05$). However, compared to each other, changes in eosinophils between visits were greater in exposed than in unexposed subjects.

Blood Analysis

White blood cell counts and blood relative inflammatory cell numbers (lymphocytes, monocytes, neutrophils, eosinophils and basophils) were not statistically different between groups for either visit, nor were they statistically different between visits within a group. Total IgE levels were statistically higher in exposed subjects compared with unexposed and normal controls for both visits (in season: 432 ± 247 , 218 ± 70 and 48 ± 27 IU/ml, respectively; $p < 0.0001$ and < 0.0001 , respectively; out of season: 364 ± 200 , 203 ± 78 and 52 ± 24 IU/ml, respectively; $p = 0.0007$ and < 0.0001 , respectively). In addition, compared to normal controls, unexposed subjects had significantly higher IgE levels both in ($p = 0.0004$) and out ($p = 0.009$) of the pollen season. No group had a significant change in IgE levels between visits.

Discussion

This study showed that non-asthmatic subjects with seasonal pollen-induced rhinitis exposed at home to animals to which they are sensitized had a higher degree of airway eosinophilia and responsiveness during and after the pollen season. We found no significant seasonal change in methacholine responsiveness within a group whether in sensitized subjects or controls. Airway eosinophilia was more marked during the pollen season, but the difference was not statistically significant. Furthermore, AHR was frequently within the asthmatic range during the pollen season in exposed subjects while it was not in unexposed ones.

Previous studies have compared the airway responsiveness of subjects with seasonal and perennial allergic rhinitis [11, 23, 24]. They observed increases of variable magnitude in airway responsiveness during the pollen season compared with out of season. In 1987, Madonini et al. [11] observed that PC_{20} to methacholine was in the asthmatic range in 11% of allergic rhinitis subjects out of pollen season, while that number increased to 48% during the pollen season. More recently, Di Lorenzo et al. [23] observed that airway responsiveness to methacholine was in the asthmatic range in 16 out of 49 (33%) allergic rhinitis subjects during the pollen season, as compared with only 8 (16%) subjects out of season. Although the mean changes in airway responsiveness between in and out of season were not significantly different in our groups of subjects, we also observed more subjects with AHR during the pollen season in the exposed group compared with the unexposed one.

The chronicity of exposure to allergens seems to be an important contributing factor in the development of AHR

[25]. In keeping with these hypotheses, avoidance of animal contacts or reduction of HDM levels has been shown to improve AHR and symptoms [26, 27]. The fact that unexposed subjects were in contact only with allergens to which they were sensitized a few months a year as compared with all year long for the exposed subjects could explain why more exposed subjects presented AHR out of season compared with in season. In addition, our results are consistent with others, and suggest that perennial rhinitis is a greater risk factor for the development of AHR than seasonal rhinitis [24]. Another considerable factor in the development of AHR is the sensitization to HDM [28, 29]. Because houses are heated and less well ventilated during fall and winter, the HDM concentrations can increase; this could have contributed to the increased AHR we saw in sensitized subjects. Nevertheless, only 2 subjects (exposed ones) out of 14 subjects sensitized to HDM presented an increase in airway responsiveness during the fall, making it unlikely that this could have contributed to AHR out of pollen season. It would have been interesting, however, to have antigen counts, in order to evaluate the contribution of the exposure to HDM and airway responsiveness or inflammation.

We previously observed that airway responsiveness and blood eosinophils significantly increased in pollen-sensitized subjects during natural exposure [10]. In this study, although there was a trend for airway responsiveness to be higher during than out of the pollen season, it did not reach statistical significance. This may be related to the number of subjects studied, the intensity of allergen exposure, or timing measures. However, as we observed fewer unexposed subjects presenting AHR out of than during the pollen season, our results still support the hypothesis that natural pollen exposure may increase airway responsiveness in non-asthmatic atopic subjects with seasonal rhinitis.

Some authors have observed an increase in airway inflammation during [15] and even out of the pollen season [30] in allergic rhinitis subjects, while others have failed to observe such variations [31]. In this study, although there was a non-significant trend in both exposed and unexposed subjects for sputum eosinophils to be lower out of than during the pollen season, we did not observe significant variations in lower airway inflammation between the two visits. However, during the pollen season, sputum eosinophils were significantly higher in exposed subjects compared with unexposed ones or normal controls. We need to gather more data comparing lower airway inflammation and changes in airway responsiveness between seasonal and perennial rhinitis. In this

regard, Gutiérrez et al. [32] compared subjects with seasonal and perennial rhinitis, but they did not compare airway inflammation between these two groups. Instead, they compared subjects on the basis of AHR and observed that subjects presenting AHR had higher sputum eosinophil percentages than subjects without AHR. If we look at our results in these terms, they are similar, since our exposed subjects had higher sputum eosinophils than our unexposed subjects. Wilson et al. [33] also compared seasonal and perennial rhinitis subjects on the basis of nasal inflammation. They found that tryptase and eosinophil cationic protein as mediators of mast cells and eosinophils activation were significantly increased in nasal lavage of perennial rhinitics compared with seasonal rhinitics. Our results, although they refer to airway inflammation, are therefore in agreement with this study. One possible explanation for why indoor allergens induce stronger airway inflammation may be that they are carried on very small particles that allow them to reach the lower airways more easily [34]. Moreover, allergens from cats and dogs can stay in suspension for a long period of time [34, 35].

We are aware that a possible weakness of our study is the small number of adequate sputum samples that were available for statistical analysis. We could have observed more statistically significant variations between visits if we had had a greater number of subjects. Nevertheless, we did observe significant differences.

It has been hypothesized that indoor allergens are more closely associated with asthma [8] while outdoor allergens are more closely related to allergic rhinitis [6, 9]. In this study, we obtained results in agreement with this hypothesis, since the subjects exposed to an animal at home had lower PC₂₀ and greater airway inflammation than did the unexposed subjects. The 'priming effect' theory could explain, in part, why indoor allergens may be closely related to asthma. In fact, while this has not been thoroughly explored, a few studies have shown that exposure to one allergen may lead to priming to another, with increased early and late asthmatic responses to the latter [36]. Moreover, it has been shown that the magnitude of allergen-induced airway responses may increase in some non-asthmatic atopic subjects when repeated at a short interval [37]. This, however, does not take into account possible protective mechanisms that could reduce the inflammatory response in non-asthmatic atopic subjects, a phenomenon described, particularly in animal models, as the development of 'tolerance' to the allergen [38].

A positive correlation has been made between total IgE concentrations and AHR and asthma [1, 9, 39]. As expected, total serum IgE was out of the normal range in

both unexposed and exposed subjects, but within the normal range for the controls. In their study, Di Lorenzo et al. [23] observed significantly higher levels of total serum IgE in pollen-sensitized subjects, both in and out of the pollen season. In addition, although they observed a tendency for total IgE to decrease out of the pollen season, levels were still out of the normal range. Our results are, therefore, in agreement with these observations, as we observed the same pattern in our subjects.

In conclusion, this study showed that subjects sensitized to both animals and pollen have a higher degree of airway responsiveness and inflammation when exposed to domestic animals, suggesting that they may be more at risk for developing AHR and asthma. This could, in part, be a consequence of the combined effect of perennial animal and seasonal pollen exposure in some non-asthmatic

atopic subjects. The consequences of persistent versus intermittent or multiple allergen exposures remain nevertheless to be further studied. We need non-invasive methods to identify atopic subjects at a higher risk of developing asthma; induced sputum analysis may provide useful measurements in this regard, although long-term studies looking at the clinical outcome of rhinitic subjects with significant airway eosinophilia will be most instructive.

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Announcement

Workshop 'Allergy Vaccination: Novel Strategies and Perspectives'

November 24–27, 2002, Sochi, Russia

Scientific Committee

Roman Khanferyan (Russia), chairman
 Alexander Chuchalin (Russia)
 Friedhelm Diel (Germany)
 Lawrence M. DuBuske (USA)
 Igor Gushchin (Russia)
 Rakhim Khaitov (Russia)
 Gabriele Pauli (France)
 Daniele Sharpin (France)
 Rudolf Valenta (Austria)

Topics:

Strategies of allergy diagnostics
 Modified allergens for immunotherapy of allergy
 DNA vaccines
 Peptide immunotherapy
 Anti-IgE therapy
 Safety of the specific immunotherapy
 New trends in the development of antimediator drugs

Important Dates

September 1, 2002 – deadline for submission of abstracts
 October 1, 2002 – final registration and hotel reservation

Scientific-Organizing Secretariat

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