

A single nasal allergen challenge increases induced sputum inflammatory markers in non-asthmatic subjects with seasonal allergic rhinitis: correlation with plasma interleukin-5

K. M. Beeh, J. Beier, O. Kornmann, C. Meier, T. Taeumer* and R. Buhl

Pulmonary Department, Internal Medicine, University Hospital Mainz, Mainz, Germany

Summary

Background Seasonal allergic rhinitis (SAR) is a risk factor for asthma in affected individuals. Nasal allergic inflammation enhances bone-marrow eosinophil production, mainly via IL-5, and rhinitis patients have increased airway inflammation during the pollen season.

Objective To assess the impact of nasal allergy on sputum inflammatory markers.

Methods In an open-labelled, randomized, placebo-controlled cross-over study with 16 non-asthmatic SAR patients (median age 25 years, 56% males), the effect of a single nasal allergen challenge performed out of season on induced sputum inflammatory parameters was evaluated. SAR patients were identified by history, skin-prick test and specific IgE. All patients had normal lung function/bronchial hyper-responsiveness out of season and a negative asthma/wheezing history. Sputum cells and supernatant levels of ECP, sICAM, IL-5 and IL-10, and plasma levels of IL-5 and ECP, were measured before and 24 h after nasal allergen challenge. After a washout period of at least 4 weeks, the procedure was repeated with placebo challenge (diluent).

Results Nasal allergen challenge led to an increase in sputum ECP (pre = 60 ± 12 , post = 212 ± 63 $\mu\text{g/L}$, $P = 0.02$ vs. placebo), and sICAM (4.8 ± 2.7 to 6.5 ± 2.9 ng/mL, $P = 0.02$ vs. placebo), whereas IL-10 decreased after provocation (44 ± 11 to 29 ± 6 pg/mL, $P = 0.06$ vs. placebo). Sputum IL-5 was undetectable in all patients. The absolute number of blood and sputum eosinophils did not change significantly after allergen or placebo challenge ($P > 0.07$, both comparisons). Plasma levels of IL-5 increased after allergen challenge (8.7 ± 2.9 to 14.5 ± 3.9 pg/mL, $P = 0.001$), and the increase in plasma IL-5 was positively correlated with the rise in sputum ECP in a subgroup of 'responders' ($n = 12$, $r = 0.71$, $P = 0.01$).

Conclusions A single nasal allergen challenge in SAR patients increased markers of allergic inflammation in the lower respiratory tract, possibly via pronounced activation of inflammatory cells through circulating immediate-type reaction cytokines like IL-5. These findings may provide additional explanatory data for the high susceptibility of SAR patients to incident asthma.

Keywords asthma, induced sputum, nasal allergen challenge, rhinitis

Submitted 24 April 2002; revised 25 November 2002; accepted 2 January 2003

Background

Bronchial asthma is characterized by a predominant eosinophilic inflammation of the lower respiratory tract [1]. Allergy is the one most common cause for asthma, and affected patients frequently suffer from coincident allergic rhinitis. However, allergic rhinitis itself may predispose for incident asthma, as epidemiologic data suggest that as much as 30 to 80% of patients with allergic rhinitis occasionally develop asthma [2]. Increased bronchial hyper-reactivity and airway inflammation

are present even in patients with sole seasonal allergic rhinitis (SAR) due to pollen sensitization. Methacholine responses consistently increase during the pollen season, and numbers of eosinophils and related mediators, e.g. eosinophilic cationic protein (ECP), are increased in the airways of affected patients [3, 4]. However, the underlying mechanisms leading to these seasonal changes are not completely understood. On the one hand, nasal congestion due to rhinitis decreases nasal airflow, thus leading to increased mouth breathing and possibly higher penetration of airborne allergens to the lung. On the other hand, it has been repeatedly demonstrated that the induction of local allergic inflammation (e.g. after bronchial or nasal allergen challenge) leads to an increased trafficking of eosinophils and progenitor cells from the bone marrow to the peripheral blood and sites of inflammation, mainly due to systemic effects of immediate-reaction cytokines like IL-5 [5, 6]. IL-5 is a cytokine

Correspondence: Kai Michael Beeh, Pulmonary Department, Internal Medicine, University Hospital, Langenbeckstr. 1, 55131 Mainz, Germany. E-mail: k.beeh@3-med.klinik.uni-mainz.de

*The presented work was part of Ms. Taeumer's doctoral thesis.

highly specific for eosinophils, leading to activation, increased maturation, chemotaxis and migration of these cells into sites of inflammation [7].

Homing of eosinophils also involves increased expression of adhesion promoting molecules, e.g. intercellular adhesion molecule (ICAM)-1 [8], and data from human studies have shown that inhalation of IL-5 leads to an increase of soluble ICAM (sICAM) in the lower respiratory tract [9]. Moreover, allergen inhalation increases sICAM in bronchoalveolar lavage [10], and nasal allergen exposure leads to increased mucosal expression of ICAM-1 [11].

IL-10 is a Th2-cytokine with a wide range of anti-inflammatory properties. In allergy, IL-10 is crucial for the termination and resolution of allergic inflammatory processes, and failure to elicit an appropriate response of IL-10 is associated with more severe allergic disease [12].

Although several authors have investigated the effect of natural nasal pollen exposure on BAL or sputum parameters, exhaled nitric oxide or bronchial hyper-responsiveness, these approaches did not fully preclude the possibility of simultaneous allergen exposure to the lower respiratory tract. Hence, the observed increase in lower airway inflammation in these studies may as well reflect local exposure to low-grade allergen concentrations leading to airway inflammation.

With this background, the present study was designed to address the question of whether nasal allergic inflammation induced by allergen challenge is also able to provoke changes in constituents of the lower respiratory tract recovered by induced sputum, either by an increase of sputum inflammatory cells, or mediators and cytokines associated with allergic inflammation, in particular eosinophilic cationic protein (ECP), IL-5, IL-10 and sICAM. Sputum parameters were further compared with peripheral blood markers to investigate a possible systemic cross-talk between the nose and the lung.

Methods

Patients

The study population consisted of 16 patients (nine males, median age 25 years, range 22 to 33 years) with a history of allergic rhinitis due to grass or birch pollen. The diagnosis was confirmed by positive skin prick test and positive (RAST class ≥ 2) specific IgE-antibodies, assessed by CAP-FEIA Immunoassay (Pharmacia, Freiburg, Germany). All patients were non-smokers, had a negative history of any asthmatic symptoms, e.g. wheezing or cough during pollen seasons, and lacked other active allergic or respiratory diseases, such as acute or chronic rhinitis, respiratory infection or atopic dermatitis, at the time of the investigation. None of the patients had active perennial allergic rhinitis during the study.

Study design

The study was performed as an open-labelled, randomized, placebo-controlled cross-over challenge trial, and consisted of four visits. At baseline (November 2000), patients underwent sputum induction and methacholine challenge, and blood samples for determination of peripheral blood eosinophils, IL-5 and ECP were drawn. Then, a nasal allergen/placebo challenge was randomly performed using individual allergens

(birch or grass pollen) or diluent, as described in detail below. Patients returned to the clinic 24 h after the challenge for induced sputum and repeated blood sampling. After a wash-out-period of at least 4 weeks, the above-described procedures except from methacholine challenge were repeated, and nasal allergen/placebo challenge was performed in an individual cross-over design. Throughout the whole study period, subjects were not allowed to take any nasal or bronchial medication, e.g. topical or systemic steroids, antihistamines, nasal or inhaled cromones, decongestants, or non-steroidal anti-inflammatory drugs.

The study design was approved by the local ethics committee. All patients were informed about the purpose of the study and gave informed written consent.

Methacholine challenge

For baseline characterization of patients, a methacholine challenge was performed to quantify non-specific bronchial hyper-responsiveness out of season. The challenge was carried out according to ATS guidelines [13] using a five-breath dosimeter protocol (MEFAR nebulizer, Markos, Monza, Italy) with purified methacholine (Provocholine, Methapharm, Brantford, Ontario, Canada).

Allergen challenge

Nasal allergen challenge was performed according to the EAACI guidelines for allergen provocations [14]. Purified grass pollen or birch allergen was obtained from ALK Scherax, Hamburg, Germany, with 100 000 standard allergen units (SQ) per ml in the stock solution. Before challenge, a spirometry was performed. Bilateral nasal administration of allergen was then started with a dose of 100 SQ units, and consequently increased with 10-fold dose increments (1000 and 10 000 SQ, respectively) until a clinical response was effectuated. A possible response was evaluated within 15 min after allergen administration and was regarded as positive when nasal itching, sneezing and hypersecretion occurred. Allergen was administered during breath-hold, and patients were carefully advised to keep their head in a forward prone position to avoid swallowing or aspiration of allergen or nasal secretions to the lower respiratory tract. In total, patients were monitored for 60 min after the last dose of allergen for safety reasons, and were dismissed when spirometry revealed no significant change in FEV₁ from baseline ($> 15\%$ decrease). Placebo challenge was performed in the same manner using allergen diluent (ALK Scherax).

Sputum induction

Sputum induction was performed according to a method previously described [15], with slight modifications [16]: patients inhaled 4% hypertonic saline delivered by an ultrasonic nebulizer device (UltraNeb 2000, DeVilbiss, West Midlands, UK) for 15 min. Patients were then told to rinse their mouth, blow their nose and carefully cough sputum into a Petri dish using forced expiratory manoeuvres. If the induction was well tolerated, the first portion of sputum was discarded, and the inhalation procedure was repeated for another 15 min.

Sputum processing and counting

Sputum plugs were carefully separated from salivary secretions, and closely examined by light microscopy to ascertain a least

possible contamination of sputum with saliva and squamous cells. An appropriate sample was then filled into a 1-mL Eppendorf cup, weighed, and mixed with twice its volume of dithiotreitol (DTT 0.1%, Calbiochem, Bad Soden, Germany). Sputum was gently vortexed and placed into a water-bath at 37 °C for 15 min to allow homogenization of the sample, with repeated mixing. Then, phosphate-buffered-saline (PBS, Gibco Life, Paisley, Scotland) was added to achieve a 10-fold dilution of the sample. Afterwards, samples were centrifuged at 400 *g* for 10 min. The supernatant was collected and immediately frozen at –20 °C for subsequent analysis, and the cell pellet was re-suspended in 200 to 400 µL PBS with 1% bovine serum albumine (BSA, Sigma Chemicals, Heidelberg, Germany). Twenty µL of cell suspension was added to 5 µL of trypan blue, vortexed, and 10 µL of the solution then counted in a Neubauer cell chamber. Viable cells were counted, and the total cell count was calculated as cell count per ml sputum. For cytopins, an adequate sample of the cell suspension was added into a cytospin-tube to achieve a total number of 20 to 30 000 cells per slide, spun for 5 min, fixated and stained with Hemacolor-staining (Merck, Darmstadt, Germany) to allow differentiation of cells. Cytospin quality was regarded adequate when contamination with squamous cells was less than 20%. Four hundred non-squamous cells were then independently counted by two blinded observers. Relative proportion of cell types was counted as percentage of all non-squamous cells.

Measurement of inflammatory markers in plasma and sputum supernatant

Sputum supernatant or plasma concentrations of IL-5, sICAM and IL-10 were measured by commercially available ELISAs (sICAM, BenderMedSystems, Vienna, Austria; IL-10 and IL-5, Pharmingen, Heidelberg, Germany) according to the manufacturer's instructions. Plates were read at a wavelength of 540 nm (IL-10 and sICAM) and 450 nm (IL-5), respectively. The lower detection limits of the assays were 7.8 pg/mL (IL-10 and IL-5) and 0.6 ng/mL (sICAM), respectively.

Sputum and plasma ECP were measured by a commercially available enzyme assay (CAP-FEIA, Pharmacia, Freiburg, Germany), with a lower detection limit of 2 µg/L.

In one patient, the amount of sputum supernatant was not sufficient to perform all analyses. Therefore, data on sputum ECP, sICAM and IL-10 following placebo challenge are derived from only 15 patients.

Statistical analysis

Statistical analysis was performed using the STATA 5.0 inter-cooled software package (Stata Corp., College Station, TX, USA) for personal computers. Unless otherwise stated, data are given as mean values with standard error of mean ($\pm$ SEM), or median (range). The Kolmogorov-Smirnov Test was used to test variables for normal distribution. Individual pre/post differences for allergen and placebo challenge, and mean differences between cross-over periods, were compared by paired (pre/post differences) or unpaired (mean challenge effect differences) non-parametric Wilcoxon-Mann-Whitney test. If applicable, pre/post comparison was performed by paired *t*-test for normally or log normally distributed values. Correlational analysis was performed using simple linear regression analysis after log transformation of log normally

distributed values. The relative change of selected parameters after challenge was expressed as the ratio of post- to pre-challenge value. Relative changes in sputum ECP, sputum sICAM and plasma IL-5 were log normally distributed in the study population and therefore log transformed for correlational analysis. A *P*-value of less than 0.05 was considered statistically significant.

Results

Patients

A summary of baseline characteristics of participating subjects is given in Table 1. Allergen challenge was safe and well-tolerated by all SAR patients. The majority of patients responded to the allergen dilution step containing 1000 SQ allergen (14/16). Only two patients responded to the first allergen dilution (100 SQ). No significant drop of FEV₁ was observed in any patient 1 and 24 h after challenge, respectively.

Induced sputum cells

Baseline values for sputum cells were comparable before allergen or placebo challenge (summary in Table 2). The overall sputum cell count remained unchanged after allergen and placebo challenge. Sputum cell differentials, either expressed as total cell number or relative proportion, also remained unchanged after allergen challenge. There was a significant decrease in sputum lymphocytes after placebo challenge, whereas macrophages, neutrophils and eosinophils were unaffected.

Sputum inflammatory markers

After allergen challenge, there was an increase in sputum ECP and sICAM, that was also statistically significant over placebo-induced changes (*P* = 0.02, both comparisons) (Figs 1 and 2). In contrast, mean IL-10 levels decreased after allergen, but not placebo challenge (difference between challenges *P* = 0.06) (Fig. 3). However, placebo baseline values for sputum ECP and IL-10, but not sICAM, differed significantly from allergen baseline values (*P* = 0.02, both comparisons). Due to the randomized cross-over design, this was not assumed to represent a carryover effect. Sputum IL-5 was below the detection limit of the assay in all patients.

There was neither a correlation of changes in sputum ECP with sputum sICAM, nor were changes in ECP and sICAM correlated with changes in sputum IL-10. Finally, the increase of sputum ECP after allergen challenge was not correlated with baseline PC₂₀ values (*P* > 0.5, all comparisons).

Peripheral blood eosinophils, plasma IL-5 and ECP

The percentage of peripheral blood eosinophils significantly increased after allergen challenge, and there was also a clear

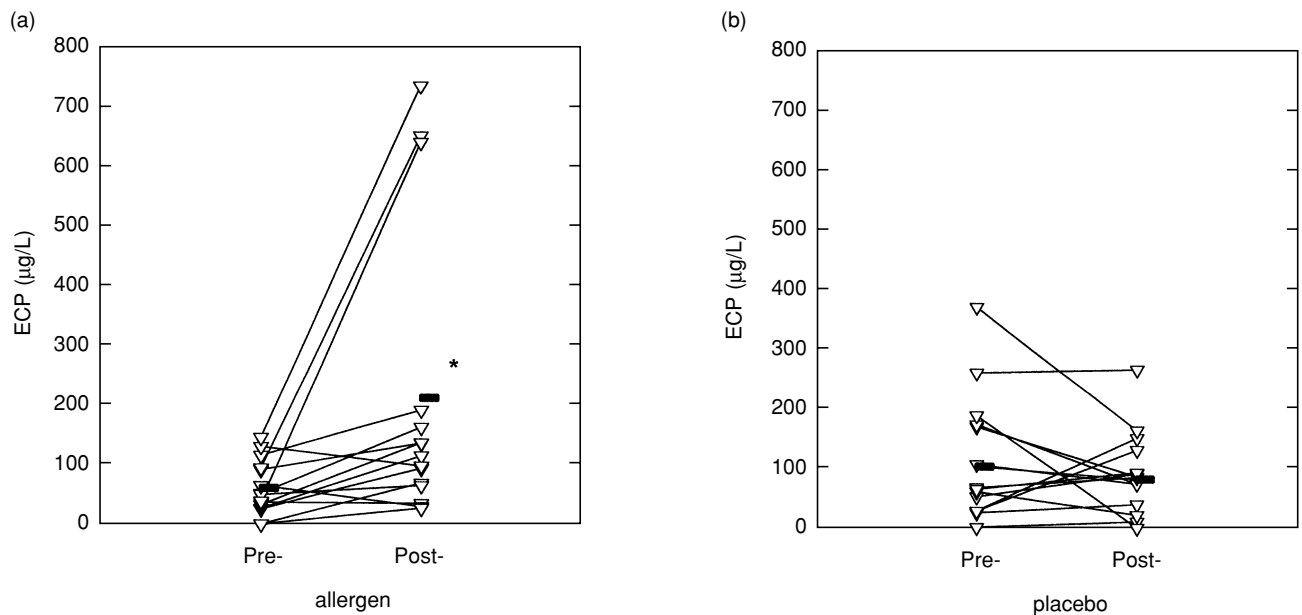
Table 1. Characteristics of subjects (*n* = 16)

Age (years, median, range)	25 (22–33)
Sex (M/F)	9/7
FEV ₁ (mean $\pm$ SEM, %predicted)	104 $\pm$ 4
PC ₂₀ methacholine (mg/mL, median, range)	8.5 (3.2–16)
Birch vs. grass sensitization (<i>n</i>)	9/7
Total IgE (kU/L, median, range)	140 (51–638)
RAST class (median, range)	3 (2–6)

Table 2. Peripheral blood and sputum markers after nasal allergen/placebo challenge

	Allergen		Placebo	
	Pre-	Post-	Pre-	Post-
Total sputum cells ($\times 10^6/\text{mL}$)	3.77 ± 1.1	3.1 ± 0.5	2.97 ± 0.45	2.6 ± 0.8
Macrophages ($\times 10^6/\text{mL}$)	2.59 ± 0.7	1.9 ± 0.27	2 ± 0.3	1.4 ± 0.29
Neutrophils ($\times 10^6/\text{mL}$)	0.97 ± 0.34	0.99 ± 0.24	0.8 ± 0.17	1.2 ± 0.57
Eosinophils ($\times 10^6/\text{mL}$)	0.014 ± 0.007	0.093 ± 0.049	0.011 ± 0.007	0.031 ± 0.018
Lymphocytes ($\times 10^6/\text{mL}$)	0.19 ± 0.06	0.15 ± 0.03	0.13 ± 0.034	$0.059 \pm 0.016^*$
Blood eosinophils ($\times 10^6/\text{mL}$)	0.16 ± 0.025	$0.19 \pm 0.028\$\$$	0.17 ± 0.03	0.17 ± 0.02
Plasma ECP ($\mu\text{g/L}$)	2.71 ± 0.65	2.68 ± 0.7	1.54 ± 0.35	2.03 ± 0.75
Plasma IL-5 (pg/mL)	8.7 ± 2.9	$14.5 \pm 3.9\%$	8.2 ± 2.3	8.2 ± 2.6
Sputum ECP ($\mu\text{g/L}$)	60 ± 12	$212 \pm 63^{**}$	112 ± 28	91 ± 19
Sputum IL-5 (pg/mL)	ND	ND	ND	ND
Sputum sICAM (ng/mL)	4.8 ± 2.7	$6.5 \pm 2.9\$\$$	6.3 ± 2.4	5.7 ± 2.1
Sputum IL-10 (pg/mL)	44 ± 11	$29 \pm 6^{***}$	23 ± 7	33 ± 8

* $P = 0.02$; ** $P = 0.004$; *** $P = 0.05$; § $P = 0.001$; §§ $P = 0.07$; ND = not detectable.

**Fig. 1.** Sputum ECP before/after allergen (a) or placebo (b) challenge. *Indicates $P = 0.004$ vs. baseline and $P = 0.02$ vs. changes after placebo.

trend indicating a rise of absolute blood eosinophils. Although blood eosinophils remained unchanged after placebo, the difference between challenges was not statistically significant (difference between challenges $P = 0.19$). In contrast, plasma IL-5 levels increased after allergen, but not placebo, challenge (difference $P = 0.003$, Fig. 4). Plasma ECP was generally low in all subjects, and no significant increase in plasma ECP was observed 24 h after allergen challenge. Further, there was no correlation of changes in sputum ECP with plasma ECP, and no correlation of sputum ECP with peripheral blood eosinophils. However, a significant correlation of the relative increase in sputum ECP, but not sputum sICAM, with changes in plasma IL-5 was found, when only responders (ratio of post/pre sputum ECP > 1 , $n = 12$) were analysed (Fig. 5).

Discussion

Seasonal allergic rhinitis (SAR) is a risk factor for incident asthma in affected subjects. SAR patients have increased inflammation in the lower respiratory tract during pollen exposure, even in the absence of clinical asthma.

Most importantly, the present study shows, that an increase in sputum markers of allergic inflammation occurs 24 h following a single nasal allergen challenge in rhinitis subjects. This increase was not related to the amount of allergen administered, as the majority of patients responded to the same dose-step of 1000 SQ. Although the time-frame of 24 h after challenge was primarily chosen for practical reasons, this choice is also supported by several investigations studying inflammatory

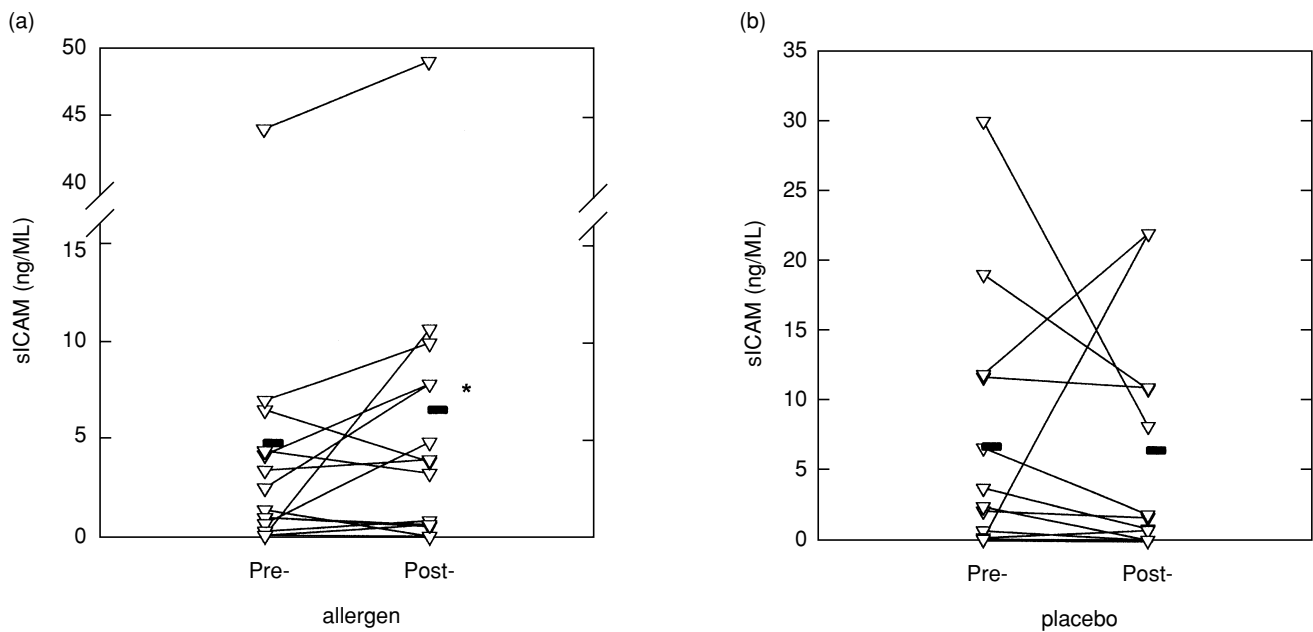


Fig. 2. Sputum sICAM before/after allergen (a) or placebo (b) challenge. *Indicates $P = 0.07$ vs. baseline and $P = 0.02$ vs. changes after placebo.

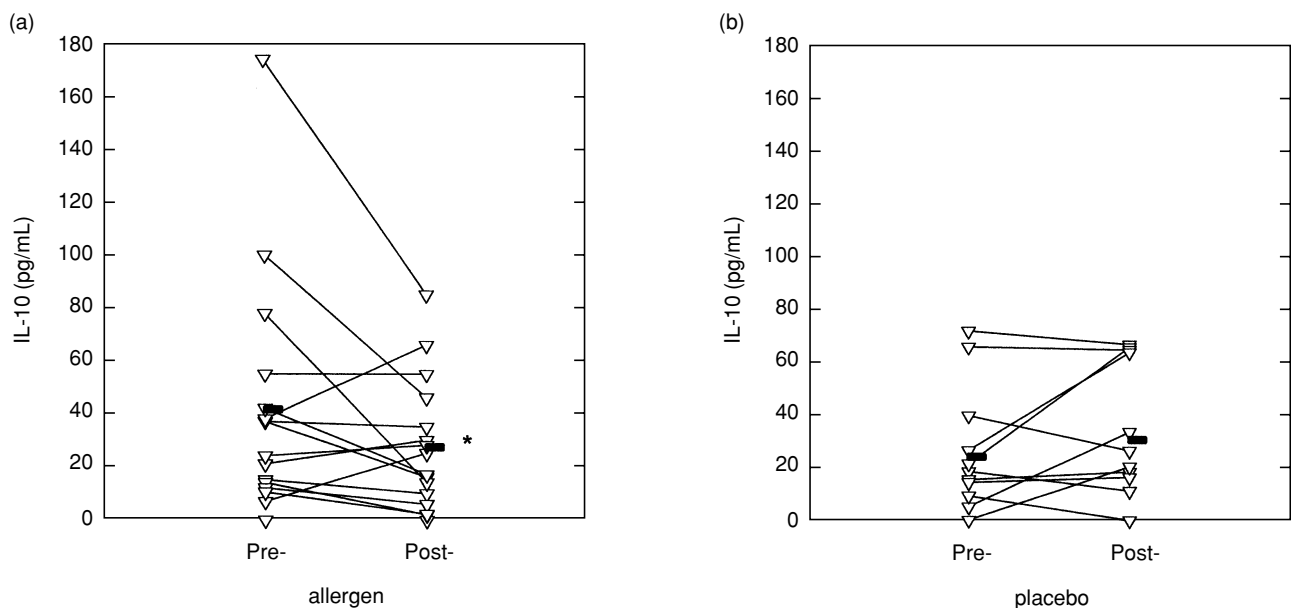


Fig. 3. Sputum IL-10 before/after allergen (a) or placebo (b) challenge. *Indicates $P = 0.08$ vs. baseline and $P = 0.06$ vs. changes after placebo.

responses after allergen exposure [11, 17]. However, we were fully aware that using a single endpoint would naturally limit the likelihood of observing significant changes in parameters of interest. Nevertheless, the findings of our study stand in line with several investigations analysing cross-talk between the nose and the lungs in allergy via direct pathophysiological mechanisms or systemic responses: nasal exposure to cold air can induce bronchoconstriction [18], and nasal provocation with methacholine leads to an increase in airway resistance in asthmatic subjects [19].

Treatment of the nose during pollen exposure in rhinitis patients, e.g. with topical corticosteroids, is also able to improve

bronchial hyper-responsiveness [20, 21]. Moreover, in a recent study by Braunstahl et al. using a quite similar study design as ours, there was an increased expression of adhesion molecules (ICAM-1, VCAM-1 and ELAM-1) and a higher number of eosinophils in bronchial biopsies from rhinitis subjects after nasal allergen challenge [11]. Using a different approach, the same authors have also demonstrated an increase in nasal mast cells and inflammatory markers after segmental bronchial allergen challenge [22]. Consistent with these observations, we have demonstrated increases in sputum sICAM, the soluble form of ICAM-1, and ECP after nasal allergen challenge. Although there was no significant increase in the overall number of

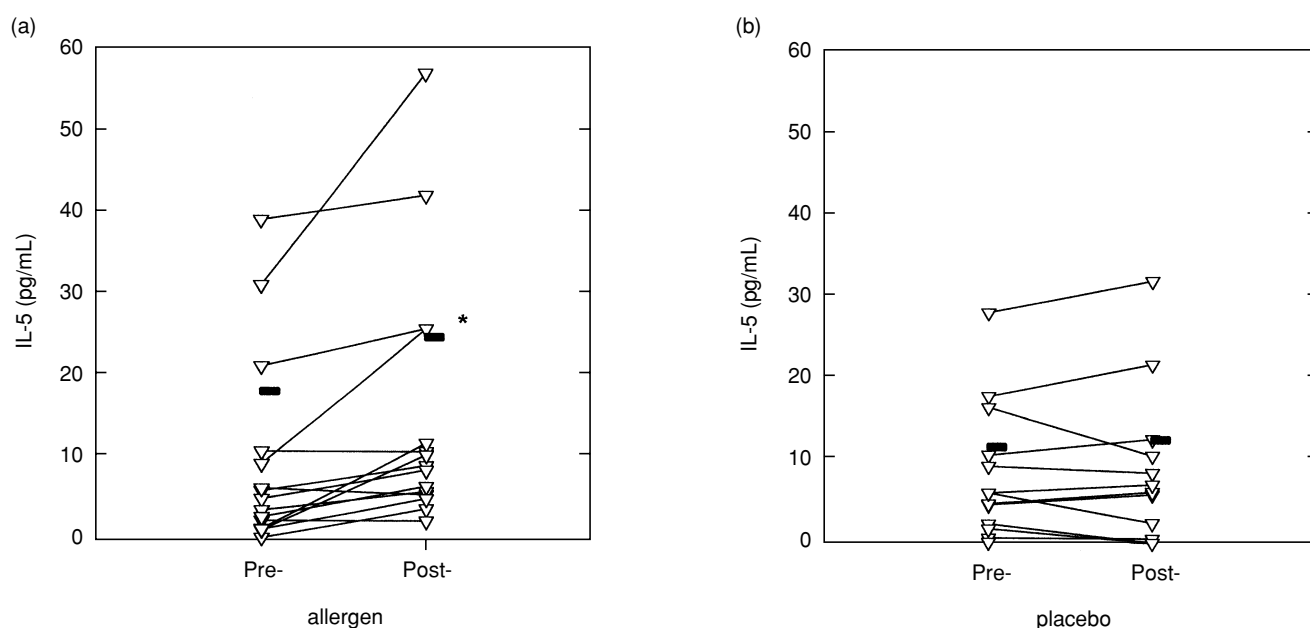


Fig. 4. Plasma IL-5 before/after allergen (a) or placebo (b) challenge; (b) shows only values of $n = 13$ (IL-5 before/after undetectable in three patients). *Indicates $P = 0.001$ vs. baseline and $P = 0.003$ vs. changes after placebo.

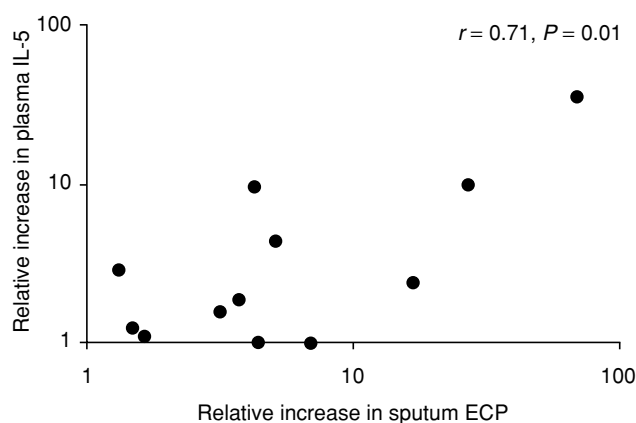


Fig. 5. Correlation of relative (post/pre value) increase in sputum ECP and relative increase (post/pre value) in plasma IL-5 ($n = 12$). $r = 0.71$, $P = 0.01$.

sputum eosinophils, the marked increase in sputum ECP indicates activation of these inflammatory cells in the lower respiratory tract following nasal challenge [23]. Adding to this, the increase of sputum ECP was closely related to the rise in plasma IL-5 in individual subjects.

Altogether, it appears reasonable that these findings result from a systemic allergic response, mediated by IL-5, as IL-5 has repeatedly been shown to activate and stimulate secretion of ECP from eosinophils [5, 7], and increase sputum levels of sICAM after inhalation [9]. However, the direct mechanisms and cells involved in the increase of sputum sICAM after allergen challenge are far less clear, as there have been no correlational studies comparing biopsies and sputum this far. In cell culture, shedding of ICAM-1 is induced by various proinflammatory cytokines, e.g. TNF- α or IL-1 α [24].

After allergen challenge, there was an increase in plasma levels of IL-5 that was not related to changes of absolute

blood eosinophil numbers. The rise in plasma IL-5 is fully consistent with other studies in allergic subjects, demonstrating local increases of IL-5 mRNA and protein, as well as systemic increases in plasma 6 to 24 h after allergen challenge [5]. In contrast, there was no significant change in the absolute number of peripheral blood eosinophils. Wood et al. have previously reported that an increase of blood eosinophils was restricted to subjects developing dual responses after allergen challenge, whereas blood eosinophils from isolated early responders remained unchanged [6]. Moreover, the authors of this study assigned this observation to an increased response of bone-marrow-derived eosinophil progenitors to circulating IL-5 in dual asthmatic responders. As the subjects studied in our investigation were non-asthmatics, as indicated by absence of bronchial hyper-responsiveness, it appears very likely that the observed rise in IL-5 without a parallel rise in blood eosinophils reflects the essential conclusion derived from the paper stated above. Moreover, the time-window of our study may have been too narrow to observe changes in eosinophils.

There was also no change in overall plasma ECP concentrations in our study population. Nevertheless, plasma ECP increased in 8/16 subjects, whereas the others remained unchanged. In this regard, Kleinau et al. have studied plasma ECP levels following bronchial allergen challenge in asthmatic children [25]. Strikingly, they observed a large variety of responses, with early increases in plasma ECP after 1 h, late increases (24 h post-challenge), or no changes at all. Thus, possible changes in plasma ECP could have been missed at least in some of our patients due to the study design.

Obviously, the diverging responses of respiratory and blood compartment to increases in plasma IL-5 also raise the question of whether eosinophils migrated to the bronchial vessels and mucosa show a distinct phenotype compared with peripheral blood. Bochner et al. have shown that the majority of eosinophils are transported in the bloodstream in a resting state, and become activated at sites of inflammation, whereas only a small

proportion of these cells is activated already in the bloodstream [26, 27]. Other authors have directly compared BAL and blood eosinophils, demonstrating increased activation and adhesion markers in BAL [28]. Hence, the sole increase of sputum, but not plasma ECP, in our study could reflect a different eosinophil phenotype and activation status. Finally, also neutrophils have been shown to store ECP [29]. Unfortunately, we did not measure markers of neutrophil activation in our study, hence this possible source of sputum ECP cannot be supported by specific data.

Unfortunately, we were not able to assess and investigate the relationship between sputum ECP and sputum IL-5, as IL-5 was not detectable in the sputum supernatant of our patients. Kelly et al. have studied the validity of IL-5 measurement in sputum, and found a poor recovery when supernatant spiked with IL-5 was analysed by enzymatic immunoassay [30]. Recently published data from the same author suggest that the presence of sputum proteases significantly influences the detection of IL-5 [31]. Moreover, given the fact that sputum levels of IL-5 reported by other authors are considerably low even in asthmatics [32], the failure to detect IL-5 in our patients' sputa would be fully compliant with these observations. However, owing to the impossibility to detect IL-5 in sputum, other mediators activating eosinophils also have to be considered.

There was a significant decrease in sputum IL-10 in our study. This is consistent with findings from other authors, who found a decrease of IL-10 positive cells 24 h after bronchial allergen challenge [17]. Moreover, Borish and colleagues have reported an absence of measurable IL-10 protein at the time of the late asthmatic reaction following segmental allergen challenge, and have suggested that this decrease reflected an inhibition of transcription [33]. Given the broad anti-inflammatory effects of IL-10 and its ability to resolve allergic inflammation *in vivo*, the findings of the present study support the hypothesis of an impaired IL-10 secretion at sites of inflammation, even in non-asthmatic patients with sole allergic rhinitis. In this context, IL-10 could particularly counteract allergen-triggered mast cell activation [34] and cytokine production [35]. With the common genetic background of asthma and rhinitis in mind, polymorphisms associated with a decreased IL-10 response to allergic stimuli, as reported in severe asthma [36], could also be a common feature of atopic subjects in general.

Although the presented evidence strongly supports the concept of a systemic cross-talk between nose and lungs, the possibility of a contamination of lower airways with nasal secretion through aspiration cannot be completely excluded. Nevertheless, the most diligent precautions were taken to avoid swallowing or inhalation of allergen and nasal secretions during and after challenge by the subjects. Patients were instructed not to swallow during nasal challenge and held their head in a forward prone position. On the following day, patients were told to thoroughly clear their throat and nose before sputum induction, and the first portion of sputum was discarded. We therefore assume that aspiration of secretions to the lung was negligible in the present study. This assumption is also supported by studies from other authors. Corren and colleagues investigated lung deposition of radiolabelled allergens after nasal challenge, and found no significant penetration of these particles into lower airways [37]. Further, postnasal drainage of inflammatory mediators has been demonstrated to play a minor role in asthmatic patients with concomitant sinusitis [38].

In summary, this study provides clear evidence that a single nasal allergen challenge in subjects with seasonal allergic rhinitis increases inflammatory parameters in the lower respiratory tract, assessed by induced sputum, and the increase of inflammatory sputum markers is associated with a rise of plasma IL-5. These data strongly support the concept of a systemic cross-talk between nose and lungs in allergic inflammation through immediate-type reaction cytokines like IL-5 and add to the understanding of mechanisms responsible for the high susceptibility of SAR patients to incident asthma. Finally, the findings of our study also underline the rationale to consequently treat lower and upper airway manifestations of allergic airway diseases.

References

- 1 Bousquet J, Chané P, Lacoste JY et al. Eosinophilic inflammation in asthma. *N Engl J Med* 1990; 323:1033–9.
- 2 Corren J. Allergic rhinitis and asthma: how important is the link? *J Allergy Clin Immunol* 1997; 99:S781–6.
- 3 Greiff L, Andersson M, Svensson C, Linden M, Wollmer P, Persson CG. Demonstration of bronchial eosinophil activity in seasonal allergic rhinitis by induced plasma exudation combined with induced sputum. *Thorax* 1999; 54:33–6.
- 4 Henriksen AH, Sue-Chu M, Lings-Holmen T, Langhammer A, Bjermer L. Exhaled and nasal NO levels in allergic rhinitis. relation to sensitization, pollen season and bronchial hyperresponsiveness. *Eur Respir J* 1999; 13:301–6.
- 5 Togias AG. Systemic immunologic and inflammatory aspects of allergic rhinitis. *J Allergy Clin Immunol* 2000; 106:S247–50.
- 6 Wood LJ, Inman MD, Watson RM, Foley R, Denburg JA, O'Byrne PM. Changes in bone marrow inflammatory cell progenitors after inhaled allergen in asthmatic subjects. *Am J Respir Crit Care Med* 1998; 157:99–105.
- 7 O'Byrne PM, Wood LJ. IL-5 and allergic inflammation. *Clin Exp Allergy* 1999; 29:573–5.
- 8 Bentley AM, Durham SR, Robinson DS et al. Expression of endothelial and leukocyte adhesion molecules intracellular adhesion molecule-1, E-selectin, and vascular cell adhesion molecule-1 in the bronchial mucosa in steady-state and allergen-induced asthma. *J Allergy Clin Immunol* 1993; 92 (6):857–68.
- 9 Shi HZ, Chen YQ, Qin SM. Inhaled IL-5 increases concentrations of soluble intracellular adhesion molecule-1 in sputum from atopic asthmatic subjects. *J Allergy Clin Immunol* 1999; 103:463–7.
- 10 Takahashi N, Liu MC, Proud D, Yu XY, Hasegawa S, Spannhake E. Soluble intracellular adhesion molecule 1 in bronchoalveolar lavage fluid of allergic subjects following segmental antigen challenge. *Am J Respir Crit Care Med* 1994; 150:704–9.
- 11 Braunstahl GJ, Overbeek SE, KleinJan A, Prins JB, Hoogsteden HC, Fokkens WJ. Nasal allergen provocation induces adhesion molecule expression and tissue eosinophilia in upper and lower airways. *J Allergy Clin Immunol* 2001; 107:469–76.
- 12 Pretolani M. Interleukin-10: an anti-inflammatory cytokine with therapeutic potential. *Clin Exp Allergy* 1999; 29 (9):1164–71.
- 13 American Thoracic Society. Guidelines for methacholine and exercise challenge testing 1999. *Am J Respir Crit Care Med* 2000; 161:309–20.
- 14 EAACI. Subcommittee on provocation tests with allergens. EAACI provocation tests with allergens. *Allergy* 1997; 52 (Suppl. 35):5–36.
- 15 Pizzichini E, Pizzichini MMM, Efthimiadis A et al. Indices of airway inflammation in induced sputum: reproducibility and validity of cell and fluid-phase measurements. *Am J Respir Crit Care Med* 1996; 154:308–17.

- 16 Beeh KM, Kornmann O, Lill J, Buhl R. Induced sputum cell profiles in lung transplant recipients with or without chronic rejection: correlation with lung function. *Thorax* 2001; 56:557–60.
- 17 Gauvreau GM, Watson RM, O'Byrne PM. Kinetics of allergen-induced airway eosinophilic cytokine production and airway inflammation. *Am J Respir Crit Care Med* 1999; 160:640–7.
- 18 Nolte ND, Berger D. On vagal bronchoconstriction in asthmatic patients by nasal irritation. *Eur J Respir Dis* 1983; 128:S110–5.
- 19 Littell NT, Carlisle CC, Millman RP, Braman SS. Changes in airway resistance following nasal provocation. *Am Rev Respir Dis* 1990; 141:580–3.
- 20 Aubier M, Levy J, Clerici C, Neukirch F, Herman D. Different effects of nasal and bronchial glucocorticosteroid administration on bronchial hyperresponsiveness in patients with allergic rhinitis. *Am Rev Respir Dis* 1992; 146 (1):122–6.
- 21 Foresi A, Pelucchi A, Gherson G, Mastropasqua B, Chiapparino A, Testi R. Once daily intranasal fluticasone propionate (200 µg) reduces nasal symptoms and inflammation but also attenuates the increase in bronchial hyperresponsiveness during the pollen season in allergic rhinitis. *J Allergy Clin Immunol* 1996; 98:274–82.
- 22 Braunstahl GJ, KleinJan A, Overbeek SE, Prins JB, Hoogsteden HC, Fokkens WJ. Segmental bronchial provocation induces nasal inflammation in allergic rhinitis patients. *Am J Respir Crit Care Med* 2000; 161:2051–7.
- 23 Venge P, Bystrom J, Carlson M et al. Eosinophil cationic protein (ECP): molecular and biological properties and the use of ECP as a marker of eosinophil activation in disease. *Clin Exp Allergy* 1999; 29 (9):1172–86.
- 24 Leung KH. Release of soluble ICAM-1 from human lung fibroblasts, aortic smooth muscle cells, dermal microvascular endothelial cells, bronchial epithelial cells, and keratinocytes. *Biochem Biophys Res Commun* 1999; 260:734–9.
- 25 Kleinau I, Niggemann B, Wahn U. Individual time-courses of ECP and EPX during allergen provocation tests in asthmatic children. *Pediatr Allergy Immunol* 1995; 6:109–18.
- 26 Bochner BS, Schleimer RP. The role of adhesion molecules in human eosinophil and basophil recruitment. *J Allergy Clin Immunol* 1994; 94:427–38.
- 27 Blom M, Tool ATJ, Mul FPJ, Knol EF, Roos D, Verhoeven AJ. Eosinophils isolated with two different methods show different characteristics of activation. *J Immunol Meth* 1995; 178:183–93.
- 28 Kroegel C, Liu MC, Hubbard WC, Lichtenstein LM, Bochner BS. Blood and bronchoalveolar eosinophils in allergic subjects after segmental allergen challenge: surface phenotype, density heterogeneity, and prostanoid production. *J Allergy Clin Immunol* 1994; 93:725–34.
- 29 Bystrom J, Garcia RC, Hakansson L et al. Eosinophil cationic protein is stored in, but not produced by peripheral blood neutrophils. *Clin Exp Allergy* 2002; 32:1082–91.
- 30 Kelly MM, Leigh R, Horsewood P, Gleich GJ, Cox G, Hargreave FE. Induced sputum: validity of fluid-phase IL-5 measurement. *J Allergy Clin Immunol* 2000; 105 (6, Part 1):1162–8.
- 31 Kelly MM, Leigh R, Carruthers S et al. Increased detection of interleukin-5 in sputum by addition of protease inhibitors. *Eur Respir J* 2001; 18 (4):685–91.
- 32 Liu LY, Swensen CA, Kelly EA, Kita H, Busse WW. The relationship of sputum eosinophilia and sputum cell generation of IL-5. *J Allergy Clin Immunol* 2000; 106 (6):1063–9.
- 33 Borish L, Aarons A, Rumblyrt J, Cvietusa P, Negri J, Wenzel SE. Interleukin-10 regulation in normal subjects and patients with asthma. *J Allergy Clin Immunol* 1996; 97:1288–96.
- 34 Royer B, Varadaradjalou S, Saas P, Guillosson JJ, Kantelip JP, Arock M. Inhibition of IgE-induced activation of human mast cells by IL-10. *Clin Exp Allergy* 2001; 31:694–704.
- 35 Fiorentino DF, Zlotnik A, Mosmann TR, Howard M, O'Garra A. IL-10 inhibits cytokine production by activated macrophages. *J Immunol* 1991; 147:3815–22.
- 36 Hobbs K, Negri J, Klennert M, Rosenwasser LJ, Borish L. Interleukin-10 and transforming growth factor-beta promoter polymorphisms in allergies and asthma. *Am J Respir Crit Care Med* 1998; 158:1958–62.
- 37 Corren J, Adinoff AD, Irvin CG. Changes in bronchial responsiveness following nasal provocation with allergen. *J Allergy Clin Immunol* 1992; 89:611–8.
- 38 Bardin PG, Van Heerden BB, Joubert JR. Absence of pulmonary aspiration of sinus contents in patients with asthma and sinusitis. *J Allergy Clin Immunol* 1990; 86:82–8.