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EXPERIMENTAL ALLERGIC RHINITIS-RELATED COUGH AND AIRWAY EOSINOPHILIA IN SENSITIZED GUINEA PIGS

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Allergic rhinitis is one of the most common causes of chronic cough. The characteristic feature of allergic rhinitis is eosinophilic nasal inflammation. This study was determined to find the relation between airway eosinophils and chemically-induced cough in guinea pigs with antigen-induced rhinitis at the early and late allergic phases. Forty animals were sensitized with ovalbumin (OVA) and divided into four separated groups. Four weeks later, the sensitized animals were either once or repeatedly (6 times at 7-day intervals) intranasally challenged with OVA to develop experimental allergic rhinitis. The control group was given saline. Cough was elicited by inhalation of citric acid aerosols and evaluated at 30 min (early phase) or 24 h (late phase) after the 1st or 6th nasal challenge (NC) in the sensitized animals. The citric acid-induced cough was significantly increased in the sensitized animals in the early allergic phase after the first and repeated NC compared with the control values [14(9-19) *vs.* 16(10-17) *vs.* 8(6-10); P=0.049], whereas there was no significant increase in the cough response tested in the late allergic phase. A correlation between the cough intensity and the number of eosinophils from nasal mucosa only (P=0.008) was found.

Key words: *allergic rhinitis, citric acid-induced cough, eosinophils, guinea pig, mechanically-induced cough, ovalbumin*

INTRODUCTION

The most common cause of chronic cough is a group of related conditions of chronic rhinitis, sinusitis, and postnasal drip (1). The underlying pathology of rhinitis, such as asthma, is airway inflammation in which eosinophilia is a characteristic feature (2). Allergic rhinitis (AR) is part of a systemic disease

complex. The relationship between AR and asthma is close, which has led to the "one airway, one disease" concept. Both conditions share common immunopathology and pathophysiology (3, 4). The pathophysiological events following allergen exposure are described as biphasic, composed of an early (mediated by mast cells-derived mediators and clinically manifested as rhinorrhoea, sneezing, pruritis and nasal congestion.) and a late phase response (occurs from 4 h to 12 h after exposure and persists to 24 h) that involves increased recruitment and activation of inflammatory cells predominantly eosinophils (5). These cells release an array of pro-inflammatory mediators, including cysteinyl leukotrienes, eosinophil cationic proteins (ECP), eosinophil peroxidase (EPO), and a major basic protein (MBP) which participate on propagation of inflammation through the airway or systemic pathways. This is one possible mechanism for lower airway dysfunction among patients with upper airway disease, such as rhinitis (6).

Eosinophilic airway inflammation is a fundmental feature of a cough variant of asthma, eosinophilic bronchitis without asthma, and eosinophilic tracheobronchitis with cough hypersensitivity associated with atopy, termed as atopic cough (7). All the three eosinophilic airway disorders have been recognized to be causes of isolated chronic cough with heightened cough sensitivity whereas a cough variant of asthma is characterized with slightly increased cough or within normal limits (8).

Our previous experimental evidence has demonstrated significantly enhanced cough during exudative allergic rhinitis in awake sensitized guinea pigs immediately after the 1st nasal antigen challenge (9). Other related papers have shown significant enhanced cough sensitivity to citric acid in guinea pigs after repeated nasal challenges, which corresponds with the early allergic phase, no significance was present in late allergic phase (10, 11). The number of coughs has increased in other studies, including a model of allergic diseases (7, 12). At present, the relationship between eosinophilic inflammation and cough response during different time phases of allergic rhinitis is still under debate. Therefore, in the present study we attempted to find a relation between airway eosinophils and allergic rhinitis-related cough in guinea pigs in the early and late allergic phases.

MATERIAL AND METHODS

Animals

Male Trik strain guinea pigs (n=47) weighing 250-350 g were obtained from the Department of Experimental Pharmacology of the Slovak Academy of Sciences (Dobra Voda, Slovakia). Animals were housed in the air-conditioning room and were given a standard laboratory diet and water *ad libitum*. The study protocol complied with the national guidelines and was approved by the Ethics Committee of Jessenius Faculty of Medicine for Animal Experiments in Martin, Slovakia. The animals were divided into four separate experimental groups according to antigen challenge procedures and cough protocol.

Ovalbumin sensitization and model of allergic rhinitis

After acclimatization to laboratory conditions, sensitization of animals was performed. All animals were actively sensitized with ovalbumin (10 μg, Sigma), administered together with aluminium hydroxide (100 mg) in saline (1 ml, i.p.), using the method described previously (13). Twenty one days later, successful sensitization was confirmed by the intradermal injection of ovalbumin solution (25 μl of 200 $\mu g.ml^{-1}$) into the dorsal back surface. The sensitized animals were used 7 days later for experiments.

In the second phase of the experiment, the sensitized animals (n=38) were used to develop a model of allergic rhinitis by either single or repeated (6 times at 7-day intervals) intranasal instillation of 0.015 ml of 0.5% OVA separately for each nostril. Control animals (n=9) were intranasally challenged with saline in the same dose as was used in the experimental ones. Allergic rhinitis was evaluated from the occurrence of typical clinical symptoms with respect to nose and eyes irritation using a scoring system reported previously (11).

Chemically-induced cough

The complete procedure of chemically-induced cough was previously described (11). Briefly, unanaesthetized animals were individually placed into a body plethysmograph box (type 855, Hugo Sachs Electronic, Germany) and were exposed to citric acid aerosol (Lachema) generated *via* a jet nebulizer (Pariprovocation test I, Pari Starneberg, Germany) in doubly increasing concentration (from 0.05 to 1.6 M) for 30 s. The interval between separate exposures was 1 min. Respiratory changes in the airflow were measured using pneumotachograph (Godart, Germany) with a Fleish head connected to the head chamber. The appearance of cough was detected with a microphone placed in the roof of the head chamber. Pneumotachograph changes and cough sounds were simultaneously recorded. Cough was evaluated on the basis of a sudden enhancement of expiratory airflow accompanied by typical cough sound. The cough sound was analyzed from software system according (12), using spectral analysis of respective sounds. To quantify the intensity of cough, the cough response was expressed as the total number of coughs during all citric acid challenges.

Mechanically-induced cough

Immediately after chemically-induced cough, the animals of all groups were anesthetized (urethane, 1.1 g/kg, i.p., Riedel-de Haën AG) and cough was induced by mechanical stimulation of tracheobronchial mucosa using a nylon fibre (14). The number of cough efforts was counted from the trace of intrapleural pressure recorded by an electromanometer. To quantify cough, the number of cough efforts during a cough bout and the intensity of cough bout (the sum of all positive deflection of intrapleural pressure during all cough efforts in the cough bout) were used.

Cough challenge protocol

Sensitized animals were divided into four separate experiments, because the purpose of our investigation was to find the relation between airway eosinophils and the cough response at different time points of allergic rhinitis. The citric acid-induced cough was elicited during the early allergic response, 30 min after 1^{st} NC (1^{st} experiment), which was repeated after the 6^{th} NC (2^{nd} experiment), and during the late allergic response, 24 h after the 1^{st} NC (3^{rd} experiment), which was repeated after the 6^{th} NC (4^{th} experiment). The control animals, challenged with saline, were similarly stimulated by the tussigen at the time points simulating the early and late phases of allergic rhinitis. Likewise, the mechanically-induced cough was provoked in all separated groups according the above time course.

Airway eosinophils in selected animals of all study groups

At the end of experiment, the animals were killed by an overdose of anaesthesia and samples from upper and lower airways were removed and processed by a standard histological method to describe histomorphological findings, including the number of airway eosinophils. Semiquantitative evaluation of eosinophils was made in separate groups (individually in selected animals of each group), as identified from 10 high power fields using 10x ocular and 40x objective microscope lens. The eosinophils were counted and their number was averaged. Light microscopic examination was performed independently by two pathologists, to identify the location and number of eosinophils. Eosinophils from the samples of the nose, larynx, trachea, bronchi, and lung of each animal were correlated with individual cough responses of the same animal.

Statistical analysis

Data on cough were expressed as medians and interquartile ranges. Data on the intensity of mechanically-induced cough bouts and the number of eosinophils were presented as means ±SD. The inter-group differences in the cough response and eosinophils were assessed with one-way analysis of variance (ANOVA) followed by the Duncan multiple range test. Correlation between the number of coughs and the number of eosinophils was made with linear regression analysis. A value of $P<0.05$ was considered significant.

RESULTS

The effect of nasal antigen challenge on citric acid- induced cough

The citric acid-induced cough was significantly increased in the sensitized animals in early allergic phase, 30 min after the 1st and 6th nasal ovalbumin challenges compared with their control value [14(9-19) *vs.* 16(10-17) *vs*. 8(6-10); P=0.049] *(Fig. 1, Panel A)*. In contrast, the intensity of cough reflex provoked in the late allergic response, 24 h after the 1st and 6th NC was without any significant change [9(4-11) *vs.* 8(7-9) *vs.* 8(5-8); P=0.689] compared with control value *(Fig. 1, Panel B)*.

Distribution of airway eosinophils and its correlation with the number of coughs in early and late allergic phases

Table 1 demonstrates the comparison of the number of eosinophils evaluated semiquantitatively from some regions of upper and lower airways in selected animals of each separate group. The results showed the most widespread eosinophilia in nasal mucosa. A significant increase ($P<0.05$) in the number of nasal eosinophils was present in all sensitized groups of animals regardless of the early allergic response after the 1st [178(98)] or 6th [257(61)] nasal antigen challenge or late allergic response after the 1st [174(137)] or 6th [248(47)] nasal antigen challenge compared with controls [29(28)]. From these results is apparent that repeated nasal ovalbumin challenges led to an increase in nasal eosinophils when compared with only one challenge. Similar effects occurred in other regions of airways.

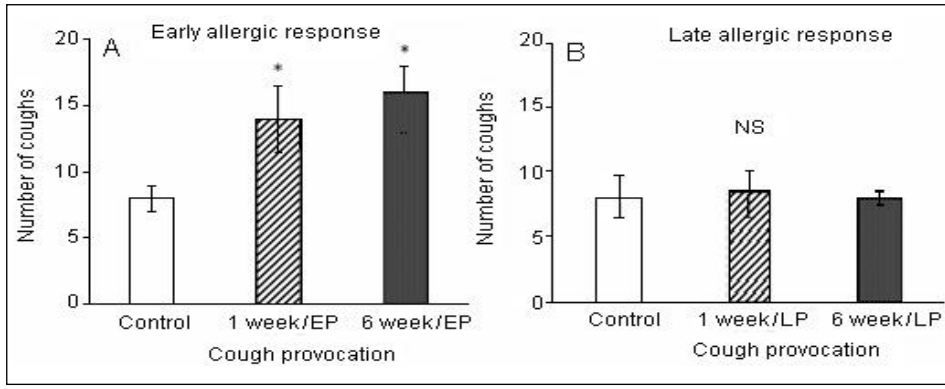

Fig. 1. Citric acid-induced cough intensity changes in sensitized groups of animals 30 min after the 1st and 6th nasal ovalbumin challenge (EP-early phase, Panel A) and 24 h after the 1st and 6th nasal ovalbumin challenge (LP-late phase, Panel B) as compared with control animals challenged with saline. Data are expressed as medians and interquartile ranges, *P<0.05 vs. to control, NS – nonsignificant.

Table 1. Distribution of airway eosinophils in control and sensitized animals with allergic rhinitis during early (EP) and late (LP) allergic phases.

	CONTROL	1 week /EP	1 week/LP	6 week/EP	6 week/LP
	n = 4	n = 5	n = 5	n = 5	n = 5
Nose	29(28)	178(98) *	174(137) *	257(61) *	248(47) *
Larynx	6(3)	94(68)	74(40)	101(72) *	123(85) *
Trachea	26(9)	98(46)	64(30)	116(72) *	108(54) *
Bronchi	38(24)	96(57)	78(69)	111(64)	81(52)
Lung	4(0.8)	6(0.7)	6(1.2)	7(0.7)	6(2.9)

Comparison of eosinophils from some regions of airways in selected animals of separate groups with one way ANOVA and Duncan multiple range tests. Data are mean ±SD. *P<0.05 *vs.* control.

Another significant increase (P<0.05) in the number of eosinophils was observed in the larynx and trachea, but it was present solely the in sensitized animals after repeated NC during the early and late allergic phases as compared with control. In the lungs, contrary to other regions of airways, there were seen only few mostly dispersed eosinophils around small bronchi. Eosinophils from the samples of the nose, larynx, trachea, bronchi, and lung of selected animals of each study group were correlated with individual chemically and mechanically-induced cough response of the same animals. Our results revealed correlation between the citric acid-induced cough intensity after each NC according to our protocol and the number of eosinophils only in the nasal mucosa (r=0.529, P=0.008) *(Fig. 2).* Other histological alterations were in accord with the findings of our previous studies using the same model of allergic rhinitis (10, 11).

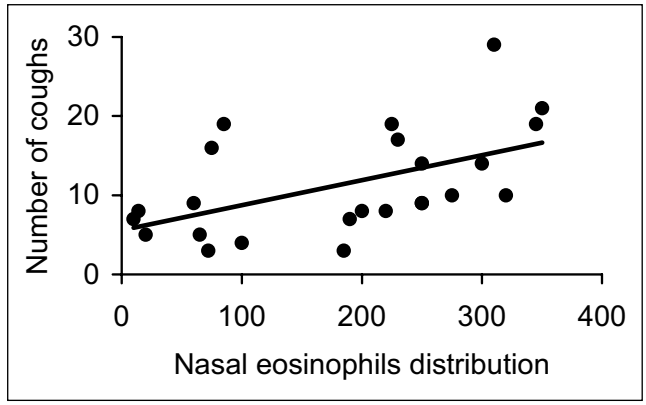

Fig. 2. Correlation between the number of coughs and the number of nasal eosinophils in selected animals of all groups of animals. Correlation coefficient = 0.529 (P=0.008)

Table 2. Changes in mechanically-induced tracheobronchial cough in early (EP) and late (LP) allergic response after the 1st and 6th nasal ovalbumin challenge compared with control animals challenged with saline.

	CONTROL	1 week/EP	1 week/LP	6 week/EP	6 week/LP
	n = 9	n = 9	n = 10	n = 10	n = 9
Number of coughs (medians + IQR)	1.5(1)	2(2)**	3(2)**	3(2)**	4(1) **
Intensity of cough bout (means ±SD)	66(29)	134(69)**	133(56)**	133(52)**	137(64)**

** P<0.01 *vs.* control

The effect of nasal antigen challenge on mechanically-induced cough

Significant increase in mechanically-induced cough from tracheobronchial mucosa was found (P<0.01) in all study groups of sensitized animals compared to control as shown in *Table 2.* The similar effect was seen in the intensity of cough bout expressed in kPa *(Table 2).*

DISCUSSION

Eosinophils are one of the major effector cells found in the lungs in patients with asthma and allergic rhinitis. Their well-documented cytotoxic potential results in damage to the airway epithelium and tissue inflammation while their release of lipid mediators such as cysteinyl leukotrienes, cytokines, reactive oxygen species and other pro-inflammatory and tussive mediators that can activate afferent airway sensory nerve endings. In allergic diseases, there are well-documented correlations between eosinophil numbers and disease severity even though eosinophils display heterogeneity in human tissue (2, 15). Moreover, there is evidence that the eosinophil is important in cough, airway remodelling,

and asthma exacerbations (16, 17). Thus there is much interest in understanding how eosinophils accumulate in allergic airway diseases.

Recently, many researchers have extensively focused on the early and late allergic phases, particularly in relation to pulmonary hyperresponsiveness and eosinophil accumulation (13, 18, 19). Other workers have reported increased cough sensitivity after a single or repeated antigen challenges in relation to airway eosinophilic inflammation (1, 7, 10-12, 20, 21). According to the association between rhinitis and lower airway dysfunction, several pathomechanisms could explain the enhanced cough sensitivity during rhinopathies: nasal obstruction leading to mouth breathing, an increased deposition of inhaled allergen in lower airways, a nasal-bronchial reflex, microaspiration of nasal secretion, postnasal drip syndrome and increased bronchial hyperresponsiveness in subjects with allergic rhinitis (1). In case of allergic rhinitis, the cough reflex may be sensitized through an action of inflammatory mediators from the nasal mucosa, a reflex sensitization of airway sensory nerves, or facilitation of the central cough generator from the nasal reflex input (1, 22, 23).

At present, the exact role of the airway eosinophils is not entirely clear with respect to the allergic disease-related cough, although many authors have described coexistence of enhanced cough and accumulation of eosinophils in bronchoalveolar lavage or distal airways obtained after antigen challenges (7, 12, 13, 20, 21). For the above-mentioned reasons, our present study was expanded to characterize the relationship between the accumulation of eosinophils after a single or repeated nasal challenges and the cough response in the early and late allergic phases in sensitized animals. The early allergic phase was studied 30 min after antigen challenge, when clinical symptoms of allergic rhinitis were most conspicuous and the late phase was considered 24 h after antigen challenge. The number of eosinophils from the airway regions examined was estimated in either phase of allergic rhinitis in all study groups. Our findings show a significantly enhanced cough sensitivity after a single or repeated nasal challenges that corresponds only with the early allergic phase that manifests full clinical symptoms of allergic rhinitis, including sneezing, rhinorrhea, and nasal cracles. These findings are in accord with those of our previous studies (9, 10, 11). Mechanically-induced cough from the tracheobronchial region also was increased in all study groups of the sensitized animals. This observation is supported by the findings that allergic airway inflammation is associated with increased excitability and mechanosensitivity of RAR fibres (24).

With respect to cough, allergic airway inflammation accompanied by increased number of eosinophils is associated with increased responsiveness to capsaicin and other tussigenic stimuli, suggesting afferent nerve fiber and central sensitization of the cough reflex (22). In our study, the number of eosinophils was increased in the nasal mucosa in each study group, regardless of the phase, early or late, of allergic rhinitis. The eosinophils in the larynx and trachea were increased only after repeated nasal challenges. In the lungs, contrary to other regions of airways, the occurrence of eosinophils was rare.

In the present study we also found a good correlation between the number of eosinophils in the nasal mucosa and the intensity of citric acid-induced cough. Ahlstrom-Emanuelsson et al (2) recently demonstrated that nasal mucosa in patients with allergic rhinitis featured increased eosinophil numbers together with markedly augmented eosinophil degranulation during natural allergen exposure. Out of pollen season, the occurrence of eosinophils in nasal mucosa was still present, but with moderate degranulation. In addition, these authors reported that eosinophil degranulation index does not correlate with the eosinophil number in the nasal mucosa. We can speculate that the increased cough reflex sensitivity during the early phase of experimental allergic rhinitis in the present study could be not only due to the accumulation of eosinophils but also their degranulation. Our finding that in the late allergic phase eosinophils accumulation was not accompanied by enhanced cough response could support this interpretation. The finding that the cough response is not enhanced in the late allergic phase also find support in clinical observations that that there is no correlation between cough reflex sensitivity and airway inflammation 24 h after allergen challenge in asthmatic patients (25).

In summary, single or repeated nasal challenges enhance cough intensity to citric acid only in the early phase of allergic response. It seems that nasal eosinophilic inflammation plays a role in enhancement of cough related to allergic rhinitis. At present, there still remains uncertainty concerning the mechanisms of eosinophilic inflammation and cough, which opens a new therapeutic approach with respect to testing antieosinophilic drugs.

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