

Short communication

The upper and lower airway responses to nasal challenge with house-dust mite *Blomia tropicalis*

Background: The house dust mite *Blomia tropicalis* (*B. tropicalis*) was found to be the most prevalent domestic mite in Singapore. However, its pathogenicity in allergic airway diseases remains to be investigated.

Methods: Twenty adults with persistent allergic rhinitis (PAR) were studied. Five had a history of asthma, and all were asymptomatic except one who was under treatment with low-dose inhaled corticosteroid. Nasal challenge was carried out by nasal spray with phosphate-buffered saline (PBS) and with increasing concentrations of crude *B. tropicalis* extracts (0.6, 6.0 and 60 µg/ml) at 15 min intervals. Subjective symptom scores and absolute number of sneezes were recorded together with objective measurements of spirometry (forced expiratory volume in 1 s, FEV₁) and acoustic rhinomanometry (volume of the nasal cavity). These were performed at baseline, 5 min after each incremental challenge, and 30 min, 1 h, 3 h, 5 h and 7 h after the last challenge. Meanwhile, concentrations of mediators in nasal secretions (tryptase, leukotriene C₄ (LTC₄) and eosinophil cationic protein (ECP)) were measured in nasal aspirate samples at similar time intervals. An identical (control) challenge procedure with PBS alone was repeated in seven patients after a washout period of at least 2 weeks.

Results: Significant increases in the subjective and objective nasal symptoms, together with a significant increase of tryptase and LTC₄ concentrations in nasal secretion, were found 5 min after each challenge with *B. tropicalis*, but not with PBS. There was no definitive pattern of the late-phase nasal response in either subjective symptoms or objective measurements. Three patients (3/5) with a history of asthma showed a fall in FEV₁ readings (33%, 22% and 11% from baseline, respectively) at 7 h post challenge with concomitant mild wheezing in the night.

Conclusions: Our study demonstrates direct evidence of allergic nasal response to *B. tropicalis* in sensitized adults. It shows that nasal provocation may also provoke concomitant asthmatic symptoms during the late-phase reaction, especially in people with a history of asthma.

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House-dust mites are the most common indoor allergens and probably contribute to the development of allergic diseases, such as allergic rhinitis and asthma. International studies have demonstrated that *Dermatophagoides pteronyssinus* (Der p), *Dermatophagoides farinae* (Der f), and *Euroglyphus maynei* (Eur m) are the most common mite species worldwide (1).

Blomia tropicalis (*B. tropicalis*), another dust mite species, was found to be the most prevalent domestic mite in Singapore (2) and our atopic population is highly sensitized to this mite (3). This study aimed to investigate the early- and late-phase nasal responses and the lower airway response to nasal challenge with *B. tropicalis* in sensitized patients with persistent allergic rhinitis (PAR) (1,4). A combined assessment was made using subjective symptoms and objective measurements, together with the

determination of inflammatory mediators and eosinophil cell counts in nasal secretions during challenge and 7 h after nasal challenge with *B. tropicalis*. This study aimed to provide direct evidence of the clinical importance of *B. tropicalis* as a trigger of nasal allergic reaction and its impact on asthma.

Material and methods

Study patients

Twenty adult patients (10 men and 10 women) aged between 21 and 44 years (mean 28 years) with ongoing PAR were recruited. Their sensitization to *B. tropicalis* was confirmed by a positive skin prick reaction. None of them had acute nasal symptoms or had taken any medication during the previous 2 weeks (for at least 30 days for any

nasal or systemic corticosteroid). However, one patient was receiving treatment with a low-dose inhaled corticosteroid for prevention of asthma. Five patients had a history of asthma, but were asymptomatic. Written informed consent was obtained from each subject. Approval to conduct this study was granted by the National Medical Research Council of Singapore and the institutional review board of the Medical Faculty of National University of Singapore.

Crude extract of *B. tropicalis*

Crude extract of *B. tropicalis* was prepared from cultured mites, as described previously (5). In brief, harvesting of mites was performed when the culture was approximately 4 weeks old. One gram of frozen or lyophilized mites was homogenized using a pestle and mortar in the presence of liquid nitrogen. Twenty-five ml of phosphate-buffered saline (PBS), 2 mM phenylmethyl-sulfonyl fluoride (PMSF) and 1 mM ethylenediamine tetraacetic acid (EDTA) was used for protein extraction at 4°C overnight. After centrifugation at 15 000 g for 15 min, the supernatant of the extract was dialyzed overnight at 4°C against PBS.

Nasal allergen challenge

Nasal allergen challenge was carried out in a double-blind manner by nasal spray, one puff (0.04 ml of allergen solution) per nostril (6). The study subject was asked to be in complete apnea during spraying. This precaution would prevent the provocation extract from entering into the lower airway. At the start, a nasal secretion sample was collected as a baseline control. Fifteen minutes later, nasal challenges with PBS (diluent of allergen extract) and subsequently with increasing concentrations of *B. tropicalis* extracts (0.6, 6.0, and 60 µg/ml) were performed at intervals of 15 min. Nasal secretions were collected 5 min after each challenge and at 0.5 h, 1 h, 3 h, 5 h and 7 h after the last challenge. Assessment of the subjective and objective symptoms was performed just before collection of each nasal secretion.

Nasal sampling procedure

The nasal secretion samples were collected by gentle aspiration from the middle meatus and from the floor of both nasal cavities into a preweighed plastic (polystyrene) sampling tube (6, 7). This was immediately followed by aspiration of a known volume (1.0 ml) of PBS (pH 7.4) including 10% Mesna (Mistabron, sodium 2-mercaptoethane; UCB Pharmaceuticals Company, Brussels, Belgium). Mesna decreases the viscosity of the samples, which is needed to enable the accurate and precise pipetting of volumes of nasal aspiration fluids. The total amount of secretion sampled was measured by weighing the collection tube and the tube with the aliquot of PBS before and after sampling. The nasal secretion samples were cooled on ice immediately after sampling, then mix in a Vortex mixer and centrifuged at 1000 g at 4°C for 15 min. Supernatants were stored at -80°C within 1 h of sampling until the assays were performed.

Subjective evaluation of the nasal symptoms

Five major groups of symptoms, e.g. itchy eyes and nose, nasal obstruction, sneezing, and rhinorrhea were recorded using a symptom severity scale: 0, none (no symptom evident); 1, mild (symptom clearly present but minimal awareness); 2, moderate (definite awareness of symptom which is bothersome but tolerable); 3, severe (symptom is hard to tolerate). A total nasal symptom score for each patient was calculated by adding these five scores together.

Measurement of acoustic rhinometry and spirometry

An SRE 2100 acoustic rhinometer (Rhinometrics A/S, Denmark) was used to measure nasal geometry. Every test consisted of three consecutive measurements on each side of the nasal cavity. Volume was defined by the transnasal volume at a distance of 2–4 cm from the nostril. Total volume for each patient was calculated by adding left and right sides together; this value changes inversely with nasal mucosal congestion.

Spirometry was carried out using a portable spirometer (Cosmed, Pony Spirometer Version 3.0, Rome, Italy). The forced expiratory volume in 1 s (FEV₁) was recorded for assessment.

Measurement of the mediators

The concentrations of tryptase, leukotriene C₄ (LTC₄), and eosinophil cationic protein (ECP) in nasal secretions were measured as described in previous studies (6, 7). Briefly, the concentrations of tryptase and ECP were measured by Pharmacia UniCAP system (Pharmacia & Upjohn, Uppsala, Sweden). LTC₄ levels were determined by enzyme immunoassay (EIA, Laboratoire des Stalergenes, Fresnes, France).

Cellular examination

After removing the supernatant of the nasal secretions, the cell pellets were resuspended in 1 ml PBS, pH 7.4, containing 5% bovine serum albumin (BSA). The cells were washed with PBS 1% BSA, and cytocentrifuged preparations were made and stained using May–Grünwald Giemsa for examination by light microscopy. Three hundred leukocytes were counted and each cell type was calculated as a percentage of the total number of leukocytes.

Statistical evaluation

We used two-tailed tests at the 1% level of significance in order to correct for multiple comparisons. Calculations were performed using the SPSS software package for windows (SPSS release 10.0.5, 27 November 1999; SPSS Inc., Chicago, IL). The Wilcoxon rank sum test was used to compare all the parameters at each sampling time with baseline values after nasal challenge with PBS and with increasing concentrations of *B. tropicalis*.

Results

All participants completed the study. After a washout period of at least 2 weeks, seven patients underwent an identical challenge procedure with only PBS, as a control.

The early-phase reaction (EPR) of the nose

All patients showed an EPR to nasal challenge with *B. tropicalis*, but not to PBS. Table 1 shows the dose-related development of nasal symptoms after nasal challenge with *B. tropicalis*. Concomitantly, there was a significant increase in the number of sneezes, and a significant decrease in the total nasal volume, together with significantly increased concentrations of tryptase

Table 1. Median individual and total symptom scores before and after nasal challenge with PBS and *Blomia tropicalis*

Symptoms	Symptom scores after nasal challenge*									
	Baseline	Phosphate-buffered saline	<i>Blomia tropicalis</i>			1/2 h	1 h	3 h	5 h	7 h
			0.6 µg/ml	6.0 µg/ml	60 µg/ml					
Itchy eyes	0 (0–1)	0 (0–1)	0 (0–3)	0 (0–3)	0 (0–3)	0 (0–2)	0 (0–2)	0 (0–1)	0 (0–1)	0 (0–1)
Itchy nose	0 (0–1)	0 (0–1)	1 (0–3)	1 (0–3)	1 (0–3)	0 (0–3)	0 (0–3)	0 (0–2)	0 (0–1)	0 (0–1)
Sneezing	0 (0–1)	0 (0–1)	1 (0–3)	2 (0–3)	2 (0–3)	0 (0–1)	0 (0–1)	0 (0–3)	0 (0–3)	0 (0–3)
Rhinorrhea	1 (0–1)	1 (0–2)	2 (1–3)	2 (0–3)	3 (0–3)	2 (0–3)	1 (0–3)	1 (0–3)	1 (0–3)	1 (0–3)
Obstruction	0 (0–1)	0 (0–1)	1 (0–2)	2 (0–3)	3 (1–3)	3 (0–3)	2 (0–3)	1 (0–3)	1 (0–3)	1 (0–3)
Total score	1 (0–4)	1 (0–3)	6 (0–13)	7 (3–14)	8 (4–15)	6 (3–9)	4 (0–8)	2 (0–7)	2 (0–10)	2 (0–8)

Figures in parentheses represent ranges.

* Assessment of the subjective symptoms was performed 5 min after each challenge and at 30 min, 1 h, 3 h, 5 h and 7 h after the last challenge.

and LTC₄ in the nasal secretions (Figs 1 and 2). Complaints of itchy eyes were uncommon in our study.

The late-phase reaction (LPR) of the nose

There was no definitive pattern of the LPR in all measurements. All subjects had a high percentage of eosinophils (median 71%) and high concentrations of ECP (median 165 ng/g) in nasal secretions at baseline. Most subjects showed a trend of increasing ECP concentrations (median 394.1 ng/g) 5 h after nasal challenge with *B. tropicalis*. This, however, did not reach statistical significance.

The lower airway response

Although all patients showed a typical EPR of the nose, there was neither concomitant wheezing/coughing nor a fall in FEV₁ of more than 20%, except for one patient who had a 37% decrease in FEV₁ from baseline 30 min after the last challenge. The median FEV₁ of all the subjects remained unchanged during the entire study period. However, three patients with a history of asthma had a decrease in FEV₁ (33%, 22% and 11%, respectively) at 7 h post challenge with *B. tropicalis* and reported mild wheezing in the night.

Discussion

This study has confirmed a previous report from the USA (8) that a typical early-phase nasal response to *B. tropicalis* challenge was observed by both subjective and objective symptoms in all patients. Meanwhile, significantly increased concentrations of tryptase and LTC₄ in

nasal secretions have confirmed the participation and activation of mast cells during the EPR (9, 10). It is not surprising that there was no definitive LPR in these patients since almost all had baseline eosinophilia (median 71%) and a high concentration of ECP (median 164 ng/g) in nasal secretions before challenge. It has been shown that a patient with ongoing allergic rhinitis is often in a continuous late-phase reaction characterized by eosinophilia and complaints mainly of nasal obstruction (9). This local phenomenon has been described by Connell (11) as a “priming effect” when the nasal mucosa becomes hyperresponsive to antigen.

During the past few years, there has been growing awareness of the association between allergic rhinitis and asthma. It is recommended that allergic rhinitis should be considered a risk factor for asthma along with other known risk factors (1). Several mechanisms have been proposed to link uncontrolled allergic rhinitis and the occurrence or worsening of asthma (12). In our study, only three patients who had history of asthma showed a fall in FEV₁ around 7 h after nasal challenge with *B. tropicalis*, which was followed by wheezing in the night. These symptoms occurred when the patients had recovered from the EPR and were free of nasal symptoms. Further, they showed similar nasal responses (in terms of the pattern and severity of nasal symptoms and release of mediators) after nasal challenges as the other patients in the study. It seems that people with a history of asthma are more susceptible to development of lower airway symptoms after nasal allergen challenge.

It was noted in our previous experience that nasal challenge with grass pollens in patients with seasonal allergic rhinitis, either within or outside the pollen season, did not result in asthmatic symptoms (6, 7, 9).

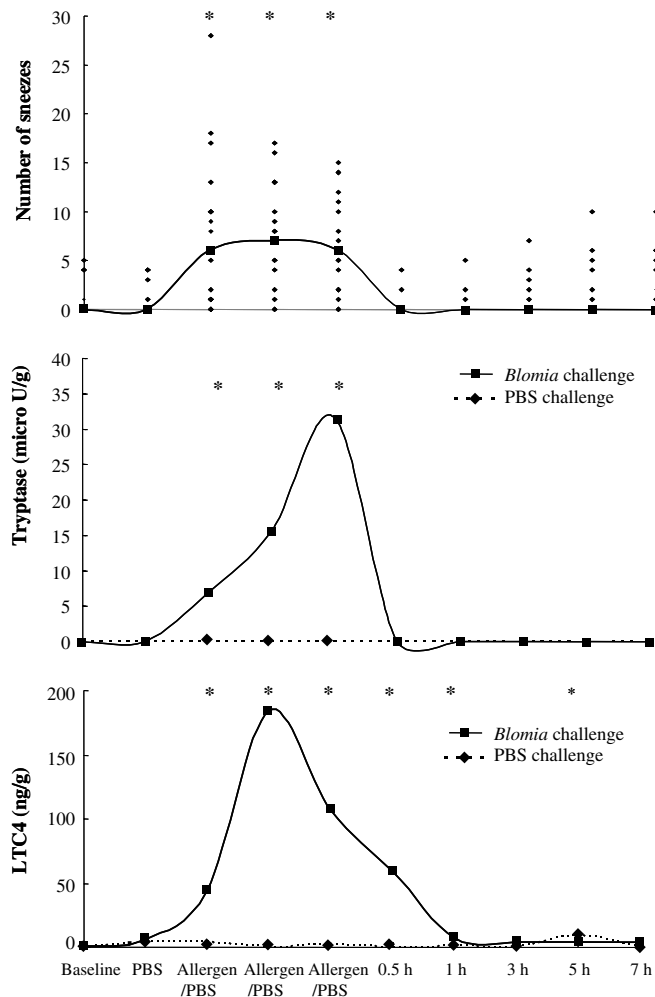


Figure 1. Nasal challenge was carried out using a nasal spray with phosphate-buffered saline (PBS) and with increasing concentrations of crude *Blomia tropicalis* extract (0.6, 6.0, and 60 μ g/ml) at 15 min intervals. An identical challenge procedure with PBS (PBS challenge) was repeated in seven patients after a washout period of at least 2 weeks. The figures show the absolute number of sneezes of individual subjects (top) and concentrations of tryptase and leukotriene C_4 (LTC_4) in nasal secretion 5 min after each challenge, and at 30 min, 1 h, 3 h, 5 h and 7 h after the last challenge. A significant ($P < 0.01$) increase in the number of sneezes was accompanied by increased concentrations of tryptase and LTC_4 after each challenge with *B. tropicalis*, confirming mast-cell activation during the early-phase reaction.

However, these studies did not include anyone with a history of asthma, which was an exclusion criterion. It has been shown that genes for atopy and airway hyperresponsiveness are present in the genomic material of individuals with asthma (13). The intricate play of these genes with each other and with the environment may ultimately determine whether an individual devel-

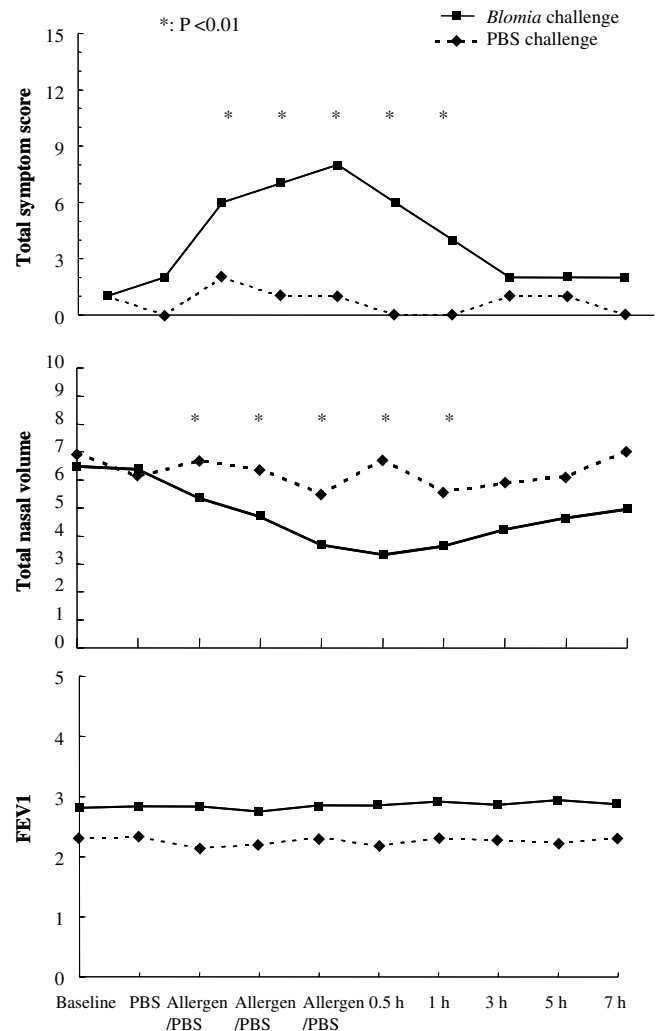


Figure 2. The same settings as Figure 1 were used. A significant increase in the total symptom score was accompanied by a significant decrease in the nasal cavity volume (due to nasal congestion) as measured by acoustic rhinomanometry. However, there was no significant change in forced expiratory volume in 1 s (FEV_1) during the entire study observation period.

ops asthma (14). It may explain why some individuals with atopic rhinitis have concomitant asthma or hyper-sensitivity of the lower airway, while others do not have this phenotype.

In conclusion, our study demonstrates that nasal challenge with *B. tropicalis* can trigger clinically important nasal allergic reactions with symptoms and mucosal inflammation in sensitized people. Nasal allergen challenge may cause concomitant asthmatic symptoms, especially in patients with coexisting asthma during the late-phase reaction. The pathogenic mechanism leading to this indirect lower airway response needs further investigation.

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