

ORIGINAL ARTICLE

Immediate and dual response to nasal challenge with *Dermatophagoides pteronyssinus* in local allergic rhinitis

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Summary

Background Local allergic rhinitis (LAR) is characterized by *in situ* production of specific IgE (sIgE) antibodies and a positive response to a nasal allergen provocation test (NAPT) in the absence of atopy.

Objective The aim of this study was to investigate the immunological mechanisms involved in the immediate and late responses after nasal exposure to *Dermatophagoides pteronyssinus* (DP) in patients with LAR.

Methods A total of 40 subjects with LAR to DP were studied and compared with 50 healthy controls. Immediate and late responses to NAPT-DP were assessed using a visual analogue scale of nasal symptoms and acoustic rhinometry. Tryptase, ECP, total and sIgE-DP were measured in the nasal lavage by immunoassay at baseline, 15 min, 1, 6 and 24 h after nasal challenge.

Results NAPT-DP was positive in all patients, with significant increases in tryptase (45%), ECP (65%) and sIgE-DP (25%) ($P < 0.05$). Sixty percent of the LAR patients presented an immediate response to NAPT-DP and 40% a dual response. Immediate responders showed a fast release of tryptase with a peak at 15 min after NAPT-DP, and a progressive increase in nasal ECP and sIgE-DP from 1 to 24 h after challenge, with a peak at 24 h. Dual responders presented persistently higher levels of tryptase from 15 min to 6 h after challenge, and a similar pattern of nasal release of ECP and sIgE-DP to immediate responders. There were no isolated late responders. NAPT-DP was negative in all healthy controls, with no increases in tryptase, ECP, or total and sIgE-DP in nasal secretions.

Conclusions The results demonstrated the existence of immediate and dual responses to a NAPT with DP in LAR patients, with the local presence of sIgE and mast cell/eosinophil activation.

Keywords ECP, local allergic rhinitis, nasal allergen provocation test, nasal specific IgE, tryptase

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Introduction

Nasal allergen provocation test (NAPT) studies over the past 15 years have improved our knowledge of the mechanisms of allergic rhinitis [1]. In 1873, Blackley was the first to describe an association between sneezing, rhinorrhoea and nasal congestion with seasonal variations in atmospheric pollen concentrations. He also described the late-phase reaction in rhinitis patients [2]. Since then, many studies have documented that NAPT in atopic patients causes immediate and late-phase nasal

reactions with nasal symptoms and a significant increase in the concentration of inflammatory mediators in nasal lavage fluids, including histamine, prostaglandin D₂, leukotriene C₄, tryptase and eosinophilic cationic protein (ECP), as well as different cytokines and chemokines, as compared with healthy controls [3–9].

The immediate response has been well described and is originated by the early release of mast cell products such as histamine and other vasoactive mediators. The late-phase response has been less well studied and is characterized by the release of different cytokines, mainly

produced by T-helper type 2 (Th2) cells and granulocytes [10].

Over recent years, an important number of patients diagnosed previously with idiopathic rhinitis have been shown to have a new form of 'local allergic rhinitis' (LAR) or 'entopy', with *in situ* production of specific IgE (sIgE) and a positive response to NAPT in the absence of atopy [11–15]. During symptomatic periods, LAR patients have a nasal inflammatory pattern similar to that seen in allergic rhinitis, with increased levels of eosinophils, basophil-mast cells, total lymphocytes, CD3⁺T cells and ECP [13, 14, 16, 17]. However, the pathophysiology of the nasal response to NAPT in LAR patients has not been sufficiently studied. We, therefore, studied the immunological mechanisms involved in the immediate and late responses to nasal exposure to *Dermatophagoides pteronyssinus* (DP) in patients with LAR. The clinical response, nasal patency and nasal release of total and sIgE, tryptase and ECP were monitored up to 24 h after a nasal challenge in these patients and compared with healthy controls.

The results demonstrated that nasal exposure to house dust mite in patients with LAR induces immediate or dual responses, with the local presence of sIgE, mast cell/eosinophil activation and increased concentrations of tryptase and ECP, plus a further stimulation of IgE production.

Methods

Study subjects

The study included 40 adult patients with LAR to DP (LAR group) and 50 healthy non-atopic subjects (control group) recruited from our outpatient clinics. The LAR patients were diagnosed by a positive NAPT-DP with negative skin prick test (SPT) plus negative serum sIgE to common environmental aeroallergens, and a negative intradermal skin test to DP. The criteria for the LAR group included a history of persistent LAR to DP for at least 2 years. Patients were excluded if they had other immunological diseases, chronic rhinosinusitis and/or nasal polyposis (CT scan), vasomotor rhinitis (clear rhinorrhoea and a response to ipratropium bromide) or a respiratory infection in the previous 4 weeks. The patients had to avoid any treatment with systemic or nasal corticosteroids for 4 weeks or systemic antihistamines or nasal vasoconstrictors for 2 weeks before the study.

The control subjects had no allergic or nasal diseases, were not pregnant or lactating and had negative SPT, serum sIgE to aeroallergens, intradermal skin test and NAPT to DP.

The study was performed in July–September, 2007 when the patients were symptom free and without treatment. The local ethics committee approved the study, and informed consent was obtained.

Skin tests

Skin prick test. The SPT was performed with the most prevalent inhalant allergens, including house dust mite (DP, *D. farinae*, *Lepidoglyphus destructor* and *Blomia tropicalis*), pollens (*poa*, *Phleum*, *Lolium*, *Cassuarina*, *Eucalyptus*, *Cupresus*, *Platanus*, *Olea*, *Helianthus*, *Chenopodium*, *Plantago*, *Artemisia*, *Parietaria judaica*, *Salsola kali*, *Rumex* and *Ricinus*), moulds (*Alternaria*, *Aspergillus*, *Cladosporium*, and *Penicillium*) and dog, cat and hamster epithelia (ALK-Abelló, Madrid, Spain).

Intradermal skin test. Intradermal skin testing was carried out with freshly reconstituted freeze-dried allergen solutions of DP (0.4 and 4 mcg/mL) (ALK-Abelló).

Total and specific immunoglobulin E in serum. Total IgE and sIgE were determined by immunoassay (UniCAP, Phadia, Uppsala, Sweden). For serum sIgE, we used the same allergens as for the SPT. The cut-off value for sIgE was 0.35 kU/L.

Nasal symptoms. Symptoms of nasal obstruction, rhinorrhoea, itching and sneezing were each recorded by placing a vertical mark on a horizontal visual analogue scale (VAS) of 100 mm. The severity was categorized as mild (0–30), moderate (31–70) or severe (71–100). The total VAS was the sum of the four VAS scores (total range 0–400).

Acoustic rhinometry. Nasal patency was assessed by acoustic rhinometry with the use of an SRE 2000 rhinometer (Rhinometrics, Lynge, Denmark) following the guidelines of the Standardization Committee on Acoustic Rhinometry [18]. The volume of the nasal cavity corresponding to the lower turbinate (VOL 2–6 cm) was analysed in each nostril.

Nasal allergen provocation test. Using a metered pump spray, symptom-free patients and healthy controls were challenged intranasally in July–September 2007 with saline to exclude nasal hyperactivity. If negative, NAPT was performed 1 week later with freshly reconstituted DP (4 µg/mL) extract (ALK-Abelló). Two puffs (100 µL) of the solution at room temperature were applied in each nasal passage. A positive NAPT was considered to be an increase $\geq 30\%$ in the total nasal symptoms and a decrease $\geq 30\%$ in the total volume of both nasal cavities from 2 to 6 cm (VOL 2–6 cm) in acoustic rhinometry compared with the baseline test. A positive response was categorized as immediate (15 min–1 h after NAPT), late (6–24 h) or dual (both immediate and late). These changes in VAS and acoustic rhinometry were validated previously in symptomatic persistent allergic rhinitis patients and using mediator release (histamine).

Nasal allergen provocation test monitoring. NAPT with DP was monitored before and 15 min, 1, 6 and 24 h after the challenge by nasal symptoms, acoustic rhinometry, measurement of inflammatory mediators and total and sIgE in nasal secretions. The subjects remained in the laboratory throughout the first hour and for 30 min before the 6- and 24-hour time-points to limit their potential exposure to changes in temperature, physical exercise or irritant odorants.

Nasal inflammatory mediators, total immunoglobulin E and specific immunoglobulin E measurements

A bilateral nasal lavage following the Naclerio method [3] was performed with 8 mL of physiological saline at room temperature with the subjects seated with their necks extended 30° from the vertical. After 10 s, the subjects flexed their necks and the sample of mucus and saline was expelled into a container. The procedure was then repeated in the other nostril.

Samples were centrifuged at 1069 g for 7 min at 4 °C, and the supernatant was stored at –20 °C for the measurement of tryptase, ECP, total IgE and sIgE to DP by immunoassay (UniCAP, Phadia). The detection limits of the immunoassays were 1 ng/mL for tryptase, 2 ng/mL for ECP and 0.35 kU/L for sIgE.

Statistical analysis

SPSS version 15.0 (Chicago, IL, USA) was used for statistical analysis. The data were expressed as the median and range. The clinical and demographic data were compared between groups by χ^2 -analysis and the Mann-Whitney *U*-test. Friedman's test was used to examine the overall differences. If significant differences occurred, the Wilcoxon's signed-ranks test was used to identify significant differences in tryptase, ECP, total IgE and sIgE in nasal secretions, VAS symptoms and VOL 2–6 cm in acoustic rhinometry, within groups. All *P*-values of <0.05 were considered statistically significant.

Results

Subjects. A summary of the demographic and clinical data of the patients and the controls is presented in Table 1. There were no differences in sex, age or city/country dwelling. Thirty-three percent of the LAR patients reported symptoms of asthma and 25% of conjunctivitis.

Nasal allergen provocation test. No significant differences were found in the recovered volumes (mean±SD) of nasal lavage between the LAR patients (5.5±0.7 mL) and the controls (5.8±0.8 mL).

Four LAR patients and one control had a positive response to saline nasal challenge, with no increase in

Table 1. Characteristics of the study subjects

Groups	LAR	Control
Subjects (<i>N</i>)	40	50
Age (years)	25 (18–63)	31 (18–67)
Disease duration (years)	3.5 (2–20)	NA
Sex, <i>N</i> (%)		
Male	12 (30)	16 (32)
Female	28 (70)	34 (68)
FHA, <i>N</i> (%)	18 (45)	24 (48)
Dwelling, <i>N</i> (%)		
City	25 (63)	34 (68)
Country	15 (38)	16 (32)
Co-morbidity, <i>N</i> (%):		
Asthma	13 (33)	NA
Conjunctivitis	10 (25)	NA
Triggering factors, <i>N</i> (%)		
House dust	36 (90)	NA

Data on age and disease duration are expressed as median and range. FHA, family history of atopy; LAR, local allergic rhinitis; *N*, number of subjects; NA, not applicable.

nasal inflammatory mediators or sIgE-DP, and were excluded from the study.

NAPT-DP was positive in all LAR patients, with significant increases in tryptase (18/40 patients; 45%), ECP (26/40 patients; 65%) and sIgE-DP (10/40 patients; 25%) in the nasal secretion after NAPT (*P*<0.05). Total IgE was not increased at any time-point. Sixty percent of the LAR patients presented an immediate response to NAPT-DP and 40% a dual response. No isolated late response was observed.

Nasal challenge with DP was negative in all healthy controls, with no increases in tryptase, ECP, total or sIgE in nasal secretions.

Local allergic rhinitis patients with an immediate response to nasal challenge. The LAR patients with an isolated immediate response to NAPT-DP presented a significant increase in VAS symptoms at 15 min after a nasal challenge (*P*<0.001), associated with a maximum reduction in nasal patency, as represented by the mean VOL 2–6 cm of the nasal cavity (Fig. 1a). The mean VOL 2–6 cm decreased significantly from 100% (baseline value) to 62% at 15 min after NAPT (*P*<0.05) and returned to normal values 1 h after the challenge (Fig. 1a).

Analysis of the frequency and intensity of individual nasal symptoms showed that rhinorrhoea and sneezing (85%) were the most frequent, and rhinorrhoea (75%) the most severe symptom reported by LAR patients during an immediate response to NAPT-DP (Table 2).

These clinical responses were associated with significant increases in nasal tryptase (11/24 patients, 46%), ECP (16/24 patients, 67%) and sIgE to DP (5/24 patients, 21%). The kinetics of tryptase production in patients with an isolated immediate response showed a fast release with

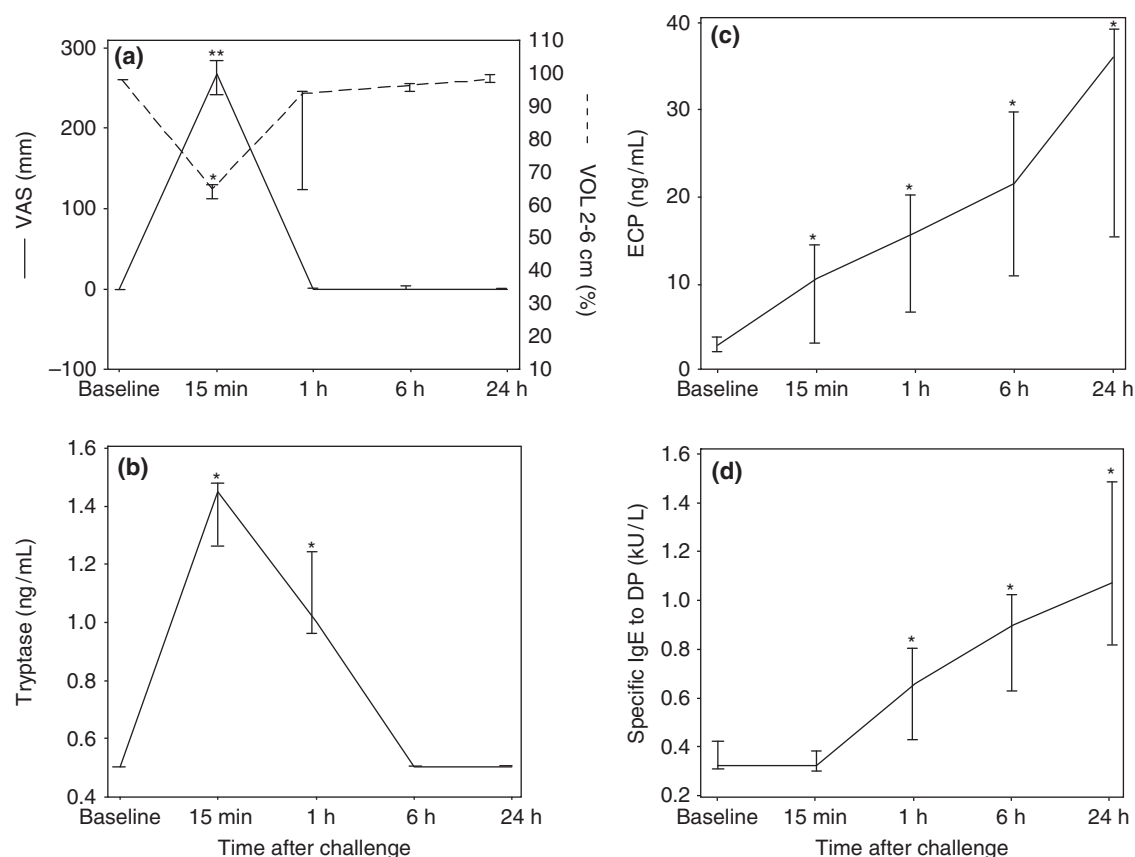


Fig. 1. Changes in total visual analogue scale (VAS) symptoms (a), VOL 2–6 cm (a) and nasal levels of tryptase (b), ECP (c) and sIgE to DP (d) in LAR patients with an immediate response to NAPT-DP. Data are expressed as median±range. Significant differences between baseline and 15 min, 1, 6 and 24 h after challenge are indicated by * $P<0.05$ and ** $P<0.001$. VOL 2–6 cm, volume of both nasal cavities from 2 to 6 cm; ECP, eosinophilic cationic protein; sIgE, specific IgE; DP, *Dermatophagoides pteronyssinus*; LAR, local allergic rhinitis; NAPT, nasal allergen provocation test.

Table 2. Frequency and intensity of nasal symptoms in immediate and late responses to nasal allergen provocation test with *Dermatophagoides pteronyssinus*

	Frequency	Severe symptom
Immediate response, <i>N</i> (%)		
Nasal obstruction	26 (65)	23 (58)
Itching	31 (78)	26 (65)
Rhinorrhea	34 (85)	30 (75)
Sneezing	34 (85)	25 (63)
Late response, <i>N</i> (%)		
Nasal obstruction	35 (88)	25 (63)
Itching	30 (75)	10 (25)
Rhinorrhea	32 (80)	31 (78)
Sneezing	14 (35)	14 (35)

significant increases at 15 min and 1 h ($P=0.02$) after nasal challenge compared with baseline (Fig. 1b). The maximum peak was observed 15 min after challenge and returned to the initial level after 6 h. Significant increases in nasal ECP were observed at 15 min, 1, 6 and 24 h after challenge ($P<0.05$). The kinetics of the nasal release of ECP were different from tryptase (Fig. 1c), with a progres-

sive nasal release from 15 min, reaching maximum levels at the final evaluation point, 24 h after challenge.

Nasal sIgE to DP was detected in 21% of patients who developed an immediate response to NAPT-DP, with significant increases at 1, 6 and 24 h ($P<0.05$) compared with baseline values (Fig. 1d); two of these patients (8%) had detectable baseline levels of nasal sIgE-DP during the asymptomatic period. The nasal values of sIgE-DP showed a progressive increase, reaching a maximum 24 h after challenge; no further determinations were made after this point.

Local allergic rhinitis patients with a dual response to nasal challenge. The patients with a dual response (40%) presented two peaks of significant increases in VAS symptoms, at 15 min and 6 h ($P<0.05$) after challenge compared with baseline (Fig. 2a). The comparison between immediate and dual responders showed similar values of VAS symptoms during the first 15 min after challenge and significantly higher values of VAS symptoms in patients with a dual response at 6 h after NAPT-DP ($P=0.01$). Analysis of individual nasal symptoms showed that nasal obstruction was the most frequent (88%) and rhinorrhea

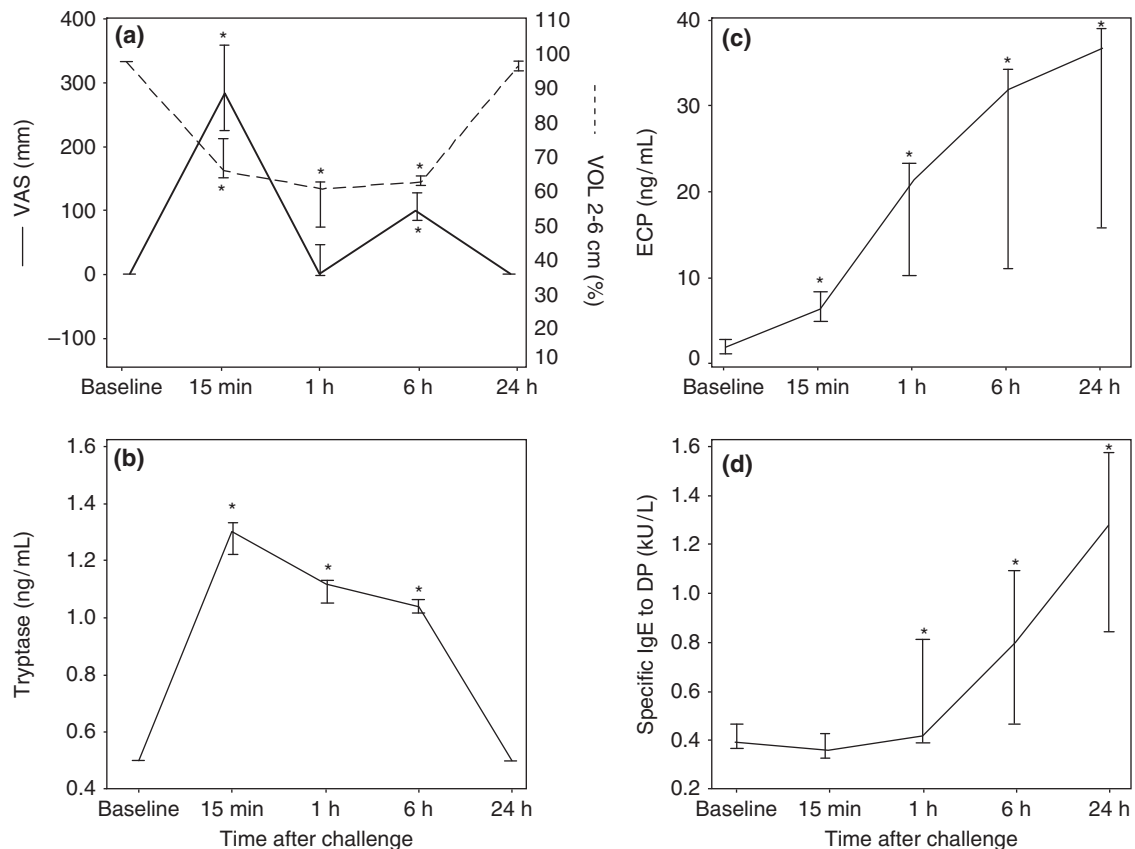


Fig. 2. Changes in total visual analogue scale (VAS) symptoms (a), VOL 2–6 cm (a) and nasal levels of tryptase (b), ECP (c) and sIgE to DP (d) in LAR patients with a dual response to NAPT-DP. Data are expressed as median $\pm$ range. Significant differences between baseline and 15 min, 1, 6 and 24 h after challenge are indicated by * $P < 0.05$ and ** $P < 0.001$. VOL 2–6 cm, volume of both nasal cavities from 2 to 6 cm; ECP, eosinophilic cationic protein; sIgE, specific IgE; DP, *Dermatophagoides pteronyssinus*; LAR, local allergic rhinitis; NAPT, nasal allergen provocation test.

the most severe (78%) during the late response (Table 2). This clinical response was associated with a reduction in the mean VOL 2–6 cm of the nasal cavity above 30% from 15 min to 6 h after challenge ($P = 0.002$) (Fig. 2a).

Significant increases were also detected in nasal tryptase (7/16 patients, 48%), ECP (10/16 patients, 63%) and sIgE to DP (5/16 patients, 31%) associated with nasal symptoms during the dual responses to NAPT-DP.

LAR patients with immediate and dual responses showed different kinetics of nasal release of tryptase (Fig. 2b). Dual responders had a significant increase in nasal secretions at 15 min, 1 and 6 h compared with baseline values ($P < 0.05$). Comparison studies between both groups showed that dual responders presented higher levels of tryptase at 6 h compared with immediate responders ($P = 0.002$).

Significant increases in nasal ECP (Fig. 2c) were observed at 15 min, 1, 6 and 24 h after NAPT-DP ($P < 0.05$), with a similar pattern to that in immediate responders. No significant differences in nasal concentrations of ECP were detected between LAR patients with immediate and dual responses.

The time course of the nasal release of sIgE-DP is shown in Fig. 2d. Significant increases in nasal sIgE-DP

were detected in 31% of the patients with a dual response at 1, 6 and 24 h after challenge compared with baseline ($P < 0.05$). Three of these (19%) presented detectable baseline levels of sIgE-DP. No significant differences were found in levels of nasal IgE-DP between LAR patients with immediate and dual responses to NAPT-DP, although the patients with an immediate response presented higher levels at 1 h and dual responders at 24 h after challenge.

*Total immunoglobulin E and specific immunoglobulin E to *Dermatophagoides pteronyssinus*.* No significant differences were found in serum and baseline nasal levels of total and sIgE to DP between LAR patients and healthy controls (Table 3).

Discussion

Idiopathic rhinitis (IR) is an entity that has not been well defined clinically and may have several aetiologies and different mechanisms. The variability in the presence of an inflammatory response in nasal mucosa from IR patients may be because studies may have included patients with a

Table 3. Serum and baseline nasal levels of total IgE and specific IgE

	LAR patients	Healthy controls
Serum levels		
Total IgE (IU/mL)	68 (54–109)	52 (48–120)
sIgE-DP (kU/L)	<0.35	<0.35
Baseline nasal levels		
Total IgE (IU/mL)	8 (5–12)	10 (7–19)
sIgE-DP (kU/L)	0.30 (0.30–0.63)	<0.35

Data represent median and range.

LAR, local allergic rhinitis; sIgE-DP, specific IgE to *Dermatophagoides pteronyssinus*.

different pathogenesis, e.g. predominantly inflammatory [16, 17, 19, 20] vs. neurogenic mechanisms [21].

Evidence has demonstrated that some IR patients with symptoms suggestive of allergic rhinitis and a negative skin test and absence of serum sIgE antibodies present a new form of 'LAR' or 'entopy' with a local inflammatory response and a local presence of sIgE and a positive response to a NAPT [11–14]. In order to identify these patients, a detailed clinical history that excludes subjects with vasomotor or infectious rhinitis is appropriate before initiating pathophysiological studies in LAR or entopy.

Most studies performed in LAR patients have been carried out during the symptomatic period [13, 14, 16, 17]. However, studies of the quantitation of inflammatory mediators and sIgE in nasal secretions during NAPT are lacking. Accordingly, our purpose was to study the kinetics of pathophysiological changes, the nasal release of inflammatory mediators (tryptase and ECP) and the local sIgE production after NAPT-DP in patients with LAR.

In this study, 60% of the LAR patients had an isolated immediate response to NAPT-DP and 40% a dual response, with the recurrence of symptoms and nasal release of inflammatory mediators 6 h after challenge. No isolated late response was observed. These data are similar to those observed in previous studies in non-allergic rhinitis [13–15], and allergic rhinitis and asthmatic patients [8, 22–27]. Although with a lower frequency, isolated late responses to NAPT have been reported in allergic rhinitis and asthmatic patients [28]. In a recent study, Wedback et al. [29] described the presence of an isolated late response in 67% of non-atopic rhinitis patients, and immediate and/or dual response in just 33%.

Our study showed significant differences between immediate and dual LAR responders to nasal challenge in the frequency and severity of the clinical symptoms and also in the kinetics of the inflammatory mediators released after a nasal challenge. Sneezing and rhinorrhoea were the most frequently reported symptoms in patients with an immediate response to NAPT-DP and nasal obstruction during the late response.

Tryptase, a specific marker for mast cell activation, has proved a useful inflammatory marker of the immediate

nasal response [6, 30–32]. Immediate responders showed a release of tryptase, with a peak from 15 min to 1 h after NAPT-DP, returning to baseline values at 6 h, with no further increase. Dual responders also presented a significant release of tryptase at 15 min, with similar values to the immediate responders, but in their case it persisted for 6 h before decreasing.

Although different studies have shown short peaks of tryptase at variable time-points during the late response to nasal challenge with DP in some allergic rhinitis patients [30, 31], indicating mast cell degranulation during this period, a high sustained release of tryptase during the late-phase of allergic reactions had not been reported. Tryptase detected in the 6 h lavage could have reached the luminal surface any time between 1 and 6 h after challenge. Further studies will require the addition of new analytical time-points of 3 and 4 h after provocation in order to detect new tryptase release.

A similar pattern of nasal release of ECP and sIgE-DP was found in immediate and dual responders. The late response in allergic rhinitis has been associated with an influx of eosinophils [9, 10, 26, 30]. In our study, LAR patients sensitized to DP presented a significant nasal release of ECP starting at 15 min after provocation, with a progressive increase to the final point 24 h after a provocation challenge. At these time-points, the pattern of response in immediate and dual responders was similar. No further determinations were made. These results indicate a recruitment of activated eosinophils at a very early stage of the process, not just during the late response, as has been reported previously in atopic patients [9, 10, 26, 30].

T lymphocytes are one of the key cells regulating and co-ordinating immune responses in allergic diseases. In atopy, Th2 cells regulate IgE synthesis and cell recruitment at the sites of inflammation [1]. In previous studies, our group has reported an increase in nasal T lymphocytes during the symptomatic period in LAR patients [13, 14]. These cells may contribute to the recruitment of eosinophils and to the production of the sIgE antibodies in LAR patients. Pawankar et al. [33] reported that both nasal T cells and mast cells are important sources of Th2-type cytokines like IL-4 and IL-13, and can induce IgE synthesis in allergic rhinitis patients.

In this study, detectable baseline levels of sIgE were observed in five LAR patients with an increase in local sIgE production in 10 of the 40 after NAPT, including the original five, with significant levels from 1 to 24 h. These results indicate that a sIgE-DP was locally present at the time of the study of the nasal fluid. In the other five patients, nasal sIgE was not detected at baseline. We believe that the reason for not detecting sIgE nasal antibodies in the other cases with a positive NAPT may be either a limitation in the sensitivity of the method for the nasal detection of sIgE or, less likely, the involvement

of another mechanism. The dilutional effect of nasal lavage may contribute to a lower sensitivity. A direct interaction between allergen and mast cells has been proposed [12], although no evidence supporting a non-IgE mechanism has been shown yet.

In previous studies, we have demonstrated an elevation of ECP and sIgE to both house dust mite [8] and grass pollen [9] during natural exposure to aeroallergens, although tryptase levels were not measured. In this study performed in LAR patients sensitized to DP, we found clear differences between immediate and late allergic responses after NAPT-DP in nasal symptoms and the release of tryptase into nasal secretions. The maximum levels of ECP and sIgE were obtained 24 h after challenge, the last evaluation time-point of this study. Whether nasal production of ECP and/or sIgE increases further beyond this time-point is a question that will be examined in future studies.

In summary, NAPT with house dust mite was a good approach to confirm the immunological mechanisms involved in LAR and to detect the presence of local sIgE antibodies. The increase in the local presence of sIgE observed in this study requires further investigation to assess the kinetics of the production of sIgE antibodies and the lymphocyte subpopulations and cytokines involved.

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