

Allergen-Induced Release of Sulphidopeptide Leukotrienes (SRS-A) and LTB₄ in Allergic Rhinitis

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Leukotrienes are a recently discovered group of arachidonic acid-derived lipid mediators. Using radioimmunoassay and high pressure liquid chromatography (HPLC), we have identified the SRS-A sulphidopeptide leukotrienes (LTC₄, LTD₄ and LTE₄) in nasal washings from patients with allergic rhinitis who underwent nasal challenge with specific allergen. Smaller, but significant, amounts of LTB₄ were also detected. The concentrations of nasal leukotrienes were directly related to the dose of allergen, and were recovered in washings in a time-dependent fashion after challenge. When the patients were subjected to methacholine nasal challenge on a control day, we found only negligible amounts of either the sulphidopeptide leukotrienes or LTB₄. These findings support the view that LTC₄, LTD₄ and LTE₄ might contribute to the pathogenesis of allergic rhinitis as a result of their recognized effects on mucous hypersecretion and vasopermeability, and that the potent chemoattractant LTB₄ might be involved in the subsequent infiltration of inflammatory cells.

Key words: allergic rhinitis; leukotrienes; nasal provocation; SRS-A.

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Hay fever and perennial allergic rhinitis result from the inhalation of specific antigenic material in those susceptible individuals who have previously made an IgE response. The interaction of allergen and IgE on the surface of cells of the mast cell/basophil series leads to the release of a variety of vasoactive, chemotactic and enzymatic mediators which in turn are presumed to be responsible for the vasodilation, oedema, increased mucous secretion, cellular recruitment and increased capillary permeability that are characteristic of the disease (23). A major role of histamine in these events is assumed since histamine is present in nasal secretions after allergen challenge in rhinitis, and treatment with antihistamines partially alleviates the symptoms (9, 17). On the other hand, the total ineffectiveness of even the newer selective H₁-antagonist in controlling the symptoms of rhinorrhea and congestion during the height of the pollen season (13, 14) indicates

that other mediators are likely to be involved in the manifestation of many of the symptoms of this disease.

The leukotrienes (LT) are a newly discovered class of lipid mediators which include the sulphidopeptide leukotrienes LTC₄, LTD₄ and LTE₄ (previously termed slow reacting substance of anaphylaxis - SRS-A) and the potent chemotactic factor LTB₄. These lipid mediators are derived from 5-lipoxygenation of arachidonic acid. LTC₄ and LTD₄ cause bronchoconstriction in man (1, 2, 10, 24), increased mucous gland secretion (16) and altered capillary permeability (6). When administered topically to the nose, LTC₄ caused an increase in nasal blood flow although a concomitant increase in nasal secretion was not found (4). Leukotriene B₄ is a potent chemoattractant and is believed to play an important role in the neutrophil and eosinophil infiltration which is characteristic of acute allergic

reactions. In the present report we show that the sulphidopeptide leukotrienes and LTB_4 were recoverable from nasal washings in patients with allergic rhinitis who have undergone nasal challenge with specific allergen, indicating that these lipid mediators may contribute to rhinorrhoea, nasal obstruction and inflammatory cell infiltrates.

MATERIAL AND METHODS

Patients

Nasal challenge was performed on 13 subjects (age 15–51) with house dust mite- or grass pollen-induced allergic rhinitis. All had more than one positive skin prick test to common allergens (1.2% w/v) and gave a positive response (weal diameter >3 mm more than response to the diluent control) to the antigen used in the challenge. The subjects had not taken oral or nasal aerosol medication for at least 2 weeks prior to the study.

Challenge procedure

Solutions were insufflated into the nose using a bulb nebuliser. Four sprays delivered a measured volume of 0.20 ± 0.01 ml (mean $\pm$ SEM, $n = 20$). The dose of allergen was divided, with two sprays being administered to each nostril.

Antigen extracts, either house dust mite (HDM) (*Dermatophagoides pteronyssinus*) or mixed grass pollen (B_2) (Bencard, Brentford, Middlesex) were used at 0.1% (w/v) dilution in saline, except during the dose response study, when the initial concentration of allergen used was half that which in an allergen dose titration produced a 3 mm weal on skin prick testing. In this part of the study doubling antigen concentrations were then administered at 30 min intervals until there was either a marked symptomatic response, or a dose of 0.1% was reached.

Methacholine bromide (Sigma, Poole, Dorset), 12.5 mg per 0.2 ml, was dissolved in saline.

All patients had a positive response to 0.1%,

or the largest dose administered, shown by ≥ 3 sneezes, rhinorrhoea with production of ≥ 0.5 ml of nasal drip fluid and itching of the nose. After methacholine the predominant symptom was nasal secretion of which ≥ 0.5 ml was collected.

Nasal washes were performed prior to and 7.5 min, 15 min, 22.5 min and 30 min intervals after the allergen and methacholine nasal challenges in the time course study, and 15 min after the challenge in the remainder of the study. Each individual was instructed to tilt his head backwards $\neq 30^\circ$ from the horizontal while in the sitting position and hold his breath. One millilitre portions of normal saline were instilled into each nostril and the individual then flexed his neck and expelled the mixture of mucus and saline into the same plastic beaker in which the nasal drip fluid had been collected on ice (20, 21). In all subjects this procedure was repeated until a total volume of $\neq 5$ ml of wash fluid was recovered. This fluid was mixed with the spontaneously produced nasal drip fluid prior to analysis.

Extraction of leukotrienes from nasal wash fluid

Nasal wash fluid was mixed with an equal volume of HPLC grade methanol (Rathburn, Walkerburn, Scotland) using a Silverson grinder (Silverson Ltd., Chesham, Bucks.). After acidification to pH 4.0 with acetic acid the precipitate was removed by centrifugation (12,000 g, 10 min, 4°C) and the supernatant was diluted with approximately 40–50 ml of double distilled H_2O to give a 10% methanol solution. The leukotrienes were then extracted using "C18 Sep-paks" (Waters Associates, Northwich, Cheshire, England) (22) and re-suspended in PBS buffer or HPLC running solvent in a nitrogen atmosphere. RP-HPLC, used to further separate the leukotrienes, was performed on a 5 micron Nucleosil C18 column (30×0.5 cm) (Jones Chromatography, Llanbradach, Glam.) in methanol:water:acetic acid (70:30:0:1) adjusted to pH 5.4 with ammonia. The column was calibrated using synthetic LTB_4 , LTC_4 , LTD_4 and LTE_4 (gifts from Dr. J. Rokach, Merck-Frosst, Quebec, Canada).

Leukotriene measurement

The measurement of LTC_4/D_4 and LTB_4 using a double antibody radioimmunoassay (with antisera being supplied by Dr. E. Hayes, Merck sharp & Dohme, Rahway, N.J., U.S.A.) has been described in detail elsewhere (8, 12, 22). Recovery experiments were performed to establish that the assay procedure did not appreciably degrade synthetic leukotrienes. After "Sep-Pak" extraction and radioimmunoassay the recovery was $82 \pm 9\%$ for LTC_4 and $100 \pm 10\%$ for LTB_4 . Furthermore the curve of percentage inhibition of antibody-radioligand binding produced by serial dilutions of the mucus in LTC_4 radioimmunoassays was similar to the sigmoid curve produced from dilutions of synthetic LTC_4 .

Statistical analysis

The Wilcoxon matched-pair signed-rank test was used to compare the results of the two challenge procedures in the same individuals.

RESULTS

Nasal washes were performed prior to and 7.5, 15, 22.5 and 30 min after antigen challenge. The amount of leukotriene C_4/D_4 extracted from these sequential washes from seven individuals challenged with *D. pteronyssinus* is shown in Fig. 1. In all situations there was an increase with time (Fig. 1b) in the amounts of LTC_4/D_4 extracted from the nasal wash fluid after antigen challenge which ranged from pre-challenge concentrations of 0.6 to 6.3 picomoles, to post-challenge values of 9.1 to 52.4 picomoles. Increased amounts of the LTC_4/D_4 were recoverable from the nose within 7.5 min of the challenge. The peak times for individual patients were at 7.5 min (3 subjects), 15 min (2 subjects) and 22.5 min (2 subjects). Leukotriene concentrations were returning towards baseline values by 30 min. In comparison, insignificant quantities of LTC_4/D_4 were found in the nasal secretions induced by methacholine, although a small increase over baseline was seen at the 7.5 min time-point.

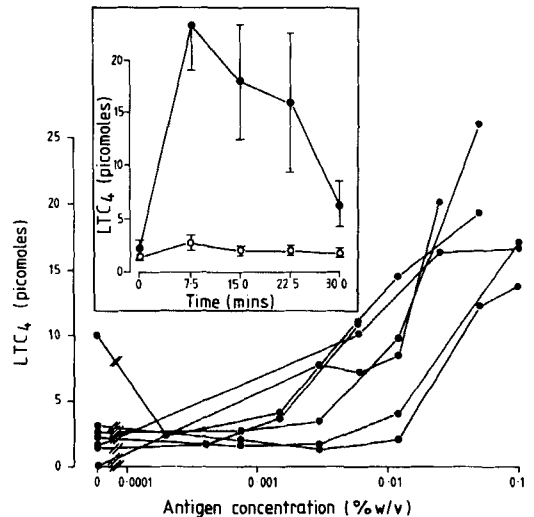


Fig. 1. a) Immunoreactive LTC_4 in the nasal secretions after increasing concentrations of antigen (HDM 6, grass pollen 1); and b) (insert) the time course of release of immunoreactive LTC_4 into the nasal secretions after antigen (HDM) (closed circle) and methacholine provocation (open circle) (mean $\pm$ SEM) ($n = 7$).

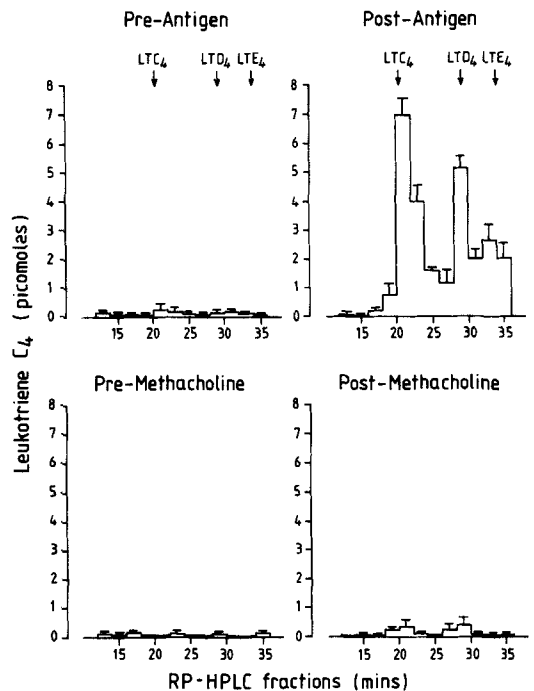


Fig. 2. LTC_4 immunoreactivity in RP-HPLC fractions of nasal secretions collected prior to, and 15 min after, antigen or methacholine nasal challenge. Three subjects were studied. The profiles are compared to the elution times of synthetic markers. The bars represent the mean $\pm$ SEM.

Table 1

Time course of release LTB₄ and LTC₄ (picomoles) into the nasal secretions after antigen and methacholine challenge

Min	LTB ₄		LTC ₄	
	Antigen	Methacholine	Antigen	Methacholine
0	1.3 (0.7–1.9)	0.6 (0.2–1.0)	2.4 (1.0–3.3)	1.4 (1.0–1.1)
7½	4.3 (2.7–6.4)*	2.2 (0.4–4.2)	21.0 (9.6–27.9)*	2.2 (1.4–3.4)
15	2.7 (1.0–4.9)*	0.6 (0.2–0.9)	18.0 (9.1–19.1)*	1.7 (1.1–2.4)
22½	2.1 (0.9–3.0)*	0.4 (0.2–0.4)	17.3 (5.5–25.7)*	1.8 (0.9–2.4)
30	1.5 (0.1–2.1)	0.4 (0.1–0.7)	6.5 (8.3–5.8)*	1.7 (0.9–2.4)

Mean (quartiles) of six experiments (**P* < 0.05).

In seven subjects the changes in LTC₄/D₄ concentration were measured 15 min after challenge with either normal saline or increasing concentrations of the *D. pteronyssinus* antigen (Fig. 1a). Low levels of LTC₄/D₄ were found in the nasal washings after normal saline insufflation and there was an antigen dose-dependent increase in LTC₄ concentration in successive challenges.

In three further experiments, the nasal wash fluid obtained prior to, and 15 min after, nasal provocation with antigen or methacholine was subjected to RP-HPLC and immunoreactive leukotriene concentrations were measured in the fractions. The results were compared with the elution profile of synthetic leukotriene markers (Fig. 2). Prior to either methacholine or antigen challenge no LTC₄/D₄ immunoreactivity was detected. After methacholine challenge there were two small peaks of immunoreactive material corresponding to the elution times of synthetic LTC₄ and LTD₄, but these were at the lower limit of detection of the assay. In the samples collected after antigen challenge there were two major peaks of LTC₄ immunoreactivity. The largest peak eluted between 20 and 22 min corresponding to LTC₄, and the second eluted between 28 and 30 min corresponding to LTD₄. A third small peak between 33 and 35 min was attributed to LTE₄.

The time-course of LTB₄ and LTC₄ recovery from nasal washings, after either antigen or methacholine challenge, is shown in Table 1. Although there was about five times more LTC₄ in the washings, LTB₄ concentrations with allergen were higher at each time point

when compared to methacholine with significant differences at 7.5, 15 and 22.5 min (*P* < 0.05). Dose-response studies were also performed (data not shown). In six patients the amounts of LTB₄ detected were highest with increasing concentrations of antigen, although in two individuals there were high baseline (saline) values. After further purification of antigen-induced nasal washing by RP-HPLC, LTB₄ immunoreactivity eluted together with the synthetic LTB₄ marker (3 experiments).

When three non-atopic, skin test negative individuals, were challenged with a 0.1% *D. pteronyssinus* antigen extract they had no clinical symptoms and no increase in extractable LTC₄/D₄ or LTB₄ in post-challenge nasal fluids.

DISCUSSION

The clinical model used in the present study is directly relevant to allergic rhinitis since antigen challenge to the nose produces symptoms similar to those occurring during "natural" exposure.

Although the radioimmunoassay measures primarily LTC₄, the cross-reactivity with LTD₄ and LTE₄ was in the ratio 100:70:8 respectively, but was minimal with other arachidonic acid metabolites (8). This accounted for the LTC₄ immunoreactivity that co-eluted with LTD₄ and LTE₄ (in addition to LTC₄) on RP-HPLC (Fig. 2) and is compatible with the recognized natural degradation of LTC₄ → LTD₄ → LTE₄ by sequential peptide cleavage (15). The present

findings are also consistent with a recent report on sulphidopeptide nasal leukotrienes in ragweed allergy which indicated that the natural degradation of LTC₄ to LTD₄ to LTE₄ occurs in nasal washes (7).

Nasal challenge with a 0.1% antigen solution was sufficient to produce moderate or severe symptoms in all subjects, and was associated with an increase in extractable leukotrienes. The mean concentrations of both LTC₄ and LTB₄ peaked at 7.5 min (Fig. 1 and Table 1), and decreased thereafter towards pre-challenge values. The first wash after antigen instillation was delayed until 7.5 min to ensure adequate exposure to antigen, and to minimize interference with potential interactions between mediators.

In the present report the specificity of allergen-induced leukotriene secretion was obtained by employing a methacholine control day. When the same individuals were challenged with a dose of methacholine sufficient to produce at least 0.5 ml of nasal drip fluid, they showed only a very modest increase in LTC₄ or LTB₄ extracted from the 7.5 min sample (Fig. 1 and Table 1), and no detectable change at other time points. This is compatible with the view that methacholine acts by direct effect on glandular cholinergic receptors (5).

The extracted leukotriene from the nasal washes obtained at 15 min after challenge increased with the higher antigen concentrations (Fig. 1). The initial amount selected produced either mild or no symptoms in all individuals, and the severity of symptoms increased in parallel with LTC₄ and LTB₄ release with increasing antigen dose.

LTC₄ immunoreactivity in the nasal mucus sample was shown to co-elute with authenticated synthetic LTs on RP-HPLC, thus confirming the specificity of the assay procedure (Fig. 2).

The increase in the chemotactic leukotriene, LTB₄, in nasal secretions 7.5, 15 and 22.5 min after antigen challenge was significantly greater than that observed after methacholine challenge. This observation is thought to have biological significance since LTB₄ is an exquisitely potent naturally occurring chemoattractant

with activities for both neutrophils and eosinophils (18).

The cellular sources of leukotrienes are uncertain. Basophil leucocytes, present within nasal secretions, or mast cells, present within the epidermis and submucosa, are the most likely origin (11, 19). Eosinophils, present in the nasal mucosa in some individuals with allergic rhinitis also have the capacity to generate LTC₄ *in vitro* (22). However, a limited examination of nasal secretions, or mast cells, present within the epidermis and submucosa, are the most likely in the nasal mucosa and LTC₄ production.

In conclusion, this study shows that house dust mite and grass pollen rhinitis, like ragweed pollinosis (7), are associated with the release of the sulphidopeptide leukotrienes. The inclusion of a methacholine control day validates the antigenic specificity of the response. Furthermore, we show that the potent chemotactic factor, LTB₄, is also generated in these local allergic reactions in the nose. Taken in conjunction with other evidence of leukotriene release into the nose (7), and the observation that peptide leukotrienes may be released into tear fluid during conjunctival provocation (3), this study supports the view that leukotrienes may be important mediators released in allergic reactions.

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