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Comparison of antigen-induced leukotriene and histamine release from nasal scrapings in allergic rhinitis*

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SUMMARY

***Background**: In the early phase response of allergic rhinitis, the nasal mucosa produces important mediators including histamine and leukotrienes.*

***Objective**: To investigate the relationship between antigen-induced leukotriene release and histamine secretion in nasal scrapings.*

***Methods**: Using nasal mucosal scrapings from patients sensitized to only house dust mite, we studied the time course of antigen-induced leukotriene release and its relationship to histamine release.*

***Results**: Cumulative peptydyl leukotriene (LT) production from nasal scrapings increased from 10 min to ~90 min following exposure to mite antigen. The rate of LT release was small (<5 pg/10 min) until 10 min following antigen exposure, increased to ~250 to 350 pg LT/10 min from 10 to 45 min post exposure, was reduced to <100 pg/10 min by 60 to 150 min, and by 180 min LT production was negligible. By contrast, histamine secretion began 30 sec after antigen exposure and was complete within ~10 min. Net antigen-induced LT secretion strongly correlated (R=0.72) with net antigen induced histamine secretion with a ratio of 1:8.7. In addition, net LT/ng histamine and total LT secretion correlated well with antigen-specific IgE in serum, and with the patients' symptoms.*

***Conclusion**: There is a close relationship between amounts of histamine and LT secretion from antigen challenged nasal mucosa, although the time course of LT release is delayed. In the early phase response, LT are likely to be generated from mucosal mast cells, and thus, mast cell activation will provide an important therapeutic target.*

Key words: leukotriene, histamine, allergic rhinitis, mast cell, RAST

INTRODUCTION

Peptidyl leukotrienes (LT), C4, D4, E4, and F4 are arachidonic acid metabolites that can play important roles in IgE-mediated inflammatory diseases [1,2]. In human nasal allergy these LT can enhance nasal vascular permeability and mucus secretion [3,4]. LT are also involved in the infiltration and activation of eosinophils into asthmatic individuals [5,6]. Many studies have reported that both histamine and LT are released into nasal secretion [7-11]. Lewis et al [12] reported that IgE-dependent release of slow-reacting substance of anaphylaxis (SRS-A) (now known to be LT), from human lung fragments or dispersed human lung cells did not begin until histamine release was almost completed. Furthermore, Orange [13] reported that IgE-dependent release of histamine could be separated pharmacologically from production and release of SRS-A.

Previously we have established that the number of formalin-sensitive or tryptase positive mast cells in the nasal epithelium increased in correlation with the severity of symptoms and antigen-specific serum IgE levels in allergic rhinitis. Using nasal scraping, we showed that antigen-induced histamine release after began within 30 sec and was over by 5 to 7 min [14,15]. In the current study we investigated the relationship between the time course of histamine release and LT release.

MATERIAL AND METHODS

Subjects

Fifty-nine patients (23 male and 36 female subjects; age range, 9 to 39 years; mean age ± SEM, 19.4 ± 1.2 yr), sensitized only by house dust (H.D) mite were studied for relationships between mite-induced LT and histamine release from nasal

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scrapings and for relationships between mediator release and clinical findings. The patients had moderate to severe perennial nasal symptoms and CAP-RAST class 1 to 6 [16] for dermatophagoides pteronyssinus (DP). Patients who had severe perennial nasal symptoms with >class 3 CAP-RAST for DP were selected to study the time course of LT (6 patients: 5 male, 1 female, aged 13.8 ± 2.6 yr), and histamine release (8 patients: 2 male, 6 female, aged 18.2 ± 1.9 yr). In addition, the effect of the mast cell stabilizer, pemirolast potassium on histamine and LT release from nasal scrapings was studied (7 patients: 2 male, 5 female, aged 16.7 ± 0.8 yr). Informed consent was obtained in all cases. The ethics committee of Nippon Medical School approved the protocol.

Nasal scrapings

Epithelial specimens of the nasal inferior turbinate were obtained by gentle scraping (without bleeding) with a curette (Nagashima Co., Tokyo, Japan) until the specimen filled the cup of the curette. With this procedure, about 6mg (wet volume) of the epithelial layer was obtained.

Antigen-induced histamine and leukotriene release

Histamine and leukotriene release from nasal scrapings was performed as previously described [15,17,18]. Nasal scrapings (6mg) from the inferior turbinate surface were placed on a small square of filter paper (16mm^2 No.4 Tokyo Roshi Co., Tokyo, Japan), and moistened with cold solution containing 10 mM N-(2-hydroxyethyl)piperazine-N'-(2-ehanesulfonic acid (HEPES)-buffered Tyrode's solution with 0.1% bovine serum albumin without Ca^{2+} and Mg^{2+} (composed of 137 mmol/L NaCl, 5.6mmol/L glucose, 2.7 mmol/L KCl, 0.4 mmol/L $NaHPO_4$, pH 7.4). Specimens were placed into a small plastic tube contained 900 µl of prewarmed HEPES-buffered Tyrode's without Ca^{2+} and Mg^{2+}, and incubated for 20 min at 37°C. To induce histamine and LT release, the specimens were placed in 450 µl of prewarmed HEPES-buffered Tyrode's solution with 1.8 mmol/L $CaCl_2$ and 0.5 mmol/L $MgCl_2$ together with 0.22 µg PN/ml (1:2x10^{4}dilution) DP mite antigen extract (gift from Torii pharmaceutical Co., Tokyo, Japan), or in HEPES-buffer as a control for 30 min at 37°C. We used the same source and dose of PD antigen in this study as previously [15]. The reaction was terminated with 900 µl of cold HEPES-buffered Tyrode's solution. The tube was centrifuged at 200x g for 3 min and 450 µl of the supernatant was separated for LT assay and frozen at –50°C, and 900 µl heated to 90°C for 10min and then used for the histamine assay. The remaining tissue was suspended in 900 µl of cold HEPES-buffered Tyrode's solution, boiled and used to assay remaining histamine.

Histamine and peptidyl-leukotriene (C4, D4, E4, F4) measurements

Antigen-induced LTs release in supernatant (A) and sham control (A') was measured by radioimmunoassay kit (DuPont NEN Research Products, Boston, USA) in duplicate. Histamine release (B) in the supernatant, and residual histamine (C), as well as histamine in sham treated control samples (B', C') in same patients were measured by the method of May et al. [19] with a modification. Histamine extracted was taken up in high performance liquid chromatography mobile phase (40% methanol in water containing 0.042 mol/l acetate buffer pH 4.0) and chromatography was performed on a YMC Packed Column A-302 (Yamamura Chemical Laboratories, Kyoto, Japan). The flow rate was 1 ml/min and the fluorescence was monitored at 460 nm with excitation at 360 nm. Percent histamine release was calculated by {(histamine in B)/(histamine in B plus C)} x 100. Antigen induced net % histamine release was calculated from ($^B/_{B+C}$ – $^{B'}/_{B'+C'}$) x 100, and antigen induced net histamine content (ng) was calculated from (B+C) x ($^B/_{B+C}$ – $^{B'}/_{B'+C'}$).

To standardize LT release from nasal scraping to metachromatic cell number in the tissues, we expressed the LT content in relation to the total histamine content (pg/ng). Antigen-induced net LT (pg)/ng histamine was calculated from (A/B+C) – (A'/B'+C') and antigen induced net LT content (pg) was from{(A/B+C) – (A'/B'+C')} x (B+C) }.

Time course of histamine and leukotriene release

To study the time of LT (6 patients: 5 male, 1 female; aged 13.8 ± 2.6 yr) and histamine (8 patients: 2 male, 6 female, aged 18.2 ± 1.9 yr) release, four nasal scrapings from one side of the nose of each patient were pooled in 3µl of HEPES-buffered Tyrode's solution with 1:20,000 dilution of DP antigen. For histamine and LT measurements, 200 µl each of supernatant was sampled at each time point.

Effect of pemirolast potassium on histamine and LT release from nasal scrapings

Five nasal scrapings were taken from one site of the nose from each 7 patients. The first specimen was incubated with HEPES buffer only, and the second with DP antigen (1:20,000 dilution); the third, fourth and fifth specimens were preincubated with 10^{-6} mol/L, 10^{-5} mol/L, 10^{-4} mol/L of pemirolast potassium (final concentration) for 10 min prior to exposure to DP antigen. Each specimen was incubated for 30 min and then samples collected as outlined above and LT and histamine release determined.

Statistical analysis

Correlation coefficients between the antigen-induced net LT (pg/ng histamine) and net percent histamine secretion, and antigen-induced net LT (pg) and histamine (ng) released were obtained by using Spearman's Rank analysis. For comparisons of antigen-induced net LT (pg/ng histamine) and net LT (pg) release between groups of CAP-RAST classes, the degrees of nasal symptoms, and for inhibition effect of pemirolast potassium, F analysis was conducted to determine if the variances were equal. If this was true, then t-tests were used. If the variances were not equal, then the non-parametric Mann-Whitney U statistic was used. Data is presented as mean ± SEM.

Table 1. Peptydyl leukotriene release per 10 minutes from nasal scrapings.

Min	0	2	5	10	20	30	45	60	90	120	150	180
Cases												
1	222.6	-24.0	411.0	774.8	658.3	196.4	481.6	54.9	-1.5	1.0	20.8	-3.8
2	0	0	0	0	315.0	549.1	70.5	-13.3	246.2	126.9	-69.8	-61.2
3	0	0	0	0	0	0	278.1	-12.7	13.5	15.1	62.4	58
4	0	0	0	1151	1087.9	203.7	167.6	109.8	-154.4	162.4	9.8	35.9
5	0	0	0	0	0	388.7	290.0	-149.5	65.0	176.0	181.4	-224.6
6	0	0	0	0	0	467.9	270.2	250.9	43.4	89.9	46.3	23.2

Each value indicates antigen induced LT product (pg per 10 minutes) from nasal scraping.
Minus value was caused from LT content measured by radioimmunoassay was less than LT content at former time

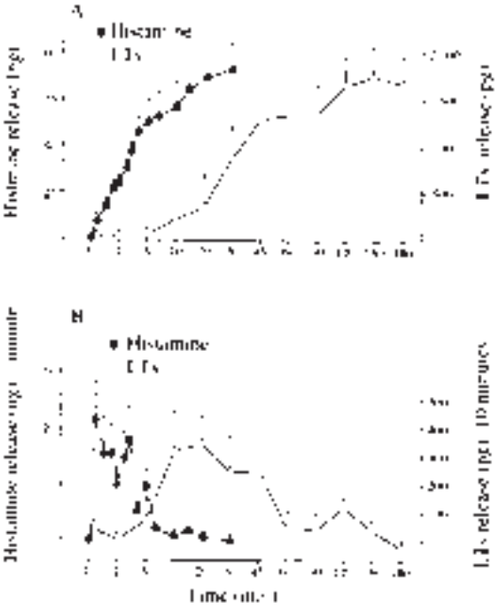

Figure 1. A) Time course of total histamine and LT release. B) Histamine release and LT were calculated per minute and 10 minutes respectively.

RESULTS

Time course of histamine and LT release

Histamine release began within 30 sec of antigen exposure and plateaued by 10 min (Figure 1A, n=8). When histamine release was calculated per min, the magnitude was high in the first 30 sec (~22 ng/min), and was sustained for up to 5 min, but thereafter stopped (Figure 1A). By contrast, antigen-induced LT release was limited at 5 min (n=6), increased by 10 min and reached a plateau by 45 min. Fifty % of the total LT released occurred within 30 min. When LT release was calculated per min (Figure 1B), maximum rate of release occurred by 10 min and was sustained until 45 min. From 60 to 180 min LT release subsided. Some variation occurred in the rate release among the patient samples (Table 1), and there were evidences for a second phase of release from 90 to 150, although this was only 24.6 ± 6.9% of the magnitude of the first sustained period of LT release (10 to 45 min).

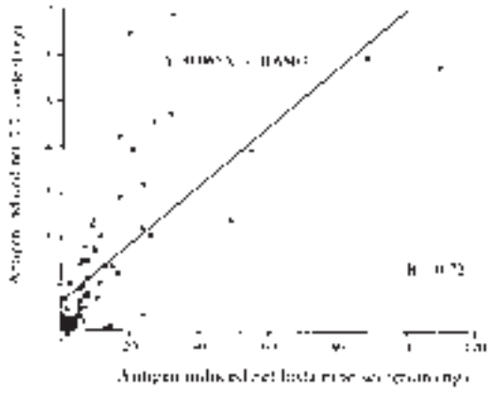

Figure 2. Correlation between antigen induced net LT content and antigen induced net histamine content.

Magnitude of antigen-induced histamine and leukotriene release from nasal scrapings

From each patient four scrapings were taken and duplicated test were done for control and antigen-induced histamine and LT release.

The total histamine content in nasal scrapings from 59 patients was 88.7 ± 12.0 ng. Antigen-induced histamine release was 19.7 ± 3.2 ng, whereas spontaneous, sham-induced releases were 7.4 ± 1.1 ng. Thus, antigen specific histamine release was 13.4% of total histamine.

Antigen-induced LT release for 30 min was 2.0 ± 0.3 ng, whereas sham-induced LT release was 0.5 ± 0.1ng. Antigen-induced LT was 22.9 ± 2.4 pg/ng histamine, while sham-induced release was 5.4 ± 0.7 pg/ng histamine. Thus, mean antigen-specific LT release was 19.1pg/ng histamine.

LT release induced by antigen correlated well with antigen-induced histamine release ($R=0.76$, $p<0.001$). Moreover, antigen-induced specific LT release/ng histamine correlated with antigen-induced specific percent histamine release ($R=0.62$, $p<0.005$). Total specific LT release correlated with antigen-induced specific total histamine release ($R=0.72$, $p<0.0001$)(Figure 2). These observations support the suggestion that LT release occurred largely from histamine containing, metachromatic cells, either mast cell or basophils.

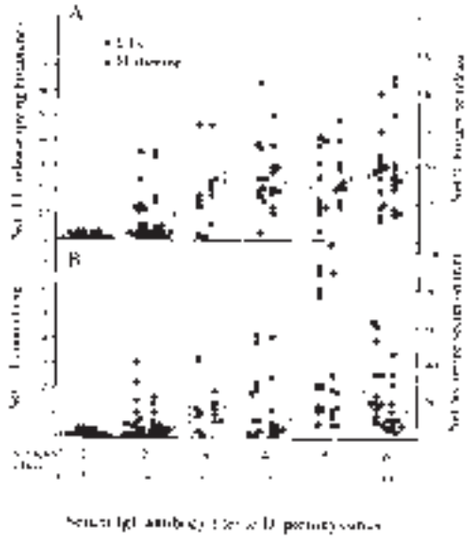

Figure 3. A) Relationships between DP-CAP-RAST class and antigen-induced LT (pg/ng histamine) or antigen induced net % histamine release. In net antigen-induced LT (pg/ng histamine); class 1 and 2-6 ($p<0.05$), class 2 and 4-6 ($p<0.01$). In net % histamine release; class 1 and 2-6 ($p<0.01$), 2 and 3-6 ($p<0.05$). B) Relationship between DP-CAP-RAST class and antigen-induced net LT content or antigen-induced net histamine release. In the net LT content; class 1 and 2, 4-6 ($p<0.05$), 2 and 5-6 ($p<0.01$), 3 and 5-6 ($p<0.05$). In the net histamine release; class 1 and 2-6 ($p<0.05$), 2 and 3, 5-6 ($p<0.02$), 4 and 5 ($p<0.05$). Bars indicate means ± SE.

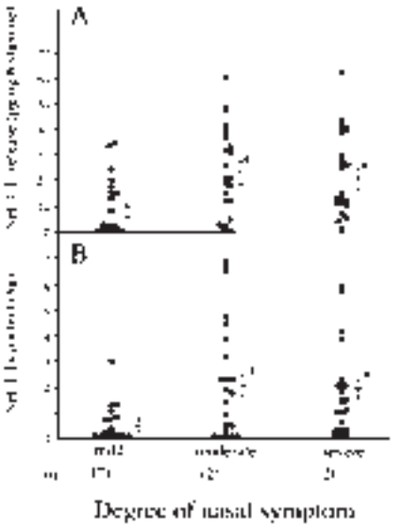

Figure 4. Comparisons of net LT (pg/ng histamine) and net LT content among severity of nasal symptoms. *Averages of antigen induced net LT (pg/ng histamine) and net LT content in groups of moderate and severe of symptoms were significantly higher than in mild group ($p<0.05$).

Relationship between antigen-induced leukotriene release, serum IgE antibody and clinical findings

Fifty-nine patients were divided into 6 groups according with CAP-RAST serum IgE antibody to DP: class 1, RAST 0.35 to 0.7, n=5; class 2, RAST 0.8 -3.5 n=14, class 3, RAST 3.6 to 17.5, n=6; class 4, RAST 17.6 to-50, n=10; class 5, RAST 51 to 100, n=10; class 6, RAST >100, n=13. Both LT and histamine increased as the RAST class group increased (Figure 3).

Patients' symptoms were assessed by a questionnaire in which sneezing, nose blowing and nasal stuffiness were graded from 0 to 3 according to the degree of each symptom. The symptom score was the sum of the grade of nasal stuffiness together with the higher score of either sneezing or nose blowing. Scores of 1 to 2 were considered mild, 3 to 4 moderate and 5 to 6 severe. Antigen-specific LT release (pg/ng histamine) was 9.9 ± 3.0 in the mild group, 22.0 ± 3.6 in the moderate group, 21.4 ± 3.6 pg/ng in the severe group (Figure 4A). Both the moderate and severe groups were statistically different than the mild group in LT release. A similar pattern of LT release was demonstrated for total specific release (Figure 4B).

Effect of pemirolast potassium on histamine and leukotriene release from nasal scrapings

Pemirolast potassium significantly ($p<0.02$) inhibited antigen specific histamine secretion at 10^{-4} M (51.2% inhibition)(Table 2). For antigen specific LT release (pg/ng histamine), significant inhibition occurred at both 10^{-5} and 10^{-4} pemirolast potassium (34% and 65% inhibition respectively).

DISCUSSION

Leukotrienes are important arachidonic acid metabolites in allergic respiratory disease, especially nasal allergy and asthma [1,2]. There is abundant evidence about the role of LT in asthma and recently it has become clear that LT play important roles in nasal allergy. Since early reports of the effects of LT

Table 2. Effect of pemirolast potassium on histamine and LT release from nasal scrapings.

	Specific histamine %	%inhibition	Specific LT(pg/ng histamine)	%inhibition
Antigen	16.6±3.0		24.2±2.9	
+10-6 M	15.6±3.8	7.1±12.9%	19.6±4.1	25.7±10.1%*
+10-5 M	14.5±4.0	13.4±16.5%	16.5±2.8	33.8±4.8%**
+10-4 M	8.8±3.2	51.2±15.1 %*	9.0±1.8	64.8±5.1%***

*$p<0.05$, **$p<0.005$, ***<0.0001

Spontaneous histamine and LT releases were 7.1±0.3 % and 4.3 (pg/ng histamine) respectively.

Pemirolast potassium was added 10 minutes before antigen addition.

on nasal physiology and pathophysiology, there have been numerous advances [20,21].

The purpose of this study was to evaluate the time course of LT and histamine release in the early phase of IgE-mediated reponses in nasal scrapings from monosensitized (HDM) patients. We had previously shown that >80% of were tryptase positive mast cells that increased in number in correlation with the severity of nasal allergic symptoms and IgE antibody levels [15].

Cysteinyl leukotrienes, LTC4, LTD4 and LTE4 are produced by activated mast cells and eosinophils. The eosinophil derived LT in the nasal scrapings could not be ignored. However mast cells release LT as well as histamine by antigen challenge in the early phase response whereas eosinophils act mostly in the late phase response and have no evidence to release LT by antigen-IgE mediated reaction.

Our current results show that whereas antigen specific histamine secretion from nasal scrapings is complete in about 5 min, LT release does not begin until about 5 min and continues over 45 min and up to 150 to 180 min. Interestingly, both histamine and LT release also correlated with DP specific IgE levels in plasma and with symptom scores. Although the time course of antigen specific histamine and LT secretion differed, their magnitude correlated, suggesting that metachromatic cells were likely to be an important source of LT, or involved in inducing/enhancing their secretion.

In support of this contention, we employed pemirolast potassium, a drug known to inhibit LT release from rat peritoneal mast cells [22] and human peripheral blood leukocytes and lung fragments [23]. Pemilolast significantly inhibited both histamine and LT release from nasal scrapings. However, given current evidence about the specificity of pemirolast potassium and cell sources of LT, it cannot be excluded that other cell types such as eosiniphils and macrophages are important sources of LT in nasal allergy, as has been shown in asthma [6]. In addition it was reported that bronchial epithelial cells of the human produced cysteinyl-LT in the RSV-infection or ozone exposure [24,25]. Our study showed that LT production was undetectable from nasal scraping with no MC or little histamine.

Sneezing appears within 1 min of antigen challenge on the surface of the inferior turbinate, and is normally over within 5 min. It is thought to be mediated by histamine [26]. Nasal obstruction occurs by 10 to 30 min after antigen provocation and it is thought to involve LT-induced alterations in the local vasculature. The time course of LT release from nasal scrapings was similar to that of nasal resistance after local antigen challenge.

Lewis et al. [12] reported that IgE-dependent release of SRS-A from either human lung fragments or enzymatically dispersed human pulmonary cells did not begin until histamine release was almost complete. Moreover, Orange et al. [13] showed that IgE-dependent histamine release can be pharmacologically separated from generation and release of SRS-A. Miadonna et al. [10] and Wang et al. [27] observed the maximal LTC4 concentration in nasal secretion at 5 to 10 min after allergen challenge. LT concentration peaked concurrently with histamine levels. However, Shaw et al. [8] stated that LT levels in nasal washings from allergic patients were elevated for up to 30 min after local allergen challenge. Shirasaki et al. [28] in studies of sensitized guinea pigs observed increased LT concentration for up to 90 min after allergen challenge, whereas histamine levels were elevated for only 10 min.

Thus, our results have detailed the time course of antigen specific histamine and LT release from nasal scrapings mono-sensitized rhinitis patients. There was a strong correlation between histamine and LT release, despite their differences in time course following allergen challenge. This study further demonstrated the value of nasal scrapings in studies of human allergic inflammation and such scrapings can be used as a tool for assessment of new anti-allergen and antigen-inflammatory agents.

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REFERENCES

1. Lewis RA, Austen KF (1981) Mediation of local homeostasis and inflammation by leukotriene and other mast cell-dependent compounds. Nature 293: 103-108.
2. Borish L (2003) Allergic rhinitis: Systemic inflammation and implication for management. J Allergy Clin Immunol 112: 1021-1031.
3. Bisgaard H, Olsson P, Bende M (1986) Effect of leukotriene D4 on nasal mucosal blood flow, nasal airway resistance and nasal secretion in humans. Clin Allergy 16: 289-297.
4. Okuda M, Watase T, Mezawa A, Liu CM (1987) The role of leukotriene D4 in allergic rhinitis. Ann Allergy 60: 537-540.
5. Laitinen LA, Laitinen A, Haahtela T, Vilkka V, Spur BW, Lee TH (1993) Leukotriene E4 and granulocytes infiltration into asthmatic airways. Lancet 1241: 989-990.
6. Seymour M L, Rak S, Åberg D, Rise G C, Penrose J F, Kanaoka Y, Austen K F, Holgate S T, Sampson A P (2001) Leukotriene and prostanoid pathway enzymes in bronchial biopsies of seasonal allergic asthmatics. Am J Respir Crit Care Med 164: 2051-2056.
7. Naclerio RM, Meier HL, Kagey-Sobotka A, Adkinson NF Jr, Meyers DA, Norman PS, Lichtenstein LM (1983) Mediator release after nasal airway challenge with allergen. Am Rev Respir Dis 128: 597-602.
8. Shaw RJ, Fitzharris P, Cromwell O, Wardlaw AJ, Kay AB (1985) Allergen-induced release of sulphidopeptide leukotriene (SRS-A) and LTB4 in allergic rhinitis. Allergy 40: 1-6.
9. Creticos PS, Adkinson NF Jr, Kagey-Sobotka A, Proud D, Meier HL, Naclerio RM, Lichtenstein LM, Norman PS (1985) Nasal challenge with ragweed pollen in hay fever patients. Effect of immunotherapy. J Clin Invest 76: 2247-2253.
10. Miadonna A, Tedeschi A, Leggieri E, Brasca C, Folco GC, Sala A, Froldi M, Zanussi C (1988) Mediators and allergic inflammation of human airways. Respiration 54 suppl 1: 78-83.
11. Bisgaard H, Robinson C, Romeling F, Mygind N, Church M, Holgate ST (1988) Leukotriene C4 and histamine in early allergic reaction in the nose. Allergy 43: 219-227.
12. Lewis RA, Wasserman SI, Goetzl EJ, Austen KF (1974) Formation of slow-reacting substance of anaphylaxis in human lung tissue and cells before release. J Exp Med 140: 1133-1146.

13. Orange RP (1975) Dissociation of the immunologic release of histamine and slow reacting substance of anaphylaxis from human lung using cytochalasins A and B. J Immun ol 114: 182-186.
14. Otsuka H, Denburg J, Dolovich J, Hitch D, Lapp P, Rajan MD, Bienenstock J, Befus D (1985) Heterogeneity of metachromatic cells in human nose: Significance of mucosal mast cells. J Allergy Clin Immunol 76: 695-702.
15. Otsuka H, Inaba M, Fujikura T, Kunitomo M (1995) Histochemical and functional characteristics of metachromatic cells in the nasal epithelium in allergic rhinitis: Studies of nasal scrapings and their dispersed cells. J Allergy Clin Immunol 96: 528-536.
16. Ewan PW, Coote D (1990) Evaluation of capsulated hydrophilic carrier polymer (the immuno CAP) for measurement of specific IgE antibodies. Allergy 45: 22-29.
17. Befus AD, Pearce FL, Gauldie J, Horsewood P, Bienenstock J (1982) Mucosal mast cells. I. Isolation and functional characteristics of rat intestinal mast cells. J Immunol 128: 2475-2480.
18. Befus AD, Dyck N, Goodacre R, Bienenstock J (1987) Mast cells from the human intestinal lamina propria: isolation, histochemical subtypes, and functional characterization. J Immunol 138: 2604-2610.
19. May CD, Lyman M, Alberto R, Cheng J (1970) Procedures for immunochemical study of histamine release from leukocytes with small volume of blood. J Allergy 46: 12-20.
20. Haberal I, Corey J P. (2003) The role of leukotrienes in nasal allergy Otolaryngol Head Neck Surge 129: 274-279.
21. Figueroa D J, Borish L, Baramki D, Philip G, Austin C P, Evans J F (2003) Expression of cysteinyl leukotriene synthetic and signaling proteins in inflammatory cells in active seasonal allergic rhinitis. Clin Exp Allergy 33: 1380-1388.
22. Bulimia H, Nakashima S, Miyata H, Onawa Y (1991) Effect of a novel ant allergic drug, pemirolast, on activation of rat peritoneal mast cells; Inhibition of exocytotic response and membrane phospholipid turnover. Int Arch Allergy Appl Immunol 96: 62-67.
23. Kawashima T, Tomioka H, Yoshida S (1988) Inhibition of chemical mediator from human leukocytes and lung fragments by TBX. Jpn J Allergol 37: 438-447.
24. Behera AK, Kumar M, Matsuse H, Lockey RF, Mohapatra SS (1998) Respiratory syncytial virus induces the expression of 5-lipoxygenase and endothelin-1 in bronchial epithelial cells. Biochem Biophys Res Commun 251: 704-709.
25. McKinnon KP, Madden MC, Noah TL, Devlin RB (1993) In vitro ozone exposure increase release of arachidonic acid products from a human bronchial cell line. Appl Pharmacol 118: 215-223.
26. Clarke PS (1984) The diagnosis of perennial rhinitis due to house dust mite (Dermatophagoides Pteronyssinus) demonstrated by nasal provocation tests. Ann Allergy 59: 25-28.
27. Wang D, Clement P, Smitz J, DeWaele M, Derde MP (1995) Correlations between complaints, inflammatory cells and mediator concentrations in nasal secretions after nasal allergen challenge and during natural allergen exposure. Int Arch Allergy Immunol 106: 278-285.
28. Shirasaki H, Asakura K, Kojima T, Sohma S, Kataura A (1992) The roles of histamine, leukotriene C4 and bradykinin on nasal vascular permeability in experimental nasal allergy of guinea pigs. Rhinology 30: 41-48.

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