
◎原 著

Enhanced leukotriene generation and bronchial hyperresponsiveness in asthmatics with allergic rhinitis

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Abstract : Rhinitis frequently precedes asthma, and treating allergic rhinitis has beneficial effects on asthma, suggesting upper airway disease is a risk factor for asthma. The aim of the present study was to investigate the influence of allergic rhinitis on serum IgE level, leukotriene generation by peripheral leukocytes, and bronchial hyperresponsiveness (BHR) to methacholine in patients with atopic asthma. Seventy-one asthmatic subjects (mean age, 59.5 ± 12.5 years; 37 women, 34 men) were recruited, and 48 asthmatics had allergic rhinitis and 23 asthmatics did not have allergic rhinitis. The $\log_{10}(\text{Dmin})$ was significantly lower for those with allergic rhinitis than those without allergic rhinitis ($p < 0.05$), implying that those with allergic rhinitis developed BHR to a greater degree than those without allergic rhinitis. LTC_4 generation from peripheral leukocytes was significantly greater for atopic asthmatics with allergic rhinitis than those without allergic rhinitis ($p < 0.05$). In contrast, the amount of LTB_4 produced from peripheral leukocytes did not significantly differ between asthmatic patients with and without allergic rhinitis. These results suggest that the presence of allergic rhinitis enhances BHR by enhancing LTC_4 production, while the presence of allergic rhinitis did not affect LTB_4 production in patients with atopic asthma.

Key words : asthma, allergic rhinitis, leukotriene generation, bronchial hyperresponsiveness

Introduction

The incidence of allergic diseases such as allergic rhinitis and asthma is increasing to epidemic proportions (allergic rhinitis : 10-50% ; and asthma : 5-15%), both in the developed and the developing world, with a reduced quality of life of the patients, lower productivity and increasing medical costs¹⁾. The increasing evidence on the links between allergic rhinitis and asthma comes from epidemiological, immunological and clinical studies²⁾. Epidemiologically, up to 40% of patients with allergic rhinitis also have asthma, and up to 80% of patients with asthma experience nasal symptoms. Rhinitis frequently precedes asthma, and treating allergic rhinitis has beneficial effects on asthma, suggesting upper airway disease is a risk factor for asthma^{3,4)}.

Both allergic rhinitis and asthma are inflammatory diseases, and their inflammatory mechanisms are similar in that they are characterized by an inflammatory infiltrate made up of eosinophils, T cells, and mast cells that release several mediators including leukotrienes (LTs), chemokines and cytokines, local and systemic immunoglobulin (Ig) E synthesis. A typical early phase and late phase response are also common to both rhinitis and asthma. LTs are potent proinflammatory mediators that participate in the pathophysiological changes in the airways of patients with asthma⁵⁾.

In previous studies, we have shown that LTC₄ production by leukocytes is associated with IgE-mediated allergy and exacerbation of asthma ; that generation of LTB₄ is closely related to bronchial hyperresponsiveness (BHR) in patients with asthma⁶⁾ ; and that cigarette smoking enhances production of IgE antibodies, BHR and generation of LTB₄ by leukocytes in

elderly asthmatics⁷⁾.

The aim of the present study was to investigate the influence of allergic rhinitis on serum IgE level, leukotriene generation by peripheral leukocytes, and BHR in patients with atopic asthma.

Subjects and Methods

SUBJECTS

Seventy-one asthmatic subjects (mean age, 59.5 ± 12.5 years ; 37 women, 34 men) were recruited from Misasa Medical Center. Asthma was diagnosed according to the definition proposed by the American Thoracic Society⁸⁾. All subjects had specific IgE antibodies against inhalant allergens. All subjects were life-long non-smokers, and stable, with no changes in asthma symptoms or medication for at least 1 month, except for the use of short-acting B₂ agonists. Nine of the 71 patients were being administered a long-term systemic glucocorticoid regimen, and had been treated with glucocorticoids for more than 2 years. No patients were treated with LT modulators or nasal steroids. Allergic rhinitis was diagnosed on the basis of clinical history, symptom and the presence of specific IgE antibodies. Serum IgE was measured using a radioimmunosorbent test (RIST), and IgE antibodies specific to 12 common aeroallergens including house dust mites, moulds, pollens and animal danders were measured using the Pharmacia CAP system (Pharmacia Diagnostics AB, Uppsala, Sweden).

Informed consent was obtained from all subjects, and the study protocol was approved by the ethics committee of our institution.

BRONCHIAL RESPONSIVENESS

Bronchial responsiveness to methacholine was measured using an Astograph (TCK6100, Chest Co). Different concentrations of methacholine (49, 98, 195, 390, 781, 1563, 3125, 6250 and

12 500 $\mu\text{g}/\text{ml}$) were prepared, and were used for bronchial challenge according to the method used by Chai et al.⁹⁾

The increase in total respiratory resistance (Rrs) after methacholine inhalation was measured using the oscillation technique. A cumulative dose of methacholine that caused a significant increase in Rrs was assessed as the minimum dose (Dmin). The units for Dmin are equivalent to 1 min of aerosol inhalation at 1 mg/mL during quiet tidal breathing. All medications were stopped 12 hours prior to examination. LEUKOTRIENE (B_4 , C_4) GENERATION

The amount of leukotrienes, LTB_4 and LTC_4 , generated by peripheral leukocytes was assessed as described previously^{6, 7, 10, 11)}. First, 5 ml of 6% dextran (molecular weight, $\sim 200,000$ kDa) (Nacalai Tesque Inc., Kyoto, Japan) was added to 20 mL of heparinized peripheral blood, and the resultant mixture was incubated for 1 hour at room temperature. The leukocyte-rich plasma supernatant was then removed and used. The number of cells was adjusted to 5×10^6 cells/mL in Tris CM buffer (30 mM Trisma [pH 7.7], 120 mmol/L NaCl, 5 mmol/L KCl, 1 mmol/L CaCl_2 , 0.5 mmol/L MgCl_2), and the cells were then incubated with 1 mg of the calcium ionophore A23187 (Sigma, St Louis, MO) for 15 minutes at 37°C . After a $4\times$ volume of prechilled ethanol (final, 80% ethanol) was added, the mixture was centrifuged at 3000 rpm for 30 minutes. A syringe filter (Toyo Roshi Co, Tokyo, Japan) was used to draw off the supernatant, and the filtrate was decompressed and dried to a solid. Quantification of LTB_4 and LTC_4 was performed using high-performance liquid chromatography (HPLC) and ultraviolet (UV) spectroscopy, following the method of Lam et al.¹²⁾. Quantities of LTB_4 and LTC_4 were expressed as nanograms per 5×10^6 cells.

PULMONARY FUNCTION TESTS

Pulmonary function tests were performed using a CHESTAC 33 system (Chest Co., Tokyo, Japan). For all subjects, the following measurements were made using the forced vital capacity (FVC) maneuver: FVC, forced expiratory volume in 1 second (FEV_1), FEV_1/FVC , mean forced expiratory flow during the middle half of the FVC (FEF_{25-75}). Measurements of FVC, FEV_1 , and FEF_{25-75} for each patient were expressed as a percentage of the predicted values¹³⁾.

STATISTICAL ANALYSIS

All statistical analyses were performed using Stat View software (SAS Institute Inc., Cary, NC, USA). Results were expressed as mean \pm SD. The differences between groups were judged with the unpaired t test or the Mann-Whitney U test for continuous data, and with chi-square tests for categorical data. A p value of <0.05 was considered to indicate statistical significance.

Results

The clinical characteristics of 48 asthmatics with allergic rhinitis and 23 asthmatics without allergic rhinitis are presented in Table 1. The dosage of medication did not differ between the asthmatic groups. There was no significant difference between those with and without allergic rhinitis in patient age, %FVC, % FEV_1 , FEF_{25-75} or FEV_1/FVC . The geometric mean of serum IgE level was 299 IU/mL in those with allergic rhinitis, and 183 IU/mL in those without allergic rhinitis. However, there was no significant difference between the asthmatic groups.

Table 1. Patient characteristics

	AR(-)	AR(+)	Difference
Number of patients	23	48	—
Female/Male	11/12	26/22	NS
Age (yrs)	62.0 ± 10.9	58.3 ± 13.2	NS
Serum IgE (IU/ml) *	183 (6–2250)	299 (20–2916)	NS
%FVC (%)	102.5 ± 22.2	102.5 ± 16.3	NS
%FEV ₁ (%)	90.4 ± 23.9	90.6 ± 22.6	NS
FEV ₁ /FVC (%)	67.3 ± 11.4	69.3 ± 12.2	NS
%FEF _{25–75} (%)	64.3 ± 33.2	60.1 ± 30.5	NS
Medication			
Inhaled BDP (mg/day)	365 ± 344	483 ± 675	NS
Systemic corticosteroids (n)	3	6	NS

Data are presented as mean ± SD.

*: Values are geometric means of serum IgE levels, with range shown in parentheses.

AR = allergic rhinitis; FVC = forced vital capacity; FEV₁ = forced expiratory volume in one second; FEF_{25–75} = mean forced expiratory flow during the middle half of the FVC; BDP = beclomethasone dipropionate.

Figure 1 shows the comparison of BHR to methacholine between atopic asthmatics with and without allergic rhinitis. The \log_{10} (Dmin) was -0.172 ± 0.568 in asthmatics with allergic rhinitis and 0.290 ± 0.540 in asthmatics without allergic rhinitis. The \log_{10} (Dmin) was significantly lower for those with allergic rhinitis than those without allergic rhinitis ($p < 0.05$), implying that those with allergic rhinitis developed BHR to a greater degree than those without allergic rhinitis.

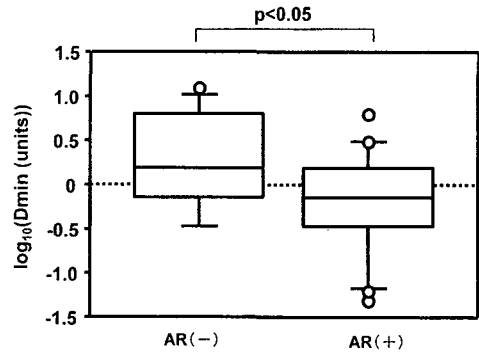


Figure 1.

Figure 1. Bronchial hyperresponsiveness to methacholine in atopic asthmatic patients with and without allergic rhinitis. AR = allergic rhinitis.

Figure 2 and 3 show the influence of allergic rhinitis on the generation of LTB₄ and LTC₄ from peripheral leukocytes in patients with atopic asthma by stimulation with the calcium ionophore A23187. The amount of LTB₄ generated by the leukocytes was 95.7 ± 23.9 ng/ 5×10^6 cells in asthmatics with allergic rhinitis and 88.2 ± 31.2 ng/ 5×10^6 cells in asthmatics without allergic rhinitis. No significant differences were found between the asthmatic groups (Figure 2). The amount of LTC₄ generated by the leukocytes was 80.5 ± 58.2 ng/ 5×10^6 cells

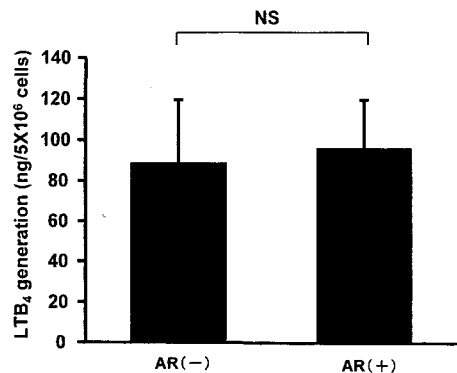


Figure 2.

Figure 2. Leukotriene B₄ generation by peripheral leukocytes in atopic asthmatic patients with and without allergic rhinitis.

in asthmatics with allergic rhinitis and 48.9 ± 38.3 ng/5x10⁶ cells in asthmatics without allergic rhinitis. The leukocytes of atopic asthma patients with allergic asthma produced significantly more LTC₄ than the leukocytes of those without allergic rhinitis ($p < 0.05$) (Figure 3).

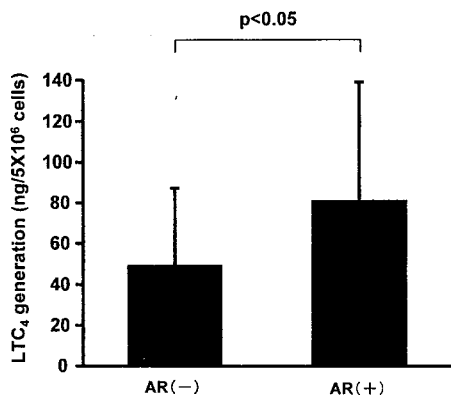


Figure 3.

Figure 3. Leukotriene C₄ generation by peripheral leukocytes in atopic asthmatic patients with and without allergic rhinitis.

Discussion

Asthma and allergic rhinitis occur frequently, are very often associated and both cause a high rate of morbidity. A recent epidemiological study of risk factors for rhinitis and asthma established that although rhinitis was a risk factor for the onset of asthma, asthma was not a risk factor for the onset of rhinitis, which suggested that in reality rhinitis precedes asthma¹⁴. Cys-LTs are important mediators in the pathophysiology of both asthma and allergic rhinitis, released in both the early and late phase of the allergic reaction, and in many ways underpin the 'one airway' concept of upper and lower airway disease¹⁵. Leukotriene receptor antagonists (LTRAs), which inhibit the actions of cys-LTs, have proved to be effective and well tolerated therapies in the treatment of asthma¹⁶ and more

recently rhinitis¹⁷. It is also well established that nonspecific BHR to methacholine is more frequent in rhinitic patients as compared to nonrhinitic subjects, independently of the presence of atopy¹⁸⁻²¹. In the present study, we examined how the presence of allergic rhinitis influences serum IgE level, BHR, and leukotriene production by peripheral leukocytes stimulated by calcium ionophore A23187, which reflects the pathogenesis of asthma, in patients with atopic asthma.

In the present study, BHR and generation of LTC₄ by peripheral leukocytes were significantly greater for atopic asthmatics with allergic rhinitis than those without allergic rhinitis. In contrast, the amount of LTB₄ produced from peripheral leukocytes did not significantly differ between asthmatic patients with and without allergic rhinitis. These results suggest that the presence of allergic rhinitis enhances BHR by enhancing LTC₄ production, while the presence of allergic rhinitis did not affect LTB₄ production in patients with atopic asthma.

Leukotrienes are lipid mediators generated by metabolism of arachidonic acid. They fall into 2 classes: Cys-LTs such as LTC₄, LTD₄, LTE₄, which induce bronchoconstriction, enhance BHR and smooth muscle hypertrophy, cause mucus hypersecretion and mucosal edema, and induce influx of eosinophils into airway tissue; and the dihydroxy acids such as LTB₄, which are neutrophil chemoattractants²². The findings of Fujimura et al. indicate that interleukin-8 induces BHR and airway neutrophil accumulation in guinea pigs *in vivo*²³, and suggest that this effect is partly mediated by the release of LTB₄.

Factors mechanistically linking upper and lower airway allergic inflammation include loss of nasal protection secondary to rhinitis-induced congestion and nasal-bronchial reflex^{24,25}. Allergen-specific IgE production is strongly

correlated with both allergic rhinitis and asthma, suggesting allergic immune responses are important in their etiology and pathogenesis^{25,26}. The inflammatory processes arise from inappropriate immune responses to otherwise innocuous aeroallergens resulting in development of an allergic immune, instead of a tolerant phenotype. Several studies have emphasized the importance of Th2-type T cells in the development and propagation of this response^{27,28}. Active inflammatory conditions in the nasosinus may result in BHR through the cholinergic nervous system induced by the direct stimulation of receptors in the nasosinus and/or indirect stimulation of receptors in the nasopharynx from inflammatory cells or products of post-nasal discharge²⁹. This nasobronchial reflex may in turn influence the pathophysiology of bronchial asthma, such as bronchial muscle tone³⁰.

In conclusion, the present results suggest that BHR and LTC₄ generation by peripheral leukocytes are influenced by co-existing allergic rhinitis. However, the mechanism leading to BHR is complex and remains unclear, and is influenced not only by airway remodeling³¹. Thus, there is a need for further detailed clinical studies to elucidate the relation between asthma and allergic rhinitis.

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アレルギー性鼻炎を合併したアトピー性喘息患者におけるロイコトリエン産生能と気道過敏性

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アレルギー性鼻炎は気管支喘息のリスクファクターの一つであると考えられている。しばしば喘息に先行して発症し、その治療を行うことは喘息の治療に有用であることが報告されている。我々は本検討において、アトピー性喘息71例(平均年齢59.5±12.5歳, 女性37例, 男性34例)を対象としアレルギー性鼻炎の有無によって2群に分類し、血清IgE値, 末梢白血球のロイコトリエン産生能, メ

サコリンに対する気道過敏性を測定し、2群間で比較検討した。対象症例中、48例はアレルギー鼻炎を合併し、23例はアレルギー鼻炎を合併していなかった。アレルギー性鼻炎非合併例に比較してアレルギー性鼻炎合併例において、気道過敏性は有意に亢進していた ($p<0.05$)。末梢白血球のLTC₄産生能も同様に、アレルギー性鼻炎非合併例に比較してアレルギー性鼻炎合併例において有意に亢進していた ($p<0.05$)。しかし、末梢白血球のLTB₄産生能は、両群間で有意の差を認めなかった。以上より、アレルギー性鼻炎の存在が末梢白血球のLTC₄産生亢進を介して、気道過敏性の亢進に関連している可能性が示唆された。

キーワード：気管支喘息, アレルギー性鼻炎, ロイコトリエン産性能, 気道過敏性