

Enhanced production of leukotrienes by peripheral leukocytes and specific IgE antibodies in patients with chronic obstructive pulmonary disease

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Background: How leukotrienes (LTs) and IgE-mediated allergy reflect clinical features in patients with chronic obstructive pulmonary disease (COPD) remains unclear.

Objective: Our goal was to determine whether LTB₄ and LTC₄ would correlate with airway obstruction and whether IgE-mediated allergy would influence the generation of LTs and bronchial hyperresponsiveness in patients with COPD.

Methods: We measured the pulmonary function, methacholine bronchial hyperresponsiveness, and generation of LTB₄ and LTC₄ from peripheral leukocytes stimulated with calcium ionophore A23187 in relation to the presence of specific IgE antibodies against inhalant allergens.

Results: The leukocytes of patients with COPD generated significantly more LTB₄ (with allergy, $P < .001$; without allergy, $P < .001$) and LTC₄ (with allergy, $P < .001$; without allergy, $P < .01$) than the leukocytes of the control subjects. LTC₄ production was significantly higher in the allergic COPD subjects than in the nonallergic COPD patients ($P < .01$), but the amount of LTB₄ generated was not significantly different. FEV₁ significantly correlated with the level of both LTB₄ (with allergy, $r = -0.556$, $P = .0375$; without allergy, $r = -0.731$, $P = .0046$) and LTC₄ (with allergy, $r = -0.764$, $P = .0043$; without allergy, $r = -0.526$, $P = .0414$) generation in COPD. The log₁₀ of the minimum dose of methacholine was significantly higher in COPD patients without allergy than in those with allergy ($P < .05$).

Conclusion: Enhanced LT generation from peripheral leukocytes is observed in patients with COPD, and the presence of specific IgE antibodies against inhalant allergens enhances LTC₄ generation, bronchial hyperresponsiveness, and the relationship between LTC₄ generation and airway obstruction. (*J Allergy Clin Immunol* 2001;107:492-8.)

Key words: COPD, IgE-mediated allergy, LTB₄, LTC₄, bronchial hyperresponsiveness

Chronic obstructive pulmonary disease (COPD) is characterized by airway inflammation and asthma.^{1,2} Studies using bronchoalveolar lavage (BAL), bronchial

Abbreviations used

BAL:	Bronchoalveolar lavage
COPD:	Chronic obstructive pulmonary disease
Cys:	Cysteinyl
DL _{CO} :	Diffusing capacity for carbon monoxide
D _{min} :	Minimum dose of methacholine
FVC:	Forced vital capacity
Gr:	Reciprocal of the respiratory resistance
LT:	Leukotriene
Rrs:	respiratory resistance
RV:	Residual volume

biopsy, and induced sputum suggest that eosinophils in asthma³⁻⁵ and neutrophils in COPD^{2,6-9} are the main cellular components of airway inflammation. Furthermore, several studies have demonstrated increased numbers of macrophages, mast cells, neutrophils, or CD8⁺ T lymphocytes in the airway walls in association with COPD.¹⁰⁻¹³ Recent studies reported the detection of eosinophils in the airways of some patients with COPD^{6,7,14} and the correlation of the eosinophilic level in their BAL fluid with the severity of airway obstruction.⁶ Eosinophilic inflammation was found to be induced by IgE-mediated allergy in asthma,^{15,16} but few studies have attempted to determine the correlation between eosinophilic inflammation and IgE-mediated allergy in COPD.

Leukotrienes (LTs) are products of arachidonic acid synthesized by the 5-lipoxygenase pathway. The cysteinyl (Cys) LTs (LTC₄, LTD₄, and LTE₄) induce contraction of human bronchial smooth muscle,¹⁷ mucus formation,¹⁸ and bronchial wall edema.¹⁹ Cys LTs are mainly generated by eosinophils, mast cells, and macrophages.^{20,21} Eosinophil localization in the airways often accompanies increased LTC₄ production,²² and the amount of Cys LT produced is related to the activation state of the eosinophils.^{23,24} LTB₄ is mainly generated by neutrophils and macrophages.²¹ LTB₄ was found to increase in patients with COPD during both bacterial exacerbation²⁵ and a stable state,²⁶ and LTE₄ was observed to be related to the severity of COPD lung.²⁷ However, it remains unclear how LTs reflect clinical features in patients with COPD.

We hypothesized that both LTB₄ and LTC₄ would correlate with airway obstruction in patients with COPD and also that IgE-mediated allergy would influence the clinical features of COPD. In this study we investigated the

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generation of LTC₄ and LTB₄ by leukocytes stimulated with calcium ionophore A23187 and the pulmonary function and bronchial hyperresponsiveness in relation to the presence of specific IgE antibodies against inhalant allergens in patients with COPD.

METHODS

Subjects

Subjects were recruited from the Misasa Medical Branch of Okayama University Medical School. Thirty-one subjects diagnosed with COPD and 15 healthy control subjects participated in the study. A diagnosis of COPD was made according to American Thoracic Society criteria, and subjects were selected according to the following inclusion criteria: clinical diagnosis of COPD based on medical history (persistent dyspnea, mainly on exertion, without sudden attacks); current smoker or ex-smoker with smoking history ≥ 20 pack-years (ie, ≥ 1 pack daily for ≥ 20 years); FEV₁ < 80% of the predicted value and FEV₁/FVC (forced vital capacity) < 70% after inhalation of bronchodilator; reversibility < 10% or 200 mL in FEV₁ after inhalation of 400 μ g salbutamol through a metered dose inhaler; no history of bronchial asthma, atopic dermatitis, allergic rhinitis, or other allergic diseases; no respiratory tract infection, exacerbation of COPD, treatment with oral or inhaled glucocorticoids, or antiallergic drugs (including LT modulator) at least 1 month before the study.

We classified the subjects into 3 groups: COPD patients without specific IgE antibodies (COPD without allergy); COPD patients with specific IgE antibodies (COPD with allergy); and control subjects. Serum total IgE and serum specific IgE were measured with the Pharmacia CAP System (Pharmacia Diagnostics AB, Uppsala, Sweden). The diagnosis of allergy was based on the presence of serum specific IgE and/or a positive skin test result for 12 common inhalant allergens, including house dust mites, *Candida albicans*, *Aspergillus fumigatus*, animal dander, and grass pollen. The patients continued to use their usual bronchodilator medications, consisting of inhaled anticholinergics, inhaled β -agonists, or a combination of these drugs. The inhaled drugs were withheld for at least 12 hours before the study. None of the control subjects had a history of pulmonary or allergic diseases, and all were nonsmokers, had normal lung function, negative skin test results, and negative specific IgE test results. Smoking history was expressed as pack-years. The protocol was approved by the hospital ethics committee, and all subjects gave their written informed consent.

Pulmonary function tests

Spirometry before and after bronchodilation was performed by means of a CHESTAC 33 (Chest Co, Tokyo, Japan). All subjects underwent an FVC maneuver including FEV₁ and FEV₁/FVC. Residual volume (RV) was measured by body plethysmography with the Autobox 2800 (Chest Co). The diffusing capacity for carbon monoxide (DL_{CO}) was measured by the single-breath technique with a CHESTAC 33. The FEV₁, RV, and DL_{CO} measurements for each patient were expressed as a percentage of the predicted values according to the prediction equations of the Japanese Society of Chest Disease.²⁸

Leukocyte stimulation and LT measurement

The amounts of LTC₄ and LTB₄ generated by the peripheral leukocytes were assessed as previously described.²⁹ A quarter volume of 6% dextran (molecular weight ~200,000 d; Nacalai Tesque Inc, Kyoto, Japan) was added to 20 mL of peripheral blood, and this was left 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 mmol/L Trisma [pH 7.7], 120 mmol/L NaCl, 5 mmol/L KCl, 1 mmol/L CaCl₂, 0.5 mmol/L MgCl₂), and 1 μ g of calcium ionophore A23187 (Sigma, St Louis, Mo) was then added to the cell suspension. The solution was mixed and incubated for 15 minutes at 37°C. After a 4 $\times$ volume of prechilled ethanol (final, 80% ethanol) was added, this was centrifuged at 3000 rpm for 30 minutes. A syringe filter (Toyo Roshi Co, Tokyo, Japan) was used to draw the supernatant, and the filtrate was decompressed and dried to a solid. Quantification of LTC₄ and LTB₄ was performed by HPLC and UV spectroscopy, with the method of Lam et al.³⁰ Quantities of LTC₄ and LTB₄ were expressed as nanograms per 5×10^6 cells.

Measurement of airway responsiveness

Bronchial responsiveness was evaluated with the TCK 6100 Astograph (Chest Co) with direct writing of the dose-response curve of respiratory resistance (Rrs) during the continuous inhalation of methacholine in stepwise incremental concentrations.³¹ Different concentrations of methacholine (49, 98, 195, 390, 781, 1563, 3125, 6250, and 12,500 μ g/mL) were prepared for the bronchial challenge, which was carried out according to the method of Chai et al³² and administered through the Astograph. We used the minimum dose of methacholine (D_{min}) as the indicator of bronchial sensitivity. The D_{min} was the amount of the cumulative dose at the inflection point where the reciprocal of Rrs (Grs) decreased linearly. When this inflection point was unclear, we selected the point of intersection of the control value and the linear slope of Grs. D_{min} was expressed in units, with 1 unit equaling 1 minute of inhalation of aerosol solution at 1.0 mg/mL during quiet tidal breathing. All medications were stopped for at least 12 hours before this examination.

Statistical analysis

Results are expressed as median values with the range given in parentheses. Differences between groups were first analyzed with the Kruskal-Wallis test. When a significant difference was found between groups, intergroup comparisons were assessed nonparametrically with the Mann-Whitney *U* test. Statistical significance was established at the *P* < .05 level after a Bonferroni correction. Corrections between variables were examined by the Spearman rank correlation coefficient for the 2 groups of patients with COPD. Only correlations having both an *r* value > 0.4 and a *P* value < .05 were considered relevant.

RESULTS

Subject characteristics

The clinical characteristics of subjects are presented in Table I. Serum IgE levels were significantly higher in COPD subjects with allergy than those without allergy. The FEV₁ and FEV₁/FVC were significantly higher in control subjects than in COPD subjects but did not differ significantly between patients with and without allergy. The COPD patients with allergy showed a significantly higher proportion of eosinophils in peripheral leukocytes than those without allergy. The proportions of neutrophils, lymphocytes, and monocytes in peripheral leukocytes did not differ among the 3 groups. The RV, the DL_{CO}, and the type and dosage of medication did not differ between the groups with COPD. Seven of the 15 patients with allergy had specific IgE antibodies against house dust mite, 6 had those against *C albicans*, and 2 had those against both.

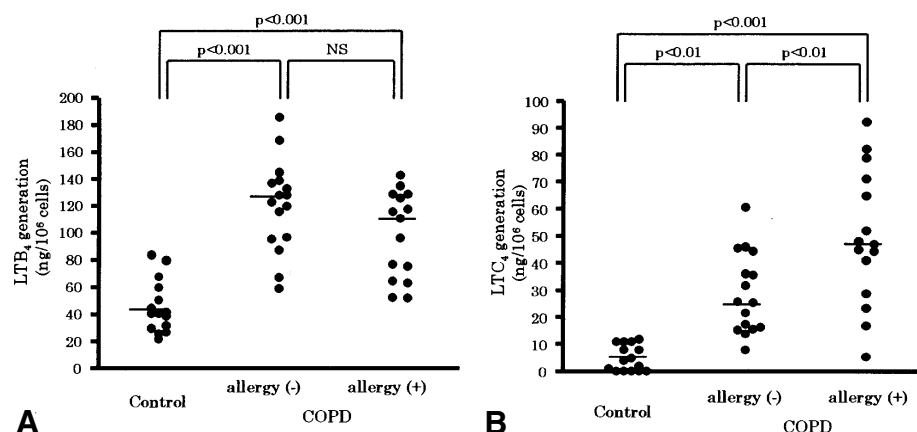


FIG 1. Amount of LTB₄ (A) and LTC₄ (B) generated by peripheral leukocytes of COPD patients and control subjects on stimulation with the calcium ionophore A23187.

TABLE I. Characteristics of control subjects and patients with COPD

	Control subjects	COPD patients	
		Without allergy	With allergy
No. of subjects	15	16	15
Sex (M/F)	5/10	16/0	15/0
Mean age (y)*	66 (53-76)	69 (50-81)	72 (56-83)
Serum IgE (U/mL) ^{†,‡}	ND	56 (3-245)	270 (21-2298)
FEV ₁ (%Pred)*§	101.8 (95.1-108.5)	53.5 (30.6-77.0)	60.3 (38.0-74.1)
FEV ₁ /FVC (%)*§	88.1 (84.4-91.8)	44.8 (35.5-58.3)	49.6 (36.3-59.5)
FEV ₁ reversibility (%)*	ND	5.8 (0.0-9.9)	7.3 (2.0-9.9)
RV (%Pred)*	ND	172.8 (123.8-221.5)	167.1 (141.3-219.6)
DL _{CO} (%Pred)*	ND	65.1 (37.7-78.7)	66.6 (41.4-84.9)
Peripheral blood leukocytes			
Total leukocyte count (×10 ⁶ /mL)*	5.8 (3.8-8.5)	6.3 (4.2-9.5)	6.5 (4.5-9.8)
Neutrophils (%)*	64.5 (55-71)	65 (55-70)	66 (54-72)
Eosinophils (%)*	2 (1-6)	3 (1-8)	6 (3-10)
Monocytes (%)*	5.5 (3-12)	3 (1-10)	3 (1-10)
Lymphocytes (%)*	26 (23-35)	25.5 (23-34)	24 (23-33)

ND, Not determined; %Pred, percentage predicted.

*Data expressed as median (range).

[†]Data expressed as geometric mean of serum IgE levels (range).

[‡]Serum IgE levels are significantly higher in COPD patients with allergy than in those without allergy ($P < .001$).

[§]Values are significantly higher in control subjects than in COPD patients with and without allergy ($P < .001$). There were no significant differences between COPD patients with and without allergy.

^{||}Values are significantly higher in COPD patients with allergy than in control subjects and COPD patients without allergy ($P < .01$).

Production of LTB₄ and LTC₄ by leukocytes

Fig 1 shows the amount of LTB₄ and LTC₄ generated by the peripheral leukocytes of the patients with COPD and control subjects on stimulation with the calcium ionophore A23187. The amount of LTB₄ generated by the leukocytes was 111.0 (52.3-143.0) ng/5 × 10⁶ cells in COPD patients with allergy, 125.5 (59.5-186.0) ng/5 × 10⁶ cells in COPD patients without allergy, and 41.0 (22.0-84.0) ng/5 × 10⁶ cells in control subjects (Fig 1, A). The amount of LTB₄ generated did not significantly differ between COPD patients with allergy and those without allergy. The leukocytes of COPD patients both with ($P < .001$) and without allergy ($P < .001$) generated significantly more LTB₄ than the leukocytes of the control

subjects. In contrast, the amount of LTC₄ generated by the leukocytes was 47.0 (5.4-92.3) ng/5 × 10⁶ cells in COPD patients with allergy, 25.7 (8.0-60.6) ng/5 × 10⁶ cells in COPD patients without allergy, and 4.0 (0-12.0) ng/5 × 10⁶ cells in control subjects (Fig 1, B). The leukocytes of COPD patients both with ($P < .001$) and without allergy ($P < .01$) generated significantly more LTC₄ than the leukocytes of the control subjects. LTC₄ production was significantly higher in the COPD patients with allergy than in those without allergy ($P < .01$).

We examined the relationship between the levels of LTB₄ and LTC₄ produced by leukocytes and FEV₁ (percentage of predicted value) in the 2 groups of patients with COPD. Fig 2 shows that FEV₁ correlated significantly with the level of both LTB₄ ($r = -0.556$, $P = .0375$)

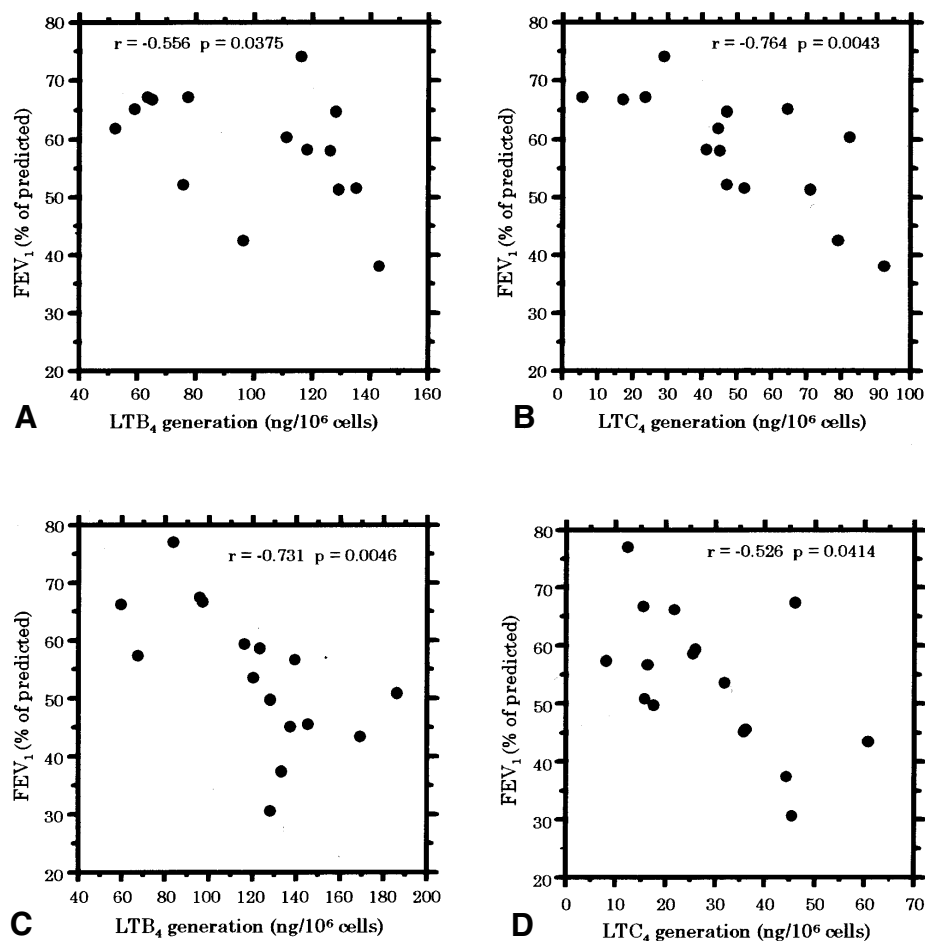


FIG 2. Relationship between levels of LTB₄ (A) and LTC₄ (B) produced by leukocytes and FEV₁ (percentage of predicted) in COPD patients with allergy, and levels of LTB₄ (C) and LTC₄ (D) produced by leukocytes and FEV₁ (percentage of predicted) in COPD patients without allergy.

(Fig 2, A) and LTC₄ ($r = -0.764$, $P = .0043$) (Fig 2, B) generation in COPD patients with allergy. The correlation was still significant to LTB₄ production, but with a much larger dispersion in comparison with LTC₄. Fig 2, C, shows a highly significant correlation between the level of LTB₄ generation and FEV₁ ($r = -0.731$, $P = .0046$) in COPD patients without allergy. The level of LTC₄ production also correlated significantly with FEV₁ in those patients with a much larger dispersion than that of LTB₄ production ($r = -0.526$, $P = .0414$) (Fig 2, D).

Relationship between bronchial hyperresponsiveness and the presence of specific IgE antibodies in patients with COPD

The bronchial hyperresponsiveness to methacholine in patients with COPD was examined in relation to and with the presence of specific IgE antibodies to common inhalant allergens. The log₁₀ of the D_{min} was significantly higher in COPD patients without allergy than in those with allergy ($P < .05$) (Fig 3).

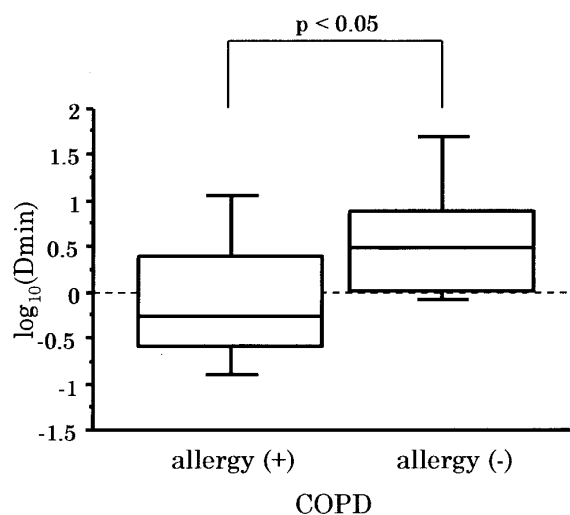


FIG 3. Relationship between bronchial hyperresponsiveness and presence of specific IgE antibodies in patients with COPD.

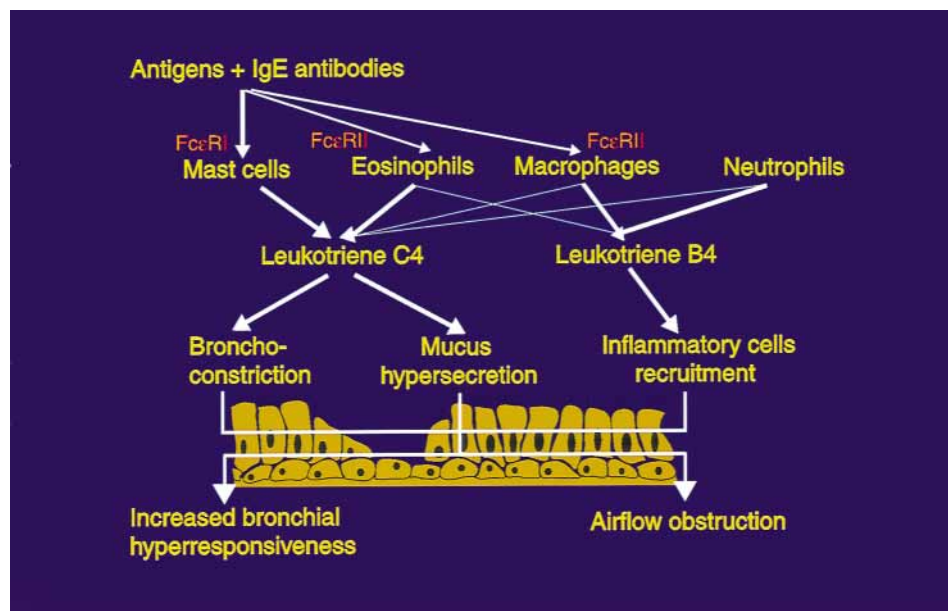


FIG 4. Influence of LTs and IgE antibodies on COPD. LTB_4 and LTC_4 generated from neutrophils, eosinophils, and macrophages can induce bronchial hyperresponsiveness and airflow limitation. Presence of specific IgE antibodies can enhance the generation of LTC_4 and LTB_4 .

DISCUSSION

Our study shows that the generation of LTC_4 , but not LTB_4 , by leukocytes stimulated with calcium ionophore A23187 is enhanced by the presence of specific IgE antibodies against inhalant allergens. A few studies have demonstrated the presence of specific IgE antibodies in COPD. Horses with chronic bronchitis/bronchiolitis are more often sensitized to some *Aspergillus fumigatus* and *Alternaria alternata* allergens than control horses.³³ In human patients with acute exacerbation of chronic bronchitis and infection of in the lower respiratory tract by *Haemophilus influenzae* or *Streptococcus pneumoniae*, IgE-mediated histamine was released by the infecting species, and bacteria-specific IgE was detected in sera.³⁴ However, to the best of our knowledge, our study is the first to classify subjects with COPD by the presence of specific IgE antibodies and to compare the synthesis of LTs by leukocytes between 2 groups.

The Cys LTs (LTC_4 , LTD_4 , and LTE_4) and LTB_4 play important roles in the airway pathophysiology of patients with bronchial asthma. Accumulation of eosinophils into the airways is often associated with increased production of LTC_4 .²² The divalent cation ionophore A23187 is one stimulus frequently used for studies of eosinophil activation or degranulation, as is *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine.³⁵ Schauer et al³⁶ reported that the generation of LTC_4 by leukocytes stimulated with calcium ionophore A23187 was significantly higher in children with asthma than in healthy control subjects. They also demonstrated that granulocytes from patients with a history of severe asthma displayed a higher LTC_4 formation than granulocytes from patients with less severe disease. LTB_4 has a chemotactic action for neu-

trophils as well as IL-8, which induces bronchial hyperresponsiveness and airway neutrophil accumulation.³⁷ However, few reports have described the generation of LTB_4 and LTC_4 by leukocytes in patients with COPD. Seggev et al³⁸ reported that serum LTB_4 concentrations are higher in patients with COPD than in patients with asthma or healthy control subjects. Shindo et al²⁷ observed that the mean LTE_4 level in healthy control subjects significantly differed from that during acute exacerbation before prednisolone treatment but did not differ in patients with COPD during remission. We determined in this study that the generation of LTB_4 and LTC_4 by leukocytes stimulated with calcium ionophore A23187 is significantly greater in patients with stable COPD than in healthy control subjects. Our result appears to be inconsistent with that of Shindo et al because they measured the plasma level of LTE_4 , a derivative of LTC_4 , but we measured the generation of LTC_4 . We also found that the proportion of neutrophils did not differ significantly among the 3 groups, and the proportion of eosinophils did not differ significantly between control subjects and COPD patients without allergy. These findings suggest that neutrophils and eosinophils might be activated in patients with COPD.

We also found a significant correlation between the amount of LT generation from peripheral leukocytes and airway obstruction. LTB_4 generation significantly correlated with FEV_1 in COPD patients both with and without specific IgE antibodies, but LTC_4 generation significantly correlated with FEV_1 in COPD patients with specific IgE antibodies. We speculate that the role of LTs for airway obstruction is important in both groups according to the presence of specific IgE antibodies.

Bronchial hyperresponsiveness is a characteristic feature not only of patients with asthma but also of those with COPD. In asthma, the main determinant of hyperresponsiveness is IgE-mediated allergy, the presence of which is usually demonstrated by positive skin test results, elevated levels of specific or total serum IgE, and eosinophilia. In our study we demonstrated that the bronchial hyperresponsiveness to methacholine is enhanced by the presence of specific IgE antibodies against inhalant allergens in patients with COPD. Renkema et al³⁹ suggested an important role for IgE in the course of COPD because higher initial serum IgE levels predict a more favorable course with regard to the annual decline of PC₂₀ (the provocative concentration causing a 20% decrease in FEV₁) histamine in nonallergic patients with COPD. Our results support those of Schmidt et al⁴⁰ because they found that elevated levels of serum IgE predict airway hyperresponsiveness to histamine in vitro and the in vitro model of passive sensitization also mimics conditions of nonspecific airway hyperactivity, which are relevant under in vivo conditions.⁴⁰

We used rigorous selection criteria to exclude the presence of asthmatic features (reversibility of obstruction) and the occurrence of exacerbated disease or anti-inflammatory treatment in order to study the generation of LTs and bronchial hyperresponsiveness of COPD. We are confident that our subjects showed no significant reversibility after bronchodilator inhalation at least twice on other days.

We conclude that enhanced LT generation from peripheral leukocytes is observed in patients with COPD and that the presence of specific IgE antibodies against inhalant allergens enhances LTC₄ generation, bronchial hyperresponsiveness, and the relationship between LTC₄ generation and airway obstruction. IgE-mediated allergy may play an important role in the pathophysiology even in COPD. We speculate how LTs and IgE influence COPD as shown in Fig 4. Further research into airway inflammation with sputum and BAL fluid is necessary, and a longitudinal study should be designed to assess the progression of disease in COPD patients with and without specific IgE antibodies.

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