

Increased Generation of Leukotriene C₄ from Eosinophils in Asthmatic Patients

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Human eosinophils with a density of over 1.095 were isolated from peripheral blood by dextran sedimentation, centrifugation with Lymphoprep and density gradients with Percoll. After the cells ($1 \times 10^5/\text{ml}$) were incubated with $1 \mu\text{g}/\text{ml}$ calcium ionophore A23187 for 20 min, leukotriene C₄ (LTC₄) content in the supernatant was measured by radioimmunoassay. The generation of LTC₄ was significantly higher in the cells from extrinsic asthmatics ($23.5 \pm 14.8 \text{ ng}/10^6 \text{ cells}$, mean $\pm$ SD, $n = 26$, $P < 0.01$) and intrinsic asthmatics ($24.6 \pm 20.6 \text{ ng}/10^6 \text{ cells}$, $n = 27$, $P < 0.01$) as compared with normal healthy subjects ($8.3 \pm 7.7 \text{ ng}/10^6 \text{ cells}$, $n = 10$). There was no significant difference in the generation of LTC₄ between intrinsic and extrinsic asthmatics. These observations indicate that eosinophils from asthmatic patients have increased ability to release LTC₄.

Key words: asthma; eosinophils; leukotriene C₄.

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Leukotriene (LT) C₄, D₄ and E₄ have a potent bronchoconstrictive activity and are being considered an important chemical mediator in bronchial asthma. LTs are synthesized from arachidonic acid as a substrate by 5-lipoxygenase pathway. We have already reported that asthmatic patients have an increased 5-lipoxygenase activity (7), and the results raise the possibility that the cells from asthmatic patients are able to generate higher amounts of LTs than those from normal healthy subjects. In this study we examined whether the increased activity is associated with a higher generation of LTC₄ in asthmatic patients.

MATERIAL AND METHODS

Subjects

All subjects investigated in this study were 26-65 years old and were classified into three groups: normal subjects, extrinsic asthmatics and intrinsic asthmatics. Normal healthy subjects had negative skin tests and no evidence of allergic

disease and they comprised eight men and two women (33.7 ± 1.1 years, mean $\pm$ SE). Fifty-three asthmatic patients comprised 26 with extrinsic (40.8 ± 1.9 years, 20 men and 6 women) and 27 with intrinsic asthma (49.0 ± 2.0 years, 16 men and 11 women). Patients with extrinsic asthma showed positive skin reactions against at least one of seven common allergens, and those with intrinsic asthma had negative skin reaction and negative allergic history. No one had taken steroids or non-steroidal anti-inflammatory drugs in the 2 weeks prior to the study. The subjects refrained from any medication at least 24 h prior to blood collection.

Isolation of eosinophils and generation of LTC₄ by calcium ionophore A23187 (Ca-I)

Blood (20 ml) was collected with 1/10 volume of 3.8% sodium citrate solution and was centrifuged at $200 \times g$ for 10 min at room temperature. After removal of platelet-rich plasma, tyrode buffer was added to the lower layer containing red blood cells and leukocytes to supple-

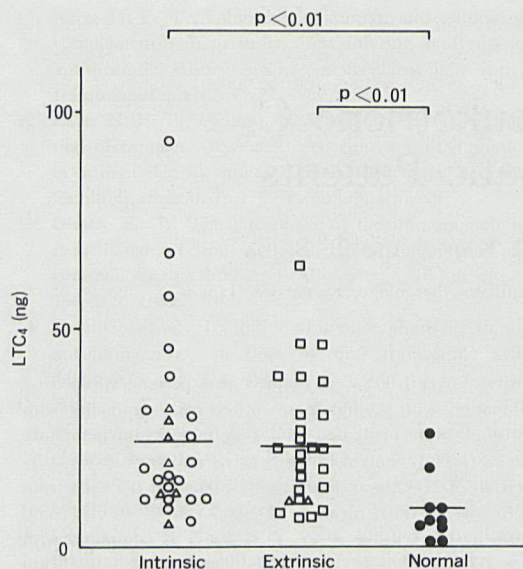


Fig. 1. Generation of LTC₄ in eosinophils from intrinsic asthmatics ○, extrinsic asthmatics □, aspirin-sensitive asthmatics Δ, and normal subjects ●. Each point represents individual values, and bars indicate mean values.

ment the volume. Leukocytes were collected by dextran sedimentation, and polymorphonuclear leukocytes (PMNs) were separated by centrifugation on Lymphoprep.

Eosinophils were isolated from PMNs by a method of Gärtner (2) modified in our laboratory (4). Eosinophils with a density of over 1.095 were used in this study. The purity of eosinophils was greater than 98 % and the viability of the cells as measured by trypan blue dye exclusion test was greater than 95 %. The eosinophils were suspended in tyrode buffer at a concentration of 1×10^5 cells/ml and incubated with 1 μ g/ml of Ca-I (Calbiochem, La Jolla, U.S.A.) at 37°C for 20 min. After centrifugation at $1700 \times g$ for 10 min, the supernatant was removed to quantitate LTC₄ by radioimmunoassay. The concentration of LTC₄ in the supernatant was determined with a commercial radioimmunoassay kit (New England Nuclear). The antiserum reacts by 55.3 % with LTD₄, 8.6 % with LTE₄ and 9.5 % with LTC₄-sulfone, and cross-reactivity for other ligands is reported to be below 0.1 % by the manufacturer. The standard curve was constructed in the range of 25 pg to 1.6 ng. The inter- and intra-assay variations were within ± 10 %.

Statistical significance of the data was assessed by Student's *t* test.

RESULTS

The amount of LTC₄ generation in response to 1 μ g/ml of Ca-I was significantly different between normal subjects and either asthmatic subgroup (intrinsic or extrinsic asthma) (Fig. 1). The amount of LTC₄ generation was significantly higher in the cells from extrinsic asthmatics (23.5 ± 14.8 ng/ 10^6 cells, mean $\pm$ SD, $n = 26$, $P < 0.01$) and intrinsic asthmatics (24.6 ± 20.6 ng/ 10^6 cells, $n = 27$, $P < 0.01$) compared with those from normal subjects (8.3 ± 7.7 ng/ 10^6 cells, $n = 10$), and there was no significant difference between the two asthmatic groups. The possibility of whether the slightly older average age of the asthmatic subjects was responsible for the increased generation of LTC₄ was ruled out, since there was no correlation between the amount of LTC₄ generation and aging (data not shown).

Aspirin-sensitive asthma patients were included in both extrinsic and intrinsic asthma groups. Although the aspirin-sensitive patients had a significantly higher LTC₄ generation (17.2 ± 3.3 ng/ 10^6 cells, $n = 9$) than the normal subjects, their LTC₄ generation was not higher than that of the other asthmatic patients.

DISCUSSION

We have already reported that PMNs from asthmatic patients have an increased 5-lipoxygenase activity (7). It is therefore possible that PMNs from asthmatic patients generate higher amounts of LTs than those from normal healthy subjects. In order to clarify this possibility, LTC₄ generation was studied in human eosinophils because it is known that eosinophils are a major source of LTC₄ in human PMNs (3, 5, 8, 9, 11, 12).

Maclouf (6) has reported that 12-HPETE derived from platelets increases the generation of LTB₄ and 5-HETE from leukocytes. This finding suggests that 12-HPETE is able to activate 5-lipoxygenase in neutrophils, as LTB₄ and 5-HETE is mainly generated from neutrophils (11). Because the effect of 12-HPETE on LTC₄

generation from eosinophils has not been established, experiments were performed to determine if generation of LTC₄ from eosinophils could also be affected by platelets. We observed that the addition of platelets to eosinophils resulted in an increase of LTC₄ generation (10).

These results suggest that the contaminating platelets could affect LTC₄ generation from eosinophils and, thus, it is important to deplete platelets from the eosinophils when comparing LTC₄ generation. Therefore, after removal of platelet-rich plasma, we isolated eosinophils by discontinuous density gradients. The platelet/eosinophil ratio was always below 1.

Since we have observed that eosinophils with different density have the capacity to generate different amounts of LTC₄ generation after stimulation with Ca-I (4), we collected eosinophils with the same density (over 1.095) for comparison.

Incubation of eosinophils with various concentrations of Ca-I for 20 min at 37°C induced a concentration-dependent generation of LTC₄ which plateaued at 5 µg/ml. However, Ca-I showed cytotoxic activity at a concentration of over 5 µg/ml. Based on these results, we used 1 µg/ml Ca-I to study LTC₄ generation. Because of the limited eosinophil counts available, the experiment at different concentrations of Ca-I could not be examined.

Analysis by high-speed liquid chromatography demonstrated that LTC₄ was the major LT generated from eosinophils, and eosinophils do not significantly metabolize LTC₄ into LTD₄ and LTE₄ under these conditions (1).

Eosinophils were isolated from two patients two times at an interval of about 3 months. There was not a wide variation of LTC₄ generation in the same subject on the two occasions (12.5 vs 12.5 and 25 vs 26 ng/10⁶ cells).

Higher generation of LTC₄ in asthmatic patients could be attributed to an increased activity of 5-lipoxygenase. LTC₄ has strong smooth muscle contractile activity and may be a mediator of various pathological processes, particularly bronchial asthma. However, definitive proof for the involvement of LTC₄ from eosinophils in asthma will require a more powerful physiological stimulus than Ca-I.

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