# Accumulation of Leukotriene C4 and Histamine in Human Allergic Skin Reactions

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## Abstract

**To determine whether lipoxygenase products of arachidonic acid metabolism are released in vivo during human allergic cutaneous reactions, we serially assayed chamber fluid placed over denuded skin sites for the presence of both C-6 peptide leukotrienes (e.g., LTC4, LTD4, and LTE4) and leukotriene B4 (LTB4), using radioimmune assay and HPLC separation, and compared it to histamine (assayed radioenzymatically) in 13 atopic and two nonatopic volunteers. Skin chamber sites challenged with ragweed or grass pollen antigen (250–750 protein nitrogen units/ml) for the first hour and phosphate-buffered saline (PBS) for the next 3 h were assayed hourly and compared to sites challenged with PBS alone. As assessed by HPLC, LTC4 composed >85% of the C-6 peptide leukotriene released at any skin site, whereas little LTD4 or LTE4 was detected. LTC4 was present in significantly greater concentrations at antigen sites as compared to PBS-challenged sites throughout the 4-h period. Minimal concentrations of LTB4 were found throughout this time period and were not different at antigen or PBS sites. Histamine was present in significantly greater concentrations at antigen rather than PBS sites, but the pattern of release was different from that of LTC4. Peak histamine release invariably occurred during the first hour and decreased progressively thereafter, whereas the greatest amounts of LTC4 were detected during the 2nd to 4th hours. The amount of LTC4 accumulating at the site was dependent upon the dosage of antigen used in the epicutaneous challenge. We have demonstrated in this study that of the leukotrienes assessed LTC4 is released in the greatest quantity in situ during in vivo allergic cutaneous reactions and that it is present at such sites for at least 4 h after antigen challenge. Since intradermal injection of LTC4 in humans induces wheal and flare responses that persist for hours, our findings support the hypothesis that LTC4 is an important mediator of human allergic skin reactions.**

## Introduction

The C6-peptide leukotrienes LTC4, LTD4, and LTE4, formerly defined by their biologic activity as slow reacting substance of anaphylaxis (SRS-A), are lipoxygenase metabolites of arachidonic acid that are thought to play an important role in human mast cell–mediated reactions. They are potent mediators of bronchoconstriction, vascular and nonvascular smooth muscle constriction, increased vascular permeability, and epithelial mucus secretion (1). Leukotriene B4 (LTB4) may also be an important allergic mediator, as it stimulates neutrophil and eosinophil chemotactic activity, as well as their aggregation, secretion of lysosomal constituents, and adherence to vascular endothelium (1). In vitro studies have demonstrated that many cells, including mast cells, neutrophils, eosinophils, basophils, and alveolar macrophages, are capable of generating leukotrienes (LTs)[1] (2). Mast cells and Langerhans cells are among the resident cell population in the skin. Our laboratory has found a prominent accumulation of neutrophils, eosinophils, and mononuclear cells during the course of cutaneous allergic reactions (3). Therefore, the skin provides an ideal site to investigate the profile and temporal pattern of LTs released during ongoing allergic reactions. We have studied the release of the C6 peptide LTs and LTB4 in the skin of atopic subjects during challenge with ragweed or grass pollen extract. We found that LTC4 is the LT present in largest amounts during such reactions; here, we compare its release to that of histamine.

## Methods

*Patients.* 13 atopic and two nonatopic healthy volunteers gave informed consent before study. Atopic subjects fulfilled the following criteria: (*a*) a history of allergic rhinitis during the fall and/or spring seasons; (*b*) a positive skin test, defined as a wheal of at least 10-mm diameter in response to an intradermal injection of 0.02 ml of 10 protein nitrogen units (PNU) per milliliter grass or ragweed pollen extract (Greer Laboratories, Lenoir, NC); and (*c*) no history of previous immunotherapy. Nonatopic subjects had no history of allergic rhinitis and had negative skin tests in response to intradermal injections of 0.02 ml of 1,000 PNU/ml grass and ragweed pollen extracts. No subject had urticaria or atopic dermatitis and no medicines were ingested for at least 72 h before study. The degree of antigen sensitivity of individual subjects was assessed by the extinction dilution skin test titer (EDST). EDST was defined as the greatest 10-fold dilution of relevant pollen extract that would elicit a positive skin test (4). Seven subjects were sensitive to an EDST of 10 PNU/ml, three to an EDST of 1 PNU/ml, and three to an EDST of 0.1 PNU/ml.

*Epicutaneous antigen challenge.* 20 epicutaneous antigen challenges were performed on 13 atopic and two nonatopic subjects according to methods described previously (4). Using mild heat and suction, two 1-cm diameter blisters were raised on each forearm. The blisters were aseptically unroofed and a sterile plastic collection chamber was taped in place over each blister base. To wash away any mediators released by induction of the blister itself, each chamber was filled with sterile isotonic phosphate-buffered saline (PBS); after 5 min, the PBS was removed and discarded. Incubations were then performed in the

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*Received for publication 7 February 1985.*

J. Clin. Invest.

0021-9738/85/08/0650/07 $1.00
Volume 76, August 1985, 650–656

1. *Abbreviations used in this paper:* EDST, extinction dilution skin test titer; HPLC, high-performance liquid chromatography; LT, leukotriene; and PNU, protein nitrogen units.

appended chambers according to the following protocols. Protocol I: simultaneously, two blister bases were incubated for 1 h with 0.3 ml of 500 PNU/ml grass or ragweed pollen extract and two control sites were incubated with PBS. The chambers were removed at the end of the 1-h incubation period; the blister bases were aspirated dry; fresh chambers were taped in place; and all chambers were filled with 0.3 ml PBS. The contents were removed after 1 h and the procedure was repeated in this way, a total of four serial 1-h incubations was performed at each site. Protocol II: chambers were removed and replaced at hourly intervals as described above. During the first hour, one site each was incubated with 0.3 ml of 250 PNU/ml pollen extract, 500 PNU/ml pollen extract, 750 PNU/ml pollen extract, or PBS. For the subsequent three incubation periods, PBS was placed in all four chambers. In protocol I, paired antigen-challenged sites and paired PBS-challenged sites, respectively, were pooled for subsequent mediator assay. In both protocols, fluids were aliquoted and frozen at −20°C for histamine assay and at −70°C after being gassed with nitrogen for LT assays.

A recovery experiment was performed to determine whether loss of chamber fluid LTB4, LTC4, or LTD4 was excessive in our model either by means of nonspecific sticking to the chamber or blister base, by degradation in the chamber fluids during incubation, or during storage before assay. In two of the atopic subjects, the washed blister bases were incubated for 1 h with synthetic LTC4, LTD4, LTB4, and PBS. Specific radioimmunoassays were performed on fluids before and after incubation; percent recovery was defined as recovered immunoreactive LT concentration/immunoreactive LT concentration before incubation. In these two subjects, 35% (5.5:16 pmol/ml) and 24% (3.3:14 pmol/ml) of incubated LTC4 was recovered; 56% (36:64 pmol/ml) and 71% (51:72 pmol/ml) of incubated LTD4 was recovered; 62% (34:56 pmol/ml) and 58% (32:55 pmol/ml) of incubated LTB4 was recovered. None of the LTs was present at the PBS site.

*Mediator assays.* Histamine: histamine concentrations were measured in triplicate by a radioenzymatic technique as previously described (5). This assay reproducibly detects histamine levels of at least 2 ng/ml in skin chamber fluids. Leukotrienes: [14,15-$^3$H(N)]LTC4 (34.0 Ci/mmol) and [14,15-$^3$H(N)]LTD4 (36.0 Ci/mmol) (New England Nuclear, Boston, MA), [5,6,8,9,11,12,14,15-$^3$H(N)]LTB4 (180–221 Ci/mmol) (Amersham Corp., Arlington Heights, IL), and Sep-Pak columns (Waters Associates, Milford, MA) were obtained. Synthetic LTB4, LTC4, LTD4, and LTE4 were provided generously by Dr. J. Rokach of Merck Frosst Laboratories, Dorval, Canada. Chamber fluids were acidified to pH 3.5 with 0.1 M acetic acid and applied to prewashed Sep-Pak columns; the columns were developed with 2 ml distilled water, 2 ml of 0.001 M acetic acid, and 2 ml of methanol and 0.001 M acetic acid (1:4, vol/vol), and the LTs were eluted in 2 ml of methanol. In selected samples, one-half of each of the eluates was dried *in vacuo* and redissolved for reverse-phase high-performance liquid chromatography (HPLC) on a 4.6 mm × 25 cm column of 10 μm octadecylsilane (Ultrasil, Altex Division of SmithKline Beckman, Inc., Berkeley, CA) that was developed isocratically with methanol: 0.03 g phosphoric acid per 100 ml of distilled water (pH 3.5 with ammonium hydroxide) (70:30, vol/vol) at a flow rate of 1 ml/min in a dual metered pump system (Beckman Instruments, Inc., San Jose, CA) (6). The elution times of synthetic LTC4, LTB4, LTD4, and LTE4, as assessed optically, were 14.1–15.6, 19.3–20.7, and 22.4–23.8 and 26.2–27.5 min, respectively (range, $n$ = 8).

Radioimmunoassays for LTs were performed as described with rabbit antisera of high specificity for LTC4 and LTB4 (7, 8) and a rabbit antiserum that recognizes all of the C-6 peptide LTs (Drs. E. Hayes and A. Rosenthal, Merck Laboratories, Inc., Rahway, NJ) (6), using [$^3$H]LTC4 and synthetic LTC4, H$^3$ of LTB4 and synthetic LTB4 and H$^3$ of LTD4 and synthetic LTC4, LTD4, or LTE4 as the respective pairs of ligands. Each value was corrected for the recovery of radiolabeled compound. The lowest limit of reliable quantification and the amount required for 50% inhibition of the radioligand, respectively, were 0.08 pmol and 0.35 pmol for the LTC4 assay (7) and 0.03 pmol and 0.15 pmol for the LTB4 assay (8). The same values for the C-6 peptide LT assay were dependent on the unlabeled LT employed to displace [$^3$H]LTD4 and were 0.1 and 0.7 pmol for LTC4, 0.2 and 1.3 pmol for LTD4, and 0.5 and 6.8 pmol for LTE4. LTC4 was used as the competing ligand to develop the standard curve from which values were calculated for analyses of samples before HPLC. Three separate standard curves appropriate for the different regions of the eluate from HPLC were developed using LTC4, LTD4, and LTE4, respectively, to displace [$^3$H]LTD4 (Fig. 1). In all cases in which sufficient fluid was available (31 of 100 fluids from antigen challenged sites and 25 of 52 fluids from PBS-challenged sites), chamber fluids were assayed for C-6 peptide LT, LTC4, and LTB4 both before and after HPLC coelution with synthetic standard LTB4, LTC4, and LTD4. A mean of 95% of the [$^3$H]LTC4 was recovered after HPLC with a correlation coefficient of 0.91 ($P < 0.001$) between LTC4 values before and after HPLC.

LT and histamine accumulations were expressed as concentrations (pmol/ml and ng/ml, respectively). The absolute amount of accumulated mediator over the 4-h period was determined by multiplying the sum of the concentrations in the hourly collections by the volume of fluid in the chambers, 0.3 ml.

## Results

Seven atopic and two nonatopic subjects were studied with Protocol I to determine whether radioimmunoreactive C-6 peptide LTs or LTB4 were released in response to cutaneous antigen challenge. Assays for immunoreactive C-6 peptide LTs were performed on all chamber fluids, while assays for immunoreactive LTB4 were performed only on fluids obtained from atopic subjects. More total (Table I) and hourly (data not shown) immunoreactive C-6 peptide LTs were recovered from chamber fluids at antigen than at PBS-challenged sites (12.24±5.93 pmol vs. 2.73±0.36 pmol, respectively). Analysis of histamine in the chamber fluid (Table I) revealed significantly greater release at antigen compared to PBS sites. Chamber fluids from subject 6, which did not contain elevated concentrations of histamine at antigen-challenged sites, also did not contain elevated concentrations of immunoreactive C-6 peptide LTs.

Chamber fluid obtained from nonatopic subjects contained equivalent amounts of immunoreactive C-6 peptide LT at

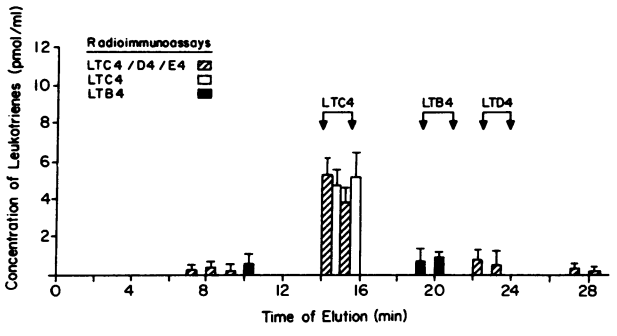


*Figure 1.* Reverse-phase HPLC resolution of LTs in cutaneous chamber fluid. 1-ml aliquots of the eluate from HPLC purification of three replicate extracts of cutaneous fluid from an antigen challenge site of one allergic subject were dried *in vacuo,* reconstituted in 0.2 ml of 0.05 M Tris-HCl (pH 8.0), and analyzed by radioimmunoassays. The values for LTC4 and LTD4 determined by the LTC4/D4/E4 assay were derived from two different standard curves of displacement of [$^3$H]LTD4 by LTC4 and LTD4, respectively. Each bar depicts the mean±SD of the results of duplicate assays of every 1-min pool of the three HPLC eluates. The arrows and brackets above represent the range of elution times of the synthetic LTs.

*Table I. C-6 Peptide LTs and Histamine at Antigen and PBS-Challenged Skin Sites*

| C-6 Peptide LTs* | | | Histamine‡ | |
|---|---|---|---|---|
| Atopic subjects | Antigen sites | PBS sites | Antigen sites | PBS sites |
| 1 | 2.94 | 1.27 | 35 | 3 |
| 2 | 47.31 | 2.07 | 81 | 2 |
| 3 | 6.75 | 3.03 | 9 | 3 |
| 4 | 10.89 | 2.94 | 26 | 7 |
| 5 | 8.37 | 3.63 | 26 | 7 |
| 6 | 3.99 | 2.94 | 5 | 5 |
| 7 | 5.46 | 3.24 | 24 | 6 |
| Mean±SEM | 12.24±5.93§ | 2.73±0.36 | 29.5±9.5‖ | 4.5±0.8 |

* Total picomoles of chamber fluid C-6 peptide LT collected over 4 h of incubation (summed concentration in pmol/ml × 0.3 ml).
‡ Total nanograms of chamber fluid histamine collected over 4 h of incubation (summed concentration in ng/ml × 0.3 ml).
§ $P < 0.02$ compared to PBS sites (Wilcoxan's test for paired differences).
‖ $P < 0.025$ compared to PBS sites (Student's $t$ test for paired values).

antigen (mean = 0.45 pmol) and PBS (mean = 0.42 pmol) challenged sites. The low concentrations of immunoreactive LTB4 found (range < 0.05–1.60 pmol/ml), in fluids of antigen-challenged sites was not significantly different from that in PBS-challenged sites.

Having ascertained that epicutaneous antigen challenge caused the release of immunoreactive C-6 peptide LTs, we next performed HPLC on selected fluids from antigen-challenged sites followed by specific radioimmunoassays (RIA) for LTB4, LTC4, and a combination, LTC4-D4-E4. As shown in one representative analysis of the eluate from HPLC (Fig. 1), most of the immunoreactive C-6 peptide LT consisted of LTC4 with little LTD4; the quantity of LTB4 also was small. The values for the LTC4-D4-E4 immunoreactivities eluting at 7–9 min and 27–28 min were calculated using the LTD4 standard curve, but probably represent sulfur oxidation products of the C-6 peptide LTs and LTE4, respectively, based on the times of elution relative to synthetic standards. Using an LTE4 standard curve, the quantity of presumptive LTE4 detected in HPLC eluates of these samples and of singlicate samples from four other patients were 0.65±0.17 pmol/ml (mean±SD) relative to 7.7±2.2 pmol/ml of LTC4. RIA of eluted LTC4 and LTD4 peaks demonstrated that a mean of 93% (range = 67–100%) of the C-6 peptide LTs consisted of LTC4 and 7% (range = 0–33%) consisted of LTD4 (Table II). In all cases involving protocols I and II in which sufficient fluid was available (31 of 100 fluids from antigen-challenged sites and 25 of 52 fluids from PBS challenged sites) assays were performed for C-6 peptide LT, LTC4, and LTB4 both before and after HPLC coelution with synthetic standard LTB4, LTC4, and LTD4. In this larger group of samples >85% of the immunoreactive C-6 peptide LT was LTC4 and 10% was LTD4, minimal amounts of LTB4 were detected.

Having determined that LTC4 is the predominant C-6 peptide LT present in chamber fluids following epicutaneous antigen challenge, we examined the time-course of LTC4 release during the 4-h study period. In five of the seven subjects in protocol I, for whom the quantity of fluid was sufficient, and in all six subjects in protocol II, the levels of specific LTC4 were assayed by a monospecific RIA in chamber fluids collected hourly at sites incubated with antigen or PBS during the first hour followed by PBS incubation at each site for the subsequent 3 h. The LTC4 levels (Table III) were significantly higher in fluids collected each hour at sites challenged with antigen than in those challenged with PBS. Although the temporal patterns of release varied among subjects, release was generally sustained throughout the 4-h collection period, with higher levels tending to occur during the second to fourth hours of incubation. At PBS challenged sites, a similar temporal pattern of LTC4 release was noted for the group as a whole, with the values at 2 h being significantly higher than at 1 h.

Chamber fluid histamine was present in significantly greater concentrations at antigen than PBS-challenged sites (Table IV). For all subjects, the highest concentration was present in the first hour with decreasing concentrations thereafter. This temporal pattern was quite different than the pattern of sustained release over 4 h of LTC4 at the same sites.

Protocol II was designed to determine whether LTC4 release is antigen dose–dependent. Six atopic subjects (numbers 8–13) were studied. As shown in Fig. 2, greater total and hourly concentrations of LTC4 were measured at antigen-challenged rather than PBS-challenged sites. The greatest LTC4 accumulations were seen at sites challenged with 500 PNU/ml antigen solutions (mean = 3.0±0.30), with significantly less total LTC4 at sites challenged with 250 PNU/ml of antigen (mean = 1.4±0.55, $P \leq 0.05$). The dose-response relationship was most apparent during hours 2–4 of collection of chamber fluids, whereas concentrations of LTC4 were similar during the first hour at all antigen-challenged sites. Unlike LTC4, histamine was not released into chamber fluids in a dose-dependent response manner within this range of antigen concentrations (Fig. 3).

At sites challenged with 500 PNU/ml antigen where accumulated LTC4 was at peak concentrations and histamine was at a plateau level, there was no correlation between hourly or total fluid histamine and LTC4 concentrations in individual chamber fluids. In addition, no correlations were found between hourly or total chamber LTC4 or histamine accumulation and subject sensitivity as measured by EDST.

## Discussion

The findings described have indicated that significantly greater amounts of C-6 peptide LT are released into skin chambers

*Table II. Concentration of LTC4 and LTD4 Determined by Radioimmunoassay after HPLC Separation*

| Subject | Hour | LTC4* | LTD4* |
|---|---|---|---|
| 3 | 1 | 6.3 (100) | <0.25 (0) |
| 4 | 2 | 7.6 (100) | <0.25 (0) |
| 4 | 3 | 6.7 (74) | 2.4 (26) |
| 4 | 4 | 3.5 (100) | <0.25 (0) |
| 5 | 4 | 6.3 (100) | <0.25 (0) |
| 6 | 4 | 5.1 (100) | <0.25 (0) |
| 7 | 2 | 5.2 (100) | <0.25 (0) |
| 7 | 3 | 2.9 (67) | 1.4 (33) |
| Mean | | (93%) | (7%) |

* pmol/ml (%).

*Table III. LTC4* Release at Antigen (500 PNU/ml) and PBS-challenged Sites*

| Antigen sites | | | | | | PBS sites | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Hours | | | | | | Hours | | | | |
| Subject | 1 | 2 | 3 | 4 | Total‡ | Subject | 1 | 2 | 3 | 4 | Total‡ |
| 3 | 6.2* | 2.3 | 3.1 | 2.0 | 4.08 | 3 | 1.2* | 2.7 | 0.6 | 1.3 | 1.74 |
| 4 | 4.6 | 11.0 | 8.1 | 4.0 | 8.31 | 4 | 1.9 | 1.7 | 0.9 | 1.2 | 1.72 |
| 5 | 3.7 | 4.3 | 3.9 | 6.0 | 5.37 | 5 | 1.0 | 3.0 | 1.2 | 3.0 | 2.46 |
| 6 | 4.1 | 5.0 | 2.7 | 2.7 | 4.35 | 6 | 1.2 | 3.3 | 2.3 | 2.7 | 2.85 |
| 7 | 3.5 | 6.2 | 2.4 | 3.0 | 4.53 | 7 | 1.6 | 2.5 | 1.5 | 3.7 | 2.79 |
| 8 | 0.8 | 3.9 | 6.0 | 3.7 | 4.32 | 8 | 0.7 | 0.9 | 0.2 | 0.2 | 0.60 |
| 9 | 1.7 | 4.7 | 2.3 | 0.3 | 2.70 | 9 | 0.9 | 1.7 | 0.8 | 0.2 | 1.08 |
| 10 | 0.8 | 2.9 | 2.0 | 5.4 | 3.33 | 10 | 0 | 0 | 1.4 | 1.2 | 0.78 |
| 11 | 0.2 | 1.6 | 2.8 | 4.0 | 2.58 | 11 | 0 | 0.6 | 0.3 | 0.2 | 0.33 |
| 12 | 0.2 | 0.5 | 3.9 | 2.7 | 2.19 | 12 | 0 | 0.6 | 0 | 0 | 0.18 |
| 13 | 1.9 | ND | 4.8 | 0.5 | 2.88 | 13 | 0 | 0 | 0.7 | 0.7 | 0.42 |
| Mean±SE | 2.51±0.60‖ | 4.24±0.92¶ | 3.81±0.56‖ | 3.11±0.53‖ | 4.05±0.51§ | Mean±SE | 0.77±0.20 | 1.54±0.36** | 0.90±0.20 | 1.30±0.38 | 1.35±0.30 |

* pmol/ml. ‡ pmoles (sum of all four hourly concentrations × 0.30 ml). § $P < 0.001$ from PBS site (Student's $t$ test for paired values). ‖ $P < 0.01$ from PBS site (Student's $t$ test for paired values). ¶ $P < 0.02$ from PBS site (Student's $t$ test for paired values). ** $P < 0.01$ from 1-h PBS site (Student's $t$ test for paired values).

*Table IV. Hourly Chamber Fluid Histamine Concentrations after Epicutaneous Antigen (500 PNU/ml) and PBS Challenge in 11 Atopic Subjects*

| | 500 PNU/ml antigen-challenged sites | | | | | PBS-challenged sites | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Hour | | | | | Hour | | | | |
| | 1 | 2* | 3 | 4 | Total | 1 | 2 | 3 | 4 | Total |
| | | | | | (ng) | | | | | (ng) |
| Mean | 49‡ | 14‡ | 9 | 5 | 23‡ | 4 | 4 | 6 | 4 | 5 |
| SEM | 9 | 3 | 2 | 1 | 3 | 1 | 1 | 3 | 2 | 2 |

* $n$ = 10 (one specimen not available). ‡ $P < 0.001$ compared to PBS sites, Student's $t$ test for paired values.

for at least 4 h after epicutaneous antigen challenge as compared with the amounts released at control skin sites. LTC4 accounted for >85% of the C-6 peptide LT with little LTD4 or LTE4 detected. Essentially, no chamber fluid LTB4 was found at either antigen- or control-challenged sites. Chamber fluid concentrations of LTC4 were significantly elevated during the entire 4 h of incubation; and although temporal patterns varied among individuals for the group as a whole, the greatest amounts were detected during the second, third, and fourth hours of incubation. This pattern of release differed from that of histamine, in which the greatest concentrations were obtained during the first hour with progressively decreasing concentrations in fluids collected during hours 2–4.

Many cell types have been demonstrated to be capable of generating C-6 peptide LTs and LTB4: human lung mast cells (9) and eosinophils (10–12) release predominantly LTC4 and little LTB4, whereas neutrophils (10–13) and alveolar macrophages (14, 15) produce a substantial quantity of LTB4. Human monocytes generate up to 3.5 times more LTB4 than LTC4 (16). Using skin biopsies, our laboratory has previously documented the timing of progressive cellular infiltration during ongoing cutaneous allergic reactions (3). Neutrophilic leukocytes begin to appear at the reaction site at 20 min after antigen challenge and continue to accumulate for a number of hours. Eosinophils do not begin to accumulate in the interstitial areas until 2 to 4 h after antigen administration. Mononuclear cells progressively infiltrate the site beginning at ~2 h, and increase in concentration over the next several hours. Basophils are not seen in significant quantities during the first 4 h of cutaneous allergic reactions. This would suggest that in our model, resident skin mast cells and perhaps accumulating eosinophils are the source of the LTC4 collected in the epicutaneous chamber fluids. Although neutrophils are able to generate LTC4, their predominant lipoxygenase pathway product is LTB4. It is possible that LTB4 is released in significant quantities during cutaneous allergic reactions but is not detected in our collection chamber model. The recovery of LTs described in the Methods section would suggest that if LTB4 diffuses into chamber fluids in concentrations greater than several picomoles per milliliter it would have been detectable. Since the oxidative degradation of LTB4 is intracellular and that of the C6-peptide LTs is extracellular (2), it

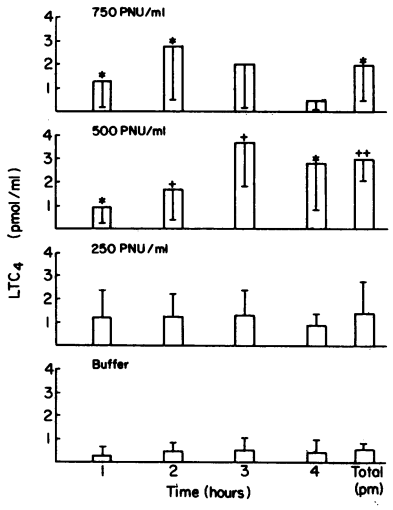


*Figure 2.* Hourly chamber fluid LTC4: dose response characteristics. Mean and standard deviation of chamber fluid LTC4 (pmol/ml) of six subjects is represented on the ordinate, the time of collection after 750, 500, and 250 PNU/ml of antigen or buffer (PBS) on the abcissa. The total LTC4 release (sum of all hours × 0.3 ml) is represented by the 5th bar. * represents $P < 0.05$ + $P < 0.01$ ++ $P < 0.001$ from buffer sites.

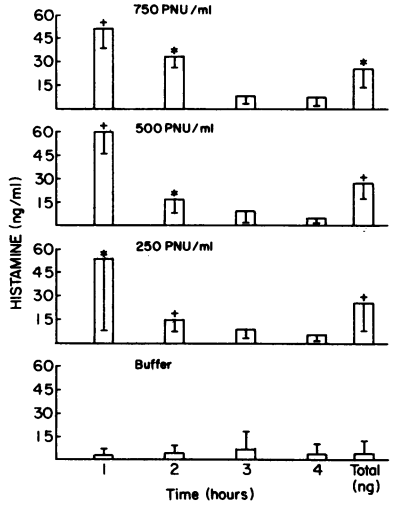


*Figure 3.* Hourly chamber fluid histamine levels: dose response characteristics. Mean and standard deviation of chamber fluid histamine levels (ng/ml) of six subjects if represented on the ordinate, the time of collection after 750, 500, and 250 PNU/ml of antigen or buffer (PBS) on the abcissa. The total histamine release (sum of all hours × 0.3 ml) is represented by the fifth bar. * represents $P < 0.01$ + $P < 0.001$ from buffer sites.

is conceivable that LTB4 might be catabolized effectively before it could diffuse into the chamber fluids in measurable amounts. Eosinophils might contribute to LTC4 detected during the third and fourth hours, but are probably not present in sufficient numbers in the first 2 h to account for the earlier accumulation of LTC4. Our laboratory has previously reported the release of histamine in progressively decreasing amounts during a 4-h period in ongoing allergic reactions (4). In that report, we suggested that the prolonged release of histamine represented continuing mast cell activity and that other mast cell–derived mediators might also be released for prolonged periods during ongoing allergic reactions. The findings reported here are additional, although circumstantial, evidence that mast cells might be the primary source of the LTC4 detected at antigen-challenged sites in the present study.

The amount of chamber fluid LTC4 that was recovered was related to the amount of antigen incubated with a peak response following the 500 PNU/ml epicutaneous antigen challenge. Previous studies in our laboratory have demonstrated that chamber fluid histamine accumulation and the degree of neutrophilic leukocyte infiltration into epicutaneous antigen-challenged skin sites are antigen dose–dependent (17). In that study, the antigen doses examined were 0, 100, 500, and 1,000 PNU/ml. Perhaps the broader dose range and larger number of subjects used in the former study detected a dose-dependent response for histamine release, not apparent with the use of narrower antigen dose range in the study reported here.

However, the temporal pattern of release of LTC4 was different from that of histamine. Whereas peak histamine release invariably occurs during the first hour and progressively decreases thereafter, LTC4 release was relatively constant after the first hour. This difference is most readily explained by the fact that histamine is present within mast cell granules as a pre-formed mediator, whereas LTC4 must be generated after perturbation of the cell membrane and release of arachidonic acid by lipoxygenase metabolism.

A substantial amount of in vitro evidence has been accumulated that suggests a major role for the lipoxygenase products of arachidonic acid metabolism in allergic as well as nonallergic inflammatory diseases. However, to clearly demonstrate a pathophysiologic role for the LTs, Koch's postulates must be fulfilled. The availability of synthetic LTs has permitted experiments which indicate that the LTs can reproduce many features of allergic disease. Inhalation of LTC4 (18, 19) and LTD4 (19, 20) by humans causes bronchoconstriction. Intracutaneous injections of LTC4, LTD4, or LTE4 cause a wheal and a flare that persists for as long as 2 and 6 h, respectively (21, 22). In addition, intracutaneous injections of LTC4 and LTD4 have been shown to be as potent as histamine in causing an increase in the microvascular cutaneous blood flow (22). Intracutaneous injection of LTB4 (21) causes a transient wheal and flare that is followed in 3–4 h by tender induration with neutrophil infiltration and fibrin deposition, histologically resembling a late cutaneous allergic reaction. Although what may have been slow reacting substance of anaphylaxis in the sputums of asthmatics was first detected by means of a bioassay using guinea pig tissue in 1930 (23), definitive proof of the presence of LTs during ongoing allergic reactions has been difficult to obtain. Reports such as ours have only become possible since the development of sensitive radioimmunoassays for the C-6 peptide LTs and LTB4. Since our experiments were completed, there have been reports that have demonstrated the presence of C-6 peptide LTs in body fluids during ongoing allergic reactions. LTC4-like immunoreactivity has been detected in human tear fluid following in vivo conjunctival antigen challenge (24). LTC4, -D4, and -E4 have been detected in nasal washings in a dose-dependent manner following intranasal pollen challenge in atopic humans (25). As suggested by the authors of this report, the presence of LTD4 and LTE4 in nasal washings may represent the metabolism by tissue or nasal secretory enzymes of mast cell–derived LTC4. In our skin model, we detected little LTD4 and no LTE4. It is possible that the LTC4 we collected in skin chamber fluids originated from cells located superficially in the denuded blister base allowing rapid diffusion into the epicutaneous fluids, where it was relatively protected from further enzymatic metabolism.

Thus, our findings, which conclusively demonstrate the presence of LTC4 in situ during ongoing cutaneous allergic reactions, as well as the other reports (24, 25) mentioned above, fulfill the second criterion of Koch's postulates. It now remains only for clinical trials of pharmacologic LTs inhibitors to demonstrate definitively the role of C-6 peptide LTs in in vivo allergic reactions in humans.

## Acknowledgments

We gratefully acknowledge the expert technical assistance of Ms. Mary Valenzano.

This work was supported by National Institutes of Health grant R01 AI 14332, HL-31809, AI 19784. S. F. Talbot is a recipient of a fellowship from the Asthma and Allergy Foundation of America.

## References

1. Goetzl, E. J., D. G. Payan, and D. W. Goldman. 1984. Immunopathogenic roles of leukotrienes in human diseases. *J. Clin. Immunol.* 4:79–84.

2. Lewis, R. A., and K. F. Austen. 1984. The biologically active leukotrienes: biosynthesis, metabolism, receptors, functions, and pharmacology. *J. Clin. Invest.* 73:889–897.

3. Atkins, P. C., G. Green, and B. Zweiman. 1973. Histologic studies on human skin test responses to ragweed, compound 48/80, and histamine. *J. Allergy Clin. Immunol.* 51:263–273.

4. Talbot, S. F., P. C. Atkins, M. Valenzano, and B. Zweiman. 1984. Correlations of in vivo mediator release with late cutaneous allergic responses in humans. I. Kinetics of histamine release. *J. Allergy Clin. Immunol.* 74:819–826.

5. Atkins, P. C., M. Valenzano, and B. Zweiman. 1982. Plasma concentrations of histamine measured by radioimmunoassay: effects of histaminase incubations. *J. Allergy Clin. Immunol.* 69:39–45.

6. Matthay, M. A., W. L. Eschenbacher, and E. J. Goetzl. 1984. Elevated concentrations of leukotrienes D4 in pulmonary edema fluids of patients with the adult respiratory distress syndrome. *J. Clin. Immunol.* 4:479–483.

7. Lindgren, J. A., S. Hammarstrom, and E. J. Goetzl. 1983. A sensitive and specific radioimmunoassay for the leukotriene C4. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 152:83–88.

8. Lee, J. Y., T. Chernov, and E. J. Goetzl. 1984. Characteristics of the epitope of leukotriene B4 recognized by a highly specific mouse monoclonal antibody. *Biochem. Biophys. Res. Commun.* 123:944–950.

9. Peters, S. P., D. W. MacGlashan, E. S. Schulman, R. P. Schleimar, E. C. Hayes, J. Rokadi, N. F. Adkinson, and L. M. Lichtenstein. 1983. Arachidonic acid metabolism in purified human lung mast cells. *J. Immunol.* 132:1972–1979.

10. Borgeat, P., B. Fruteau de Laclos, H. Rabmovich, S. Picard, P. Braquet, J. Herbert, and M. Laviolette. 1984. Eosinophil-rich human polymorphonuclear leukocyte preparations characteristically release leukotriene C4 on ionophore A23187 challenge. *J. Allergy Clin. Immunol.* 74:310–315.

11. Weller, P. F., C. W. Lee, D. W. Foster, E. J. Corey, K. F. Austen, and R. A. Lewis. 1983. Generation and metabolism of 5-lipoxygenase pathway leukotrienes by human eosinophils: predominant production of leukotriene C4. *Proc. Natl. Acad. Sci. USA.* 80:7626–7630.

12. Shaw, R. J., O. Cromwell, and A. B. Kay. 1984. Preferential generation of LTC4/D4 by human eosinophils and LTB4 by neutrophils: evaluation by specific radioimmunoassay. *J. Allergy Clin. Immunol.* 73:192. (Abstr.)

13. Ford-Hutchinson, A. W., M. A. Bray, M. V. Dorg, M. E. Shipley, and M. J. H. Smith. 1980. Leukotriene B, a potent chemokinetic and aggregating substance released from polymorphonuclear leukocytes. *Nature (Lond.).* 286:264–265.

14. Fels, A. O. S., N. A. Pawlowski, E. B. Cramer, T. K. C. King, Z. A. Cohn, and W. A. Scott. 1982. Human alveolar macrophages produce leukotriene B4. *Proc. Natl. Acad. Sci. USA.* 79:7866–7870.

15. Godard, P., M. Damon, F. B. Michael, E. J. Corey, K. F. Austen, and R. A. Lewis. 1983. Leukotriene B4 production from human aveolar macrophages. *Clin. Res.* 31:548A. (Abstr.)

16. Pawlowski, N. A., E. Abraham, A. Hamill, and W. A. Scott. 1984. The cyclooxygenase and lipoxygenase activities of platelet-depleted human monocytes. *J. Allergy Clin. Immunol.* 74:324–330.

17. Bedard, P.-M., P. C. Atkins, and B. Zweiman. 1983. Antigen-induced local mediator release and cellular inflammatory responses in atopic subjects. *J. Allergy Clin. Immunol.* 71:394–398.

18. Weiss, J. W., J. M. Drazen, N. Coles, E. R. McFadden, P. F. Weller, E. J. C. Corey, R. A. Lewis, and K. F. Austen. 1982. Bronchoconstrictor effects of leukotriene C in humans. *Science (Wash. DC).* 216:196–198.

19. Holroyd, M. D., R. E. C. Altomyan, M. Cole, M. Dixon, and E. V. Elliott. 1981. Bronchoconstriction produced in man by leukotrienes C and D. *Lancet.* 2:17–18.

20. Weiss, J. W., J. M. Drazen, E. R. McFadden, P. Weller, E. J. Corey, R. A. Lewis, and K. F. Austen. 1983. Airway constriction in normal humans produced by inhalation of leukotriene D. Potency, time course, and effect of aspirin therapy. *JAMA (J. Am. Med. Assoc.).* 249:2814–2817.

21. Soter, N. A., R. A. Lewis, E. J. Corey, and K. F. Austen. 1983. Local effects of synthetic leukotrienes (LTC4, LTD4, LTE4, and LTB4) in human skin. *J. Invest. Dermatol.* 80:115–119.

22. Bisgaard, H., J. Kristensen, and J. Sondengaard. 1982. The effect of leukotriene C4 and D4 on cutaneous blood flow in humans. *Prostaglandins.* 23:797–801.

23. Harkavy, J. 1930. Spasm-producing substance in the sputum of patients with bronchial asthma. *Arch. Int. Med.* 45:641–646.

24. Bisgaard, H., A. W. Ford-Hutchinson, S. Charleson, and E. Tandorf. 1984. Detection of leukotriene C4-like immunoreactivity in tear fluid from subjects challenged with specific allergen. *Prostaglandins.* 27:369–374.

25. Creticos, P. S., S. P. Peters, N. F. Adkinson, R. M. Naclerio, E. C. Hayes, P. S. Norman, and L. M. Lichtenstein. 1984. Peptide leukotriene release after antigen challenge in patients sensitive to ragweed. *N. Engl. J. Med.* 310:1626–1630.