Proc. Natl. Acad. Sci. USA
Vol. 79, pp. 7866–7870, December 1982
Immunology

Human alveolar macrophages produce leukotriene B_4

(arachidonic acid/lipoxygenase/zymosan/chemotaxis/calcium ionophore A23187)

ANNA O. S. FELS*, N. A. PAWLOWSKI†, E. B. CRAMER†, T. K. C. KING*, Z. A. COHN†, AND W. A. SCOTT†

†The Rockefeller University and *New York Hospital–Cornell Medical Center, New York, New York 10021

Contributed by Zanvil A. Cohn, September 29, 1982

ABSTRACT **Human alveolar macrophages obtained by bronchoalveolar lavage were labeled overnight with [^{3}H]arachidonic acid. The cells were stimulated with calcium ionophore A23187, and the 20:4 oxygenated metabolites released into the culture medium were identified by reverse-phase HPLC. Leukotriene B_4 was the major 20:4 metabolite produced by these cultures. Leukotriene B_4 was identified by its reverse-phase HPLC elution time, its UV spectrum, and its chemotactic and chemokinetic activities for neutrophils. In addition, the macrophage- and neutrophil-derived leukotriene B_4 free acids and methyl esters were found to have identical HPLC retention times.**

The leukotrienes are a recently discovered class of biologically active compounds that are formed from arachidonic acid (20:4) via the lipoxygenase pathway. They include leukotriene B_4 [LTB_4, (5*S*,12*R*)-dihydroxy-6,14-*cis*-8,10-*trans*-icosatetraenoic acid] (1), a compound with multiple proinflammatory actions, including chemotactic activity for neutrophils (2), and the slow-reacting substances, leukotrienes C_4 [(5*S*)-hydroxy-(6*R*)-*S*-glutathionyl-7,9-*trans*-11,14-*cis*-icosatetraenoic acid] and D_4 [(5*S*)-hydroxy-(6*R*)-*S*-cysteinylglycine-7,9-*trans*-11,14-*cis*-icosatetraenoic acid] (3, 4), which have vasoactive properties in addition to their contractile activity on select smooth muscle.

All major classes of granulocytes have been shown to produce leukotrienes *in vitro*. However, recent studies with murine pulmonary and peritoneal macrophages indicated that macrophages may be a particularly rich source of these compounds, with LTC_4 being the major lipoxygenase product (5, 6). To date, the macrophage is the only cell type known to synthesize substantial quantities of leukotrienes in response to inflammatory stimuli such as zymosan (5), and IgG (7) and IgE (8) immune complexes.

In this paper, we report the capacity of human alveolar macrophages to produce leukotrienes. Pulmonary alveolar macrophages represent a large population of resident leukocytes in the lung and as such are believed to be the first line of defense against inhaled material. These cells therefore are ideally positioned to initiate inflammatory and allergic reactions in the lung. The available evidence suggests that human alveolar macrophages generate a low molecular weight chemotactic factor for neutrophils that may be a lipid (9). Our work identifies one such molecule and demonstrates that LTB_4 is the major lipoxygenase product of these cells when they are stimulated with calcium ionophore A23187.

MATERIALS AND METHODS

Isolation of Human Alveolar Macrophages. Human alveolar macrophages were obtained by fiberoptic bronchoscopy with bronchoalveolar lavage in patients who had given informed consent. Lidocaine (2%) was used to anesthetize the nose and upper airways. The bronchoscope (model FB-19D, Pentex Precision Instrument, Norwood, NJ) was then passed transnasally and wedged into a subsegmental bronchus of the right middle lobe or lingula. Lidocaine (1%) was used as needed to suppress cough. Sterile saline, 100 to 150 ml in 50-ml aliquots, was instilled into and then withdrawn from the lung.

Macrophages from three patients were used for the initial quantification of 20:4 release and metabolism in response to a stimulus of opsonized zymosan or calcium ionophore A23187. In two of the patients, fiberoptic bronchoscopy was performed to evaluate a solitary pulmonary nodule; in these cases the lavage was performed on the lung contralateral to the side of the lesion. These patients were smokers, and the diagnosis was adenocarcinoma of the lung. The third patient, a nonsmoker, underwent fiberoptic bronchoscopy as a part of the diagnostic work-up of a single episode of hemoptysis. The procedure revealed no abnormalities and the patient's symptom has not returned. Alveolar macrophages from an additional eight patients were used for the separation of LTB_4 and the (5*S*,12*S*)-dihydroxyicosatetraenoic acid (di-HETE) stereoisomer as described below.

Characterization of Macrophage Cultures. Between 10 and 60×10^6 nucleated cells were obtained per procedure. Differential cell counts of Wright's stained preparations after cytocentrifugation indicated that 90% of the cells from each lavage were macrophages.

Release of 20:4 by Macrophage Cultures. After centrifugation at $800 \times g$ for 10 min, cells were suspended in phosphate-buffered saline (P_i/NaCl), centrifuged again, and finally resuspended in RPMI 1640 medium (GIBCO) containing 20% fresh human serum and penicillin at 100 units/ml plus streptomycin at 100 μg/ml.

Approximately 5×10^6 cells (3 ml) were added to 60-mm plates. After 2 hr at 37°C in 95% air/5% CO_2, the cultures were washed three times with P_i/NaCl and incubated for 16 hr in fresh medium containing 10% serum and [5,6,9,11,12,14,15-^{3}H]20:4 ([^{3}H]20:4; 95.4 Ci/mmol; New England Nuclear; 1 Ci = 3.7×10^{10} becquerels) at 5 μCi/ml.

At the end of the 16-hr labeling period, the cultures were washed three times with P_i/NaCl and overlaid with fresh RPMI 1640 medium without serum. A single culture was incubated for 4 hr at 37°C in the absence of a stimulus (control culture). The remaining cultures were divided into equal portions; one-half was exposed to calcium ionophore A23187 (Calbiochem-Behring) at 10 μg/ml for 20 min. The other cultures were incubated for 4 hr with zymosan at 500 μg/ml that had been op-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: 20:4, arachidonic acid; LTB_4, (5*S*,12*R*)-dihydroxy-6,14-*cis*-8,10-*trans*-icosatetraenoic acid; LTC_4, (5*S*)-hydroxy-(6*R*)-*S*-glutathionyl-7,9-*trans*-11,14-*cis*-icosatetraenoic acid; LTD_4 (5*S*)-hydroxy-(6*R*)-cysteinylglycine-7,9-*trans*-11,14-*cis*-icosatetraenoic acid; P_i/NaCl, phosphate-buffered saline; [^{3}H]20:4, [5,6,8,9,11,12,14,15-^{3}H]20:4; mono-HETE, monohydroxyicosatetraenoic acid; di-HETE, dihydroxyicosatetraenoic acid.

sonized at 37°C for 20 min with 50% fresh human serum in saline.

Extraction and Separation of 20:4 Metabolites. For the characterization of 20:4 metabolites released by macrophages, aliquots (50 μl) of the medium were taken for radioactivity measurements and the medium was placed on ice. The cells were washed and then scraped twice into 750-μl portions of 0.05% Triton X-100 (Rohm and Haas). Portions (50 μl) of the Triton X-100 cell lysates were assayed for radiolabel content, and protein was determined by the method of Lowry *et al.* (10) with bovine serum albumin as a standard.

The 20:4 metabolites were extracted from the culture medium by the method of Unger *et al.* (11). Briefly, the medium was made 50% (vol/vol) in ethanol and adjusted to pH 3 with formic acid (10 μl/ml of medium), and the resulting solution was extracted twice with 1 vol each of chloroform containing 0.005% butylated hydroxytoluene (BHT; Sigma). The chloroform phases were taken to dryness under a stream of nitrogen, and the 20:4 metabolites were resuspended in 400 μl of the appropriate starting buffer for HPLC. The 20:4 metabolites were further purified and separated by reverse-phase HPLC on 5-μm-particle Ultrasphere columns (4.6 mm × 25 cm; Altex, Rainin Instruments, Woburn, MA). Fractions (1 ml) were collected at a flow rate of 1 ml/min. Sequential elution was carried out with 60 ml of methanol/water/acetic acid, 65:34.9:0.1 (vol/vol), adjusted to pH 5.4 with ammonium hydroxide, followed by 40 ml of methanol/water/acetic acid, 75:25:0.01 (vol/vol), and 40 ml of methanol/acetic acid, 100:0.01 (vol/vol). The absorbance of column effluents was monitored at 280 nm by using a Kratos UV monitor (Spectroflow Monitor SF 770, Kratos, Schoeffel Instrument Division, Westwood, NJ). After the removal of 50-μl aliquots for radioactivity determinations, ultraviolet spectra of the appropriate fractions were obtained with a Perkin–Elmer 557 double-wavelength double-beam spectrophotometer. Quantities of LTB_4 were calculated from the absorbance at 281 nm [ε = 39,500 M^{-1} cm^{-1} (1)]. Radioactivity was measured by liquid scintillation counting in Hydrofluor (National Diagnostics, Somerville, NJ). As described previously (12), the elution characteristics of prostaglandins, monohydroxyicosatetraenoic acids (mono-HETEs), and 20:4 were determined by the use of radiolabeled standards. Those of dihydroxyicosatetraenoic acids (di-HETEs), including LTB_4 and its stereoisomers, were elucidated by comparison with corresponding 20:4 metabolites produced by neutrophils (1).

Human neutrophils were isolated and labeled with [^{3}H]20:4 as described (13). After a 15-min incubation with calcium ionophore A23187, the reaction was terminated by the addition of 1.5 vol of cold methanol and the medium was extracted by the method of Borgeat and Samuelsson (14). The medium extracts were dried under nitrogen and subjected to silicic acid chromatography (15). LTB_4 was purified from the appropriate silicic acid column fraction by reverse-phase HPLC as described above for the macrophage-derived LTB_4.

Separation of LTB_4 and (5*S*,12*S*)-di-HETE by Formation of Methyl Esters. Alveolar macrophages obtained from an additional eight patients were labeled with [^{3}H]20:4 and exposed to calcium ionophore as described above. After extraction from culture media, the 20:4 metabolites were separated by reverse-phase HPLC. The contents of fractions containing leukotrienes (as assessed by UV spectra) with elution times characteristic of LTB_4 were pooled and dried under reduced pressure. A total of 7.6 μg of leukotrienes was recovered as determined by the absorbance at 281 nm.

LTB_4-containing fractions from reverse-phase HPLC were dried completely under reduced pressure and exposed to diazomethane in diethyl ether (16). The methyl esters were subjected to normal-phase HPLC on a μPorasil column (10-μm particles, 3.9 mm × 30 cm, Waters Associates). Sequential elution was carried out with 60 ml of isopropyl alcohol/hexane/acetic acid, 5:95:0.01 (vol/vol), at a flow rate of 1 ml/min. The UV absorbance of column effluents was monitored at 280 nm, and UV spectra of the appropriate fractions were determined as described above. Preparations of LTB_4 from human neutrophils labeled with [^{3}H]20:4 and treated with calcium ionophore A23187 were similarly treated.

Chemotaxis and Chemokinesis Assays. Neutrophils were isolated from the citrated blood of normal human volunteers by Hypaque/Ficoll and dextran sedimentation techniques (17, 18). The resulting cell fraction contained approximately 98% neutrophils with 98–99% viability as determined by trypan blue exclusion. Prior to use, neutrophils were suspended in Gey's balanced salt solution (M A Bioproducts) containing 2% (wt/vol) bovine serum albumin, penicillin at 63 units/ml, and streptomycin at 138 μg/ml.

Neutrophil chemotaxis and chemokinesis assays were performed in a 48-well microchemotaxis assembly (19) (Neuroprobe, Bethesda, MD) according to the method of Harvath *et al.* (20). Neutrophils were stimulated to traverse the 10-μm-thick polyvinylpyrrolidine-free polycarbonate filter sheet (3-μm pore) by the addition of LTB_4 (1,000, 100, 10, 1 nM) to either the lower compartment (chemotaxis, eight wells for each LTB_4 concentration) or both compartments (chemokinesis, two wells for each LTB_4 concentration). After a 20-min incubation at 37°C in a 95% air/5% CO_2 atmosphere, the filters were removed and stained with Diff-Quick (Harle, Gibbstown, NJ).

The cells that had migrated were counted with an Artek counter (Artek Systems, Farmingdale, NY). The lower filter surface was examined at a magnification of ×50 and four or five fields were counted per well (each field was 0.8 mm^2). These were compared to neutrophils stimulated to undergo chemotaxis or chemokinesis in response to 100 nM fMet-Leu-Phe (Peninsula Laboratories, San Carlos, CA). Controls consisted of cells incubated by Gey's balanced salts solution without additions.

The LTB_4 derived from the alveolar macrophages of each of the three patients described above was separately assayed for chemotactic activity. The chemokinetic assay was performed on the LTB_4 from a single patient.

RESULTS

Human alveolar macrophages maintained in serum took up 83–98% of the [^{3}H]20:4 supplied in the culture medium. Of the incorporated radiolabel, 4–6% (n = 3) was released on exposure of the cultures to calcium ionophore A23187 at 10 μg/ml for 20 min. In contrast, the quantity of radiolabel released by cultures challenged with a maximal phagocytic load of opsonized zymosan (0–4%, n = 2) was not significantly greater than that of control cultures (0–1% in 20 min and 1–2% in 4 hr, n = 3), although opsonized zymosan was readily ingested by these cultures with maximal uptake of approximately 10 particles per cell by 1 hr.

Using HPLC conditions that allow the mutual separation of cyclooxygenase and lipoxygenase products, we examined the total 20:4 metabolites synthesized by human alveolar macrophages challenged with calcium ionophore A23187. A representative chromatogram is shown in Fig. 1. Di-HETEs, as evidenced by the prominent peaks of radiolabel eluting at 25–50 min, constitute a major species of 20:4 metabolites released by these cells. Based on elution times of radiolabeled standards, other 20:4 metabolites include cyclooxygenase products (4–16 min), mono-HETEs (90–105 min), and unreacted 20:4 (110–120 min). The metabolites with elution times of 55–90 min remain to be identified. However, these may include additional

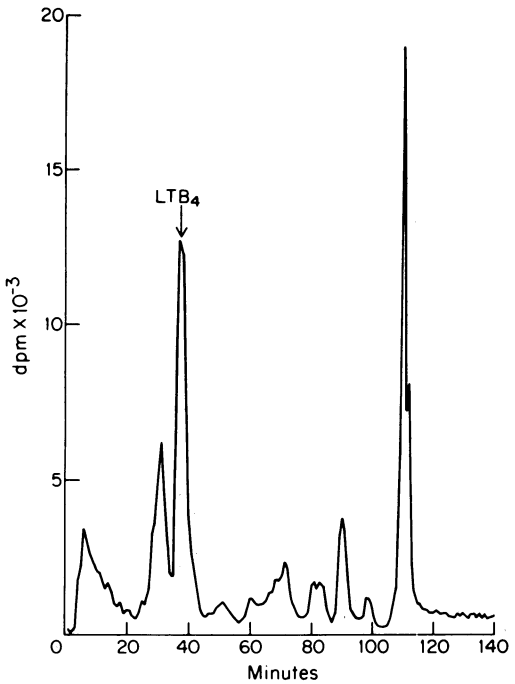

FIG. 1. Reverse-phase HPLC profiles of 20:4 metabolites released by [^{3}H]20:4-labeled human alveolar macrophages exposed to calcium ionophore A23187 at 10 μg/ml for 20 min. The 20:4 metabolites were extracted from the medium and subjected to HPLC. The UV absorbance profile of the column effluent was monitored at 280 nm, and the radiolabel content of fractions (1 ml) was determined. The elution times for the relevant standards are cyclooxygenase products, 4–16 min; di-HETEs, 25–50 min; mono-HETEs, 90–105 min; and unreacted 20:4, 110–120 min.

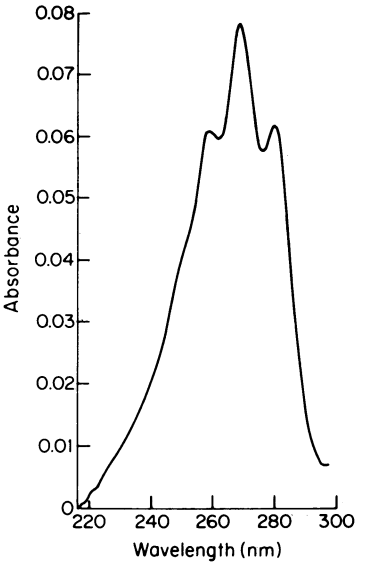

FIG. 2. UV absorbance spectrum of LTB_4 produced by human alveolar macrophages stimulated with calcium ionophore A23187. HPLC fractions 35–45 (Fig. 1) were pooled and the UV spectrum was recorded.

di-HETEs and also 12*L*-hydroxy-5,8,10-heptadecatrienoic acid (HHT), a product of the cyclooxygenase pathway.

The 20:4 metabolites in fractions 35–45 (Fig. 1, 16–24% of the total recovered radiolabel) have elution characteristics of LTB_4. The UV spectrum of this material (Fig. 2) indicated an absorbance maximum at 270 nm with shoulders at 260 and 280 nm, confirming the presence of a conjugated triene structure. The yield of this triene from calcium ionophore-stimulated macrophages was 96 ± 42 nmol/10^9 cells or 0.67 ± 0.16 pmol/μg of cell protein.

Because the reverse-phase HPLC elution and UV spectral characteristics of the material in fractions 35–45 were identical to those of LTB_4 isolated from human neutrophils (data not shown), the chemotactic and chemokinetic activities of these compounds were examined as a further means of identification. The macrophage-derived leukotriene produced a neutrophil chemotactic response that was concentration dependent (Fig. 3). Activity was evident at the lowest concentration (10^{-9} M) of the leukotriene tested. At 10^{-7} M in three separate experiments, the chemotactic response was 55–85% of that elicited by fMet-Leu-Phe (10^{-7} M). The control cells incubated in buffer showed 11–16% of the response obtained with 10^{-7} M fMet-Leu-Phe. Chemokinesis was evident at concentrations of 10^{-7} to 10^{-8} M of the macrophage product and had 33% of the activity of 10^{-7} M fMet-Leu-Phe.

The chemotactically inactive (5*S*,12*S*) stereoisomer of LTB_4 chromatographs with LTB_4 on the reverse-phase HPLC system used in this study. LTB_4 may be separated from this isomer by normal-phase HPLC after conversion to methyl esters (16). Because neutrophils release this inactive triene in addition to LTB_4 when stimulated with calcium ionophore, the possibility existed that the LTB_4 isolated from alveolar macrophages might be similarly contaminated. The normal-phase HPLC profiles of the neutrophil- and alveolar macrophage-derived LTB_4 methyl esters are shown in Fig. 4. Two major peaks of radiolabel that also showed absorbance at 280 nm (Fig. 4*A*) were obtained from the neutrophil material as described (16). UV spectrom-

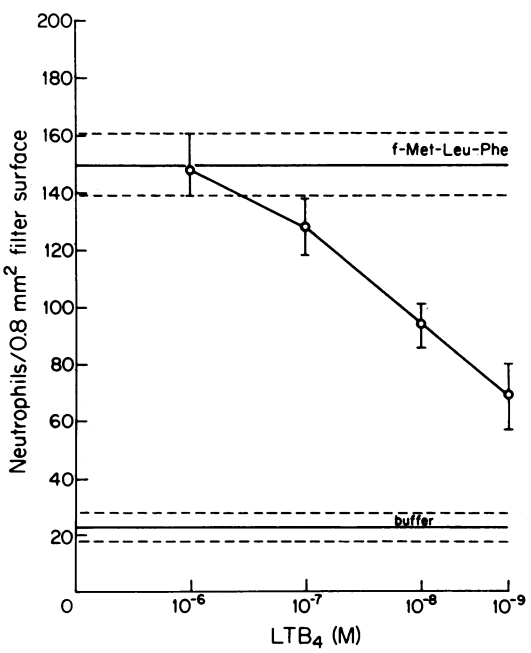

FIG. 3. Chemotactic activity of alveolar macrophage-derived LTB_4. Macrophages were stimulated with calcium ionophore A23187 and the LTB_4 was isolated by reverse-phase HPLC (fractions 35–45, Fig. 1). Neutrophils were stimulated to traverse a 10-μm-thick polycarbonate sheet by the addition of LTB_4 or fMet-Leu-Phe to the lower compartment of the chemotaxis chamber. Controls consisted of cells incubated in buffer without additions. After 20-min incubation at 37°C, the filters were removed and stained and the neutrophils that had migrated to the lower filter surface were counted. Solid and broken horizontal lines indicate the mean ± SD, respectively, of the chemotactic response of neutrophils to fMet-Leu-Phe (10^{-7} M, eight wells) or buffer. ○, Chemotactic response to the indicated concentrations of LTB_4 (eight wells for each concentration).

etry indicated that the compound in peak 1 (retention time, 16–18 min) had an absorbance maximum at 269 nm with shoulders at 259 and 278 nm, indicating that the compound contained a conjugated triene structure. These UV absorbance and elution characteristics suggest that this compound is the (5S,12S) stereoisomer of LTB_4 (16). The compound in peak 2 (retention time, 23–25 min), the major radiolabeled compound, had an UV maximum at 271 nm and shoulders at 261 and 281 nm. The absorbance maxima at higher wavelengths plus the longer elution time is consistent with this compound's being LTB_4 as previously described (1, 16).

The normal-phase elution profile of the alveolar macrophage-derived LTB_4 methyl esters is shown in Fig. 4*B*. The single major radiolabeled product had a retention time (23–25 min) identical to that of the neutrophil-derived LTB_4 (peak 2, Fig. 4*A*). The ratio of the (5S,12S)-di-HETE to LTB_4, as estimated from the radiolabel content of the appropriate fractions, was 1:3 for the neutrophil material but 1:14 for the macrophage material. Interestingly, the chemotactic activity of the alveolar macrophage LTB_4 isolated by reverse-phase HPLC is higher than for the neutrophil-derived LTB_4 similarly isolated. The lower ratio (1:14) of the (5S,12S) isomer to LTB_4 in the macrophage preparation may account for this difference because the (5S,12S) compound exhibits weak or no chemotactic activity (21). In summary, the chromatographic data presented above together with the chemotactic activity of the macrophage leukotriene strongly suggest that human alveolar macrophages release LTB_4 in response to a calcium ionophore stimulus.

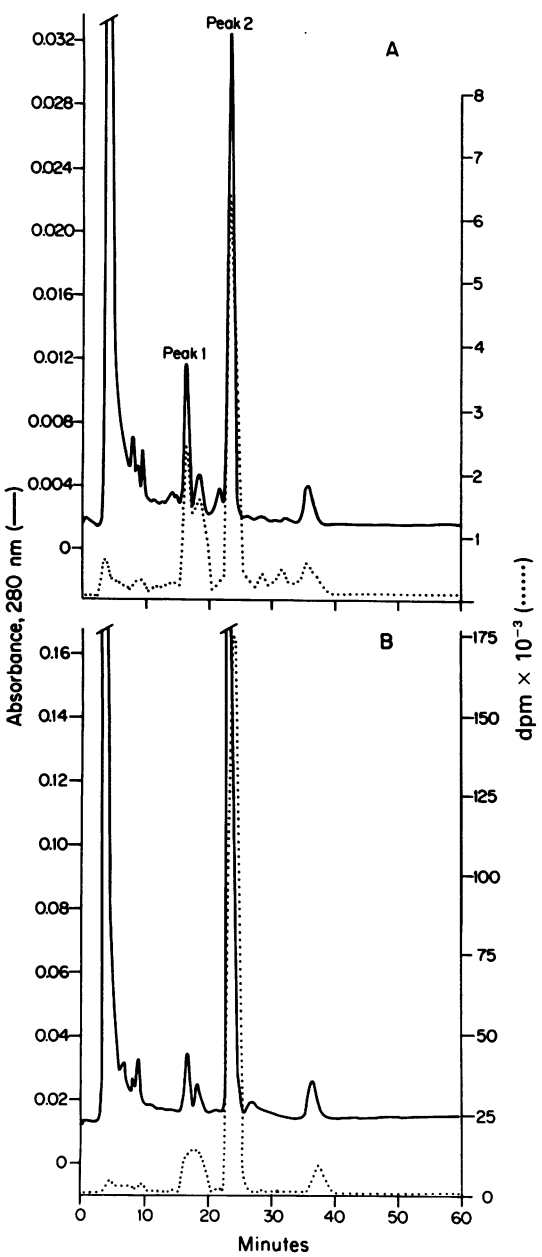

FIG. 4. Normal-phase HPLC of neutrophil- (*A*) and alveolar macrophage- (*B*) derived LTB_4 isolated by reverse-phase HPLC. LTB_4 (fractions 35–45, Fig. 1) was converted to the methyl ester by exposure to diazomethane in ether. LTB_4 and the (5S,12S)-di-HETE methyl esters were separated by normal-phase HPLC. Column effluents were monitored at 280 nm and the radiolabel content of fractions (1 ml) was determined.

DISCUSSION

The data presented in this report indicate that human alveolar macrophages release a variety of 20:4 oxygenated metabolites on challenge with calcium ionophore A23187. The major portion of the released 20:4 is metabolized by the lipoxygenase pathway, with LTB_4 being the predominant product. From HPLC profiles we estimate that LTB_4 represents approximately 28% of the total 20:4 metabolites and 16–24% of the total radiolabel recovered from these cells. The identification of the macrophage di-HETE as LTB_4 rests on its coelution with neutrophil-derived LTB_4 on reverse-phase HPLC, the coelution of its methyl ester with the methyl ester of the neutrophil-derived LTB_4 on normal-phase HPLC, and the chemotactic and chemokinetic activity of the macrophage di-HETE for human neutrophils.

LTB_4 has several activities that are distinct from those of the slow-reacting substances, leukotrienes C and D. LTB_4 is chemotactic and chemokinetic for human neutrophils with an activity comparable to that of fMet-Leu-Phe on a molar basis (2, 22). LTB_4 also causes margination of neutrophils *in vivo* (23) and promotes neutrophil aggregation *in vitro* (22); it has been suggested that these effects of LTB_4 on neutrophils may be mediated by a calcium influx (24). In the presence of a vasodilator such as prostaglandin E_2, LTB_4 has been shown to cause a significant increase in vascular permeability (25). In addition to these proinflammatory actions, LTB_4 causes contraction of pulmonary parenchymal strips at concentrations significantly less than histamine but greater than those of the closely related leukotrienes C_4 and D_4 (26). This action of LTB_4 is thought to be mediated by prostaglandins (27). Thus, LTB_4 may be an important mediator of inflammation and may, in addition, cause bronchospasm of the small airways.

The question of what role macrophage-derived LTB_4 might play in inflammatory diseases of the lung is an intriguing one. Pulmonary macrophages are present in the interstitium and in the alveoli. These cells therefore are ideally positioned to respond to systemic or inhaled antigenic challenges to the lung. The release of a potent chemotactic factor by pulmonary macrophages could be important not only in the initiation of acute inflammatory reactions but also in the pathogenesis of certain chronic lung diseases. In many pulmonary diseases, as diverse as adult respiratory distress syndrome and emphysema, the influx and subsequent degranulation of neutrophils have been postulated to be primary causes of damage to the lung parenchyma. Whether LTB_4 is an important mediator of these inflammatory changes requires further investigation.

The authors gratefully acknowledge the physicians who supplied the human alveolar macrophages used in this study: Dr. Robert Garrett, Dr. Daniel Libby, Dr. J. P. Smith, and Dr. Diane Stover. This work

was supported by National Institutes of Health Grants CA-30198, HL-27186, and AI-07012, Grant 79-1009 from the American Heart Association, and a grant-in-aid from the Squibb Institute for Medical Research. A.O.S.F. is the recipient of a Stony-Wold Herbert Fellowship. N.A.P. is the recipient of Postdoctoral Fellowship HD-0578 from the National Institute of Child Health and Human Development.

1. Borgeat, P. & Samuelsson, B. (1979) *J. Biol. Chem.* **254,** 2643–2646.
2. Smith, M. J. H., Ford-Hutchinson, A. W. & Bray, M. A. (1980) *J. Pharm. Pharmacol.* **32,** 517–518.
3. Murphy, R. C., Hammarström, S. & Samuelsson, B. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 4275–4279.
4. Orning, L., Hammarström, S. & Samuelsson, B. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 2014–2017.
5. Rouzer, C. A., Scott, W. A., Cohn, Z. A., Blackburn, P. & Manning, J. M. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 4928–4932.
6. Rouzer, C. A., Scott, W. A., Hamill, A. L. & Cohn, Z. A. (1982) *J. Exp. Med.* **155,** 720–733.
7. Rouzer, C. A., Scott, W. A., Kempe, J. & Cohn, Z. A. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 4279–4282.
8. Rouzer, C. A., Scott, W. A., Hamill, A. L., Liu, F.-J., Katz, D. H. & Cohn, Z. A. (1982) *J. Exp. Med.* **156,** 1077–1086.
9. Merill, W. W., Naegel, G. P., Matthay, R. A. & Reynolds, H. Y. (1980) *J. Clin. Invest.* **65,** 268–276.
10. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265–275.
11. Unger, W. G., Stamford, I. F. & Bennet, A. (1971) *Nature (London)* **233,** 336–337.
12. Scott, W. A., Pawlowski, N. A., Andreach, M. & Cohn, Z. A. (1982) *J. Exp. Med.* **155,** 535–547.
13. Pawlowski, N. A., Scott, W. A., Andreach, M. & Cohn, Z. A. (1982) *J. Exp. Med.* **155,** 1653–1664.
14. Borgeat, P. & Samuelsson, B. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 2148–2152.
15. Borgeat, P. & Samuelsson, B. (1979) *J. Biol. Chem.* **254,** 1653–1664.
16. Lindgren, J.-A., Hansson, G. & Samuelsson, B. (1981) *FEBS Lett.* **128,** 329–335.
17. Boyum, A. (1968) *Scand. J. Clin. Invest. Suppl.* **97,** 77–89.
18. Clark, R. A. & Kimball, H. R. (1971) *J. Clin. Invest.* **50,** 2645–2652.
19. Falk, W., Goodwin, R. & Leonard, E. (1980) *J. Immunol. Methods* **33,** 239–247.
20. Harvath, L., Falk, W. & Leonard, E. (1980) *J. Immunol. Methods* **37,** 39–45.
21. Malmsten, C. L., Palmblad, J., Radmark, O., Uden, A.-M., Engstedt, L. & Samuelsson, B. (1980) *Acta Physiol. Scand.* **110,** 449–451.
22. Ford-Hutchinson, A. W., Bray, M. A., Doig, M. V., Shipley, M. E. & Smith, M. J. H. (1980) *Nature (London)* **286,** 264–265.
23. Dahlén, S.-E., Björk, J., Hedqvist, P., Arfors, K.-E., Hammarström, S., Lindgren, J.-Å. & Samuelsson, B. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 3887–3891.
24. Naccache, P. H., Molski, T. F. P., Becker, E. L., Borgeat, P., Picord, S., Vallerand, P. & Sha'afi, R. I. (1982) *J. Biol. Chem.* **257,** 8608–8611.
25. Bray, M. A., Ford-Hutchinson, A. W. & Smith, M. J. H. (1981) in *SRS-A and Leukotrienes,* ed. Piper, P. J. (Wiley, New York), pp. 253–270.
26. Hannson, G., Lindgren, J. A., Dahlén, S.-E., Hedqvist, P. & Samuelsson, B. (1981) *FEBS Lett.* **130,** 107–112.
27. Sirois, P., Borgeat, P., Jeanson, A., Roy, S. & Girard, G. (1980) *Prostaglandins Med.* **5,** 429–444.