Proc. Natl. Acad. Sci. USA
Vol. 79, pp. 7904–7908, December 1982
Medical Sciences

Radioimmunoassay for leukotriene B_4

(rat mast cell/human neutrophil/lipoxygenase/chemotactic factor)

ROBERT A. LEWIS*, JEAN-MICHEL MENCIA-HUERTA*, ROY J. SOBERMAN*, DENNIS HOOVER†, ANTHONY MARFAT†, E. J. COREY†, AND K. FRANK AUSTEN*‡

*Department of Medicine, Harvard Medical School and Department of Rheumatology and Immunology, Brigham and Women's Hospital, Boston, Massachusetts 02115; and †Department of Chemistry, Harvard University, Cambridge, Massachusetts 02138

Contributed by K. Frank Austen, September 28, 1982

ABSTRACT **A rabbit immunized with leukotriene B_4 [LTB_4; (5*S*,12*R*)-6,14-*cis*-8,10-*trans*-icosatetraenoic acid] coupled to bovine serum albumin via the 12-oxy function of the lipid produced antibodies having an average association constant (K_a) for [14,15-^{3}H]LTB_4 of 3.2×10^9 M^{-1} at 37°C and in a concentration of 0.37 μg/ml of the immune plasma. When 10 μl of anti-LTB_4 and 3.9 nCi of [14,15-^{3}H]LTB_4 (28 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels) were incubated in a volume of 250 μl, 50% inhibition of radioligand binding was achieved with 0.31 ng of LTB_4 and with 1.95 ng of (5*S*,12*S*)-6-*trans*-8-*cis*-LTB_4. The sulfidopeptide leukotrienes, LTC_4 and LTD_4, displaced the radioligand from this antibody with less than 1/100th the activity of LTB_4, and the diastereoisomers of 6-*trans*-LTB_4, 5-L-hydroxy-6-*trans*-8,11,14-*cis*-icosatetraenoic acid (5-HETE), and three prostaglandins were minimally effective. The specificity of this radioimmunoassay was further shown by assessment of the immunoreactive products generated from calcium ionophore (A23187)-activated rat serosal mast cells and human neutrophils after reversed-phase HPLC. Resolution of the supernatants from each cell type yielded a single immunoreactive peak that coeluted with synthetic LTB_4 and quantitatively correlated with the physical measurement by integrated A_{269} in that peak; UV-absorbing peaks eluting at other retention times were not immunoreactive. The immunoreactive LTB_4 generated averaged 4.6 ng per 10^6 rat mast cells and resolution of the supernatants by reversed-phase HPLC without a prior extraction step gave a recovery of 54%, validating the direct applicability of this sensitive and specific assay for LTB_4, a highly potent chemotactic factor, to unfractionated biologic fluids.**

The 5-lipoxygenase pathway for oxidative metabolism of arachidonic acid generates a number of biologically active end products, including 5-L-hydroxy-6-*trans*-8,11,14-*cis*-icosatetraenoic acid (5-HETE) (1); the C-6 sulfidopeptide leukotrienes C_4 (LTC_4), D_4 (LTD_4), and E_4 (LTE_4) (2–6); and an exceedingly potent granulocyte chemotactic factor (7), (5*S*,12*R*)-dihydroxy-6,14-*cis*-8,10-*trans*-icosatetraenoic acid, LTB_4 (8–10). Because each of these products has unique biological effects at relatively low concentrations (11), specific, sensitive, and quantitative assays are needed to study their biosynthesis, catabolism, and presence in disease states. A class-specific radioimmunoassay (RIA) for LTC_4, LTD_4, and LTE_4 has been described (12) and has been used to quantitate the generation of these products with and without resolution by reversed-phase HPLC (RP-HPLC) from a number of cultured cell types (12, 13) as well as to evaluate their metabolism (14, 15). LTB_4 has previously been measured spectrophotometrically (16, 17) and biologically as a chemotactic factor (10, 18) after purification by RP-HPLC. Because neither of these methods is applicable for quantitating LTB_4 from an unresolved mixture of cell products, a specific and sensitive RIA has been developed.

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.

MATERIALS AND METHODS

Materials. Methanol (HPLC grade; Burdick and Jackson Laboratories, Muskegon, MI), Ficoll/Hypaque and macromolecular dextran (Pharmacia, Uppsala, Sweden), Hanks' balanced salt solution (Microbiological Associates, Bethesda, MD), *p*-nitrophenylchloroformate (Aldrich Chemical, Metuchen, NJ), bovine serum albumin (crystallized, globulin free; Sigma, St. Louis, MO), and the calcium ionophore A23187 (Calbiochem–Behring) were purchased from the manufacturers. Prostaglandin (PG) E_2, $PGF_{2\alpha}$, and PGD_2 were obtained from John Pike (Upjohn, Kalamazoo, MI). 14,15-Ditritiated LTB_4 was prepared by catalytic tritiation (Lindlar catalyst) of 14,15-dehydro-LTB_4 [synthesized in a manner analogous to that of LTB_4 itself (19)]; the radioligand, [14,15-^{3}H]LTB_4 (28 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels), purified by RP-HPLC, was supplied by New England Nuclear (Boston, MA) as was [14,15-^{3}H]LTC_4 (40 Ci/mmol). LTB_4, (5*S*,12*S*)-6-*trans*-LTB_4, (5*S*,12*R*)-6-*trans*-LTB_4, (5*S*,12*S*)-6-*trans*-8-*cis*-LTB_4, 5-HETE, LTC_4, LTD_4, and LTE_4 were prepared as described (3, 9, 19–22).

Production of Immunogen and Antibodies. The immunogen was a urethane derivative in which linkage had been established between a lysine amino group of bovine serum albumin and the 12-oxy function of LTB_4 through a carbonyl group, as represented by the formula LTB_4-O-CO-NH-albumin. The leukotriene derivative used for preparation of the immunogen was the 5-benzoate of LTB_4 methyl ester, which is available by base-catalyzed E2′ elimination of methyl (5*S*)-benzoyloxy-11(*R*),(12*R*)-epoxy-6,9,14-*cis*-icosatriene (19). The 5-benzoate of LTB_4 methyl ester (1.8 mg), which had been dried by azeotropic distillation of toluene under reduced pressure, was treated at 0°C with 4.03 mg (5 equivalents) of *p*-nitrophenylchloroformate in 240 μl of anhydrous pyridine. The resulting solution was brought to 23°C and maintained at that temperature for 10 min. TLC on Et_3N-treated silica gel plates developed with ether/hexane, 1:1 (vol/vol)/1% Et_3N showed essentially complete conversion of the 5-benzoate of LTB_4 methyl ester (R_f, 0.17) to its 12-*p*-nitrophenoxycarbonyl derivative (R_f, 0.42). Et_3N (5 drops) was added to the reaction mixture, and the resulting solution was directly applied to a silica gel column equilibrated with ether/hexane, 1:2 (vol/vol)/1% Et_3N at 0°C and eluted in 30–45 min under the same conditions. After removal of solvent at reduced pressure from the product-containing fractions, 2.46 mg (yield, >90%) of the 12-*p*-nitrophenoxycarbonyl de-

Abbreviations: 5-HETE, 5-L-hydroxy-6-*trans*-8,11,14-*cis*-icosatetraenoic acid; LTB_4, leukotriene B_4; LTC_4, leukotriene C_4; LTD_4, leukotriene D_4; LTE_4, leukotriene E_4; RP-HPLC, reversed-phase HPLC; RIA, radioimmunoassay; PGE_2, prostaglandin E_2; $PGF_{2\alpha}$; prostaglandin $F_{2\alpha}$; PGD_2, prostaglandin D_2.

‡ To whom reprint requests should be addressed.

rivative of LTB_4-5-benzoate methyl ester was recovered, as assessed at the wavelength (272 nm) of maximum UV absorbance (in methanol), and calculated with the appropriate molar extinction coefficient (ϵ, 59,700 cm^{-1} M^{-1}). The product was homogeneous by TLC and showed the expected proton magnetic resonance spectrum. It could be stored for several weeks in dry benzene at −20°C under argon without noticeable decomposition.

A solution was prepared by stirring 33.3 mg of albumin at 4°C with 170 μl of 0.2 M aqueous borate buffer (pH 9.0) and then adding sufficient dimethyl sulfoxide to bring the final volume to 1 ml. A 450-μl portion of this solution was added with constant stirring, at 4°C, to a 10-ml flask containing the 12-*p*-nitrophenoxycarbonyl derivative of LTB_4-5-benzoate methyl ester (2.46 mg) as a thin film over the walls of the flask. The film disappeared after about 4 hr; after 52 hr, the clear yellow solution was treated with 6 ml of chilled MeOH/0.15 M aqueous K_2CO_3 (1:3). The resulting solution was gently stirred for 60 hr at 25°C to selectively saponify the methyl ester and benzoate groups and then brought to pH 6.8–7.0 by addition of 1 M HOAc. After dilution with 1 ml of H_2O, the solution was chromatographed on Sephadex G-25 equilibrated with H_2O/MeOH, 9:1 (vol/vol). The immunogen-containing fractions, as assessed by UV analysis, were eluted just after the void volume, combined, concentrated to a small volume at reduced pressure, diluted to 15.5 ml with H_2O, and dialyzed against distilled H_2O at 4°C for 18 hr. UV analysis of the dialyzed immunogen at 275 nm (assuming ε = 51,300 for bound LTB_4 and subtracting the albumin absorption, using ε = 35,000 at 275 nm and M_r 60,000) indicated that an average of 10.9 lysine amino groups of albumin, out of a total of 59, were coupled to LTB_4 in the immunogen; i.e., 920 μg of LTB_4 was bound to 15 mg of albumin. The dialyzed solution of immunogen was divided into aliquots and sealed in vials under argon and stored at 4°C until use.

Five 5-month-old New Zealand White rabbits each received intramuscular injections of 1 mg of LTB_4-albumin conjugate in complete Freund's adjuvant, followed by subcutaneous and intramuscular injections 3 wk later with a total of 500 μg of the conjugate in adjuvant. The rabbits were bled 10 days later. Blood was collected in citric acid/citrate/dextran and centrifuged at 400 × *g*, and the plasma was separated. The single rabbit producing anti-LTB_4 antibodies was injected subcutaneously and intramuscularly with 500 μg of the same immunogen preparation at 8 and 12 wk and was bled twice weekly until the antibody titer fell by 50%.

RIA for LTB_4. The RIA was carried out in 3.5-ml polypropylene test tubes (Sarstedt, Princeton, NJ). The diluent for all reagents was Tris isogel buffer (0.1 M Tris·HCl, pH 7.4/0.14 M NaCl/0.1% gelatin). [14,15-^{3}H]LTB_4, appropriately diluted immune rabbit plasma to LTB_4-albumin, and either standard compounds or unknown samples were combined in test tubes in a total volume of 250 μl with mixing after each addition; the reaction mixtures were incubated at 37°C for 60 min. A 100-μl portion of goat anti-rabbit IgG plasma (previously titrated to antibody excess with respect to the rabbit IgG in the immune plasma and in the nonimmune plasma control) was added, the mixture was shaken, and the rabbit IgG–goat anti-rabbit IgG complex was precipitated overnight at 4°C. The immunoprecipitates were centrifuged at 1,500 × *g* for 60 min at 4°C, and the supernatant fluids were decanted. The precipitates were dissolved in 200 μl of 0.1 M NaOH and 2.5 ml of scintillation fluid was added to each tube. The tubes were stoppered with polyethylene push-in stoppers (Sarstedt) and thoroughly mixed, and the radioactivity was measured in a liquid scintillation counter (Mark III, Tracor Analytic, Elk Grove, IL) with an efficiency of 70%.

Isolation and Activation of Cells. Rat serosal mast cells were isolated by lavage of the pleural and peritoneal cavities and purified to >97% purity with isopyknic and velocity gradients in metrizamide solutions as described (23). Replicate samples of 10^6 cells were suspended in 0.4 ml Tyrode's solution/0.1% gelatin, equilibrated for 5 min at 37°C, and then challenged in duplicate tubes with the divalent cation ionophore A23187 at final concentrations of up to 2.5 μM for 20 min at 37°C. Time-course experiments were carried out under the same conditions at intervals of up to 20 min with 1 μM A23187. The reactions were terminated by addition of EDTA (pH 7.4) to a final concentration of 2 mM and by sedimentation of the cells at 400 × *g* for 5 min at 25°C. The supernatants were stored under argon at −70°C until assayed for LTB_4.

Neutrophils from healthy human volunteers were harvested from venous blood, which was collected into citric acid/citrate/dextran and allowed to sediment over 2 hr at 25°C. The leukocyte-containing plasma layer was aspirated, and the cells were sedimented at 400 × *g* for 10 min at 25°C. The cells were then suspended in 9 ml of distilled H_2O and immediately treated with 3 ml of 0.6 M KCl, with rapid Vortex mixing after each step to lyse the remaining erythrocytes. This procedure was repeated, and the cells were then suspended in calcium-free Hanks' balanced salt solution at a final concentration of 5×10^7/ml. Then, 2-ml portions were overlayered on 3 ml of Ficoll/Hypaque, and the neutrophils were purified to >97% by sedimentation through the Ficoll/Hypaque cushions at 400 × *g* for 30 min at 25°C (7). The neutrophils (2×10^8) were suspended in 10 ml of phosphate-buffered saline/0.8 mM $CaCl_2$, pH 7.4, warmed to 37°C over 5 min, and then challenged by addition of A23187 to a final concentration of 2 μM. After incubation at intervals of up to 5 min, 2-ml portions were removed from the cell suspension into precooled test tubes in an ice bath and sedimented at 400 × *g* for 5 min at 4°C. Portions of these supernatants were stored under argon at −70°C until assayed for LTB_4.

RP-HPLC. Five replicate samples of 1×10^6 rat mast cells in a final volume of 0.5 ml were each challenged for 20 min with 1 μM A23187 at 37°C and the spun supernatants were combined and mixed with 4 vol of EtOH for 30 min at 4°C. This mixture was then centrifuged at 1,500 × *g* for 10 min at 4°C to remove precipitated proteins. The supernatant was evaporated to dryness at reduced pressure, dissolved in 1 ml of MeOH/H_2O, 1:1 (vol/vol), and injected for resolution by RP-HPLC.

A 1.8-ml portion of each cell-free supernatant from one of the two time-course studies of LTB_4 generation by human neutrophils was extracted by a modification of the method of Bligh and Dyer (24). The supernatants were adjusted to pH 3 by dropwise addition of 2 M citric acid, followed by addition of 6.6 ml of MeOH/$CHCl_3$, 2:1 (vol/vol) to each. After sedimentation at 400 × *g* for 5 min at 25°C, the supernatant from each sample tube was mixed with 17.6 ml of MeOH/$CHCl_3$/H_2O, 4.5:6.8:6.3, (vol/vol) and allowed to form two phases. The extractable lipids partitioned into the lower (organic) phase, which was separated and washed with 1 vol of saturated NaCl solution and dried with anhydrous Na_2SO_4, and the solvent was removed under a stream of nitrogen. The dried material was dissolved in 1 ml of MeOH/H_2O, 1:1 (vol/vol) and injected for resolution by RP-HPLC.

RP-HPLC was carried out on an $Altex_{C18}$ column at a flow rate of 1 ml/min in an isocratic solvent of MeOH/H_2O/HOAc, 65:34.9:0.1 (vol/vol), pH 5.6. The A_{269} value was monitored on line and the peak absorbance areas were integrated on a Shimadzu C RIA Data Processor (Kyoto, Japan). One-milliliter fractions of the eluate were evaporated to dryness in a Speed Vac Concentrator (Savant Products, Hicksville, NY); the

residues were dissolved in Tris isogel buffer/EtOH, 97.5:2.5 (vol/vol) for radioimmunoassay of the sulfidopeptide leukotrienes (12) and of LTB_4.

RESULTS

Coprecipitation of [14,15-^{3}H]LTB_4 with the anti-LTB_4 rabbit plasma goat–anti-rabbit IgG complex increased as the volume of rabbit anti-LTB_4 plasma increased (Fig. 1). Of the 5,925 cpm of [14,15-^{3}H]LTB_4 added in two experiments, an average of 868 cpm (15%) was specifically bound by 10 μl of immune rabbit plasma/goat anti-rabbit IgG.

The coprecipitation of various amounts of [14,15-^{3}H]LTB_4 by 10 μl of the rabbit anti-LTB_4 plasma was analyzed in two separate experiments and is presented in Fig. 2 as a reciprocal plot containing all data points from both experiments. The combining sites of the antibodies in 10 μl of the immune plasma were saturated with 2,050 cpm of [14,15-^{3}H]LTB_4. At a counting efficiency of 70%, 10 μl of the immune plasma contained 0.024 pmol of [14,15-^{3}H]LTB_4-binding IgG molecules and each ml of undiluted rabbit immune plasma contained 2.4 pmol (0.37 μg) of specific antibody. An average association constant, K_a, was calculated to be 3.2×10^9 M^{-1} at 37°C.

Serologic Specificity of the [14,15-^{3}H]LTB_4–Anti-LTB_4 Reaction. Inhibition of [14,15-^{3}H]LTB_4 binding by several arachidonic acid metabolites was assessed in three separate experiments with [14,15-^{3}H]LTB_4 (5,800–6,025 cpm), 10 μl of rabbit immune plasma, and various amounts of unlabeled test compounds (Fig. 3). The mixtures were incubated as usual in a volume of 250 μl for 60 min at 37°C before addition of the goat anti-rabbit IgG. Fifty percent inhibition (ID_{50}) of binding of [14,15-^{3}H]LTB_4 was achieved with 0.31 ng of LTB_4 and with 1.95 ng of (5*S*,12*S*)-6-*trans*-8-*cis*-LTB_4. At 10 ng, inhibition by a racemic mixture of (5*S*,12*S*)- and (5*S*,12*R*)-6-*trans*-LTB_4 was only 19%, by LTC_4 was 28%, and by LTD_4 was 15%; and 5-HETE, PGD_2, PGE_2, and $PGF_{2\alpha}$ did not inhibit.

Generation of LTB_4 from Rat Mast Cells and Human Neutrophils. In four experiments, 1×10^6 rat serosal mast cells generated increasingly large quantities of LTB_4, as measured by RIA, as the concentration of A23187 increased (up to 1 μM).

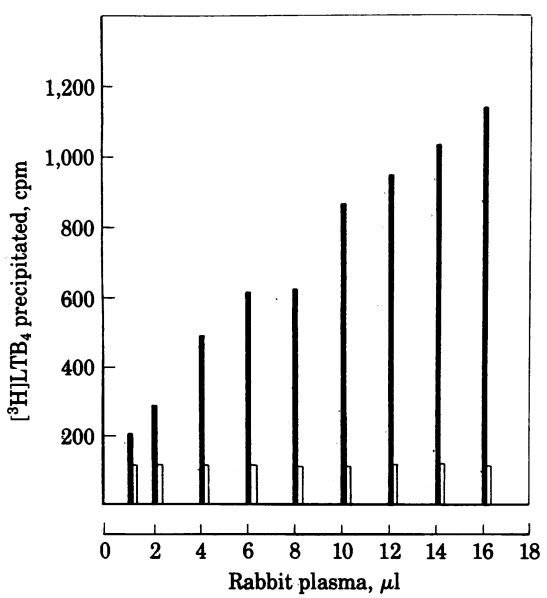

FIG. 1. Coprecipitation of [^{3}H]LTB_4 by various quantities of anti-LTB_4 rabbit plasma (■) or nonimmune rabbit plasma (□) complexed with goat anti-rabbit IgG. Each point is the mean of duplicate analyses, which varied by 3.0 ± 2.5% (mean ± SD, n = 9).

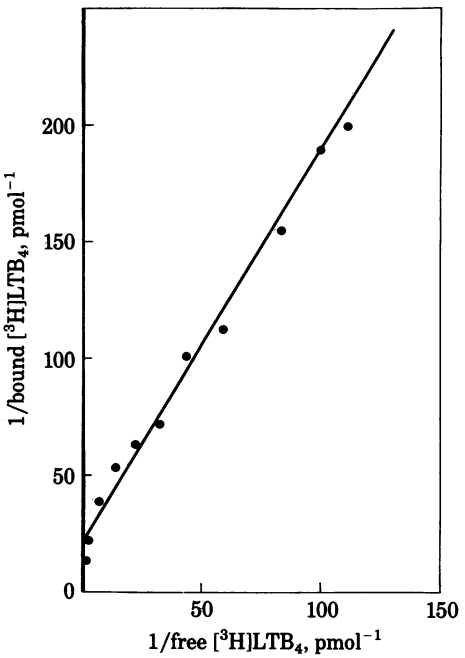

FIG. 2. Coprecipitation of various amounts of [^{3}H]LTB_4 as a function of pmol added to 10 μl of rabbit anti-LTB_4 plasma. Each point in the double reciprocal plot is the mean of duplicate analyses and all points are included from two separate experiments. The duplicate values differed by 5.0 ± 2.9% (mean ± SD, n = 11).

Maximal biosynthesis of LTB_4 was 4.63 ± 1.02 ng per 10^6 cells (mean ± SD) (Fig. 4*A*). At all tested ionophore concentrations, the combined generation of LTC_4, LTD_4, and LTE_4 was below the limits of detection (0.1 ng per 10^6 cells) for the class-specific sulfidopeptide leukotriene RIA (12).

In three additional experiments, 1×10^6 rat mast cells were activated with 1 μM A23187 for times up to 20 min. LTB_4 generation increased over the initial 5 min after challenge and then plateaued. Maximal LTB_4 biosynthesis in these experiments was 4.07 ± 1.03 ng per 10^6 cells (mean ± SD) (Fig. 4*B*).

In two experiments, human neutrophils at 2×10^7/ml were activated with 2 μM A23187 for periods of up to 5 min, and the LTB_4 was measured directly from the supernatants by RIA. The immunoreactive LTB_4 increased over time up to 2 min in each experiment. Maximal generation of LTB_4 for the cells from two

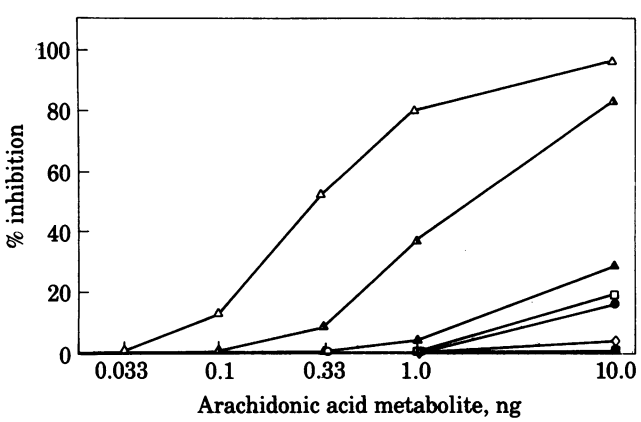

FIG 3. Inhibition of [^{3}H]LTB_4 rabbit anti-LTB_4 binding by LTB_4 (△); (5*S*,12*S*-6-*trans*-8-*cis*-LTB_4 (▲); LTC_4 (▲); racemic 6-*trans*-LTB_4 (□); LTD_4 (●); 5-HETE (◇); and PGD_2, PGE_2, and $PGF_{2\alpha}$ (■). Each point represents mean inhibition of binding for duplicate analyses; the duplicate values agreed to within 5.5 ± 4.7% (mean ± SD, n = 98).

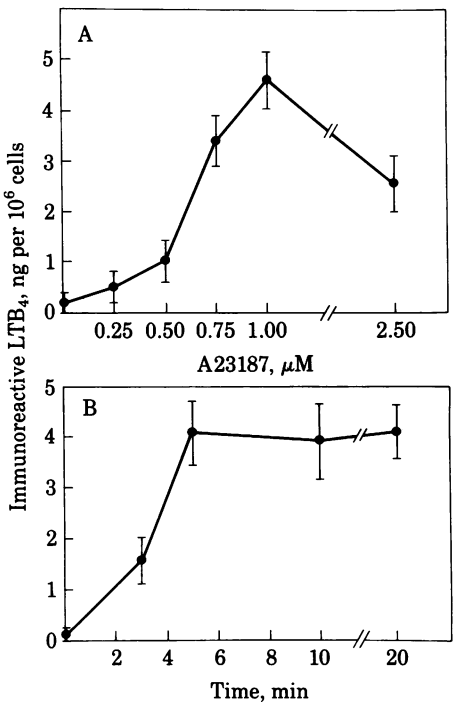

FIG. 4. (*A*) Dose-dependent generation and release of immunoreactive LTB_4 from purified rat serosal mast cells stimulated with A23187 for 20 min at 37°C. Results are mean ± SD of four experiments. (*B*) Time-dependent generation and release of immunoreactive LTB_4 from purified rat serosal mast cells stimulated with 1 μM A23187 at 37°C. Results are mean ± SD of three experiments.

different donors was 40.0 and 28.7 ng per 10^6 cells, respectively (Fig. 5).

Immunochromatography of LTB_4. Five replicate suspensions of 1×10^6 rat serosal mast cells were activated with 1 μM A23187 for 20 min at 37°C and sedimented, and the pooled supernatants contained 18.5 ng of LTB_4 by RIA. After ethanol precipitation, evaporation, and RP-HPLC, all of the immunoreactive material recognized by the LTB_4 RIA eluted in three tubes, which corresponded to the retention time of authentic LTB_4. In this experiment, the calculated LTB_4 recovered from the HPLC, using the integrated A_{269} value as standardized with known quantities of LTB_4, was 12.5 ng and that calculated from the RIA was 10.0 ng. The overall recovery was 54% as compared with a recovery of 65% on chromatography of 100 ng of synthetic LTB_4 when all measurements were by RIA.

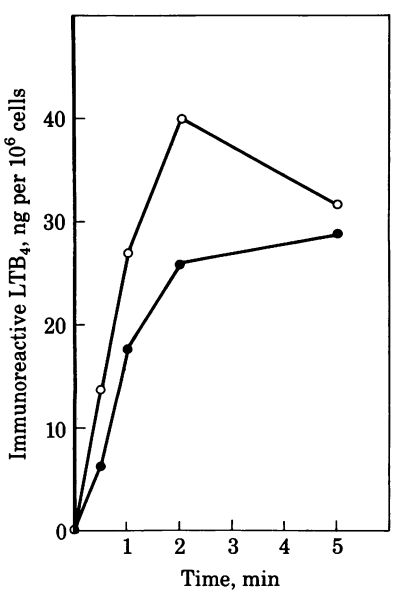

FIG. 5. Time-dependent generation and release of immunoreactive LTB_4 from human neutrophils from two donors (○, ●) stimulated with 2 μM A23187 at 37°C. Results are means of duplicate assays.

RP-HPLC of the supernatants from the time-dependent generation of LTB_4 by human neutrophils (Fig. 5) showed that the anti-LTB_4-reactive products for each interval were present as a single A_{269} peak at the retention time of authentic LTB_4. No immunoreactivity was detectable in the 269 nm-absorbing doublet peak eluting just before LTB_4 and corresponding to the retention time of the 6-*trans*-LTB_4 diastereoisomers or in the elution front, which contains ω-oxidized metabolites of LTB_4 (25). No immunoreactive LTB_4 was detected in the eluted fractions from the zero-time incubation, and the increase of LTB_4 synthesis with time (Fig. 6) was congruent with that assessed before RP-HPLC (Fig. 5). Recoveries of LTB_4, as assayed by RIA and the integrated A_{269} value after extraction and chromatography were 39.2 ± 7.3% (mean ± SD, $n = 4$) and 37.6 ± 13.0% (mean ± SD), respectively.

DISCUSSION

The conjugate through the 12-oxy function of LTB_4 with albumin emulsified in Freund's adjuvant elicited 0.37 μg of specific antibodies/ml of plasma in a single rabbit, with a calculated K_a for [14,15-^{3}H]LTB_4 at 37°C of 3.2×10^9 M^{-1} (Fig. 2). Both the

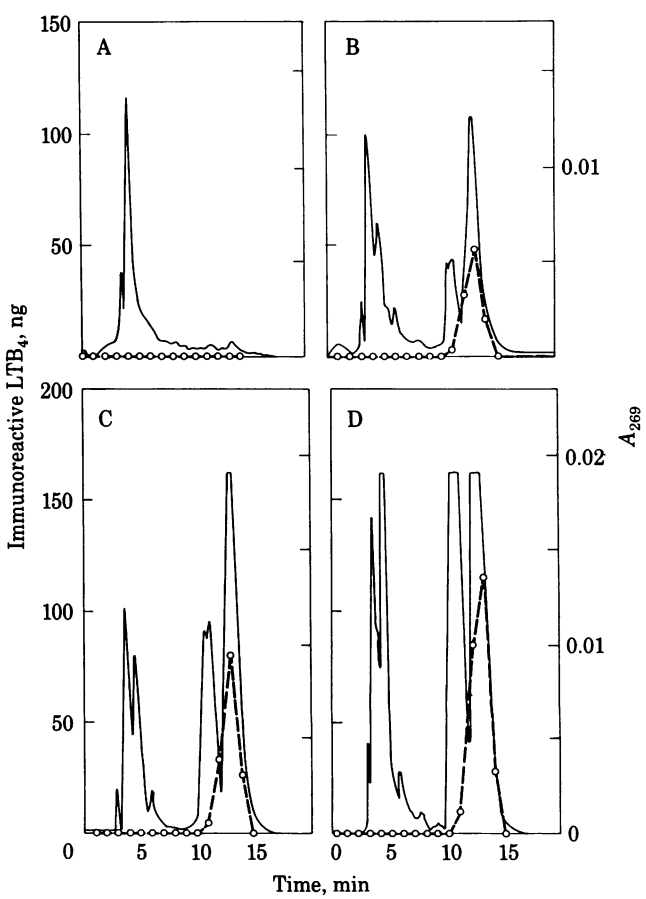

FIG. 6. RP-HPLC of LTB_4 released from human neutrophils after stimulation with 2 μM A23187 at 37°C. Immunoreactive LTB_4 (○---○) and A_{269} (——) values are shown for 0 min (*A*), 0.5 min (*B*), 1 min (*C*), and 5 min (*D*) of incubation.

quantity of antibody per ml and its K_a are comparable with those (0.32 μg and $2.8 \times 10^9\ M^{-1}$, respectively) previously reported for a class-specific rabbit immune plasma raised against a LTD_4-albumin conjugate formed via the icosanoid carboxyl group (12). Of the naturally occurring metabolites of arachidonic acid tested, LTB_4 was the most effective inhibitor of [14,15-^{3}H]LTB_4 binding by rabbit anti-LTB_4 plasma and exhibited a ID_{50} of 310 pg in the RIA (Fig. 3). The immunoreactive role of the C-5 and C-12 hydroxyl groups, with their relative positions determined by the 6-*cis*-8,10-*trans* triene, is indicated by the lack of reactivity of the immune rabbit plasma with PGD_2, PGE_2, and $PGF_{2\alpha}$, the lack of reactivity with 5-HETE, which possesses identical C-1 to C-5 and C-13 to C-20 segments, and the relative lack of reactivity with LTC_4 and LTD_4, both of which possess identical C-1 to C-5 and C-13 to C-20 segments and a conjugated triene unit.

The lack of crossreactivity (<0.1%) of the 12*R* and 12*S* diastereoisomers of 6-*trans*-LTB_4 (10) (Fig. 3) is especially noteworthy, because these substances are rapidly produced from LTA_4 by nonenzymatic hydrolysis (8) and potentially could interfere with analyses for LTB_4. The low affinity of 6-*trans*-LTB_4 for the anti-LTB_4 also indicates a high degree of stereospecificity of the antibody for the C-5 to C-12 segment of the LTB_4 structure. That this specificity is not absolute, however, is indicated by the degree of crossreactivity (16%) observed with (5*S*,12*S*)-di-HETE, an enzymatically produced icosanoid that results from sequential 5- and 12-lipoxygenation (26). The greater affinity of the anti-LTB_4 for (5*S*,12*S*)-di-HETE as compared with the 12*R* and 12*S* diastereoisomers of 6-*trans*-LTB_4 may be related to the fact that the most stable conformers of LTB_4 and (5*S*,12*S*)-di-HETE are nearly superimposable, whereas LTB_4 and the 6-*trans*-LTB_4 diastereoisomers are not (10).

In the present study, the LTB_4 RIA was used to assess LTB_4 generation from rat mast cells after dose-dependent activation by A23187 (Fig. 4*A*) and from rat mast cells and human neutrophils over a time course after activation by A23187 at a single concentration (Figs. 4*B* and 5). The unresolved cell-free supernatants of activated rat serosal mast cells contained a mean maximum of immunoreactive LTB_4 of 4.63 ng per 10^6 cells (Fig. 4*A*) but were devoid of measurable immunoreactive sulfidopeptide leukotrienes (<0.1 ng per 10^6 cells). The LTB_4 RIA showed that the maximal time-dependent generation of LTB_4 from A23187-activated human neutrophils from two different donors was 28.7 and 40.0 ng per 10^6 cells. This quantitation is in general agreement with the values of 16.2 and 20.2 ng per 10^6 A23187-activated human neutrophils, as assessed by gas chromatography/mass spectrometry (8), and is higher than the amounts of 8.9, 8.6, and 6.2 ng per 10^6 cells generated by ionophore-activated horse eosinophils, mouse mastocytoma cells, and rat neutrophils, respectively, and quantitated by UV absorbance after RP-HPLC (27–29).

The identity of the biologically generated immunoreactive material from both rat serosal mast cells and human neutrophils as LTB_4 was established by coelution with synthetic LTB_4 from RP-HPLC and by the correspondence between the measurements by RIA and by integrated UV absorbance after RP-HPLC. Immunochromatography of the biologically generated material from each cell type further indicated the specificity of the RIA in that the antibody did not recognize other peaks of absorbance at 269 nm, particularly including those corresponding in retention time to the diastereoisomers of 6-*trans*-LTB_4 (Fig. 6), or those at shorter retention times, which are reported to include ω-oxidation products of LTB_4 (25). The combination of a high degree of specificity and correspondence to a physical method of LTB_4 measurement indicates that, as compared with UV absorbance and chemotaxis assays, the RIA can be used to quantitate LTB_4 generation without prior chromatography.

This work was supported by Grants AI-07722, AI-10356, AM-05577, HL-17382, and RR-05669 from the National Institutes of Health; a grant from the New England Peabody Home for Crippled Children; a grant from the Lillia Babbitt Hyde Foundation; and a grant from the National Science Foundation. R.A.L. is the recipient of Allergic Diseases Academic Award AI-00399 from the National Institutes of Health. J.-M.M.-H. is a Research Fellow of the Institut National de la Santé et de la Recherche Médicale, France.

1. Borgeat, P., Hambert, M. & Samuelsson, B. (1976) *J. Biol. Chem.* **251**, 7816–7820, and correction (1977) **252**, 8772.
2. Murphy, R. C., Hammarström, S. & Samuelsson, B. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 4275–4279.
3. Lewis, R. A., Austen, K. F., Drazen, J. M., Clark, D. A., Marfat, A. & Corey, E. J. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 3710–3714.
4. Morris, H. R., Taylor, G. W., Piper, P. J. & Tippins, J. R. (1980) *Nature (London)* **285**, 104–105.
5. Orning, L., Hammarström, S. & Samuelsson, B. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 2014–2017.
6. Lewis, R. A., Drazen, J. M., Austen, K. F., Clark, D. A. & Corey, E. J. (1980) *Biochem. Biophys. Res. Commun.* **96**, 271–277.
7. Goetzl, E. J. & Pickett, W. C. (1980) *J. Immunol.* **125**, 1789–1791.
8. Borgeat, P. & Samuelsson, B. (1979) *J. Biol. Chem.* **254**, 2643–2646.
9. Corey, E. J., Marfat, A., Goto, G. & Brion, F. (1980) *J. Am. Chem. Soc.* **102**, 7984–7985.
10. Lewis, R. A., Goetzl, E. J., Drazen, J. M., Soter, N. A., Austen, K. F. & Corey, E. J. (1981) *J. Exp. Med.* **154**, 1243–1248.
11. Lewis, R. A. & Austen, K. F. (1981) *Nature (London)* **293**, 103–108.
12. Levine, L., Morgan, R. A., Lewis, R. A., Austen, K. F., Clark, D. A., Marfat, A. & Corey, E. J. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 7692–7696.
13. Razin, E., Mencia-Huerta, J.-M., Lewis, R. A., Corey, E. J. & Austen, K. F. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 4665–4667.
14. Lee, C. W., Lewis, R. A., Corey, E. J., Barton, A., Oh, H., Tauber, A. I. & Austen, K. F. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 4166–4170.
15. Lee, C. W., Lewis, R. A., Corey, E. J. & Austen, K. F. *Immunology*, in press.
16. Borgeat, P. & Samuelsson, B. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 2148–2152.
17. Klickstein, L., Shapleigh, C. & Goetzl, E. J. (1980) *J. Clin. Invest.* **66**, 1166–1171.
18. Goetzl, E. J. & Pickett, W. C. (1981) *J. Exp. Med.* **153**, 482–487.
19. Corey, E. J., Marfat, A., Munroe, J. E., Kim, K. S., Hopkins, P. B. & Brion, F. (1981) *Tetrahedron Lett.* **22**, 1070–1080.
20. Corey, E. J., Clark, D. A., Goto, G., Marfat, A., Mioskowski, C., Samuelsson, B. & Hammarström, S. (1980) *J. Am. Chem. Soc.* **108**, 1436–1439.
21. Rådmark, O., Malmsten, C., Samuelsson, B., Clark, D. A., Goto, G., Marfat, A. & Corey, E. J. (1980) *Biochem. Biophys. Res. Commun.* **92**, 954–961.
22. Corey, E. J. & Hashimoto, S. (1981) *Tetrahedron Lett.* **22**, 299–302.
23. Lewis, R. A., Holgate, S. T., Roberts, L. J., II, Maguire, J. F., Oates, J. A. & Austen, K. F. (1979) *J. Immunol.* **123**, 1663–1668.
24. Bligh, E. G. & Dyer, W. J. (1959) *Can. J. Biochem. Physiol.* **37**, 911–917.
25. Hansson, C., Lindgren, J. A., Dahlén, S. E., Hedqvist, P. & Samuelsson, B. (1981) *FEBS Lett.* **130**, 107–112.
26. Borgeat, P., Fruteau de Laclos, B., Picard, S., Drapeau, J., Vallerand, P. & Corey, E. J. (1982) *Prostaglandins* **23**, 713–724.
27. Jorg, A., Henderson, W. P., Murphy, R. C. & Klebanoff, S. J. (1982) *J. Exp. Med.* **155**, 390–402.
28. Murphy, R. C., Pickett, W. L., Culp, B. R. & Lands, W. E. M. (1981) *Prostaglandins* **22**, 613–622.
29. Ford-Hutchinson, A. W., Bray, M. A., Cunningham, F. M., Davidson, E. M. & Smith, M. J. H. (1981) *Prostaglandins* **21**, 143–152.