Leukotriene B4 Stimulates Rac-ERK Cascade to Generate Reactive Oxygen Species That Mediates Chemotaxis*

Received for publication, May 24, 2001, and in revised form, November 26, 2001
Published, JBC Papers in Press, December 27, 2001, DOI 10.1074/jbc.M104766200

Chang-Hoon Woo‡, Hye-Jin You†‡, Sung-Hoon Cho‡§, Young-Woo Eom‡, Jang-Soo Chun‡, Yung-Joon Yoo§, and Jae-Hong Kim‡¶

From the ‡Graduate School of Biotechnology, Korea University, 5-1 Anam-dong, Seoul 136-701 and the §Department of Life Science, Kwangju Institute of Science and Technology, 1 Oryong-dong, Kwangju 500-712, Korea

Leukotriene B4 is a potent chemoattractant known to be involved mainly in inflammation, immune responses, and host defense against infection, although the exact signaling mechanisms by which it exerts its effects are not well understood. Here we show that exogenous leukotriene B4 induces reactive oxygen species (ROS) generation via a Rac-dependent pathway, and that stable expression of RacN17, a dominant negative Rac1 mutant, completely blocks leukotriene B4-induced ROS generation. In addition, leukotriene B4-induced ROS generation is selectively blocked by inhibition of ERK or cytosolic phospholipase A2, but not p38 kinase, which is indicative of its dependence on ERK activation and synthesis of arachidonic acid. Consistent with these findings, leukotriene B4 Rac-dependently stimulates ERK and cytosolic phospholipase A2 activity, and transient transfection with plasmid expressing RacV12, a constitutively activated Rac1 mutant, also dose-dependently stimulates ERK activity. Our findings suggest that ERK and cytosolic phospholipase A2 are situated downstream of Rac, and we conclude that Rac, ERK, and cytosolic phospholipase A2 all play pivotal roles in mediating the ROS generation that appears to be a prerequisite for leukotriene B4-induced chemotaxis and cell proliferation.

LTs are potent biological mediators of inflammation generated from arachidonic acid via the 5-LO pathway (1, 2). Among them, LTB4 is one of the most potent chemoattractants known, acting mainly on neutrophils and eosinophils, but also on mast cells and endothelial cells (3–5). LTB4 stimulates a number of cellular functions in addition to chemotaxis, including release of lysosomal enzymes and production of ROS (6–8); it also promotes cell adhesion to vascular endothelial cells and transmigration, which amplifies inflammatory responses. Although LTB4-induced leukocyte recruitment is thought to play a protective role in the host defense against various pathogens, it is also involved in the pathogenesis of such inflammatory diseases as bronchial asthma (9, 10), inflammatory bowel diseases (11, 12), and psoriasis (13, 14).

Despite many reports on the cellular functions of LTB4, the exact signaling pathway along which its biological activities are transduced remains largely unknown. It is known, however, that LTB4 acts via two G protein-coupled receptors, BLT1 and BLT2 (15–20). The former is a high affinity LTB4 receptor expressed mainly in polymorphonuclear leukocytes, whereas the latter is a ubiquitous, low affinity receptor whose expression is highest in spleen (17, 18). The details of the cellular functions of BLT1 and BLT2 are still largely unknown. Recently, however, LTB4-induced chemotaxis was shown to be completely inhibited in cells pretreated with PTX (100 ng/ml), indicating the participation of a PTX-sensitive G protein in LTB4 signaling to chemotaxis (16). LTB4 also elicits increases in intracellular free Ca2+ and inositol 1,4,5-triphosphate, but these are apparently not involved in the chemotactic response by LTB4 (15). In addition to the LTs, LTB4 can also bind to and activate the intranuclear transcription factor peroxisome proliferator-activated receptor-α, resulting in the activation of genes that terminate inflammatory processes (21, 22).

We previously observed that LTB4 plays a role in mediating TNF-α-induced ROS generation in Rat-2 fibroblasts (23). LTB4 likewise induces ROS generation in neutrophils, eosinophils, and other fibroblasts (6–8, 23, 24), but the signaling pathway via which this effect is exerted as well as the precise cellular function of the increased ROS levels remains largely unknown. Although NADPH oxidase was proposed to play a role in the generation of ROS in response to LTB4 in eosinophils, the detailed signaling mechanism is still unclear (7). Previously, we and others have shown that Rac, a member of the Rho family GTPases, plays a crucial role in ROS generation in fibroblasts (23–25). Additionally, the generation of ROS by Rac was shown to be mediated mainly by cPLA2-linked cascade (24), suggesting a possible role of cPLA2 as a downstream mediator of Rac in the signaling to ROS generation. Therefore, in an effort to broaden our understanding of LTB4-induced signaling, we studied the pathway via which exogenous LTB4 induces the generation of ROS in Rat-2 fibroblasts. Our results suggest that Rac, ERK, and cPLA2 all play pivotal roles in the LTB4-induced generation of ROS required for the chemotactic activity and proliferation elicited by exogenous LTB4.

* This work was supported by Grant R01-1999-00097 from the Interdisciplinary Research Program of Korea Science and Engineering Foundation, by a grant from the Life Phenomena and Function Research Group program of the Ministry of Science and Technology, by Grant 01-P2-P04-J201P701-0007 from the Korea Health 21 research and development project of the Ministry of Health & Welfare, and by a grant from the Brain Korea 21 program.

‡ To whom correspondence should be addressed: Graduate School of Biotechnology, Korea University, 5-1 Anam-dong, Sungbuk-gu, Seoul 136-701, Korea. Tel.: 82-2-3290-3452; Fax: 82-2-927-9028; E-mail: jhongkim@korea.ac.kr.

§ The abbreviations used are: LT, leukotriene; 5-LO, 5-lipoxygenase; LTB4, leukotriene B4; ROS, reactive oxygen species; BLT, leukotriene B4 receptor; PTX, pertussis toxin; TNF-α, tumor necrosis factor-α; ERK, extracellular signal-regulated kinase; cPLA2, cytosolic phospholipase A2; DPI, diphenylene iodonium; NAC, N-acetylcysteine; FBS, fetal bovine serum; DMEM, Dulbecco’s modified Eagle’s medium; PBS, phosphate-buffered saline; BSA, bovine serum albumin; cyclLT, cysteinyl leukotriene; COX, cyclooxygenase; PAK, p21-activated protein kinase; GST, glutathione S-transferase; PI 3-kinase, phosphatidylinositol 3-kinase; LPA, lysophosphatidic acid; DCF, 2′,7′-dichlorofluorescein; PBD, Pak1-binding domain.
Role of Rac-cPLA2 Cascade in ROS Generation by LTB4

EXPERIMENTAL PROCEDURES

Chemicals and Plasmids—2′,7′-Dichlorofluorescein diacetate was purchased from Molecular Probes. MK-886, genistein, herbimycin, and AACOCF2 were from BIOMOL. LTB4 and cysLTs were from Cayman Chemical Co. LPA, LY294002, wortmannin, DPI, and NAC were from Sigma. FBS, DMEM, phenol red-free DMEM, gentamicin, and nonesSENTIAL amino acids were from Invitrogen. ZK 158252, a specific BLT antagonist, was provided by Dr. Claudia Giesen (Experimental Dermatology, Schering AG, Berlin, Germany). All other chemicals were from standard sources and were molecular biology grade or higher.

PEXV and pEXV-RacC171 plasmids were gifts from Dr. A. Hall (University College, London, United Kingdom).

Cell Culture and DNA Transfection—Rat-2 fibroblasts were obtained from the American Type Culture Collection (ATCC, CRL 1764), and the cells were cultured in DMEM supplemented with 0.1 mM nonessential amino acids, 10% FBS, penicillin (50 units/ml), and streptomycin (50 μg/ml) at 37 °C under a humidified 95%, 5% (v/v) mixture of air and CO2. Stable Rat-2-RacN17 clones expressing Rac1C17, a dominant negative Rac1 mutant, were described previously (23, 26). Transient transfection was carried out by plating 5 × 104 cells in 100-mm dishes for 24 h and then adding calcium phosphate:DNA precipitates prepared with 20 μg of DNA/dish. To opt for variations in transfection number and transfection efficiency, all clones were cotransfected with 1 μg of pCMV-β-GAL, a eukaryotic expression vector containing the Escherichia coli β-galactosidase (lacZ) structural gene under the transcriptional control of the cytomegalovirus promoter. The quantity of DNA used in each transfection was held constant (20 μg) by adding sonicated calf thymus DNA (Sigma). To measure ERK kinase activity with PathDetect trans-reporting substrates (PerkinElmer) and ZEtak fused to a Rac1C17 plasmid was co-transfected with pFR-Luc reporter plasmid according to the manufacturer’s protocol. After incubating with the calcium phosphate:DNA precipitates for 6 h, the cells were rinsed twice with PBS before incubating them in DMEM supplemented with 0.5% FBS for an additional 24 h. Each dish of cells was then rinsed twice with PBS and lysed in 0.1 ml of lysis solution (0.2 M Tris, pH 7.6, plus 0.1% Triton X-100). Supernatants were assayed for luciferase activity as well as protein concentration and β-galactosidase activity.

Measurement of Intracellular H2O2—Intracellular H2O2 was measured as a function of DCF fluorescence using the procedures of Ohba et al. (27). Briefly, cells were grown on coverslips for 2 days and then serum-starved in DMEM supplemented with 0.5% FBS for an additional 2 days. They were then stabilized in serum-free DMEM containing 10% FBS and then exposed to the appropriate agonist for the indicated times. The medium was replaced twice with medium supplemented with 0.1 mM nonessential amino acids, 10% FBS, and then for 1 h with horseradish peroxidase-conjugated secondary antibody prior to development using an ECL kit. Bands corresponding to cPLA2, p38, and ERKs on XAR-5 film (Eastman Kodak Co.) were measured by densitometry.

Translocation of cPLA2—To visualize the localization of endogenous cPLA2, cells were plated on coverslips and grown for 24 h in DMEM containing 10% FBS. They were then washed in serum-free DMEM for 16 h before exposure to an agonist. Thereafter the cells were washed with cold PBS, cells were fixed with 4% paraformaldehyde, permeabilized with 0.1% Triton X-100, blocked in 1% BSA, and labeled with mouse anti-cPLA2 antibody (1:150). The immunolabeled cells were then washed with PBS and labeled with a tetramethylrhodamine B isothiocyanate-conjugated, anti-mouse secondary antibody (1:200). After washing again with cold PBS, the cells were mounted on a slide glass for observation under fluorescence microscope.

Subcellular Fractionation of Cell Lysates—Rat-2 cells were serum-starved in DMEM containing 0.5% FBS for 24 h and then exposed to the appropriate agonist for the indicated times. The medium was replaced twice with medium supplemented with 0.1 mM nonessential amino acids, 10% FBS, and then for 1 h with horseradish peroxidase-conjugated secondary antibody prior to development using an ECL kit. Bands corresponding to cPLA2, p38, and ERKs on XAR-5 film (Eastman Kodak Co.) were measured by densitometry.

Chemotaxis Assay—The chemotactic motility of Rat-2 or Rat2-RacN17 cells was assayed using Transwell chambers with 6.5-mm diameter polycarbonate filters (8-μm pore size). Briefly, the lower surfaces of the filters were coated with 10 μg/ml gelatin (Collaborative Biomedicals) in HEPES-buffered RPMI 1640 medium for 2 h at 37 °C. Dry coated filters containing various amounts of LTb4 were placed in the lower wells of the Transwell chambers, after which 100 μl of Rat-2 or Rat2-RacN17 cells in DMEM containing 1% FBS were loaded into the top wells, yielding a final concentration of 1×105 cells/ml. If necessary, inhibitors were applied to the cells for 20 min at room temperature before seeding. After incubation at 37 °C in 5% CO2 for 3 h, the filters were disassembled, and the upper surface of each filter was scraped free of cells by wiping it with a cotton swab. Cells that had migrated to the underside of the filter were stained with 0.1% crystal violet and harvested by microcentrifugation, and resuspended in 0.2 ml of Rat-2 or Rat2-RacN17 sample was assayed in duplicate, and the assays were repeated twice.

Cell Growth Assay—To assess cell growth, Rat-2 or Rat2-RacN17 cells were plated onto 60-mm plates (106 cells/plate) in DMEM containing 10% FBS. The next day, the medium was replaced with serum-free medium or serum-free medium containing LTb4 or LPA. The number of viable cells was then counted after an additional 48 h.

Data Analysis and Statistics—Data are expressed as means ± S.D. or as percentages ± S.D. of control. Statistical comparisons between groups were made using Student’s t test. Values of p < 0.01 were considered significant.

RESULTS

LTB4 Initiates ROS Generation in Rat-2 Fibroblasts—To assess whether LTB4 induces ROS generation, Rat-2 fibroblasts were serum-starved for 48 h and then exposed to exogenous LTB4 for 3 min. The resultant ROS generation was monitored as a function of H2O2-sensitive DCF fluorescence. As shown in Fig. 1A, 0.3 μm LTb4 elicited a significant (2.5-fold) increase in the levels of ROS; no further increases were seen at LTb4 concentrations up to 1 μm (data not shown). This effect was completely inhibited by ZK158252, a specific BLT antagonist.
Exogenous LTB₄ induces ROS generation in Rat-2 fibroblasts. A and B, Rat-2 cells were serum-starved for 2 days and then exposed to 0.3 μM LTB₄ (3 min), 1 μM cysLTs (3 min) or 20 ng/ml TNF-α (10 min). To assess the effects of PTX inhibition, cells were pretreated with ZK158252 (3 μM) for 30 min before the addition of LTB₄. DCF fluorescence, reflecting the relative levels of ROS (arbitrary units), was imaged using a confocal laser scanning microscope (A) and then quantified as described under “Experimental Procedures” (B). Data are expressed as means ± S.D. (n = 30 cells) from three independent experiments. Statistical significance of ROS measurements was assessed with unpaired t tests (p < 0.01).

It has been reported that BLTs are closely coupled to a PTX-sensitive G protein (15, 16). Consistent with that idea, pretreatment with PTX (100 ng/ml) also completely blocked PTX-sensitive generation of ROS by LTB₄ (15, 16). Consistent with that idea, the ROS response elicited by TNF-α (~2.6-fold increase) was completely abolished by ZK158252 (Fig. 1B), which is consistent with the previous report suggesting the mediatory role of BLTR in TNF-α signaling to ROS generation (23).

Essential Roles of Rac and cPLA₂ in the LTB₄ Signaling to ROS Generation—Previously, we and others have reported that Rac1 plays a crucial role in ROS generation in fibroblasts (23–25). To examine whether Rac1 is involved in LTB₄ signaling to ROS generation, we compared the effects of LTB₄ in Rat-2 and Rat2-RacN¹⁷ cells, which express a dominant negative Rac1 mutant (26). Although substantial ROS generation (a ~2.3-fold increase over control) was observed within 3 min of exposing Rat-2 cells to 0.3 μM LTB₄, little effect was observed in Rat2-RacN¹⁷ cells under the same conditions (Fig. 3A and B). This result prompted us to test directly the extent to which exposure to LTB₄ alters cellular Rac1 activity; we found that indeed LTB₄ induced significant, time-dependent increase in Rac1 activity, consistent with the proposed mediatory role of LTB₄ signaling (Fig. 3C).

We recently reported that cPLA₂ serves as a key downstream mediator of Rac in Rat-2 cells (23, 24, 30, 31). We therefore assessed the effects of AACOCF₃, a specific cPLA₂ inhibitor, on LTB₄-induced ROS generation and found that pretreatment with 10 μM AACOCF₃ almost completely blocked LTB₄-induced ROS generation (~90% inhibition) (Fig. 4A). By contrast, MK-886, a specific 5-LO inhibitor (Fig. 4A), and indomethacin, a nonspecific COX inhibitor, had little or no inhibitory effect (data not shown). It thus appears that activation of cPLA₂, without subsequent metabolism of arachidonic acid by 5-LO or COX, is required for LTB₄-induced ROS generation.

The role of cPLA₂ in LTB₄ signaling was further confirmed by our observation that LTB₄ evoked translocation of cPLA₂ to the membrane compartment (e.g. nuclear envelope area) in Rat-2, but not Rat2-RacN¹⁷ cells (Fig. 4B), as well as time-dependent increases (up to 10 min) in levels of cPLA₂, in the particulate fraction of Rat-2 cell lysates (Fig. 4C). Interestingly, we also observed that, with more prolonged incubations (e.g. 30 min after LTB₄ treatment), the level of cPLA₂ in the particulate fraction was significantly diminished, whereas that in the soluble fraction increased (Fig. 4C).

PTX-sensitive generation of ROS by LTB₄—Serum-starved Rat-2 cells were exposed to 0.3 μM LTB₄ for 5 min in the presence or absence of PTX (100 ng/ml), EGTA (2 mM), U73122 (0.1 μM), or calphostin C (30 nm). Inhibitors were added 20 min prior to the addition of LTB₄, with the exception of PTX, which was added 18 h earlier. DCF fluorescence was quantified as described under “Experimental Procedures”. Data are expressed as means ± S.D. (n = 30 cells) from three independent experiments. Statistical significance of ROS measurements was assessed using unpaired t tests (p < 0.01).

LTB₄-induced ROS Generation Requires ERK—To investigate the possible involvement of MAP kinases in LTB₄ signaling to ROS generation, we tested the effects of 10 μM PD098059, a specific MEK inhibitor, and 10 μM SB203580, a specific p38 kinase inhibitor, on the production of intracellular ROS. We found that, although pretreatment with the former inhibited LTB₄-induced ROS generation, pretreatment with the latter did not (Fig. 5A). This finding suggested the presence of ERK in the LTB₄ signaling pathway, which prompted us to test whether ERK is indeed activated in cells exposed to LTB₄. We found that exposing serum-starved Rat-2 cells to 0.3 μM LTB₄ for 5 min significantly elevated levels of the activated (phosphorylated) ERK form in manner that was entirely dependent on Rac activity, as the effect was absent in Rat2-RacN¹⁷ cells (Fig. 5B). Addition of epidermal growth factor, by contrast, evoked virtually the same level of ERK activation in both Rat-2 and Rat2-RacN¹⁷ cells. ERK activation was also dose-dependently elevated by transient transfection with plasmids expressing RacV¹², a constitutively active form of Rac1 (Fig. 5C). Similarly, RacV¹² elevated Elk-luciferase activity in a dose-dependent manner (Fig. 5D), confirming the ERK activation by Rac1.

PI 3-Kinase Activity Is Required for the LTB₄ Signaling to ROS—To investigate further mediators involved in the LTB₄-induced ROS generation, we assessed the effects of inhibitors of PI 3-kinase on production of intracellular ROS. As shown in Fig. 6A, LTB₄-evoked ROS generation was completely blocked...
by specific PI 3-kinase inhibitor LY294002 (10 μM) or wortman-
in (50 nM). Besides PI 3-kinase, we observed that tyrosine
kinase inhibitors, herbimycin and genistein, also dramatically
diminished LTB4-evoked ROS generation. Moreover, when we
tested whether LTB4-induced ERK activation is affected by PI
3-kinase inhibition, we found the effect of LTB4 on ERK to be
highly dependent on PI 3-kinase and tyrosine kinase(s) activi-
ties, as ERK activation was clearly diminished by pretreatment
with LY294002, wortmannin (Fig. 6B), herbimycin, or genis-
tein (data not shown). Administration of 1 μM LTB4-APA, a
specific BLT antagonist, confirmed that LTB4 elicited ERK
activation via BLT (Fig. 6B). Together, our results strongly
suggest the mediatory roles of PI 3-kinase and tyrosine ki-
nase(s) in the LTB4 signaling pathway to ROS production,
acting upstream of ERK. This result is consistent with our previ-
sor report (32), suggesting the role of PI 3-kinase acting
upstream of Rac1 in Rat-2 fibroblasts. Additionally, PI 3-kinase
or tyrosine kinase activities were shown to be essential for
mediating the chemotaxis in response to LTB4 (8, 16).

**DISCUSSION**

The present study demonstrates the central role played by a
Rac-linked cascade in LTB4-signaling to ROS generation in
Rat-2 fibroblasts. Supporting that conclusion are the observa-
tions that exposure to LTB4 stimulates Rac activity and that
stable expression of RacN17 dramatically inhibits LTB4-evoked
ROS generation. In addition, our findings suggest that ERK
and cPLA2 are situated downstream of Rac1, mediating LTB4
signaling to ROS generation.

Levels of ERK and cPLA2 activation were both Rac-depend-
tently increased following treatment with LTB4, as demon-
strated by ERK phosphorylation and translocation of cPLA2 to
the nuclear envelope (Figs. 4B and 5B). It was previously

**FIG. 3.** LTB4 stimulates ROS generation via a Rac-dependent pathway. A, serum starved Rat-2 or Rat2-RacN17 cells were exposed to LTB4 for 3 min, after which DCF fluorescence was imaged using a confocal laser scanning microscope. B, DCF fluorescence levels reflecting of the relative levels of ROS (arbitrary units) quantified as described under “Experimental Procedures.” Data are expressed as means ± S.D. (n = 30 cells) from three independent experiments. Statistical significance of ROS measurements was assessed with unpaired t tests (p < 0.01). C, Rat-2 cells were serum-starved for 36 h prior to exposure to 0.3 μM LTB4 for the indicated times. Cell lysates were incubated with GST-PAK-PBD coupled to glutathione-agarose beads. Bound Rac-GTPase was eluted, resolved by 15% SDS-PAGE, and transferred to a polyvinylidene difluoride membrane, which was then probed with an anti-Rac1 antibody. The results shown are representative of at least three independent experiments.

**FIG. 4.** Rac-mediated cPLA2 activation is critical for LTB4 signaling to ROS generation. A, serum-starved Rat-2 cells were pretreated with 10 μM AOCOCF3 or 50 nM MK886 and then exposed to 0.3 μM LTB4. DCF fluorescence was quantified as described under “Experimental Procedures.” Data are expressed as means ± S.D. (n = 30 cells) from three independent experiments. Statistical significance of ROS measurements was assessed with unpaired t tests (p < 0.01). B, images of cells exposed to 0.3 μM LTB4 for 10 min and then labeled first with anti-cPLA2 antibody and then with a tetramethylrhodamine B isothiocyanate-conjugated anti-mouse secondary antibody. C, Rat-2 cells were exposed to 0.3 μM LTB4 for the indicated times (0, 5, 10, and 30 min) and lysed, after which the cytosolic and particulate fractions were prepared as described under “Experimental Procedures.” Cell lysates were analyzed for the level of cPLA2 by Western blotting of equal amounts of cellular protein. The results shown are representative of at least three independent experiments. The relative intensity was measured and expressed as percentages ± S.D. of control from three independent experiments.
shown that cPLA₂ translocation from cytosol to particulate fraction represents an activation of cPLA₂ (33, 34). Furthermore, expression of constitutively active Rac¹²² also elicited dose-dependent activation of ERK (Fig. 5, C and D). Consistent with our findings are earlier reports of increased ERK activity in response to LTB₄ and of ERK's involvement in the LTB₄ signaling to eosinophil activation (7, 8, 35, 36). cPLA₂ mediates a variety of cellular activities (e.g. stimulation of c-fos serum response element or c-Jun amino-terminal kinase, among others) induced by Rac activation, suggesting stimulation of cPLA₂ by Rac1 (23, 24, 30, 31). Most germane to the present study, Rac-dependent ROS generation was shown to be mediated largely by a cPLA₂-linked cascade (24). The downstream signaling pathway via which cPLA₂ activation leads to ROS generation is completely unknown, however. Nevertheless, because no detectable inhibition of LTB₄-induced ROS generation was observed with treatment with MK-886 or indomethacin, we predict that eicosanoid synthesis by 5-LO or COX is likely not involved.

Our results clearly demonstrate that EGTA has no inhibitory
Throughout this experiment, LTB4 showed pharmacological properties distinct from those of BLT2. BLT-specific primers (39) (data not shown). In addition, the expression of BLT2 was well detected in Rat-2 fibroblasts as well as other cell lines, including A549 epithelial cells using RT-PCR analysis with each primer. This is because, whereas BLT1 expression has not been detected in Rat-2 fibroblasts, we suspect that BLT2 occupation is possibly involved.

LTB4 evokes rapid, transient increases in Ca\(^{2+}\) mobilization in the presence of 0.3 \(\mu\)M LTB4 in the presence and absence of 2 \(\mu\)M DPI or 2 mM NAC. Numbers of viable cells were counted 48 h later. The results are expressed as percentages \(\pm\) S.D. of control from three independent experiments. Statistical significance of growth measurements was assessed using unpaired \(t\) tests \((p < 0.01)\). Rat-2 or Rat2-Rac\(^{N17}\) cells were plated on 60 mm plates for 24 h and then incubated with buffer alone or containing 0.3 \(\mu\)M LTB4 or 10 \(\mu\)M LPA. Numbers of viable cells were counted 48 h later. The results are expressed as percentages \(\pm\) S.D. of control from three independent experiments.

LTB4-induced ROS generation was completely abolished by pretreatment with ZK 158252, a potent inhibitor antagonizing both BLT1 and BLT2 (17). The action of ZK 158252 was quite specific to the LTB4 receptor, as ROS generation elicited by an LTC4/D4/E4 mixture was not affected by this compound (Fig. 1). In any event, consistent with the proposed action of BLT as a receptor for LTB4 in Rat-2 fibroblasts, we observed that ROS generation by LTB4 is completely abolished by pretreatment with ZK 158252, a potent inhibitor antagonizing both BLT1 and BLT2 (17). The action of ZK 158252 was quite specific to the LTB4 receptor, as ROS generation elicited by an LTC4/D4/E4 mixture was not affected by this compound (Fig. 1). In support of our finding, increasing evidence points to the existence of a signaling link between LTB4 and ROS generation. For example, Li et al. (6) and Lindsay et al. (7, 8) showed that LTB4 activates ROS generation in neutrophils and eosinophils (6–8), respectively. Further, NADPH oxidase appears to be involved in LTB4 signaling to H2O2 generation in guinea pig eosinophils (7), and, although details of the mechanism are still unclear, cPLA2 appears to play an essential role in the activation of NADPH oxidase in human phagocyte myeloid cells (41).

The generation of ROS in response to LTB4 does not appear to cause cytotoxicity. Usually, production of ROS in non-phagocytes is only 1–2% of that seen in phagocytes, which produce large amounts of \(O_2^-\) as part of the body’s defense against disease processes (42). Instead, our findings suggest that, at least in fibroblasts, ROS generation elicited by LTB4 functions in the regulation of intracellular signaling cascades leading to chemotaxis and cell proliferation. Future studies aimed at clar-
ifying the linkage between cPLA₂ and ROS will likely be pivotal to our complete understanding of LTB₄-evoked intracellular signaling and inflammatory responses.

Acknowledgment—We thank Dr. Claudia Giesen for providing a BLT antagonist, ZK 158252.

REFERENCES

1. Brock, T. G., McNish, R. W., and Peters-Golden, M. (1995) J. Biol. Chem. 270, 21652–21658
2. Left, A. R. (2000) Am. J. Respir. Crit. Care Med. 161, S125–S132 (review)
3. Mackarel, A. J., Russell, K. J., Brady, C. S., FitzGerald, M. X., and O’Connor, C. M. (2000) Am. J. Respir. Cell Mol. Biol. 23, 154–161
4. Matsukawa, A., Hogaboom, C. M., Lukacs, N. W., Lincoln, P. M., Strieter, R. M., and Kunel, S. L. (1999) J. Immunol. 163, 6148–6154
5. Tager, A. M., Dufour, J. H., Goodarzi, K., Bercuary, S. D., von Andrian, U. H., and Luster, A. D. (2000) J. Exp. Med. 192, 439–446
6. Li, Y., Perrante, A., Pouls, A., and Harvey, D. P. (1996) J. Clin. Invest. 97, 1605–1609
7. Lindsay, M. A., Perkins, R. S., and Giembycz, M. A. (1998) J. Immunol. 160, 4526–4534
8. Lindsay, M. A., Haddad, E. B., Roussel, J., Teixeira, M. M., Hellewell, P. G., Barnes, P. J., and Bailleul, P. G., and Giembycz, M. A. (1998) J. Leukocyte Biol. 64, 555–562
9. Turner, C. R., Breslow, R., Conklyn, M. J., Arndt, C. J., Patterson, D. K., Lopez-Araya, A., Owens, B., Lee, P., Watson, J. W., and Showell, H. J. (1996) J. Clin. Invest. 97, 381–387
10. Shinoh, K., Kaide, K., and Fukumura, M. (1997) Thorax 52, 1024–1029
11. Schmidt, C., Baumeister, B., Kipnowski, J., Schiermeyer-Dunkhase, B., and Vetter, H. (1996) Hepatogastroenterology 43, 1508–1512
12. Endres, S., Lorenz, R., and Loescheke, K. (1999) Curr. Op. Clin. Nutr. Care 2, 117–120
13. Iversen, L., Kragballe, K., and Ziboh, V. A. (1997) Skin Pharmacol. 10, 169–177
14. Van Pelt, J. P., De Jong, E. M., Seijger, M. M., Van Hooijdonk, C. A., De Bakker, E. S., Van Vlijmen, I. M., Parker, G. L., Van Erp, P. E., and Van De Kerkhof, P. C. (1998) Br. J. Dermatol. 139, 396–402
15. Yokomizo, T., Iizumi, T., Chang, K., Taka, W., and Shimizu, T. (1997) Nature J. Biol. 397, 460–464
16. Haribabu, B., Zhlev, D. V., Pringle, B. C., Richardson, R. M., All, H., and Snyderman, R. (1999) J. Biol. Chem. 274, 37087–37092
17. Yokomizo, T., Kato, K., Terawaki, K., Iizumi, T., and Shimizu, T. (2000) J. Exp. Med. 192, 421–431
18. Toda, A., Yokomizo, T., Masuda, K., Nakao, A., Iizumi, T., and Shimizu, T. (1999) Biochem. Biophys. Res. Commun. 262, 806–812
19. Kamohara, M., Takasaki, J., Matsumoto, M., Saito, T., Ohishi, T., Ishii, H., and Furuuchi, K. (2000) J. Biol. Chem. 275, 27090–27094
20. Martin, V., Ronde, P., Unett, D., Wong, A., Hoffman, T. L., Edinger, A. L., Doms, R. W., and Funk, C. D. (1999) J. Biol. Chem. 274, 8579–8603
21. Devchand, P. R., Keller, H., Peters, J. M., Vazquez, M., Gonzalez, F. J., and Wahl, W. (1996) Nature 384, 39–43
22. Chinetti, G., Fruchart, J.-C., and Staels, B. (2000) Inflamm. Res. 49, 479–505
23. Woo, C.-H., Eom, Y.-W., Yoo, M.-H., You, H.-J., Han, H.-J., Song, W.-K., Yoo, Y.-J., Chun, J.-S., and Kim, J.-H. (2000) J. Biol. Chem. 275, 32357–32362
24. Woo, C.-H., Lee, Z.-W., Kim, B.-C., Ha, K.-S., and Kim, J.-H. (2000) Biochem. J. 348, 525–530
25. Sundaresan, M., Yu, Z. X., Ferrans, V. J., Salcine, D. J., Gutkind, J. S., Iriani, K., Goldschmidt-Clermont, P. J., and Finkel, T. (1996) Biochem. J. 318, 379–382
26. Kim, B.-C., Yose, Y.-J., Yi, S.-J., Shin, I.-C., Ha, K.-S., Hwang, S.-B., and Kim, J.-H. (1999) Mol. Cells 8, 90–95
27. Ohno, M., Shihanuma, M., Kuruki, T., and Nose, K. (1994) J. Cell Biol. 126, 1079–1088
28. Akasaki, T., Koga, H., and Sumimoto, H. (1999) J. Biol. Chem. 274, 18655–18659
29. Devchand, P. R., Hibi, A. K., Perroud, M., Scheunig, W. D., Spiegelman, B. M., and Wahl, W. (1999) J. Biol. Chem. 274, 23341–23348
30. Kim, B.-C., and Kim, J.-H. (1997) Biochem. J. 326, 333–337
31. Yokomizo, T., Izumi, T., and Shimizu, T. (2001) J. Biol. Chem. 276, 431–437
32. Kim, B.-C., Yi, S.-J., Yi, S.-J., Shin, I.-C., Ha, K.-S., Hwang, S.-B., and Kim, J.-H. (2001) Mol. Cells 10, 90–95
33. Schievella, A. R., Regier, M. K., Smith, W. L., and Lin, L.-L. (1996) J. Biol. Chem. 270, 30749–30754
34. Schonhardt, T., and Ferber, E. (1987) Biochem. Biophys. Res. Commun. 149, 769–775
35. O’Donnell, V. B., Spychler, S., and Azzi, A. (1995) Biochem. J. 310, 133–141
36. Araki, R., Komada, T., Nakatani, K., Naka, M., Shima, T., and Tanaka, T. (1995) Biochem. Biophys. Res. Commun. 210, 837–843
37. Perkins, R. S., Lindsay, M. A., Barnes, P. J., and Giembycz, M. A. (1995) Biochem. J. 310, 795–806
38. Subramanian, N. (1992) Biochem. Biophys. Res. Commun. 187, 670–675
39. Yokomizo, T., Iizumi, K., and Shimizu, T. (2001) Life Sci. 68, 2027–2032
40. Yokomizo, T., Kato, K., Hagiya, H., Iizumi, T., and Shimizu, T. (2001) J. Biol. Chem. 276, 12454–12459
41. Cross, A. R., and Jones, O. T. G. (1991) Biochim. Biophys. Acta 1057, 281–298
Leukotriene B₄ Stimulates Rac-ERK Cascade to Generate Reactive Oxygen Species That Mediates Chemotaxis
Chang-Hoon Woo, Hye-Jin You, Sung-Hoon Cho, Young-Woo Eom, Jang-Soo Chun, Yung-Joon Yoo and Jae-Hong Kim

J. Biol. Chem. 2002, 277:8572-8578.
doi: 10.1074/jbc.M104766200 originally published online December 27, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M104766200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 42 references, 22 of which can be accessed free at http://www.jbc.org/content/277/10/8572.full.html#ref-list-1