Published in final edited form as:

Shock. 2011 March ; 35(3): 240–244. doi:10.1097/SHK.0b013e3181faceb3.

Leukotriene B₄ and its Metabolites Prime the Neutrophil Oxidase and Induce Pro-Inflammatory Activation of Human Pulmonary Microvascular Endothelial Cells

John C. Eun, Ernest E. Moore, Anirban Banerjee, Marguerite R. Kelher, Samina Y. Khan, David J. Elzi, Nathan J.D. McLaughlin, and Christopher C. Silliman

The Department of Surgery, Denver Health Medical Center, Denver, CO the Departments of Surgery and Pediatrics, School of Medicine University of Colorado Denver, Aurora, CO and the Research Department, Bonfils Blood Center, Denver, CO

Abstract

Background—Leukotrienes are pro-inflammatory lipid mediators, derived from arachidonic acid via 5-lipoxygenase (5-LO). Leukotriene B₄ (LTB₄) is an effective neutrophil (PMN) chemoattractant, as well as being a major product of PMN priming. LTB₄ is rapidly metabolized into products that are thought to be inactive, and little is known about the effects of LTB₄ on the pulmonary endothelium. We **hypothesize** that LTB₄ and its metabolites are effective PMN priming agents and cause pro-inflammatory activation of pulmonary endothelial cells.

Methods—Isolated PMNs were primed (5 min, 37°C) with serial concentrations 10⁻¹¹–10⁻⁵M of LTB₄ and its metabolites: 6-trans-LTB₄, 20-OH-LTB₄, and 20-COOH-LTB₄ and then activated with fMLP. Primary human pulmonary microvascular endothelial cells (HMVECs) were incubated with these lipids (6 hrs, 37°C, 5% CO₂) and intercellular adhesion molecule-1 (ICAM-1) was measured by flow cytometry. PMN adhesion was measured by myeloperoxidase assays and to ensure that these reactions were specific to the LTB₄ receptors BLT1 and BLT2 were antagonized with CP105,696 (BLT1) or silenced with siRNA (BLT1 and BLT2).

Results—LTB₄ and its metabolites primed PMNs over a wide range of concentrations depending upon the specific metabolite. In addition, at high concentrations these lipids also caused increases in the surface expression of ICAM-1 on HMVECs and induced HMVEC-mediated adhesion of PMNs. Silencing of BLT2 abrogated HMVEC activation and blockade of BLT1 inhibited the observed PMN priming activity. We **conclude** that LTB₄ and its ω-oxidation and non-enzymatic metabolites prime PMNs over a range of concentrations and activate HMVECs. These data have expanded the repertoire of causative agents in ALI and post-injury multiple organ failure.

Keywords

Lipids; ICAM-1; LTB₄ receptors; BLT1; BLT2

INTRODUCTION

Leukotrienes are lipid mediators derived from the metabolism of arachidonic acid by the enzyme, 5-lipoxygenase (5-LO) (1,2). Leukotrienes derive their name from the fact that “leukocytes” were initially recognized as the major source of these compounds as well as the

fact that they all contained a “triene”, 3 conjugated double bonds, in their structure (3,2). After activation by 5-lipoxygenase activating protein (FLAP), 5-lipoxygenase (5-LO) cleaves arachidonic acid resulting in the production of 5-hydroperoxyeicosatetraenoic acid (5-HpETE) which is then cleaved again by 5-LO and FLAP to produce leukotriene A₄ (LTA₄). LTA₄ is then cleaved by either LTA₄ hydrolase to produce leukotriene B₄ (LTB₄) or by LTC₄ synthase to produce or leukotriene C₄ (LTC₄) (1,4,3,2).

LTB₄ signals through either the high affinity receptor BLT1 or the low affinity receptor BLT2 (3,5). Ligand activation by LTB₄ induces PMN adherence to endothelial cells, promotes PMN chemotaxis, stimulates the generation and release of superoxide anion, and increases 5-LO activation in PMNs to produce more LTB₄ (1,2,5). Elevated levels of LTB₄ have also been seen in several inflammatory diseases such as psoriasis, inflammatory bowel disease, and acute respiratory distress syndrome (ARDS) (1,2).

The role of the PMN in acute lung injury (ALI), transfusion-related lung injury (TRALI), and ARDS is a well studied phenomenon and is associated with a two-event hit model of events (6,7,8). The first hit (such as trauma, infection) causes endothelial activation/adherence leading to PMN sequestration in the lung as well as activation or “priming” of the PMN that leads to increased adherence to endothelial cells, and a “hyper functioning” PMN which provides an exaggerated release of antibacterial factors upon activation (6,7,8). A second event, such as sepsis, hypotension, or transfusion, then activates these primed PMNs to cause endothelial damage and capillary leak, resulting in ALI and eventual multi-organ failure (MOF) (6,7,8).

The vascular endothelium is a part of innate immunity and an active participant in PMN:endothelial interactions (9,8). As PMNs move through the pulmonary vasculature, they are slowed in the pulmonary capillaries by the interaction between L-selectin on the PMN and P- and E-selectins on the endothelial cell (9,8). Once a PMN is primed, the L-selectins are shed and the conformation of the β₂-integrins are changed from non-adhesive to adhesive which promotes firm adherence to intercellular adhesion molecule-1 (ICAM-1) or other adhesion molecules on the surface of the activated endothelium (9,8). This pro-inflammatory activation of the vascular endothelium has caused adherence of primed PMNs, also known as pulmonary sequestration. PMNs now either marginate from the vasculature to the tissues in response to a chemotactic gradient to a nidus of infection or a second event may occur, such as infection or transfusion, that causes activation of the PMN’s microbicidal arsenal resulting in endothelial cell damage, papillary leak and organ injury (9,8). LTB₄ induces PMN adherence to Human umbilical vein endothelial cells HUVECs) via intercellular adhesion molecule-1 (ICAM-1); however, LTB₄ receptor activation of HUVECs is controversial in that a number of authors have ascribed the same activity through ligation of both BLT1 and BLT2 (10,11,12,5).

LTB₄ primes PMNs; however, breakdown products along the 5-LO pathway induce priming/activation of PMNs, including: 5-12-dihydroxy-6,8,10,14-eicosatetraenoic acid (5,12-diHETE), a breakdown product of LTA₄, and 5-hydroxy-6,8,11,14-eicosatetraenoic acid (5-HETE), a breakdown product of 5-HpETE (13,14,15) (13,3,15). Furthermore, although the ω-oxidation products of LTB₄, 20-OH-LTB₄ and 20-COOH-LTB₄, are reported ligands of BLT1 on PMNs, they did not activate the NADPH oxidase at the narrow range of concentrations employed (14). We hypothesize that LTB₄ and its metabolites can prime PMNs and induce pro-inflammatory activation of vascular endothelium. Thus, LTB₄ and its metabolites may represent another important mediator in the development of PMN-induced ALI and MOF.

MATERIALS AND METHODS

Materials

Materials—All chemicals, unless otherwise specified, were purchased from Sigma Chemical Corporation, St. Louis, MO, including the endotoxin (LPS from *S. enteritidis*). All solutions and buffers were made from sterile water for human injection, United States Pharmacopeia (USP), or sterile 0.9% saline for intravenous administration, USP, purchased from Baxter Healthcare Corp., Deerfield, NY, as reported previously, followed by sterile filtering with Nalgene™ MF75 series disposable sterilization filter units purchased from Fischer Scientific Corp., Pittsburgh, PA. All lipids were purchased from Cayman Chemical (Ann Arbor, MI) and were isolated by reverse phase high pressure liquid chromatography with ≥97% purity. Human pulmonary microvascular endothelial cells, media and all related reagents were obtained from Lonza Corporation (San Diego, CA). A fluorescein isothiocyanate-linked antibody (clone YN1/1.7.4) to ICAM-1/CD54 and antibodies against BLT1 and BLT2 were purchased from Beckman Coulter (Brea, CA) and Cayman Chemical (Ann Arbor, MI), respectively.

PMN isolation

Blood from healthy volunteers was drawn into a heparinized syringe and the PMNs were isolated by a dextran sedimentation followed by Ficoll gradient centrifugation, and hypotonic lysis of contaminating red blood cells, as previously described (16). The isolated PMNs were then washed once with Kreb's Ringer phosphate with dextrose at pH 7.35 (KRPd) and resuspended at a final concentration of 2.5×10^7 cells/ml (16). The final cell population was >than 99% PMNs by differential staining with >99% viability by trypan blue exclusion (16).

Priming of the PMN oxidase

Superoxide anion generation by PMNs was measured by superoxide dismutase-inhibitable cytochrome *c* reduction at 550 nm in 96-well microplates as described previously (16). Briefly, isolated PMNs (3.75×10^5) were incubated with albumin (control), LTB₄, 20-OH-LTB₄, 20-COOH-LTB₄, or 6-trans-LTB₄ at concentrations from 0.1–10 μM for 5 minutes at 37°C. The respiratory burst was initiated with the addition of 1 μM fMLP and was measured as the superoxide dismutase-inhibitable reduction of cytochrome *c* at 550 nm (nmol O₂^{•−}/min) (16,17). Priming is defined as the augmentation of the albumin-primed, fMLP-activated controls (16,17).

Endothelial cell culture

Human pulmonary microvascular endothelial cells (HMVEC) were grown on 12-well plates in EBM-2 growth media supplemented with EGM-2MV. After confluence was assessed microscopically, the cells were treated with LTB₄, 20-OH-LTB₄, 20-COOH-LTB₄, and 6-trans-LTB₄ at concentrations of 1 and 10 μM for 6 hours (17). The cells were then trypsinized (0.25 mg/ml trypsin + ethylenediaminetetraacetic acid) for 3 to 4 minutes at 37°C. After dissociation from the wells, trypsin neutralizing solution was placed in the wells and the cells were transferred to flow cytometry tubes (17). HMVECs were >99% viable by trypan blue staining (17).

ICAM-1 surface expression

Human microvascular endothelial cells (HMVECs) were incubated with LTB₄, 20-OH-LTB₄, 20-COOH-LTB₄ or 6-trans-LTB₄ for 6 hours at 37°C and 7.5% CO₂. Briefly, the cells were trypsinized, washed (200g for 7 minutes), and incubated with 1 μg of a FITC-labeled monoclonal antibody to ICAM-1 (Beckman-Coulter) for 30 min at 4°C in the dark

(17). The HMVECs were fixed with fresh 5% paraformaldehyde, diluted, and ICAM-1 surface expression was measured by flow cytometry as published (17). The data are presented as the mean fluorescence intensity (MFI) with the isotype fluorescence subtracted from each experimental group prior to any analyses. Importantly, the buffer-treated controls from each group are set at an MFI of 10, and the voltage and other flow parameters remain unchanged for all measurements from this experimental group (17).

PMN Adherence

PMN adherence was measured by measuring the amount of myeloperoxidase activity present in the PMN:HMVEC co-culture (17). Briefly, the plates were forcefully decanted from a height of 100 cm onto absorbent material, the firmly adherent cells were lysed with 1% Triton-X and MPO was measured as previously described (17).

RNA silencing

A vector-based inducible system was purchased from GenScript. Using their design tool, we employed the following 3 sequences to silence BLT2: GGACCATGGAGCTCCGAACCTA, CCTTCTTCAGTTCTAGCGTCA, and CTCTACGTCTTCACCGCTGGA, which was blast filtered prior to use. The GenScript system employs a neomycin resistance gene, and the inducible promoter is the human H1.2, containing the tetracycline operator. The vector also contains a cGFP gene to track transfection efficiency. Transfection was completed following the manufacturer's directions and optimized for HMVECs. Optifect was used as the transfection reagent due to the lower toxicity as compared to Lipofectamine 2000. For further characterization, a disparate mixture of 4 separate siRNAs was employed as a second reagent: LTB₄R2 Accell siRNA SMARTpool with non-targeting siRNAs employed as controls purchased from Dharmacon (Lafayette, CO). For BLT1 silencing, the LTB₄R1 Accell siRNA SMARTpool containing a mixture of 4 separate siRNAs was purchased with non-targeting siRNAs employed as controls purchased from Dharmacon and used as detailed above for LTB₄R2.

Statistics

All data are expressed as the mean $\pm$ the standard error of the mean. Statistical significance was determined by a paired analysis of variance ANOVA followed by a Bonferroni or other *post hoc* test depending upon the equality of variance. When the data were normalized, significance was determined at the $p < .01$ level for normalized data and at the $p < .05$ level for data that were not normalized.

RESULTS

LTB₄ and its derivatives prime PMNs

Human PMNs were incubated with PAF, LTB₄, the LTB₄ metabolites, 20-OH-LTB₄ and 20-COOH-LTB₄ through ω -oxidation, and 6-trans-LTB₄, a non-enzymatic breakdown product of LTA₄. These PMNs were then activated with fMLP and the maximal rate of superoxide was measured by the SOD-inhibitable reduction of cytochrome c and were then compared to the albumin-primed controls. LTB₄ primed the PMN oxidase from 10⁻⁹M to 10⁻⁵M (Table 1). The 20-OH-LTB₄ and the 6-trans-LTB₄ caused significant priming from 10⁻⁹M to 10⁻⁵M and 10⁻⁸M to 10⁻⁵M, respectively. In contrast, the 20-COOH-LTB₄ derivative only primed PMNs at 10⁻⁵M, the highest concentration tested.

LTB₄ and its derivatives activate endothelial cells

HMVECs were incubated with LTB₄, 20-OH-LTB₄, 20-COOH-LTB₄, and 6-trans-LTB₄ at concentrations of 10⁻⁵M–10⁻⁶M for 6 hours at 37°C and 7.5% CO₂. ICAM-1 surface

expression was measured by flow cytometry. At 10 μ M LTB₄ and its metabolites: 6-trans-LTB₄, 20-OH-LTB₄ and 20-COOH-LTB₄ all elicited increased surface expression of ICAM-1 as compared to albumin-treated controls (Fig. 1). However at concentrations $\leq$ 1 μ M LTB₄ or its metabolites were not able to significantly increase ICAM-1 surface expression.

To determine if LTB₄-mediated increases in ICAM-1 resulted in pro-inflammatory activation of HMVECs, the ability of these activated HMVECs to cause PMN adherence was investigated. As compared to vehicle-treated HMVECs, incubation of HMVECs for 6 hours with 10⁻⁵M LTB₄ induced significant PMN adherence as determined by MPO levels: LTB₄ 10.5 $\pm$ 0.5%* vs. albumin-treated controls 5.4 $\pm$ 0.3% and similar to identical HMVECs treated with 2 μ g/ml of LPS 13.2%* (*= p <.05 vs. albumin-treated controls, n =3) (Table 2).

LTB₄ antagonism: native antagonists, receptor blockade and RNA silencing

Lipoxin A₄, a natural antagonist of LTB₄ inhibited both the LTB₄-mediated increase in ICAM-1 on HMVECs by 84 $\pm$ 10% (n =5) and the LTB₄-induced priming activity; LTB₄: 44.1 $\pm$ 7%, 20-OH-LTB₄: 28.6 $\pm$ 3%, 20-COOH-LTB₄: 97 $\pm$ 4%, and 6-trans-LTB₄: 55 $\pm$ 9% (both p <0.05 versus buffer pre-treated controls, n =5) (18,19) (Table 3). In addition, pre-incubation of PMNs with CP-105,696, a BLT1 receptor antagonist, inhibited the priming activity of LTB₄ (79.1 $\pm$ 10%) and all of the metabolites including: 6-trans-LTB₄ (98 $\pm$ 6%), 20-OH-LTB₄ (100 $\pm$ 5%), and 20-COOH-LTB₄ (100 $\pm$ 4%) (p <.05, n =5) (Table 3). In contrast, pre-incubation of HMVECs with CP-105,696 had no effects on the LTB₄-elicited increases in ICAM-1 surface expression (Table 3).

RNA silencing of BLT2, the low affinity LTB₄ receptor, but not the high affinity BLT1 receptor decreased the BLT2 immunoreactivity present in western blots of whole cell HMVEC lysates with an antibody specific for BLT2 (Fig. 2). To confirm that this decrease in immunoreactivity was biologically relevant, these silenced HMVECs were incubated with 10⁻⁵M LTB₄ for 6 hours and the ICAM-1 surface expression was measured by flow cytometry. RNA silencing using two different siRNA's was completed and demonstrated that the LTB₄ induced increase in ICAM-1 was inhibited by the siRNAs alone but even better by the combination (Table 4).

DISCUSSION

These results demonstrate that LTB₄, its ω -oxidation products, 20-OH-LTB₄ and 20-COOH LTB₄, and the non-enzymatic metabolite 6-trans-LTB₄ demonstrated concentration-dependent PMN priming. Because LTB₄ is a well-known agent of inflammation, specifically in the recruitment of PMNs, it is not surprising that LTB₄ primes PMNs at low concentrations. While the metabolites of LTB₄ are not as effective as LTB₄, the inflammatory capacity attributed to LTB₄ may not be mediated by LTB₄ alone; rather, it may be due to a mixture of LTB₄ and its metabolites. These data also imply that the inflammatory stimulation of LTB₄ may extend well beyond its half-life of LTB₄ for the half-life of the metabolites is much longer and that even enzymatic degradation and hydrolysis may not stop the pro-inflammatory effects of these compounds.

Another novel finding in this study is the PMN priming ability of 6-trans-LTB₄, a non-enzymatic breakdown product of LTA₄. This implies that the pro-inflammatory products of the 5-lipoxygenase pathway are not totally dependent on the action of the enzymes 5-LO and LTA₄-hydrolase. In situations, such as hemorrhagic shock where there is an abundance of arachidonic acid (AA) due to increase amounts of secretory phospholipase A₂ (sPLA₂), the pro-inflammatory action of leukotriene synthesis can extend beyond the kinetics of LTA₄-hydrolase action (20). The enzymatic activity of sPLA₂ cleaves AA from the sn-2

position of the phospholipid, thus allowing AA to be further metabolized into leukotrienes, prostaglandins, or other inflammatory mediators (13,4,3). Therefore, these increases in AA may result in increases in pro-inflammatory mediators both through activation of 5-lipoxygenase, e.g. LTB₄, and via non-enzymatic production of such agents through oxidative mechanisms (20).

The LTB₄ and its metabolites also increase CD54 (ICAM-1) surface expression on human pulmonary microvascular endothelial cells (HMVECs). LTB₄ (10 μM) 20-OH-LTB₄, (10 μM) and its metabolites 20-COOH-LTB₄, or 6-trans-LTB₄ significantly increased the surface expression of ICAM-1 on HMVECs. The interaction of β₂-integrin on the PMN and ICAM-1 on the endothelial cell is a critical step in inflammation and is important to clear infection in the affected organ or may predispose the individual to PMN-mediated organ injury (9,8). In addition, LTB₄ and its metabolites caused increased PMN adherence to the EC surface which highlighted the ability to cause PMN sequestration in the lung and the capillary beds of other organs.

Priming of PMNs by LTB₄ and its metabolites occurred through activation of the dominant, chemotactic, high affinity BLT1 receptor as shown by the use of the BLT1 receptor antagonist CP-105,696 (21,10) (22,23), which significantly inhibited their PMN priming activity. These data confirmed that the BLT1 receptor is not specific for LTB₄ alone but demonstrated that receptor activation induced priming of the PMN oxidase (14). In addition, LXA₄, a natural antagonist of LTB₄, significantly inhibited both the PMN priming activity and activation of HMVECs by 50±7% and 42±4%, respectively (24,25). In contrast the BLT1 receptor antagonist CP-105,696 had no effect on LTB₄ pro-inflammatory activation of HMVECs, nor did silencing of BLT1 in HMVECs. Distinct from PMNs, pro-inflammatory activation of HMVECs occurs through ligand activation of the low affinity BLT2 receptor as demonstrated by silencing this receptor, which inhibited HMVEC activation by LTB₄ and its metabolites. These data are not surprising for the BLT2 receptor is expressed by many diverse cell types, and although PMNs do express BLT2 which in comparison has lower affinity and has no documented cellular function (21,10,5). These data may provide potential therapeutic avenues in the critically ill patient in which selective receptor blockade could inhibit the pro-inflammatory actions of LTB₄ and its metabolites on either PMNs or HMVECs.

In summary, various products of the leukotriene pathway cause pro-inflammatory changes in innate immunity, e.g. PMNs and the vascular endothelium. While the end product of leukotriene synthesis, LTB₄, is a well-known pro-inflammatory agent, little has been done in regards to the metabolites of LTB₄. We have shown that the enzymatic breakdown products of LTB₄, 20-CO-LTB₄, and 20-COOH-LTB₄ as well as the non-enzymatic breakdown product of LTA₄, 6-trans-LTB₄, have similar activity and signal through one of the two described LTB₄ receptors depending upon the cell type. Inactivation of this pathway appears more complicated than previously described because the metabolites retain activity, although higher concentrations are required (14,3). Thus, the 5-LO pathway has alternate means of producing pro-inflammatory mediators, both enzymatic or not, that can lead to the development of ALI and subsequent MOF.

Acknowledgments

This work was supported by Bonfils Blood Center and grant #GM49222 from NIGMS, NIH.

References

1. Crooks SW, Stockley RA. Leukotriene B₄. Int J Biochem Cell Biol 1998;30(2):173–178. [PubMed: 9608670]

2. Sala A, Zarini S, Bolla M. Leukotrienes: lipid bioeffectors of inflammatory reactions. *Biochemistry (Mosc)* 1998;63(1):84–92. [PubMed: 9526099]
3. Murphy RC, Gijon MA. Biosynthesis and metabolism of leukotrienes. *Biochem J* 2007;405(3):379–395. [PubMed: 17623009]
4. Gijon MA, Zarini S, Murphy RC. Biosynthesis of eicosanoids and transcellular metabolism of leukotrienes in murine bone marrow cells. *J Lipid Res* 2007;48(3):716–725. [PubMed: 17179116]
5. Tager AM, Luster AD. BLT1 and BLT2: the leukotriene B(4) receptors. *Prostaglandins Leukot Essent Fatty Acids* 2003;69(2–3):123–134. [PubMed: 12895595]
6. Moore FA, Moore EE. Evolving concepts in the pathogenesis of postinjury multiple organ failure. *Surg Clin North Am* 1995;75(2):257–277. [PubMed: 7899997]
7. Partrick DA, Moore FA, Moore EE, Barnett CC Jr, Silliman CC. Neutrophil priming and activation in the pathogenesis of postinjury multiple organ failure. *New Horiz* 1996;4(2):194–210. [PubMed: 8774796]
8. Silliman CC. The two-event model of transfusion-related acute lung injury. *Crit Care Med* 2006;34(5 Suppl):S124–S131. [PubMed: 16617256]
9. Silliman CC, Kelher M. The role of endothelial activation in the pathogenesis of transfusion-related acute lung injury. *Transfusion* 2005;45(2 Suppl):109S–116S. [PubMed: 16086797]
10. Medoff BD, Tager AM, Jackobek R, Means TK, Wang L, Luster AD. Antibody-antigen interaction in the airway drives early granulocyte recruitment through BLT1. *Am J Physiol Lung Cell Mol Physiol* 2006;290(1):L170–L178. [PubMed: 16126787]
11. Palmblad J, Lerner R, Larsson SH. Signal transduction mechanisms for leukotriene B4 induced hyperadhesiveness of endothelial cells for neutrophils. *J Immunol* 1994;152(1):262–269. [PubMed: 8254196]
12. Palmblad JE, Lerner R. Leukotriene B4-induced hyperadhesiveness of endothelial cells for neutrophils: relation to CD54. *Clin Exp Immunol* 1992;90(2):300–304. [PubMed: 1358491]
13. Bokoch GM, Reed PW. Effect of various lipoxygenase metabolites of arachidonic acid on degranulation of polymorphonuclear leukocytes. *J Biol Chem* 1981;256(11):5317–5320. [PubMed: 6787038]
14. Jackson RH, Morrissey MM, Sills MA, Jarvis MF. Comparison of antagonist and agonist binding to the leukotriene B4 receptor intact human polymorphonuclear neutrophils (PMN). *J Pharmacol Exp Ther* 1992;262(1):80–89. [PubMed: 1320692]
15. O'Flaherty JT, Wykle RL, Lees CJ, Shewmake T, McCall CE, Thomas MJ. Neutrophil-degranulating action of 5,12-dihydroxy-6,8,10,14-eicosatetraenoic acid and 1-O-alkyl-2-O-acetyl-sn-glycero-3-phosphocholine Comparison with other degranulating agents. *Am J Pathol* 1981;105(3):264–269. [PubMed: 6274199]
16. Partrick DA, Moore EE, Moore FA, Barnett CC, Silliman CC. Lipid mediators up-regulate CD11b and prime for concordant superoxide and elastase release in human neutrophils. *J Trauma* 1997;43(2):297–302. [PubMed: 9291376]
17. Wyman TH, Bjornsen AJ, Elzi DJ, Smith CW, England KM, Kelher M, Silliman CC. A two-insult in vitro model of PMN-mediated pulmonary endothelial damage: requirements for adherence and chemokine release. *Am J Physiol Cell Physiol* 2002;283(6):C1592–C1603. [PubMed: 12388071]
18. Levy BD, Clish CB, Schmidt B, Gronert K, Serhan CN. Lipid mediator class switching during acute inflammation: signals in resolution. *Nat Immunol* 2001;2(7):612–619. [PubMed: 11429545]
19. Takata S, Matsubara M, Allen PG, Janmey PA, Serhan CN, Brady HR. Remodeling of neutrophil phospholipids with 15(S)-hydroxyeicosatetraenoic acid inhibits leukotriene B4-induced neutrophil migration across endothelium. *J Clin Invest* 1994;93(2):499–508. [PubMed: 7906693]
20. Partrick DA, Moore EE, Silliman CC, Barnett CC, Kuypers FA. Secretory phospholipase A2 activity correlates with postinjury multiple organ failure. *Crit Care Med* 2001;29(5):989–993. [PubMed: 11378609]
21. Kavelaars A, Vroon A, Raatgever RP, Fong AM, Premont RT, Patel DD, Lefkowitz RJ, Heijnen CJ. Increased acute inflammation, leukotriene B4-induced chemotaxis, and signaling in mice deficient for G protein-coupled receptor kinase 6. *J Immunol* 2003;171(11):6128–6134. [PubMed: 14634128]

22. Cunha JM, Sachs D, Canetti CA, Poole S, Ferreira SH, Cunha FQ. The critical role of leukotriene B₄ in antigen-induced mechanical hyperalgesia in immunised rats. *Br J Pharmacol* 2003;139(6): 1135–1145. [PubMed: 12871832]
23. Trandafir CC, Nishihashi T, Ji X, Wang A, Kurahashi K. Cysteinyl leukotrienes and leukotriene B mediate vasoconstriction to arginine vasopressin in rat basilar artery. *Clin Exp Pharmacol Physiol* 2005;32(12):1027–1033. [PubMed: 16445567]
24. Samuelsson B, Dahlen SE, Lindgren JA, Rouzer CA, Serhan CN. Leukotrienes and lipoxins: structures, biosynthesis, and biological effects. *Science* 1987;237(4819):1171–1176. [PubMed: 2820055]
25. Serhan CN, Hamberg M, Samuelsson B. Lipoxins: novel series of biologically active compounds formed from arachidonic acid in human leukocytes. *Proc Natl Acad Sci USA* 1984;81(17):5335–5339. [PubMed: 6089195]

Figure 1. LTB₄ induces increased surface expression of ICAM-1 on HMVECs

Panel A: the surface expression of CD54 (ICAM-1) measured by flow cytometry of HMVECs incubated with a fluorescently-labeled (FITC) monoclonal antibody to CD54 is depicted as a function of treatment group. As compared to albumin-treated controls, HMVECs incubated with LTB₄ and its metabolites (5-OH-LTB₄, 5-COOH-LTB₄, and 6-trans-LTB₄) induced ICAM-1 surface expression at 10 μM but not 1 μM (*=*p*<.05 vs. albumin-treated controls, *n*=7). Panel B shows a representative tracing of the change in MFI between HMVECs treated with albumin or 10⁻⁵M LTB₄.

Figure 2. Silencing of the BLT2 receptor surface expression on HMVECs

HMVECs were incubated with media, Optifect alone (Vehicle), Optifect with non-targeting si-RNA (NT) or with commercially available si-RNA for BLT2 from GenScript (BLT2#1), from Accell (BLT2#2), or both (Both). The immunoblot below depicts BLT2 immunoreactivity in whole cell lysates from HMVECs (25 µg protein/well) and is representative of 2 identical experiments.

Table 1

The concentration-dependent priming activity of LTB₄ and its metabolites.

Priming Agent	10 ⁻⁵ M	10 ⁻⁶ M	10 ⁻⁷ M	10 ⁻⁸ M	10 ⁻⁹ M	10 ⁻¹⁰ M	10 ⁻¹¹ M
PAF	4.1±0.4 ^{*/I}	4.3±0.3 [*]	3.9±0.6 [*]	3.2±0.7 [*]	2.5±0.6 [*]	2.4±0.9	
LTB ₄	3.1±0.5 [*]	2.2±0.4 [*]	2.5±0.5 [*]	2.4±0.5 [*]	2.8±0.4 [*]	2.7±0.9	1.2±0.2
6-trans-LTB ₄	3.1±0.3 [*]	2.8±0.4 [*]	2.2±0.2 [*]	2.1±0.6 [*]	1.6±0.5	1.1±0.1	1.1±0.2
20-OH-LTB ₄	2.1±0.2 [*]	1.9±0.3 [*]	1.9±0.3 [*]	2.3±0.4 [*]	2.0±0.6	1.7±0.4	1.0±0.3
20-COOH-LTB ₄	2.1±0.1 [*]	1.6±0.4	1.1±0.2	0.9±0.1	1.0±0.2	1.3±0.1	0.9±0.2

^I The data is expressed as the mean ± the standard error of the mean of the fold increase over albumin-primed PMNs activated with 1 μM fMLP.

^{*} p<0.01 versus the fMLP controls, n=8.

Table 2

PMN adherence to activated HMVECs.

Agonist	% of Total PMNs added
Buffer Control	5.4±0.3%
10 ⁻⁵ M LTB ₄	10.5±0.5% *
2 µg/ml LPS	13.2±0.8% *

The data is presented as the mean±SEM of the % of myeloperoxidase present in the wells versus the total MPO of the PMNs added.

*
p<0.05 versus the buffer-treated controls (n=3).

Table 3Antagonism of LTB₄ and its metabolites on PMN priming and HMVEC activation.

Inhibitors/Lipids	LTB ₄	6-trans LTB ₄	20-OH-LTB ₄	20-COOH-LTB ₄
<i>PMN Priming</i>				
LXA ₄ [100nM]	44.1±7% *	55±9% *	28.6±3% *	97±4% *
CP-105,696 [100 nM]	79.1±10% *	98±6% *	100±5% *	100±4% *
<i>HMVEC Activation</i>				
LXA ₄	84±10%			
CP-105,696	8±6%	10±7%	9±8%	6±8%

The data is presented as the percent inhibition of 1) the augmentation above fMLP of the maximal rate of the SOD-inhibitable O₂[−] production ± SEM for PMN priming or for HMVEC activation the surface expression (MFI) of ICAM-1 on HMVECs ± SEM.

* p<0.05 vs. cells, PMNs or HMVECs treated with buffer + the lipid agonist.

Table 4

RNA silencing of BLT2 in HMVECs inhibited the LTB₄-mediated increases in ICAM-1.

siRNA/Treatment	Albumin	10 ⁻⁵ M LTB ₄
Non-targeting	10.1±1.4 ^I	14.2±2.0 [*]
siRNA BLT2 #1	10.4±1.6	9.8±2.0 [#]
siRNA BLT2 #2	9.1±1.2	8.9±1.4 [#]
Both	10.2±1.3	10.4±1.7 [#]

^IThe data is presented as the mean ± the standard error of the mean of the mean fluorescent intensity (MFI) of the surface expression of ICAM-1 measured by flow cytometry.

^{*}p<.05 vs. buffer-treated HMVECs loaded with non-targeting siRNA.

[#]p<.05 versus the LTB₄-induced increase in ICAM-1 in HMVECs loaded with non-targeting siRNA.