Remodeling of Neutrophil Phospholipids with 15(S)-Hydroxyeicosatetraenoic Acid Inhibits Leukotriene B_4–induced Neutrophil Migration across Endothelium

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Abstract

5-Lipoxygenase products, such as leukotrienes, are important stimuli for leukocyte-mediated tissue injury in acute inflammation. 15-Hydroxyeicosatetraenoic acid (15-HETE) is an eicosanoid generated by a variety of cell types via the actions of 15-lipoxygenases and, in addition, cyclooxygenases and epoxygenases. 15-HETE levels are frequently elevated at sites of inflammation, and extracellular 15(*S*)-HETE is esterified rapidly into neutrophil (PMN) phospholipids in vitro to levels that are comparable with arachidonic acid. We present evidence that remodeling of PMN phospholipids with 15(*S*)-HETE stereoselectively inhibits PMN migration across endothelium in response to leukotriene B_4 (LTB_4) and other chemoattractants. Esterified 15(*S*)-HETE causes a striking reduction in the affinity of LTB_4 cell-surface receptors for their ligand and inhibition of LTB_4-triggered stimulus–response coupling. As a result of these actions, esterified 15(*S*)-HETE attenuates the cytoskeletal rearrangements and CD11/CD18-mediated adhesive events that subserve directed locomotion of PMN across endothelium. These observations indicate that products of the 5-lipoxygenase and 15-lipoxygenase pathways can exert counterbalancing influences on PMN trafficking across endothelium. They suggest that 15(*S*)-HETE may be a potent endogenous inhibitor of PMN–endothelial interactions in vivo and serve to limit or reverse acute inflammation. (*J. Clin. Invest.* 1994. 93:499–508.) Key words: cell adhesion • eicosanoids • chemoattractants • receptors • cytoskeleton

Introduction

Polymorphonuclear neutrophils (PMNs) are important effectors of tissue injury in many inflammatory diseases (for review see reference 1). PMN chemotaxis and adhesion to endothelial cells are central events in the recruitment of circulating PMNs during inflammation (for review see references 1–3). Chemotaxis is initiated by engagement of chemoattractants with PMN cell-surface receptors and activation of signal transduction events that provoke dramatic rearrangements of the PMN cytoskeleton and directed locomotion (for review see references 2, 4). Most chemoattractants also promote PMN adhesion to endothelial cells by enhancing the avidity of PMN cell-surface adhesion molecules for endothelial ligands (for review see references 5–8). Initial adhesion appears to be supported by interaction of PMN or endothelial cell selectins with carbohydrate-containing counterreceptors. Selectin-mediated adhesion, which is relatively resistant to shear stress, tethers PMNs, causes them to roll on endothelium, and facilitates their immobilization by engagement of PMN CD11/CD18 β_2 integrins with endothelial cell ligands such as intercellular adhesion molecule-1 (ICAM-1).[1] Adhesion of PMNs through CD11/CD18 is essential for normal PMN trafficking and host defense (for review see reference 5), and also may promote tissue injury in inflammation by priming PMN oxidative bursts and degranulation responses (9, 10) and by promoting the transcellular biosynthesis of lipid mediators during cell–cell interactions (11).

Leukotrienes are generated by the initial actions of 5-lipoxygenase on arachidonic acid and serve as mediators of inflammation in vitro and in vivo (for review see references 12, 13). Leukotriene B_4 (LTB_4), in particular, is a potent stimulus for PMN chemotaxis and CD11/CD18-mediated PMN–endothelial cell adhesion. Inhibitors of LTB_4 biosynthesis attenuate injury in many leukocyte-dependent diseases (13). The potential roles of lipoxygenase-derived products as inhibitors of PMN trafficking are less well defined. 15(*S*)-hydroxy-5,8,11-*cis*-13-*trans*-eicosatetraenoic acid (15[*S*]-HETE) is a potential candidate in this regard (14–22). 15-HETE is generated from unesterified sources of arachidonic acid by a variety of mammalian cells including PMNs, endothelial cells, and some epithelial cells via reactions catalysed by 15-lipoxygenases, cyclooxygenases, and/or cytochrome P-450 epoxygenases (for review see reference 16). 15-HETE has been detected in submicromolar concentrations in sera of normal human blood and in higher concentrations after cell activation (17). 15-HETE levels are increased in tissues and exudates in a variety of experimental and human diseases including asthma (18), arthritis (19), and psoriasis (20). Furthermore, administration of exogenous 15(*S*)-HETE reduces tissue injury in human psoriasis vulgaris (21) and carrageenan-induced experimental arthritis (22). The mechanism by which 15(*S*)-HETE attenuates tissue injury under these circumstances is not completely understood. Recently it was shown that extracellular 15(*S*)-HETE is esterified rapidly and stored in phospholipids of PMNs for later use (14). In this study, we assessed the influence of 15(*S*)-HETE remodeling of PMN phospholipids on LTB_4-induced migration of PMNs across monolayers of hu-

Portions of this work were presented at the American Society of Nephrology 25th annual meeting in Baltimore, MD on 15–18 November 1992, and have appeared in abstract form (1992. *J. Am. Soc. Nephrol.* 3:617*a*).

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Received for publication 10 August 1993 and in revised form 16 September 1993.

1. *Abbreviations used in this paper:* BCS, bovine calf serum; B_{max}, maximal binding capacity; HETE, hydroxyeicosatetraenoic acid; HETE-ME, methylated derivative of HETE; HUVEC, human umbilical vein endothelial cells; ICAM-1, intercellular adhesion molecule-1; IP_3, inositol trisphosphate; LTB_4, leukotriene B_4; LXA_4, lipoxin A_4; MK886, an inhibitor of 5-lipoxygenase activity; ONO-4057, an LTB_4 receptor antagonist; PKC, protein kinase C.

J. Clin. Invest.

0021-9738/94/02/0499/10 $2.00
Volume 93, February 1994, 499–508

man umbilical vein endothelial cells (HUVEC) as an in vitro model of PMN trafficking during acute inflammation.

Methods

PMN isolation and labeling with ^{111}In. PMNs were isolated at 4°C from heparinized venous blood drawn from normal volunteers by standard procedures of Ficoll-Hypaque (Organon Teknika Corp., Durham, NC) density gradient centrifugation and dextran sedimentation, as described previously (11, 23). Contaminating red blood cells were removed by hypotonic lysis. The final pellet was suspended in Dulbecco's PBS, pH 7.4, and contained 96±3% PMNs, as determined by light microscopy. Suspensions in which PMNs showed signs of cellular activation (> 10% of PMNs in clumps of two or more cells) or loss of membrane integrity (< 97% of PMNs excluding trypan blue) were routinely discarded. For studies of transmigration and adhesion, PMNs were radiolabeled with ^{111}In oxine (1 μCi/10^6 cells; Amersham Corp., Mediphysics, Arlington Heights, IL) for 30 min at room temperature, were washed three times in PBS to remove extracellular ^{111}In oxine, and were resuspended in PBS/1% bovine calf serum (BCS).

Expression of CD11/CD18. PMNs (5×10^6) were fixed by the addition of 2 vol of ice cold PBS/1% paraformaldehyde and were kept overnight at 4°C. After washing with ice cold PBS, fixed PMNs were incubated with saturating concentrations of anti-CD18 monoclonal antibody (R15.7, 10 μg/ml) at 4°C for 30 min followed by FITC-conjugated goat F(ab')2-anti-mouse IgG (Caltag Laboratories, San Francisco, CA) at 4°C for 30 min. After a final washing with ice cold PBS, CD11/CD18 expression was assessed in a fluorescence-activated cell sorter (Epics 750; Coulter Electronics Inc., Hialeah, FL).

Right angle light scattering. The scattering intensity of 2.5×10^6 PMNs in PBS was determined at room temperature with a fluorescence spectrophotometer (LS-50; Perkin-Elmer Corp., Norwalk, CT) at a wavelength of 340 nM. Cells were incubated for 5 min with gentle stirring (low setting) in a quartz cuvette with 1-cm path length before addition of LTB_4 (10^{-7} M). For each sample, scattering intensity was normalized to that of the baseline before addition of LTB_4.

F-actin content. The relative amounts of F-actin in PMNs before and after addition of LTB_4 were determined by quantitating the amount of rhodamine-phalloidin bound to fixed extracted cells by slight modification of a previously described technique (24). Specifically, 5×10^6 cells/ml were incubated with stirring at room temperature. 200-μl aliquots were removed at various times before and after addition of 10^{-7} M LTB_4 and were added to 200 μl of 7% formaldehyde, 0.1% TX-100, and 0.1 μM rhodamine-phalloidin (Sigma Chemical Co., St. Louis, MO) in TBS, pH 7.8. Cells were fixed overnight at 4°C in the dark, were washed twice with TBS, and were extracted twice with 1 ml MeOH. Extracted rhodamine-phalloidin was quantitated with a fluorescence spectrophotometer (LS-5a; Perkin-Elmer Corp.) with excitation and emission wavelengths of 540 and 570 nM, respectively. The fluorescence intensity of each sample was measured three times, and the results presented are the average of three experiments.

Human umbilical vein endothelial cells. HUVEC were isolated and propagated as described previously (25). Briefly, cells were dispersed by 0.1% collagenase (CLS 3; Worthington Biochemical Corp., Freehold, NJ) and were propagated in cell culture medium (RPMI 1640; BioWhittaker Inc., Walkersville, MD) supplemented with 15% BCS (Hyclone Laboratories, Logan, UT), 15% NU-serum (Collaborative Research Inc., Lexington, MA), 50 μg/ml endothelial mitogen (Biomedical Technologies, Inc., Stoughton, MA), 8 U/ml heparin, 50 U/ml penicillin, and 50 μg/ml streptomycin (HUVEC medium). HUVEC were characterized based on morphological criteria and by indirect immunofluorescence using a specific antiserum to human Factor VIII antigen, and were studied at passage levels 2 and 3.

PMN migration across HUVEC monolayers. For studies of transmigration, HUVEC were grown to confluence in HUVEC medium on gelatin-coated (1%) polycarbonate filters containing 3-μm pores (Transwell; Costar Corp., Cambridge, MA), which were inserted into 24-well tissue culture plates (Costar Corp.). The HUVEC monolayer and filter divided each well into an upper luminal (100 μl) and a lower abluminal compartment (600 μl). Before the study, monolayers were grown in RPMI 1640/10% BCS for 24 h and were washed with PBS/1% BCS. Transmigration was initiated by addition of ^{111}In-labeled PMNs (100 μl, 3×10^5 PMNs) to the luminal compartment and of chemoattractant to the abluminal compartment. Assays were performed in a humidified atmosphere containing 5% CO_2/95% air at 37°C and were terminated by aspiration of nonadherent PMNs from the luminal compartment, by removing Transwell inserts (Costar Corp.) from the abluminal compartment, and by washing PMNs that were loosely adherent to the undersurface of filters into the abluminal chamber with 1 ml PBS (4°C) without Ca^{2+} and Mg^{2+}. The number of PMNs that migrated to the abluminal compartment was calculated by lysing cells with 0.5% Triton X-100, measuring the radioactivity with a gamma counter (Clinigamma; LKB Instruments, Inc., Gaithersburg, MD), and comparing counts to the specific activity of the original PMN suspension. Unless stated otherwise, results are expressed as the percentage of ^{111}In-labeled PMNs added to the luminal compartment that migrated to the abluminal compartment. LTB_4-induced transmigration was calculated by subtraction of transmigration observed in the presence of diluent.

PMN adhesion to HUVEC monolayers. For studies of adhesion, HUVEC were grown to confluence in HUVEC medium on gelatin-coated (1%) 96-well tissue culture plates (Costar Corp.) and then in RPMI 1640/10% BCS for 24 h before the study. Monolayers were washed once with 0.2 ml of PBS/1% BCS and were coincubated with 0.2 ml ^{111}In-labeled PMN suspension (4×10^5 PMNs/well) in a humidified atmosphere containing 5% CO_2/95% air at 37°C. PMNs were allowed to settle for 10 min before the addition of agonists and then were coincubated with HUVEC monolayers for a further 30 min in the presence of agonists. Assays were terminated by removal of nonadherent cells by aspiration of medium and by washing of monolayers with 0.2 ml PBS/1% BCS. The contents of each well were solubilized with 0.01% SDS/0.025 N NaOH, and the radioactivity was measured in a gamma counter (Clinigamma; LKB Instruments, Inc.). The number of adherent PMNs (PMNs per square millimeter of HUVEC monolayer) was calculated from the specific activity of each PMN preparation. For each experiment, LTB_4-stimulated adhesion was calculated by subtraction of adhesion observed in the presence of diluent alone.

PMN adhesion to adsorbed ICAM-1. ICAM-1 was adsorbed overnight at 4°C onto 24-well tissue culture plates (Costar Corp.). Plates were then incubated with 1% human serum albumin at 37°C for a further 30 min. After aspiration, plates were treated with PBS containing 1% Tween for 2 min and were washed five times with PBS. ^{111}In-labeled PMNs were added, were allowed to settle for 5 min, and then were incubated with LTB_4 (10^{-7} M) or diluent for 5 min. Nonadherent PMNs were removed by aspiration, and the plate was washed once with PBS. Adherent PMNs were solubilized, and adhesion was calculated as for PMN adhesion to HUVEC.

Measurement of inositol trisphosphate (IP_3) levels in PMNs. For assessment of IP_3 levels in PMNs, incubations (10^7 PMNs/1 ml PBS) were terminated by the addition of 200 μl 100% trichloroacetic acid and were kept on ice for 15 min. Samples were centrifuged, and the supernatant was collected and mixed vigorously with 2 ml 1,1,2-trichloro-1,2,2-trifluoroethane-trictylamine mixture (3:1, vol/vol). After separation (~ 3 min), the clear aqueous top layer was removed and analyzed for IP_3 content with an IP_3 radioreceptor assay kit (New England Nuclear, Boston, MA).

Binding of [^{3}H]LTB_4 and [^{3}H]15(S)-HETE to PMNs. For assessment of LTB_4 receptor function, PMN suspensions (2.5×10^6 cells/0.5 ml) were incubated with [^{3}H]LTB_4 (1 nM) at 4°C in the presence or absence of various concentrations of unlabeled LTB_4 (10^{-10}–10^{-6} M). For assessment of specific binding of 15(S)-HETE, PMNs (3×10^6 cells/0.3 ml) were incubated with [^{3}H]15(S)-HETE (0.2 nM) at 4°C in the presence or absence of 1,000-fold excess of unlabeled 15(S)-HETE. After the specified time, PMNs were centrifuged (~ 1 min at 12,000 *g*) through silicon oil (0.4 ml), and the radioactivity associated with cell pellets was determined in a scintillation counter, as described previously (26). The K_d and maximal binding capacity

(B_{max}) of the high affinity LTB_4 receptors were determined by Scatchard analysis. Specific binding of [^{3}H]15(*S*)-HETE was calculated from the difference between cell-associated radioactivities in the presence or absence of unlabeled 15(*S*)-HETE.

Permeability of HUVEC monolayers to ^{125}I-BSA. This was assessed as an index of the integrity of the HUVEC monolayer during PMN–HUVEC interactions. To this end, migration of ^{125}I-BSA (0.3 μCi/100 μl, New England Nuclear) from the luminal compartment to the abluminal compartment was measured and was expressed as: (cpm in abluminal compartment/cpm in luminal compartment) × 100.

Reagents and mAbs. FMLP, PMA, and A-23,187 were obtained from Sigma Chemical Co. LTB_4, 5(*S*)-HETE, 12(*S*)-HETE, 15(*S*)-HETE, and lipoxin A_4 (LXA_4) were obtained from BIOMOL Research Labs Inc. (Plymouth Meeting, PA). The purity of each eicosanoid was checked before incubation with cells by ultraviolet spectroscopy, and their concentrations were determined from their extinction coefficients. [^{3}H]15(*S*)-HETE and [^{3}H]LTB_4 were obtained from New England Nuclear. The following mAbs were used to assess adhesion molecule expression or function: mAb R15.7 (IgG_1) recognizes the CD18 subunit of CD11/CD18 integrins (gift of Dr. R. Rothlein, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT); mAb Hu5/3 (IgG_1) recognizes a functional epitope on human ICAM-1 (gift of Dr. F. W. Luscinskas, Harvard Medical School, Boston, MA); mAb anti-LAM1.3 (IgG_1) recognizes a functional epitope on L-selectin (gift of Dr. T. F. Tedder, Harvard Medical School). ICAM-1 was a gift of Dr. M. Lawrence and Dr. T. A. Springer (Harvard Medical School). MK886 (27) was a gift of Dr. A. W. Ford-Hutchinson (Merck Frosst Inc., Pointe Claire-Dorval, Canada), and ONO-4057 (28) was a gift of Dr. Tsumoru Miyamoto (Ono Pharmaceutical Co., Ltd., Osaka, Japan).

Statistical analysis. Results were expressed as mean±SE. A two-tailed Student's *t* test was used for statistical analysis. A *P* value of < 0.05 was taken to represent a statistically significant difference between group means.

Results

15(S)-HETE remodeling of PMN phospholipids inhibits their migration across HUVEC in response to chemoattractants. PMN migration across confluent HUVEC monolayers appears to be a reasonable in vitro model of chemoattractant-stimulated PMN recruitment during inflammation (3). In the present study, HUVEC monolayers, grown on polycarbonate filters containing 3-μm pores, prevented the passage of most ^{111}In-labeled PMNs and ^{125}I-labeled albumin during 30 min of PMN–endothelium coincubation (migration in 30 min: 2.5±0.4% [$n = 5$] and 3.27±0.31% [$n = 3$], respectively). The addition of LTB_4 to the abluminal compartment induced a rapid and concentration-dependent migration of PMNs from the luminal compartment ($ED_{50} = 4 \times 10^{-10}$ M; maximal at 10^{-8} M: 42±5% within 30 min), which was inhibited by prior exposure of PMNs to ONO-4057 (10^{-5} M, 37°C, 5 min), an LTB_4 receptor antagonist (Fig. 1). Transmigration was not observed after addition of 15(*S*)-HETE (30 μM) to the abluminal compartment (PMN migration: 2.60±0.16%, $n = 4$). LTB_4-induced transmigration was not associated with morphologic evidence of injury to the HUVEC monolayers, or an increase in the permeability of the monolayers to ^{125}I-albumin (^{125}I-albumin migration in 30 min: vehicle 3.27±0.31%; LTB_4 2.78±0.32%, $n = 3$). LTB_4-induced transmigration was inhibited by prior exposure of PMNs to saturating concentrations (10 μg/ml) of mAbs (mAb R15.7) against the common CD18 subunit of CD11/CD18 integrins (transmigration at 30 min: LTB_4 alone 48.1±0.68%; LTB_4 plus anti-CD18 mAb 4.95±0.77%, $n = 4$, $P < 0.001$).

This system was used to determine the influence of 15(*S*)-

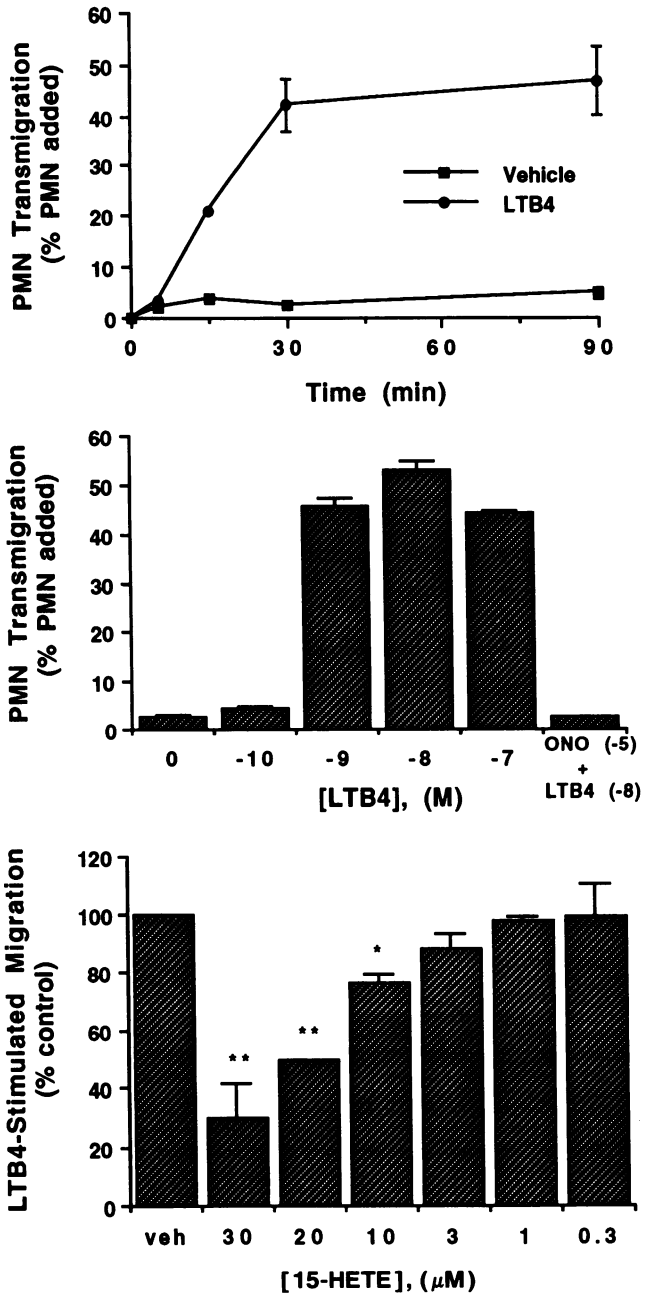

Figure 1. Inhibition of LTB_4-induced PMN migration across HUVEC monolayers after remodeling of PMN phospholipids with 15(*S*)-HETE. LTB_4-induced migration of ^{111}In-labeled PMNs (3×10^5/well) across confluent monolayers of HUVEC grown on gelatin-coated polycarbonate filters was assessed as described in Methods. (*Top*) Studies of time dependence in which transmigration was assessed 5–90 min after addition of 10^{-8} M LTB_4 ($n = 3$–5). (*Middle*) Studies of dose dependence in which LTB_4 was added at concentrations of 10^{-10}–10^{-7} M and transmigration was assessed after 30 min ($n = 3$). ONO, ONO-4057, an LTB_4 receptor antagonist. (*Bottom*) Studies to define the influence of 15(*S*)-HETE remodeling of PMN phospholipids on LTB_4-induced transmigration (10^{-8} M, 30 min) in which ^{111}In-labeled PMNs were exposed to different concentrations of 15(*S*)-HETE or its diluent for 30 min at 37°C and were washed twice before coincubation with HUVEC. $^*P < 0.005$; $^{**}P < 0.001$. The loading concentration of 15(*S*)-HETE that was required to inhibit transmigration diminished with increasing time of exposure (threshold concentration for inhibition: 10 μM at 30 min, 0.3 μM at 60 min (see text). PMN transmigration is expressed as the percentage of PMNs added to the luminal compartment that migrated to the abluminal compartment. Data are means±SE.

HETE on chemoattractant-stimulated PMN–endothelial cell interaction. ^{111}In-labeled PMNs were incubated with 15(*S*)-HETE (0.3–30.0 μM, 30 min, 37°C) or its diluent, were washed twice with PBS, and LTB_4-induced transmigration (10^{-8} M, 30 min) was assessed. Using these experimental conditions, PMNs rapidly esterify 15(*S*)-HETE into membrane phospholipids to levels that are comparable with arachidonic acid (14). ^{111}In levels were not different in 15(*S*)-HETE–remodeled and control PMNs. LTB_4-induced transmigration was reduced dramatically after remodeling of PMN phospholipids with 15(*S*)-HETE, and the degree of inhibition was dependent on the loading concentration of 15(*S*)-HETE (Fig. 1, *bottom*). The loading concentration of 15(*S*)-HETE that was required to inhibit transmigration diminished with increasing time of exposure. Thus, while the threshold concentration for inhibition was 10 μM at 30 min, doses as low as 0.3 μM afforded a significant reduction in transmigration at 1 h (11.5±3.7% inhibition, $n = 9$).

While these data supported an action of esterified 15(*S*)-HETE with PMNs, some 15(*S*)-HETE is released from esterified sites upon activation of PMNs (14) and could potentially inhibit migration via actions with HUVEC. To explore the latter possibility, HUVEC were treated with 15(*S*)-HETE (30 μM, 30 min, 37°C), were washed twice with PBS, and transmigration was assessed. LTB_4-induced transmigration (10^{-8} M, 30 min) was not altered under these experimental conditions (LTB_4-stimulated transmigration: 15(*S*)-HETE–treated HUVEC 43.5±1.2%; diluent-treated HUVEC 42.6±0.8%; $n = 4$).

The influence of 15(*S*)-HETE on transmigration induced by the complement component C5a and the synthetic peptide FMLP was assessed to determine if this action of 15(*S*)-HETE was restricted to LTB_4 or applied to other receptor-mediated chemoattractants. As with LTB_4, these agonists provoked rapid migration of PMNs without affecting the permeability of HUVEC monolayers to ^{125}I-albumin. 15(*S*)-HETE (30 μM, 30 min, 37°C) also attenuated PMN migration induced by these stimuli (10^{-8} M, 30 min) (PMN transmigration: C5a 55.1±2.35%, C5a plus 15(*S*)-HETE 26.5±1.21%, $P < 0.001$; FMLP 36.8±1.81%, FMLP plus 15(*S*)-HETE 16.0±3.64%, $P < 0.025$; $n = 3$).

Chemical requirements for inhibition of transmigration. The pattern of esterification of 15(*S*)-HETE into PMN phospholipids differs from the profiles of incorporation of other monohydroxy derivatives of arachidonic acid such as 5(*S*)-HETE and 12(*S*)-HETE (14, 29). In the present experiments, 5(*S*)-HETE and 12(*S*)-HETE (30 μM, 30 min, 37°C) afforded less inhibition of transmigration than equimolar concentrations of 15(*S*)-HETE (Fig. 2, *top*). The actions of 15(*S*)-HETE and 15(*R*)-HETE were compared to define the chirality requirements for inhibition of transmigration. 15(*S*)-HETE afforded significantly greater inhibition of LTB_4-induced transmigration than equimolar concentrations of 15(*R*)-HETE (Fig. 2, *bottom*). While most 15(*S*)-HETE is esterified into PMN phospholipids within seconds (14), some nonesterified 15(*S*)-HETE could potentially alter PMN function by intercalating into PMN cell membrane lipid bilayers.

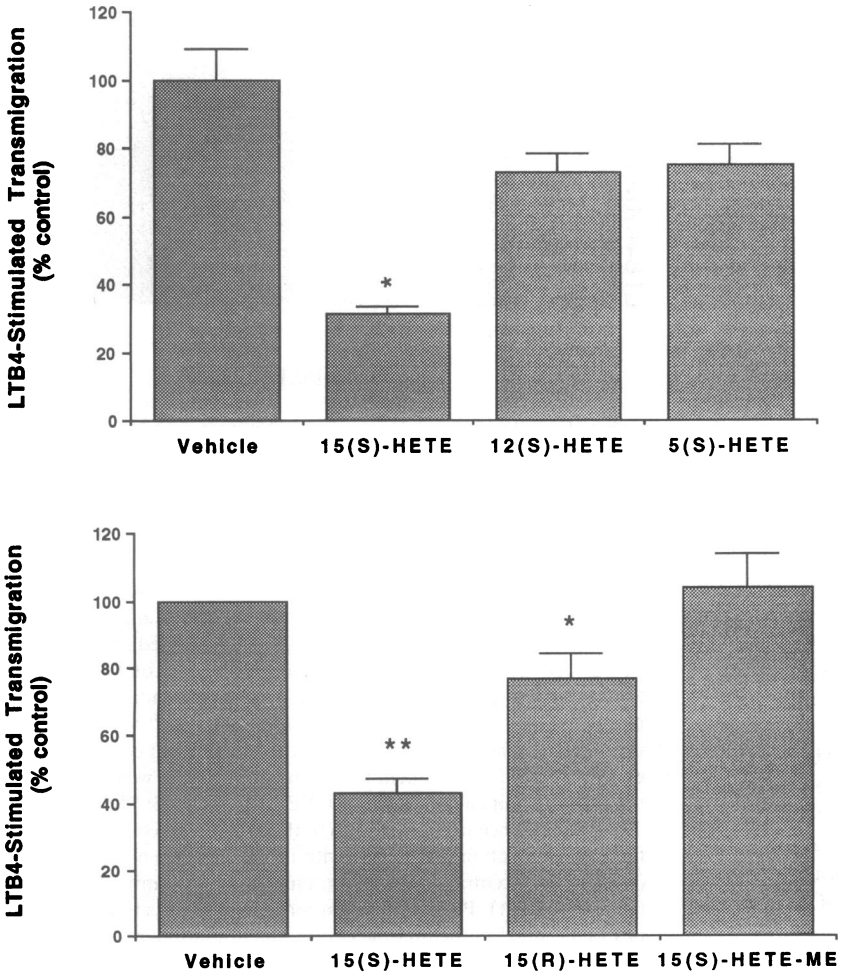

Figure 2. Inhibition of LTB_4-induced PMN migration across HUVEC monolayers by 15(*S*)-HETE: stereoselectivity and structure–activity relationships. (*Top*) Studies of regioselectivity in which ^{111}In-labeled PMNs were loaded with 15(*S*)-HETE (30 μM) or equimolar concentrations of 5(*S*)-HETE or 12(*S*)-HETE for 30 min and were washed twice before coincubation with HUVEC. $*P < 0.005$. (*Bottom*) Studies of stereoselectivity in which ^{111}In-labeled PMNs were loaded with equimolar concentrations (30 μM) of 15(*S*)-HETE, 15(*R*)-HETE, or 15(*S*)-HETE-ME for 30 min and were washed twice before coincubation with HUVEC. $*P < 0.025$; $**P < 0.001$. PMN transmigration is expressed as a percentage of LTB_4-induced transmigration observed with diluent-treated PMNs. Data are means±SE of 3–11 experiments.

15(*S*)-HETE and the carboxy terminus methylated derivative of 15(*S*)-HETE (15[*S*]-HETE-ME) were compared to evaluate this possibility (Fig. 2, *bottom*). 15(*S*)-HETE-ME did not affect LTB_4-induced transmigration, in contrast to the striking inhibition by 15(*S*)-HETE. These data indicate that the inhibitory action of 15(*S*)-HETE in PMNs is stereoselective and is dependent on esterification of 15(*S*)-HETE into PMN phospholipids.

15(S)-HETE–remodeled PMNs display impaired F-actin assembly and right angle light scattering responses upon stimulation with LTB_4. PMN trafficking across endothelium, as mentioned above, involves an orchestrated series of biochemical and morphologic events that include reversible polymerization of F-actin, membrane ruffling, polarization upon adherence, and directed motility. F-actin content was monitored before and after stimulation of PMNs with LTB_4, as an index of these cytoskeletal rearrangements. LTB_4 stimulated significantly less polymerization of F-actin in 15(*S*)-HETE–remodeled PMNs than in control cells, as determined by spectroscopic analysis of rhodamine-phalloidin staining (Fig. 3, *inset*). PMNs undergo a biphasic right angle light scatter response upon stimulation with chemoattractants (30), and the first phase (maximal at 10–15 s) appears to be tightly coupled to transient polymerization of actin (31). This initial response was assessed as another index of cytoskeletal reorganization in LTB_4-stimulated PMNs and, in agreement with the assays of rhodamine-phalloidin staining, was inhibited after remodeling of PMNs with 15(*S*)-HETE (Fig. 3). These data suggest that esterified 15(*S*)-HETE inhibits the rapid alterations in cytoskeletal architecture that subserve directed PMN migration across endothelium.

15(S)-HETE remodeling impairs LTB_4-induced CD11/CD18-mediated PMN adhesion to endothelium and adsorbed ICAM-1. The influence of 15(*S*)-HETE on LTB_4-induced PMN adhesion to confluent HUVEC monolayers was also assessed, since adhesion is another critical step in directed PMN migration across endothelium in vitro and in PMN recruitment during inflammation in vivo (3). Under the static conditions used in this study, LTB_4 provoked concentration-dependent PMN adhesion (threshold 3×10^{-10} M, maximal $\geq 10^{-7}$ M) that was inhibited by mAb against CD18 (mAb R15.7, 10 μg/ml) but not anti–L-selectin (anti-LAM1.3 mAb), as observed previously by other investigators (32–34). 15(*S*)-HETE (3 μM, 30 min) did not induce PMN adhesion (data not shown). As with transmigration, LTB_4-induced adhesion was markedly attenuated after remodeling of PMNs with 15(*S*)-HETE (IC_{50} = 2.66 μM; Fig. 4, *top*). Again, the magnitude of inhibition was significantly greater with 15(*S*)-HETE than with either 12(*S*)-HETE or 5(*S*)-HETE (Fig. 4, *bottom*).

LTB_4 provokes CD11/CD18-mediated PMN–endothelial cell adhesion by increasing the avidity of CD11/CD18 integrins for endothelial ligands, such as ICAM-1 (3, 34). In keeping with these previous observations, LTB_4-induced PMN adhesion and transmigration were inhibited by 65.0±11.0% and 42.6±14.0%, respectively, by anti–ICAM-1 mAb (Hu5/3; 25 μg/ml) in this study (n = 4). LTB_4-stimulated PMN adhesion to ICAM-1 immobilized on plastic was monitored as an additional measure of CD11/CD18 avidity for cognate ligands. As with adhesion to HUVEC, PMN adhesion to adsorbed ICAM-1 was markedly inhibited after 15(*S*)-HETE remodeling of PMNs (Fig. 5). This inhibitory action of 15(*S*)-HETE did not appear to be restricted to interactions of CD11/CD18 with ICAM-1 as 15(*S*)-HETE–remodeled (30 μM, 30 min) PMNs also displayed reduced adhesiveness for inert substrates (tissue culture plastic coated with 1% gelatin) upon stimulation with LTB_4 (10^{-7} M, 30 min) (54±7% inhibition, n = 3), a CD11/CD18-dependent and ICAM-1–independent event. In contrast to its attenuation of adhesion, 15(*S*)-HETE did not inhibit LTB_4-induced upregulation of CD11/CD18 surface expression on PMNs, as determined by FACS® analysis (Fig. 5, *inset*). Taken together, these data suggest esterified 15(*S*)-HETE inhibits LTB_4-induced migration of PMNs across endothelial monolayers by attenuating PMN chemotaxis and CD11/CD18-dependent adhesion in a dose-dependent and stereoselective manner.

15(S)-HETE does not inhibit LTB_4-induced transmigration by interaction with 15(S)-HETE receptors or altering the profile of eicosanoids generated by activated PMNs. The results described above strongly suggest that 15(*S*)-HETE blunts PMN responsiveness to chemoattractants after its esterification

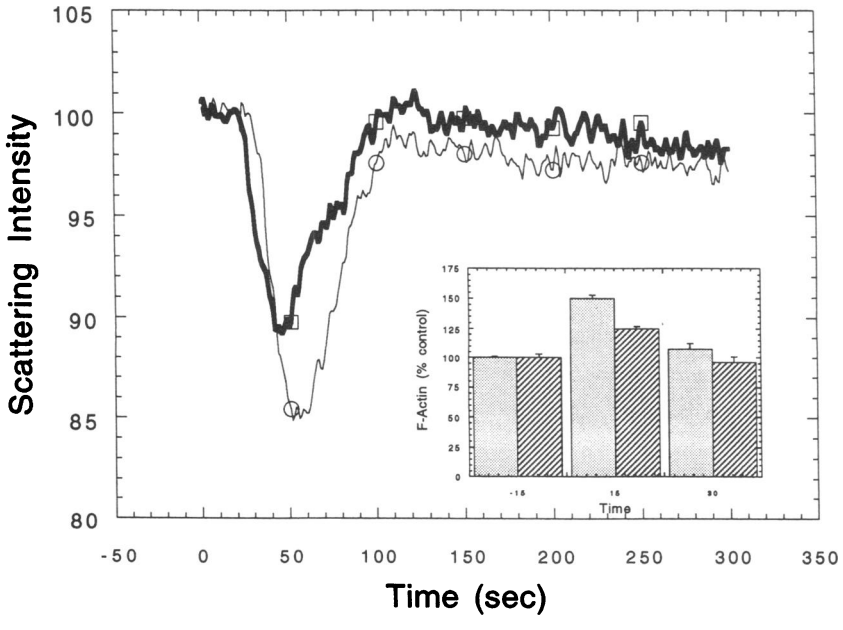

Figure 3. Esterified 15(*S*)-HETE inhibits LTB_4-stimulated cell shape change and actin polymerization in PMNs. The right angle light scattering of control (*thin line*) and 15(*S*)-HETE–treated (*thick line*) PMNs was examined in response to stimulation with 10^{-7} M LTB_4 as described in Methods. There was a consistent inhibition of the initial loss of scattering intensity in 15-HETE–remodeled PMNs (n = 9; representative experiment shown here). (*Inset*) To confirm that this altered light scattering response was due to cytoskeletal changes, the relative concentration of F-actin in control □ and 15(*S*)-HETE–▨ treated cells, stimulated with LTB_4 for 15 or 30 s, was measured. 15(*S*)-HETE pretreatment blunted the LTB_4-stimulated increase in total F-actin content. Data are means±SE of six experiments.

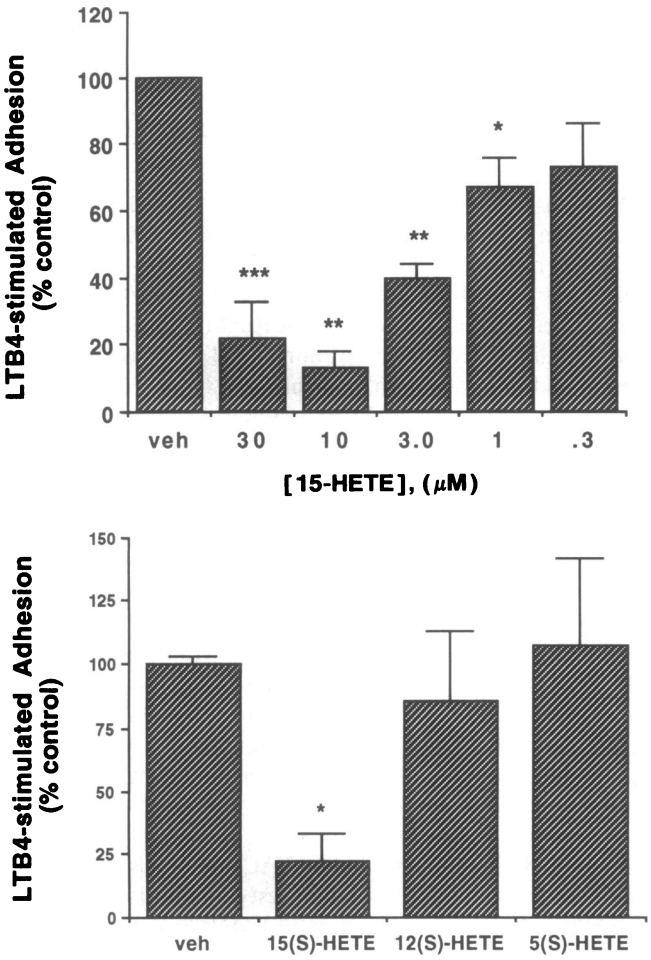

Figure 4. Remodeling of PMN phospholipids with 15(*S*)-HETE inhibits LTB_4-induced CD11/CD18-mediated PMN adhesion to HUVEC. (*Top*) Studies on the influence of 15(*S*)-HETE on LTB_4-stimulated adhesion in which ^{111}In-labeled PMNs were exposed to 15(*S*)-HETE (0.3–30 μM) or its diluent for 30 min at 37°C and were washed twice before coincubation with HUVEC and LTB_4 (10^{-7} M, 30 min) (n = 3; *$P < 0.005$; **$P < 0.001$; ***$P < 0.05$). (*Bottom*) Studies of stereoselectivity in which ^{111}In-labeled PMNs were exposed to equimolar concentrations (30 μM) of 15(*S*)-HETE, 12(*S*)-HETE, or 5(*S*)-HETE and were washed twice before coincubation with HUVEC and LTB_4 (10^{-7} M, 30 min) (n = 3; *$P < 0.01$). Data are expressed as a percentage of LTB_4-induced adhesion observed with diluent-treated PMNs (152.3±35.8 PMN/mm^2, n = 4).

into PMN phospholipids. However, some 15(*S*)-HETE is released from esterified sites upon activation of PMNs and could potentially inhibit chemotaxis and adhesion by interaction with recently reported specific 15(*S*)-HETE receptors (35) and activation of signal transduction events. We monitored the binding of [^{3}H]15(*S*)-HETE to PMNs to explore this possibility. Assays were performed at 4°C to differentiate ligand–receptor interactions from enzymatic esterification of [^{3}H]15(*S*)-HETE into PMN phospholipids. Specific binding of [^{3}H]15(*S*)-HETE was not observed, even after a 3-h incubation, suggesting that PMNs do not display specific cell-surface receptors for 15(*S*)-HETE (Table I).

15(*S*)-HETE–remodeled PMNs also generate an alternative profile of eicosanoids upon activation with chemoattractants, including increased LXA_4 generation and reduced LTB_4 synthesis (14). The influence of LXA_4 on LTB_4-induced (10^{-8} M, 30 min) transmigration was assessed to explore the possibility that 15(*S*)-HETE remodeling inhibits this response through the formation of LXA_4. To this end, PMNs were incubated with LXA_4 (10^{-7}–10^{-12} M) for a short duration (5 min) before coincubation with HUVEC because, when generated by release of esterified 15(*S*)-HETE, LXA_4 is likely to modulate transmigration rapidly. LXA_4 did not inhibit LTB_4-induced PMN transmigration at this time interval (LTB_4-stimulated transmigration: LTB_4 alone 55.5±3.1%; LXA_4 (10^{-7} M) plus LTB_4 62.9±1.0%, n = 3). To determine if 15(*S*)-HETE remodeling inhibits PMN–endothelial cell interactions by inhibiting LTB_4 generation by activating PMNs, transmigration was assessed after treatment of PMNs with MK886 (10^{-6} M, 10 min), a potent inhibitor of 5-lipoxygenase activity (27). LTB_4-induced PMN transmigration was not affected by this agent (PMN transmigration: LTB_4 alone 33.7±2.8%; MK866 plus LTB_4 29.7±4.1%). Taken together, these data suggest that the inhibitory effects of 15(*S*)-HETE remodeling were due to an action of esterified 15(*S*)-HETE within PMN cell membranes, and were not the result of actions of deacylated 15(*S*)-HETE released from phospholipid stores or the altered profile of lipoxygenase products generated by 15(*S*)-HETE–remodeled PMNs.

15(S)-HETE remodeling inhibits PMN–endothelial adhesion induced by LTB_4, but not A-23,187 or PMA. LTB_4 stimulates many PMN functional responses, including CD11/CD18-mediated adhesion, by activating specific cell-surface

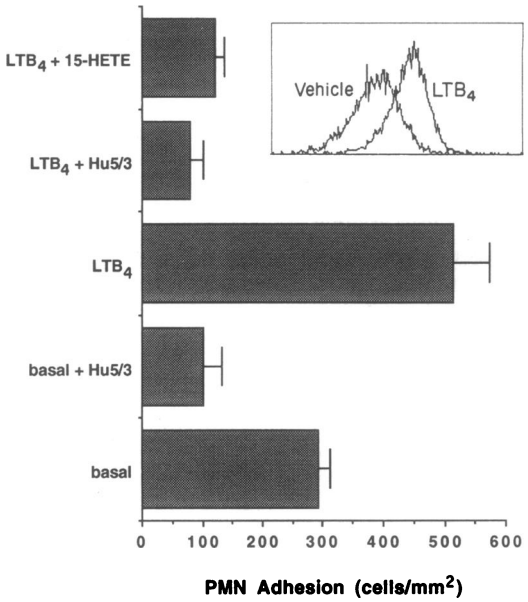

Figure 5. Influence of 15(*S*)-HETE on LTB_4-stimulated PMN adhesion to adsorbed ICAM-1. LTB_4-induced adhesion (10^{-7} M) of 15(*S*)-HETE–remodeled (30 μM, 30 min, 37°C) PMNs or diluent-treated PMNs to ICAM-1 adsorbed onto tissue culture plastic was assessed as described in Methods. ICAM-1–dependent adhesion was calculated by incubating PMN- and ICAM-1–coated plates with anti–ICAM-1 mAb (Hu5/3; 25 μg/ml) for 10 min before and during coincubations. Data are means±SE of six experiments. *$P < 0.05$. CD11/CD18 surface expression was assessed by FACS® analysis on 15(*S*)-HETE–remodeled (30 μM) (*inset*) and diluent-treated (not shown) PMNs using an anti-CD18 mAb (R15.7, 10 μg/ml). PMNs were studied in their basal state and after stimulation with LTB_4 (10^{-7} M). LTB_4 provoked similar upregulation of CD11/CD18 surface expression on 15(*S*)-HETE–remodeled and diluent-treated PMNs. Indeed, basal and LTB_4-stimulated CD11/CD18 levels were always higher with 15(*S*)-HETE–remodeled PMNs (not shown).

Table I. Assessment of Specific Binding of [^{2}H]15(S)-HETE to PMNs

Incubation time	Cell-associated radioactivity (%)	
	[^{3}H]15(*S*)-HETE	[^{3}H]15(*S*)-HETE plus unlabeled 15(*S*)-HETE
30 min	2.75±0.08	2.40±0.30
60 min	2.54±0.04	2.56±0.03

PMNs (3×10^6/0.3 ml) were incubated with [^{3}H]15(*S*)-HETE (0.2 nM) at 4°C in the presence or absence of excess unlabeled 15(*S*)-HETE (0.2 μM) ($n = 3$). Cell-associated radioactivity was measured as in Methods and expressed as percentage of total radioactivity added.

receptors and signal transduction events including activation of phospholipase C, generation of IP_3 and diacylglycerol, mobilization of intracellular calcium, and activation of protein kinase C (PKC) (5, 36). Lipids may also modulate the avidity of integrins, including CD11/CD18 integrins, directly within PMN membranes (37, 38). A-23,187 and PMA provoke PMN–endothelial cell adhesion through CD11/CD18 integrins by raising the intracellular calcium concentration and activating PKC, respectively. These agents were used to distinguish between inhibition of membrane-associated signal transduction events by esterified 15(*S*)-HETE and a more direct action of esterified 15(*S*)-HETE on integrin function. In striking contrast to its effect on LTB_4-induced adhesion, 15(*S*)-HETE remodeling did not inhibit PMN adhesion stimulated by either A-23,187 or PMA (Table II). Since A-23,187 and PMA also influence endothelial cell adhesiveness for PMNs, potentially complicating the interpretation of these studies, the influence of 15(*S*)-HETE on PMN adhesion to paraformaldehyde-fixed HUVEC was also tested. As with adhesion to viable HUVEC, neither A-23,187- nor PMA-induced adhesion was inhibited by 15(*S*)-HETE (Table II). These data suggest that 15(*S*)-HETE inhibits LTB_4-induced signal transduction events at or between receptor binding and activation of phospholipase C in PMNs.

15(S)-HETE remodeling increases the K_d of high affinity LTB_4 receptors on PMNs and inhibits LTB_4-induced IP_3 generation. PMN motility responses are initiated by the interaction of LTB_4 with specific high affinity cell-surface receptors on PMNs (39; for review see reference 40). Binding of [^{3}H]LTB_4 to 15(*S*)-HETE–remodeled and vehicle-treated PMNs was assessed, and was evaluated by Scatchard analysis to determine the influence of esterified 15(*S*)-HETE on LTB_4 receptor function. Control PMNs expressed high affinity binding sites for [^{3}H]LTB_4 with a mean dissociation constant of 0.93×10^{-9} M and mean densities of 289 fmol/10^7 PMNs, consistent with reports by other investigators (39). The affinity of this receptor was reduced significantly after 15(*S*)-HETE remodeling of PMNs (Table III).

LTB_4-induced IP_3 generation was monitored to further define the influence of esterified 15(*S*)-HETE on receptor-triggered signal transduction events. LTB_4 provoked a rapid increase in IP_3 formation in vehicle-treated PMNs (Fig. 6, *top*), as reported by other investigators (41). IP_3 levels were maximal after 5 s of exposure and declined to basal levels within 1 min (IP_3 levels in picomoles per 10^6 PMNs: basal 1.16±0.36; 5 s after LTB_4 stimulation 2.42±0.41, $n = 7$, $P < 0.001$). Basal levels of IP_3 were not different in 15(*S*)-HETE–remodeled and vehicle-treated PMNs (not shown). In contrast, 15(*S*)-HETE–remodeled PMNs generated significantly less IP_3 upon stimulation with LTB_4 (35.3±11.8% inhibition, $n = 7$, $P < 0.025$; Fig. 6, *bottom*). Taken together, these results suggest that 15(*S*)-HETE remodeling causes striking downregulation of the LTB_4 receptors that subserve PMN migration, and inhibition of stimulus–response coupling with PMN cell membranes.

Discussion

Here, we present evidence that 15(*S*)-HETE is a potent and stereoselective inhibitor of LTB_4-stimulated PMN migration in vitro. This effect was dependent on esterification of 15(*S*)-HETE into PMN phospholipids, could be overcome by agents that bypass membrane signaling events, and was associated with a fivefold reduction in the affinity of specific PMN cell-surface receptors for LTB_4. Importantly, 15(*S*)-HETE inhibited PMN transmigration at concentrations that are likely to be found in circulating blood during inflammation (17). While our studies focused on the influence of 15(*S*)-HETE on LTB_4-induced PMN responses, it is noteworthy that 15(*S*)-HETE also blocked transmigration induced by C5a and FMLP and therefore may modulate the activity of other receptor-mediated stimuli for PMN recruitment during acute inflammation. These observations suggest a mechanism for the observed antiinflammatory activity of 15(*S*)-HETE in inflammatory diseases and support a role for 15(*S*)-HETE as a potential endogenous chalone which may serve to limit or reverse inflammation in vivo.

15(*S*)-HETE is esterified rapidly into PMN phospholipids

Table II. 15(S)-HETE Remodeling of PMN Phospholipids Is Associated with Inhibition of PMN–Endothelial Cell Adhesion Induced by LTB_4, but Not A-23,187 or PMA

Stimulus	Inhibition of PMN adhesion (%)		
	LTB_4 (0.1 μM)	PMA (0.1 μM)	A-23,187 (5 μM)
Viable HUVEC	83.0±19.5 ($n = 4$)*	−2.3±6.8 ($n = 5$)	16.1±12.3 ($n = 6$)
PFA-treated HUVEC	93.7±12.3 ($n = 3$)‡	−12.9±11.7 ($n = 4$)	21.2±9.7 ($n = 4$)

^{111}In-labeled PMNs were exposed to 15(*S*)-HETE (3 μM) for 30 min at 37°C and were washed twice to remove extracellular 15(*S*)-HETE. Adhesion of 15(*S*)-HETE–remodeled PMNs (4×10^5/0.2 ml/well) to confluent HUVEC monolayers was assessed after stimulation (30 min) with either LTB_4, PMA, A-23,187, or diluent. PFA-treated HUVEC, HUVEC monolayers that were treated with paraformaldehyde (1%) overnight at 4°C and washed three times in PBS before coincubation with PMNs. Adhesion of 15(*S*)-HETE–remodeled PMNs is expressed as percentage inhibition of adhesion observed with PMNs that were exposed to diluent. Data are means±SE of three to six experiments, each performed in quadruplicate. * $P < 0.025$; ‡ $P < 0.005$.

Table III. Influence of 15(S)-HETE Remodeling on Binding of [^{3}H]LTB$_4$ to PMNs

Condition	K_d	B_{max}
	nM	*fmol/10^7 PMNs*
Vehicle-treated PMNs	0.93±0.21	289±25
15(S)-HETE–remodeled PMNs	4.69±0.86*	336±46

PMNs were exposed to 15(S)-HETE (30 μM) (15(S)-HETE–remodeled PMNs) or its diluent (vehicle-treated PMNs) for 30 min at 37°C and were washed twice with PBS before incubation with [^{3}H]LTB$_4$ (1 nM) for 20 min at 4°C in the presence or absence of various concentrations (10^{-10}–10^{-6} M) of cold LTB$_4$. The K_d and B_{max} of the high affinity LTB$_4$ receptor were calculated by Scatchard analysis. Data are the means of four different experiments. * $P < 0.05$.

(for review see reference 16). It is noteworthy that the carboxy terminus (carbon-1) methyl ester derivative of 15(S)-HETE was relatively ineffective in attenuating transmigration (Fig. 2), suggesting that esterification of 15(S)-HETE is a requirement for its inhibitory activity. The pattern of esterification of 15(S)-HETE into PMN phospholipids differs markedly from that of 5(S)-HETE or 12(S)-HETE (14, 29). Under the remodeling conditions used in this study, PMNs esterify ~ 20% of [^{3}H]15(S)-HETE into inositol-containing phospholipids, while uptake into other phospholipid classes, cholesterol ester, and triglycerides is < 4% (14). In contrast, PMNs incorporate ~ 0.6% of 5(S)-HETE into phosphatidylinositol, ~ 1.5% into phosphatidylcholine, and ~ 0.8% into phosphatidylethanolamine, and ~ 8% of added radiolabel is incorporated into triglycerides (14). In the case of 12(S)-HETE, PMNs incorporate ~ 2% into phosphatidylcholine, negligible amounts into other phospholipid classes, and ~ 23% into triglycerides (29). In this study 15(S)-HETE was a significantly more potent inhibitor of transmigration than either 5(S)-HETE or 12(S)-HETE (Fig. 2). These results suggest that the pattern of phospholipid remodeling is an important determinant in the action of 15(S)-HETE on PMN transmigration and that remodeling of the inositol-containing phospholipid pool may be particularly important in this regard.

The inhibitory action of 15(S)-HETE proved stereoselective; 15(S)-HETE was significantly more potent than the R-isomer as an inhibitor of transmigration (Fig. 2). 15(S)-HETE is the major product of leukocyte 15-lipoxygenase (for review see reference 16). In contrast, cyclooxygenases and epoxygenases generate the carbon-15 alcohol with racemic amounts of the R and S enantiomeric forms. Taken together, these results suggest that lipoxygenase-derived 15(S)-HETE may be the most effective inhibitor of PMN–endothelial cell interactions in vivo.

Esterified 15(S)-HETE appeared to inhibit PMN migration, in part, by blunting the cytoskeletal changes that subserve chemotaxis. In support of this hypothesis, the LTB$_4$-stimulated increase in F-actin content was reduced after remodeling of PMN phospholipids with 15(S)-HETE, as assessed by rhodamine-phalloidin binding (Fig. 3). Furthermore, treatment of PMNs with 15(S)-HETE also reduced the PMN right angle light scatter response induced by LTB$_4$ (Fig. 3), another index of cytoskeletal rearrangement (30, 31). Several lines of evidence suggested that esterified 15(S)-HETE also blocked PMN transmigration by inhibiting PMN–endothelial cell adhesion mediated by interactions of CD11/CD18 integrins with endothelial cell ligands. Both transmigration and adhesion were CD11/CD18-dependent events, as determined by mAb-blocking studies, and were attenuated dramatically by 15(S)-HETE. These responses also were inhibited by mAb against ICAM-1, suggesting that ICAM-1 was an important endothelial ligand for CD11/CD18 under these conditions. Furthermore, 15(S)-HETE, in addition to blocking PMN adhesion to endothelium, also blocked LTB$_4$-stimulated adhesion of PMNs to purified ICAM-1 (Fig. 5). Interestingly, 15(S)-HETE did not prevent the rapid increase in CD11/CD18 surface expression that occurs upon stimulation of PMNs with LTB$_4$ (Fig. 5, *inset*). These observations provide further evidence that chemoattractants provoke CD11/CD18-mediated PMN adhesion, in large part, by increasing CD11/CD18 avidity rather than through changes in CD11/CD18 surface expression (for review see reference 5). Furthermore, they suggest that these events may be mediated by different signal transduction pathways (vide infra).

Some 15(S)-HETE is mobilized from esterified sites upon activation of PMNs and is transformed to other bioactive eicosanoids (14). Other cell types, such as mast/basophil PT-18 cells, appear to express cell-surface receptors for 15(S)-HETE (35). The presence of putative 15(S)-HETE–specific cell-surface receptors would be an attractive hypothetical site for 15(S)-HETE–evoked signaling responses. However, we were unable to demonstrate specific cell-surface binding sites for 15(S)-HETE with PMNs at 4°C (Table I), making it unlikely

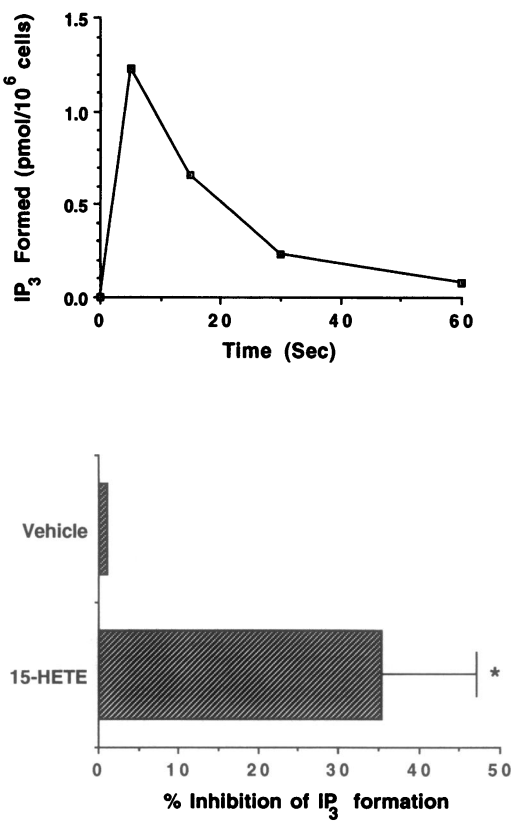

Figure 6. LTB$_4$-stimulated IP$_3$ formation by PMNs: inhibition by esterified 15(S)-HETE. (*Top*) Time course of IP$_3$ formation by PMNs upon stimulation with LTB$_4$ (10^{-7} M, 5 s, 37°C). Representative of two experiments, each performed in duplicate. (*Bottom*) Inhibition of LTB$_4$-stimulated (10^{-7} M, 5 s, 37°C) IP$_3$ formation in 15(S)-HETE–remodeled PMNs (30 μM, 30 min, 37°C). Data are mean±SE of seven experiments. * $P < 0.025$.

that 15(S)-HETE inhibited PMN–endothelial cell interactions in our study through receptor-triggered actions of deacylated 15(S)-HETE with PMNs. In this regard, it is also noteworthy that 15(S)-HETE itself, at the concentrations used to remodel PMN phospholipids, did not induce PMN adhesion or transmigration, suggesting that 15(S)-HETE does not activate and, thus, desensitize LTB_4 receptors. Some of the esterified 15(S)-HETE, upon release from PMN phospholipids, is transformed to a variety of bioactive products including 5(S), 15(S)-diHETE, LXA_4, and lipoxin B_4 (14). While LXA_4 can inhibit a variety of PMN responses including LTB_4-induced IP_3 generation (42), chemotaxis (43), and LTB_4-induced PMN–endothelial cell adhesion (44) and other cell types (23), this eicosanoid did not inhibit LTB_4-induced transmigration of PMNs under the experimental conditions (i.e., 5-min pretreatment) used in this study. This does not, however, preclude a role for lipoxins as modulators of leukocyte–endothelial cell interactions within different temporal frameworks. Similarly, 15(S)-HETE did not appear to inhibit PMN–endothelial cell interaction(s) by impairing the ability of PMNs to generate LTB_4, as MK886, a potent and specific inhibitor of 5-lipoxygenase activity (27), did not influence LTB_4-induced transmigration. This series of observations suggests that 15(S)-HETE did not blunt PMN–endothelial cell interactions through changes in eicosanoid biosynthesis by activated PMNs.

Esterified 15(S)-HETE, by altering the composition and biophysical properties of PMN cell membrane lipids, could potentially influence the avidity of CD11/CD18 integrins for endothelial ligands. Along these lines, the function of other integrins such as the vitronectin receptor has been shown to be modulated by the surrounding phospholipid environment (37). Furthermore, Hermanowski-Vosatka et al. (38) have recently demonstrated CD11/CD18 modulatory activity in the lipids of activated PMNs. Alternatively, 15(S)-HETE could influence LTB_4-induced adhesion and transmigration of PMNs by blocking LTB_4-triggered signal transduction events. In the present study, A-23,187 and PMA were useful pharmacologic tools for distinguishing among these possibilities in that they bypass receptor–ligand interactions. 15(S)-HETE remodeling did not inhibit adhesion induced by either of these agonists, in marked contrast to its dramatic effects on LTB_4-induced PMN–endothelial cell interactions (Table II). These data provided compelling evidence that esterified 15(S)-HETE inhibits LTB_4-induced adhesion by interfering with LTB_4 signal transduction pathways within PMN cell membranes. Importantly, they also indicate that 15(S)-HETE–remodeled PMNs are viable and respond appropriately to stimuli which bypass initial signal transduction events.

Assessment of specific binding of [^{3}H]LTB_4 (Table III) and LTB_4-induced IP_3 generation (Fig. 6) confirmed that 15(S)-HETE dramatically impairs stimulus–response coupling in PMN cell membranes. Concurrent with the reduction in CD11/CD18-mediated PMN adhesion and transmigration, 15(S)-HETE remodeling caused a striking decrease in the affinity of the high affinity LTB_4 cell-surface receptors that subserve these responses (39). Furthermore, IP_3 generation, a pivotal event in LTB_4-triggered signal transduction events in PMN cell membranes (41), was inhibited in 15(S)-HETE–remodeled cells. The mechanism by which 15(S)-HETE esterification modulates the affinity of LTB_4 receptors can now be addressed in future studies. Interestingly, alterations in membrane lipid composition have been reported to alter the mobility of membrane-associated proteins (45), to influence the conformation and/or density of many different receptors, and to modulate their accessibility and affinity for soluble ligands (37, 46–48). Indeed, the omega-3 fatty acids, eicosapentaenoic acid and docosahexaenoic acid, also reduce the expression of LTB_4 receptors on PMNs (49), a mechanism that may potentially account for their antiinflammatory activity in vivo.

Other cellular events that may potentially be modulated by esterified 15(S)-HETE and, thus, account for its inhibitory actions on LTB_4-triggered PMN responses include LTB_4 coupling to G-proteins, and activity of phospholipase C, actin-binding proteins, and PKC. Along these lines, it is intriguing to note that 15(S)-HETE–remodeled cells generate 15(S)-HETE–remodeled diacylglycerol (50). Furthermore, arachidonate depletion impairs recognition of both phosphatidylinositol phosphate and bisphosphate by phospholipase C in pituitary GH3 cells (51), and phosphatidylinositol phosphate and bisphosphate are important regulators of gelsolin, profilin, and several actin-binding proteins that modulate PMN actin assembly and motility (for review see references 2, 4).

In summary, the results of this study indicate that 15(S)-HETE remodeling of PMN phospholipids stereoselectively reduces the affinity of PMN LTB_4 receptors for their cognate ligands and causes striking inhibition of LTB_4-triggered stimulus–response coupling, cytoskeletal rearrangements, and PMN–endothelial cell interactions. These observations, when viewed in the context of the previously documented inhibitory actions of 15(S)-HETE on PMN superoxide generation in vitro (52) and PMN-mediated inflammation in vivo (21, 22), provide compelling evidence that 15(S)-HETE may be an important endogenous inhibitor of inflammation. They raise the intriguing possibility that products of 5-lipoxygenase and 15-lipoxygenase pathways may exert counterbalancing modulatory influences on PMN trafficking, in a manner analogous to the opposing regulatory actions of the cyclooxygenase-derived products prostacyclin and thromboxane A_2 on hemostasis and thrombosis. Further elucidation of the cellular and molecular actions of 15-lipoxygenase–derived eicosanoids may reveal additional novel sites for therapeutic intervention in inflammation.

Note added in proof: Recent studies have shown that 15-HETE derived from cyclooxygenase-2 in the presence of aspirin is in the R configuration (Smith, W., et al. 3rd International Conference on Eicosandoids and Other Bioactive Lipids in Cancer, Inflammation and Radiation Injury, Washington, DC, 13–16 October 1992) and that 15(S)-HETE also inhibits PMN degranulation stimulated by receptor-specific agonists (Smith R. J., et al. 1993. *Proc. Natl. Acad. Sci. USA.* 90:7270–7274.)

Acknowledgments

The authors thank Drs. M. E. Brezinski, F. W. Luscinskas, T. P. Stossel, and B. M. Brenner for helpful discussions.

These studies were supported by National Institutes of Health grants DK-44380 to H. R. Brady, GM-38765 to C. N. Serhan, and AR-38910 to P. A. Janmey. C. N. Serhan is the recipient of an Established Investigator Award from the American Heart Foundation. H. R. Brady is the recipient of an award from the William F. Milton Fund. M. Matsubara is the recipient of a Postdoctoral Research Fellowship Award from the Juvenile Diabetes Foundation. P. G. Allen is the recipient of grant 13-402-920 from the American Heart Association, Massachusetts affiliate.

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