Leukotriene B4 Receptor Antagonism Reduces Monocytic Foam Cells in Mice

Robert J. Aiello, Patricia-Ann Bourassa, Saralyn Lindsey, Weifan Weng, Ann Freeman, Henry J. Showell

Abstract—Leukotriene B4 (LTB4) is a potent chemotactic agent that activates monocytes through the LTB4 receptor (BLTR). We tested the hypothesis that LTB4 receptor blockade would slow atherosclerotic progression by inhibiting monocyte recruitment. Homozygous low-density receptor knockout (LDLr−/−) mice and apolipoprotein E deficient (apoE−/−) mice were treated with a specific LTB4 receptor antagonist, CP-105,696, for 35 days. In apoE−/− mice, treatment with the LTB4 antagonist did not affect plasma lipid concentrations but significantly reduced CD11b levels both in vascular lesions and whole blood. Compared with age-matched controls, lipid accumulation and monocyte infiltration were significantly reduced in treated apoE−/− mice at all time points tested. Lesion area reduction was also demonstrated in LDLr−/− mice maintained on a high-fat diet. LTB4 antagonism had no significant effect on lesion size in mice possessing the null alleles for another chemotactic agent, monocyte chemotactant protein-1 (MCP-1−/−×apoE−/−), suggesting MCP-1 and LTB4 may either interact or exert their effects by a common mechanism. These results demonstrate that in a preclinical model of atherosclerosis LTB4 receptor blockade reduces lesion progression and further suggest a previously unrecognized role for LTB4 or other oxidized lipids recognized by the BLTR receptor in the pathogenesis of this disease. (Arterioscler Thromb Vasc Biol. 2002;22:443-449.)

Key Words: leukotriene B4  ■  atherosclerosis  ■  monocyte chemoattractant protein-1

The pathogenesis of atherosclerosis is a complex process, which involves the recruitment and activation of monocytes in the developing atherosclerotic lesion.1 It is thought to initiate when circulating monocytes are first attracted to a site of vascular injury through the upregulation of adhesion molecules and chemotactic factors in the lesion.2,3 Once attached to the vessel wall, monocytes, attracted by a gradient of chemotactic factors, migrate between the endothelial cells into the subendothelial space where they differentiate into macrophages and become lipid-laden foam cells. A growing body of evidence suggests that the recruitment of monocytes is governed by cell-specific chemotactants. Several molecules have chemotactic activity for monocytes, including N-formylmethionyl peptides exemplified by N-formylmethionyl-leucyl-phenylalanine (FMLP), the complement fragment C5a, the arachidonic acid metabolite leukotriene B4 (LTB4), 12-hydroxyeicosatetraenoic acid (12-HETE), and the chemotactant monocyte chemotactic protein-1 (MCP-1).5

MCP-1 is the prototype of the CC chemokine β subfamily and exhibits the most potent chemotactic activity for monocytes.5 MCP-1 is highly expressed in human atheromatous plaques,6 and its overexpression contributes to the development of atherosclerosis in mouse models.7 In hypercholesterolemic mice, a deficiency in either MCP-18 or the MCP-1 receptor (CCR2)9 results in a marked decrease in atheromas and fewer monocytes in vascular lesions. However, at later stages of lesion development in these mice, differences in lesion size relative to wild type become less pronounced. These data suggest that other chemotactic factors are likely compensating for the lack of MCP-1.

LTB4, a product of the 5-lipoxygenase pathway of arachidonic acid metabolism, is also a potent chemoattractant and proinflammatory mediator thought to be involved in the pathogenesis of several inflammatory diseases.10,11 Ex vivo, human vascular plaques have been shown to produce leukotrienes,12 but the evidence for a specific role of 5-lipoxygenase in atherosclerosis is only circumstantial. However, most cell types, including macrophages found in lesions, are capable of nonenzymatically producing isomers of LTB4, such as isoleukotrienes, by free radical oxidation.13 These oxygenated products of arachidonic acid are known to modulate diverse biological processes including leukocyte recruitment and activation.

LTB4 and B4 isoleukotrienes induce their effects by binding to the high-affinity LTB4 receptor (BLTR)14 which is expressed on inflammatory cells such as neutrophils, eosinophils, and macrophages. Moreover, LTB4 receptor antagonists have been shown to inhibit the recruitment of macro-

See page 361 and cover

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plagues, neutrophils, and eosinophils in preclinical disease models such as experimental autoimmune encephalomyelitis, collagen-induced arthritis, and allograft transplantation.15-17 Thus, LTβ4 or other ligands that bind to the LTβ4 receptor may play a proatherogenic role because of its ability to promote the adhesion and chemotaxis of leukocytes across the endothelium. To explore the potential pathophysiological role of LTβ4 in atherosclerosis, we tested a potent specific LTβ4 receptor antagonist, CP-105,696 on lesion progression in several murine models of atherosclerosis. CP-105,696 was chosen because it was previously shown to inhibit 3H-LTB4 binding to murine receptors,18 with nanomolar potency and to selectively block LTβ4-mediated functional responses of murine leukocytes in vitro and in vivo.16,17

Methods

Mouse line derivation

All animal experiments were performed according to protocols approved by the institutional committee for use and care of laboratory animals. Genetically modified apolipoprotein E–deficient (ApoE /−), LDL receptor-deficient (LDLr−/−), and MCP–1–deficient (MCP–1−/−) mice used in the present study have been previously described and characterized.19,20 To obtain mice homozygous for both the apoE and MCP–1 (ScyA2) null alleles, male apoE /− mice on a C57B/6 background (C57E/H11002) were mated with heterozygous female MCP–1+/− mice on a C57B/6 background. The resulting apoE+/−×MCP–1−/− progeny were identified by Southern blot analysis and inbred to produce apoE−/−×MCP–1−/− (C57E−/−×MCP–1−/−) and control apoE−/−×MCP–1+/− (C57E−/−×MCP–1−/−) mice. ApoE−/− mice on the mixed genetic background 1290la×C57B/6 (129E−/−) were bred in-house and derived from brother and sister matings. All mice were weaned at 21 days, maintained on a 1-hour light/dark cycle, and fed either a chow or a Western-type diet.21

Administration of Compound

CP-105,696 was evaluated for its effects on lesion development in age-matched groups of mice (15 to 30 weeks old at time of sacrifice) with a 35-day treatment protocol. Subgroups of mice were treated with CP-105,696 for shorter times (7 to 14 days) for evaluation of acute changes in tissue gene expression as noted in the table and figure legends. In all study groups, the compound was administered by oral gavage once daily in a vehicle composed of 0.6% Tween 80 and 50 μg/mL of fluorescein-conjugated rat IgG. After 30 minutes, rat anti-mouse MOMA-2 was applied at a concentration of 50 μg/mL, and rat anti-mouse CD11b was applied at a concentration of 25 μg/mL, and rat anti-mouse CD11b was applied at a concentration of 5 μg/mL. Incubation with primary antibodies was followed by a biotinylated donkey anti-rat IgG secondary antibody (1.4 μg/mL, Jackson ImmunoResearch), and incubation with horseradish peroxidase–conjugated streptavidin (1:500). A nonspecific rat IgG2a antibody (Pharmingen) was used as a negative control. Antibody binding was visualized with 3,3′-diaminobenzidine, and all sections were counterstained with Gill III hematoxylin. Results are expressed as the percent of the total cross-sectional vessel wall stained with Oil red O. For each animal, the average lesion size of 12 to 16 sections was determined, and data are expressed as lesion size or mean percent lesion area ±SD.

Immunohistochemical Staining of Macrophages

Serial paraformaldehyde–fixed, OCT-embedded sections of aortic valves were immunostained for macrophages using rat monoclonal antibodies (IgG1a) against MOMA–2 (BioSource) and CD11b (Pharmingen). Endogenous biotin and peroxidase activity were blocked by incubating each section with an avidin/biotin solution (Vector Laboratories) and 0.3% H2O2 in 1% bovine serum, respectively. Rat anti-mouse MOMA–2 was applied at a concentration of 25 μg/mL, and rat anti-mouse CD11b was applied at a concentration of 5 μg/mL. Incubation with primary antibodies was followed by a biotinylated donkey anti-rat IgG secondary antibody (1.4 μg/mL, Jackson ImmunoResearch), and incubation with horseradish peroxidase–conjugated streptavidin (1:500). A nonspecific rat IgG2a antibody (Pharmingen) was used as a negative control. Antibody binding was visualized with 3,3′-diaminobenzidine, and all sections were counterstained with Gill III hematoxylin. Results are expressed as the percent of the total cross-sectional vessel wall area (normal + diseased area/section, excluding the lumen) stained with diaminobenzidine.

Plasma Lipoproteins and Lipids

Separation of plasma lipoproteins with fast protein liquid chromatography (Pharmacia LKB Biotechnology, Inc) was performed as previously described.21 Total plasma cholesterol and triglycerides were measured by using colorimetric methods with commercially available Cholesterol/HP (Boehringer-Mannheim) and Triglycerides G (Wako Chemical) kits.

Aortic Tree Analysis

In some experiments, mice were anesthetized as above and perfusion-fixed with 4% paraformaldehyde, and the entire aortic tree was removed including the brachiocephalic region and the carotid, subclavian, and femoral branches. The tissue was cleaned of adventitia and laid out on a piece of polystyrene, and a digital image was obtained with a digital camera (Sony) attached to a copy stand. The carotid and subclavian branches were removed, and the brachiocephalic region was isolated for further processing. The percent of the aortic surface covered by lesions in the remaining portion of the aortic tree was determined by using an en face preparation as previously described.21 Each aorta was evaluated for lesion area by direct-image capture and displayed on a Trinitron monitor (Sony). The lesion area was determined in unstained tissue with ImagePro 3.1 image analysis (Image Processing Solutions). Areas of atherosclerotic plaques in aortas cleaned of adventitia appear as yellowish-white opaque areas compared with translucent non-lesioned areas. This area was quantified by manually setting thresholds for shades of black (background), gray (normal tissue), and white (lesion area).

Lesion Analysis

To determine cross-sectional lesion area, hearts were perfusion-fixed in 4% paraformaldehyde, infiltrated with 30% gum sucrose for 24 hours at 4°C, embedded in Tissue-Tek OCT compound (Sakura Finetek) and sectioned in 10 μm, −18°C, as previously described.21 The sections were stained with Oil red O (Polysciences) and counterstained with Gill III hematoxylin (Sigma). Oil red–stained sections of the aortic sinus and valve regions were captured directly from a digital camera attached to a Leitz Laborlux S (Leica) light microscope and displayed on a Trinitron monitor. The aortic sinus begins with the first appearance of the valve cusps delineating the lumen into 3 distinct areas. At the end of the aortic sinus, the coronaries branch off, the valve cusps no longer divide the lumen, and the perimeter of the aorta is round rather than bulging. The aortic valve begins at this point and continues to the junction of the aorta, approximately 300 μm. Image analysis was performed with ImageJ software analysis (Image Processing Solutions). Results are expressed as the average lesion size per section or as the percent of the total cross-sectional vessel wall stained with Oil red O. For each animal, the average lesion area of 12 to 16 sections was determined, and data are expressed as lesion size or mean percent lesion area ±SD.
TABLE 1. Effect of LTB4 Antagonism on Lesions in Atherosclerotic Mice

<table>
<thead>
<tr>
<th>Strain</th>
<th>N</th>
<th>Sex</th>
<th>Age, wk</th>
<th>Body Weight, gm</th>
<th>Dose, mg/kg</th>
<th>TPC, mg/dL</th>
<th>TG, mg/dL</th>
<th>Sinus, %lesion</th>
<th>Valve, %lesion</th>
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<td>129E0</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>10 F</td>
<td>15</td>
<td>25±1</td>
<td>0</td>
<td>739±59</td>
<td>72±13</td>
<td>22.5±4</td>
<td>24.9±3</td>
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<td>120±15</td>
<td>34.6±2</td>
<td>41.0±3</td>
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<td>780±51</td>
<td>138±21</td>
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<td>0</td>
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<td>59±12</td>
<td>43.5±3</td>
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<td>8 M</td>
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<td>38.8±3</td>
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<td>18</td>
<td>34±2</td>
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<td>30</td>
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<td>532±205</td>
<td>5.6±2*</td>
<td>6.5±3*</td>
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</tbody>
</table>

ApoE-deficient mice on a mixed 129/01/B6 background (129E0) or a C57B1/6 background (C57E0) and homozygous LDLR−/− mice were treated for 35 days orally with CP-105,696 at doses indicated: 0, 20, 50, or 100 mg/kg. LDLr-knockout mice were fed a Western diet; all other strains were maintained on chow diet. The mean percent lesion area in 10-μm sections of the aortic sinus and valve region was calculated after Oil red O staining. Values are reported as means ± SD. TPC indicates total plasma cholesterol; TG, triglycerides.

*P<0.01, †P<0.05 significant difference from vehicle-treated group.

Peritoneal Macrophage Chemotaxis Assay

On the termination of the study, a subset of mice from each group was injected intraperitoneally with 1 mL of sterile 6% casein. After four days, peritoneal exudate cells were harvested by washing the peritoneal cavity with Hanks’ Balanced Salt Solutions (Life Technologies), supplemented with 1% fetal bovine serum. Peritoneal cells then were washed three times in RPMI-1640 supplemented with 0.1% bovine serum albumin. For chemotaxis, cells were suspended at a concentration of 2×10⁶ cells/mL in chemotaxis buffer (RPMI-1640 supplemented with 0.1% bovine serum albumin and 20 mmol/L HEPES, pH 7.4). The cell suspension was loaded in the upper chamber of a 48-well microtaxis chamber. Murine MCP-1 or LTB4 (10 mmol/L) was added to the lower chamber, which was separated from the upper chamber by a 5-μm polycarbonate membrane. The peritoneal macrophages attached to the underside of the membrane were fixed and stained using the Diff-Quick stain set (Dade Behring Inc). The results were expressed as the mean number of cells that migrated in four high power fields (×20) in three replicate samples.

Statistics

Statistical significance was determined with unpaired Student’s t test by using the StatView statistical program (Abacus Concepts, Inc). Results are reported as mean±SD.

Results

Analysis of Lesions

The contribution of BLTR antagonism to atherosclerosis at different stages of lesion development was studied in genetically modified murine models of atherosclerosis. Because inflammatory responses to leukotrienes have been previously shown to differ among mouse strains with different genetic backgrounds, two strains of apoE−/− mice on a C57B/6 background (C57E0) and apoE−/− mice on a mixed background of 129/01a/BalbC (129E0) and LDLr−/− mice on a C57B/6 background were treated with a LTB4 antagonist (CP-105,696). The two different strains of apoE−/− (C57E0−/− and 129E0−/−) mice had similar lesion development (Table 1), and the extent of their atherosclerotic lesions increased significantly with age. The spontaneous lesions that developed in the aortic root (Figure 1) of the chow-fed apoE−/− were significantly (P<0.05) reduced in both strains of apoE−/− mice after treatment with the LTB4 antagonist CP-105,696 (Table 1). Lesions were also reduced throughout the entire aortic tree (Figure 2C and 2D). Representative sections from the brachiocephalic region of 20-week-old...
CD11b expression

Previous studies have shown that CP-105,696 inhibits the LTB4-mediated upregulation of CD11b on neutrophils and monocytes in vitro. To determine whether CP-105,696 was specifically affecting monocyte function, we studied the effects of CP-105,696 on monocyte CD11b cell surface expression in vivo. As shown in Figure 3, the levels of CD11b expression in circulating monocytes were decreased in a dose-dependent manner after treatment with CP-105,696 compared with vehicle-treated controls. The decrease in the percentage of cells staining for CD11b suggest that the amount of CD11b/cell was decreased. In contrast, neither the percent total monocytes or the number of monocytes in whole blood determined as peroxidase-positive stained cells

![Figure 3](http://atvb.ahajournals.org/)
by flow cytometric analysis was affected by treatment. Together these data suggest that the LTB4 antagonist decreased the level of CD11b expressed at the plasma membrane on circulating monocytes.

**Plasma Lipids**
The administration of CP-105,696 had no significant effect on plasma lipids or body weight (Table 1) in the chow fed apoE-deficient strains of mice. However, the plasma cholesterol levels in the 129E<sup>−/−</sup> mice fed a standard chow diet ranged between 600 and 800 mg/dL, which was significantly higher than levels found in the C57E<sup>−/−</sup> strain maintained on a chow diet (300 to 500 mg/dL). Plasma triglycerides were unchanged with treatment and averaged ≈150 mg/dL for all genotypes. LDLr<sup>−/−</sup> mice made hypercholesterolemic by being fed a Western diet exhibited plasma cholesterol levels ranging between 800 and 1200 mg/dL. These markedly elevated lipid levels were reduced as was body weight in LDLr<sup>−/−</sup> mice treated with CP-105,696 compared with control mice. A specific mechanism contributing to this effect on body weight and plasma lipids is unclear. No adverse effects or reduction in feed intake has been previously reported in animals studies after administration of CP-105,696. It is important to note that no effect was observed in any of the other strains of mice fed the chow diet. Size separation of plasma lipoproteins with fast protein liquid chromatography demonstrated there was a nonsignificant increase in VLDL cholesterol in mice treated with CP-105,696, but otherwise the lipoprotein profiles for treated and control mice were identical (data not shown).

**MCP-1 Null Allele**
MCP-1 deficient mice were bred onto the C57E<sup>−/−</sup> background to determine the relative contribution of LTB4 antagonism and MCP-1 depletion on lesion formation. Consistent with previous findings<sup>6</sup> at 16 weeks of age, mice homozygous for the MCP-1 null allele on a C57E<sup>−/−</sup> background (C57E<sup>−/−</sup>×MCP-1<sup>−/−</sup>) showed a significant but highly variable 50% reduction in lesion area in the aortic valve and sinus (Table 3) compared with C57E<sup>−/−</sup> (Table 1). As previously reported,<sup>6</sup> these apparent differences in lesion size resulting from MCP-1 depletion were not maintained as lesion size and complexity increased with age. Although there was a significant reduction in lesions observed in the apoE<sup>−/−</sup> strains treated with CP-105,696 (Table 1), there was no significant effect on lesion size in the mice homozygous for the MCP-1 null alleles (C57E<sup>−/−</sup>×MCP-1<sup>−/−</sup>) at any time points tested (Table 3).

**LTB4 and CCR2 Expression**
Because atherosclerosis in C57E<sup>−/−</sup>×MCP-1<sup>−/−</sup> mice was not decreased in response to the LTB4 antagonist CP-105,696, it was important to determine that these animals expressed functional LTB4 receptors. Both C57E<sup>−/−</sup> and C57E<sup>−/−</sup>×MCP-1<sup>−/−</sup> mice expressed similar levels of CCR2 and LTB4 receptor mRNA levels in peritoneal macrophages, liver, lung, and spleen, and these levels were unchanged with treatment (data not shown). Reagents were not available to determine receptor protein concentrations. To demonstrate functional LTB4 receptors on macrophages, the chemotactic response to both LTB4 and MCP-1 was measured by using elicited cells. The ability of elicited cells obtained from C57E<sup>−/−</sup>×MCP-1<sup>−/−</sup> mice (24±3 cells/×20 field) to migrate in response to LTB4 was identical to cells obtained from C57E<sup>−/−</sup> mice (25±4 cells/×20 field). The chemotactic response to 10 nmol/L MCP-1 was also similar for cells elicited from either C57E<sup>−/−</sup>×MCP-1<sup>−/−</sup> or C57E<sup>−/−</sup> mice (33±4 versus 27±5 cells/×20 field, respectively).

**Discussion**
We investigated the potential role for LTB4 and/or other ligands that bind to the LTB4 receptor in atherosclerosis using inbred strains of LDLr<sup>−/−</sup> and two strains of apoE<sup>−/−</sup> mice. We show that in both strains of apoE<sup>−/−</sup> mice and in LDLr<sup>−/−</sup> mice, lipid staining and foam cell accumulation were significantly reduced in the aortic valve, sinus, brachiocephalic region, and throughout the entire aortic tree after treatment with a specific LTB4 receptor antagonist. Blockage of the LTB4 receptor was achieved through the use of a very specific LTB4 antagonist, CP-105,696 which has robust potency and kinetics in mice.<sup>16,17,23</sup>

LTB4, a product of the 5-lipoxygenase pathway, induces a complex cascade of molecular and cellular events including chemotaxis, the promotion of leukocyte adherence to the vascular endothelium, stimulation of superoxide radical formation, and gene expression.<sup>10</sup> Monocyte recruitment into the subendothelial space is the hallmark of atherosclerosis and thus chemotactic factors are likely to play a major role in both the initiation and progression of this disease. LTB4 was originally purified from neutrophils and shown to be a major chemotactic factor for neutrophils.<sup>24</sup> However, neutrophils are seldom observed in atherosclerotic lesions, and their role

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**Table 3. Effect of CP-105,696 on Atherosclerotic Lesions in MCP-1–Deficient Mice**

<table>
<thead>
<tr>
<th>N</th>
<th>Sex</th>
<th>Age, wk</th>
<th>Body Weight, gm</th>
<th>Dose, mg/kg</th>
<th>TPC, mg/dL</th>
<th>TG, mg/dL</th>
<th>Sinus, %lesion</th>
<th>Valve, %lesion</th>
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Mice homozygous for the null alleles of MCP-1 and apoE<sup>−/−</sup> (MCP-1<sup>−/−</sup>×apoE<sup>−/−</sup>) on a C57bl/6 background (C57E0) were treated for 35 days orally with CP-105,696 at doses indicated: 0, 30, or 100 mg/kg. The mean percent lesion area in 10-μm sections of the aortic valve and sinus area was calculated after Oil red O staining. Values are reported as means±SD.
is less clear than in other inflammatory states. Besides neutrophils, LTB4 and some of its metabolites have also been shown to activate and attract a variety of other cell types found in atherosclerotic tissues including lymphocytes, natural killer cells, and most notably, monocytes. LTB4 is one of the most potent chemoattractants produced in atherosclerotic lesions, and our data suggest that it may be proatherogenic because of its ability to promote the adhesion to, and chemotaxis of, monocytes and leukocytes across the endothelium.

LTB4 produced by 5-lipoxygenase and other eicosanoids that interact with the LTB4 receptor have been implicated in a variety of human inflammatory diseases although their role in atherosclerosis has not been established. Considerable attention has been directed to the role of lipoxygenase and particularly 12- and 15-lipoxygenase in LDL oxidation and subsequent atherosclerosis. Cyros et al demonstrated that the absence of 12/15-lipoxygenase expression decreases lipid peroxidation and atherogenesis in apoE-deficient mice. Other circumstantial evidence has been provided that suggests lipoxygenases have a pathogenic role in the modification of LDL found in atherosclerotic lesions. Others have found that 5-lipoxygenase activity is not essential for human monocyte–mediated LDL oxidation.

Other LTB4 receptor ligands can be generated by lipid peroxidation occurring in atherosclerotic lesions by a variety of enzymatic and nonenzymatic reactions. Harrison and Murphy demonstrated that free radical oxidation can lead to the formation of numerous isomers of LTB4, B4 isoleukotrienes, which also activate inflammatory cells by binding to the LTB4 receptor. In addition to neutrophils, LTB4 is a major product of aortic endothelial cells and activated monocytes. Moreover, LTB4 and B4 isoleukotrienes are produced in atherosclerotic lesions, and the levels of LTB4 have been correlated with the degree of leukocyte infiltration in human atherosclerotic plaques.

Although the source of LTB4 in atherosclerotic lesions is unclear, one measurable effect of antagonizing the LTB4 receptor in our atherosclerotic mice was the reduced surface expression of the β2 integrin CD11b in both vascular lesions and whole blood. The recruitment of monocyctic cells into an area of inflammation is dependent on a set of highly regulated events. This sequence includes the coordinate induction of chemotactic factors as well as adhesion factors known as integrins. The integrin family of adhesion molecules actively participates and is required for cellular recruitment of cells. In addition to inducing chemotaxis through its receptor, LTB4 also stimulates leukocytes through modulation of integrin expression. The LTB4 antagonist CP-105,696 has been previously shown to inhibit monocyte chemotaxis in vivo and treatment with CP-105,696 significantly decreased macrophage CD11b expression in murine cardiac allograft transplant studies. It was unclear, however, if changes in tissue CD11b staining represented changes in monocyte numbers or activation. In the present study, antagonism of LTB4 receptors clearly decreased whole blood monocyte CD11b upregulation in apoE−/− mice without affecting total circulating monocyte numbers. Therefore, it is tempting to speculate that one protective effect of LTB4 antagonism is via modulation of CD11b interaction with its cognate ligands (eg, ICAM-1) which is upregulated in lesion prone sites of apoE-deficient mice. Nageh et al demonstrated a 50% to 70% reduction in atherosclerotic fatty streaks in mice with homozygous mutations for ICAM-1. Patel et al demonstrated antibodies against the ICAM-1 adhesion factor that reduced macrophage recruitment to atherosclerotic plaques in apoE-deficient mice. However, the potential redundancy among the intracellular adhesion ligands makes it difficult to demonstrate cause and effect relationships. For example, Manka et al demonstrated that the absence of ICAM-1 does not prevent against neointima formation after arterial injury whereas P selectin deficiency is critical for monocyte recruitment in this model. One possibility is that diminished CD11b expression, and thus the potential impairment of migratory and activation events of macrophages, lead to a reduction in lesion development. Kubo et al further reported that leukocyte CD11b expression may not be essential for the development of atherosclerosis in mice. Nevertheless, reductions in CD11b expression in the presence of CP-105,696 suggest that the activation state of monocytes is significantly attenuated in atherosclerotic lesions as manifested in whole blood.

In this study, blockade of the LTB4 receptor reduced the mononuclear infiltrate in vascular lesions in both chow-fed apoE−/− and fat-fed LDLr−/− mice. The magnitude of this response is comparable to the reduction in lesions observed in mice after MCP-1 gene disruption, or the MCP-1 receptor (CCR2). It is interesting to note that although MCP-1 has a powerful effect on monocyte recruitment, MCP-1–deficient mice still mount a significant tissue macrophage infiltrate, especially in latter stages of lesion development. Interestingly, we found that blockade of the LTB4 receptor did not eliminate or decrease the residual mononuclear infiltrate seen in MCP-1–deficient animals. The absence of any notable effect on lesion size or development may suggest that the antiatherosclerotic effects of LTB4 antagonism either require MCP-1 or interrupt a pathway common to both MCP-1 and LTB4. Matsukawa et al suggested that MCP-1 amplifies the atherosclerotic effects of LTB4 antagonism either require MCP-1 or interrupt a pathway common to both MCP-1 and LTB4. Our results show that LTB4 receptor antagonism with CP-105,696 prevents lesion progression in a preclinical animal model of atherosclerosis and suggests that LTB4 receptor antagonists may have clinical value in the treatment of atherosclerosis in man.

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References

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