Inhibition of Atherogenesis in BLT1-Deficient Mice Reveals a Role for LTB4 and BLT1 in Smooth Muscle Cell Recruitment

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**Background**—It is known that 5-lipoxygenase and its product, leukotriene B4 (LTB4), are highly expressed in several human pathologies, including atherosclerotic plaque. LTB4 signals primarily through its high-affinity G protein–coupled receptor BLT1, which is expressed on specific leukocyte subsets. BLT1 receptor expression and function on other atheroma-associated cell types is unknown.

**Methods and Results**—To directly assess the role of the LTB4-BLT1 pathway in atherogenesis, we bred BLT1−/− mice into the atherosclerosis-susceptible apoE−/− strain. Compound-deficient apoE−/−/Blt1−/− mice fed a Western-type diet had a marked reduction in plaque formation compared with apoE−/− controls. Immunohistochemical analysis of atherosclerotic lesions in compound-deficient mice revealed a striking decrease in smooth muscle cells (SMCs) and significant decreases in macrophages and T cells. We report here novel evidence of the expression and function of BLT1 on vascular SMCs. LTB4 triggered SMC chemotaxis, which was pertussis toxin sensitive in Blt1−/− SMCs and absent in Blt1−/− cells, suggesting that BLT1 was the dominant receptor mediating effector functions through a G protein–coupled signaling pathway. Furthermore, BLT1 colocalized with SMCs in human atherosclerotic lesions.

**Conclusions**—These new findings extend the role of inducible BLT1 to nonleukocyte populations and suggest an important target for intervention to modulate the response to vascular injury. (Circulation. 2005;112:578-586.)

**Key Words:** atherosclerosis ■ inflammation ■ leukotrienes ■ muscle, smooth

Leukotriene B4 (LTB4) is an eicosanoid lipid derivative of the arachidonic acid signaling pathway generated by 5-lipoxygenase (5-LO) and leukotriene A4 hydrolase. LTB4 is a proinflammatory mediator that activates multiple leukocyte subsets, leading to cell recruitment, production of reactive oxygen species, and induction of gene expression.1−8 LTB4 signals primarily through its high-affinity G protein–coupled receptor BLT1, which is highly expressed in specific subsets of circulating peripheral blood leukocytes and present in much lower amounts in spleen, thymus, bone marrow, lymph nodes, heart, skeletal muscle, brain, and liver. A low-affinity receptor, BLT2,9−12 is also ubiquitously expressed at low levels in many human tissues.12,13 Interestingly, recent studies have demonstrated marked induction of BLT1 expression by proinflammatory cytokines such as those that govern the transition of naïve to effector T cells.14 Such studies raise the possibility that BLT1 expression may be increased in other cell types during inflammatory processes.

A mounting body of correlative evidence has linked LTB4 to several chronic inflammatory conditions, including atherosclerosis. We know that 5-LO and its derivatives are highly expressed within human carotid, aortic, and coronary artery plaques.15,16 Genetic studies have associated particular variants of 5-LO and its accessory protein, 5-LO activating protein, with stroke and myocardial infarction in humans.17,18 Two recent murine studies suggest a causal role for the LTB4-BLT1 axis in atherosclerotic lesion formation. Treatment of the atherosclerosis-susceptible Ldlr−/− and apoE−/− mice with a small-molecule BLT1 antagonist reduced plaque burden,19 a finding that was confirmed more recently in apoE−/−/Blt1−/− compound-deficient mice.20 Both groups observed diminished macrophage accumulation and suggested...
that it was the primary contribution of LTB4 and BLT1 to atherogenesis.

Atherosclerotic lesions are marked by the recruitment of not only macrophages but also other leukocytes such as memory T cells, as well as nonleukocytes, including vascular smooth muscle cells (SMCs). In light of recent data demonstrating functionally significant cytokine-induced expression of BLT1 in previously unanticipated leukocyte subsets, we generated apoE−/−/Blt1−/− compound-deficient mice to evaluate the pleiotropic effects of the LTB4-BLT1 pathway on the multiple cell types involved in atherosclerotic lesion formation. We report here the expression and function of BLT1 on SMCs in vitro and in vivo. These findings extend the role of inducible BLT1 to nonleukocyte populations and suggest an important target for intervention to modulate the response to vascular injury.

Methods

Animals

Blt1−/− mice were backcrossed 10 times into a C57/BL6J background and subsequently bred with apoE−/− mice (Jackson Laboratory). Intercrosses of resulting apoE−/−/Blt1−/− mice yielded offspring that entered the study. Genotyping for BLT1 and apoE was performed by PCR. The mice were fed a high-fat Western-type diet (0.15% cholesterol, 21% fat; Harlan Teklad TD88137) beginning at 4 weeks of age for 6 or 12 weeks.

Blood and Plasma Analysis

Blood samples were obtained by cardiac puncture at death. Plasma samples were analyzed for total cholesterol and triglyceride levels (Roche Diagnostics) in the MGH Clinical Laboratories.

En Face Lesion Analysis

Aortas were dissected from mice of the indicated age, stained with oil red O (ORO), and analyzed as previously described. Images were obtained with a Nikon Coolpix camera attached to an inverted microscope. The percentage of ORO-stained lesion area was determined by Image Pro Plus image analysis software. Lesion analysis was conducted by a single observer blinded to the genotype of the mice.

Murine Aortic Root Lesion Analysis and Immunohistochemistry

The heart and aortic root were dissected and removed as described above and embedded in OCT. Serial sections were cut through the aorta at the level of the aortic valve leaflets, and every third section (5-μm intervals) through the aortic sinus (400-μm total length) was stained with ORO and hematoxylin. Immunohistochemistry was performed with antibodies to identify SMCs (α-actin, 1:50; DAKO), macrophages (F4/80, 1:25; Serotec), and T cells (CD4, 1:50; BD Biosciences) detected with either DAKO ARK peroxidase (DakoCytomation) or goat anti-rat HRP-conjugated IgG (1:50; BD Biosciences) as indicated. Negative controls were prepared by substitution with an isotype control antibody. Stained sections were viewed using 3-amino-9-ethyl carbazole (Vector Laboratories) according to the recommendations provided by the supplier. Cell types were characterized using the following cell-specific antibodies: anti-muscle actin mAb for SMCs (Enzo Diagnostics), anti-CD31 mAb for endothelial cells (DAKO Corp), and anti-CD68 mAb for Mg (DAKO Corp).

Murine Vascular SMC Isolation

Murine aortas were dissected, cut into ~1 mm² segments, and then placed as explants in T25 flasks containing 1 mL RPMI with 20% FBS (2 to 3 aortas per flask). Flasks were incubated with 3 to 4 mL RPMI with 20% FBS on the SMC outgrowth well become >50% confluent. Staining with FITC-labeled anti-mouse smooth muscle α-actin (1:50; DAKO) confirmed >99% SMC purity (not shown).

RNA Isolation and Quantitative PCR

Total RNA was isolated from either cultured SMCs or aortas from mice perfused with RNA Later (Ambion) using Trizol reagent (Invitrogen) and RNeasy columns (Qiagen). Briefly, after DNA digestion, 2 μg RNA from each sample was reverse transcribed using Taqman reverse-transcription reagents, including oligo(dT)15, random hexamers, and Multiscribe reverse transcriptase (Applied Biosystems). Quantitative (Q) RT-PCR reactions were conducted using the Multiplex QPCR system as described. Amplification plots were analyzed with MX4000 software, version 3.0. Gene expression was normalized to GAPDH as an internal control.

LTB4 Signaling Studies and Western Blotting

After 24 hours of cytokine activation (tumor necrosis factor [TNF]-α [20 ng/mL] and interferon [IFN]-γ [1000 U/mL]), SMCs were stimulated with either vehicle control or 100 nmol/L LTB4, in RPMI with 1% FBS for 5, 30, or 60 minutes. Reactions were stopped with ice cold 1 × PBS without Ca²⁺ and Mg²⁺, and Western analysis of cell lysates was performed using the indicated antibodies as previously described. Rabbit anti-mouse phospho–focal adhesion kinase (FAK) and mouse anti-phospho-ERK 1/2 antibodies were obtained from Cell Signaling Technologies, Inc. Rabbit anti-mouse total FAK and total ERK 1/2 antibodies were purchased from Transduction Laboratories.

Vascular SMC Migration Assays

Murine aortic SMCs were activated with TNF-α (20 ng/mL) and INF-γ (100 U/mL) for 24 hours in RPMI with 1% FBS. Once suspended, 20,000 cells were added to the top wells of Costar Transwell modified Boyden chambers (6.5-mm-diameter tissue culture–treated polycarbonate membranes containing 8-μm pores). LTB4 was added to the top or bottom wells as indicated. For inhibitor studies, SMCs were pretreated with the BLT1 receptor antagonist CP-105,696 (Pfizer; 10 μmol/L, 30 minutes, 37°C), pertussis toxin (250 ng/mL, 1 hour, 37°C), or vehicle control. After incubation of the chemotaxis chambers at 37°C for 6 hours, membranes were fixed in methanol and stained with hematoxylin and eosin. Cells adherent to the bottom of the membranes were counted by phase contrast microscopy (×100 magnification).

Vascular SMC Proliferation Studies

Murine SMCs grown on 24-well plates underwent growth arrest for 3 days in 1% FBS, followed by stimulation with LTB4 (100 nmol/L, replaced daily), 20% FBS, or both during days 4 to 7. The relative
Analysis of Baseline Characteristics Between apoE<sup>−/−</sup> and apoE<sup>−/−</sup>/Blt1<sup>−/−</sup> Mice Shows No Statistically Significant Differences in Weight, White Blood Cell Count, Total Cholesterol, or Triglycerides

<table>
<thead>
<tr>
<th></th>
<th>apoE&lt;sup&gt;−/−&lt;/sup&gt;</th>
<th>apoE&lt;sup&gt;−/−&lt;/sup&gt;/Blt1&lt;sup&gt;−/−&lt;/sup&gt;</th>
</tr>
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<tr>
<td>Weight, g (n)</td>
<td>23.7±3.82 (31)</td>
<td>25.2±3.2 (30)</td>
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<td>White blood cell count, 1000/mL (n)</td>
<td>11.3±2.9 (20)</td>
<td>10.2±3.7 (27)</td>
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<td>Total cholesterol, mg/dL (n)</td>
<td>1720±330 (10)</td>
<td>1794±235 (14)</td>
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<tr>
<td>Triglycerides, mg/dL (n)</td>
<td>197±41 (10)</td>
<td>193±69 (10)</td>
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cell numbers were subsequently determined using a fluorescent nucleic acid probe and quantitative epifluorescence<sup>29</sup> (Cyquant).

Flow Cytometry

Human coronary artery SMCs used for flow cytometry experiments were a generous gift from Dr Mukesh Jain (Brigham & Women’s Hospital). Cells were either left untreated or activated with TNF-α (20 ng/mL) and IFN-γ (1000 U/mL) for 48 hours as indicated. Adherent cells were placed in suspension as previously described,<sup>30</sup> incubated with mouse anti-human FITC-labeled BLT1 antibody or mouse anti-human FITC-labeled IgG2a isotype control (both from Serotec), and then fixed in 1X PBS/2% paraformaldehyde. Samples were analyzed with a Becton-Dickinson FACS and CellQuest software.

Statistical Analysis

The null hypothesis for the variables measured in the 2 groups of animals or cell culture conditions was initially tested by Student t tests. If the data did not fit the constraints of a parametric test, they were analyzed by 1-way ANOVA. A value of \( P<0.05 \) was considered significant. All data are presented as mean±SEM or SD as indicated.

Results

To directly assess the role of BLT1 in atherogenesis, we backcrossed Blt1<sup>−/−</sup> mice 10 times onto a C57BL/6 background and then bred them to apoE<sup>−/−</sup> mice to generate compound-deficient apoE<sup>−/−</sup>/Blt1<sup>−/−</sup> and control apoE<sup>−/−</sup>/Blt1<sup>+/+</sup> mice. Mice were placed on a high-fat, high-cholesterol Western diet for 6 or 12 weeks. There were no significant differences in total cholesterol or triglyceride levels between the 2 groups (the Table). Circulating white blood cell counts were also comparable, thus excluding any obvious confounding variables.

Lesion area was first quantified in aortas pinned out and stained with ORO. There was a striking reduction in aortic plaque burden at 6 weeks in the compound-deficient apoE<sup>−/−</sup>/Blt1<sup>−/−</sup> mice compared with the apoE<sup>−/−</sup> animals (1.72% versus 3.82% of total aortic area; 55% reduction; \( P=0.00003; \) Figure 1A and 1B). These findings were concordant across the aortic arch (53% reduction; \( P=0.00004), \) thoracic aorta (72.4% reduction; \( P=0.00004), \) and abdominal aorta (51.0% reduction; \( P=0.005), \) and abdominal aorta (72.4% reduction; \( P=0.001; \) Figure 1C). We observed decreased lesion formation in both male (52.9% reduction; \( P=0.001), \) and female (57.5% reduction; \( P=0.008; \) females: apoE<sup>−/−</sup>/Blt1<sup>−/−</sup>, n=7; apoE<sup>−/−</sup>/Blt1<sup>+/+</sup>, n=5, \( *P=0.008; \) females: apoE<sup>−/−</sup>/Blt1<sup>−/−</sup>, n=6; apoE<sup>−/−</sup>/Blt1<sup>+/+</sup>, n=5, \( P=0.001). \)

![Figure 1](http://circ.ahajournals.org/)

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P = 0.008) and female (56.7% reduction; P = 0.001; Figure 1D) mice without significant intergender differences (P = NS). En face analysis revealed a trend toward lesion reduction at the 12-week time point as well (Figure 1E).

We performed cross-sectional analysis of aortic roots in parallel with the en face studies. After 6 weeks on the Western-type diet, the average cross-sectional lesion area for apoE−/−/Blt1−/− mice was 214.7 μm² as opposed to 480.3 μm² for the apoE−/− controls (P = 0.006; Figure 2A and 2B), concordant with the quantitative analysis of total aortic area involvement by surface lipid staining. A consistent decrease in aortic sinus lesion area was observed in the apoE−/−/Blt1−/− mice after 12 weeks on the Western-style diet, indicating that loss of BLT1 continues to affect lesion development in older mice (Figure 2C) (n = 8 for each genotype; *P = 0.008).

Next, we performed immunohistochemical analysis to further delineate the effects of BLT1 on lesion formation (Figure 3A through 3C). Analyses were performed on the mice fed a Western-style diet for 6 weeks and were normalized to lesion area in light of the significant differences in plaque size between the 2 groups as noted above. Intimal area was decreased in cross sections of aortic root lesions from apoE−/−/Blt1−/− mice. There was a significant decrease in both monocyte (39.2% reduction; n = 6; P = 0.01) and T-cell (30.6%; n = 6; P = 0.03) accumulation in the plaques of the double-knockout mice compared with apoE−/− controls. The most unanticipated finding, however, was a striking inhibition (42.6% reduction; n = 6; P = 0.0000017) of SMC accumulation in the apoE−/−/Blt1−/− mice (Figure 3C). Staining for α-actin was reduced both in the media and in the fibromuscular caps overlying the lesions. These findings were...
confirmed by a concordant 2-fold decrease in α-actin mRNA in the aortic lesions as assessed by QPCR (Figure 3D).

The markedly diminished number of SMCs in the vessel wall of apoE<sup>−/−</sup>/Blt1<sup>−/−</sup> mice suggested that BLT1 may play a direct role in SMC recruitment and/or proliferation. We next assessed BLT1 expression in isolated murine aortic SMCs by PCR. Although resting SMCs showed no evidence of BLT1 expression, activation with TNF-α and/or IFN-γ markedly enhanced expression over 24 hours (Figure 4A). QPCR confirmed a >25-fold increase in BLT1 expression in the TNF-α- and IFN-γ-stimulated SMCs. (Figure 4B). Using a recently generated anti-human BLT1 mAb, we then examined surface expression of the protein on human coronary artery SMCs. Flow cytometry studies revealed low baseline staining, but >50% of SMCs expressed BLT1 after cytokine activation (Figure 4C). BLT1 expression was 8-fold higher than BLT2 expression, which was assessed by QPCR only because no antibody exists for this receptor (not shown).

To assess the potential functional effects of LTB₄ on vascular SMCs, we performed migration assays in modified Boyden chambers. We observed robust, bell-shaped chemotaxis to LTB₄ with a concentration-dependent response curve similar to recently published findings in activated T cells<sup>14</sup> (Figure 5A). Simultaneous addition of LTB₄ to upper and lower wells abrogated cell migration, confirming that our findings were indeed due to chemotaxis, not merely chemokinesis (Figure 5B). Pretreatment of SMCs with pertussis toxin also ablated LTB₄-triggered chemotaxis, consistent with the involvement of a G<sub>i</sub>-coupled signaling pathway (Figure 5A). The selective BLT1 antagonist CP-105,696 efficiently inhibited SMC migration, suggesting that BLT1 was the primary receptor mediating the observed effect (Figure 5A). This finding was then confirmed by chemotaxis assays with BLT1-deficient SMCs, which also had markedly diminished migration to LTB₄ (Figure 5A). Although LTB₄ was a potent agonist for SMC chemotaxis, it did not stimulate SMC proliferation (Figure 5C and 5D). At higher serum doses, there was neither a synergistic nor adverse effect on proliferation.

We next performed signaling studies to characterize pathways downstream of BLT1 in SMCs. FAK is a key modulator of cell-matrix interactions and cytoskeletal rearrangement leading to SMC migration.<sup>32</sup> Exposure to LTB₄ consistently increased FAK activation, as assessed by Western blotting with a phospho-specific antibody (Figure 6A). Further experiments evaluated the impact of LTB₄ stimulation on the ERK 1/2 pathway, which is more closely tied to SMC proliferation<sup>33,34</sup> (Figure 6B). Interestingly, this pathway was suppressed 1 hour after LTB₄ stimulation, a finding consistent with the lack of response to LTB₄ observed in the proliferation assays.

In light of our data demonstrating the expression and function of SMC BLT1 in vitro, we performed immunohistochemistry on human atherosclerotic plaques using a recently developed anti-human BLT1 Ab<sup>31</sup> (Figure 7A through 7C). Our analysis revealed the expression of BLT1+ cells within atherosclerotic lesions, which was particularly notable in lipid-rich areas. Adjacent sections of the same atherosclerotic tissue stained for BLT1 showed colocalization with SMCs, particularly in the area of the fibrous cap. Macrophages within the lesion had even more robust BLT1 expression. Interestingly, in areas of neovascularization, we also saw evidence for BLT1 expression on CD31+ endothelial cells and apparently in the SMCs of the walls of the neovessels. The isotype-matched control antibody showed no signal.

**Discussion**

Emerging evidence has implicated the LTB₄-BLT1 pathway in chronic inflammatory diseases, including atherosclerosis. Here we have shown that the absence of BLT1 confers a >2-fold decrease in early lesion formation as assessed by both aortic en face and cross-sectional analyses of plaque burden in apoE<sup>−/−</sup>/Blt1<sup>−/−</sup> mice compared with apoE<sup>−/−</sup> controls. Reduction in lesion formation was evident in all parts of the aorta and was similar in both male and female mice. Histochemical analysis revealed a remarkable reduction in SMCs in BLT1-deficient mice, which was confirmed by a reduction in SMC-specific α-actin message in the lesions. We report here novel evidence of the expression and function of
BLT1 on SMCs in vitro and the presence of BLT1 on SMCs in atherosclerotic lesions in humans.

Our study agrees with a report in which a small-molecule BLT1 antagonist reduced plaque burden in Ldlr−/− and apoE−/− mice,19 a finding that was confirmed more recently in apoE−/−/Blt1−/− compound-deficient mice.20 Both studies suggested that loss of BLT1-mediated macrophage activation accounted for the diminished atherogenesis. Results observed in the compound-deficient mice led investigators to study LTB4-triggered gene regulation in a rat basophilic cell line, which revealed increased levels of the scavenger receptor CD36 and the chemokine monocyte chemoattractant protein (MCP)-1. However, LTB4-triggered augmentation in CD36 and/or MCP-1 mRNA seen in the rat basophilic cell line cells could not be confirmed in primary macrophages or macrophage cell lines. Thus, the mechanisms by which BLT1 contributes to lesion formation have remained incompletely defined. Furthermore, the notion that BLT1 may function in the recruitment of other cellular plaque constituents has not been previously appreciated. Neither of the above reports specifically evaluated the effects of the LTB4-BLT1 pathway on the SMC component in atherosclerotic lesions.

The prevailing dogma is that chemoattractants contribute to atherosclerotic lesion development by chemokine-triggered leukocyte firm adhesion and chemotaxis into inflamed tissues. Although chemokines are best described for their role in host immunity, a growing body of evidence suggests a broader range of biological targets than was first appreciated. For example, mice deficient in the chemokine stromal derived factor-1α or its receptor CXCR436,37 have lethal defects of the cardiac intraventricular septum and aberrant migration of cerebellar neurons. In terms of vascular pathology, prior studies have implicated chemokine-dependent responses in SMCs. Roque et al38 evaluated the response to arterial injury in animals lacking the MCP-1 receptor CCR2. One month after the insult, CCR2-deficient mice had preserved leukocyte accumulation but a profound reduction in intimal lesion area, suggesting CCR2-dependent effects on SMC recruitment. More recent work has also demonstrated that SMCs within the neointima of atherosclerotic lesions express the fractalkine receptor CX3CR1 and that cultured vascular SMCs undergo chemotaxis to fractalkine that can be inhibited by G-protein inactivation with pertussis toxin.39 We therefore extend these prior observations by providing novel evidence for the induction of the lipid chemoattractant receptor BLT1 on SMCs. The present study, coupled with our recent findings demonstrating the marked induction of BLT1 in the cytokine-induced transition from

Figure 5. Effects of LTB4 on SMC chemotaxis and proliferation. A, Cultured SMCs from either Blt1−/− or Blt1−/− mice were placed in modified Boyden chamber and chemotaxis assessed to LTB4. When indicated, cells were pretreated with either pertussis toxin (PTX) (n=4; *P=0.000002 vs untreated SMCs at 100 nmol/L LTB4) or CP-105,696, a selective BLT1 antagonist (n=4; *P=0.00001 vs untreated SMCs at 100 nmol/L LTB4). Note that these inhibitors were used only at 10 and 100 nmol/L LTB4 concentrations. For Blt1−/− SMC chemotaxis, comparison is made with Blt1−/− SMCs at 100 nmol/L LTB4 (n=4; *P=0.003). B, When indicated, chemotaxis assays were performed with simultaneous addition of LTB4 to upper and lower wells (n=4). C, D, As detailed in Methods, cultured SMCs were subjected to growth arrest by serum deprivation for 72 hours. Cells were then treated with indicated concentration of serum-containing media and/or hydroxyurea and/or LTB4. Cumulative data are expressed as mean±SEM for indicated number of experiments.
naïve to effector T cells, suggests that BLT1 may have more widespread effects in inflammation than originally postulated. We hypothesize that there may be even more profound effects of BLT1 deletion in models in which SMCs dominate the response to injury such as after arterial wall mechanical injury.

Although murine strains such as apoE mice have important features of atherosclerotic disease, they do not replicate the complex lesions seen in humans. Modulation of the LTB₄-BLT1 pathway in humans might be more complex than previously suggested because of unanticipated effects on SMCs. In early atherosclerotic lesions, there is intense vessel wall remodeling involving the recruitment and proliferation of SMCs, which in turn contribute to extracellular matrix formation and overall plaque volume. In late-stage lesions in humans, however, SMCs form fibrous caps that are believed to inhibit plaque rupture. Thus, early intervention in the LTB₄-BLT1 pathway might be most beneficial both to capture the more robust effects early in lesion development, as suggested by our study, and to avoid potential unintended effects on plaque stability of more mature lesions.

Our studies suggest a role for LTB₄ in SMC migration but no effect on SMC proliferation. Concordant with the functional assays, signaling studies revealed activation of focal adhesion-associated kinase and inhibition of the proliferation-associated ERK 1/2 pathway. However, a thorough assessment of the functionally relevant pathways governing BLT1-dependent cell movement remains the focus of future investigation. Our studies, of course, do not preclude a role for BLT1 in proliferative processes requiring additional signals that might be present in the vessel wall.

The chemotactic response of SMCs to LTB₄ resembled that of leukocytes. These findings agree with the markedly diminished lesional SMC accumulation observed in the apoE/BLT1 mice. However, our studies do not preclude BLT1-dependent modulation of smooth muscle responses in the vessel wall through its effects on monocytes or T cells. The initial studies of MCP-1 and CCR2 deletion in proatherogenic strains did not specifically evaluate SMC content in the lesions. More recent work using anti-MCP-1 gene therapy diminished macrophage accumulation but actually increased lesional SMC content. Deletion of the fractalkine receptor, which mediates monocyte recruitment and potentially SMC responses, had no effect on SMC recruitment to atherosclerotic plaques. Thus, mediators of monocyte recruitment do not necessarily directly or indirectly modulate vessel wall SMC biology as well. Bone marrow transplantation studies between BLT1 knockout and wild-type mice are necessary to dissect primary leukocyte versus nonleukocyte contributions to lesion formation.

Multiple lines of investigation have implicated the LTB₄-BLT1 axis in atherogenesis in both murine systems and...
humans. As with classic peptide chemokines, the pleiotropic effects of lipid chemoattractants in disease contexts are increasingly appreciated. Our new findings expand a role for BLT1 in regulating all major vessel wall constituents, thus underscoring its therapeutic promise for a variety of vascular diseases.

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References

CLINICAL PERSPECTIVE

Leukotriene B4 (LTB4) is an eicosanoid lipid derivative of the arachadonic acid signaling pathway and a potent leukocyte activator. LTB4 is highly expressed in atherosclerotic lesions. To test whether this molecule contributes in a causal manner to lesion formation, we generated mice that were deficient for the LTB4 receptor (BLT1) and then crossed these mice into the atherosclerosis-susceptible apoE-knockout strain. Mice that lacked BLT1 had a significant reduction in atherosclerosis as opposed to mice with BLT1 intact. Analysis of the lesions in mice lacking BLT1 demonstrated a reduction in leukocyte accumulation as we had hypothesized. However, there was an even more striking reduction in smooth muscle cells in the lesions of the mice without BLT1. In this article, we go on to show the previously unanticipated expression and function of the BLT1 receptor on smooth muscle cells. These findings are clinically relevant because small-molecule BLT1 antagonists are under development and would target multiple cell constituents relevant to atherosclerosis. Furthermore, these studies may be particularly applicable to clinical situations in which smooth muscle cells dominate the response to injury such as restenosis after percutaneous intervention.
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