Identification of 5-Lipoxygenase as a Major Gene Contributing to Atherosclerosis Susceptibility in Mice

Margarete Mehrabian, Hooman Allayee, Jack Wong, Weibin Shih, Xu-Ping Wang, Zory Shaposhnik, Colin D. Funk, Aldons J. Lusis

Abstract—We previously reported the identification of a locus on mouse chromosome 6 that confers almost total resistance to atherogenesis, even on a hypercholesterolemic (LDL receptor–null) background. 5-Lipoxygenase (5-LO) is the rate-limiting enzyme in leukotriene synthesis and was among the chromosome 6 locus candidate genes that we examined. The levels of 5-LO mRNA were reduced about 5-fold in a congenic strain, designated CON6, containing the resistant chromosome 6 region derived from the CAST/Ei strain (CAST), as compared with the background C57BL/6J (B6) strain. 5-LO protein levels were similarly reduced in the CON6 mice. Sequencing of the 5-LO cDNA revealed several differences between CON6 and the B6 strain. To test whether 5-LO is responsible for the resistant phenotype, we bred a 5-LO knockout allele onto an LDL receptor–null (LDLR null) background. On this background, the mice bred poorly and only heterozygous 5-LO knockout mice were obtained. These mice showed a dramatic decrease (26-fold; P<0.0005) in aortic lesion development, similar to the CON6 mice. Immunohistochemistry revealed that 5-LO was abundantly expressed in atherosclerotic lesions of apoE−/− and LDLR−/− deficient mice, appearing to colocalize with a subset of macrophages but not with all macrophage-staining regions. When bone marrow from 5-LO−/− mice was transplanted into LDLR−/−, there was a significant reduction in atherogenesis, suggesting that macrophage 5-LO is responsible, at least in part, for the effect on atherosclerosis. These results indicate that 5-LO contributes importantly to the atherogenic process and they provide strong presumptive evidence that reduced 5-LO expression is partly responsible for the resistance to atherosclerosis in CON6 mice. (Circ Res. 2002;91:820–827.)

Key Words: mouse ▪ atherosclerosis ▪ genetics ▪ inflammation ▪ 5-lipoxygenase

Atherosclerosis is initiated by the trapping and oxidation of low-density lipoproteins (LDL) in the subendothelial layer of the artery wall, resulting in the formation of biologically active species that stimulate vascular cells to produce inflammatory molecules.1,2 This signals a cascade of leukocyte recruitment and chemotaxis of other activated species that stimulate vascular cells to produce inflammation and fibroproliferation. To identify genes that contribute to this complex process, we previously constructed a cross between a resistant strain, B6. A major locus for atherosclerosis was identified on mouse chromosome 6 and was subsequently confirmed with the congenic strain designated CON6 in which the central region of chromosome 6 from CAST was bred onto a B6 background.3 These CON6 mice had reduced insulin levels and dramatically decreased lesion formation when bred onto an LDL receptor–null (LDLR−/−) background and fed an atherogenic diet. Moreover, bone marrow transplantation studies indicated that the resistant phenotype was conferred in part by bone marrow–derived cells.

In examining the congenic region for potential positional candidate genes, we observed that 5-lipoxygenase (5-LO) mapped directly underneath the linkage peak for the locus. 5-LO is the rate-limiting enzyme in leukotriene (LT) biosynthesis4 and is expressed primarily in leukocytes, including monocytes and macrophages.5 Leukotrienes are potent proinflammatory lipid mediators derived from arachidonic acid and have been shown to affect several pathophysiological conditions.6 Therefore, 5-LO could potentially contribute to the development of atherosclerosis through lipoprotein oxidation and/or inflammatory processes. In the present study, we examined the contribution of 5-LO to atherosclerotic lesion formation based on its location within the chromosome 6 congenic region, its role in inflammation, and its expression in leukocytes. The results indicate that 5-LO participates in atherogenesis in mouse models and are consistent with 5-LO deficiency contributing to the resistant phenotype of CON6 mice.

Materials and Methods

Animal Husbandry

Mice were purchased from the Jackson Laboratories, Bar Harbor, Maine, and housed 4 per cage at 25°C on a 12-hour light/dark cycle.

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They were maintained either on a chow diet or a high-fat, high-cholesterol diet containing 15% fat, 1.25% cholesterol, and 0.5% cholic acid (diet No. 90221, Harlan-Teklad). The mice used in the experiments described below were of both sexes and between 4 to 6 months of age. All procedures were in accordance with current National Institutes of Health guidelines and were approved by the UCLA Animal Research Committee.

5-LO mice on a B6 background were generated as described previously.1 To generate double knockout animals, 5-LO+/− mice were first bred to LDLR−/− mice (also on a B6 background), and the F1 progeny were backcrossed to LDLR−/− mice to produce 5-LO−/−/LDLR−/− mice. These mice were then intercrossed to generate double knockout animals. Although a small number of 5-LO−/−/LDLR−/− mice were obtained, they did not produce offspring. Therefore, the experiments described herein were performed with 5-LO−/−/LDLR−/− mice. The segregation of the 5-LO−/− mutation was followed using PCR primers specific for the targeted allele (neo primer) 5′-ATCCGCTTCTTGACGAGTTC-3′, primer specific to the normal allele 5′-TGCAACCCAGTACTCATCAACG-3′; downstream primer in intron 6 for both the wild-type allele and the targeted allele 5′-GCAGGAAGTCACGTCGTGACCCATGGCGA-3′. PCR primers used to genotype the LDLR locus for the normal allele were 5′-ACCCCAAGAGTGCTCCAGGATG-3′ and 5′-CGCAGTGCTCTCATTGACTGT-3′; and for the targeted allele were 5′-AGAGATCTCGTCGTGAACCTGCGGCA-3′ and 5′-GAGCGGCGATACCGTGAAAGCAGCGG-3′.

**Phospholipid and Insulin Measurements**

Mice were fasted overnight and bled retro-orbitally under isoflurane anesthesia. Enzymatic assays for plasma cholesterol levels were performed as described previously. Insulin levels were measured in duplicate by ELIZA (Crystal Chemical IUSKRO20).

**Northern Blot Analysis**

Total RNA was isolated from bone marrow cells using Trizol reagent (Life Technologies Inc). The RNA (10 μg) was run on a 1% agarose formaldehyde gel, transferred to nylon membrane, and hybridized with a 700-bp mouse-specific probe from the 3′ end of the 5-LO cDNA. The blots were stripped and probed for GAPDH as an internal control. Levels of 5-LO mRNA were quantitated by phosphorimaging and are expressed as the ratio of 5-LO to GAPDH mRNA.

**Western Blot Analysis**

Homogenates of bone marrow cells (80 μg protein) in SDS sample buffer were subjected to electrophoresis on NuPAGE 4% to 12% precast SDS polyacrylamide gradient gels (Novex) under reducing conditions as suggested by the manufacturer. Proteins were transferred to nitrocellulose membranes, incubated (1:3000 dilution) overnight with antibodies to 5-LO, LTA4 hydrolase, or LTB4 (Cayman Chemical), and visualized by ECL detection (Amersham, Little Chalfont). Image-Quant software (Molecular Dynamics) was used for the quantification of bands, which were normalized to GAPDH.

**Measurement of LTB4 Levels**

LTB4 levels were determined in duplicate using a commercially available ELIZA kit (Cayman Chemical). Assays were performed on bone marrow cells (25 μg protein) homogenized in 10 mmol/L Tris, pH 8.0.

**Sequence Analysis of 5-LO cDNA**

RNA was isolated from peritoneal macrophages of CAST and B6 mice and cDNA was prepared using the Superscript RT-PCR kit (Invitrogen). The PCR primers used for sequencing in both directions were as follows: 5′-ATCCGCTTCTTGACGAGTTC-3′, 5′-GCAGGAAGTCACGTCGTGACCCATGGCGA-3′, 5′-CTACCGATTCAAGTGATCGACTG-3′, 5′-GCACGACTTGAGCTGGAAG-3′. Therefore, the experiments described herein were performed with 5-LO−/−/LDLR−/− mice. The segregation of the 5-LO−/− mutation was followed using PCR primers specific for the targeted allele (neo primer) 5′-ATCCGCTTCTTGACGAGTTC-3′, primer specific to the normal allele 5′-TGCAACCCAGTACTCATCAACG-3′; downstream primer in intron 6 for both the wild-type allele and the targeted allele 5′-GCAGGAAGTCACGTCGTGACCCATGGCGA-3′. PCR primers used to genotype the LDLR locus for the normal allele were 5′-ACCCCAAGAGTGCTCCAGGATG-3′ and 5′-CGCAGTGCTCTCATTGACTGT-3′; and for the targeted allele were 5′-AGAGATCTCGTCGTGAACCTGCGGCA-3′ and 5′-GAGCGGCGATACCGTGAAAGCAGCGG-3′.

**Isolation of Bone Marrow Cells and Peritoneal Macrophages**

Bone marrow cells were flushed from mouse femurs with DMEM/5% fetal calf serum (FCS) and centrifuged at 1500 RPM for 15 minutes (3 repetitions of washing and centrifugation). Peritoneal macrophages were isolated after lavage with DMEM/5% FCS, as described for bone marrow cells.

**Measurement of 5-LO by Immunohistochemistry**

Immunostaining was performed on aortic lesion cryostat sections from apolipoprotein E−/− (apoE−/−) and LDLR−/− mice, as described below. Alternate sections were fixed with formaldehyde, washed with PBS, and incubated in blocking buffer, followed by either rabbit anti-human 5-LO (Cayman Chemical, Mich) or rat anti-mouse MOMA-2 (Accurate Chemical, NY) antiseraum. The sections were then washed and incubated with biotinylated goat anti-rabbit IgG at a dilution of 1:200. After extensive washing, the macrophages and 5-LO protein were visualized by alkaline phosphatase using Vector Red as substrate. Appropriate control experiments, including omission of primary antibody, were performed.

Peritoneal monocyte/macrophages were harvested with 20 ml DMEM/5% FCS 3 days after 4% thioglycolate (DIFCO, MI) injection. The cells were centrifuged at 1500 rpm, washed 3 times with media, and cultured overnight on glass slides. The slides were stained with a 1:200 dilution of rabbit anti-human 5-LO and hematoxylin.

**Aortic Lesion Analysis**

After 8 weeks on a high-fat, high-cholesterol diet, mice were euthanized and the upper portion of the heart and proximal aorta were removed, embedded in OCT compound (Miles Laboratories), and stored at −70°C. Serial 10 μm-thick cryosections from the middle portion of the left ventricle of the aortic arch were collected and mounted on poly-d-lysine–coated plates. Sections were stained with oil red O and hematoxylin, and the lipid staining areas were counted in a blinded fashion by light microscopy.

**Bone Marrow Transplantation**

Four-month-old LDLR−/− mice were used as recipients for bone marrow transplanted from either 3-month-old 5-LO−/−/LDLR−/− mice or control LDLR−/− mice. Recipient mice were lethally irradiated and then injected with 107 bone marrow cells through the tail vein, as described previously. Four weeks after transplantation, DNA from blood-derived leukocytes was analyzed for the presence of the targeted 5-LO allele, and the animals were placed on the high-fat, high-cholesterol diet for 8 weeks.

**Statistical Analyses**

Differences in measured variables between groups of mice were determined by ANOVA (Statview version 5.0). Values are expressed as mean±SEM, and differences were considered statistically significant at P<0.05.

**Results**

**CON6 Mice Have Reduced Expression of 5-LO**

Quantitative trait locus mapping of a cross between resistant CAST and susceptible B6 mice for atherosclerotic lesion development revealed a locus with a powerful effect on atherosclerosis on mouse chromosome 6. Subsequently, a congenic strain, CON6, containing the locus derived from CAST on the background of B6 was constructed. The congenic strain was almost entirely resistant to atherosclerosis, even when an LDL receptor–null mutation was introduced. These studies defined the critical region of the gene to between 45 cm and 74 cm on mouse chromosome 6. Fine mapping of the locus has been initiated, but phenotypic...
characterization of the progeny for lesion development requires many months. In addition, because lesion formation has a very high coefficient of variation, progeny testing will be required. To complement this approach, we have also tested various candidate genes within the locus. The 5-LO gene is located near the middle of the congenic region, at 53 cM. Given the proinflammatory properties of 5-LO and leukotrienes, we reasoned that variation in the 5-LO gene could be involved in the resistance to atherosclerosis of the CON6 mice.

To examine this possibility, we quantitated the expression of mRNA for 5-LO in bone marrow cells, a tissue previously shown to synthesize 5-LO. Northern blot analysis was performed using a mouse 5-LO cDNA probe and the signal was quantitated using a PhosphorImager and GAPDH as an internal control. As shown in Figure 1A, CON6 mice on an LDL receptor–null background exhibited only about 15% of the mRNA levels of LDLR<sup>−/−</sup> mice. Similarly, CON6 mice exhibited a very significant reduction in 5-LO protein levels compared with B6 controls, as determined by immunoblot analysis (Figure 1B). Densitometric analysis of the blots indicated that CON6 mice have approximately 25% of the levels of 5-LO protein as B6 mice.

5-LO catalyzes the oxidation of arachidonic acid to 5-HPETE and LTA<sub>4</sub>, which is then converted to LTB<sub>4</sub> by LTA<sub>4</sub> hydrolase. As measured by ELIZA assay, LTB<sub>4</sub> levels in CON6 mice were only a few percent of those in B6 mice (Figure 2A), demonstrating that 5-LO activity is also reduced in CON6 mice. LTA<sub>4</sub> hydrolase and LTB<sub>4</sub> ω-hydroxylase are 2 downstream enzymes from 5-LO and were also examined in bone marrow cells by immunoblot analysis (Figure 2B). The protein levels of LTA<sub>4</sub> hydrolase and LTB<sub>4</sub> ω-hydroxylase were both increased in CON6 versus B6 mice, suggesting that these enzymes are upregulated in response to decreased 5-LO levels.

**Sequence Variation Between B6 and CAST 5-LO cDNA**
We sequenced the 5′UTR and coding region of 5-LO cDNA from B6 and CAST mice to examine possible variations that
could influence the enzyme’s synthesis and/or function (online Table, which can be found in the online data supplement available at http://www.circresaha.org). The 5-LO sequence is highly conserved between the 2 strains with only 6 nucleotide changes, 4 of which did not result in amino acid substitution. The 2 amino acid changes occurred at residue 645, where B6 has an isoleucine and CAST has a valine, and at 646, where B6 has a valine and CAST has an isoleucine. It is not known whether these substitutions influence 5-LO expression and/or function.

5-LO Is Expressed in Atherosclerotic Lesions and in Macrophages

To determine whether 5-LO is present in atherosclerotic lesions, immunohistochemical studies of mouse aortic sections were performed. The proximal aortas (from the aortic root up to the aortic arch) of apoE\(^{−/−}\) and LDLR\(^{−/−}\) mice were sectioned and stained with antibody to either 5-LO or the macrophage-specific marker, MOMA-2 (Figure 3). As expected, staining with oil red O (data not shown) revealed the presence of large lipid-filled areas and a necrotic core within the lesions of both apoE\(^{−/−}\) and LDLR\(^{−/−}\) mice. Staining with MOMA-2, a macrophage-specific marker also revealed sites of infiltration of monocyte/macrophages into the subendothelial space (3A-C). The adjacent sections, stained with 5-LO antibody, revealed abundant 5-LO protein staining that appeared to colocalize, at least in part, with a subset of monocyte/macrophages (Figures 3D through 3F). Interestingly, 5-LO staining was not present in all regions containing monocyte/macrophages, as evident from the LDLR\(^{−/−}\) sections (3C and F). We next tested whether the expression of 5-LO in macrophages from CON6 and B6 mice by immuno-

staining the cells with antiserum against 5-LO. As shown in Figure 4, B6 macrophages exhibited significant 5-LO staining, whereas CON6 macrophages had dramatically reduced staining.

Deficiency of 5-LO Dramatically Reduces Atherosclerosis in an LDLR\(^{−/−}\) Model

Given the dramatically reduced size of aortic lesions and reduced expression of 5-LO in CON6 mice, we assessed the involvement of 5-LO in lesion development by examining atherosclerosis in 5-LO knockout mice, previously constructed by Funk and colleagues.\(^7\) We bred 5-LO–null mice, on a B6 background, with LDL receptor–null mice, also on a B6 background. The frequency of double knockout mice was much lower than expected based on Mendelian segregation, presumably because the two mutations are incompatible with life (data not shown). Because the CON6 mice exhibited reduced, but not absent, 5-LO activity, we examined mice heterozygous for the 5-LO–null mutation on an LDL receptor–null background. 5-LO protein levels did not differ in LDL\(^{−/−}\) mice fed either a chow or high-fat, high-cholesterol diet (Figure 5). However, there were decreased levels of 5-LO mRNA (Figure 1A) and protein (Figure 5) in the 5-LO\(^{+/−}\)/LDL\(^{−/−}\) mice compared with LDLR\(^{−/−}\) mice on a chow diet, which was less than the 50% that would be expected from heterozygotes. An explanation for this apparent transregulation is unknown. Because homozygous double knockout mice were not obtained either, it is possible that there is an interaction between LDLR and 5-LO such that disruption of both leads to altered expression of one or both genes as well as incompatibility with life.

After feeding of an atherogenic diet for 8 weeks, we observed a striking effect of 5-LO on atherosclerosis. As
expected, the 5-LO$^{+/+}$/LDLR$^{-/-}$ control mice had large advanced lesions, with an average area of 153,080 ± 21,010 μm$^2$. The 5-LO$^{+/+}$/LDLR$^{-/-}$ mice, on the other hand, had an aortic lesion area of only 5830 ± 2080 μm$^2$ (Figure 6A). Thus, mice heterozygous for the 5-LO–null mutation had over a 26-fold decrease ($P<0.0005$) in lesion size despite having cholesterol levels similar to LDLR$^{-/-}$ mice, exceeding 500 mg/dL (Figure 6B). This reduction in atherosclerosis was very similar to what we previously observed when the CON6 locus was transferred onto the LDLR$^{-/-}$ background and indicates that 5-LO has a dose-dependent effect on lesion size.

5-LO$^{+/+}$ Mice on an LDLR$^{-/-}$ Background Have Reduced Insulin Levels
In our previous studies of the CAST×B6 intercross, we observed a significant quantitative trait locus for insulin levels on chromosome 6 that was coincident with the locus for lesion formation. Moreover, the CON6 strain exhibited decreased insulin levels as compared with B6 mice. To examine whether 5-LO could also account, in part, for the linkage of insulin to this locus, we measured insulin levels in the 5-LO$^{+/+}$/LDLR$^{-/-}$ mice. Analogous to the lesion results, heterozygosity for a 5-LO–null allele on an LDLR$^{-/-}$ background decreased insulin levels 3-fold compared with 5-LO$^{+/+}$/LDLR$^{-/-}$ controls (Figure 7). This suggests that variations of the 5-LO gene may also have a role in regulation of insulin levels associated with this locus.

Bone Marrow Transplantation of the 5-LO$^{+/+}$ Allele Confers Resistance to Atherosclerosis
We previously demonstrated that transplantation of CON6 bone marrow into B6 mice resulted in an approximate 2-fold decrease in lesion formation, consistent with the concept that the genetic variation between CON6 and B6 strains is due, in part, to leukocyte functions. To test whether 5-LO$^{+/+}$ mice exhibited a similar bone marrow–dependent effect on atherosclerosis, we transplanted either 5-LO$^{+/+}$/LDLR$^{-/-}$ or 5-LO$^{+/+}$/LDLR$^{-/-}$ bone marrow into LDL receptor–deficient mice. Successful transplantation was confirmed 4 weeks after the

Figure 3. 5-LO is present in atherosclerotic lesions of apoE$^{-/-}$ and LDLR$^{-/-}$ mice. A through C, Staining of aortic sections with macrophage-specific MOMA-2 showed large advanced lesions. D through F, Adjacent proximal sections stained with anti–5-LO antibody showing colocalization of 5-LO (arrow) with a subset of macrophages surrounding the necrotic core (NC) but not with all regions staining for macrophages. A and D and B and E are from two 1-year-old apoE$^{-/-}$ mice on a chow diet. C and F are representative of lesions from a 4- to 6-month-old LDLR$^{-/-}$ mouse on a high-fat, high-cholesterol diet for 8 weeks. I indicates intima; L, lumen; and M, media.
procedure, as previously described. After 8 weeks on a high-fat, high-cholesterol diet, 5-LO mRNA remained significantly decreased in peritoneal macrophages from LDLR \(^{-/-}\) mice transplanted with 5-LO \(^{+/+}\)/LDLR \(^{-/-}\) marrow, indicative of successful bone marrow transplantation. Consistent with the CON6 findings that we reported previously, LDLR \(^{-/-}\) mice receiving 5-LO \(^{+/+}\) bone marrow exhibited a 2-fold decrease in atherosclerosis compared with controls (Figures 8A and 8B), suggesting that the 5-LO in macrophages is involved in lesion formation. Presumably, artery wall cells other than those derived from bone marrow are also involved in atherosclerosis, which may explain why trans-

![Figure 5](image)

**Figure 5.** Decreased 5-LO protein in 5-LO \(^{+/+}/\)LDLR \(^{-/-}\) mice compared with LDLR \(^{-/-}\) controls. Immunoblot analysis of bone marrow cells stained with rabbit anti-human 5-LO antibody indicates that 5-LO \(^{+/+}/\)LDLR \(^{-/-}\) mice (n=3) have an approximately 90% reduction in 5-LO protein compared with control LDLR \(^{-/-}\) mice (n=3). Data are from 4- to 6-month-old animals on a chow or high-fat diet. Levels of 5-LO protein did not differ in LDLR \(^{-/-}\) mice on either a chow or high-fat (HF) diet.

![Figure 6](image)

**Figure 6.** 5-LO–null mutation decreases aortic lesions even in the presence of elevated total cholesterol levels. Four- to six-month-old 5-LO \(^{+/+}\) (n=4) and 5-LO \(^{+/+}\) (n=4) mice on an LDLR \(^{-/-}\) background fed a high-fat, high-cholesterol diet for 8 weeks have over a 26-fold decrease in lesion formation, despite cholesterol levels that exceeded 500 mg/dL. There were no significant differences in the levels of LDL/VLDL-cholesterol.

![Figure 7](image)

**Figure 7.** Plasma insulin levels in 5-LO \(^{+/+}/\)LDLR \(^{-/-}\) mice compared with LDLR \(^{-/-}\) controls. Insulin levels from 4- to 6-month-old 5-LO \(^{+/+}/\)LDLR \(^{-/-}\) (n=5) and 5LO \(^{+/+}/\)LDLR \(^{-/-}\) (n=5) mice on a chow diet were determined by immunoassay as described in Materials and Methods. Mice fed a high-fat, high-cholesterol diet (n=5 for each genotype) had similar differences (data not shown).
Discussion

In the present study, we provide compelling evidence that the positional candidate gene, 5-LO, is involved in the development of atherosclerotic lesions in mice. Most significantly, heterozygous deficiency for the enzyme in a knockout model decreased lesion size in LDLR<sup>−/−</sup> mice by about 95%, an effect far greater than any other gene, with the possible exception of macrophage colony stimulating factor (MCSF).<sup>10</sup> The enzyme was expressed abundantly in macrophage-rich regions of atherosclerotic lesions, suggesting that 5-LO and/or its products may act locally to promote lesion development. There are a number of potential mechanisms by which these products may act. These include the following: seeding of LDL by oxidation products produced by the 5-LO pathway; the production of natural ligands for nuclear receptors, such as peroxisome proliferator-activated receptor α (PPARα); and various autocrine and paracrine effects mediated through G protein-associated primary receptors for leukotrienes. Such effects could potentially influence specific immunity functions, such as the differentiation and migration of other cells.

It is interesting to note that transplantation of 5-LO–deficient bone marrow does not decrease lesions to the same extent as global disruption of 5-LO<sup>−/−</sup>.

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The congenic region of CON6 spans approximately 30 cM and contains hundreds of genes, and it is possible that another gene in this region, independent of 5-LO, contributes to the atherosclerosis resistant phenotype in CON6 mice. For example, PPARγ, a transcription factor involved in adipocyte differentiation and insulin/glucose homeostasis,<sup>11,12</sup> is also located in the congenic region. We previously demonstrated that CON6 mice have reduced PPARγ mRNA levels, raising the possibility that this gene also contributes to lesion development in CON6 mice. However, recent studies have demonstrated significantly increased aortic lesion formation in LDLR<sup>−/−</sup> mice transplanted with PPARγ-deficient bone marrow.<sup>13</sup> These latter results suggest that the lack of PPARγ accelerates lesion development and do not support the notion that PPARγ protects CON6 mice from atherosclerosis. Given the role that PPARγ plays in insulin/glucose metabolism and its decreased expression in CON6 mice, it is possible that PPARγ contributes to insulin levels in CON6 mice. Whether other genes in the congenic region contribute to lesion development will require the analysis of subcongenic strains.

Although the most straightforward explanation for the effect of 5-LO on atherosclerosis in CON6 mice is the decreased expression, it is possible that structural differences also contribute. Sequencing of B6 and CAST 5-LO cDNA revealed two amino acid differences between the two strains at positions 645 (CAST/Val; B6/Ile) and 646 (CAST/Ile; B6/Val). 5-LO is highly conserved among mammals and the human and rat sequences are identical with that of B6 at position 645 and 646. It is not known whether these substitutions influence 5-LO function but they may influence the cellular trafficking of 5-LO. For example, these two residues are within a conserved region of basic amino acids, from...
positions 639 to 656, found in many proteins that translocate from the cytosol to the nucleus.\textsuperscript{14} Lepley and Fitzpatrick\textsuperscript{14} used a synthetic 639 to 656 fusion peptide to show that this potential nuclear localization sequence (NLS) in 5-LO acts as a regulatory domain involved in the nuclear translocation of the enzyme from the cytosol. In contrast, using a synthetic fusion peptide containing the last 90 amino acids of the 5-LO C-terminus, Funk and Chen\textsuperscript{3} did not find this peptide able to translocate into the nucleus. Due to the high conservation of this sequence in proteins containing a NLS, these amino acid substitutions could be potentially important in regulating the translocation of 5-LO to the nucleus.

The process by which atherosclerosis develops in the artery wall is complex and involves a variety of steps, such as lipid oxidation and leukocyte migration/proliferation.\textsuperscript{15,16} Studies in mice suggest that 12/15-LO is an important mediator of atherosclerosis, presumably due to “seeding” LDL LDL.\textsuperscript{17} The eicosanoid products of 5-LO could similarly promote lipoprotein oxidation, thereby contributing to inflammation and foam cell formation. 5-LO and its products have also been implicated in the chemotaxis of leukocytes, which may provide another mechanism for its proinflammatory role in atherosclerosis. For example, 5-HETE exhibits chemotactic activity, although only at relatively high concentrations.\textsuperscript{18} In neutrophils, dendritic cells, and monocyte/macrophages, 5-HETE can be converted to 5-oxo-ETE,\textsuperscript{19,20} which is 10-fold more potent than 5-HETE in stimulating monocyte migration.\textsuperscript{21,22} Moreover, both 5-oxo-ETE and 5-HETE have been shown to synergistically induce monocyte migration in response to monocyte chemotactic protein-1 (MCP-1).\textsuperscript{23} These results become more relevant because LTBA and MCP-1 levels have been shown to cross-regulate each other. Studies have shown that intraperitoneal injection of MCP-1 induces production of LTBA, whereas MCP-1 stimulates the production of LTBA from mouse peritoneal macrophage in a dose-dependent manner.\textsuperscript{23} These studies suggest that 5-LO and its product LTBA could promote atherosclerosis by recruiting monocytes to the vessel wall.

Several mechanisms have been proposed for LTBA activation of inflammatory responses, including the binding and activation of PPARα and direct G protein signaling pathways mediated by the leukotriene receptors.\textsuperscript{24–27} PPARα is expressed in all vascular cells and could play a role in vascular inflammation.\textsuperscript{28} For example, PPARα mediates MCP-1 synthesis in mouse aortic endothelial cells when stimulated with minimally modified LDL or oxidized phospholipids.\textsuperscript{29} LTBA is one of the ligands that activates PPARα\textsuperscript{24} and binds with an affinity in the nanomolar range.\textsuperscript{28} In addition, each leukotriene has a specific high-affinity G protein–coupled cell surface receptor, which can influence differentiation, migration, and immune functions.\textsuperscript{6,25} For example, LTB4 receptor–null mutants had significant defects in neutrophil and macrophage recruitment and exhibited altered cellular function, such as changes in calcium flux.\textsuperscript{30,31} Thus, 5-LO and its metabolites may play an important role in atherosclerosis either as natural nuclear receptor ligands or through receptor-mediated inflammatory signaling pathways.

In a preliminary study of randomly ascertained individuals, we have observed evidence for an association between 5-LO polymorphisms and carotid artery intima-media thickness, a validated surrogate marker for atherosclerosis.\textsuperscript{32} This raises the possibility that genetic variation in 5-LO could also affect human heart disease. In this regard, it is noteworthy that the acute inflammatory responses of leukotrienes are significantly influenced by genetic background.\textsuperscript{33} The results of this study provide further evidence for the important role that inflammatory mediators could play in atherosclerosis and may provide an avenue for implementing novel therapeutic strategies, because effective inhibitors of 5-LO have already been developed. Additional studies will be required to gain a more in-depth understanding of 5-LO’s role in the cellular and biochemical processes leading to the development and progression of atherosclerosis. Future work with other mouse models and other human populations may help to dissect the molecular interactions underlying this process.

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