Background—Atherosclerosis has features of an inflammatory disease. Because cyclooxygenase (COX)-2 is expressed in atherosclerotic lesions and promotes inflammation, we tested the hypotheses that selective COX-2 inhibition would reduce early lesion formation in LDL receptor–deficient (LDLR−/−) mice and that macrophage COX-2 expression contributes to atherogenesis in LDLR−/− mice.

Methods and Results—Treatment of male LDLR−/− mice fed the Western diet with rofecoxib or indomethacin for 6 weeks resulted in significant reductions in atherosclerosis in the proximal aorta (25% and 37%) and in the aorta en face (58% and 57%), respectively. Rofecoxib treatment did not inhibit platelet thromboxane production, a COX-1–mediated process, but it significantly reduced the urinary prostacyclin metabolite 2,3-dinor-6-keto-PGF1α. Fetal liver cell transplantation was used to generate LDLR−/− mice null for expression of the COX-2 gene by macrophages. After 8 weeks on the Western diet, COX-2+/−→LDLR−/− mice developed significantly less (33% to 39%) atherosclerosis than control COX-2+/+→LDLR−/− mice. In both the inhibitor studies and the transplant studies, serum lipids did not differ significantly between groups.

Conclusions—The present studies provide strong pharmacological and genetic evidence that COX-2 promotes early atherosclerotic lesion formation in LDLR−/− mice in vivo. These results support the potential of anti-inflammatory approaches to the prevention of atherosclerosis. (Circulation. 2002;105:1816-1823.)

Key Words: atherosclerosis | inflammation | mice | macrophage | transplantation

Atherosclerosis, the underlying cause of myocardial infarction and ischemic stroke, has features of an inflammatory disease.1 Cyclooxygenase (COX) plays a key role in inflammation as the rate-limiting enzyme in the conversion of arachidonic acid to prostaglandins. COX exists as 2 isoforms derived from 2 separate genes.2 COX-1 is constitutively expressed by most tissues and mediates normal physiological functions, whereas COX-2 is rapidly induced at sites of inflammation. Aspirin and nonsteroidal anti-inflammatory drugs (NSAIDs) are nonselective inhibitors of both COX isoenzymes.3 COX-2 selective inhibitors were developed to provide the anti-inflammatory and analgesic effects of NSAIDs with minimal gastrointestinal toxicity. Recent studies have shown that COX-2 is expressed by endothelial cells, smooth muscle cells, and macrophages in human atherosclerotic lesions.4,5 These observations suggest the hypothesis that selective inhibition of COX-2 might inhibit atherogenesis through its anti-inflammatory effects.

Eicosanoids modulate physiological processes in atherosclerosis and thrombosis, such as leukocyte–endothelial cell adhesion, vasorelaxation, and platelet aggregation.6 Activation of platelets leads to the formation of free arachidonic acid (AA), which can be converted by COX-1 into thromboxane (Tx) A2, a potent inducer of vasoconstriction and platelet aggregation.2 The efficacy of low-dose aspirin in the secondary prevention of cardiovascular events is attributed to inhibition of TxA2 production by platelets, a COX-1–specific effect.7 Prostacyclin (PGI2) is the dominant PG produced by endothelial cells and is the main product of arachidonic acid in all vascular tissues.2 In contrast to TxA2, PGI2 is a potent vasodilator and an inhibitor of platelet aggregation and leukocyte adhesion; thus, PGI2 is believed to be vasoprotective.2 COX-2 contributes significantly to systemic PGI2 synthesis in humans,8 but COX-1 also plays an important role in the production of PGI2 by vascular cells.9,10 It has been suggested that COX-2 inhibition may upset the balance between prostacyclin and Tx...
and promote thrombosis. Concerns have been raised that COX-2 inhibitors may increase the risk of cardiovascular events, but this issue is controversial and will likely require additional clinical trials to be resolved.6-11-13 Although thrombosis is the immediate cause of acute coronary events, atherosclerosis is the chronic disease process underlying most myocardial infarctions and strokes. PGs have been implicated in a variety of processes that promote atherosclerosis; therefore, inhibition of COX-2 may retard atherogenesis.

Within the vessel wall, monocytes differentiate into activated macrophages that secrete cytokines, growth factors, and PGs.1 Activation of macrophages by interferon γ, lipopolysaccharide, or interleukin-1β induces expression of COX-2,14 resulting in the production of proinflammatory eicosanoids. For example, PGE2 has been shown to induce production of the inflammatory cytokine interleukin-6.15 Matrix metalloproteinases (MMPs) have been implicated in macrophage migration,16 and COX-2 expression and PGE2 have been shown to promote the release and activation of MMPs.17 Therefore, COX-2 inhibition might interfere with macrophage migration by reducing release and activation of MMPs.16 Thus, COX-2-mediated eicosanoid production by activated macrophages may promote atherosclerosis through several mechanisms, including activation of chemotaxis, increasing vascular permeability, propagation of the inflammatory cytokine cascade, stimulation of macrophage and smooth muscle cell migration.5,18 On the basis of these observations, we hypothesize that COX-2 inhibition would reduce the proatherosclerotic effects of eicosanoids produced by macrophages.

In the present studies, we have used both pharmacological and genetic approaches to examine the hypothesis that COX-2 promotes early atherosclerotic lesion formation, and we demonstrate that both selective COX-2 inhibition and the absence of macrophage COX-2 retard early atherogenesis in LDL receptor–deficient (LDLR−/−) mice. These studies provide strong pharmacological and genetic evidence for a role for COX-2–dependent prostanoid formation in promoting atherogenesis.

Methods

Animal Procedures

Apolipoprotein E–deficient (ApoE−/−), LDLR−/−, C57BL/6 mice and COX-2−/− heterozygous mice were originally purchased from Jackson Laboratories (Bar Harbor, Maine). All mice in atherosclerosis studies were at the 10th backcross into the C57BL/6 background. All mice were maintained on a rodent chow diet containing 4.5% fat (PMI No. 5010). The Western diet contained 21% fat and 0.15% cholesterol (Teklad). Plasma levels of rofecoxib and indomethacin were determined by high-pressure liquid chromatography (HPLC)/mass spectroscopy and HPLC/photodetection analysis, as described.21 Urine was collected over 72 hours in metabolic cages with 3 mice per cage after 6 to 8 weeks of treatment with COX inhibitors and analyzed by GC/MS for 2,3-dinor-6-keto-PGF1α and 11-dehydro TxB2, by the Eicosanoid Analysis Core at Vanderbilt University.

Fetal Liver Cell Collection and Transplantation

Female and male COX-2−/− mice were mated. On day 14 of gestation, embryos were dissected free from the placenta and yolk sac, and a single-cell suspension of fetal liver cell (FLC) was prepared as described.20 Fetal tails were used for COX-2 genotyping and sex determination by PCR using primer sets specific for mouse COX2 gene and Zfy gene of the Y chromosome.20,22 Recipient mice were lethally irradiated (9 Gy) from a cesium γ source, and, 4 hours later, 5 × 10⁶ cells in 300 μL of RPMI 1640 media were injected into the orbital venous plexus.

Verification of Macrophage COX-2 Genotype Change in Recipient Mice

Peritoneal macrophages were plated at a density of 2 × 10⁶ cells/well in 12-well plates. After 2 hours, nonadherent cells were removed and fresh DMEM media containing 10% FBS and 1 μg/mL lipopolysaccharide was added for 5 hours. Total RNA was extracted using Atlas pure total RNA isolation kit (Clontech Laboratory, Inc). cDNA was obtained by reverse transcription using Moloney murine leukemia virus reverse transcription in the presence of RNase inhibitor and random hexamers (all from Promega) at 37°C for 60 minutes. After cDNA generation, primers specific for mouse COX-2 (TCA AAA GTG CTG GAA AAG GTT and TCT ACC TGA GTG TTG ACT GTG) and β-actin (TCA GAA GGA CTC CTA TGT GG and TCT CTT TGA TGT CAC GCA CG) genes were used to amplify products of 296 and 500 bp, respectively.

Quantitation of Arterial Lesions

At euthanasia, mice were perfused gently with 30 mL PBS through the left ventricle. The aorta was dissected from the aortic arch to the iliac bifurcation, and en face analysis was performed as described.20 The heart and aortic root were embedded in OCT and snap frozen in liquid N2. Cryosections of 10-μm thickness were taken from 300 μL of the proximal aorta starting from the end of the aortic sinus.23 Cryosections were stained with oil-red O and counterstained with hematoxylin, and quantitative analysis of lipid-stained lesions was performed using an imaging system KS 300 (Release 2.0, Kontron Electronik GmbH).20 Sections were also stained for collagen content with Masson’s trichrome.

Immunocytochemistry

To detect COX-2 protein and macrophages in the arteriolar lesions, 5-μm serial cryosections of the proximal aorta were fixed in cold acetone and incubated with either rabbit antisera to murine COX-2

Serum Cholesterol and Triglyceride Analysis

The concentration of total cholesterol and triglycerides was determined using Sigma kit numbers 352 and 339 adapted for microtiter plate assay. Serum was subjected to fast performance liquid chromatography (FPLC) analysis using a Supersose 6 column (Pharmacia) on an HPLC system model 600 (Waters). A 100-μL aliquot of serum was separated with a buffer containing 0.15 mol/L NaCl, 0.01 mol/L NaHPO4, and 0.1 mmol/L EDTA, pH 7.5, at a flow rate of 0.5 mL/min. Forty 0.5-mL fractions were collected, and tubes 11 through 40 were analyzed for cholesterol.20
COX-2 Expression in Mouse Atherosclerotic Lesions

We used immunocytochemistry and in situ hybridization to examine atherosclerotic lesions in the proximal aorta of apoE−/− mice for expression of COX-2. Immunocytochemical studies revealed that COX-2-expressing cells were localized exclusively in atherosclerotic lesions and not in unaffected areas of the artery. COX-2-expressing cells were detected in early, intermediate, and advanced lesions. Similar to the results in humans,4,5 COX-2 expression was detected in endothelial cells, smooth muscle cells, and monocyte/macrophages in murine atherosclerotic lesions (Figure 1 and data not shown). In lesions containing predominantly macrophage-derived foam cells, occasional monocyte macrophages were noted to express COX-2 in early lesions from 11-week-old apoE−/− mice (Figures 1a and 1b); however, most macrophage-derived foam cells did not express COX-2 in 19-week-old apoE−/− mice with larger foam cell lesions (Figures 1c and 1d). In situ hybridization studies confirmed that in fatty streak lesions of the apoE−/− mice, COX-2 mRNA-expressing cells were found exclusively in atherosclerotic lesions, and they were distributed as occasional cells surrounding the foam cell lesion area (Figures 1f and 1h). Consistent with the results of immunocytochemistry, most macrophage-derived foam cells did not express COX-2 mRNA.

Results

Impact of COX Inhibitors on Platelet Thromboxane Production and Urinary Prostaglandin Metabolites

To evaluate the selectivity of the COX inhibitors at the dosage regimens of our atherosclerosis studies, we measured platelet TxB2 production, a COX-1 mediated process, in an ex vivo assay.3 To induce platelet TxB2 production, blood was incubated with 55 μmol/L Ca2+ ionophore A23187.25 There was no evidence of inhibition of COX-1 activity by rofecoxib 10 mg/kg BID, whereas indomethacin 1.5 mg/kg BID inhibited platelet TxB2 production by 98% (Figure 2a). Furthermore, treatment of male apoE−/− mice with rofecoxib 10 mg/kg BID for 8 weeks did not reduce urinary 11-dehydro TxB2 excretion (Figure 2b). Next, we examined the ability of rofecoxib to inhibit excretion of the urinary prostacyclin metabolite 2,3-dinor-6-keto-PGF1α as an index of systemic prostacyclin production. Treatment of LDLR−/− mice with
rofecoxib 10 mg/kg BID and indomethacin 1.5 mg/kg BID resulted in significant 33% and 92% reductions in urinary 2,3-dinor-6-keto-PGF1α, respectively (Figure 2c).

**COX-2 Inhibition at Early Stages of Atherosclerotic Lesion Formation**

To assess the role of COX-2 in early lesion development, we examined the ability of treatment with rofecoxib 10 mg/kg BID and indomethacin 1.5 mg/kg BID to inhibit the development of atherosclerosis in male LDLR−/− mice fed a Western diet. Drug treatment was initiated 2 weeks after starting the Western diet and continued for 6 weeks. As expected, no significant differences in serum cholesterol and triglyceride levels were observed between groups at any time point (Table 1). Furthermore, HDL cholesterol levels at euthanasia did not differ between the LDLR−/− mice treated with vehicle, rofecoxib, or indomethacin (160.3±16.8, 163.9±9.0, or 166.2±10.0 mg/dL±SEM, respectively). Immunocytochemistry of cross-sections of the proximal aorta demonstrated that the lesions consisted almost exclusively of macrophage-derived foam cells (Figure 3). Occasional monocytes or macrophages expressing COX-2 were present, but most macrophage-derived foam cells did not express COX-2 (Figure 3). A small amount of collagen was present in the lesions, and the distribution was similar between groups (data not shown). The mean lesion area in the proximal aorta was decreased by 25% (P=0.02) and 37% (P=0.003) in mice receiving rofecoxib and indomethacin, respectively (Figure 4a), but the difference between the rofecoxib and indomethacin groups was not significant (P>0.05). En face analysis of the aortas revealed striking 58% (P=0.002) and 57% (P=0.01) reductions in lesion area in LDLR−/− mice receiving rofecoxib or indomethacin, respectively (Figure 4b). These data indicate that inhibition of prostaglandin synthesis with a selective COX-2 inhibitor delays the progression of atherogenesis in LDLR-deficient mice during fatty streak lesion formation.

**Fetal Liver Cell Transplantation Studies**

The role of macrophage COX-2 expression in atherosclerotic lesion formation was examined in mice under dietary conditions inducing fatty streak atherosclerotic lesions. Seven-week-old male LDLR−/− mice were lethally irradiated and transplanted with FLC from either male COX-2−/− (n=13, experimental group) or COX-2−/+ (n=14, control group) embryos. Eight weeks after transplantation, when reconstitution was completed, these mice were challenged with the Western diet for 8 weeks. The change in macrophage genotype of recipient mice was verified at the end of the experiments, because COX-2 expression was detected by PCR technique in macrophages from COX-2−/+ → LDLR−/− but not from COX-2−/− → LDLR−/− mice (Figure 5).

Serum lipid levels did not differ between experimental and control groups in LDLR−/− mice fed a chow diet or a high-fat diet (Table 2). Examination of the cholesterol distribution by

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**Figure 2.** Effect of rofecoxib and indomethacin on Ca²⁺ ionophore-stimulated platelet thromboxane production, urinary thromboxane metabolite 11-dehydro TxB₂, and urinary prostacyclin metabolite 2,3-dinor-6-keto-PGF₁α. a, C57BL/6 mice were dosed twice daily for 3 days with vehicle (n=5), 10 mg/kg BID rofecoxib (n=5), or 1.5 mg/kg BID indomethacin (n=5), and after the morning dose on the fourth day, the Ca²⁺ ionophore-stimulated platelet TxB₂ production was measured by GC/MS. Values are mean±SEM. One-way ANOVA, P<0.0001; Dunnett’s post-test, *P<0.01 vs control. b, apoE−/− mice were given vehicle (n=12) or 10 mg/kg rofecoxib (n=9) twice daily by gavage for 8 weeks. Urine was collected in metabolic cages with 3 mice in each cage and analyzed by GC/MS for 11-dehydro TxB₂. c, Male LDLR−/− mice were given vehicle (n=9), 10 mg/kg rofecoxib (n=12), or 1.5 mg/kg indomethacin (n=12) twice daily for 8 weeks. Urine was collected in metabolic cages with 3 mice in each cage and analyzed by GC/MS for 2,3-dinor-6-keto-PGF₁α. One-way ANOVA, P<0.0001; Dunnett’s post-test, *P<0.01 vs control. Indo indicates indomethacin.

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**Table 1. Total Serum Cholesterol and Triglyceride Levels in Male LDLR−/− Mice**

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum Lipids</th>
<th>Baseline</th>
<th>2 Weeks</th>
<th>6 Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle, n=15</td>
<td>Cholesterol</td>
<td>621±22</td>
<td>839±75</td>
<td>935±53</td>
</tr>
<tr>
<td></td>
<td>Triglycerides</td>
<td>224±22</td>
<td>251±29</td>
<td>397±45</td>
</tr>
<tr>
<td>Rofecoxib, n=20</td>
<td>Cholesterol</td>
<td>607±27</td>
<td>844±38</td>
<td>857±23</td>
</tr>
<tr>
<td></td>
<td>Triglycerides</td>
<td>194±18</td>
<td>198±10</td>
<td>317±13</td>
</tr>
<tr>
<td>Indomethacin, n=10</td>
<td>Cholesterol</td>
<td>621±25</td>
<td>853±81</td>
<td>791±66</td>
</tr>
<tr>
<td></td>
<td>Triglycerides</td>
<td>194±21</td>
<td>204±13</td>
<td>330±26</td>
</tr>
</tbody>
</table>

Values are mg/dL (mean±SEM).
FPLC in LDLR<sup>−/−</sup> mice transplanted with FLC and fed the Western diet for 8 weeks revealed accumulation of cholesterol in VLDL, LDL, and IDL ranges with a small HDL peak and no differences between the main groups of the experiment (Figure 6).

Immunocytochemical analysis of cross-sections of the proximal aorta from the COX-2<sup>−/−</sup>→LDLR<sup>−/−</sup> mice demonstrated that the lesions contained predominantly macrophage-derived foam cells that did not contain COX-2 protein, and smooth muscle cells expressing COX-2 were evident in the media (data not shown). Staining for collagen content with Masson’s trichrome revealed very little collagen in the lesions (data not shown). Quantitative analysis of the extent of atherosclerosis in the LDLR<sup>−/−</sup> mice reconstituted with COX-2<sup>−/−</sup> macrophages revealed that the mean atherosclerotic lesion area in the proximal aorta was reduced by 39% compared with control COX-2<sup>−/−</sup>→LDLR<sup>−/−</sup> mice (Figure 7a; 23088±3982 versus 37672±3979 μm²/section±SEM, respectively, P<0.016). The area staining for macrophages was significantly smaller in the COX-2<sup>−/−</sup>→LDLR mice than in COX-2<sup>−/−</sup>→LDLR mice, 21178±637 versus 34621±2469 μm², respectively (P<0.002). A similar reduction (33%) was detected in en face analysis of pinned-out aortas in COX-2<sup>−/−</sup>→LDLR<sup>−/−</sup> mice compared with COX-2<sup>−/−</sup>→LDLR<sup>−/−</sup> mice (Figure 7b; 0.41±0.05% versus 0.61±0.06%, respectively, P<0.015). Thus, LDLR<sup>−/−</sup> mice reconstituted with macrophages null for COX-2 were significantly protected from atherosclerotic lesion formation.

### Discussion

We have examined the distribution of COX-2 expression in atherosclerotic lesions of apoE<sup>−/−</sup> mice using immunocytochemistry and in situ hybridization and have found that COX-2 is expressed by endothelial cells, smooth muscle cells, and monocyte/macrophages in mouse atherosclerotic lesions but not in normal arteries. A surprising finding of our studies was the fact that most macrophage-derived foam cells do not express COX-2. Furthermore, we have found a similar lack of COX-2 expression in macrophage-derived foam cells of human coronary atherosclerotic lesions (Linton et al, unpublished data, 1999). This observation is consistent with the in vitro observation that oxidized LDL suppresses COX-2 expression in human monocyte macrophages. Transformation of macrophages into foam cells in response to acetylated LDL is associated with decreasing production of PGI<sub>2</sub> and PGE<sub>2</sub>. Decreased production of PGE<sub>2</sub> during macrophage foam cell formation may downregulate COX-2 expression, because PGE<sub>2</sub> has been shown to regulate the stability and level of COX-2 mRNA in a positive-feedback loop mediated by EP4 receptor activation and cAMP elevation. In addition, peroxisome proliferator-activated receptor γ, which is highly expressed in macrophage-derived foam cells in human imaging system, and data are represented as the percent lesion area for each mouse and horizontal lines represent the mean level for each group. Wilcoxon rank sum test was used to analyze the data. *P=0.002 and **P=0.01 vs control. Number of animals in each group is represented by n. Indo indicates indomethacin.
The PCR products were resolved by agarose gel electrophoresis. Our results indicate that foam cell formation by the macrophage is associated with downregulation of COX-2 expression in vivo. In contrast, macrophage activation is associated with increased expression of COX-2 and has been implicated in the pathogenesis of atherosclerosis.

In the inhibitor studies, we provide evidence that rofecoxib was indeed selective for COX-2, because there was no inhibition of platelet TxA2 production, a COX-1–mediated process. In contrast, indomethacin dramatically inhibited platelet TxA2 production. Rofecoxib was used at a dose (10 mg/kg BID) superior to that required to achieve maximal anti-inflammatory effects in rats (ED50=0.7 to 1.5 mg/kg) and comparable with that reported to inhibit polyp synthesis in mice (14.7 mg/kg). To confirm the efficacy of rofecoxib in our atherosclerosis studies, we examined the ability of rofecoxib 10 mg/kg BID to inhibit systemic prostacyclin metabolite. Therefore, we have provided evidence of selectivity and efficacy for the COX-2 inhibitor used in the present studies.

Two decades ago, NSAIDs were reported to decrease atherosclerosis in cholesterol-fed rabbits. Pratico et al have recently examined the effects of selective inhibition of COX-2 with nimesulide and nonselective inhibition of COX-2 with indomethacin on atherosclerosis in LDLR−/− mice. Indomethacin reduced the extent of atherosclerosis in en face preparations by 55%, whereas nimesulide produced an impressive trend for an ∼30% reduction in atherosclerosis, which was not significant. On the basis of these results, the authors concluded that COX-1–dependent prostanoid formation accelerates atherogenesis in LDLR−/− mice. Although this work presents an intriguing hypothesis, it does not rule out a contribution of COX-2 to atherogenesis. In fact, the authors suggest that the failure of nimesulide to retard atherogenesis may reflect incomplete suppression of the inflammatory response or features of the model and the timing of the evaluation. Nimesulide is a less selective COX-2 inhibitor than rofecoxib. The 18-week duration of the Western diet would have produced more advanced lesions, and an effect of COX-2 inhibition may have been lost. Furthermore, the analysis of atherosclerosis was performed in groups that combined data from both male and female mice. Sex differences, which are known to influence the extent of atherosclerosis in murine models, could have obscured the significance of the effect of nimesulide. In the present studies, the significant reduction in atherosclerosis in the LDLR−/− mice treated with rofecoxib demonstrates a role for COX-2 dependent prostanoid formation in promoting atherogenesis.

### Table 2. Total Serum Cholesterol and Triglyceride Levels in LDLR−/− Mice After Transplantation With COX-2−/− or COX-2+/− Fetal Liver Cells

<table>
<thead>
<tr>
<th>Group of Animals</th>
<th>Serum Lipids</th>
<th>8 Weeks of Western Diet</th>
<th>8 Weeks of Western Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Chow Diet</td>
<td>COX-2−/−→LDLR−/−</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>n=14</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cholesterol 233±5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>221±4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>657±26</td>
</tr>
<tr>
<td></td>
<td>Triglycerides</td>
<td></td>
<td>170±12</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>290±12</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cholesterol 236±6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>211±7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>647±27</td>
</tr>
<tr>
<td></td>
<td>Triglycerides</td>
<td></td>
<td>162±10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>293±14</td>
</tr>
</tbody>
</table>

Values are mg/dL (mean±SEM). No differences reached statistical significance at any time point.
A limitation in interpreting studies of pharmacological inhibitors is the potential for the drugs to exert pharmacological actions on unintended targets. For example, indomethacin and several other NSAIDs have been reported to bind to peroxisome proliferator-activated receptors and therefore might affect atherosclerosis indirectly. Therefore, we sought a genetic approach to additionally implicate COX-2 in atherogenesis. Fetal liver cell transplantation was used to examine the role of macrophage COX-2 expression in LDLR/− mice transplanted with COX-2+/− or COX-2+/+ FLC and then fed the Western diet for 8 weeks. The lesion area in COX-2+/−→LDLR mice was significantly (33% to 39%) reduced compared with the COX-2+/+→LDLR mice. These results provide genetic evidence that strongly corroborate our studies using selective COX-2 inhibitors by implicating macrophage COX-2 in promoting early atherogenesis. We believe it is unlikely that donor-derived COX-2+/− endothelial cells, smooth muscle cells, or T-cells contributed to the reduction in atherosclerosis. Mounting evidence supports the existence of bone marrow–derived stem cells for endothelial cells and smooth muscle cells. However, the contribution of donor-derived endothelial cells and smooth muscle cells to aortic atherosclerotic lesions in LDLR-deficient mice after bone marrow transplant is <1% of the respective cell type (Linton et al, unpublished data, 2002). Although T-cells have been reported to express COX-2 in vitro, prostaglandin production is undetectable, and evidence for a physiological role for T-cell COX-2 expression in vivo is lacking. In contrast, activated macrophages express COX-2 abundantly, and this expression has been shown to play a crucial role in the inflammatory response. Given that macrophage-derived foam cells do not express COX-2, the reduction in atherosclerosis seen in the transplant studies is likely attributable to an effect of COX-2 expression on atherogenesis before foam cell formation. Macrophage COX-2 may promote atherogenesis through activation of chemotaxis, propagation of the inflammatory cytokine cascade, or macrophage migration.

Our results do not directly address the issue raised in the VIGOR trial, which showed an increase in cardiovascular events in rheumatoid arthritis patients treated with rofecoxib versus naproxen. In contrast, a recent analysis of 23 phase IIb through V rofecoxib studies containing >28 000 patients did not show any evidence for increased cardiovascular events in patients treated with rofecoxib versus placebo or NSAIDs. Furthermore, the CLASS trial of 8059 patients with osteoarthritis showed no difference in the incidence of cardiovascular events between patients taking celecoxib or NSAIDs. These studies highlight the need for clinical trials to assess the effects of selective COX-2 inhibition combined with antiplatelet agents on cardiovascular events. Coronary events occur as a result of atherosclerotic plaque rupture and thrombosis. Although the mouse is a widely used model for the investigation of atherosclerosis, the absence of plaque rupture and coronary thrombosis leading to myocardial infarction are clear limitations of the mouse as a model for human coronary heart disease. However, our results suggest that by decreasing atherosclerosis, COX-2 inhibitors might reduce the substrate for thrombotic cardiovascular events.

Mounting evidence supports an important role for inflammation in atherosclerosis and plaque rupture. We present evidence that pharmacologic inhibition of COX-2 reduces atherosclerosis in the LDLR-deficient mouse model of atherosclerosis, and we provide genetic evidence indicating that macrophage COX-2 promotes early atherogenesis. These results demonstrate the potential of COX-2 inhibitors in the treatment of atherosclerosis and support the potential of anti-inflammatory approaches to the prevention of coronary heart disease.

Acknowledgments
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