Absence of Myeloid COX-2 Attenuates Acute Inflammation but Does Not Influence Development of Atherosclerosis in Apolipoprotein E Null Mice

Ajay J. Narasimha, Junji Watanabe, Tomo-o Ishikawa, Saul J. Priceman, Lily Wu, Harvey R. Herschman, Srinivasa T. Reddy

Objective—The role of myeloid cell cyclooxygenase-2 (COX-2) in the progression of atherosclerosis has not been clearly defined.

Methods and Results—We investigated the role of COX-2 expressed in the myeloid lineage in the development of atherosclerosis using a myeloid-specific COX-2⁻/⁻ (COX-2⁻⁻/⁻) mouse on a hyperlipidemic apolipoprotein (apo) E⁻⁻ background (COX-2⁻⁻/⁻ M/apoE⁻⁻/⁻). Myeloid COX-2 depletion resulted in significant attenuation of acute inflammation corresponding with decreased PGE₂ levels in an air pouch model. COX-2 depletion in myeloid cells did not influence development of atherosclerosis in COX-2⁻⁻/⁻ M/apoE⁻⁻/⁻ when compared to apoE⁻⁻/⁻ littermates fed either chow or western diets. The unanticipated lack of contribution of myeloid COX-2 to the development atherosclerosis is not attributable to altered maintenance, differentiation, or mobilization of myeloid and lymphoid populations. Moreover, myeloid COX-2 depletion resulted in unaltered serum prostanoid levels and cellular composition of atherosclerotic lesions of COX-2⁻⁻/⁻ M/apoE⁻⁻/⁻ mice.

Conclusions—Our results suggest that COX-2 expression in myeloid cells, including macrophages, does not influence the development of atherosclerosis in mice. (Arterioscler Thromb Vasc Biol. 2010;30:260-268.)

Key Words: atherosclerosis ■ cyclooxygenase-2 ■ inflammation ■ myeloid cells ■ prostanoids

Atherosclerosis is the primary mechanism underlying the development of coronary heart disease.¹ Atherosclerosis is a chronic, inflammatory disease that is often correlated with increased low-density lipoprotein (LDL) and decreased high-density lipoprotein levels.² During the progression of the disease, LDL is oxidized and becomes trapped in the aortic subendothelial space, resulting in increased inflammation and leukocyte recruitment. The lesions that develop in the aortic root and proximal aorta are characterized by infiltration of macrophages and other immune cells, including polymorphonuclear neutrophils (PMN) and lymphocytes.³

Macrophages are one of the major cell types found in atherosclerotic lesions. In addition to their role in the inflammatory response, macrophages play an important antiatherogenic role by preventing lipid accumulation through lipid efflux to acceptors such as high-density lipoprotein and apolipoprotein A-I (apoA-I).⁴ An imbalance between lipid uptake and efflux often results in increased lipid accumulation in macrophages, resulting in a proatherogenic formation of foam cells and early fatty streaks.⁵

Whereas macrophages are the most well-studied inflammatory cells in atherosclerotic plaques, other myeloid cells, including PMN, impact the progression of atherosclerosis.⁶ PMN release myeloperoxidase at the lesion site, which may participate in oxidative modification of LDL, accelerating the development of the atheroma.⁷

Cyclooxygenase-2 (COX-2) is the inducible form of the COX enzyme and is responsible for the rate-limiting step in the conversion of arachidonic acid to prostanoids (prostacyclin, prostaglandins, and thromboxanes).⁸ COX-2 is highly expressed in macrophages, PMN, and other leukocytes in acute and chronic inflammation, and its expression is well-characterized in chronic inflammatory disorders such as atherosclerosis.⁹ COX-2 is induced in human¹⁰ and mouse¹¹ atherosclerotic lesions and is expressed in macrophages, endothelial cells, and smooth muscle cells.¹²

Clinical data have implied a potential antiatherogenic role for COX-2 because prolonged use of COX-2–specific inhibitors results in an increase in cardiovascular events, such as heart attacks and strokes.¹²–¹⁴ Although these data clearly implicate COX-2 in cardiovascular disease, no

Received August 4, 2009; revision accepted October 29, 2009.

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Arterioscler Thromb Vasc Biol is available at http://atvb.ahajournals.org

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DOI: 10.1161/ATVBAHA.109.198762
mechanism has been proposed to fully explain the role of COX-2 in atherosclerosis, which is the underlying cause of coronary heart disease. Although there have been a large number of studies performed to attempt to elucidate the mechanism, there is no consensus in the literature. COX-2 inhibition has been shown to decrease15-17 increase,18 or have no effect19-21 on the progression of atherosclerosis in mice, depending on the mode and duration of COX-2 inhibition, as well as the types of mouse models used. Similarly, the role of macrophage COX-2 in atherosclerosis has been explored but has not been conclusively elucidated.15,16,22

In addition to their role in lipid metabolism at the site of the atherosclerotic lesions described, macrophages, as well as other myeloid cells, play a large role in the regulation of cytokine production and other inflammatory mediators, including COX-2–derived prostanooids. In this article, we describe using a myeloid cell-specific COX-2–/− (COX-2–/−/M) mouse to determine whether COX-2 in myeloid cells, including macrophages, plays a role in the development of atherosclerotic lesions in mice.

Materials and Methods

Animals

COX-2 floxed “knock-in” mice, in which exons 4 and 5 of mouse COX-2 are flanked by loxP sites, have been described previously.23 Lysozyme M (LysM) Cre mice24 on a C57BL6/J background were generously provided by Dr Yibin Wang. The apoE/H9251 mice on an 129Sv/J background were obtained from Jackson Labs (Bar Harbor, Me). To obtain COX-2–/−/M mice on an apoE–/−/H9251 background (COX-2–/−/M/apoE–/−), COX-2 floxed mice were crossed to LysM Cre mice to obtain COX-2+/−/M (COX-2+/−/M/LysM+/−) mice and wild-type (COX-2+/+/M) littermates. COX-2–/−/M mice were further crossed with apoE–/−/H9251 mice to obtain COX-2–/−/M/apoE–/− (COX-2–/−/M/LysM+/−, apoE–/−/M) mice. The apoE–/−/M controls used in experiments were COX-2+/−/M, LysM+/−, and apoE–/−/M littermates. All mice were maintained on a standard chow (6% fat) diet. In atherosclerosis studies, female mice were maintained on chow diet for 14 weeks or female mice were maintained on chow diet for 18 weeks, starting at the age of 6 weeks. Serum samples were isolated from overnight-fasted mice, cryopreserved in 10% sucrose, and kept at −80°C for further analysis of cytokines and prostanooids.

Aortic Root Lesions

Atherosclerotic lesion area in the aortic root was determined as described previously.26 Briefly, heart and proximal aorta from mice were obtained and embedded in OCT compound. Serial 10-μm-thick cryosections from the middle portion of the ventricle to the aortic arch were collected, mounted on precoated slides, and stained with Oil Red O and hematoxylin. The lipid-containing area on each section, centered on the aortic valves, was determined with a blinded fashion, using an ocular with a 20×20-μm2 grid on a light microscope. The average lesion area per aorta, calculated from 5 to 10 sections of each aorta, was scored.

Serum Lipids

Serum lipids were determined as described previously.27

Immunohistochemistry

Fresh-frozen aortic root sections were stained for CD68, α-actin, and COX-2. Briefly, after fixation in ice-cold acetone, sections were blocked in 4% bovine serum albumin plus 10% goat serum for 3 hours at room temperature. Rat antimouse CD68 (1:100; Serotec), rabbit antihuman α-actin (1:100; Spring Bioscience), and rabbit antihuman COX-2 (1:200; Cayman Chemical) were used with an overnight incubation at 4°C. Goat antirat and goat antirabbit alkaline phosphatase secondary antibodies (Jackson ImmunoResearch) were used at 1:200 with a 1-hour incubation at room temperature. Immunostaining was visualized using Vector Red substrate plus levamisole to inhibit endogenous alkaline phosphatase activity (Vector Laboratories). Immunostaining was...
Flow Cytometry

Single-cell suspensions were prepared from each tissue before performing flow cytometry. Spleens and periaortic lymph nodes were gently dissociated between 2 glass slides for single-cell isolation. Peripheral blood was isolated directly into BD Vacutainer K2 EDTA tubes (BD Biosciences) at room temperature and red blood cells were lysed (Sigma-Aldrich). Mouse bone marrow cells were harvested by flushing the tibias and femurs of the mice, followed by red blood cell lysis. Single-cell suspensions were filtered and incubated for 30 minutes on ice with the following purified and FITC-conjugated, Phycoerythrin (PE)-conjugated, Allophycocyanin (APC)-conjugated, PerCP-Cy5-5–conjugated, PE-Cy7–conjugated, and APC-Cy7–conjugated antibodies: CD45, CD11b, Gr-1, F4/80 (purchased from eBioscience), Ly6C, CD4, and 7-AAD (purchased from BD Biosciences). Cells were washed twice before analysis on the BD LSR-II flow cytometer (BD Biosciences).

For sorting experiments, single-cell suspensions of bone marrow were either untreated or treated with lipopolysaccharide (2 μg/mL; Sigma) in DMEM 10% fetal bovine serum at 37°C for 4 hours. Cell suspensions were then incubated with CD11b, Ly6C, and Gr-1 antibodies as described. Cells were sorted through a BD FACSAriaII cell sorter (BD Biosciences) and genomic DNA isolation. Peripheral blood was isolated directly into BD Vacutainer K2 EDTA tubes (BD Biosciences) at room temperature and red blood cells were lysed (Sigma-Aldrich). Mouse bone marrow cells were harvested immediately.

Genomic Polymerase Chain Reaction

Cre-mediated recombination of genomic DNA was detected using the following primers flanking the COX-2 floxed region and designed for COX-2 introns 3 and 5, respectively: 5′- AAAGTTCAGCCATTGTACAGCAGGG-3′ and 5′- GAATCTCC-TAGAACTGACTGG-3′. Polymerase chain reaction conditions used were 95°C for 3 minutes, 30 cycles of 95°C for 30 seconds, 60°C for 1 minute, and 72°C for 2 minutes. The larger band (2067 bp) corresponds to the nonrecombined allele and the smaller band (651 bp) corresponds to a recombined allele.

Statistics

Statistical significance was determined by Student t test. Statistical significance was defined as P<0.05.

Results

To characterize the COX-2−/− mouse model, peritoneal macrophages were isolated from wild-type and COX-2−/− mice, and COX-2 protein expression and PGE_2 levels were determined. Because COX-2 is induced by inflammatory stimuli, macrophages were treated with 50 ng/mL TNF-α for 4 hours to determine COX-2 protein expression in cell lysates, and for 18 hours to determine accumulation of PGE_2 in the culture supernatants. Macrophages from COX-2−/− mice did not express COX-2 protein or produce PGE_2 when stimulated with TNF-α (Figure 1), suggesting that the COX-2 gene was successfully knocked out in macrophages of COX-2−/− mice. COX-2−/− mice were crossed with hyperlipidemic apoE−/− mice to obtain COX-2−/−/apoE−/− mice. All of the subsequent experiments were performed using COX-2−/−/apoE−/− mice and apoE−/− littermates.

Macrophages and other myeloid cells regulate a variety of acute inflammatory processes, primarily through the production of PGE_2. To determine if myeloid COX-2 affects acute inflammation in vivo, the air pouch model of acute inflammation was studied in COX-2−/−/apoE−/− mice and apoE−/− littermates. Air pouches made on the backs of either COX-2−/−/apoE−/− mice or apoE−/− controls were injected with either zymosan or saline, and inflammatory exudates were collected 24 hours later. Exudates from saline-treated air pouches contained only negligible numbers of infiltrating cells and concentrations of inflammatory mediators (Figure 2). Injection of zymosan into air pouches resulted in a large infiltration of leukocytes; however, the total number of leukocytes in zymosan-injected air pouches in COX-2−/−/apoE−/− mice was different from those in air pouches of apoE−/− controls (Figure 2A). Both adherent (mostly myeloid) cells and nonadherent (mostly nonmyeloid) cells obtained from the air pouch exudates of apoE−/− mice injected with zymosan express COX-2 protein. In contrast and as expected, COX-2 protein expression was only detectable in nonmyeloid cells from COX-2−/−/apoE−/− mice, whereas COX-2 protein was depleted in the infiltrating myeloid cells (Figure 2B). Moreover, PGE_2 in COX-2−/−/apoE−/− exudates is decreased compared to exudates from control mice, suggesting a reduction in overall COX-2 activity, potentially related to specific COX-2 knockdown in myeloid cells (Figure 2C). Furthermore, the acute inflammatory mediator TNF-α was significantly decreased in exudates obtained from COX-2−/−/apoE−/− mice (Figure 2D), demonstrating an attenuation of acute inflammation correlated with loss of COX-2 expression and activity in myeloid cells. Unlike TNF-α, IL-1β and IL-6 levels were not affected in COX-2−/−/apoE−/− mice in the air pouch model of acute inflammation (data not shown).

To determine whether COX-2 depletion in myeloid cells modulates chronic inflammatory conditions such as atherosclerosis, COX-2−/−/apoE−/− and apoE−/− control fe-
males were fed either a standard chow diet or the western diet, and atherosclerotic lesion development was determined in all groups (see Materials and Methods for details). Both groups of mice, with and without western diet, had typical atherosclerotic lesions develop as determined by Oil Red O staining (Figure 3A, B, D, E). However, there were no significant differences in atherosclerotic lesion development between COX-2 \(^{-/-}\)/apoE \(^{-/-}\) mice and COX-2 \(^{-/-}\)/apoE \(^{-/-}\) control mice fed either the standard chow diet or the western diet (Figure 3C,F). Moreover, lipid levels in circulation, including high-density lipoprotein and LDL levels, were unaffected by diet, and atherosclerotic lesion development was determined by Oil Red O (Figure 3B). Open circles are averaged lesion size from individual mice and black bars represent average lesion area of group. Lesion composition, in addition to lesion size, is another indicator of susceptibility to atherosclerosis. In general, an increase in macrophage number and decreased smooth muscle cell proliferation, as well as the presence of thin fibrous cap encasing the lesion, suggest a more unstable, rupture-prone plaque. To determine if lesions in COX-2 \(^{+/}\)/apoE \(^{+/}\) mice contained differences in phenotype, compositions of macrophages and smooth muscle cells were quantified in atherosclerotic lesions by immunohistochemistry using antibodies to CD68 and \(\alpha\)-actin, respectively (Figure 4). Figure 4A through 4D (CD68) and 4G and 4H (\(\alpha\)-actin) show representative abundance of macrophages and smooth muscle cells, respectively, from both diets.
apoE−/− and COX-2−M−/−apoE−/− mice. Lesions from COX-2−M−/−/apoE−/− showed no significant differences in macrophage or smooth muscle cell expression regardless of diet (Figure 4E,F,I), suggesting that lesions from these mice, in addition to being similar in size to those from apoE−/− mice, also are similar in composition and phenotype.

COX-2 and its products have been implicated in the differentiation of myeloid cells, including macrophages,[32,33] however, it is not clear whether the lack of COX-2 in myeloid cells alters differentiation programs that are most likely mediated by paracrine or transcellular mechanisms. We examined if there were differences in maintenance, differentiation, or mobilization of myeloid and lymphoid cells in COX-2−M−/−/apoE−/− mice. Flow cytometry was performed on bone marrow, spleen, lymph nodes, and peripheral blood harvested from COX-2−M−/−/apoE−/− mice and apoE−/− controls (Figure 5). We observed negligible changes in percentages of monocytes/macrophages, PMN, and CD4+ lymphocytes in any of the tissues assayed (Figure 5E–H). These data suggest that depletion of myeloid COX-2 does not influence maintenance of myeloid or lymphoid cells in the bone marrow or percentages of myeloid and some lymphoid cells in peripheral tissues or in circulation.

To further confirm that COX-2−M−/−/apoE−/− have specific depletion of COX-2 in myeloid cells, bone marrow was isolated from apoE−/− and COX-2−M−/−/apoE−/−, and Cre-mediated recombination of the COX-2 gene as well as lypopolysaccharide-induced COX-2 protein expression was determined in monocytes/macrophages, PMN, and CD11b+ (nonmyeloid) cells (Figure 6). The floxed COX-2 cassette was specifically deleted in monocytes/macrophages and PMN but not in nonmyeloid cells isolated from the bone marrow of COX-2−M−/−/apoE−/− mice (Figure 6A). Furthermore, lypopolysaccharide-induced COX-2 expression was noticeably absent in monocytes/macrophages and PMN but was retained in nonmyeloid cells isolated from the bone marrow of COX-2−M−/−/apoE−/− mice, confirming the specificity of the COX-2−M−/−/apoE−/− mouse model (Figure 6B).

To determine patterns of COX-2 expression in the atherosclerotic lesion, immunohistochemistry was performed on lesions from COX-2−M−/−/apoE−/− and apoE−/− controls fed western diet (supplemental Figure I, available at http://atvb.ahajournals.org). In apoE−/− lesions, there is qualitative colocalization between macrophages (CD68) and COX-2 staining, confirming that macrophages at the site of lesions express COX-2. Macrophages and COX-2 expression do not seem to colocalize in COX-2−M−/−/apoE−/− lesions. To determine if serum levels of COX products are affected by myeloid depletion of COX-2, levels of PGE2, 6-keto-PGF1α (stable metabolite of prostacyclin), and TXB2 (stable metabolite of TXA2) were assayed (Figure I) in serum samples obtained from COX-2−M−/−/apoE−/− and apoE−/− controls fed western diet. There were no differences in serum levels of prostanoids between the 2 groups of mice (Figure I).

Discussion

Atherosclerosis, a disease of the large arteries that is the primary cause of coronary heart disease and stroke, is a multifactorial, chronic inflammatory disease in which low plasma levels of high-density lipoprotein and high plasma
levels of LDL are strong predictors of the condition. COX enzymes and their products, prostanoids, (prostacyclin, prostaglandins, and thromboxanes), play a key role in acute and chronic inflammation, including atherosclerosis. The inducible form of COX, COX-2, is involved in stimulus-induced prostanoid synthesis and is the target for the class of nonsteroidal antiinflammatory drugs termed COX-2–specific inhibitors. Defining the cardiovascular effects of COX-2–specific inhibitors has taken an increasing clinical relevance because of an increased risk of myocardial infarction and stroke, which led to the voluntary withdrawal of 2 classes of COX-2–specific inhibitors from the market. The issue remains intensely controversial, mainly because of lack of consensus in the literature about the role of COX-2 in the development of atherosclerosis.

Macrophages are the hallmark of inflammatory diseases, including atherosclerosis. Based on their ability to robustly express COX-2, we reasoned that macrophages present an attractive mechanism to study the role of COX-2 in atherosclerosis. In this study, a mouse model in which COX-2 was deleted in only myeloid cells was developed and the role of macrophages and other myeloid cells in acute inflammation and atherosclerosis was investigated. As expected, macrophage COX-2 contributes to PGE₂ formation during acute inflammation in vitro (Figure 5).
demonstrate that COX-2 in myeloid cells, including macrophages, is not involved in the progression of atherosclerosis in mice.

There was also no change in lesion phenotype observed in lesions from COX-2−/−/apoE−/− mice (Figure 4). Lesion phenotype, as it relates to plaque stability, is an important index of risk of atherosclerosis. Increased macrophage abundance as well as decreased smooth muscle cell proliferation are indicative of an unstable, rupture-prone plaque. However, in the current study, COX-2 depletion in myeloid cells did not affect macrophage infiltration, smooth muscle cell proliferation, or characteristics of the fibrotic cap encasing the lesion. Although macrophage COX-2 has been shown to regulate the activity of matrix metalloproteinases, which can contribute to plaque rupture, the current studies do not demonstrate regulation of plaque phenotype by myeloid COX-2.

Lipid levels in circulation were also unchanged (Table I), further supporting the lack of impact of myeloid COX-2 on atherosclerosis development. We previously showed absence of COX-2 alters lipoprotein metabolism in mice; however, those results were obtained from total COX-2−/− mice. Our current studies suggest that the hyperlipidemia observed in the total COX-2−/− mice is attributable to cell types from a nonmyeloid lineage. It follows that other cell types expressing COX-2 could play a role in lipid regulation in circulation and will be the focus of further studies.

Myeloid cells play a large role in inflammation, but we observed no effect of myeloid COX-2 depletion on important processes in atherosclerotic inflammation, including macrophage recruitment to the lesion site. Because it has been hypothesized that COX-2 is involved in the differentiation of myeloid cells, we hypothesized that the lack of significant differences in lesion macrophage recruitment may be explained by relative differences in differentiation or maintenance of leukocyte populations in COX-2−/− mice. Our current studies suggest that the hyperlipidemia observed in the total COX-2−/−/apoE−/− mice when compared to COX-2−/−/apoE−/− mice (Figure 5). Moreover, a closer inspection of leukocyte populations, including monocytes/macrophages, PMNs, and CD4+ T cells, also demonstrated a lack of effect of myeloid COX-2 depletion on maintenance of leukocytes in bone marrow and peripheral lymphoid tissues, as well as mobilization of leukocytes into circulation (Figure 5).

COX-2 knockout in the entire myeloid compartment has no effect on lesion formation. An explanation for this unexpected finding could be the prominence of COX-2 staining at the lesion itself, even in COX-2−/−/apoE−/− animals (Figure I). There is functional overlap in prostanoid production between various cell types in many biological processes. In addition, although prostanooids are relatively unstable, they can function in a paracrine fashion in the vasculature and circulation. Moreover, intermediate COX metabolites, including PGH2, can be processed transcellularly by various downstream prostanoid synthases, resulting in local prostanoid production by distal cells. Other modes of transcellular prostanoid formation include the release of secretory phospholipase A2 by...
distal cells that could initiate arachidonic acid metabolism in local cell populations.45

In the current studies, COX-2 is expressed robustly not only in macrophages in the lesion but also in endothelial cells, smooth muscle cells, and lymphocytes as well (data not shown). Therefore, in an atherosclerotic lesion, where many cell types exist in close proximity, prostanooids produced by COX-2 in various cell types could compensate for COX-2 depletion in myeloid cells or even act in a paracrine fashion on myeloid cells themselves, maintaining proinflammatory-dependent myeloid cell functions. Another possibility is that myeloid cells lacking COX-2 could still process exogenous PGH2 in a transcellular process, masking any effects of COX-2 depletion. In addition, COX-2–deficient myeloid cells could still produce proinflammatory isoprostanes, generated from nonenzymatic, free-radical–catalyzed peroxidation of arachidonic acid,46 thereby affecting atherosclerosis progression independently of COX activity.

The hypothesis that compensatory mechanisms may help explain the lack effect of myeloid depletion of COX-2 on atherosclerosis is further supported by the finding that serum levels of prostanooids, PGE2, 6-keto-PGF1α, and TXB2 are unchanged in COX-2–/- mice (Fig. 1). Therefore, the depletion of COX-2 in myeloid cells and the postulated resulting decrease in PGE2 production by myeloid cells is unable to influence the progression of chronic inflammation in atherosclerosis potentially because of sufficient prostanooid production by other cell types or unaltered prostanooid production by myeloid cells via transcellular mechanisms.

The human clinical data suggest that COX-2 activity is antiatherogenic. This study proves that when modeled in mice, myeloid COX-2, including macrophage COX-2, is not responsible for this antiatherogenic activity. The use of other conditional COX-2–/- mice, including those made to deplete COX-2 from endothelial cells or smooth muscle cells, could be fruitful in terms of elucidating an anti-atherogenic role for COX-2 that would help propose a mechanism to explain the clinical data.

Acknowledgments

The authors thank Feng Su, Victor Grijalva, and Ani Shabbazian for their expert technical assistance.

Sources of Funding

This work was supported by NHLBI grants 1R01HL71716 (S.T.R.) and R01HL082823 (S.T.R.).

Disclosure

None.

References


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Arterioscler Thromb Vasc Biol. 2010;30:260-268; originally published online November 19, 2009;
doi: 10.1161/ATVBAHA.109.198762

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Absence of Myeloid COX-2 Attenuates Acute Inflammation But Does Not Influence Development of Atherosclerosis in Apolipoprotein E Null Mice

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Supplemental Figure Legends

Supplemental Figure I. Lack of COX-2 expression in myeloid cells does not affect total COX-2 expression or activity. Lesions from apoE<sup>-/-</sup> mice (A, B) and COX-2<sup>M/-M</sup>/apoE<sup>-/-</sup> mice (C, D) on western diet mice were immunostained for CD68 (A, C) and COX-2 (B, D) (red staining) as described in Methods. Black arrows denote areas of co-localization between CD68 and COX-2 staining and red arrows denote CD68 positive areas lacking COX-2 staining. Levels of PGE<sub>2</sub> (C), 6-keto-PGF<sub>1α</sub> (D), and TXB<sub>2</sub> (E) were determined in individual serum samples from apoE<sup>-/-</sup> and COX-2<sup>M/-M</sup>/apoE<sup>-/-</sup> mice on chow and western diet (n=13-16). Open circles are concentrations from individual mice and black bars represent average concentration of group.
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<th>Total-C</th>
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<td><strong>apoE&lt;sup&gt;-/-&lt;/sup&gt; (Chow)</strong></td>
<td>581.6 ± 207.6</td>
<td>23.5 ± 6.6</td>
<td>534.2 ± 211.6</td>
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<td>23.9 ± 8.5</td>
<td>476.3 ± 115.3</td>
<td>150.9 ± 49.5</td>
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<td>1202.8 ± 524.8</td>
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<td><strong>COX-2&lt;sup&gt;-/-/-&lt;/sup&gt;/ apoE&lt;sup&gt;-/-&lt;/sup&gt; (Western)</strong></td>
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All values are in mg/dL. Values are average ± SD. N=13-17 mice. Chow = chow diet, Western = western diet. Total-C = Total cholesterol, HDL-C = HDL cholesterol, LDL-C = LDL cholesterol, Free-C = Unesterified Cholesterol, FFA = Free Fatty Acids, TRIG = Triglycerides