Apolipoprotein C-I Is Crucially Involved in Lipopolysaccharide-Induced Atherosclerosis Development in Apolipoprotein E–Knockout Mice

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Background—Lipopolysaccharide (LPS), which is released from Gram-negative bacteria on multiplication or lysis, aggravates atherosclerosis in humans and rodents by inducing inflammation via toll-like receptors. Because apolipoprotein C-I (apoCI) enhances the LPS-induced inflammatory response in macrophages in vitro and in mice, we investigated the effect of endogenous apoCI expression on LPS-induced atherosclerosis in mice.

Methods and Results—Twelve-week-old apoe−/− apoc1−/− and apoe−/− apoc1+/+ mice received weekly intraperitoneal injections of LPS (50 μg) or vehicle for a period of 10 weeks, and atherosclerosis development was assessed in the aortic root. LPS administration did not affect atherosclerotic lesion area in apoe−/− apoc1−/− mice but increased it in apoe−/− apoc1+/+ mice. In fact, apoCI expression increased the LPS-induced atherosclerotic lesion area by 60% (P<0.05), concomitant with an increase in LPS-induced plasma levels of fibrinogen and E-selectin. This indicated that apoCI expression increased the LPS-induced inflammatory state, both systemically (ie, fibrinogen) and at the level of the vessel wall (ie, E-selectin). In addition, both macrophage-derived apoCI and HDL-associated apoCI increased the LPS-induced tumor necrosis factor-α response by macrophages in vitro.

Conclusions—We conclude that apoCI is crucially involved in LPS-induced atherosclerosis in apoe−/− mice, which mainly relates to an increased inflammatory response toward LPS. We anticipate that apoCI plasma levels contribute to accelerated atherosclerosis development in individuals who have chronic infection. (Circulation. 2007;116:2173-2181.)

Key Words: apolipoproteins • atherosclerosis • inflammation • lipoproteins • hypercholesterolemia • leukocytes

Circulatory disease (CVD) is the principal cause of death in Europe, the United States, and much of Asia. The main cause of CVD is atherosclerosis.1–3

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Although hypertension and hyperlipidemia are common risk factors for atherosclerosis, it is also evident that bacterial infections worsen the outcome of atherosclerosis by maintaining a heightened state of inflammatory response, thus propagating this inflammatory disease.1–4 Lipopolysaccharide (LPS) is a highly inflammatory constituent of the outer membrane of Gram-negative bacteria. When these bacteria multiply or lyse, LPS is released and then activates the MD-2/toll-like receptor 4 (TLR4) receptor complex on endothelial cells, neutrophils, and macrophages, which results in a proinflammatory response through activation of the nuclear factor-κB pathway.5 TLR4 is expressed in macrophages and endothelial cells within atherosclerotic lesions,6,7 and indeed, impaired LPS signaling in human carriers of the TLR4 polymorphisms Asp299Gly or Thr399Ile is associated with LPS hyporesponsiveness,8 and the Asp299Gly mutation is associated with decreased CVD.4 Conversely, infection with the Gram-negative bacterium Chlamydia pneumoniae has been associated with increased CVD in humans.9–11

In rodents, bacterial infection and LPS also promote atherogenesis. For example, local delivery of C pneumoniae in the vessel wall of carotid arteries increases the development of atherosclerosis in low-density lipoprotein (LDL) receptor gene–deficient (ldlr−/−) mice,12 and infection with C pneumoniae accelerates the development of atherosclerosis in...
apolipoprotein (apo) E gene–deficient (apoE−/−), ldlr−/−, and APOE*3-Leiden transgenic mice.13–15 In addition, LPS accelerates intimal lesion development in a periadventitial cuff model in wild-type mice, which is largely decreased when TLR4 expression is absent.16 Furthermore, repeated intravenous and intraperitoneal administration of LPS accelerates atherosclerosis in rabbits and apoE−/− mice, respectively.17,18

Until recently, apolipoprotein C-I (apoCI) was known primarily for its role in lipoprotein metabolism. ApoCI circulates in plasma with a concentration of 6 mg/dL and is mainly bound to the lipoproteins very-low-density lipoprotein (VLDL), chylomicrons, and high-density lipoprotein (HDL).19 ApoCI inhibits lipoprotein lipase (LPL),20,21 and endogenous apoCI expression was associated with modest hyperlipidemia in apoE−/− mice.22 Recently, we have discovered that apoCI strongly binds to LPS, thereby augmenting the inflammatory response to LPS and the Gram-negative bacterium Klebsiella pneumoniae in mice in vivo and to LPS in macrophages in vitro.22

In the present study, we investigated the effect of endogenous apoCI on the development of LPS-induced atherosclerosis in apoE−/−apoCI−/− versus apoE−/−apoCI+/+. We found that LPS-induced atherosclerosis is enhanced in apoCI-expressing mice, in association with a higher inflammatory state achieved with LPS.

**Methods**

For an extended version of the Methods section, see the online-only Data Supplement.

**Animals**

ApoE−/−apoCI−/− and apoE−/−apoCI+/+ mice were back-crossed at least 8 times to the C57Bl/6 background. ApoE−/−apoCI−/− and apoE−/−apoCI+/+ littersmates were generated, and female mice were used for experiments, housed under standard conditions with a 12-hour light cycle (7 AM to 7 PM), and fed ad libitum with regular chow.

**Analysis of LPS Response in Time**

Twelve-week-old apoE−/−apoCI−/− and apoE−/−apoCI+/+ littersmates (n=8 per genotype) received an intraperitoneal injection of LPS (50 μg; Escherichia coli serotype 055:B5, Sigma, St Louis, Mo) in 200 μL of PBS. Twenty-four hours before LPS injection (t=0) and every 24 hours thereafter for a period of 1 week, plasma samples were taken after a 4-hour fast. TC was measured, and the values shown indicate the increase compared with t=0. Basal levels of TC were 7.0±0.5 and 9.3±1.8 mmol/L in apoE−/−apoCI−/− and apoE−/−apoCI+/+ mice, respectively. Values are mean±SD; n=8.

**HDL Analysis**

To determine the apoCI level on HDL relative to HDL lipid concentrations, HDL from apoE−/−apoCI−/− mice that received either vehicle or LPS was isolated via precipitation of apoB-containing lipoproteins,27 and HDL phospholipids, HDL cholesterol, and apoCI23 were determined.

**Plasma Analysis of Fibrinogen and E-Selectin**

Plasma fibrinogen levels were determined with a homemade ELISA,28 and plasma E-selectin levels were ascertained via a commercially available ELISA (R&D Systems Europe, Abingdon, UK).

**Incubation of Macrophages With LPS In Vitro**

ApoE−/−apoCI−/− and apoE−/−apoCI+/+ peritoneal macrophages were isolated and cultured27 and then incubated with LPS (0.1 to 100 ng/mL) in Dulbecco’s Modified Eagle Medium supplemented with 0.01% human serum albumin (4 hours at 37°C). Tumor necrosis factor (TNF)-α in the medium was determined. Murine RAW 264.7 macrophages were cultured22 and incubated with LPS (100 ng/mL) with or without HDL isolated from apoE−/−apoCI−/− and apoE−/−apoCI+/+ littersmates (24 hours at 37°C), and TNF-α was determined in the medium.22

**Atherosclerotic Lesion Analysis**

For determination of atherosclerosis development, mice were euthanized at 24 hours after the tenth injection. Hearts were fixed and embedded in paraffin, and sections were made.23 Lesion area and composition with regard to adherent monocytes, macrophages, collagen, smooth muscle cells (SMCs), and T cells were determined.29

**Statistical Analysis**

The Mann-Whitney nonparametric test for 2 independent samples was used to define differences between data sets from experimental groups. The criterion for significance was set at P<0.05. Linear regression analysis was used to evaluate correlations between HDL apoCI levels versus HDL lipid parameters. Statistical analyses were performed with SPSS version 11.5 (SPSS Inc, Chicago, Ill).

The authors had full access to the data and take full responsibility for its integrity. All authors have read and agree to the manuscript as written.

**Results**

**Effect of ApoCI on Plasma Lipid Levels After LPS Injection**

To examine the effect of LPS on plasma lipid levels, we measured plasma levels of TC and TG before and during a
The level of TC decreased thereafter to return to its starting value after 7 days (Figure 1). LPS only modestly increased levels of TG, by 0.1 mmol/L, and this also returned to starting values within 7 days (results not shown). ApoCI expression apparently did not affect the total LPS-induced increase in TC (Figure 1) and TG levels (results not shown).

We next investigated the effect of repeated injections of LPS or vehicle on plasma TC and TG levels in apoe−/−apoC1−/− and apoe−/−apoC1+/− mice. LPS or vehicle was injected weekly for a period of 10 weeks, and plasma lipid levels were determined 24 hours before the first injection and 24 hours after the first, fifth, and tenth injections. The lipid response to the first injection was similar to that observed in the previous experiment. The response declined somewhat after the subsequent injections up to ∼50% after the tenth injection, which indicates that repeated LPS injections led to tolerance to some extent (data not shown). Over the entire period of these injections, LPS increased TC exposure for both genotypes by only ∼2.5 mmol/L. The level of TC decreased thereafter to return to its starting value after 7 days (Figure 1). LPS only modestly increased levels of TG, by ∼0.1 mmol/L, and this also returned to starting values within 7 days (results not shown). ApoCI expression apparently did not affect the total LPS-induced increase in TC (Figure 1) and TG levels (results not shown).

ApoC-I is crucial for LPS-induced atherosclerosis.

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apoCI mainly circulates on HDL, we next examined the effect of repeated LPS injections on apoCI levels in plasma. Values are mean ± SD. n = 8.

Effect of ApoCI on LPS-Induced TNF-α Response of Macrophages

Because we found previously that exogenous apoCI increased the LPS-induced TNF-α response in macrophages, ApoCI mainly circulates on HDL, we next examined the effect of HDL (0.1 and 1 μg protein/mL) from apoCI- and apoCI-/+ mice on the LPS-induced TNF-α response by macrophages. ApoCI presence on HDL increased the TNF-α response by 77% (P < 0.05; HDL = 0.1 μg protein/mL) and 66% (P < 0.05; HDL = 1 μg protein/mL) as evident from a comparison of the response between apoCI- and apoCI-/+ HDL (Figure 5A). In addition, we investigated the effect of apoCI expression by macrophages on the LPS-induced TNF-α response using apoCI- and apoCI-/+ peritoneal macrophages. Endogenous apoCI expression by macrophages increased the TNF-α response elicited by 1, 10, and 100 ng/mL LPS, which reached significance with 100 ng/mL LPS (45%; P < 0.05; Figure 5B). Collectively, both apoCI on HDL and macrophage-derived apoCI contributed to enhancement of the LPS-induced TNF-α response by macrophages.

Effect of ApoCI on LPS-Induced Atherosclerosis Development

We next examined the effect of repeated LPS injections on atherosclerosis development and the effect of apoCI on LPS-induced atherosclerosis. For this purpose, mice were euthanized 24 hours after the tenth LPS injection, and the development of atherosclerosis was studied at the level of the aortic root. Representative lesions are shown in Figure 6A through 6D, and the data of all mice are summarized in Figure 6E. LPS increased atherosclerotic lesion area by 53% (P < 0.05) in apoCI- mice, as derived from the comparison between vehicle- and LPS-treated apoCI- mice (Figure 6C versus 6D). In contrast, LPS did not affect the atherosclerotic lesion area in apoCI- mice (Figure 6A versus 6B). Furthermore, apoCI expression in...
creased LPS-induced atherosclerosis by 60% (P<0.05), as evident from a comparison of LPS-injected apo<sup>e−/−</sup>apoC<sup>1−/−</sup> and apo<sup>e−/−</sup>apoC<sup>1−/−</sup> mice (Figure 6B versus 6D). In contrast, apoCI expression did not affect atherosclerosis in mice injected with vehicle (Figure 6A versus 6C). These data thus indicate that endogenous apoCI expression is a strong determinant of the LPS-induced increase in atherosclerotic lesion size (Figure 6E).

Figure 4. Effect of apoCI on plasma inflammatory markers after LPS injection. Twelve-week-old apo<sup>e−/−</sup>apoC<sup>1−/−</sup> (●) and apo<sup>e−/−</sup>apoC<sup>1−/−</sup> (○) mice received an injection of LPS (50 μg). Twenty-four hours before LPS injection (t=0) and every 24 hours thereafter for a period of 4 days, plasma samples were taken after a 4-hour fast. Fibrinogen (A) and E-selectin (B) were measured, and the values shown indicate the increase compared with t=0. Values are mean±SD; n=8. *P<0.05, significant difference between genotypes.

Figure 5. Effect of apoCI on the LPS-induced TNF-α response in macrophages. RAW 264.7 macrophages were incubated with LPS (100 ng/mL) in Dulbecco’s Modified Eagle Medium supplemented with 0.01% human serum albumin, in the presence or absence of HDL isolated from apo<sup>e−/−</sup>apoC<sup>1−/−</sup> or apo<sup>e−/−</sup>apoC<sup>1−/−</sup> littermates (0.1 and 1 μg HDL protein/mL). After 24 hours, cells were washed and lysed, and TNF-α was determined in the medium (A). Peritoneal macrophages from apo<sup>e−/−</sup>apoC<sup>1−/−</sup> and apo<sup>e−/−</sup>apoC<sup>1−/−</sup> littermates were incubated without or with LPS (0.1 to 100 ng/mL) in Dulbecco’s Modified Eagle Medium supplemented with 0.01% human serum albumin. After 4 hours, cells were washed and lysed, and TNF-α was determined in the medium (B). Values are represented relative to cell protein content as mean±SD; n=4. *P<0.05.

Figure 6. Effect of apoCI on LPS-induced atherosclerosis development in the aortic root. Twelve-week-old apo<sup>e−/−</sup>apoC<sup>1−/−</sup> (solid symbols) and apo<sup>e−/−</sup>apoC<sup>1−/−</sup> (open symbols) mice received weekly injections of LPS (50 μg; circles) or vehicle (squares) for a period of 10 weeks and were euthanized after the last injection. Hearts were isolated, cross-sectioned (5 μm) throughout the aortic root, and stained with hematoxylin-phloxin-saffron. Representative pictures are shown (A through D). Atherosclerotic lesion area was measured in 4 sections per mouse at 40-μm intervals. Each data point represents the mean per mouse. Each line indicates the mean of the data points (E). n=8 to 11. *P<0.05.
Effect of ApoCI on LPS-Induced Atherosclerotic Lesion Composition

To study whether LPS treatment affected lesion composition, we characterized the atherosclerotic lesions with respect to monocyte adhesion, the content of CD3+ T cells, macrophages, SMCs, and collagen. In mice of both genotypes, LPS treatment tended to stimulate monocyte adhesion (Figure 7A) and T-cell recruitment (Figure 7B), although the effects did not reach statistical significance. These effects are consistent with previous observations that LPS stimulates monocyte adhesion and T-cell recruitment.5,18 In mice deficient in apoCI, LPS treatment did not affect macrophage area (Figure 7C), SMC area (Figure 7D), or collagen area (Figure 7E), as evident from a comparison of vehicle-treated and LPS-treated apoe-/-/apoCI-/-/ mice. In contrast, in mice expressing apoCI, LPS increased the macrophage area significantly (*P<0.05; Figure 7C), tended to increase SMC area (Figure 7D), and significantly increased collagen area (*P<0.05; Figure 7E), as evident from a comparison of vehicle-treated apoCI-/-/apoCI-/-/ mice and LPS-treated apoCI-/-/apoCI-/-/ mice.

In both the vehicle- and LPS-treated groups, apoCI expression did not affect monocyte adhesion (Figure 7A), yet it showed a tendency to increase T-cell recruitment (Figure 7B) and macrophage area (Figure 7C), as evident from a comparison of the vehicle- or LPS-treated apoCI-/-/apoCI-/-/ mice and the vehicle- or LPS-treated apoCI-/-/apoCI-/-/ mice. Furthermore, in the LPS-treated groups, apoCI expression significantly increased SMC (Figure 7D) and collagen (Figure 7E) area, as evident from a comparison of LPS-treated apoCI-/-/apoCI-/-/ mice and LPS-treated apoCI-/-/apoCI-/-/ mice.

Taken together, apoCI expression accelerated atherosclerosis after treatment with LPS. As a result, the atherosclerotic lesions increased in size and concomitantly contained more SMCs and collagen, which reflected the progression of atherosclerosis.

Discussion

Gram-negative bacteria such as *C pneumoniae* release LPS on multiplication or lysis during infections, which leads to a chronic inflammatory state that accelerates atherosclerosis in humans and rodents.9–18 We have shown previously that apoCI binds to LPS, thereby augmenting the inflammatory response to LPS and *K pneumoniae* in mice and in macrophages in vitro.22 In the present study, we investigated the significance of these observations for a chronic inflammatory disease, atherosclerosis, by assessing the effect of apoCI expression on LPS-induced atherosclerosis in apoCI-/- mice and in vivo. We found that endogenous apoCI increased atherosclerosis development in apoCI-/- mice induced by chronic treatment with LPS.

We showed that injection of LPS similarly increased the TC content of IDL/LDL and HDL in both apoCI-/-/apoCI-/-/ and apoCI-/-/apoCI-/-/ mice and marginally increased TG levels. This response was transient, which indicates that the
effects were induced after LPS injection, returning to their starting value before the next LPS injection. It is tempting to speculate about the mechanism underlying the lipid changes in the present study after LPS injection. Serum amyloid A and apoA, which were both elevated after LPS injection, can contribute to an increased HDL plasma level by stimulating HDL generation.\(^3\,\text{3}\) In addition, serum amyloid A can inhibit HDL clearance.\(^3\) In contrast to the present findings, HDL cholesterol decreases in humans upon challenge with LPS.\(^3\text{5}\) We speculate that the increased HDL cholesterol in the present mouse model is mainly related to reduced clearance of HDL as a consequence of the absence of apoE that mediates the clearance of HDL, as reported previously.\(^3\text{6}\) The increased cholesterol content in IDL/LDL might be secondary to reduced LPL activity, because LPS injection decreases LPL activity.\(^3\text{7}\) The effects of LPS on lipid levels were transient and similar in both mouse models, and because the increased levels of plasma cholesterol contributed only \(\approx 5\%\) to the total cholesterol exposure over time until atherosclerosis assessment (not shown), it is unlikely that the transient increase in plasma lipid levels after LPS injection was a primary contributor to development of atherosclerosis.

The augmenting effect of apoCI on LPS-induced atherosclerosis is related to a greater inflammatory status in LPS-treated \(\text{apoC-I}^{+/+}\)/\(\text{apoC-I}^{-/-}\) mice than in \(\text{apoC-I}^{-/-}\)/\(\text{apoC-I}^{-/-}\) mice. We showed that apoCI expression enhanced the effect of LPS on fibrinogen and E-selectin. Fibrinogen is an acute-phase protein that is secreted from the liver and that reflects the general inflammatory status of mice and putatively participates in atherosclerotic lesion development.\(^3\text{8}\) Fibrinogen is primarily regulated by interleukin-6.\(^3\text{9}\) E-selectin is a target gene of the nuclear factor–κB pathway in macrophages and reflects the inflammatory state of the vessel wall.\(^4\text{0}\) ApoCI thus increased LPS-induced inflammatory responses both systemically and at the vascular level. Although the difference in magnitude of the LPS-induced inflammatory response was relatively small, it has recently been stated in humans that small effects on inflammatory responses contribute substantially to CVD.\(^4\text{1}\) ApoCI expression per se did not affect these inflammatory markers in vehicle-treated mice, an observation that is in agreement with the observed comparable atherosclerotic lesion area in vehicle-treated \(\text{apoC-I}^{-/-}\)/\(\text{apoC-I}^{-/-}\) and \(\text{apoC-I}^{-/-}\)/\(\text{apoC-I}^{-/-}\) mice. In addition, apoCI expression by \(\text{apoC-I}^{-/-}\) macrophages per se increased the LPS-induced inflammatory response, and apoCI on HDL from \(\text{apoC-I}^{-/-}\) mice was crucial for increasing the inflammatory response of macrophages toward LPS.

A recent human study showed that HDL cholesterol negatively correlates with the response to a single administration of LPS.\(^4\text{2}\) Because apoCI is mainly localized on HDL, it may have been expected that apoCI would be associated with a decreased LPS response in the present mouse study. However, although HDL apoCI correlated with HDL phospholipids, owing to the distribution of both components in the particle shell, HDL apoCI did not correlate at all with HDL cholesterol. Therefore, the present study does not exclude the possibility that apoCI also increases the LPS response in humans.

We thus conclude that apoCI accelerates LPS-induced atherosclerosis progression in \(\text{apoC-I}^{-/-}\) mice mainly as a consequence of increasing inflammation. This is in line with the finding that LPS-treated \(\text{apoC-I}^{-/-}\) mice expressing human apoA-IV show reduced production of proinflammatory cytokines and reduced atherosclerosis compared with their \(\text{apoC-I}^{-/-}\) littermates.\(^2\text{5}\)

LPS treatment did not affect lesion composition of mice deficient in apoCI, yet it increased macrophage and collagen area in mice expressing apoCI. The increased macrophage content might have been the consequence of the increased inflammation in macrophages after apoCI expression, as we showed in vitro. However, comparison of lesions from both vehicle groups and LPS-injected groups with the same atherosclerotic lesion area revealed no differences regarding atherosclerotic lesion composition (not shown). Also, in advanced lesions, we observed adventitial infiltrates of activated lymphocytes, which have been reported to be caused by LPS injections in \(\text{apoC-I}^{-/-}\) mice.\(^1\text{8}\) Because we only observed these infiltrates in advanced atherosclerotic lesions, we suggest that these infiltrates were the consequence of the severity of the lesion rather than being caused by LPS specifically. In addition, it has been reported that apoCI increases apoptosis in human aortic SMCs by recruiting neutral sphingomyelinase.\(^4\text{2}\) It is unlikely that this effect contributed to atherosclerosis in the aortic root in the present study, because apoCI did not affect SMCs in vehicle-treated mice.

At first glance, the finding that apoCI expression did not significantly affect atherosclerosis in the vehicle-treated mice appears to be in contrast to our previous data showing that apoCI accelerates atherosclerosis as related to increased VLDL lipid levels in mice of 26 weeks of age.\(^2\text{6}\) However, in the present study, VLDL lipid levels were somewhat increased on apoCI expression, yet the absolute values in both mouse genotypes were \(\approx 20\%\) lower than in our previous study. In fact, the relatively low lipid levels in the present study led to a relatively slow progression of atherosclerosis that was not different between mice of both genotypes, and this enabled us to investigate the effect of apoCI on LPS-induced atherosclerosis specifically. Indeed, mice need to express a certain level of hyperlipidemia for LPS to have an effect on atherosclerosis development, as was demonstrated in former studies using \(C\) \(\text{pneumoniae}\), which mainly exerts its effects via LPS.\(^1\text{5}\text{-4}\text{3}\text{-4}\text{4}\) We speculate that the effects of apoCI on LPS-induced atherosclerosis are mediated via TLR4 in macrophages, because TLR4 is primarily activated by LPS\(^4\text{5}\) and has been demonstrated to be involved in LPS-induced atherosclerosis development in mice.\(^1\text{6}\) The mechanism behind the augmenting effect of apoCI on the response toward LPS is currently under investigation.

We conclude that apoCI is crucially involved in LPS-induced atherosclerosis in \(\text{apoC-I}^{-/-}\) mice, mainly as a consequence of enhancing the inflammatory response. We anticipate that in humans who suffer from chronic inflammation, plasma apoCI may enhance atherosclerosis development and CVD.

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Disclosures

None.

References


CLINICAL PERSPECTIVE

Accumulating evidence indicates that Gram-negative bacteria such as Chlamydia pneumoniae and Porphyromonas gingivalis are involved in cardiovascular disease. Infection with these pathogens may cause a chronic inflammatory state as a result of the release of endotoxins such as lipopolysaccharide (LPS), which accelerates the development of atherosclerosis in humans and rodents. It has been generally assumed that high-density lipoprotein (HDL) binds LPS, thereby attenuating the inflammatory response, which may contribute to the antiatherosclerotic potential of HDL. However, we have shown previously that apolipoprotein C-I (apoC-I), a small and highly positively charged surface apolipoprotein that is mainly HDL-associated, binds LPS and augments the inflammatory response toward LPS and Gram-negative bacteria in mice and macrophages. In the present study, we investigated the significance of these observations for atherogenesis. We assessed the effect of endogenous apoC-I expression on atherosclerosis in apoE-knockout mice that were treated chronically with LPS. Although the present study confirmed that LPS itself aggravates atherosclerosis in apoE-knockout mice, apoC-I appeared to be a crucial determinant of LPS-induced aggravation of atherosclerosis. Endogenous apoC-I expression enhanced inflammation both systemically and at the level of the vessel wall. In addition, HDL-associated apoC-I increased the inflammatory response toward LPS in macrophages in vitro. These data thus indicate that plasma apoC-I may increase the cardiovascular disease risk associated with Gram-negative bacterial infections. Furthermore, the present study underscores the importance of the apolipoprotein composition of HDL for the antiatherosclerotic potential of HDL, which should be considered in the design and analysis of HDL-raising strategies aimed at reducing cardiovascular disease risk.
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