Effect of Overexpression of Human Apo A-I in C57BL/6 and C57BL/6 Apo E–Deficient Mice on Lipoprotein-Associated Enzymes, Platelet-Activating Factor Acetylhydrolase and Paraoxonase

Comparison of Adenovirus-Mediated Human Apo A-I Gene Transfer and Human Apo A-I Transgenesis

Bart De Geest, Dominique Stengel, Michèle Landeloois, Marleen Lox, Laurence Le Gat, Désiré Collen, Paul Holvoet, Ewa Ninio

Abstract—Various mechanisms may contribute to the antiatherogenic potential of apolipoprotein A-I (apo A-I) and high density lipoproteins (HDLs). Therefore, the effect of adenovirus-mediated human apo A-I gene transfer or human apo A-I transgenesis on platelet-activating factor acetylhydrolase (PAF-AH) and arylesterase/paraoxonase (PON1) was studied in C57BL/6 and C57BL/6 apo E−/− mice. Human apo A-I transgenesis in C57BL/6 mice resulted in a 4.2-fold (P<0.0001) increase of PAF-AH and a 1.7-fold (P=0.0012) increase of PON1 activity. The apo E deficiency was associated with a 1.6-fold (P=0.008) lower PAF-AH and a 2.0-fold (P=0.012) lower PON1 activity. Human apo A-I transgenesis in C57BL/6 apo E−/− mice increased PAF-AH and PON1 activity by 2.1-fold (P=0.01) and 2.5-fold (P=0.029), respectively. After adenovirus-mediated gene transfer of human apo A-I into C57BL/6 apo E−/− mice, a strong correlation between human apo A-I plasma levels and PAF-AH activity was observed at day 6 (r=0.92, P<0.0001). However, PON1 activity failed to increase, probably as a result of cytokine-mediated inhibition of PON1 expression. In conclusion, this study indicates that overexpression of human apo A-I increases HDL-associated PAF-AH activity. PON1 activity was also increased in human apo A-I transgenic mice, but not after human apo A-I gene transfer, a result that was probably related to cytokine production induced in the liver by the adenoviral vectors. Increased levels of these HDL-associated enzymes may contribute to the anti-inflammatory and antioxidative potential of HDL and thereby to the protection conferred by HDL against atherothrombosis. (Arterioscler Thromb Vasc Biol. 2000;20:e68-e75.)

Key Words: HDL n gene transfer n platelet-activating factor acetylhydrolase n paraoxonase

Plasma levels of apo A-I and HDL cholesterol are negatively correlated with the risk of ischemic cardiovascular disease, the leading cause of death in Western countries.1,2 Studies in human apo A-I–transgenic mice and rabbits have demonstrated that increased HDL cholesterol inhibits the progression of atherosclerosis.3–6 Various mechanisms may contribute to the antiatherogenic potential of HDL. Reverse cholesterol transport, as originally proposed by Glomset,7 includes the extraction of cholesterol from extrahepatic tissues by HDL and the delivery of cholesterol and cholesterol esters to hepatocytes, which can secrete HDL-derived cholesterol into bile as free cholesterol or as bile acids. The antiatherogenic potential of HDL may also be related to its anti-inflammatory and antioxidative properties, which are mediated by several mechanisms. First, reactive oxidized lipids may be transferred from LDL to HDL,8 which may inhibit the propagation of an oxidation cascade in LDL. HDL has indeed been shown to be the predominant carrier of cholesterol ester hydroperoxides in humans.9 After transfer to HDL, cholesterol ester hydroperoxides are taken up much more efficiently by hepatocytes than are native cholesterol esters,10,11 providing a link between the antioxidative properties of HDL and reverse cholesterol transport.

Second, several HDL-associated enzymes may protect LDL against oxidative modification. LDL oxidation has been shown to induce fragmentation of the sn-2 residue of phospholipids,12 generating oxidized phospholipids with potent proinflammatory effects. Platelet-activating factor acetylhydrolase...
The recombinant adenovirus AdapoA-I, containing the cytomegalovirus promoter/enhancer, the endogenous 256-bp apo A-I promoter, and the genomic apo A-I sequence, was generated by cotransfecting the rescue plasmid pJM17 and the shuttle plasmid pLPa into 293 cells as described previously.26 To generate the pACCMVpLpa vector containing the genomic apo A-I DNA with inclusion of the 256-bp minimal apo A-I promoter, the pBluescript vector containing the genomic apo A-I DNA was restricted with XbaI and EcoRI. The genomic apo A-I DNA fragment was blunted at 37°C with Klenow DNA polymerase (Boehringer Mannheim). The pACCMVpLpa vector was opened with XbaI, dephosphorylated at 37°C for 1 hour with alkaline phosphatase (Boehringer Mannheim), and blunted by using Klenow DNA polymerase. Ligation was performed with T4 DNA ligase (Boehringer Mannheim). The correct orientation of the genomic apo A-I was determined by restriction with Hinfl and was confirmed by DNA sequencing. The human tissue-type plasminogen activator virus (Ad-HA) has been described before.27

### Animal Experiments
All experimental procedures in animals were performed in accordance with protocols approved by the Institutional Animal Care and Research Advisory Committee. All mice used in this study were female and 5 to 6 months of age. Apo E−/− mice were backcrossed for 10 generations into the C57BL/6J background and had 99.9% C57BL/6J background. Human apo A-I transgenic mice used in this study were originally described by Rubins et al.28 The human apo A-I-transgenic apo E−/− mice used here have been described before.29 Mice were fed normal chow ad libitum. Virus administration was performed by tail vein injection. Different doses of recombinant adenovirus were administered in a final volume of 300 μL.

### Isolation of Lipoproteins by Gel Filtration
Mice were killed after an overnight fast, and maximal blood volume was obtained by puncture of the inferior vena cava. To obtain plasma, anticoagulation was performed with 0.1 volume of 4% trisodium citrate. Separation of lipoproteins by gel filtration in a fast pressure liquid chromatography system (Waters Associates) was performed as described previously.26 For gel filtration of serum, an isotonic saline buffer (10 mmol/L Tris-HCl [pH 7.4], 137 mmol/L NaCl, 5 mmol/L KCl, 1 mmol/L CaCl2, and 1 mmol/L MgCl2) was used.

A pool of fractions 10 to 17 containing VLDL-, LDL-, and HDL-size lipoproteins was used to determine PAF-AH and arylesterase activity of non-HDL-size lipoproteins. Similarly, fractions 18 to 25 and 26 to 33, corresponding to large-size and small-size HDL lipoproteins, respectively, were pooled, and PAF-AH and arylesterase activities were determined on these pools.

For determination of cholesterol levels, cholesterol of fractions obtained after gel filtration was extracted with methanol/chloroform (2:1, vol/vol). Esterified and unesterified cholesterol levels were quantified by high-performance liquid chromatography on a reversed-phase column (Zorbax ODS, Du Pont de Nemours) essentially as described by Vercaesel et al.31

### Human Apo A-I ELISA
Human apo A-I levels were determined by sandwich ELISA. In brief, polylysine microtiter plates (Costar) were coated with a rabbit anti-human apo A-I polyclonal antibody. Diluted plasma samples (1:25 000, 1:50 000, 1:100 000, and 1:200 000) were added to the wells for 2 hours. After being washed, a 1:15 000 dilution of the murine monoclonal antibody A2A4 was conjugated with peroxidase and placed on the wells for 2 hours. Peroxidase reaction was performed by adding H2O2 and o-phenylenediamine. Finally, absorbance was measured at 492 nm.

### Determination of PAF-AH Activity
Hexadecyl PAF, obtained as a powder from Sigma Chemical Co, was dissolved at a final concentration of 20 mmol/L in ethanol (80% vol/vol). This solution was mixed with 1-O-hexadecyl-2-[1H-acetyl]-sn-glycero-3-phosphocholine (10 Ci/mmol, DuPont–New England Nuclear), dried under a stream of N2, and redissolved in a solution containing fatty acid–free bovine serum albumin (0.25% wt/vol in saline) to obtain a 50 μmol/L [1H-acetyl]PAF solution. AH activity was measured by the trichloroacetic acid precipitation procedure as previously described.32 In brief, the pH of the HEPES–EDTA (2 mmol/L) buffer was adjusted to 7.4, and routine assays were performed for 10 minutes at 37°C in a total volume of 100 μL. Plasma was diluted 2000-fold and lipoprotein fractions were diluted 10-fold in HEPES buffer before addition of 10 μL of [1H-acetyl]PAF (50 μmol/L; specific activity, 5000 disintegrations per minute per nanomole).
Cholesterol Levels of Lipoproteins Isolated by Gel Filtration

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Adt-PA</th>
<th>AdapoA-I</th>
<th>Human apoA-I Transgenic</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>69±2.7</td>
<td>68±3.9</td>
<td>120±8.4*</td>
<td>210±19†</td>
</tr>
<tr>
<td>Non-HDL cholesterol</td>
<td>13±0.8</td>
<td>18±2.9</td>
<td>18±1.9</td>
<td>15±2.5</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>56±2.8</td>
<td>50±3.0</td>
<td>103±7.7*</td>
<td>200±18†</td>
</tr>
</tbody>
</table>

Cholesterol values represent mean±SEM of 3 different mice and are expressed in mg/dL.
*P<0.05, †P<0.01 vs control mice.

**Determination of Arylesterase and Paraoxonase Activity of PON1**

Arylesterase activity was measured by using phenylacetate as a substrate. Initial rates of hydrolysis were determined spectrophotometrically at 270 nm in a Power Wave 200 microplate scanning spectrophotometer (Bio-Tek Instruments). The assays were performed in a final volume of 250 µL containing 1 mmol/L phenylacetate and 2 mmol/L CaCl₂ in 20 mmol/L Tris-HCl buffer, pH 8.0, in the presence of 0.1 µL of mouse serum or 10 µL of lipoprotein fraction for 5 minutes. The extinction coefficient at 270 nm for the reaction was 17 000 mol/L cm⁻¹ for 1 micromole of phenylacetate hydrolyzed per minute.

The rate of hydrolysis of paraoxon was assessed by measuring liberation of p-nitrophenol at 405 nm at 25°C. The assays were performed in a final volume of 250 µL containing 5.5 mmol/L paraoxon and 2 mmol/L CaCl₂ in 100 mmol/L Tris-HCl buffer, pH 8.0, in the presence of 2 to 4 µL of mouse serum for 4 minutes. The extinction coefficient at 405 nm for the reaction was 17 000 mol/L cm⁻¹ for 1 nanomole of p-nitrophenol converted per minute.

**Evaluation of an Acute-Phase Response and Cytokine Production After Adenoviral Gene Transfer**

High-resolution electrophoresis of serum was carried out by using Hydragel 15 HR (Sebia Benelux) according to the instructions of the manufacturer. Albumin, α₂-globulins, and complement component C₃ were quantified by densitometric scanning. Plasma levels of interleukin-1 (IL-1β) and interleukin-6 (IL-6) were determined by using the Quantikine M immunoassays (R&D Systems Europe).

**Statistical Analysis**

All data are expressed as mean±SEM. Significance of differences in cholesterol levels were assessed by a 2-tailed, unpaired, alternate Welch t test with the INSTAT V2.05a statistical program (Graph Pad Software). Comparison of PAF-AH activity, arylesterase activity, and paraoxonase activity was performed by the nonparametric Mann-Whitney U test. The correlation between arylesterase and paraoxonase activity of PON1 and the correlation between PAF-AH activity and human apo A-I was calculated by using the nonparametric Spearman rank correlation in the INSTAT V2.05a statistical program. A two-sided P value of <0.05 was considered statistically significant.

**Results**

**Effect of Human Apo A-I Overexpression on Cholesterol Levels in C57BL/6 and C57BL/6 Apo E⁻/⁻ Mice**

Cholesterol levels of lipoprotein fractions isolated by gel filtration are represented in the Table. HDL cholesterol was 1.6-fold (P<0.05) lower in C57BL/6 apo E⁻/⁻ mice than in C57BL/6 mice. Gene transfer with 10⁹ plaque-forming units (pfu) of AdapoA-I increased HDL cholesterol levels at 6 days after gene transfer by 1.8-fold (P<0.05) and 2.1-fold (P<0.05) in C57BL/6 and C57BL/6 apo E⁻/⁻ mice, respectively. HDL cholesterol levels in human apo A-I C57BL/6 and human apo A-I C57BL/6 apo E⁻/⁻ transgenic mice were 3.5-fold (P<0.01) and 3.1-fold (P<0.01) higher, respectively, than in nontransgenic control mice. HDL cholesterol levels in human apo A-I C57BL/6 and human apo A-I C57BL/6 apo E⁻/⁻ transgenic mice were 1.9-fold (P<0.05) and 1.5-fold (P<0.05) higher, respectively, than in AdapoA-I-treated C57BL/6 and AdapoA-I–treated C57BL/6 apo E⁻/⁻ mice, respectively (the Table). Less than 5% of HDL cholesterol and as much as 15% of human apo A-I was present in small-size HDL particles.

**PAF-AH Activity in C57BL/6 and C57BL/6 Apo E⁻/⁻ Mice**

PAF-AH activity in C57BL/6 control mice (n=16), in C57BL/6 mice 6 days after gene transfer with 10⁹ pfu of Adt-PA adenovirus (n=12) or AdapoA-I adenovirus (n=11), and in human apo A-I transgenic (TG)–mice (n=5). B) PAF-AH activity in C57BL/6 apo E⁻/⁻ control mice (n=9), in C57BL/6 apo E⁻/⁻ mice 6 days after gene transfer with 10⁹ pfu of Adt-PA (n=11) or AdapoA-I adenovirus (n=12), and in human apo A-I–transgenic C57BL/6 apo E⁻/⁻ mice (n=4).

![Figure 1](http://atvb.ahajournals.org/Downloaded from)
mice treated with Adt-PA control virus. PAF-AH activity increased 2.0-fold ($P<0.0001$) after AdapoA-I transfer and was 4.2-fold ($P<0.0001$) elevated in human apo A-I-transgenic mice. PAF-AH activity was 2.1-fold ($P=0.0009$) higher in human apo A-I C57BL/6 transgenic mice than in AdapoA-I-treated mice.

PAF-AH activity in apo E$^{2/-}$ control mice ($n=9$), in apo E$^{2/-}$ mice 6 days after gene transfer with $10^5$ pfu of Adt-PA adenovirus ($n=11$) or AdapoA-I adenovirus ($n=12$), and in human apo A-I apo E$^{2/-}$ transgenic mice ($n=4$) is shown in Figure IB. PAF-AH activity was 1.6-fold ($P=0.008$) lower in apo E$^{2/-}$ than in C57BL/6 mice. No significant alteration of PAF-AH activity was observed after Adt-PA transfer in apo E$^{2/-}$ mice. PAF-AH activity increased 1.8-fold ($P=0.0024$) after AdapoA-I gene transfer and was 2.1-fold ($P=0.010$) elevated in human apo A-I apo E$^{2/-}$ transgenic mice.

To investigate the association between human apo A-I overexpression and increase in PAF-AH activity in C57BL/6 apo E$^{2/-}$ mice, 3 different doses of AdapoA-I adenovirus ($5\times10^8$, $10^9$, and $2\times10^9$ pfu; $n=4$ for each dose) were administered, and PAF-AH activity was determined 6 days after gene transfer. The correlation between human apo A-I plasma levels and PAF-AH activity was 0.92 ($P<0.0001$; Figure II).

To investigate the kinetics of increased PAF-AH activity after adenovirus-mediated gene transfer, $2\times10^9$ pfu of AdapoA-I was administered to C57BL/6 apo E$^{2/-}$ mice, and PAF-AH activity was determined at days 3, 6, 14, and 21 after gene transfer. Figures IIIA and IIIB illustrate human apo A-I plasma levels and plasma PAF-AH activity, respectively. PAF-AH activity was increased 2.5-fold at day 3 ($P=0.0001$), 3.3-fold at day 6 ($P=0.0001$), 1.7-fold at day 14 ($P<0.0001$), and 1.4-fold at day 21 ($P=0.01$).

**Lipoprotein Distribution of PAF-AH Activity**

Figure IV illustrates the lipoprotein distribution of PAF-AH activity in C57BL/6 (A) and apo E$^{2/-}$ (B) mice, respectively. In C57BL/6 control mice, 83% of PAF-AH activity was recovered in large-size HDL particles and 13% in small-size HDL particles. This distribution was similar after Adt-PA gene transfer. PAF-AH activity in large-size HDL and small-size HDL particles increased 1.8-fold ($P=0.0005$) and 3.7-fold ($P=0.0005$), respectively, after gene transfer with $10^9$ pfu of AdapoA-I. PAF-AH activity in large-size HDL and small-size HDL particles of human apo A-I transgenic mice was 2.1-fold ($P=0.0015$) higher, respectively, than in control mice. The activity associated with small-size HDL particles contributed 25% and 56% of total lipoprotein-associated activity in AdapoA-I–treated C57BL/6 mice and human apo A-I transgenic mice, respectively.

In C57BL/6 apoE$^{2/-}$ control mice, 52% of PAF-AH activity was present in large-size HDL and 41% in small-size HDL. PAF-AH activity increased 2.2-fold ($P=0.014$) in large-size HDL after AdapoA-I gene transfer, whereas no significant change occurred in small-size HDL. PAF-AH activity in large-size HDL and small-size HDL of human apo A-I transgenic mice was 2.1-fold ($P=0.0028$) higher, respectively, than in control mice.

**Arylesterase and Paraoxonase Activity of PON1 in C57BL/6 and C57BL/6 Apo E$^{2/-}$ Mice**

Arylesterase and paraoxonase activities of PON1 in C57BL/6 control mice ($n=7$), in C57BL/6 mice 6 days after gene
and paraoxonase activities were 1.4-fold \((P=0.042)\) and 2.0-fold \((P=0.012)\) lower, respectively, in apo \(E^{-/-}\) mice than in C57BL/6 mice. No significant alteration of arylesterase activity was observed after Adt-PA or AdapoA-I gene transfer. Compared with apo \(E^{-/-}\) control mice, arylesterase and paraoxonase activity increased 1.8-fold \((P=0.029)\) and 2.5-fold \((P=0.029)\), respectively, in human apo A-I apo \(E^{-/-}\) transgenic mice. Arylesterase and paraoxonase activities of PON1 were highly correlated \((r=0.90, P=0.0002)\).

Figure IIIIC illustrates the time course of arylesterase activity after gene transfer with \(2\times10^9\) pfu of AdapoA-I in C57BL/6 apo \(E^{-/-}\) mice. Compared with baseline, arylesterase was 1.2-fold lower at day 3 \((P<0.05)\), 1.8-fold lower at day 6 \((P<0.0001)\), 2.2-fold lower at day 14 \((P<0.0001)\), and was not significantly different at day 21.

**Lipoprotein Distribution of Arylesterase Activity**

Figure VI illustrates the lipoprotein distribution of arylesterase activity in C57BL/6 (A) and apo \(E^{-/-}\) (B) mice. Activities were determined on fractions obtained after gel filtration of pooled serum samples. In C57BL/6 control mice, 91% of arylesterase activity was present in large-size HDL particles and 8% in small-size HDL particles. The 1.8-fold decrease in serum arylesterase activity after Adt-PA
and AdapoA-I gene transfer corresponded to a 1.5-fold and a 2.2-fold decrease, respectively, of arylesterase activity associated with large-size HDL particles. The 1.5-fold higher serum arylesterase in human apo A-I–transgenic mice corresponded to a 1.7-fold increase of activity associated with large-size HDL particles.

In apo E<sup>−/−</sup> mice, 88% of arylesterase activity was present in large-size HDL particles and 6% in small-size HDL particles. The 1.8-fold increase in serum arylesterase activity in human apo A-I–transgenic apo E<sup>−/−</sup> mice corresponded to a 1.5-fold increase of arylesterase activity in large-size HDL particles.

**Plasma Cytokines and Acute-Phase Response Proteins After Gene Transfer**

IL-1β concentration in plasma was below detection (7.5 pg/mL) in control C57BL/6 mice (n = 4) and was 70±44 pg/mL 6 days after gene transfer with AdapoA-I in C57BL/6 mice. IL-6 was below detection (15.6 pg/mL) in both control mice and 6 days after gene transfer with AdapoA-I. High-resolution electrophoresis of serum proteins in C57BL/6 mice at baseline (n = 9) and 6 days after human apo A-I gene transfer (n = 6) showed a 15% (P = 0.0004) decrease of albumin, a 48% (P = 0.0008) increase of α<sub>2</sub>-globulins, and a 13% (P = 0.040) increase of the complement component C₃.

**Discussion**

The main findings of the present study are that (1) PAF-AH, arylesterase, and paraoxonase activities are significantly lower in apo E<sup>−/−</sup> mice than in wild-type C57BL/6 mice, indicating an impaired anti-inflammatory and antioxidative potential of HDL in C57BL/6 apo E<sup>−/−</sup> mice; (2) overexpression of human apo A-I by transgenesis or adenovirus-mediated gene transfer increases HDL-associated PAF-AH activity in both C57BL/6 and C57BL/6 apo E<sup>−/−</sup> mice, and a strong correlation exists between human apo A-I plasma levels and PAF-AH-activity; (3) the arylesterase and paraoxonase activity of PON1 is increased in human apo A-I–transgenic C57BL/6 and apo E<sup>−/−</sup> mice but not in AdapoA-I–treated mice. This decrease is probably related to the inflammatory response induced by E<sub>1</sub>-deleted first-generation adenoviral vectors.

PAF-AH was predominantly associated with HDL, and only a minor fraction of the activity was associated with non-HDL. In contrast, approximately two thirds of PAF-AH activity in humans is associated with LDL and one third with HDL. Recently, Stafforini et al. demonstrated that amino acids 205, 115, and 116 are important for the binding of human PAF-AH to LDL and that the carboxyl terminus of apo B-100 plays a key role in the association of PAF-AH with LDL. When residues 115 and 116 of human PAF-AH were introducted into murine PAF-AH, the mutant murine PAF-AH associated with LDL. Therefore, both the amino acid sequence of murine PAF-AH and low levels of apo B-100 in mice may contribute to the predominant association of murine PAF-AH with HDL.

Human apo A-I overexpression in C57BL/6 transgenic mice and in mice treated with the human apo A-I adenovirus was associated with a relative increase in PAF-AH activity in small HDL compared with large HDL. These small, human apo A-I–containing HDL particles isolated by gel filtration may correspond to the very high density lipoprotein-1 subfraction (VHDL-1) isolated by isopycnic density-gradient ultracentrifugation, which has previously been shown to preferentially bind PAF-AH. In contrast to C57BL/6 control mice, a significant amount of PAF-AH activity in control apo E<sup>−/−</sup> mice was associated with small HDL. It is possible that the absence of apo E in apo E<sup>−/−</sup> mice affects murine PAF-AH distribution and may cause lower PAF-AH activity associated with HDL in these mice.

In contrast to PAF-AH, PON1 was predominantly associated with large-size HDL particles, and only a small fraction of activity was in small-size HDL particles. The association of PON1 with HDL is mediated through the binding of its N-terminal leader sequence to HDL phospholipids and does not involve a direct association with apo A-I, which may contribute to the observed differences between the distribution of PAF-AH and PON1. Arylesterase
and paraoxonase activities increased in human apo A-I–transgenic C57BL/6 and apo E\(^{-/-}\) mice but decreased after human apo A-I gene transfer in C57BL/6 and apo E\(^{-/-}\) mice. This discrepancy may be explained by the production of inflammatory cytokines in the liver after gene transfer with the first-generation E\(_2\)-deleted adenoviral vectors. Paraoxonase activity and PON1 mRNA levels in the liver have indeed been shown to decrease after tumor necrosis factor-\(\alpha\) and IL-1 administration in Syrian hamsters.\(^{38}\) The presence of detectable II-\(\beta\)F in plasma after gene transfer, the decrease of the negative acute-phase response protein albumin, and the increase of \(\alpha_2\)-globulins and complement component C\(_3\) indicate that cytokine production was induced in the liver after gene transfer. Thus, high liver concentrations of cytokines after gene transfer may have resulted in decreased PON1 expression. It remains to be investigated whether human apo A-I overexpression induced by gene transfer with a new-generation, nontoxic adenoviral vector can increase paraoxonase activity in C57BL/6 and apo E\(^{-/-}\) mice.

Previously, Castellani et al\(^{19}\) reported that the PAF-AH and arylesterase activity is similar in HDL isolated by ultracentrifugation from the plasma of C57BL/6 mice and human apo A-I–transgenic mice, at least when data are normalized for total HDL protein. The PAF-AH and arylesterase activity in HDL isolated by gel filtration in the present study represents the total activity, which was not normalized for HDL protein. Thus, our data in C57BL/6 and human apo A-I–transgenic mice are in accordance with those of Castellani et al.\(^{39}\) The present study also demonstrates that PAF-AH and arylesterase/paraoxonase activity in C57BL/6 apo E\(^{-/-}\) mice was significantly lower than in C57BL/6 mice. Decreased paraoxonase activity in apo E\(^{-/-}\) mice has been previously described by Hayek et al.,\(^{40}\) although the extent of decrease (1.4-fold) was lower than in this study (2.0-fold). This may be related to differences in genetic background or in age of the mice.

The increase in PAF-AH and paraoxonase activity in human apo A-I C57BL/6 mice is equal to or above those of C57BL/6 mice may significantly contribute to the inhibition of progression of atherosclerosis.\(^{4,5}\) by restoring an effective anti-inflammatory and antioxidant activity of HDL. Theilmeier et al\(^{41}\) recently demonstrated that human apo A-I transgenesis is associated with reduced oxidative stress in apo E\(^{-/-}\) mice, reduced \(\beta\)-VLDDL–triggered endothelial cytosolic Ca\(^{2+}\) signaling through PAF-like bioactivities, and diminished ex vivo leukocyte adhesion. Furthermore, adenoviral gene transfer of PAF-AH reduced in vivo macrophage homing in the absence of increased HDL cholesterol, indicating the potential physiological significance of elevated PAF-AH activity.

In conclusion, this study provides evidence that overexpression of human apo A-I increases HDL-associated PAF-AH activity. In contrast to higher paraoxonase activity in human apo A-I–transgenic mice, paraoxonase activity after human apo A-I gene transfer decreases, probably due to cytokine-mediated inhibition of PON1 expression. Increased levels of these HDL-associated enzymes may improve the anti-inflammatory and antioxidative potential of HDL and may directly contribute to the protection conferred by HDL against atherothrombosis.

Acknowledgments

This work was supported by the Interuniversitaire Attractiepolen Program (P4/34) and by the Fonds voor Wetenschappelijk Onderzoek-Vlaanderen (program G.0110.98). Bart De Geest is a Postdoctoral Fellow of the Fonds voor Wetenschappelijk Onderzoek-Vlaanderen. These studies were partially supported by INSERM and by a research grant from the Actions Intégrée Franco-Belge “Tournesol.”

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Arterioscler Thromb Vasc Biol. 2000;20:e68-e75
doi: 10.1161/01.ATV.20.10.e68

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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