Human Apolipoproteins A-I and A-II in Cell Cholesterol Efflux Studies With Transgenic Mice

Giulia Chiesa, Cinzia Parolini, Monica Canavesi, Nicoletta Colombo, Cesare R. Sirtori, Remo Fumagalli, Guido Franceschini, Franco Bernini

Abstract—The first step in reverse cholesterol transport is the movement of cholesterol out of cells onto lipoprotein acceptors in the interstitial fluid. The contribution of specific lipoprotein components to this process remains to be established. In this study, the role of human apolipoproteins (apo) A-I and A-II in the efflux of cellular cholesterol was investigated in transgenic mouse models in which the expression of murine apoA-I was abolished due to gene targeting (A-IKO). Serum from A-IKO mice and from mice expressing human apoA-I and/or human apoA-II was incubated with \([3\text{H}]\)cholesterol-labeled Fu5AH rat hepatoma cells for 4 hours at 37°C. The cholesterol efflux to the serum of A-IKO mice was markedly lower than that to the serum of mice transgenic for human apoA-I (5.0±1.5% versus 25.0±4.0%). Expression of human apoA-II alone did not modify the cholesterol efflux capacity of A-IKO mouse serum. Cholesterol efflux to serum of mice expressing human apoA-II together with human apoA-I was significantly lower than that to human apoA-I mouse serum (20.0±2.3% versus 25.0±4.0%). Regression analysis of cholesterol efflux versus the lipid/apolipoprotein concentrations of mouse serum suggested that 3 independent factors contribute to determine the cholesterol efflux potential of serum: the apolipoprotein composition of HDL, the serum concentration of HDL phospholipids, and the presence of a small fraction of particles containing apoA-I. (Arterioscler Thromb Vasc Biol. 1998;18:1417-1423.)

Key Words: reverse cholesterol transport ■ HDL ■ tissue culture cells ■ phospholipids ■ atherosclerosis

Several case-control and prospective studies in different populations have shown that plasma HDL levels are negatively correlated with the incidence of cardiovascular disease.\(^1\) Although the basis of this relationship remains poorly understood, it is widely accepted that the protective role of HDL against atherosclerosis is mostly related to its function in the so-called reverse cholesterol transport, the process by which excess cholesterol is removed from peripheral tissues, including the arterial wall, and transported to the liver for excretion.\(^2,3\)

The efflux of cholesterol from peripheral cells is the first step in reverse cholesterol transport and strongly depends on the presence of an acceptor in the extracellular space.\(^4\) Among extravascular lipoproteins, the smallest HDL particles should be the best acceptors because of the greatest probability of their reaching cell surfaces, consistent with the observation that a minor subfraction of small HDL particles containing apolipoprotein (apo) A-I is mainly involved in the initial phases of cholesterol efflux from cultured fibroblasts.\(^5\) Studies using immunofluorescence chromatography have shown that lipoproteins containing apoA-I without apoA-II (LpA-I) were able to remove excess cholesterol from cholesterol-loaded adipocytes, whereas particles containing both apoA-I and apoA-II (LpA-I:A-II) promoted little or no efflux.\(^6\) However, under different experimental conditions, both LpA-I and LpA-I:A-II particles were effective promoters of cholesterol efflux.\(^7\)

Experiments with reconstituted HDL have shown that several factors, eg, apolipoprotein composition\(^8,9\) and structure,\(^10,11\) phospholipid (PL) composition,\(^12\) and particle size,\(^13,14\) affect the capacity of the acceptor particle for cell cholesterol uptake. Studies with reconstituted HDL are extremely useful for investigating the molecular mechanisms involved in cell cholesterol efflux but provide little insight into the physiology of the whole process. To solve this issue, Rothblat and coworkers (de la Llera Moya et al\(^15\)) developed an assay to evaluate the cholesterol efflux ability of whole serum and investigated the importance of various serum factors in determining cholesterol efflux from cells. When samples from individuals affected with varying degrees of hyperlipidemia were tested, cell cholesterol efflux was best correlated with HDL cholesterol (HDL-C) concentration, and LpA-I demonstrated a greater association with efflux than did LpA-I:A-II.\(^15\)
The development of a large variety of transgenic animals has provided the opportunity to determine how the expression of specific factors influences the ability of serum to promote the efflux of cellular cholesterol. Studies in mice and rats demonstrated the primary role of apoA-I in promoting cholesterol efflux from cells, because the deletion of the apoA-I gene caused a 75% lower cholesterol efflux potential than in control serum, and overexpression of human apoA-I resulted in increased cholesterol efflux. However, these studies highlighted the fact that the HDL component that best reflects serum’s efflux efficiency is not the concentration of apoA-I, but the amount of PLs associated with HDL particles.

In the current study, we have investigated the role of human apoA-I and apoA-II in cell cholesterol efflux by using sera from transgenic mice lacking murine apoA-I, but expressing human apoA-I and/or human apoA-II.

Methods

Animals

All transgenic lines used in this study do not express murine apoA-I. This was achieved by crossing transgenic mice with C57BL/6 mice lacking murine apoA-I (A-IKO) due to gene targeting, kindly provided by Dr Nobuyo Maeda. In particular, human apoA-II/A-IKO (hA-II) and human apoA-I/A-IKO (hA-I) mice were obtained by multiple crosses between transgenic mice expressing human apoA-II or human apoA-I, kindly provided by Dr Edward M. Rubin, and A-IKO mice. hA-II and hA-I mice were bred to each other to obtain hA-II/hA-I/A-IKO (hA-II/hA-I) mice. C57BL/6 mice from Charles River (Milano, Italy) were used as controls. All experiments were performed on 16- to 24-week-old mice of both sexes. Animals in each group were matched for age and sex.

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Lipid/Lipoprotein Analyses

Serum total and unesterified cholesterol and PL levels were determined by enzymatic methods. Triglyceride concentrations as low as 1.5 mg/dL, using sheep antisera against human apoA-II (Hoffmann–La Roche, Basel, Switzerland) and human apoA-I (Boehringer Mannheim, Mannheim, Germany) were observed with murine apoA-II.

Total mouse lipoproteins were isolated by salt gradient ultracentrifugation. Aliquots (500 μL) of pooled sera were adjusted to a density of 1.215 g/mL with solid KBr and centrifuged for 6 hours at 4°C at 100 000 rpm in a Beckman TL100 ultracentrifuge equipped with a Beckman TL100.5 rotor. The isolated lipoproteins were dialyzed against 150 mM NaCl (pH 7.4). Aliquots were loaded onto a 13.5% SDS-polyacrylamide gel and electrophoresed for 2 hours at 80 V (Miniprotein apparatus, Bio-Rad) in the discontinuous buffer system of Shagger and von Jagow. Proteins were stained with Coomassie R-250. The lipoprotein-free fraction (d>1.215 g/mL) was dialyzed against 150 mM NaCl (pH 7.4) to measure the human apolipoprotein content.

HDL particle size distribution was determined by nondenaturing polyacrylamide gradient gel electrophoresis (GGE). Aliquots of the total lipoprotein fraction (d>1.215 g/mL) were loaded onto a nondenaturing 4% to 16% polyacrylamide gradient gel and electrophoresed for 18 hours at 125 V at 4°C. Proteins were stained with Coomassie R-250, and HDL particle size was determined by densitometry (Ultrorcan, LKB), using thyroglobulin (17.1 nm), ferritin (12.2 nm), catalase (10.4 nm), LDH (8.15 nm), and albumin (7.1 nm) as protein standards.

Two-dimensional electrophoresis was carried out as follows: 3 μL of serum was electrophoresed in a Paragon agarose gel (Beckman) according to the manufacturer’s instructions. Electrophoresis in the second dimension was performed on a nondenaturing 3% to 16% polyacrylamide gradient gel, and lipoproteins were then transferred to nitrocellulose membranes (Amersham). The filters were incubated with a sheep antisera against human apoA-I (Hoffmann–La Roche, Basel, Switzerland) followed by a rabbit anti-sheep IgG conjugated with horseradish peroxidase (DAKO, Glostrup, Denmark). Membranes were developed with a chemiluminescence Western blotting detection system kit (Amersham) and exposed to Hyperfilm enhanced chemiluminescence (Amersham film).

Endogenous Cholesterol Esterification in Mouse Plasma

Plasma was collected from individual mice after an overnight fast. One plasma aliquot from each animal was immediately frozen at −20°C, and the remaining aliquots were incubated in a water bath at 37°C. After 2 hours, incubation, aliquots were removed and stored at −20°C, until measurement of free cholesterol by enzymatic method. Results are expressed as a percentage of the initial free cholesterol that was esterified.

Efflux Assay

The cholesterol efflux potential of serum from individual mice was assayed as described by de la Llera Moya et al. by incubating diluted serum with [3H]cholesterol-labeled FuSAH rat hepatoma cells for 4 hours at 37°C. Cells were seeded in Corning 24-well (15.5 mm/well) plates at 20 000 cells per well and grown in DMEM with 5% FCS for 2 days. Lipids were radiolabeled by adding 2 nCi/mL of 1,2-[3H]cholesterol (Amersham) to 25% FCS in DMEM. Cells were grown in the presence of radiolabeled cholesterol for 2 additional days to obtain confluent monolayers. The labeling medium was replaced with DMEM containing 1% essential fatty acid–free albumin for 18 to 20 hours to allow equilibration of the label. Cells were then washed 2 times with PBS and incubated with control medium or serum diluted in DMEM with 1% essential fatty acid–free albumin for 4 hours. At the end of this period, the medium was removed, collected into tubes, and centrifuged for 5 minutes at 2000 rpm to remove any floating cells. An aliquot of the medium was then counted for [3H]cholesterol radioactivity (Formula 989, Packard). Cellular lipids were extracted with 2-propanol by overnight incubation at room temperature, and radioactivity was measured in an aliquot of the extract (Insta-Fluor, Packard). The fractional cholesterol efflux was calculated as the amount of label released to the medium divided by the total label in each well. In preliminary experiments, efflux assays were performed at different time points (0.5 to 4 hours) and with different dilutions of serum from each group of mice (ranging between 0.625% and 5%), and the efflux was found proportional to both time and dilution used; the efflux assays were then performed for 4 hours with 2.5% serum dilution. All efflux values are reported as the average of 3 determinations in different wells. Human and murine serum pools (1 of each) were included in each assay. The relative efflux of the human
to the murine pool was quite constant in each experiment (1.43 ± 0.05; coefficient of variation 3.5%). The results obtained in different experiments were normalized by relating them to the average efflux obtained with the murine serum pool.

### Statistical Analyses

Results are reported as mean ± SD, if not otherwise stated. Group differences in continuous variables were determined by ANOVA. Linear correlation coefficients were used to describe relations between fractional efflux and serum lipid and lipoprotein values. Regression lines for different data sets were compared by testing the identity of the slopes using multiple regression analysis. Group differences or correlations with \( P < 0.05 \) were considered statistically significant.

### Results

#### Serum Lipid and Lipoprotein Profile

Deletion of the mouse apoA-I gene in A-IKO mice resulted in extremely low serum cholesterol levels (Table 1), as already described; HDL cholesterol and PL concentrations were also markedly low. Expression of human apoA-II in the A-IKO background resulted in serum apoA-II levels (Table 1) similar to those usually reported in human serum. Total cholesterol concentrations were similar to those of A-IKO mice (Table 1). Serum unesterified cholesterol levels were significantly higher in hA-II than A-IKO mice, leading to an increased unesterified/esterified cholesterol ratio (0.75 ± 0.14 versus 0.51 ± 0.16; \( P < 0.01 \)). Endogenous cholesterol esterification, assayed on plasma from 5 hA-II and 5 A-IKO mice, was remarkably lower in hA-II than A-IKO mice (12.0 ± 5.3% versus 29.4 ± 1.6%, respectively). Serum triglycerides were also significantly elevated in hA-II compared with A-IKO mice (Table 1). HDL-C and HDL-PL concentrations were not significantly different between A-IKO and hA-II mouse sera. In hA-II mice, HDL-PL concentrations were positively and significantly correlated with human apoA-II levels (\( r = 0.87 \)) (Figure 1A). A significant correlation was also observed with HDL-C levels (\( r = 0.80 \)). The human apoA-II content in the lipoprotein-free fraction (\( d > 1.215 \text{ mg/mL} \)) was <6% of the total serum apolipoprotein. The expression of human apoA-II in the A-IKO background resulted in an almost complete disappearance of murine apoA-II from serum (Figure 2); moreover, the serum content of other apolipoproteins, apoA-IV and apoE, was reduced significantly. A GGE analysis of HDL particle distribution (Figure 3A) disclosed a dramatic difference between hA-II and A-IKO background mice; the latter showed a predominance of large particles, with a diameter ranging between 10.0 and 13.0 nm, whereas hA-II mice displayed a single population of small HDL particles, with a diameter of 7.5 nm.

The serum lipid profile of hA-I and hA-II/hA-I mice is shown in Table 1. To avoid interference due to variations in serum human apoA-I levels between the 2 groups, studies were performed on 10 hA-I and 10 hA-II/hA-I mice matched for serum apoA-I concentration. As expected, the lipid profile of hA-I and hA-II/hA-I mice was very different from that of

### Table 1. Lipid and Apolipoprotein Levels of A-IKO, hA-II, hA-I, and hA-II/hA-I Mouse Serum

<table>
<thead>
<tr>
<th>Mouse Line</th>
<th>A-IKO</th>
<th>hA-II</th>
<th>hA-I</th>
<th>hA-II/hA-I</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>8</td>
<td>7</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>19.8 ± 6.7</td>
<td>23.7 ± 4.7</td>
<td>125.2 ± 40.7</td>
<td>122.9 ± 15.9</td>
</tr>
<tr>
<td>Unesterified cholesterol, mg/dL</td>
<td>6.5 ± 2.3</td>
<td>10.1 ± 1.8*</td>
<td>35.6 ± 12.3</td>
<td>30.5 ± 5.6</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>10.4 ± 2.7</td>
<td>18.5 ± 8.8†</td>
<td>34.1 ± 14.1</td>
<td>33.2 ± 11.4</td>
</tr>
<tr>
<td>HDL-C, mg/dL</td>
<td>10.5 ± 6.6</td>
<td>10.3 ± 2.3</td>
<td>111.9 ± 37.0</td>
<td>111.0 ± 14.8</td>
</tr>
<tr>
<td>HDL-PL, mg/dL</td>
<td>41.8 ± 15.8</td>
<td>47.4 ± 7.9</td>
<td>187.0 ± 56.4</td>
<td>152.0 ± 50.6</td>
</tr>
<tr>
<td>hA-I, mg/dL</td>
<td>. . .</td>
<td>. . .</td>
<td>268.2 ± 76.9</td>
<td>270.9 ± 63.2</td>
</tr>
<tr>
<td>hAII, mg/dL</td>
<td>. . .</td>
<td>41.5 ± 9.1</td>
<td>. . .</td>
<td>137.1 ± 31.6</td>
</tr>
</tbody>
</table>

Table 1. Lipid and Apolipoprotein Levels of A-IKO, hA-II, hA-I, and hA-II/hA-I Mouse Serum

Data are mean ± SD. *\( P < 0.01 \) versus A-IKO; † \( P < 0.05 \) versus A-IKO.
A-IKO mice and was characterized by elevated total and HDL cholesterol levels (Table 1). The mean serum total and HDL cholesterol levels were similar in hA-I and hA-II/hA-I mice. HDL-PL concentrations were lower in hA-II/hA-I than in hA-I mice, but the difference did not reach statistical significance (P=0.16). In both mouse lines, HDL-PL concentrations were positively and significantly correlated with human apoA-I (r=0.77 in hA-I and r=0.83 in hA-II/hA-I mice) (Figure 1B). No correlation was observed between HDL-PL and human apoA-II in hA-II/hA-I mice (Figure 1A). Similarly, HDL-C levels were significantly correlated with human apoA-I concentrations in both groups (r=0.73 in hA-I and r=0.84 in hA-II/hA-I mice) but were not correlated with human apoA-II in hA-II/hA-I mice. The amount of human apolipoproteins in the lipoprotein-free fraction was <7% of the serum apolipoprotein levels. The apolipoprotein distribution in serum from hA-II/hA-I and hA-I mice (Figure 2) was very close, other than the presence of human apoA-II in the former, where the expression of the human apolipoprotein did not affect the serum content of murine apoA-II. Both hA-II/

hA-I and hA-I mice displayed a heterogeneous distribution of HDL particles, migrating within the same size range (Figure 3B). The GGE profiles of hA-II/hA-I and hA-I mice were characterized by a major HDL subpopulation, with diameters of 9.5 and 9.8 nm, respectively, a “shoulder” corresponding to larger HDL particles (10.3 nm in diameter), and a subpopulation of small particles with a diameter of 8.6 nm. The distribution of the various particles in the 2 transgenic lines was very close (Figure 3B). hA-II/hA-I and hA-I mouse sera were also analyzed for the presence of apoA-I–containing lipoprotein particles of pre-β mobility, considered the first acceptors of cellular cholesterol.16 Two-dimensional electrophoresis, followed by exposure to a specific anti-human apoA-I antibody, disclosed the presence of significant amounts of pre-β particles in both groups (Figure 4).

Cholesterol Efflux to Mouse Sera

The sera from A-IKO mice showed a marked reduction in the average fractional efflux compared with that of control mice (5.0±1.5% versus 20.2±1.9%), ie, consistent with previous findings in similar animals.16 The additional expression of human apoA-II into the A-IKO background did not affect fractional cholesterol efflux (5.0±0.8%) (Figure 5). By contrast, the mean fractional efflux to sera from the 2 transgenic lines expressing human apoA-I was much higher than sera from hA-II and A-IKO background mice; efflux to hA-II/hA-I sera was, however, 20% lower than efflux to hA-I sera (20.0±2.3% versus 25.0±4.0%; P<0.005) (Figure 5). Correlations between cholesterol efflux and serum apolipoprotein/lipid levels in the 4 mouse lines are shown in Table 2. The cholesterol efflux to hA-II mouse sera was strongly and positively correlated with serum concentrations of human apoA-II, whereas no significant correlation was observed for apoA-II in hA-II/hA-I mice. In both lines expressing human apoA-I, cholesterol efflux was strongly and positively corre-
lated with apoA-I levels. In all mouse lines, a significant linear correlation was found between fractional cholesterol efflux and serum HDL-PL or HDL-C levels. No significant differences were observed among the slopes of the regression lines in the 4 groups; however, when human apoA-I–expressing mice (hA-I and hA-II/hA-I) were compared with nonexpressers (A-IKO and hA-II), the regression lines between cholesterol efflux and HDL-PL displayed a significantly different intercept value (11.82% versus 1.54%, respectively) (Figure 6).

Discussion

The lipid/lipoprotein profile of A-IKO mice, which represents the genetic background of all mouse lines used in this study, is characterized by dramatically low HDL-C levels and by the appearance of large HDL particles not observed in other mouse lines, therefore confirming previous observations in similar animals. The expression of human apoA-II into a murine apoA-IKO background has not been described previously. The same human apoA-II transgene, when expressed into a normal mouse background, had no relevant effect on serum lipid and lipoprotein levels, murine apoA-I and apoA-II concentrations, or HDL particle size distribution. In the absence of murine apoA-I, we observed that the expression of human apoA-II caused significant differences in the serum lipid/lipoprotein profile, compared with that of background mice, probably consequent to changes in the apolipoprotein pattern (Table 1, Figures 2 and 3). A strong reduction in mouse apoA-II was observed in hA-II transgenic serum. This effect may be explained by a lower lipid-binding capacity of murine versus human apoA-II, resulting in a displacement of the former from HDL particles and consequent accelerated catabolism. The apoE content of hA-II mouse serum is also reduced, possibly because of the HDL size restriction induced by hA-II expression, with a decrease of apoE-containing HDL. Despite the dramatic changes in HDL particle size and apolipoprotein composition, HDL-PL and HDL-C levels were not modified by human apoA-II expression. By contrast, the serum concentrations of unesterified cholesterol were increased significantly in hA-II mice, a result consistent with the lower cholesterol esterification efficiency of hA-II mouse plasma. This may be due to a reduced ability of human apoA-II in activating lecithin:cholesterol acyl transferase compared with murine apoA-II or apoA-IV, whose serum content is also reduced in hA-II compared with A-IKO mice.

The expression of human apoA-II into a human apoA-I transgenic mouse background, instead, does not substantially change the apolipoprotein distribution of mouse lipoproteins, including the serum content of mouse apoA-II. This confirms previous observations that in the presence of either mouse or human apoA-I, human apoA-II does not modify murine apolipoprotein levels. This is probably due to the elevated serum HDL concentrations in human apoA-I–expressing mice, which allow the association with lipids of apolipoproteins with even low binding affinity. The HDL particle size distribution of hA-II/hA-I and hA-I mice observed in this study is similar to that of mice expressing the same human

### Table 2

<table>
<thead>
<tr>
<th>Mouse Line</th>
<th>hA-II</th>
<th>HDL-PL</th>
<th>hA-I</th>
<th>HDL-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-IKO</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>hA-II</td>
<td>0.94</td>
<td>0.002</td>
<td>0.09</td>
<td>0.001</td>
</tr>
<tr>
<td>hA-I</td>
<td>...</td>
<td>0.007</td>
<td>0.04</td>
<td>0.001</td>
</tr>
<tr>
<td>hA-II/hA-I</td>
<td>0.59</td>
<td>0.093</td>
<td>0.04</td>
<td>0.001</td>
</tr>
</tbody>
</table>

**Figure 5.** Percentage efflux of labeled cholesterol from Fu5AH hepatoma cells incubated for 4 hours at 37°C with 2.5% dilution of serum from genetically engineered mice. Each data point represents an individual animal, and the value is the average of triplicate determinations. Line illustrates average percentage efflux for each group of animals.

**Figure 6.** Relationship between percentage cholesterol efflux from Fu5AH hepatoma cells and HDL-PL levels in genetically engineered mice. Percentage efflux was measured by using standard assay conditions (2.5% dilution of serum, 4 hours, 37°C). Dashed line represents linear regression observed in human apoA-I expressing mice (● indicates hA-I; ○, hA-II/hA-I mice); solid line represents linear regression in mice not expressing human apoA-I (□ indicates A-IKO; †, hA-II mice).
apolipoproteins into a normal background, where mouse apoA-I levels are dramatically downregulated by human apoA-I expression.20

In the present study, cell cholesterol efflux was measured as originally described by de la Llera Moya et al15 for human serum; the same authors subsequently applied this approach to transgenic animal studies.16,17 The assay measures the release of radiolabeled cholesterol from cells, but it does not quantify the net movement of cholesterol between cells and serum lipoproteins. However, this method provides clear indications on cholesterol release from cells to extracellular acceptors, allowing the establishment of correlations between cell cholesterol efflux and the different apolipoproteins and lipoproteins in serum.

The cholesterol efflux to serum of mice lacking apoA-I was markedly lower than that to serum of mice expressing human apoA-I, therefore confirming the determinant role of this apolipoprotein as a mediator of cellular cholesterol efflux to serum.6,21 Because cholesterol efflux is strongly correlated with serum HDL-PL concentrations (Table 2), which in turn are correlated with the concentrations of apoA-I (Figure 1), the role played by apoA-I in cholesterol efflux apparently is strongly linked to its ability to increase HDL-PL levels, as supported by Fournier et al.17 Therefore, the HDL-PL concentration is a major determinant of the serum cholesterol efflux potential. In agreement with this hypothesis is the observed correlation between efflux and HDL-PL in A-IKO and hA-II mice, which lack apoA-I (Figure 6). Although such results fix the main role of HDL-PL as a determinant of serum efflux potential, our study highlights other factors, not related to HDL-PL, playing an important role in such a process.

A major finding arises from the observation that, when corrected for serum HDL-PL concentration, cholesterol efflux was higher in mice expressing human apoA-I compared with nonexpressers (0.142±0.033 versus 0.116±0.017; \( P=0.01 \)), indicating that at every HDL-PL concentration, the efflux potential of sera from mice expressing human apoA-I (with or without human apoA-II) is higher than that of mouse sera without apoA-I (both from hA-II and A-IKO mice). Such increased efflux potential cannot be explained by a higher efficiency of HDL particles containing apoA-I versus those lacking apoA-I. Indeed, the slopes of the correlation lines between cholesterol efflux and HDL-PL in the different groups of animals with and without apoA-I are similar. By contrast, the intercept value, which represents cholesterol efflux independent of HDL-PL, is 8-fold higher in animals expressing human apoA-I than in those without apoA-I (Figure 6). This suggests that the enhanced efflux potential in the former involves a particle containing apoA-I, which contributes little to the serum concentration of HDL-PL but displays a high capacity for cholesterol efflux. This particle apparently is not present in sera of animals lacking apoA-I, where the intercept is near zero, suggesting an almost complete dependence of cholesterol efflux potential on HDL-PL. The nature of this particle with elevated efflux potential was not investigated in this study. However, one can hypothesize that this particle is the same as the small HDL, containing apoA-I as the sole apolipoprotein component and with pre-\( \beta \) mobility, originally described by Castro and Fielding,5 which is present at low concentrations in human serum but is endowed with a very potent efflux activity. Consistent with this hypothesis, we identified pre-\( \beta \) HDL in significant amounts in human apoA-I–expressing mice, a result previously reported with similar animals.16,22 Interestingly, the HDL particles of the hA-II mice described in the present study have a size similar to that of apoA-I–containing pre-\( \beta \) HDL, but the cholesterol efflux capacity of sera from these animals was similar to that of A-IKO background mice, in which very large HDL particles were observed. This finding indicates that apolipoprotein composition and particle size differently and independently modulate the cholesterol efflux capacity of HDL not only in well-defined reconstituted systems (Guido Franceschini, PhD, unpublished data, 1997), but also in a more physiological experimental setup.

A second major observation from this study indicates that in an in vivo system, the concomitant expression of human apoA-II has an important effect on apoA-I–mediated cholesterol efflux, which is independent of changes in serum HDL-PL concentrations. This observation is in agreement with the results of studies with reconstituted HDL as cell cholesterol acceptors, which demonstrate an impaired efflux capacity of particles containing apoA-II and apoA-I compared with particles with apoA-I alone.6 Altogether, these results indicate that apoA-II, in the presence of apoA-I, has a negative effect on the cholesterol efflux potential of serum. In transgenic mice, the presence of apoA-II on HDL diminishes the antiatherogenic effect of apoA-I,25 consistent with the protective role of HDL containing only apoA-I (LpA-I), but not of those with apoA-I and apoA-II (LpA-I:A-II), against arterial disease in humans.33 The observed effect of apoA-II on apoA-I–mediated cholesterol efflux might explain this putative proatherogenic role of human apoA-II.

In conclusion, the present results suggest that 3 independent factors play a role in determining cholesterol efflux potential of serum: the apolipoprotein composition of HDL, with human apoA-II negatively modulating the effect of apoA-I on cholesterol efflux from cells; the concentration of HDL PL; and the presence of a quantitatively small fraction, but with high efflux potential, of particles containing apoA-I.

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References


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