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ApoE Induces Serum Paraoxonase PON1 Activity and Stability Similar to ApoA-I[†]

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ABSTRACT: Serum paraoxonase (PON1) is an anti-atherogenic interfacially activated lipo-lactonase that was shown to selectively bind high-density lipoprotein (HDL) carrying apolipoprotein A-I (apoA-I). ApoA-I binding occurs with nanomolar affinity and induces a dramatic increase in enzyme stability and lactonase activity. This study examined the association of PON1 with reconstituted HDL (rHDL) carrying apolipoprotein E, and its consequences on the stability and enzymatic activity of PON1, and on its anti-atherogenic potential. The results indicate that reconstituted HDL particles prepared with two most common isoforms of apoE (apoE3 and apoE4) associate with rePON1 in a manner and affinity similar to those of apoA-I. Binding to apoE-HDL stimulates the lactonase activity and stabilizes the enzyme, although the latter occurs to a >10-fold lesser extent compared to apoA-I-HDL particles. The anti-atherogenic potential of PON1, measured by inhibition of LDL oxidation and stimulation of macrophage cholesterol efflux, was also stimulated by apoE-HDL, at levels of 40−96% compared to apoA-I-HDL. Overall, reconstituted apoE-HDL exhibits properties similar to those of apoA-I-HDL, but with a lower capacity to stabilize PON1 and to induce its anti-atherogenic functions. ApoE, apoA-I, and to a lesser degree apoA-IV show distinct structural and functional similarities but little sequence homology. That these apolipoproteins, but not apoA-II, bind PON1 with high affinity and stimulate its activity suggests that PON1−HDL recognition is based primarily on surface properties of the apolipoproteins and that specific protein−protein interactions may play only a secondary role.

Human serum paraoxonases (PONs) are HDL-associated enzymes with a wide range of hydrolytic activities such as lactonase, arylesterase, and organophosphate hydrolase. The PON family consists of three genes, PON1,[1] PON2, and PON3, with ∼65% identity at the amino acid level. PON1, and to a lesser degree PON3, are expressed in the liver and are primarily found bound to plasma HDL (*1*, *2*). PON1 has been isolated from human plasma in association with HDL-containing apolipoproteins A-I, A-II, and J (*3*−*5*). Indeed, apoA-I is the major protein constituent of HDL, and PON1 in association with apoA-I-containing HDL exhibits various anti-atherogenic effects (*6*, *7*). In vivo, there is a positive correlation between the association of PON1 with apoA-I and its enzymatic activities. The arylesterase activity of PON was reported to be associated mostly with either apoA-I or apoE containing HDL (*8*). Indeed, in vitro, PON1 was shown to bind apoA-I-HDL with nanomolar affinity. Binding to apoA-I-HDL significantly stabilizes PON1 and stimulates the lactonase activity that is presumed to be the primary enzymatic function of PON1 (*9*, *10*). PON1 incorporated into apoA-I-HDL also inhibits LDL oxidation and increases the HDL-mediated cholesterol efflux (*11*). These potentially anti-atherogenic functions of PON1 appear to be mediated by the lipolactonase activity of the enzyme (*12*) which in in vitro and ex vivo assays was shown to be selectively stimulated by apoA-I-HDL, and not by other apolipoproteins such as apoA-II (*13*).

In contrast to the in vitro results, PON1 has also been shown to be associated with serum HDL particles isolated from human plasma containing both apoA-I and apoA-II, with higher stability of PON1 and stronger resistance to inactivation by oxidation (*5*). Thus, the complexity of HDL composition, and the range of effects exerted by various apolipoproteins, considerably complicate the analysis of plasma particles. Indeed, HDLs are heterogeneous in size and density and are subject to substantial compositional variations under both normal and pathological conditions (*14*, *15*). In addition to apoA-I and A-II, apoEs comprise another type of soluble apolipoproteins that appear in plasma HDLs at a fraction of ∼10% (*16*, *17*). There are three common isoforms of apoE (apoE2, apoE3, and apoE4) that differ by cysteine and arginine content at positions 112 and 158 (*18*, *19*). ApoE3 (Cys112, Arg158) is the most common isoform of apoE and is primarily found on smaller HDLs, whereas apoE4 (Arg112, Arg158) preferentially binds to large LDL particles (*20*). ApoE participates in the clearance of

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[1]Abbreviations: apoA-I, apolipoprotein A-I; apoE, apolipoprotein E; BSA, bovine serum albumin; ELISA, enzyme-linked immunosorbent assay; FC, free (unesterified) cholesterol; HDL, high-density lipoprotein; rHDL, reconstituted HDL; PBS, phosphate-buffered saline; POPC, 1-palmitoyl-2-oleoylphosphatidylcholine; PON1, serum paraoxonase; rePON1, recombinant PON1; SD, standard deviation; TBARS, thiobarbituric acid-reactive substance; TBBL, 5-thiobutyl butyrolactone; TBS, Tris-buffered saline.

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lipoprotein remnants of intestinal or hepatic origin by the liver (*21*−*23*). ApoE was shown to promote cholesterol efflux ex vivo and maintains lipid homeostasis and thus may exhibit anti-atherogenic functions (*24*−*28*). In contrast to the proposed protective role of apoE3, apoE4 is known to be associated with a higher risk of cardiovascular disease and of neurodegenerative disease in particular. Although or perhaps because it comprises only a minor constituent of HDL under physiological conditions, apoE is also a candidate for therapeutical interventions because of its anti-atherogenic potential (*29*).

As opposed to apoA-I, very little is known about apoE and its association with PON1, and how the latter could affect the anti-atherogenic potential. In this study, we analyzed the association of reconstituted HDL particles carrying anti- or pro-atherogenic variants of apoE (apoE3 or -E4, respectively) with PON1, and the effects of apoE-rHDL binding on the stability, enzymatic activity, and anti-atherogenic potential of PON1.

MATERIALS AND METHODS

Preparation of rHDL Particles. Recombinant PON1 variant rePON1-G3C9 (gi 40850544) and its variant that lacks the N-terminal helix (Δ20-rePON1) were used in this study. rePON1-G3C9 has an intact N-terminus that is essentially identical in sequence and length to rabbit and human PON1s. Both G3C9 and the truncated version carry an eight-His tag at the C-terminus. This tag has no effect on PON1's enzymatic activity and HDL binding. The rePON1 variants were expressed and purified as described previously (*13*). The human apoA-I expression plasmid kindly provided by M. Oda (Oakland Research Institute, Oakland, CA) (*30*) was expressed in *Escherichia coli* as described previously (*13*). rHDL-apoA-I particles were prepared by the cholate dialysis method as previously described (*13*). Human apoE was expressed from the pET20b vector kindly provided by V. I. Zannis (Boston University School of Medicine, Boston, MA) (*31*) and subsequently purified as described previously (*32*). Discoidal rHDL particles containing 1-palmitoyl-2-oleoylphosphatidylcholine (POPC), free cholesterol (FC), and apolipoprotein (apoA-I, apoE3, or apoE4), at a starting molar ratio of 100/5/1, were prepared by the cholate dialysis method as previously described (*13*). The final apolipoprotein concentration was 0.5 mg/mL, and the rePON1 concentration was 0.2 mg/mL.

ELISA. The binding of rePON1 to rHDL was analyzed by an enzyme-linked immunosorbent assay (ELISA). Briefly, 96-well plates (Nunc MaxiSorp) were coated via incubation of 10 μg/mL apoA-I−, apoE3−, apoE4−, or POPC/FC−rHDL for 3 h at room temperature, followed by blocking with 10 mg/mL bovine serum albumin (BSA) in phosphate-buffered saline (PBS; BSA/PBS). rePON1 was diluted in PBS and added at different concentrations, and plates were incubated at 37 °C for 3 h. After the plates had been washed with 0.1% CHAPS in BSA/PBS, anti-PON1 polyclonal serum (1:4000 in BSA/PBS) was added, followed by peroxidase-linked goat anti-mouse antibody. Plates were developed using 3,3′,5,5′-tetramethylbenzidine (TMB) substrate chromogen (Dako). The reaction was stopped by addition of 1 M H_2SO_4, and the optical density was measured at 450 nm.

Stimulation of rePON1 Activity by rHDL. PON1 enzymatic stimulation by rHDL was assayed with freshly delipidated rePON1 as described previously (*33*). Briefly, rePON1 (0.1 μM) was delipidated using Bio-Beads SM-2 (Bio-Rad) and incubated with rHDLs (10 μM) at 37 °C for 3 h. The esterase and lactonase activities were assayed using phenyl acetate and 5-thiobutyl butyrolactone (TBBL), respectively, and compared to the activity of the same concentration of delipidated rePON1 in buffer.

Inactivation of rePON1 by Calcium Chelation. Freshly delipidated rePON1 (0.1 μM) was incubated with rHDL samples (10 μM) for 2 h at 37 °C in TBS [10 mM Tris (pH 8.0) and 150 mM NaCl] supplemented with 1 mM $CaCl_2$. An equal volume of inactivation buffer [3 mM EDTA with 6 mM β-mercaptoethanol in 50 mM Tris (pH 8.0)] was added to the enzyme samples. Aliquots of these inactivation reaction mixtures were taken at different time points, and the residual PON1 activity was determined with 2 mM phenyl acetate as described previously (*13*, *33*).

Human LDL and HDL Preparation. LDL and HDL were prepared from human serum of fasted normolipidemic volunteers by discontinuous density gradient ultracentrifugation (*34*). The HDLs were then dialyzed against 150 mM NaCl with 1 mM $CaCl_2$ (pH 7.4), and their protein content was determined with the Folin phenol reagent.

Copper Ion-Induced LDL Oxidation. LDL (1 mg/mL) was dialyzed against PBS prior to the oxidation studies. J774A.1 macrophages were incubated with LDL (100 μg of protein/mL) in the absence (control LDL) or presence of 50 μg of rHDL (based on protein determination) composed of apoA-I, apoE3, apoE4, or POPC/FC only, in the absence or presence of rePON1 (20 μg) in a total volume of 0.5 mL of RPMI (without phenol red) (*11*). A similar volume was used for the POPC/FC−rePON1 sample. To initiate oxidation, 5 μM $CuSO_4$ was added to the LDL samples, and the samples were further incubated for 2.5 h at 37 °C. Samples were incubated under the same conditions in a cell-free system. At the end of the incubation period, the extent of LDL oxidation was determined by the thiobarbituric acid-reactive substance (TBARS) assay (*35*).

HDL-Mediated Cholesterol Efflux from J774A.1 Macrophages. J774A.1 cells (1×10^6 per milliliter) were labeled with [^{3}H]cholesterol for 1 h at 37 °C (*11*). The cells were washed and further incubated with no addition, or with the addition of rHDL (50 μg of protein/mL) composed of apoA-I, apoE3, apoE4, or POPC/FC only, in the absence or presence of rePON1 (20 μg/mL). Cellular and medium ^{3}H labels were quantified, and HDL-mediated cholesterol efflux was calculated as the ratio of the ^{3}H label in the medium to the sum of the ^{3}H label in the medium and the ^{3}H label in the cells.

RESULTS

Interaction of PON1 with ApoE-rHDL. The association of reconstituted HDL particles containing POPC, free cholesterol, and apoE3 (the most common apoE isoform) or apoE4 (the rare form) with rePON1 was analyzed with an ELISA. In this experiment, and all the experiments described below, we used recombinant apolipoproteins and a recombinant form of PON1. The latter (rePON1) was engineered for bacterial expression and is highly homologous in sequence to rabbit and human PON1s (*36*). Its enzymatic and HDL binding properties and anti-atherogenic properties were also shown to reproduce those of human PON1 (*10*, *11*, *13*).

The results indicated that HDL particles carrying either apoE3 or E4 bind rePON1 with an affinity that is much stronger than that of POPC/FC with no apolipoprotein. Further, the binding affinity is comparable to that of apoA-I-rHDL (Figure 1). The ELISA results indicate 50% binding to apoA-I-rHDL at ~1 nM PON1 and are therefore in agreement with surface plasmon

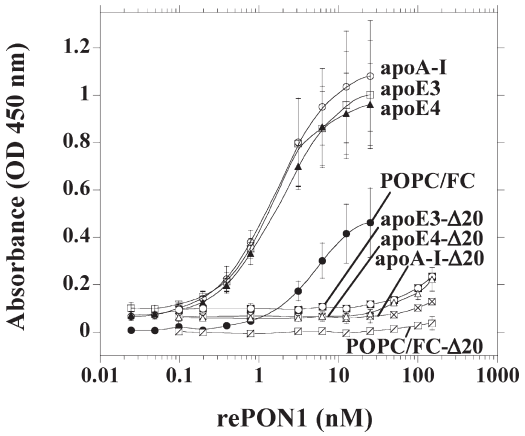

FIGURE 1: Binding of PON1 to HDL. ELISA measurements of the association of rePON1 and the truncated Δ20-rePON1 variant with rHDL composed of apoA-I, apoE3, apoE4, or no apolipoprotein (POPC/FC). Plates were coated with various HDL samples, and rePON1 and Δ20-rePON1 were added at different concentrations. Binding was detected with polyclonal anti-PON1 sera. Each binding curve represents the mean ± SD of at least three independent experiments.

resonance binding studies that showed PON1 binds apoA-I-rHDL particles with an affinity of ≤0.75 nM (*13*). The ELISA results also indicate that removal of the N-terminus of PON1 reduces the binding affinity by ≥100-fold, as previously observed for apoA-I-rHDL (the measured affinity for Δ20-rePON1 binding to apoA-I-rHDL is 1.0×10^{-7} M) (*13*, *33*). The same degree of reduction in binding upon removal of the N-terminus is observed for apoEs, suggesting that the binding affinity and mode of binding are the same as for apoA-I.

Stimulation of Enzymatic Activity. The effect of various rHDL preparations on the lactonase and arylesterase activities of PON1 was analyzed by incubating the delipidated enzyme with varying concentrations of rHDL (Figure 2A,B). The association of rePON1 with apoE3- and apoE4-rHDL stimulated both the lactonase and esterase activities of rePON1, by 11.4- and 12.5-fold for apoE3 and 12.3- and 11.7-fold for apoE4, respectively. The stimulation effect is specific to the apolipoproteins and significantly greater than that observed with POPC/FC (by 1.9- and 1.75-fold, respectively), in a manner similar to that of apoA-I-rHDL (*13*).

Stability of PON1−HDL Complexes. PON1 is a calcium-dependent enzyme that possesses a high-affinity structural calcium and a low-affinity catalytic calcium. The removal of the structural calcium ion by chelators irreversibly denatures the enzyme. Previous studies from this lab have established that PON1 undergoes rapid inactivation in the presence of EDTA, whereas slow inactivation was observed when the enzyme was incorporated into rHDL particles containing apoA-I (*13*, *33*). The association of rePON1 with rHDL containing different apolipoproteins was therefore tested by monitoring its inactivation rate in the presence of EDTA (Figure 3). As previously reported, apoA-I-rHDL-associated PON1 exhibited a mono-exponential curve corresponding to a single form of a tightly bound and stable PON1−HDL complex. Delipidated rePON1 in the absence of HDL behaved like POPC/FC-bound PON1 and exhibited a large fraction [~50% (Table 1)] of a loosely bound, unstable form that exhibits a rapid inactivation rate (*13*, *33*). Complexes of PON1 with apoE3- or apoE4-rHDL also exhibited two inactivation phases indicating a mixture of the two

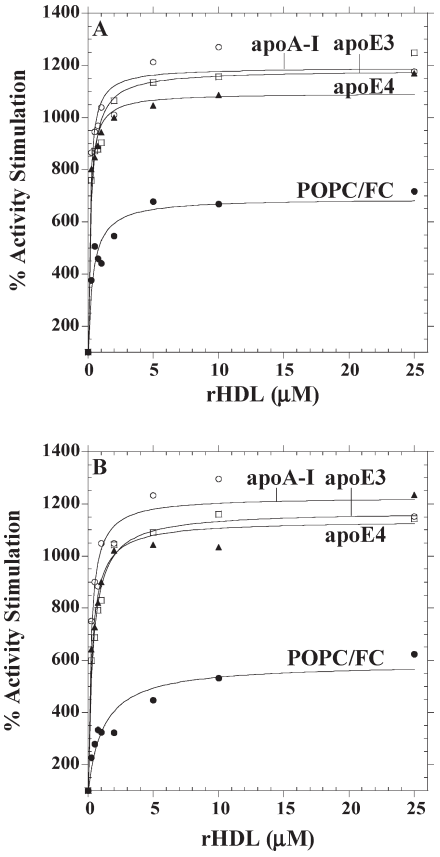

FIGURE 2: Stimulation of the enzymatic activity of PON1 by HDL. Delipidated rePON1 was incubated with increasing concentrations of different rHDLs at rHDL/rePON1 molar ratios of 2.5−250. The initial rates of esterase and lactonase hydrolysis were measured with phenyl acetate (A) and TBBL (B), respectively (using substrate concentrations of 1 mM). Activity is presented as the percentage of the initial activity relative to the delipidated enzyme assigned as 100%. Data were fitted to Langmuir saturation curve, and the activation factor (V_{max}) and apparent K_d values were derived (*13*). Each curve represents the mean of at least three independent experiments. Standard deviations were less than 10% of the parameter values.

forms, with 33 or 20% of the loosely bound, unstable form, respectively.

The rate of PON1 inactivation therefore provides a measure of the effect of HDL binding on the enzyme's configurational stability. The double-exponential pattern obtained with saturating concentrations of the apoE variants indicates the presence of the loosely bound unstable form of the enzyme along with the more stable form (Table 1). The tightly bound, stable form of PON1 (slow inactivation phase) was significantly larger in apoE4-HDL (80%) as compared to apoE3-HDL (67%), but much lower than for apoA-I-HDL (~100%).

Anti-Atherogenic Functions. We performed two ex vivo tests aimed at assessing the anti-atherogenic potential of PON1−rHDL complexes containing apoEs and comparing them to the routinely tested apoA-I-rHDL. As a control, we also tested the anti-atherogenic activity of rHDL composed of apoA-I, apoEs, or no apolipoprotein (POPC/FC), in the absence of PON1. The first test examined the inhibition of copper ion-induced LDL oxidation (Figure 4). The addition of POPC/FC to LDL resulted in a very mild inhibition of its oxidation (~5%). Slightly greater inhibition (4.5%) was observed when rePON1 was added to POPC/FC (Figure 4). The extent of LDL oxidation inhibition increased to ~10% with apoE4-rHDL and to ~15%

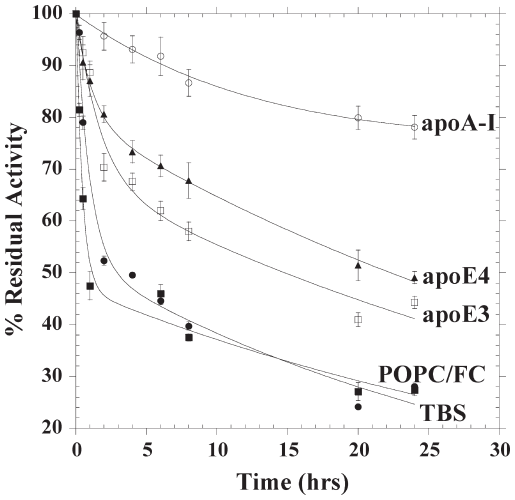

FIGURE 3: Rates of PON1 inactivation in the presence of different HDL types. Delipidated rePON1 was incubated with a 100-fold excess of rHDL composed of apoA-I, apoE3, apoE4, or POPC/FC only, or in TBS buffer, and subjected to inactivation in the presence of EDTA (3 mM) and β-mercaptoethanol (6 mM) at 37 °C. Residual activity at various time points was determined by initial rates of phenyl acetate hydrolysis (2 mM) and plotted as a percentage of the activity at time zero. Data were fitted to a double-exponential curve, from which the amplitudes of the two phases were derived (Table 1) (*13*). Each curve represents the mean and SD of at least three independent experiments.

Table 1: Inactivation Parameters of rePON1 in Buffer and on rHDL Particles[a]

sample	A_1 (fast phase)
delipidated rePON1	0.5
POPC/FC−rePON1	0.54
apoA-I-rHDL−rePON1	0.02
apoE3-rHDL−rePON1	0.33
apoE4-rHDL−rePON1	0.20

[a]Amplitudes of the first [fast (A_1)] and second [slow (A_2)] inactivation phases were derived by fitting the kinetics of rePON1 inactivation by the calcium chelator EDTA to a double-exponential curve (Figure 3). A_1 and A_2 correspond to the loosely-bound unstable form and tightly-bound stable form of PON1, respectively ($A_1 + A_2 = 1$). Each value represents the mean of three independent experiments. Standard deviations were less then 10% of parameter values.

with apoA-I or apoE3-rHDL. However, when PON1 was added to apoA-I-rHDL, the extent of inhibition of LDL oxidation was further increased by 20% as compared to that with apoA-I-rHDL alone. The addition of PON1 to apoE3-rHDL or apoE4-rHDL resulted in a 13.5 or 14.5% increase in the level of inhibition of oxidation, respectively, relative to the same HDL particles with no PON1 (Figure 4).

The second test measured the HDL-mediated cholesterol efflux from J774A.1 macrophages. POPC/FC particles with no apolipoprotein increased the cholesterol efflux from macrophages by ~2-fold as compared to the levels of cholesterol efflux in cells incubated with medium only (Figure 5). PON1 bound to POPC/FC particles increased the cholesterol efflux by an additional 12%. rHDL particles composed of apoA-I and apoE3 increased the macrophage cholesterol efflux by ~4-fold as compared to that of cells incubated in medium, and apoE4-rHDL increased the cholesterol efflux by ~3-fold. When rePON1 was added to apoA-I-rHDL, the extent of cholesterol efflux was further increased by 58% as compared to that of apoA-I-rHDL. The addition of rePON1 to apoE3-rHDL or apoE4-rHDL resulted in an additional increase of 24 or 56%, respectively, relative to the same rHDL samples without PON1. In comparison to the effect of the apoA-I-rHDL−PON1 complex, there was a reduction of 17 or 27% in the stimulation of cholesterol efflux upon using the apoE3-rHDL−PON1 or apoE4-rHDL−PON1 complex, respectively. The similar cholesterol efflux efficiency of the apoE3- and apoE4-rHDL−PON1 complexes is not surprising as apoE isoforms show similar binding for ABCA1 and exhibit identical kinetics in their abilities to induce ABCA1-dependent cholesterol efflux (*37*).

DISCUSSION

The anti-atherogenic properties of PON1 have been primarily associated with apoA-I-HDL (*38*−*42*). Indeed, the antioxidative and anti-atherogenic activities of HDL are largely attributed to apoA-I (*15*, *41*, *43*). ApoA-I promotes cellular cholesterol efflux, as opposed to apoA-II, for example (*44*). Nonetheless, plasma HDL particles contain a variety of apolipoproteins with anti-atherogenic potentials that could also stimulate the action of PON1. ApoE, for example, may protect against atherosclerosis by mediating the clearance of lipoproteins via the LDL receptor (*45*, *46*), or via binding to cell surface heparan sulfate proteoglycans (*47*). This study indicates that PON1 binds to apoE3- and apoE4-HDL particles with affinities and modes similar to those of apoA-I-HDL. However, our results also indicate that these apoEs show a significantly lower capacity to stabilize PON1 and to induce its anti-atherogenic potential (as indicated by the inhibition of LDL oxidation and induction of cholesterol efflux from macrophages).

A recent report on the mechanism of apoE binding to lipoproteins indicated differences in the ability to be accommodated on the lipoprotein surface (*48*). ApoE4 requires more interfacial area and favors VLDL, whereas apoE3 is primarily accommodated by small HDL_3 particles. Further, although apoE3 renders protection against cardiovascular risks, the apoE4 isoform is associated with the risks for atherosclerosis and Alzheimer's disease (*46*). However, our studies showed no substantial or consistent differences in the mode and effects of interactions of PON1 with these two forms. We also tried to include the rarest allelic form of apoE, apoE2, yet for reasons that remain unclear at present, we failed to express and purify this isoform in *E. coli*. Future studies might indicate whether PON1's interaction with apoE2 resembles the interactions with apoE3 and apoE4, as expected by their similar lipoprotein binding preferences (*46*).

Our results also shed some light on the mode of interaction of PON1 with HDL. Structural and biochemical studies indicated that HDL anchoring of PON1 is mediated by the N-terminal helix H1, and an additional amphipathic helix H2 that comprises part of the active site (*49*). The interface of PON1 with HDL is thought to involve an aromatic belt rich in tryptophan and tyrosine side chains and a lysine side chain on H1 (*49*). Clearly, the composition of HDL with respect to phospholipids influences the level of PON1 activity associated with HDL (*50*), and large differences in the ability of various apolipoproteins to stabilize and catalytically stimulate PON1 have been observed in vitro (*13*). However, why different apolipoproteins exhibit different effects on PON1 is at present unknown. The similar properties of apoA-I and apoE may,

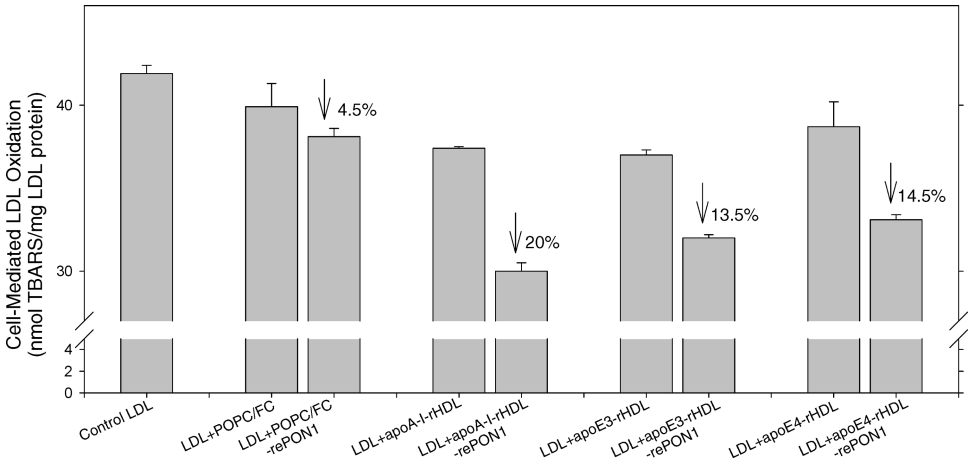

FIGURE 4: Inhibition of cell-mediated LDL oxidation. J774A.1 macrophages were incubated with LDL in the absence (control LDL) or presence of rHDL samples composed of apoA-I, apoE3, apoE4, or POPC/FC only, with or without rePON1. LDL oxidation was induced by $CuSO_4$. The extent of LDL oxidation was determined after 2.5 h. Each bar represents the mean and SD of three independent experiments. Arrows above the bars designate the increase in activity observed in the rHDL−rePON1 samples relative to the same rHDL particles without rePON1.

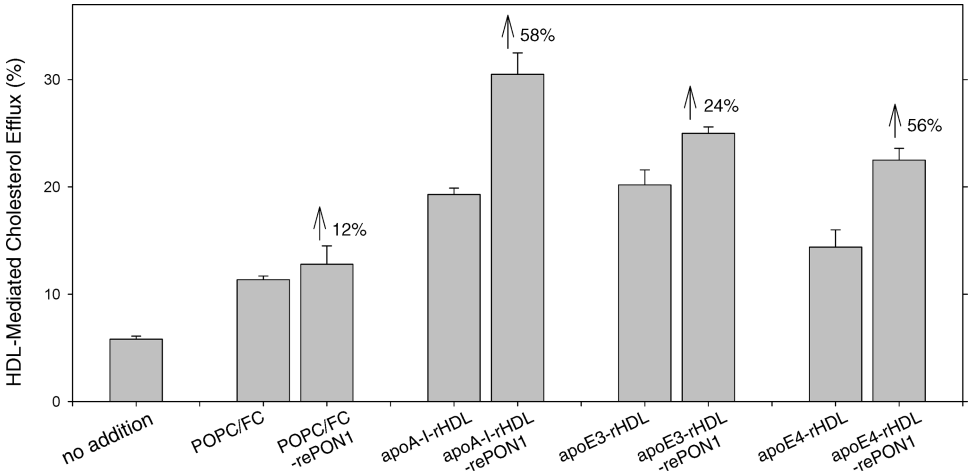

FIGURE 5: Stimulation of macrophage cholesterol efflux. J774A.1 macrophages, prelabeled with [^{3}H]cholesterol, were incubated with rHDL samples composed of apoA-I, apoE3, apoE4, or POPC/FC only with or without rePON1. The extent of rHDL-mediated cholesterol efflux was determined after incubation for 3 h at 37 °C. Each bar represents the mean and SD of three independent experiments. Arrows above the bars designate the increase in activity observed in the rHDL−rePON1 samples relative to the same rHDL particles without rePON1.

however, provide a clue regarding the mode of interaction. Exchangeable apolipoproteins including apoA-I and apoE are members of the same multigene family (*51*). ApoA-I and apoE adopt similar conformations in which the amino- and carboxyl-terminal regions form separate domains (*52*). The carboxyl-terminal domain is composed of a series of proline-punctuated amphipathic α-helical repeats and is primarily responsible for lipid binding. In both apolipoproteins, the lipid-free form is a helix bundle, and lipid binding involves a conformational change whereby the hydrophobic faces of the helixes that are buried in the bundle become accessible to interactions with the lipids (*53*−*55*). That both apoA-I and apoE induce high-affinity binding of PON1 suggests that, despite the relatively high affinity (≤1 nM), recognition is not based on specific protein−protein interactions between PON1 and the protein component of HDL but, rather, on certain surface properties of the particles. These properties might be similar as both apolipoproteins wrap around HDL particles via amphipathic α-helices. The divergence in sequence of the two apolipoproteins may account for the differences seen in PON1 stability and anti-atherogenic potential.

The hypothesis described above is also supported by the previous findings showing that apoA-IV-HDL binds PON1 with nanomolar affinity similar to that of apoA-I-HDL, although its ability to stimulate the lactonase activity of PON1 was significantly weaker than that of apoA-I-HDL, and not much stronger than that of POPC/FC particles with no apolipoprotein (*13*). Similar to apoA-I and apoE, apoA-IV contains multiple 22-amino acid repeats punctuated by proline residues that are predicted to form amphipathic α-helices (*52*, *56*). ApoA-IV also exhibits a large helix bundle domain that is similar to the N-terminal helix bundle domain of apoA-I and apoE (*56*). Indeed, apoA-IV can mimic certain roles of apoA-I, including inhibition of LDL oxidation, mediation of cholesterol efflux, and activation of lecithin cholesterol acyltransferase (LCAT) (*15*, *52*, *57*). Further, PON1 activity in the apoA-I deficient mice was associated with the VHDL fractions that contain, among other apolipoproteins, apoA-IV (*58*), suggesting that these particles can act as secondary PON1 carriers (*59*).

In contrast to these apolipoproteins, the structure of apoA-II and its contribution to lipid metabolism are considerably different. The highly hydrophobic nature of apoA-II and its

association with smaller HDL particles probably cause a dramatic reduction in the binding affinity of PON1 (≥60-fold), and in the stimulation of its lactonase activity (*13*). Interestingly, PON1 is present in vivo in both apoA-I-containing and apoA-I- and apoA-II-containing subfractions of HDL with no detrimental effects of apoA-II on PON1 activity and stability (*5*). Thus, while apoA-II alone has a negative impact on PON1, it may complement the stabilizing effect of apoA-I if not present at excess levels, probably through its ability to stabilize HDL structure (*60*).

On the basis of the comparison given above of the effects of different apolipoproteins on PON1 binding to HDL and the comparison of its biochemical properties, it can be hypothesized that the presence of amphipathic α-helices of approximately 22 amino acids is the key structural feature that enables HDL particles to tightly bind, stabilize, and enzymatically stimulate PON1. It can be suggested that other apolipoproteins or peptide mimetics having similar amphipathic structure and mode of interaction with lipids will also generate HDL particles with high PON1 affinity. Indeed, apoA-I and apoJ (clusterin) mimetics composed of short amphipathic peptides taken from apolipoprotein sequences promote the formation of HDL particles with increased PON1 activity (*61*). Yet, despite the similarly high binding affinity of various apolipoprotein-containing HDL particles, the most efficient PON1 stabilization and enzymatic stimulation is generated by apoA-I-HDL, with which PON1 is mainly associated in vivo. The effects of apoA-I are also manifested in a higher anti-atherogenic potential as indicated by the ex vivo tests.

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