Deletion of Amino Acids Glu146→Arg160 in Human Apolipoprotein A-I (ApoA-$I_{Seattle}$) Alters Lecithin:Cholesterol Acyltransferase Activity and Recruitment of Cell Phospholipid†

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ABSTRACT: Human apolipoprotein A-I (apoA-I) has an important role in the efflux of cholesterol from peripheral cells, the first step in reverse cholesterol transport. Deletion of amino acids Glu146→Arg160 in apoA-I (apoA-$I_{Seattle}$) removes a large section of a lipid binding helix and is associated in vivo with an atherogenic lipoprotein profile characterized by a deficiency in high-density lipoproteins (HDL). In the present study, we asked whether apoA-$I_{Seattle}$ had normal ability to recruit lipids from cells and to form nascent high-density lipoprotein (HDL) particles and whether the altered secondary structure affected lecithin:cholesterol acyltransferase (LCAT) activity. Wild-type apoA-I and apoA-$I_{Seattle}$ expressed in transfected Chinese hamster ovary cells formed nascent HDL particles with similar density distribution and protein-to-lipid ratio. Phospholipid subclass distribution of apoA-$I_{Seattle}$ nascent HDL demonstrated a significant increase in sphingomyelin and phosphatidylethanolamine compared to wild type. ApoA-$I_{Seattle}$ nascent HDL had a unique size distribution compared to wild-type nascent HDL; large (9−20 nm) particles predominated while there were virtually no small (7.5 nm) particles. LCAT reactivity was impaired by apoA-$I_{Seattle}$ nascent HDL where cholesterol esterification was only half that of wild-type complexes. The apoA-$I_{Seattle}$ conformation on nascent HDL was studied with a panel of monoclonal antibodies (Mabs) specific for apoA-I. Mabs that recognize the putative LCAT activation site, residues 95−122, had normal reactivity. As expected, the Mabs that recognized residues 141−164 were unreactive because of the 146−160 deletion; in addition, there was low reactivity with a Mab that recognizes residues 220−242. The data suggest that apoA-I residues 146−160 and/or 220−242 partake in normal LCAT activation and that cooperative interactions between helices may be important for maximal cholesterol esterification.

Plasma levels of high-density lipoproteins (HDL)[1] and its major protein constituent apolipoprotein A-I (apoA-I) are inversely related to the risk of development of coronary artery disease (CAD). Human wild-type apoA-I (apoA-I_{WT}) is a 243 amino acid protein containing a series of 11 and 22 amino acid repeats, some with a high degree of homology. The repeats form class A amphipathic helices (*1*) with a high binding capacity for lipids, thus making it possible for apoA-I to recruit phospholipids and cholesterol from cells. The highest lipid binding capacity is associated with helices toward the carboxy terminus of the protein (*2*). ApoA-I also serves as an activator for the plasma enzyme lecithin: cholesterol acyltransferase (LCAT). LCAT has antiatherogenic properties; it esterifies cholesterol on HDL, thus increasing the capacity of HDL to accept cholesterol from peripheral cells. Studies with monoclonal antibodies have identified a region in the amino acid sequence 95−122 (*3*, *4*) as the putative LCAT activation site of apoA-I.

We (*5*) and others (*6*) have demonstrated that lipid-free apoA-I can recruit phospholipid and cholesterol from cell membranes. In Chinese hamster ovary (CHO) cells, lipid recruitment results in the formation of nascent HDL with a unique phospholipid composition (*5*). These nascent HDL activate LCAT as judged by the esterification of cholesterol associated with the particles. For these reasons, it has been suggested that apoA-I plays an important role in preventing macrophage cholesterol deposition and foam cell formation.

Numerous natural mutations of apoA-I have been reported in human subjects. Nonsense mutations including truncation (*7*) or a changed reading frame (*8*) result in very low levels of apoA-I and are associated with an increased risk for premature CAD (*9*). Several point mutations in apoA-I have been described; such mutations are associated with low levels of plasma apoA-I and HDL but are not necessarily associated

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[1] Abbreviations: apoA-I, apolipoprotein A-I; apoA-$I_{Seattle}$, apoA-I(ΔGlu146−Arg160); apoA-I_{WT}, wild-type apoA-I; CAD, coronary artery disease; CHO cells, Chinese hamster ovary cells; HDL, high-density lipoprotein(s); LCAT, lecithin:cholesterol acyltransferase; LDL, low-density lipoprotein(s); PC, phosphatidylcholine; PE, phosphatidylethanolamine; SM, sphingomyelin.

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with increased risk for CAD. One frequently studied mutation, apoA-I_{Milano} (Arg173→Cys), results in low plasma HDL levels (30% of normal), but affected individuals seem to have little or no risk for CAD (*10*). Another mutation, Lys107→0 (apoA-$I_{Helsinki}$), induces a moderate decrease in HDL cholesterol and possibly an increased risk for CAD (*11*). Unlike the above mutations, a Leu159→Pro substitution (apoA-$I_{Zavalla}$) is associated with extremely low levels of plasma HDL and a significantly increased risk for premature CAD (*12*).

How each mutation in apoA-I affects HDL levels and properties is not fully determined. Recent studies from our laboratory with CHO cells expressing apoA-I_{Milano} revealed that this point mutation, compared to apoA-I_{WT}, is associated with a decreased ability to recruit cholesterol from cells as demonstrated by formation of unusually small nascent HDL particles with a low cholesterol-to-phospholipid ratio (*13*). However, this mutation did not impair LCAT activation.

The apolipoprotein Glu146→Arg160 deletion mutation, the apoA-$I_{Seattle}$ mutant, was described by Deeb and co-workers (*14*) in a heterozygote proband with high levels of plasma cholesterol and triacylglycerol and low levels of HDL cholesterol (only 15% of normal). The plasma apoA-I level in the Seattle proband was 12% of control whereas LCAT mass was decreased to about one-third of normal. The 15 amino acid deletion in the apoA-$I_{Seattle}$ mutation removes a large section of one of apoA-I's amphipathic helices which may alter the overall secondary structure of apoA-I. In the present study, we examined if this deletion affects apoA-$I_{Seattle}$'s ability to function in two key antiatherogenic events: the recruitment of lipids from cell membranes and the activation of LCAT.

MATERIALS AND METHODS

Expression of ApoA-$I_{Seattle}$ and ApoA-I_{WT}. The Seattle mutation (deletion of residues Glu146→Arg160) was prepared (Mutagen Kit, Bio-Rad) in a 2.2 kb *Sma*I fragment of the human apoA-I gene. The mutation was confirmed by DNA sequencing and the gene inserted into the pcDNA3 expression vector. CHO K1 cells were stably transfected with the construct by electroporation. Clonal cell lines were prepared by the infinite dilution method, and a high-expressing clone was selected. The CHO cell line PMT AIR 143 expressing human apoA-I_{WT} was used to generate control apoA-I complexes as previously described (*15*).

Isolation of Nascent HDL. Cells constitutively expressing apoA-$I_{Seattle}$ were grown in McCoy's medium with 10% Fetal Clone II (Hyclone Laboratories, Logan, UT) at 37 °C in an atmosphere of 5% CO_2/95% air. In a typical experiment, confluent cells in 40, 265 cm^2 expanded surface flasks were rinsed 3 times with Hank's balanced salt solution and incubated overnight with serum-free McCoy's medium to remove residual serum lipoproteins. The latter medium was removed and fresh serum-free McCoy's medium added. Conditioned medium was collected after 24 and 48 h. Cell debris was removed by centrifugation 10 min at 1000g. The medium was supplemented with 2.7 mM EDTA, 0.5 mM phenylmethanesulfonyl fluoride (PMSF), and 50 μg/mL gentamicin. Conditioned medium was concentrated 100-fold using a Minitan ultrafiltration system (Millipore Corp., Bedford, MA) fitted with a 10 000 MW cutoff cellulose membrane. Lipoprotein fractions, d < 1.063 g/mL and 1.063−1.21 g/mL ("nascent HDL"), were isolated from conditioned medium by sequential ultracentrifugation (*16*). Samples were dialyzed against saline/EDTA (0.15 mM NaCl, 2.7 mM EDTA), pH 7.4, for further analyses.

Chemical Analyses. The protein content of CHO cells pooled from several flasks was determined with Lowry analyses as described by Markwell (*17*). The protein content of the nascent HDL fraction was measured with a Bradford protein reagent (Bio-Rad Laboratories, Hercules, CA). Cholesterol was measured by the method of Salé et al. (*18*). Phospholipids were extracted and subclasses were separated with thin-layer chromatography (TLC) on Silica gel H plates (chloroform/methanol/water/acetic acid 100:60:16:8). Total phospholipid and phospholipid subclasses were quantified as described by Bartlett (*19*).

Quantification of ApoA-I. The amounts of apoA-I in the lipoprotein fractions and in the infranatant lower density fractions were quantified by radial immunodiffusion using polyclonal antisera against apoA-I_{WT} (*20*). The amount of "floating" apoA-I, i.e., material of d < 1.21 g/mL, was calculated as percent of total media apoA-I.

Size Distribution, Agarose Gel Electrophoretic Mobility, and Morphology of Nascent HDL. ApoA-$I_{Seattle}$ protein was identified with SDS−polyacrylamide gel electrophoresis (4−20%) with apoA-I_{WT} as standard (*21*). Nascent HDL particle size distribution was determined by nondenaturing (4−30%) polyacrylamide gradient gel electrophoresis essentially as described by Nichols et al. (*22*). Relative charge properties of apoA-$I_{Seattle}$ and wild-type complexes were determined on agarose gels (Beckman, Paragon Lipogel) with human HDL and apoA-I_{WT} for comparison. The morphology of nascent HDL was assessed by negative-staining electron microscopy as described previously (*23*).

LCAT Reactivity of Nascent HDL Assembly Products. LCAT was purified from human plasma by the method of Chen and Albers (*24*). Nascent-HDL particles were labeled with [^{14}C]cholesterol and incubated with purified LCAT enzyme as previously described (*13*). Saturating substrate concentrations of nascent HDL were used to yield maximal rates of [^{14}C]cholesterol esterification. Reactions also contained LDL as an exogenous source of cholesterol to ensure substrate concentrations were not limiting. Nascent HDL (0.15 mg of cholesterol/mL) were incubated (4 °C) with [^{14}C]-cholesterol-labeled LDL (0.30 mg of cholesterol/mL) for 5 days to allow equilibration of [^{14}C]cholesterol with nascent HDL; the specific activity of the LDL was 1 mCi of [^{14}C]-cholesterol/mg of LDL unesterified cholesterol. Rates of [^{14}C]cholesterol esterification were evaluated in reaction mixtures (0.8 mL final volume) containing 0.05 mg of cholesterol/mL of nascent HDL, 0.1 mg of cholesterol/mL of LDL, 1.5% human serum albumin, 50 mg/mL gentamicin, and sufficient purified LCAT to esterify 7 nmol of cholesterol per hour. At specified times, aliqouts were removed and cooled to 4 °C, and lipids were extracted with hexane. Cholesterol and cholesteryl ester in lipid extracts were separated by TLC, and the radioactivity associated with each was quantified by liquid scintillation counting. Results were expressed as a percentage of the initial [^{14}C]cholesterol which was esterified.

In one set of studies, the extent of cholesterol esterification in LCAT reactions was quantified by measuring the mass

of cholesteryl ester being formed. For these experiments, unlabeled nascent HDL was incubated with purified LCAT exactly as described above except LDL was omitted from reactions. After 24 h, unesterified cholesterol and cholesteryl ester were quantified via the enzymatic procedure described by Salé (*18*).

Antibody Binding to Nascent HDL. Competitive solid phase immunoassays were used to assess the binding of monoclonal antibodies to the isolated nascent HDL. These antibodies, which are specific for human apoA-I, have been described previously and their epitopes on apoA-I_{WT} documented (*25*, *26*). The immunoassays were performed as described (*26*). Briefly, isolated human plasma HDL (50 μL of 5 μg/mL) were immobilized onto 96-well Falcon 3911 Microtest III flexible assay plates. After coating, increasing amounts of nascent apoA-$I_{Seattle}$ or apoA-I_{WT} complexes (25 μL) diluted in PBS with 3% normal goat serum were added to wells in the presence of ascites fluid (25 μL) containing a limiting amount of monoclonal antibody (typically a 1×10^{-4} to 1×10^{-6} dilution). Competitor concentrations listed in the figures represent the final concentrations (μg/mL) in the 50 μL reaction mixture. The plates were incubated overnight at 4 °C, and, after washing, antibody binding to the immobilized plasma HDL antigen was detected by incubation at 37 °C with ^{125}I-labeled goat anti-mouse IgG for 1 h. Data are expressed as B/B_0 where B is the cpm bound in the presence of competitor, and B_0 is the cpm bound in the absence of competitor. To compare the affinity of an antibody for apoA-I_{WT} or apoA-$I_{Seattle}$ nascent HDL competitors, the slopes of the straight lines drawn through logit-transformed B/B_0 ratios were obtained by linear regression and subjected to tests of equality.

Statistics. Statistical significance was calculated with unpaired Student's *t*-test.

RESULTS

Secretion of ApoA-$I_{Seattle}$ from Transfected CHO Cells. The CHO clonal cell line used in these studies expressed relatively high levels of apoA-$I_{Seattle}$; secretion rates were 4.6 ± 1.6 μg of A-I/mg of cell protein/24 h ($n = 6$) compared to 1.7 ± 0.7 μg/mg of cell protein/24 h ($n = 7$) for cells expressing apoA-I_{WT}. Western blots of SDS−PAGE gels demonstrated, as expected, that the nascent HDL formed by apoA-$I_{Seattle}$ contained a protein with a slightly lower molecular weight than apoA-I_{WT} (data not shown).

We have previously shown that CHO cells secreting wild-type apoA-I form lipid/protein complexes, i.e., nascent HDL, in the medium that float at $d < 1.21$ g/mL (*5*, *15*). To determine whether apoA-$I_{Seattle}$ has the ability to form lipidated complexes, the percent of secreted apoA-$I_{Seattle}$ floating in conditioned media at $d < 1.21$ g/mL was quantified and compared with that of wild type. Ultracentrifugal isolation procedures revealed that 21 ± 6% ($n = 6$) of apoA-$I_{Seattle}$ protein was associated with lipid compared to 16 ± 2% ($n = 6$) for apoA-I_{WT}; this difference was not statistically significant. These results indicate that the 15 amino acid deletion in apoA-$I_{Seattle}$ did not impair its ability to recruit membrane lipids and to form nascent HDL.

Composition and Morphology of ApoA-$I_{Seattle}$ Nascent HDL Particles. The protein and lipid compositions of apoA-I_{WT} and apoA-$I_{Seattle}$ nascent HDL are quite similar as seen in Table 1. ApoA-$I_{Seattle}$ nascent HDL have an unesterified cholesterol to phospholipid mole ratio of 0.89 ± 0.14, compared to 1.03 ± 0.10 for apoA-I_{WT} nascent HDL, suggesting normal recruitment of cholesterol.

Table 1: Composition (Mass Percent) of ApoA-$I_{Seattle}$ and ApoA-I_{WT} Nascent HDL Isolated from Conditioned CHO Cell Medium after 24 h Incubation[a]

	protein	cholesterol	phospholipid
apoA-I_{WT}	34.4 ± 4.6	25.2 ± 0.7	40.4 ± 4.9
apoA-$I_{Seattle}$	30.7 ± 3.9	24.0 ± 5.2	45.3 ± 4.5

[a] Complexes were isolated at density 1.063−1.21 g/mL. Data represent mean ± SD of 5 experiments.

Table 2: Phospholipid Subclass Distribution (Mass Percent) of ApoA-$I_{Seattle}$ and ApoA-I_{WT} Nascent HDL Isolated from CHO Cell Medium after 24 h Incubation[a]

	PC	SM	PE	lyso-PC
apoA-I_{WT}	79.8 ± 2.1	14.9 ± 1.0	5.3 ± 1.4	ND
apoA-$I_{Seattle}$	62.8 ± 4.5***	24.0 ± 5.5**	11.4 ± 5.4*	1.8 ± 1.6

[a] Complexes were isolated at density 1.063−1.21 g/mL. Data represent mean ± SD of 5 experiments. PC, phosphatidylcholine; SM, sphingomyelin; PE, phosphatidylethanolamine; lyso-PC, lysophosphatidylcholine; ND, not detectable. (***) $P < 0.005$; (**) $P < 0.001$; (*) $P < 0.05$, compared to apoA-I_{WT} nascent HDL.

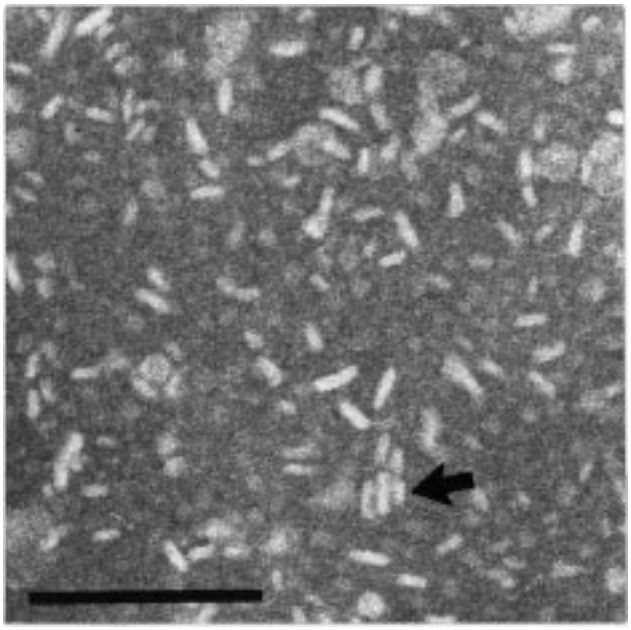

FIGURE 1: Electron micrograph of apoA-$I_{Seattle}$ nascent HDL isolated at density 1.063−1.21 g/mL from CHO cell conditioned medium after 24 h incubation. The bar marker represents 100 nm. The arrow points out a "stack" of discoidal particles with varying diameter.

The phospholipid composition of apoA-$I_{Seattle}$ nascent HDL is significantly different from that of apoA-I_{WT} complexes (Table 2). Sphingomyelin (SM) and phosphatidylethanolamine (PE) content is approximately 2-fold greater in apoA-$I_{Seattle}$ than in apoA-I_{WT} nascent HDL particles. Phosphatidylcholine, on the other hand, is significantly reduced in the apoA-$I_{Seattle}$ nascent HDL particles.

The electron micrograph (Figure 1) shows that the apoA-$I_{Seattle}$ nascent HDL are discoidal in shape; long narrow images "discs on edge" which in some instances form short stacks of disks are noted (arrow). Few, if any, spherical particles were noted. The appearance of discoidal particles

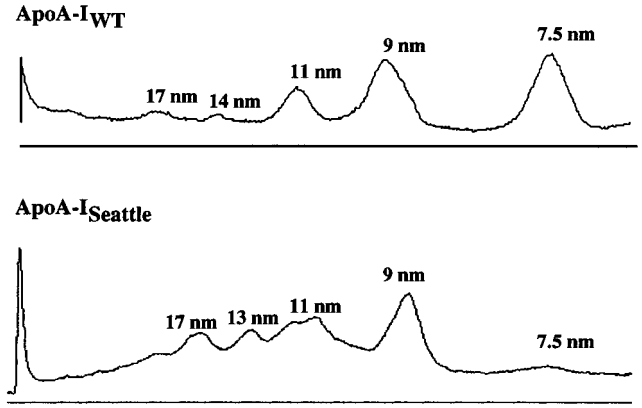

FIGURE 2: Size distribution of nascent HDL particles isolated at density 1.063−1.21 g/mL from CHO cell conditioned medium after 24 h incubation. ApoA-I$_{Seattle}$ and wild-type apoA-I nascent HDL particle size was determined by nondenaturing polyacrylamide gradient (4−30%) gel electrophoresis. The figure show scans of representative Coomassie G-250 stained gels.

is consistent with the composition of the particles which contain no core lipids, as previously described for discoidal apoA-I$_{WT}$ complexes isolated from conditioned CHO cell medium (*15*).

Relative Charge Properties of ApoA-I$_{Seattle}$ Complexes. We have previously demonstrated that apoA-I$_{WT}$ nascent HDL have pre-alpha mobility on agarose gels (*27*). ApoA-I$_{Seattle}$ nascent HDL had pre-alpha mobility similar to apoA-I$_{WT}$ nascent particles (data not shown), suggesting that the amino acids deleted in apoA-I$_{Seattle}$ did not affect the overall charge of the nascent particles.

Size Distribution of ApoA-I$_{Seattle}$ Nascent HDL. Nondenaturing gradient gel electrophoresis was carried out to determine the particle size distribution of nascent HDL. A representative gradient gel profile (Figure 2) shows major differences between the mutant and the wild-type nascent particles. Unlike apoA-I$_{WT}$ complexes that have well-defined peaks at 7.5 nm (39% of total peak area), 9 nm (31%), and 11 nm (16%) diameters, the apoA-I$_{Seattle}$ nascent HDL have virtually no 7.5 nm (5%) complexes but exhibit larger sized complexes, i.e., 13 nm (15%), 17 nm (16%), and 20 nm (7%) nm complexes in addition to 9 nm (28%) and 11 nm (28%) complexes.

LCAT Reactivity of ApoA-I$_{Seattle}$ Nascent HDL. The rate of ^{14}C-cholesterol esterification was measured over a 24 h period (Figure 3). Throughout the incubation period, LCAT activity was lower with apoA-I$_{Seattle}$ nascent particles as substrate than with wild type although the difference was not significant until the 24 h time point; activation after 24 h was nearly 2-fold higher with apoA-I$_{WT}$ particles than with apoA-I$_{Seattle}$ nascent HDL. This difference was confirmed by direct measurement of cholesteryl ester formed by incubation of the nascent HDL with purified LCAT for 24 h. Under these conditions, 8.8 ± 5.1% of cholesterol in nascent apoA-I$_{Seattle}$ particles (n = 6) was converted to cholesteryl ester compared with 24.2 ± 5.9% (n = 5) conversion for apoA-I$_{WT}$ (P < 0.005). These data demonstrate that cholesterol esterification is impaired in apoA-I$_{Seattle}$ nascent HDL.

ApoA-I Epitope Expression on ApoA-I$_{Seattle}$. Because LCAT activity was impaired by the deletion mutation, we asked whether specific epitopes in apoA-I$_{Seattle}$ were affected

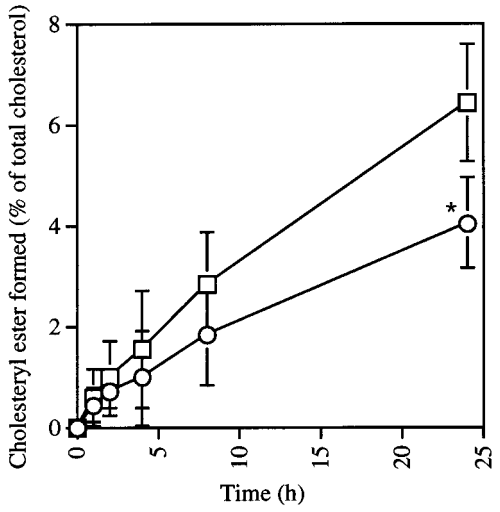

FIGURE 3: LCAT activity with apoA-I$_{Seattle}$ (open circles) compared to apoA-I$_{WT}$ (open squares) nascent HDL labeled with [^{14}C]-cholesterol as substrate. Enzyme activity is shown as cholesteryl ester formed at each time point. Data represent mean ± SD of 3 experiments. Asterisks P < 0.05.

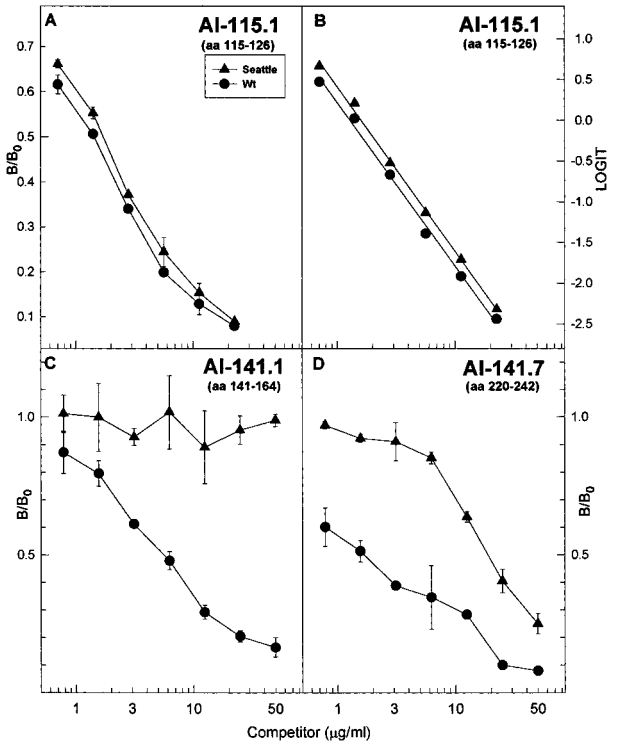

FIGURE 4: Reactivity of human apoA-I specific monoclonal antibodies to apoA-I$_{Seattle}$ (filled triangles) and apoA-I$_{WT}$ (filled circles) nascent HDL compared to human HDL. (A) Antibody AI-115.1 binding. (B) Logit-transformed function of the A-I 115.1 binding curves to confirm that the affinity (slope) of this antibody for the two apoA-I competitors is the same. (C) Antibody AI-141.1 binding; the antibody only binds to apoA-I$_{WT}$ particles. (D) Antibody AI-141.7 binding; reactivity with A-I$_{Seattle}$ nascent HDL is severely reduced.

by putative changes in structure. It is possible that the deletion affects the protein conformation not only directly surrounding the missing residues but also further from it. A panel of monoclonal antibodies (Mabs) specific for apoA-I$_{WT}$ were tested for their reactivity with apoA-I$_{Seattle}$ and apoA-I$_{WT}$ nascent HDL. The results presented in Figure 4 indicate that the conformation of the N-terminal end of apoA-

$I_{Seattle}$ is only marginally affected by the deletion mutation. There was no significant difference in the affinity for Mabs AI-1.2 (residues 1−19), AI-11 (residues 96−111), AI-4 (residues 99−121), AI-115.1 (residues 115−126; Figure 4A,B), or AI-119.1 (residues 119−144) between apoA-$I_{Seattle}$ and apoA-I_{WT}; all of these except AI-1.2 have earlier been shown to have reactivity with the putative LCAT activation site (*25*, *26*). Two Mabs binding to epitopes close to, or at, the deleted sequence in apoA-$I_{Seattle}$, Mabs AI-137.1 (residues 137−147) and AI-141.1 (residues 141−164; Figure 4C), showed little or no reactivity for apoA-$I_{Seattle}$. The Seattle deletion mutation also appeared to induce a change in conformation of the C-terminal portion of the molecule, because Mab AI-141.7 (residues 220−242) had considerably lower reactivity for apoA-$I_{Seattle}$ than for apoA-I_{WT} (Figure 4D).

DISCUSSION

We demonstrated that, even though nascent HDL formed by apoA-$I_{Seattle}$ had a similar protein-to-lipid ratio as apoA-I_{WT} nascent HDL, there was a significant difference in phospholipid recruitment where sphingomyelin (SM) and phosphatidylethanolamine (PE) were elevated compared to wild type. This suggests that loss of amino acids Glu146→Arg160, and thereby loss of one lipid binding helix, alters the interaction of the protein with membrane phospholipids. In addition to changes in phospholipid composition, we noted that LCAT reactivity was significantly decreased with apoA-$I_{Seattle}$ nascent HDL compared to wild type, suggesting that loss of this lipid binding helix induces conformational changes that can impair LCAT activation.

The cholesterol-to-phospholipid mole ratio of nascent apoA-$I_{Seattle}$ complexes is compatible with the formation of discoidal particles noted in this study; similar discoidal structures were previously reported for apoA-I_{WT} nascent particles isolated from CHO cell medium (*15*). The particle size distribution of apoA-$I_{Seattle}$ determined by nondenaturing gradient gel electrophoresis was different from that of wild type; that is, there were proportionally greater numbers of larger complexes. Deletion of amino acids 146→160 may alter the conformation of apoA-I so that formation of larger complexes is favored. However, we cannot rule out the possibility that it is the unusual phospholipid composition that affects formation and stabilization of larger sized protein−lipid complexes. The increase in SM and PE is noteworthy because it suggests that changes in the primary structure of the lipid binding domains of apoA-I can alter the selectivity for membrane phospholipids, which in turn may alter functional aspects of HDL.

A major finding in the present study was that apoA-$I_{Seattle}$ impaired LCAT activity compared to apoA-I_{WT}. We have repeatedly seen low levels of cholesteryl ester in apoA-$I_{Seattle}$ nascent HDL after incubation with LCAT, and this is consistent with a study by Sorci-Thomas and co-workers (*28*), where each apoA-I helical repeat was sequentially deleted and the removal of all but two (amino acids 44−64 and 88−98) repeats resulted in significantly decreased LCAT activation. Deletion of amino acids 143−164 or 165−186 resulted in 2.6% or 2.0% of apoA-I_{WT} LCAT activation, respectively (*28*). Studies with peptides mimicking apoA-I helices have shown that two amphipathic helices joined by a β-turn are necessary for full LCAT activation (*29*), suggesting that cooperativity between the helices is a requirement for optimal cholesterol esterification by LCAT. It has been suggested (*30*) that LCAT inserts a helix between two of the helices in apoA-I. By doing so, the LCAT enzyme may anchor itself to the nascent HDL and access the lipid substrate in an optimal conformation for enzyme activity. In apoA-$I_{Seattle}$ nascent HDL, this putative close fit between activator (apoA-I) and enzyme may have been altered, thus impairing LCAT activation. However, we cannot exclude the possibility that the unusual phospholipid composition of the nascent apoA-$I_{Seattle}$ particles also contributed to the low LCAT reactivity. A negative correlation between SM content and LCAT reactivity has been demonstrated in proteoliposomes (*31*) and in reconstituted HDL containing SM (*32*). Because SM is elevated in apoA-$I_{Seattle}$ nascent particles compared to wild type, decreased LCAT reactivity using apoA-$I_{Seattle}$ nascent HDL as substrate may, in part, be associated with the elevated SM/PC ratio in these particles.

ApoA-I epitope studies were performed to determine whether there are major structural changes in the apoA-$I_{Seattle}$ molecule compared to wild type. Mabs that recognize the putative LCAT activation site, e.g., AI-4, surprisingly showed no difference in binding to apoA-$I_{Seattle}$ compared to wild type, suggesting that this site is normally exposed on apoA-$I_{Seattle}$. At first glance, this appears to be a contradiction to the observation that LCAT activity is impaired in apoA-$I_{Seattle}$. One possible interpretation is that, because of conformational changes induced by the absence of amino acid sequence 146→160, the helix containing amino acids 95−122 may be oriented in such a way that the insertion of a helix from LCAT into apoA-I and the formation of a tight bundle of this helix with two helices from apoA-I cannot occur optimally. As expected, Mabs close to the deletion site had reduced binding, confirming the loss of structural integrity at this site. Interestingly, there was a considerable decrease in binding of Mab AI-141.7 to apoA-$I_{Seattle}$ compared to apoA-I_{WT}. This antibody recognizes apoA-I amino acids 220−242, suggesting that the deletion of amino acids 146→160 has far reaching conformational effects at the C-terminal end of the molecule.

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