Unique Epitope of Apolipoprotein A-I Expressed in Pre-β-1 High-Density Lipoprotein and Its Role in the Catalyzed Efflux of Cellular Cholesterol†

Phoebe E. Fielding,*,‡ Mikihiko Kawano,‡ Alberico L. Catapano,§ Adele Zoppo,§ Santica Marcovina,∥ and Christopher J. Fielding⊥

Cardiovascular Research Institute and Departments of Medicine and Physiology, University of California, San Francisco, California 94143-0130, Institute of Pharmacological Sciences, University of Milan, Milan, Italy, and Department of Medicine, Northwest Lipid Research Laboratories, University of Washington, Seattle, Washington 98103

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ABSTRACT: The ability of mouse anti-apolipoprotein A-I (apo A-I) monoclonal antibodies to recognize pre-β-HDL species in native plasma was determined. An antibody identifying residues 137–144 of the mature protein uniquely recognized pre-β-1 HDL, an HDL species of low molecular weight implicated in early cholesterol transport from cell membranes to plasma [Castro, G. R., & Fielding, C. J. (1988) *Biochemistry 27*, 25–29]. Incubation of plasma with this antibody significantly inhibited the efflux of labeled cholesterol from cultured fibroblast monolayers. A second antibody, binding to residues 93–99 of apo A-I, recognized a second pre-β-HDL species (pre-β-2 HDL) but not pre-β-1 HDL and did not inhibit cholesterol efflux. Several other antibodies had broad specificity for HDL (including pre-β-1 HDL). This research suggests that apo A-I residues 137–144 are adjacent to or part of a structural site in pre-β-1 HDL active in promoting the efflux of cellular cholesterol and that this site is not exposed in other HDL species.

The high-density lipoprotein (HDL) fraction of human plasma is highly heterogeneous. While all HDL contain at least one copy of apolipoprotein A-I, its major protein, they differ in the number of apo A-I polypeptides, the presence or absence of other apolipoproteins, and the ratio of protein to lipids (Fielding & Fielding, 1991). HDL of otherwise similar compositions can also differ in electrophoretic mobility, as changes in protein conformation modify net charge (Sparks & Phillips, 1992).

Among HDL, a minor fraction of small HDL containing only apo A-I and with an unusual slow (pre-β-) electrophoretic mobility appears to play a special role in the transfer of labeled cholesterol out of cell membranes into plasma (Castro & Fielding, 1988; Kawano et al., 1993). This radioactive cholesterol subsequently appears in a larger, probably discoidal pre-β-migrating HDL species, pre-β-2 HDL. The further metabolism of this cholesterol by lecithin–cholesterol acyltransferase (LCAT) can drive the reverse transport of cholesterol from the peripheral tissues (Fielding & Fielding, 1981; Davis et al., 1982; Miller et al., 1985).

Several laboratories have used monoclonal antibodies to identify exposed epitopes of apo A-I either in synthetic discoidal recombinant HDL or in the major spherical HDL fraction isolated from plasma by centrifugation (Curtis & Smith, 1988; Marcovina et al., 1990; Marcel et al., 1991; Collect et al., 1991). Centrifugation is now known to remove pre-β-HDL from the spherical HDL fraction (Asztalos et al., 1993). No studies have been reported on the structure of HDL in native plasma or specific fractions such as pre-β-HDL.

In this research, we have used anti-apo A-I monoclonal antibodies to map several epitopes of pre-β-1 HDL as well as other HDL in plasma and to correlate structure with function in the promotion of cholesterol efflux from cell membranes. Because of the instability of pre-β-1 HDL (Ishida et al., 1990; Neary et al., 1991; Miida et al., 1992; Asztalos et al., 1993), we have developed a procedure to assay epitopes of individual HDL species in native plasma.

EXPERIMENTAL PROCEDURES

Blood was taken from normal donors into ice-cooled plastic tubes containing streptokinase (150 IU/mL) as an anticoagulant (Miida et al., 1992). Following centrifugation (0–2 °C, 20 min, 2000*g*), the supernatant plasma was recovered and used immediately in the experiments described below.

Production and Characterization of Monoclonal Antibodies (Mabs). Antibodies used in this study were obtained from the injection of isolated delipidated human apo A-I into Balb/c mice (Marcovina et al., 1990). Cloned cells were injected into pristane-primed mice. Ascites fluid was purified by protein A–agarose affinity chromatography (Bio-Rad, Richmond, CA).

The sequence within apo A-I recognized by each antibody was determined by solid-phase pin technology (Cambridge Research Biochemicals, Cambridge, U.K.) (Geysen et al., 1984) using overlapping nonapeptides synthesized on polypropylene pins from Fmoc–amino acid derivatives as described in the manufacturer's instructions. Pins were incubated with the corresponding monoclonal antibody, and bound antibody was detected with peroxidase-labeled rabbit anti-mouse IgG. Assignment to an epitope of 4–10 amino acids was possible in each case (Table 1). In one case (Mab-16), interaction with a second sequence was also detected. This probably identifies a nonlinear or complex epitope. A similar finding was reported previously for several other anti-apo A-I monoclonal antibodies (Marcel et al., 1991). The other antibodies used in this study recognized only a single epitope.

Nondenaturing Two-Dimensional Electrophoresis. The procedure used was slightly modified from one previously described in detail (Castro & Fielding, 1988). Plasma (20 μL) was first electrophoresed in 0.75% w/v agarose in 50 mM

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‡ Department of Medicine, University of California.
§ Institute of Pharmacological Sciences.
∥ Northwest Lipid Research Laboratories.
⊥ Department of Physiology, University of California.

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Table 1: Assignment of Epitopes Recognized by Anti-apo A-I Monoclonal Antibodies[a]

antibody	major epitope	secondary interaction
Mab-9	LQEKLSPL (137–144)	
Mab-15	VKAKVQP (93–99)	
Mab-16	ELYRQKVEPLRAELQE (113–128)	LSPLGEEM (141–148)
Mab-19	DEPPQSPWDR (1–10)	
Mab-57	SDELRQRL (167–174)	

[a] Epitopes were identified for each antibody in terms of reactivity with overlapping nonapeptides covering the apo A-I primary sequence as described under Experimental Procedures. Immobilized peptide was reacted with Mab and detected with peroxidase-labeled rabbit anti-mouse IgG read at 405 nm. Absorbance with sequences indicated was greater by >5-fold than with any other apo A-I sequence.

barbital buffer (pH 8.6). In the second dimension, separation was on a 2–15% w/v gradient of polyacrylamide gel in 0.025 M Tris–glycine buffer (pH 8.3). A vertical 1-cm divider was placed midway across the top of the gradient gel. A 1-cm sample of low-melting agarose (SeaPlaque, FMC, Rockland, ME) containing 20-100 μg of purified monoclonal antibody was formed over half of the gradient gel. As a control, 1 cm of the same agarose gel containing mouse nonimmune IgG was formed over the other half of the gel. Gels were run for 1 h (200 V) at 4 °C and then for 3 h (400 V) at the same temperature. HDL migrating in the second dimension passed through the antibody-containing agarose layer before being fractionated on the polyacrylamide gradient. After completion of electrophoresis, the separated proteins in the gel were electrotransferred overnight to nitrocellulose (Sartorius, 0.45 μm). Apo A-I-containing lipoproteins were detected with rabbit anti-human apo A-I polyclonal antibody, which had been purified on protein G–agarose and then labeled with ^{125}I (Markwell, 1982). Individual labeled nitrocellulose areas were identified by autoradiography on Kodak XOMAT AR film at −70 °C and then excised. Contained radioactivity was then determined by γ spectrometry. Radioactivity in each HDL species, including pre-β-1 and pre-β-2 HDL as well as several subfractions of α-migrating HDL, was measured after interaction with each monoclonal antibody. This was compared with the label recovered in the same HDL species when nonspecific mouse IgG at the same concentration was used in the control half of the gel in place of monoclonal antibody and assayed simultaneously. In some experiments, electrotransferred nitrocellulose sheets were incubated with goat anti-mouse IgG labeled with ^{125}I as described above. The location of specific Mab-apo A-I complexes was then determined by autoradiography.

Cell Experiments. Cultured human skin fibroblasts were grown in 3.5-cm dishes in 10% fetal calf serum. Prior to utilization (48 h) in studies of cellular cholesterol efflux, the dishes (containing 2–2.5 μg of cell cholesterol) were labeled with 0.05 mCi of [^3H]-1,2-cholesterol complexed with fetal calf serum (Miida et al., 1992). Unlabeled plasma (0.3 mL) was preincubated (16 h, 0–2 °C) either with nonimmune mouse IgG or with purified monoclonal antibody IgG (150 μg) in a final volume of 0.43 mL. Electrophoresis of this mixture as described above indicated complete removal of predicted pre-β HDL target fractions.

To measure the rate of cholesterol efflux into unlabeled plasma, dishes were washed (×5) with phosphate-buffered saline (pH 7.2) and then incubated with 0.43 mL of native plasma–IgG mixture for 1–3 min at 37 °C. Duplicate samples were taken at 1, 2, and 3 min for analysis of contained radioactivity, using β-scintillation spectrometry. Cell cholesterol specific activity (1.5–2.5 × 10^6 cpm μg^{-1}) was determined following the extraction of lipids with chloroform and methanol (1/1, v/v). Cholesterol mass was measured fluorimetrically (Fielding, 1984), and cholesterol radioactivity was measured as described above. The rate of cholesterol efflux was linear as a function of time. In some experiments, synthetic peptide corresponding to residues 136–150 of mature apo A-I (H-ELQEKLSPLGEEMRD-OH) (Research Genetics, Huntsville, AL) was included in the incubation medium.

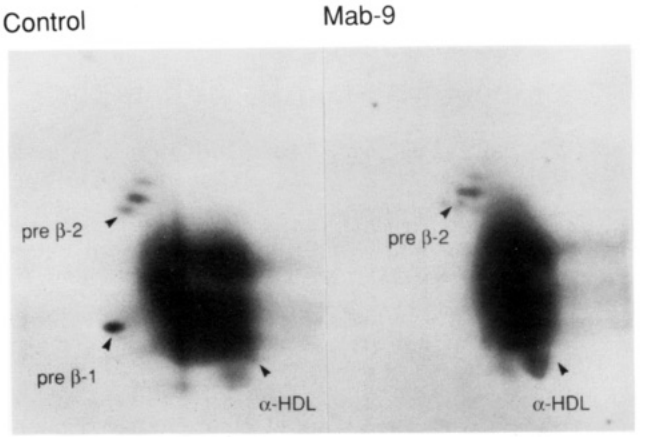

FIGURE 1: Two-dimensional electrophoresis of plasma passed through nonimmune IgG (left) or Mab-9 (right). HDL species were electrotransferred after separation, and apo A-I-containing lipoproteins were identified with ^{125}I-labeled rabbit anti-apo A-I IgG as described under Experimental Procedures. A sample of 100 μg of antibody was included in the 1.0 mL of low-melting agarose solution added above the polyacrylamide gradient. The nomenclature of pre-β-HDL species is that of Castro and Fielding (1988).

RESULTS

Recognition of Pre-β-1 HDL by Anti-apo A-I Mabs. Pre-β-1 HDL consisted as previously described (Castro & Fielding, 1988) of one to two closely migrating HDL species. Two-dimensional electrophoresis of plasma through antibody or nonimmune IgG indicated that some antibodies completely removed the whole pre-β-1 HDL, while others were completely ineffectual. As shown in Figure 1, when Mab-9 was present pre-β-1 HDL was selectively removed from plasma. There was no detectable loss of other species by the antibody. As the concentration of antibody in the gradient gel was increased, the proportion of pre-β-1 HDL decreased (Figure 2) so that at maximum concentration (100 μg gel^{-1}) no detectable pre-β-1 HDL remained. This finding indicated that in all pre-β-1 HDL the sequence of apo A-I between residues 137 and 144 of the mature protein was accessible for recognition by antibody Mab-9. It also suggested that this sequence was not accessible on other HDL species (Figure 1). Several other antibodies recognized pre-β-1 HDL as well as other HDL fractions. Mab-19 and Mab-57 recognized pre-β-1 HDL as well some of the major α-migrating HDL fraction. On the other hand, antibody Mab-15 did not recognize pre-β-1 HDL at any antibody concentration tested. This suggested that residues 93–99 of mature apo A-I, the sequence recognized by this antibody, was inaccessible in pre-β-1 HDL. Mab-16, which recognized residues 113–128 and 141–148 of apoA-I, completely removed pre-β-1 but unlike Mab-9 also recognized some HDL within the α-migrating subfraction (Figure 3).

Recognition of Pre-β-2 HDL by Anti-apo A-I Mabs. Like pre-β-1 HDL, pre-β-2 HDL consists of several closely adjacent apo A-I-containing species following nondenaturing two-dimensional electrophoresis (Castro & Fielding, 1988).

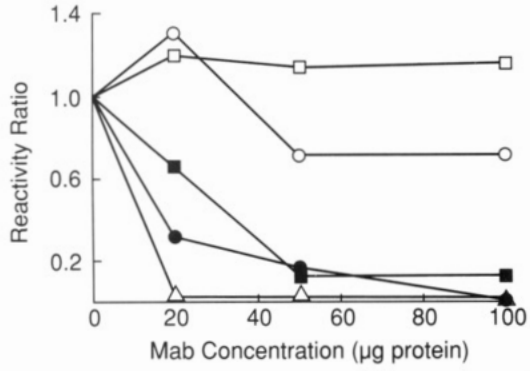

FIGURE 2: Effects of antibody concentration on the proportion of pre-β-1 HDL removed during electrophoresis. Antibody concentration was as described in the legend to Figure 1. This proportion is expressed as a reactivity ratio (cpm in pre-β-1 HDL with Mab/cpm in pre-β-1 HDL with nonimmune IgG). The reactivity ratio represents the proportion of original radioactivity in each HDL species displaced in the presence of antibody. Mab-9, closed squares; Mab-15, open squares; Mab-16, open triangles; Mab--19, open circles; Mab-57, closed circles.

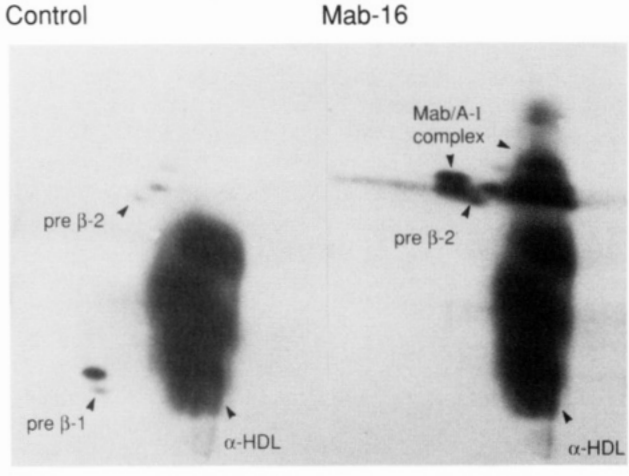

FIGURE 3: Two-dimensional electrophoresis of plasma passed through nonimmune IgG (left) or Mab-16 (right). Separation and detection of HDL species was as described in the legend to Figure 1.

Epitopes recognized on pre-β-2 HDL differed significantly from those recognized on pre-β-1 HDL by the same antibodies. All species of pre-β-2 HDL in native plasma reacted with antibody Mab-15, which had no reaction with pre-β-1 HDL. The vertical shift of pre-β-2 HDL in reaction with Mab-15, seen as the new slowly migrating species in Figure 4 (right panel), was confirmed by identifying the complex with ^{125}I-labeled goat anti-mouse IgG. Mab-9 and Mab-16, which recognized pre-β-1 HDL, had no reaction with this larger pre-β-HDL species, even at the highest antibody concentrations tested (Figure 5). Mab-19 and Mab-57 recognized pre-β-2 HDL in addition to other pre-β- and α-migrating HDL species. The reaction of Mab-19 with pre-β-1 and pre-β-2 HDL was markedly less effective at the same antibody concentration than Mab-57.

Recognition of α-HDL Fractions by Anti-apo A-I Monoclonal Antibodies. Several of the antibodies tested complexed apo A-I from α-HDL. The relative affinity of the major α-migrating HDL subfractions (HDL_3, HDL_{2a}, and HDL_{2b}) (Anderson et al., 1977; Miida et al., 1990) was estimated from the proportions of each remaining as the concentration of antibody in the gel was increased. As shown in Table 2, there was no significant difference in the proportion of label recovered in the gel (compared to that recovered in the presence of nonimmune IgG) in the three α-HDL species, which

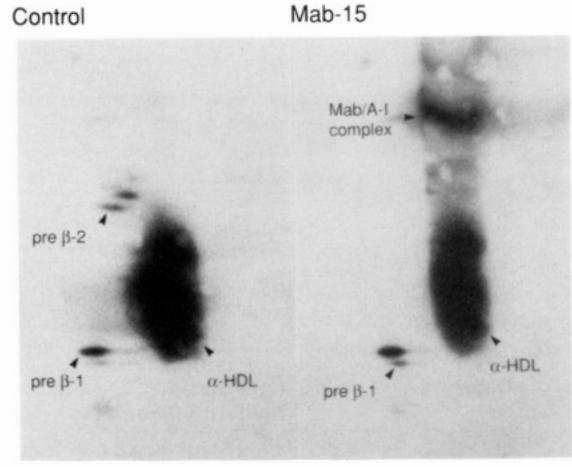

FIGURE 4: Two-dimensional electrophoresis of plasma passed through nonimmune IgG (left) or Mab-15 (right). Antibody concentration, separation, and detection of HDL species were as described in the legend to Figure 1.

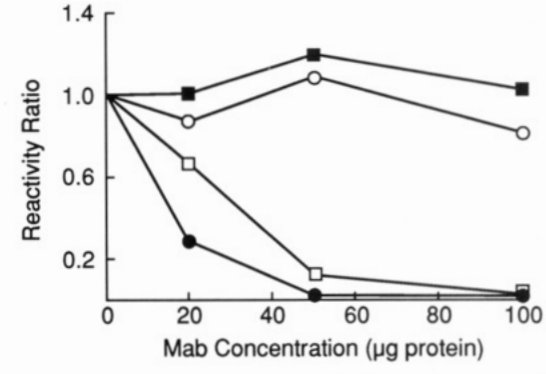

FIGURE 5: Effects of antibody concentration on the proportion of pre-β-2 HDL removed during electrophoresis. The reactivity ratio for pre-β-2 HDL was determined as described in the legend to Figure 2. Mab-9, closed squares; Mab-15, open squares; Mab-19, open circles; Mab-57, closed circles. Pre-β-2 HDL can be identified in gels containing Mab-16, but the presence of retarded material from α-HDL nearby prevents its accurate estimation.

Table 2: Effects of Anti-apo A-I Monoclonal Antibodies on α-HDL Fractions in Native Plasma[a]

antibody	HDL_3	HDL_{2a}	HDL_{2b}
Mab-9	0.95 ± 0.24	1.02 ± 0.16	1.06 ± 0.20
Mab-15	0.91 ± 0.32	0.80 ± 0.04	0.97 ± 0.03
Mab-16	1.17 ± 0.32	0.88 ± 0.14	0.96 ± 0.10
Mab-19	0.93 ± 0.19	0.98 ± 0.05	0.93 ± 0.15
Mab-57	0.89 ± 0.34	0.93 ± 0.07	0.95 ± 0.09

[a] Values shown are means ± 1 SD of three to four experiments and represent the ratio between ^{125}I-radioactivity recovered in each HDL region from gels containing monoclonal antibody and gels containing nonimmune IgG. The proportion of radioactivity in the α-HDL fractions of control gels was 0.26, 0.42, and 0.26 for HDL_3, HDL_{2a}, and HDL_{2b}, respectively.

together accounted for about 90% of the total HDL, including particles whose diameters vary between 7.5 and 11.5 nm (Anderson et al., 1977; Miida et al., 1990; Asztelos et al., 1993). These HDL species also differ significantly in lipid/protein ratio, but this appeared not to influence the reactivity of these antibodies with α-migrating HDL.

In summary, with the monoclonal antibodies used in this study, pre-β-1 and pre-β-2 HDL particles differed in epitopes expressed both from each other and from the major α-HDL fraction; while α-HDL did not differ appreciably among themselves.

Table 3: Effects of Anti-apo A-I Monoclonal Antibodies on Cholesterol Efflux from [^{3}H]Cholesterol Fibroblast Monolayers

antibody	relative rate of efflux[a]
Mab-9	0.63 ± 0.02[b]
Mab-15	0.97 ± 0.10
Mab-16	0.65 ± 0.07[c]
Mab-19	0.95 ± 0.14
Mab-57	1.03 ± 0.03

[a] Cholesterol efflux was determined relative to the rate observed when cells were incubated with plasma containing nonimmune IgG (150 μg in 0.5 mL of plasma) in place of mouse anti-human apo A-I IgG. Efflux was determined over 3 min at 37 °C as described under Experimental Procedures and was linear over the assay period. Efflux rate in the absence of antibody was 17.9 ± 6.7 ng min^{-1} mL^{-1}, comparable to previous values (Kawano et al., 1993). Values shown are means ● 1 SD for four to five experiments. [b] $p < 0.01$ relative to control. [c] $p < 0.02$ relative to control.

Effects of Anti-apo A-I Mabs on Cholesterol Efflux. Pre-β-1 HDL appears to play a major role in the early processing of cell-derived cholesterol (Castro & Fielding, 1988; Hara & Yokoyama, 1991; Kawano et al., 1993). Pre-β-2 HDL was involved in subsequent processing, while α-HDL played a relatively minor role in the initial efflux of cholesterol into native plasma.

Plasma was preincubated (16 h, 0–2 °C) with monoclonal antibodies to apo A-I or with nonimmune IgG. This mixture was then incubated at 37 °C with [^{3}H]cholesterol labeled fibroblast monolayers. Compared to the rate of efflux measured with nonimmune IgG, cholesterol efflux was significantly reduced (–37%, $p < 0.02$) in the presence of Mab-9, and similarly (–35%, $p < 0.01$) in the presence of Mab-16 (Table 3). There was no significant reduction in the rate of efflux under the same experimental conditions by either Mab-57 and Mab-19, which had broad specificity among HDL, nor by Mab-15, which recognized pre-β-2 HDL. Combinations of antibodies at the same concentration resulted in no significant further decrease in efflux. When synthetic polypeptide corresponding to residues 136–150 of apo A-I was included in the culture medium at a 100-fold molar excess relative to Mab-9, the decrease in cholesterol efflux became insignificant (–3%, $p > 0.05$). These data provide further evidence of the specific involvement of residues 137–144 of apo A-I in cholesterol efflux in this system.

DISCUSSION

Monoclonal antibodies raised against apo A-I identify epitopes whose expression can differ significantly depending on the composition of HDL (Marcel et al., 1991). Significant differences have been reported in the expression of individual epitopes as the lipid composition of α-HDL was modified in vitro (Collet et al., 1991). A different type of heterogeneity is identified here, in which individual pre-β-HDL species were recognized in plasma. This was not observed in an earlier study with these antibodies (Marcovina et al., 1990), probably because the centrifugal isolation procedure used is now known to remove pre-β-HDL from the HDL density fraction (Asztelos et al., 1993).

It is well recognized that apo A-I, in common with other apolipoproteins of related sequence, contains a series of 22 amino acid repeats with predicted amphipatic α-helical conformation separated by β-turns (Segrest et al., 1992). In synthetic discoidal HDL complexes, convincing physical data has been obtainned to indicate that apo A-I has a serpentine form in which six or seven consecutive 22 amino acid repeats are aligned antiparallel with their hydrophobic faces turned to lipid with the bulk of charged residues directed out to the aqueous phase (Sparks et al., 1992). Ionic interactions between adjacent strands are believed to stabilize this structure at the edge of the lipid disc (Brasseur et al., 1990). When apo A-I is disposed on the surface of spherical (α-migrating) HDL, evidence from monoclonal antibody studies supports the concept [originally proposed by Cheung et al. (1987)] that the central part of the polypeptide chain forms a flexible or "hinge" region responsive to local changes in the lipid environment of the particle (Collet et al., 1991). The N- and C-terminal regions of the 243 amino acid apo A-I polypeptide chain appear to be much less ordered. Two different regions of apo A-I implicated in the LCAT reaction with discoidal recombinant HDL [residues 95–121, Banka et al. (1991); residues 148–186, Minnich et al. (1992)] are within this central region.

The unique epitope now identified in pre-β-1 HDL by Mab-9 (residues 137–144) and implicated in the efflux of cholesterol from cell membranes to pre-β-1 HDL is also within the central part of apo A-I. Because pre-β-1 HDL is much smaller and more lipid-poor than discoidal HDL, it is not surprising that the spatial organization of apo A-I in this particle should differ significantly from that of other HDL. This epitope makes up the predicted β-turn between two adjacent amphipathic helical segments, which is presumably displaced from its orientation in discoidal or spheroidal HDL owing to the smaller content of lipid in pre-β-1 HDL. Residues 137–144 include two charged amino acids. Rather than itself representing a cholesterol binding site, this sequence is more likely sterically adjacent to such a site, whose three-dimensional structure could include strands from several discrete sequences of the apo A-I polypeptide chain. It is also possible that interaction of Mab-9 with residues 137–144 results in a conformational change at a more distant site. This last explanation appears less likely because of the highly ordered structure predicted for apo A-I in lipoprotein complexes (Cheung et al., 1987).

A protease-sensitive site unique to pre-β-HDL located near residue 120 may also contribute to this structure (Kunitake et al., 1990). A site in the C-terminal half of apo A-I that is involved in the binding of apo A-I to cell-surface sites may represent another contributor (Allen et al., 1993). When Mab-9 interacted with its epitope, the inhibition of cholesterol efflux was significant but incomplete. Earlier studies from this laboratory suggested that 55–60% of total efflux from cell membranes involved pre-β-1 HDL (Kawano et al., 1993). The inhibition of efflux (37%) associated with the binding of Mab-9 to pre-β-1 HDL is thus about half of the maximum predicted. A second antibody (Mab-16), which has a similar effect, recognizes sequences 113–128 and 141–148; the latter overlaps the sequence recognized by Mab-9, but whether residues 113–128 constitute an additional functional region cannot be determined from the antibodies presently available. Two other antibodies that also bound pre-β-1 HDL as well as to other HDL (Mabs 19 and 57) did not inhibit cholesterol transfer to the medium. These data, taken together, clearly indicate that the inhibition associated with the binding of Mab-9 and Mab-16 is not a nonspecific effect of antibody-HDL binding but identifies a region of apo A-I functional in cholesterol efflux in pre-β-1, the major initial acceptor of cell-derived cholesterol in native plasma.

A different epitope (residues 93–99) is exposed in pre-β-2 but not in pre-β-1 HDL. Based on its molecular weight and composition, pre-β-2 HDL is likely to represent a discoidal HDL, comparable to the synthetic HDL recombinants studied by several laboratories. In native plasma, pre-β-2 HDL did

not play a significant role as an initial acceptor of cell-derived cholesterol (Castro & Fielding, 1988). It is therefore not surprising that Mab-15, which recognizes this sequence, did not inhibit cholesterol efflux into native plasma. Discoidal HDL, like spherical HDL, phospholipid dispersions, and lipoprotein surfaces in general at sufficient concentration, will bind cell-derived cholesterol by a nonspecific probably diffusional pathway to the extent of about 40% of total efflux to native plasma (Kawano et al., 1993). Such transfer is generally not linked to esterification via the LCAT reaction and, as a result, probably contributes less effectively to cholesterol net transport. The present study suggests that the efflux of cholesterol mediated by the pre-β-1 HDL-dependent and nonspecific pathways to plasma HDL may involve not only different lipoprotein particles but also different sequences in apo A-I.

Measurement of reactivity of pre-β-1 HDL with other monoclonal or site-directed antibodies, together with data from physical techniques such as [^{13}N]lysine NMR spectroscopy (Sparks & Phillips, 1992) and computer modeling (Brasseur et al., 1990) can all be expected to contribute to a fuller three-dimensional picture of pre-β-1 HDL. The present study identifies a unique epitope of pre-β-1 HDL which appears to be closely linked to its major physiological function, the early transfer of cell membrane cholesterol, and provides a starting point for such structural studies.

REFERENCES

Allan, C. M., Fidge, N. H., Morrison, J. R., & Kanellos, J. (1993) *Biochem. J. 290*, 449–455.

Anderson, D. W., Nichols, A. V., Forte, T. M., & Lindgren, F. T. (1977) *Biochim. Biophys. Acta 493*, 55–68.

Asztalos, B. F., Sloop, C. H., Wong, L., & Roheim, P. S. (1993) *Biochim. Biophys. Acta 1169*, 291–300.

Banka, C. L., Bonnet, D. J., Black, A. S., Smith, R. S., & Curtiss, L. K. (1991) *J. Biol. Chem. 266*, 23886–23892.

Brasseur, R., De Muetter, J., Vanloo, B., Goormaghtigh, E., Ruysschaert, J. M., & Rosseneu, M. (1990) *Biochim. Biophys. Acta 1043*, 245–252.

Castro, G. R., & Fielding, C. J. (1988) *Biochemistry 27*, 25–29.

Cheung, M. C., Segrest, J. P., Albers, J. J., Cone, J. T., Brouillette, C. G., Chung, B. H., Kashyap, M., Glasscock, M. A., & Anantharamaiah, G. M. (1987) *J. Lipid Res. 28*, 913–929.

Collet, X., Perret, B., Simard, G., Raffai, E., & Marcel, Y. L. (1991) *J. Biol. Chem. 266*, 9145–9152.

Curtis, L. K., & Smith, R. S. (1988) *J. Biol. Chem. 263*, 13779–13785.

Davis, R. A., Helgerud, P., Dueland, S., & Drevon, C. A. (1982) *Biochim. Biophys. Acta 689*, 410–414.

Fielding, C. J. (1984) *Methods Enzymol. 111*, 267–274.

Fielding, C. J., & Fielding, P. E. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 3911–3914.

Fielding, P. E., & Fielding, C. J. (1991) In *Biochemistry of Lipids, Lipoproteins and Membranes* (Vance, D. E., & Vannce J., Eds.) pp 427–459, Elsevier Press, Amsterdam.

Geysen, H. M., Meloen, R. H., & Barteling, S. J. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 3998–4002.

Hara, H., & Yokoyama, S. (1991) *J. Biol. Chem. 266*, 3080–3086.

Ishida, B. Y., Albee, D., & Paigen, B. (1990) *J. Lipid Res. 31*, 227–236.

Kawano, M., Miida, T., Fielding, C. J., & Fielding, P. E. (1993) *Biochemistry 32*, 5025–5028.

Kunitake, S. T., Chen, G. C., Kung, S. F., Schilling, J. W., Hardman, D. A., & Kane, J. P. (1990) *Arteriosclerosis 10*, 25–30.

Marcel, Y. L., Provost, P. R., Koa, H., Raffai, E., Dac, N. V., Fruchart, J.-C., & Rassart, E. (1991) *J. Biol. Chem. 266*, 3644–3653.

Marcovina, S., Fantappie, S., Zoppo, A., Franceschini, G., & Catapano, A. L. (1990) *J. Lipid Res. 31*, 375–384.

Markwell, M. A. K. (1982) *Anal. Biochem. 125*, 427–432.

Miida, T., Fielding, C. J., & Fielding, P. E. (1990) *Biochemistry 29*, 10469–10474.

Miida, T., Kawano, M., Fielding, C. J., & Fielding, P. E. (1992) *Biochemistry 31*, 11112–11117.

Miller, N. E., La Ville, A., & Crook, D. (1985) *Nature 314*, 109–111.

Minnich, A., Collet, X., Roghani, A., Cladaras, C., Hamilton, R. L., Fielding, C. J., & Zannis, V. I. (1992) *J. Biol. Chem. 267*, 16553–16560.

Neary, R., Bhatnagar, D., Durrington, P., Ishola, M., Arrol, S., & Mackness, M. (1991) *Atherosclerosis 89*, 35–48.

Segrest, J. P., Jones, M. K., De Loof, H., Brouillette, C. G., Venkatachalapathi, Y. V., & Anantharamaiah, G. M. (1992) *J. Lipid Res. 33*, 141–166.

Sparks, D. L., & Phillips, M. C. (1992) *J. Lipid Res. 33*, 123–130.

Sparks, D. L., Phillips, M. C., & Lund-Katz, S. (1992) *J. Biol. Chem. 267*, 25830–25838.